Neuropsychopharmacology (2017) 42, 1458–1470

www.neuropsychopharmacology.org

The Trace Amine-Associated Receptor 1 Agonist RO5256390 Blocks Compulsive, Binge-like Eating in Rats

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Compulsive, binge eating of highly palatable food constitutes a core feature of some forms of obesity and eating disorders, as well as of the recently proposed disorder of food addiction. Trace amine-associated receptor 1 (TAAR1) is a highly conserved G-protein-coupled receptor bound by endogenous trace amines. TAAR1 agonists have been shown to reduce multiple behavioral effects of drugs of abuse through their actions on the mesocorticolimbic system. In this study, we hypothesized that TAAR1 may have a role in compulsive, binge-like eating; we tested this hypothesis by assessing the effects of a TAAR1 agonist, RO5256390, in multiple excessive feeding-related behaviors induced by limiting access to a highly palatable diet in rats. Our results show that RO5256390 blocked binge-like eating in rats responding 1 h per day for a highly palatable sugary diet. Consistent with a palatability-selective effect, drug treatment selectively reduced the rate and regularity of palatable food responding, but it did not affect either baseline intake or food restriction-induced overeating of the standard chow diet. Furthermore, RO5256390 fully blocked compulsive-like eating when the palatable diet was offered in an aversive compartment of a light/dark conflict box, and blocked the conditioned rewarding properties of palatable food, as well as palatable food-seeking behavior in a second-order schedule of reinforcement. Drug treatment had no effect on either anxiety-like or depressive-like behavior, and it did not affect control performance in any of the tests. Importantly, rats exposed to palatable food showed decreased TAAR1 levels in the medial prefrontal cortex (mPFC), and RO5256390 microinfused into the infralimbic, but not prelimbic, subregion of the mPFC-reduced binge-like eating. Altogether, these results provide evidence for TAAR1 agonism as a novel pharmacological treatment for compulsive, binge eating.
Neuropsychopharmacology (2017) **42,** 1458–1470; doi:10.1038/npp.2016.233; published online 2 November 2016

INTRODUCTION

Binge eating constitutes a core feature of forms of obesity and eating disorders (eg binge eating disorder, bulimia nervosa, and anorexia nervosa of the binge/purge type), as well as of the recently proposed disorder of food addiction (APA, 2013; Gearhardt *et al*, 2014). During a typical binge eating episode, individuals consume large amounts of highly processed, palatable foods in short periods of time, resulting in a very rapid eating behavior (APA, 2013; Micioni Di Bonaventura *et al*, 2014; Murray *et al*, 2014). Binge eating episodes also occur when not feeling physically hungry and can be triggered by conditioned environmental stimuli, even in absence of food *per se* (Giuliano and Cottone, 2015). Binge eaters experience lack of control over eating, an attribute which underlines the compulsive nature of this maladaptive feeding behavior (APA, 2013).

Trace amine-associated receptor 1 (TAAR1) is a G-protein-coupled receptor bound and activated by trace amines, a group of endogenous amines related to classical neurotransmitters, such as dopamine and serotonin (Borowsky *et al*, 2001; Bunzow *et al*, 2001; Burchett and Hicks, 2006; Grandy *et al*, 2016). TAAR1 is the only subtype of the TAAR family (TAAR1–9) to be phylogenetically conserved in all species studied, including humans, and it is sensitive to all trace amines (Borowsky *et al*, 2001; Bunzow *et al*, 2001; Lindemann and Hoener, 2005).

Growing evidence suggests that TAAR1 has a major role in regulating the behavioral actions of drugs of abuse, in particular those of psychostimulants. Indeed, both gene deletion and pharmacological studies have shown that TAAR1 activation reduces the reinforcing and rewarding effects of cocaine and methamphetamine, their locomotor stimulant effects, as well as reinstatement of seeking behavior

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Received 14 June 2016; revised 28 September 2016; accepted 30 September 2016; accepted article preview online 6 October 2016

(Harkness *et al*, 2015; Liu *et al*, 2016; Pei *et al*, 2015; Sukhanov *et al*, 2016; Thorn *et al*, 2014; Wolinsky *et al*, 2007). These actions are thought to occur via a TAAR1-mediated negative modulation of dopaminergic transmission in the mesocorticolimbic system (Espinoza *et al*, 2015; Miller *et al*, 2005; Xie *et al*, 2007); particularly, lack of TAAR1 has been shown to result in an elevated dopaminergic firing rate, whereas TAAR1 activation suppresses such firing (Bradaia *et al*, 2009; Lindemann *et al*, 2008; Revel *et al*, 2011). As a result, TAAR1 agonism has been proposed as a potential therapeutic option for addictive disorders (Pei *et al*, 2016).

On the basis of the above described observations, as well as the well-established role of dopaminergic transmission in food reward (Michaelides *et al*, 2012), we hypothesized that TAAR1 agonism may reduce compulsive, binge-like eating. To test this hypothesis, we first assessed whether the systemic activation of TAAR1 would block the excessive feeding-related behaviors induced by limiting access to highly palatable food in rats (Blasio *et al*, 2014; Velazquez-Sanchez *et al*, 2014). Next, we determined the role played by the mesocorticolimbic TAAR1 system in excessive eating of palatable food first by measuring TAAR1 expression levels in selected brain regions (ie, medial prefrontal cortex (mPFC), dorsal striatum, and nucleus accumbens) and, then, based on the information acquired, by site-specifically microinfusing the TAAR1 agonist into the relevant brain regions (ie, infralimbic (IL) and prelimbic (PrL) subregions of the mPFC).

MATERIALS AND METHODS

Subjects

One hundred sixty four male Wistar rats, 45-day old, and triple-housed upon arrival (200–225 g; Charles River, Wilmington, MA), were housed in a 12 : 12 h reverse light cycle (lights off at 1100 hours), in a humidity- and temperature-controlled vivarium. Rats were given access to corn-based chow (Harlan Teklad LM-485 Diet 7012 (65% (kcal) carbohydrate, 13% fat, 21% protein, 341 cal per 100 g); Harlan, Indianapolis, IN) and water *ad libitum*, unless otherwise specified. Experimental procedures were performed during rats' active cycle (dark cycle). Procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Boston University Medical Campus Institutional Animal Care and Use Committee (IACUC).

Drugs

The highly selective TAAR1 full agonist RO5256390 (Revel *et al*, 2012) was synthesized by F. Hoffman-La Roche. Doses for Experiment 1 (0, 1, 3, 10 mg/kg) were based on a previously published report (Pei *et al*, 2014). In the other experiments, the most effective dose from Experiment 1 was used. For intracranial administration, RO5256390 was administered at the doses of 0, 1.5, 5 and 15 μg per side; these doses were chosen based on preliminary observations obtained in our laboratory. For intraperitoneal (i.p.) administration, RO5256390 was freshly dissolved in 0.3% Tween 80 and 0.9% saline; for intracranial administration, RO5256390 was freshly dissolved in a mixture of ethanol: cremophor:saline (2 : 2 : 18 ratio). For within-subject drug testing, 2–4 intervening days were allowed, until subjects' performance returned to baseline conditions. All drug treatments were counterbalanced, unless otherwise specified.

Apparatus for Self-administration Procedures

The individual operant test chambers (Med Associates Inc., St Albans, VT) had a grid floor and were located in ventilated, sound-attenuating enclosures (Cottone *et al*, 2012; Smith *et al*, 2015). Food reinforcers were delivered by a pellet dispenser into a head entry magazine and water reinforcers by a solenoid into a head entry liquid cup magazine. Two retractable levers were placed on the opposite wall of the chamber. 28 V stimulus cue-lights were located above each lever and above the food magazine. In all the procedures, pellet acquisition occurred following a nose poke response, except for the food seeking experiment in which food pellets delivery occurred following responding on a lever based on a second order schedule of reinforcement.

Operant Binge-like Eating Procedure in *Ad libitum*-fed Rats

Training. As previously described (Blasio *et al*, 2014; Velazquez-Sanchez *et al*, 2014), rats were allowed to self-administer food pellets (45-mg precision pellets, 5TUM: 65.5% (kcal) carbohydrate, 10.4% fat, 24.1% protein, 330 cal per 100 g; TestDiet, Richmond, IN) and water (100 μl) during daily 1 h experimental sessions under a Fixed Ratio 1 (FR1) schedule of reinforcement in the operant chambers; head entry within the food magazine was detected by a photobeam, which resulted in the delivery of a food pellet within the same food magazine. A 1.0 sec timeout period, where no additional pellets were delivered, was used. Spillage is negligible (4.8% of total responses, $n = 23$). At the end of the self-administration procedure, rats were returned to their home cages and fed with the same standard chow diet provided in the operant procedure, but in a 5 g format Cottone *et al*, 2012.

Escalation of palatable food intake. After stable baseline training performance was achieved, rats were divided into a Chow control group, which received the same 45-mg chow pellets offered during the training phase, and a Palatable group, which received a nutritionally complete, chocolate-flavored, high sucrose (50% kcal) AIN-76A-based diet (45-mg precision pellets, chocolate-flavored, 5TUL: 66.7% (kcal) carbohydrate, 12.7% fat, 20.6% protein, metabolizable energy 344 cal per 100 g; formulated as 45-mg precision food pellets; TestDiet). It was previously shown that the 5TUL diet is strongly preferred over the standard chow diet by all rats (91.2% ± 3.7 preference; Cottone *et al*, 2008, 2009).

Experiment 1: effects of the TAAR1 agonist RO5256390 on operant binge-like eating. Rats in the Chow and Palatable food groups ($n = 12$ per group) were administered the TAAR1 agonist RO5256390 (0, 1, 3, 10 mg/kg, i.p.) 30 min prior to the operant sessions.

High Rate of Responding for Standard Chow Induced by Food Restriction

To reach a higher rate of responding for chow during the operant sessions, rats were food restricted in their home

cages for 12 days (to reach a total daily intake equal to 70% of a rat's daily intake; Cottone *et al*, 2012).

Experiment 2: effects of the TAAR1 agonist RO5256390 on high rate of responding for standard chow induced by food restriction. Food-restricted rats ($n=9$ per group) were administered the TAAR1 agonist RO5256390 (0, 10 mg/kg, i.p.) 30 min prior to the operant sessions.

Rate and Regularity of Sustained Eating: Inter-feeding Intervals and Return Map Analyses

Inter-feeding interval analysis. To identify between group differences in the rate and regularity of sustained eating, analysis of the ln-transformed duration of consecutive inter-feeding intervals (IFIs) was performed (Cottone *et al*, 2012). IFI, defined as the time between two consecutive food nosepoke responses, is a variable inversely correlated to eating rate, and it was automatically recorded by the computer. The mean, total IFI time, entropy, kurtosis, and skewness of the ln-transformed duration of each subject's consecutive IFIs were individually determined and then averaged across subjects.

Decreases in the IFI mean indicate a faster pellet-to-pellet responding and, therefore, an increased eating rate, which has been proposed to be an index of food palatability (Cottone *et al*, 2007; Iemolo *et al*, 2015). Decreases in the total IFI time mean a decrease in the total pellet-to-pellet time. Decreases in the histogram entropy (a measure of categorical variability, reflected in a decreased number of populated histogram bins, each with less similar event frequencies) indicate an increased regularity of intake. Conversely, an increase in the kurtosis of the IFI distribution (a measure of the distribution's 'peakedness', reflected in a more peaked top and smaller tails of the distribution) is consistent with an increased regularity of pellet-to-pellet responding. Finally, a significant increase in the skewness (a measure of the distribution's symmetry, reflected in a selective increase of the frequency of the IFI falling to the left of the histogram) is consistent with a selective increase of the fast pellet-to-pellet responding. Total IFI time was calculated as the sum of all the IFIs within a session.

Return map analysis. For return map analysis, each IFI in the time series was scatter-plotted against its subsequent IFI (IFI+1) in a Cartesian plane (Cottone *et al*, 2007; Iemolo *et al*, 2015).

Experiment 3: effects of the TAAR1 agonist RO5256390 on rate and regularity of sustained eating. Rats in the Chow, food-restricted Chow, and Palatable food groups ($n=7$–10 per group) were administered the TAAR1 agonist RO5256390 (0, 10 mg/kg, i.p.) 30 min prior to the operant sessions.

Compulsive-like Eating of Palatable Food: Light/Dark Box Conflict Test

In this test, a light/dark rectangular box ($50\times100\times35$ cm) was used, in which the aversive, bright compartment ($50\times70\times35$ cm) was illuminated by a 60 lux light. The dark compartment ($50\times30\times35$ cm) had an opaque cover and ~0 lux of light. The two compartments were connected by an open doorway, which allowed the subjects to move freely between the two. A shallow, metal cup containing a pre-weighed amount of the same food received during self-administration (45-mg chow or 45-mg chocolate pellets for rats in the Chow or Palatable food group, respectively) was positioned in the center of the light compartment. Apparatus-naive rats were habituated to an ante-room 2 h prior to testing. Under normal, control conditions, eating behavior is typically suppressed when a rat is in the aversive, bright compartment; a significant increase in food intake in spite of the adverse conditions, as compared with control conditions, was operationalized as a construct of 'compulsive-like eating' (Cottone *et al*, 2012; Velazquez-Sanchez *et al*, 2014).

Experiment 4: effects of the TAAR1 agonist RO5256390 on compulsive-like eating. Rats in the Chow and Palatable food groups ($n=21$–23 per group) were administered the TAAR1 agonist RO5256390 (0, 10 mg/kg, i.p.) 30 min prior to the testing session, using a between-subjects design.

Conditioned Food Reward: Conditioned Place Preference Test

The conditioned place preference (CPP) procedure was performed as previously described (Velazquez-Sanchez *et al*, 2015).

Experiment 5: effects of the TAAR1 agonist RO5256390 on conditioned food reward. Rats in the Chow and Palatable food groups ($n=16$–22 per group) were administered the TAAR1 agonist RO5256390 (0, 10 mg/kg, i.p.) 30 min prior to the post-conditioning phase, using a between-subjects design.

Food-seeking Behavior: Second-order Schedule of Reinforcement

The second-order schedule of reinforcement procedure was performed as previously described (Smith *et al*, 2015; Velazquez-Sanchez *et al*, 2015).

Experiment 6: effects of the TAAR1 agonist RO5256390 on food-seeking. Rats in the Chow and Palatable food groups ($n=6$–10 per group) were administered the TAAR1 agonist RO5256390 (0, 10 mg/kg, i.p.) 30 min prior to the second-order sessions.

Anxiety-like Behavior: Defensive Withdrawal Test

For this 10-min test (Cottone *et al*, 2009; Parylak *et al*, 2012), rats were placed into a withdrawal chamber (2 l Pyrex beaker wrapped in black tape) within an open field facing the rear. Latency to first emerge (all four paws in the open field) and withdrawal time were used as indices of anxiety-like behavior, although the number of entries into the chamber was used as an index of locomotor activity (Cottone *et al*, 2009; Parylak *et al*, 2012).

Experiment 7: effects of the TAAR1 agonist RO5256390 on anxiety-like behavior. Rats in the Chow and Palatable food groups ($n = 10$–12 per group) were administered the TAAR1 agonist RO5256390 (0, 10 mg/kg, i.p.) 30 min prior to the testing session, using a between-subjects design.

Depressive-like Behavior: Forced Swim Test

Rats were placed into a clear acrylic cylinder (25 cm diameter) filled with water (24 ± 1 °C; 42 cm deep) for 15 min during the pre-test day and 5 min during the test day 24 h later. Rats were administered the drug twice: at the end of the 15-min pre-test session, and again, 30 min prior to the test. Immobility time is a validated measure of depressive-like behavior (Seiglie *et al,* 2015).

Experiment 8: effects of the TAAR1 agonist RO5256390 on depressive-like behavior. Rats in the Chow and Palatable food groups ($n = 9$–12 per group) were administered the TAAR1 agonist RO5256390 (0, 10 mg/kg, i.p.) at the end of the 15-min pre-test session, and again, 30 min prior to the test, using a between-subjects design.

Western Blot

Brain punches from a separate cohort of rats trained in the operant binge-like eating procedure were processed for western blotting as previously described (Cottone *et al*, 2012). Membranes were incubated overnight at 4 °C with a primary anti-TAAR1 mouse polyclonal antibody (1:5000; provided by Roche Diagnostics) and primary anti-β-tubulin mouse monoclonal antibody, (1:30 000; sc-53140, Santa Cruz Biotechnology). The anti-TAAR1 primary antibody was validated previously (Harmeier *et al*, 2015). Membranes were incubated at room temperature for 1 h with a secondary anti-mouse IgG-HRP antibody (1:5000 or 1:10 000; sc-2004, Santa Cruz Biotechnology, for TAAR1 and β-tubulin, respectively).

Experiment 9: effects of exposure to palatable food on TAAR1 protein levels in mesocorticolimbic brain areas. mPFC, dorsal striatum, and nucleus accumbens from rats in Chow and Palatable food groups ($n = 10$–12 per group) were compared for TAAR1 protein levels.

Intracranial Surgeries, Microinfusion Procedure, and Cannula Placement

Rats were stereotaxically implanted with bilateral, intracranial cannulas targeting the IL and the PrL subregions of the mPFC, as described previously (Dore *et al*, 2013; Sabino *et al,* 2007).

Experiments 10–11: effects of microinfusion of the TAAR1 agonist RO5256390 into the IL cortex and PrL cortex on operant binge-like eating. Rats in the Chow and Palatable food groups were microinfused with the TAAR1 agonist RO5256390 (0, 1.5, 5, 15 µg per side) into either the IL ($n = 8$–10 per group) or the PrL ($n = 11$–12 per group) cortex 10 min prior to the operant sessions.

Statistical Analyses

Data were analyzed by simple or factorial ANOVAs followed by Bonferroni *post hoc* test. Statistical significance level was set at $p \leqslant 0.05$. The software/graphic packages used were SigmaPlot 11.0, Statistica 7.0, and Origin 8.5.

RESULTS

Experiment 1: Effects of the TAAR1 Agonist RO5256390 on Operant Binge-like Eating

Rats, initially trained to respond for the standard chow diet, were split in two matched groups and assigned to either the Chow or the Palatable condition ($M \pm$ SEM of last four self-administration sessions, 18.6 ± 1.3 and 18.8 ± 0.8 kcal for Chow and Palatable respectively, $t_{22} = 0.13$, $p = 0.897$, not shown). Rats allowed to self-administer the sugary, highly palatable diet 1 h per day markedly escalated food intake, unlike the control Chow group, whose intake remained stable across the 15 days of observation (Diet, $F_{1,22} = 49.83$, $p = 0.001$; Diet × Day, $F_{14,31} = 16.95$, $p = 0.001$; Figure 1a).

When the effects of the TAAR1 agonist RO5256390 on food intake were tested, results showed that rats from the Palatable food group consumed significantly more food compared with the Chow controls under vehicle conditions (Diet, $F_{1,22} = 62.72$, $p = 0.001$; Figure 1b). Drug treatment selectively and dose dependently blocked the binge-like eating of the Palatable food group in the operant task, without affecting intake of the control Chow group (Dose, $F_{3,66} = 22.17$, $p = 0.001$; Diet × Dose, $F_{3,66} = 10.02$, $p = 0.001$). In rats fed with the Palatable diet, RO5256390 treatment significantly decreased palatable food responding, compared with vehicle, at all doses tested. At the highest dose tested (10 mg/kg), drug treatment fully blocked binge-like eating, as treated rats' intake in the Palatable food group did not differ from either Chow/Vehicle or Chow/RO5256390 10 mg/kg. Figure 1c shows the time course of responding (Diet × Dose, $F_{3,66} = 3.41$, $p = 0.022$).

Experiment 2: Effects of the TAAR1 Agonist RO5256390 on High Rate of Responding for Standard Chow Induced by Food Restriction

Figure 1d shows the escalation of the standard chow responding during daily home-cage food restriction (Day, $F_{11,88} = 30.17$, $p = 0.001$). Responding for the standard chow diet in food-restricted rats was comparable to the responding of vehicle-treated *ad libitum*-fed rats in the Palatable food group in the RO5256390 administration FR1 study ($t_{20} = 0.21$, $p = 0.832$). Systemic treatment with the 10 mg/kg dose of RO5256390 had no effect on the high rate of responding for standard chow in the operant FR1 food intake task in food-restricted rats ($t_8 = 0.53$, $p = 0.610$; Figure 1c).

Experiment 3: Effects of the TAAR1 Agonist RO5256390 on Rate and Regularity of Sustained Eating

As shown in Figure 2, vehicle-treated binge-eating rats in the Palatable food group showed a significantly smaller IFI mean, but no different total IFI time compared with *ad libitum*-fed rats in the Chow food group; surprisingly,

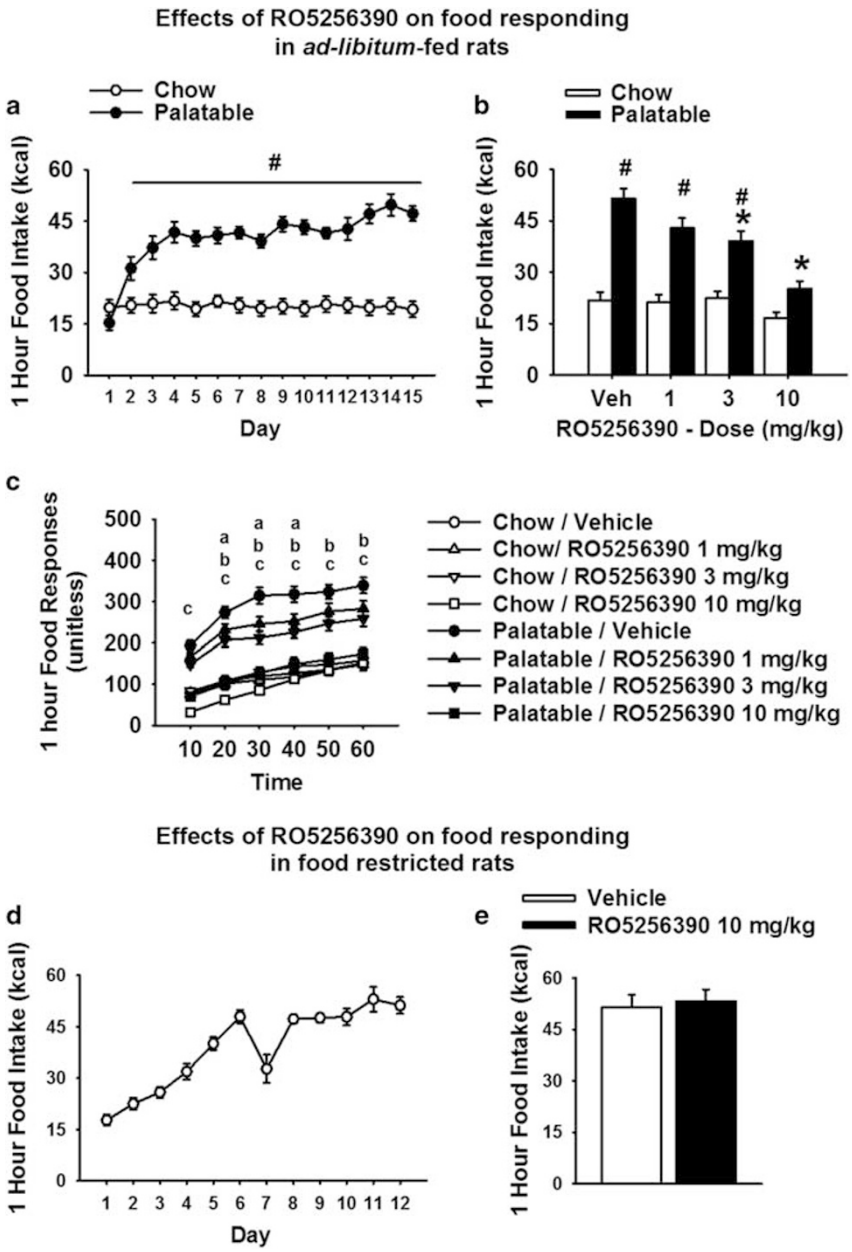

Figure 1 TAAR1 activation blocks binge-like eating without affecting either chow intake or food restriction-induced chow overeating. (a) Effects of daily 1 h self-administration of either a standard chow or a highly palatable diet on food responding in male Wistar rats ($n = 12$ per group). (b) Effects of systemic administration of the TAAR1 agonist RO5256390 (0, 1, 3, 10 mg/kg, i.p.) on 1 h food self-administration of either a standard chow or a highly palatable diet ($n = 12$ per group). (c) Time course of food responding. (d) Effects of food restriction on daily 1 h self-administration of a standard chow diet on food responding in male Wistar rats ($n = 9$ per group). (e) Effects of systemic administration of the TAAR1 agonist RO5256390 (0, 10 mg/kg, i.p.) on high rate of responding for standard chow induced by food restriction. Panels represent $M \pm$ SEM. $^{\#}p \leqslant 0.05$ *vs* Chow (a) or Vehicle Chow (b); $^{*}p \leqslant 0.05$ *vs* Vehicle Palatable; (a) RO5256390 1 mg/kg Palatable *vs* Vehicle Palatable $p \leqslant 0.05$; (b) RO5256390 3 mg/kg Palatable *vs* Vehicle Palatable $p \leqslant 0.05$; (c) RO5256390 10 mg/kg Palatable *vs* Vehicle Palatable $p \leqslant 0.05$.

vehicle-treated food-restricted chow rats showed a similar IFI mean but a much higher total IFI time than control *ad libitum*-fed rats in the Chow food group (IFI mean: Diet, $F_{2,23} = 18.78$, $p = 0.001$; total IFI time: Diet, $F_{2,23} = 20.12$, $p = 0.001$; Figure 2a and b). Therefore, even though both Palatable and food-restricted group rats ate significantly more pellets than control *ad libitum*-fed rats in the Chow food group (Diet, $F_{2,23} = 37.71$, $p = 0.001$; not shown), rats fed with the palatable diet consumed food at a higher rate within a similar time, while food-restricted chow rats ate at a similar rate but in a longer time. In addition, both rats in the Palatable food group and food-restricted chow rats showed an increased regularity of sustained eating as revealed by a decreased entropy, compared with *ad libitum*-fed Chow controls (entropy: Diet, $F_{2,23} = 49.85$, $p = 0.001$; Figure 2c). However, although rats' IFI distribution in the Palatable food group was more skewed and leptokurtic than that of *ad libitum*-fed rats in the Chow food group, the shape of the

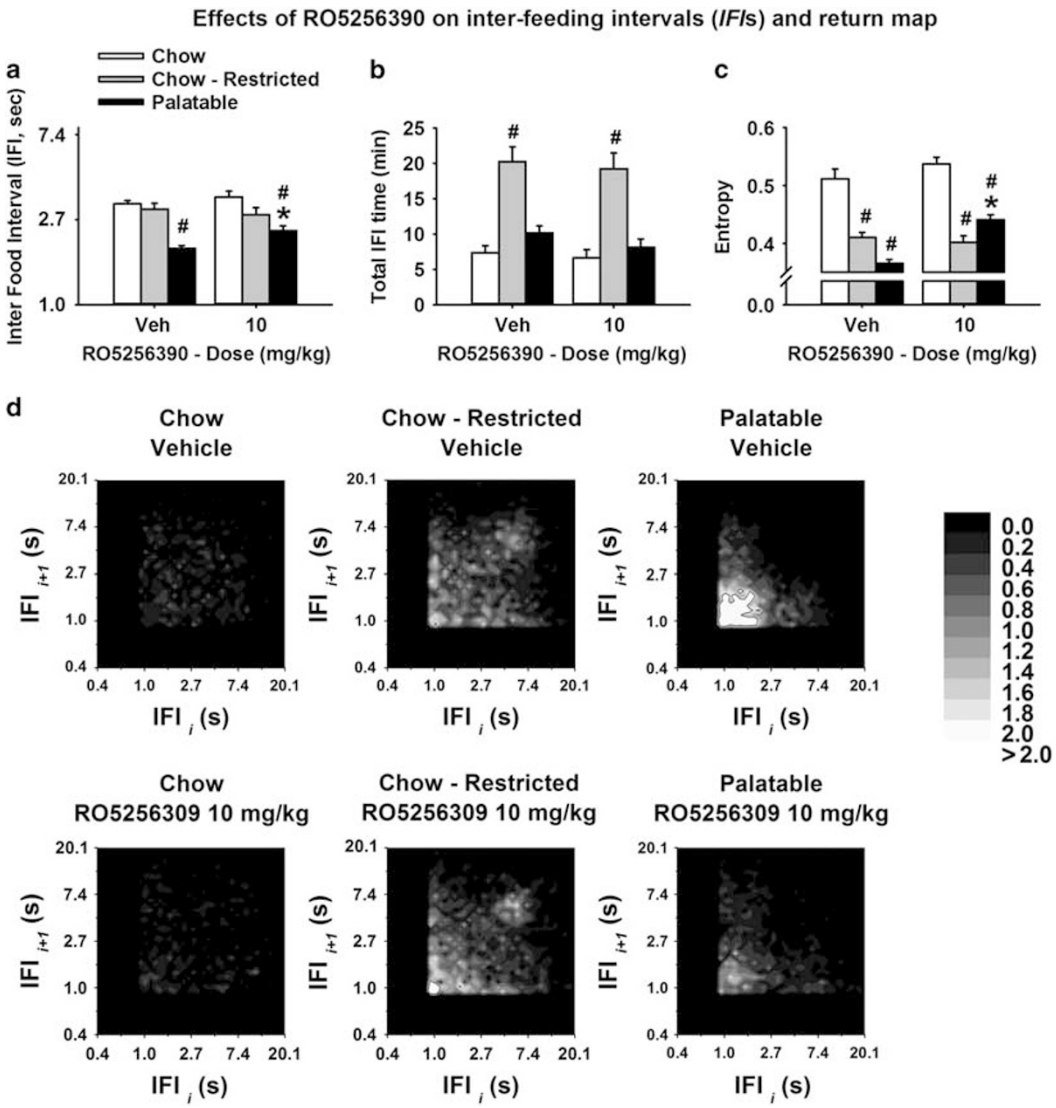

Figure 2 TAAR1 activation reduces the rate and the regularity of responding in binge eating rats. Effects of systemic administration of the TAAR1 agonist RO5256390 (0, 10 mg/kg, i.p.) on (a) inter-feeding interval (IFI) mean, (b) total IFI time, and (c) entropy, in daily 1 h self-administration of either a standard chow (in *ad libitum* or food restricted regimens) or a highly palatable diet in male Wistar rats ($n = 7–10$ per group). (d) Return map of normalized probability density plots representing successive IFIs from all subjects. Increasing gray-scale intensity (from white to black; or color intensity from black to red in the color figure) represents increasing probabilities (from 0 to 2) of successive eating events and, therefore, increased regularity of eating from pellet-to-pellet occurring at the corresponding IFI duration. Panels represent $M \pm$ SEM. $^{\#}p \leqslant 0.05$ *vs* Vehicle Chow; $^{*}p \leqslant 0.05$ *vs* Vehicle Palatable. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

distribution of IFIs in food-restricted chow rats was no different than controls (skewness: Diet, $F_{2,23} = 4.55$, $p = 0.022$; kurtosis: Diet, $F_{2,23} = 3.51$, $p = 0.047$; Supplementary Figure S1A and B). In the return map analysis, when compared with the homogeneous distribution across a wide range of IFI values of Chow controls, food responding of vehicle-treated rats in the Palatable food group was characterized by a denser cluster of IFIs skewed in the bottom left of the map, while responding in food-restricted chow rats was characterized by an overall increase of events across the entire range of IFIs, with sporadic clustered events (Figure 2d).

When RO5256390 (10 mg/kg) was administered, only Palatable food group responding was affected by drug treatment. Indeed, although the drug treatment had no effect on either *ad libitum*-fed Chow rats or food-restricted chow rats, rats in the Palatable food group RO5256390 increased the IFI mean (Dose, $F_{1,23} = 5.82$, $p = 0.024$; Diet × Dose, $F_{2,23} = 7.34$, $p = 0.003$; Figure 2a) and increased entropy (Dose, $F_{1,23} = 28.91$, $p = 0.001$; Diet × Dose, $F_{2,23} = 24.16$, $p = 0.001$; Figure 2c). *Post hoc* comparisons revealed that drug treatment did not meaningfully affect total IFI time, skewness, or kurtosis (total IFI time: Dose, $F_{1,23} = 5.24$, $p = 0.032$; Diet × Dose, $F_{2,23} = 0.59$, $p = 0.562$; skewness: Dose, $F_{1,23} = 1.58$, $p = 0.221$; Diet × Dose, $F_{2,23} = 4.99$, $p = 0.016$; kurtosis: Dose, $F_{1,23} = 0.55$, $p = 0.467$; Diet × Dose, $F_{2,23} = 1.39$, $p = 0.268$; Figure 2b and Supplementary Figure S1A and B). As already observed in Experiment 1, RO5256390 decreased the number of pellets eaten only in the Palatable food group (Dose, $F_{1,23} = 12.84$, $p = 0.002$; Diet × Dose, $F_{2,23} = 10.74$, $p = 0.001$, not shown). In the return map analysis (Figure 2d), the effects of

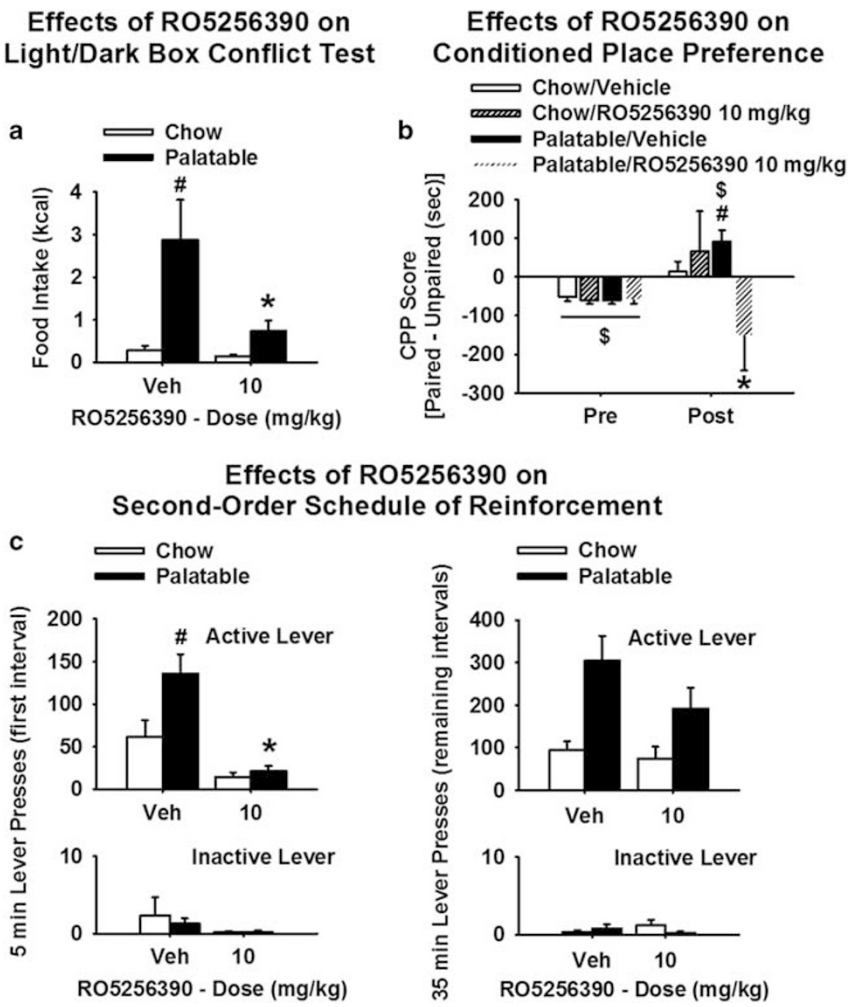

Figure 3 TAAR1 activation blocks palatable food-driven compulsive eating, conditioned food reward, and food-seeking of palatable food in binge eating rats. Effects of systemic administration of the TAAR1 agonist RO5256390 (0, 10 mg/kg, i.p.) on (a) food intake during the light/dark conflict test ($n = 21$–23 per group), (b) CPP score ($n = 16$–22 per group), (c) active and inactive lever presses during the first interval (left) and the remaining intervals (right) of a second-order schedule of reinforcement in male Wistar rats with a history of daily 1 h self-administration of either a standard chow or a highly palatable diet. Panels represent $M \pm$ SEM. $^{\#}p \leqslant 0.05$ *vs* Vehicle Chow; $^{*}p \leqslant 0.05$ *vs* Vehicle Palatable; $^{\$}p \leqslant 0.05$ *vs* 0.

RO5256390 treatment resulted in a more diffuse and less skewed pellet-to-pellet responding, as compared with the vehicle condition. Therefore, RO5256390 treatment tended to normalize the rate and the regularity of responding in bingeing of rats in the Palatable food group.

Experiment 4: Effects of the TAAR1 Agonist RO5256390 on Compulsive-like Eating

When food was placed in the bright, aversive compartment of the light/dark apparatus, control Chow intake was suppressed. Under vehicle conditions, rats in the Palatable food group consumed 10-fold more food as compared with the control Chow group (Diet, $F_{1,83} = 5.34$, $p = 0.023$; Figure 3a (see Supplementary Figure S2 for the grams of food consumed). In addition, RO5256390, administered at the 10 mg/kg dose, fully blocked compulsive-like eating of the palatable diet (Dose, $F_{1,83} = 10.37$, $p = 0.002$; Diet × Dose, $F_{1,83} = 4.10$, $p = 0.046$), as drug-treated rats' intake in the Palatable food group did not differ from that of Chow controls.

Experiment 5: Effects of the TAAR1 Agonist RO5256390 on Conditioned Food Reward

Rats in all conditions were matched to spend a similar amount of time in the least preferred compartment, as revealed by similar preconditioning CPP scores (Diet, $F_{1,71} = 0.27$, $p = 0.635$; Dose, $F_{1,71} = 0.38$, $p = 0.541$; Diet × Dose: $F_{1,71} = 0.31$, $p = 0.577$; Figure 3b). The analysis of the post-conditioning CPP scores indicated that, under vehicle condition, rats in the Palatable, but not Chow food group, displayed place conditioning, as they spent significantly more time in the initial non-preferred compartment as compared with their score in the pre-conditioning phase (biased protocol). However, RO5256390, injected at the 10 mg/kg dose, fully blocked the expression of the place preference in rats in the Palatable food group, as their post-conditioning score did not significantly differ from their pre-conditioning

score, although it significantly differed from the post-conditioning score of vehicle-treated rats in the same group (Diet × Dose: $F_{1,71} = 4.73$, $p = 0.032$; Figure 3b).

Experiment 6: Effects of the TAAR1 Agonist RO5256390 on Food-Seeking

In the second order schedule of reinforcement, the analysis of the first interval, which occurs before food ingestion, revealed that the rats in the Palatable food group showed a higher number of active lever responses compared with the Chow control rats (Diet, $F_{1,14} = 6.52$, $p = 0.023$; Figure 3c/left). RO5256390 administration selectively blocked active lever responding in rats fed with the palatable food, but not in controls (Dose, $F_{1,14} = 19.74$, $p < 0.001$; Diet × Dose, $F_{1,14} = 3.42$, $p = 0.086$; Figure 3c/left). Conversely, in the remaining intervals, which occur after food ingestion, although a main effect of Diet was observed (Diet, $F_{1,14} = 9.06$, $p = 0.009$ Figure 3c/right), no effect of either Dose or interaction Diet × Dose was detected (Dose, $F_{1,14} = 1.85$, $p = 0.194$; Diet × Dose, $F_{1,14} = 0.87$, $p = 0.365$, Figure 3c/right).

Inactive lever responding was not affected by either the food provided or the drug treatment at any point during the second order session (first interval: Diet, $F_{1,14} = 0.26$, $p = 0.618$; Dose, $F_{1,14} = 2.62$, $p = 0.128$; Diet × Dose, $F_{1,14} = 0.28$, $p = 0.605$; remaining intervals: Diet, $F_{1,14} = 0.20$, $p = 0.661$; Dose, $F_{1,14} = 0.11$, $p = 0.741$; Diet × Dose, $F_{1,14} = 4.27$, $p = 0.057$; Figure 3c).

Experiment 7: Effects of the TAAR1 Agonist RO5256390 on Anxiety-like Behavior

When anxiety-like behavior was assessed in Chow and Palatable food groups using the defensive withdrawal test, under vehicle conditions the two diet groups did not differ in the latency to exit the sheltered chamber (Diet, $F_{1,39} = 0.28$, $p = 0.600$; Figure 4a/left). In addition, latency was not affected by RO5256390 treatment (Dose, $F_{1,39} = 1.07$, $p = 0.600$; Diet × Dose, $F_{1,39} = 0.01$, $p = 0.916$). Furthermore, vehicle-treated Chow and Palatable food groups rats did not differ in the time spent in the sheltered chamber (Diet, $F_{1,39} = 1.64$, $p = 0.207$; Figure 4a/right). Drug treatment did not affect the time spent in the sheltered chamber either (Dose, $F_{1,39} = 0.01$, $p = 0.981$; Diet × Dose, $F_{1,39} = 0.03$, $p = 0.863$). Entries to the chamber did not differ between either diet or dose groups (Diet, $F_{1,39} = 0.86$, $p = 0.359$; Dose, $F_{1,39} = 0.06$, $p = 0.812$; Diet × Dose, $F_{1,39} = 0.10$, $p = 0.747$; not shown).

Experiment 8: Effects of the TAAR1 Agonist RO5256390 on Depressive-like Behavior

When depressive-like behavior was assessed in Chow and Palatable food groups using the forced swim test, under vehicle conditions the two diet groups did not differ in immobility time (Diet, $F_{1,38} = 0.92$, $p = 0.342$; Figure 4b/left), swimming time (Diet, $F_{1,38} = 0.19$, $p = 0.669$; Figure 4b/right) or climbing time (Diet, $F_{1,38} = 2.96$, $p = 0.094$; not shown). In addition, RO5256390 treatment did not affect immobility time (Dose, $F_{1,38} = 0.06$, $p = 0.806$; Diet × Dose, $F_{1,38} = 0.50$, $p = 0.484$; Figure 4b/left), swimming time (Dose, $F_{1,38} = 0.04$, $p = 0.848$; Diet × Dose, $F_{1,38} = 1.03$, $p = 0.315$; Figure 4b/right), or climbing time (Dose, $F_{1,38} = 0.07$, $p = 0.792$; Diet × Dose, $F_{1,38} = 0.06$, $p = 0.811$; not shown).

Experiment 9: Effects of Exposure to Palatable Food on TAAR1 Protein Levels in Mesocorticolimbic Brain Areas

TAAR1 protein levels were decreased in the mPFC ($t_{19} = 2.56$, $p = 0.019$; Figure 5a–c), although no differences were observed in either the dorsal striatum ($t_{21} = 0.38$, $p = 0.709$) or the nucleus accumbens ($t_{20} = 0.59$, $p = 0.563$) (Supplementary Figure S3).

Experiments 10–11: Effects of Microinfusion of the TAAR1 Agonist RO5256390 into the IL Cortex and PrL Cortex on Operant Binge-like Eating

Microinfusion of the TAAR1 agonist RO5256390 into the IL subregion of the mPFC selectively and dose dependently reduced food responding in rats in the Palatable food group (Diet, $F_{1,16} = 94.27$, $p = 0.001$; Dose, $F_{1,48} = 7.09$, $p = 0.001$; Diet × Dose, $F_{1,48} = 7.77$, $p = 0.001$). A significant effect was observed at both the 5 and 15 μg per side doses as compared with vehicle condition (23.0% reduction observed with the 15 μg per side dose). No effect was observed in control Chow rats, Figure 5d–f). Conversely, when RO5256390 was microinfused in the PrL cortex, drug treatment exerted no effect in either Chow or Palatable food responding (Diet, $F_{1,21} = 32.41$, $p = 0.001$; Dose, $F_{1,63} = 0.58$, $p = 0.632$; Diet × Dose, $F_{1,63} = 1.39$, $p = 0.253$; Supplementary Figure S4).

DISCUSSION

RO5256390 Blocks Binge-like Eating and Reduces the Rate of Palatable Food Intake

In this study, we found that the selective TAAR1 agonist RO5256390 dose dependently blocked binge eating of highly palatable food induced by a 1 h per day limited access procedure. Drug treatment was effective at all doses tested, and the highest dose (10 mg/kg) reduced palatable food intake by 51.2 ± 4.4% ($M \pm$ SEM). This effect was selective for the palatable diet, as the standard chow control intake was unaffected by drug treatment. Therefore, drug effect was not secondary to an overall behavioral deficit and was specific for palatability-rather than energy homeostatic-dependent food consumption.

Consistent with a palatability-selective effect, the highest dose (10 mg/kg) of RO5256390 did not affect standard chow diet overeating induced by a food restriction procedure, excluding the alternative interpretation that drug effects were dependent on a high rate of responding. Notably, our food restriction protocol was performed over 12 days before any pharmacological treatment, a time sufficient to induce neurochemical and behavioral adaptations in the reward system, which increase susceptibility to drugs of abuse (D'Cunha *et al*, 2013). Further evidence of a palatability-selective effect of RO5256390 was provided by the analysis of the IFIs. As a result of the increased palatability of the diet, responding in binge eating rats was characterized by highly clustered and short IFIs, as opposed to the much more diffuse and long IFIs of chow control rats. The increased

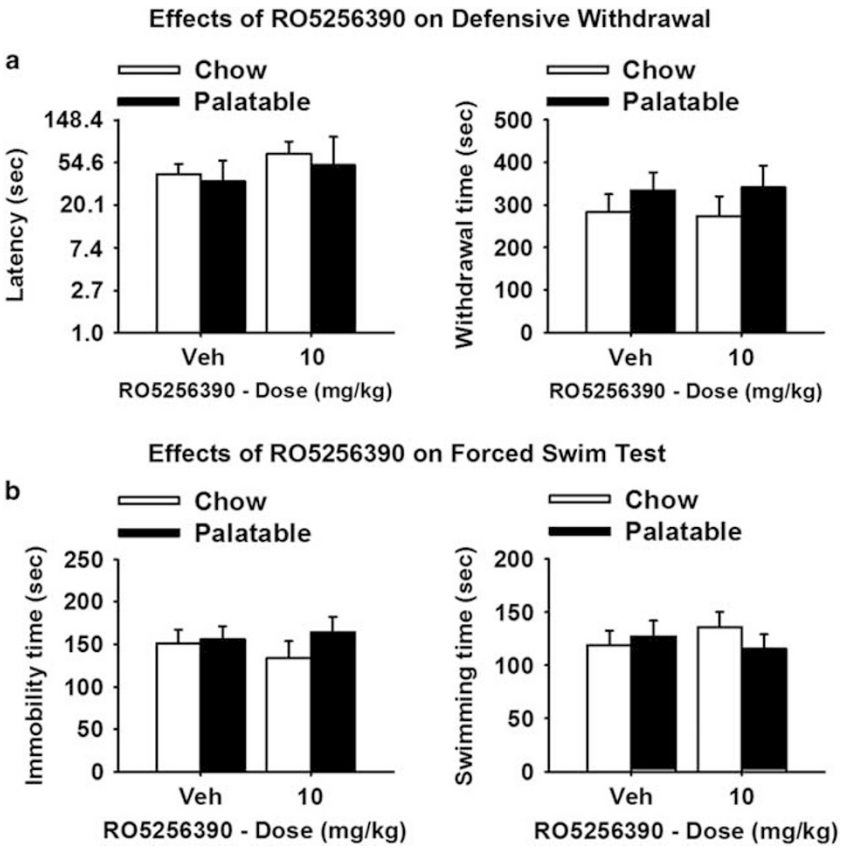

Figure 4 TAAR1 activation does not affect anxiety-like or depressive-like behavior in binge eating rats. Effects of systemic administration of the TAAR1 agonist RO5256390 (0, 10 mg/kg, i.p.) on (a, left) latency to first emerge, (a, right) withdrawal time in the chamber in the defensive withdrawal test ($n = 10–12$ per group); (b, left) immobility time, (b, right) swimming time in a forced swim test ($n = 9–12$ per group), in male Wistar rats with a history of daily 1 h self-administration of either a standard chow or a highly palatable diet. Panels represent $M \pm$ SEM.

sustained eating rate was independent from the high rate of response, as food-restricted rats, which ate the same number of pellets as rats bingeing on the palatable diet, ate much more slowly and in a longer time frame. Treatment with RO5256390 selectively decreased the rate and the regularity of pellet-to-pellet responding in binge eating rats, without affecting responding for standard chow in either *ad libitum* fed or food-restricted rats. Thus, agonism of TAAR1 specifically disrupted the ability of palatable food, but not standard chow (consumed at any rate of response or in any feeding state) to sustain clustered feeding responses. Overall, these findings strengthen the concept that, despite being quantitatively equivalent, binge eating induced by an increased palatability of the food is different at a behavioral, pharmacological, and neurobiological level than overeating induced by increased energy needs due to food restriction/deprivation (Cottone *et al*, 2012).

The observed decrease in palatable food responding following RO5256390 treatment is in line with previous reports obtained with full agonists of TAAR1. RO5256390 was able to decrease, as well as slow down, responding for chocolate flavored pellets at the 10, but not the 3 mg/kg dose (Pei *et al*, 2014). Here we observed that the decrease in palatable food responding occurs at all the doses tested (1, 3 and 10 mg/kg). However, in the previous study the rats' intake was capped to 40 food pellets per session (Pei *et al*, 2014), whereas here rats obtained an average of 332.8 ± 19.0 ($M \pm$ SEM) palatable pellets. Furthermore, our data are consistent with a recent study showing that the TAAR1 full agonist RO5166017 was able to reduce the intake of a high-fat diet in diet-induced obesity mice (Raab *et al*, 2016).

RO5256390 Blocks Compulsive-like Eating

We observed that RO5256390 blocked compulsive-like eating behavior in a light/dark box conflict test. Compulsivity is a behavioral construct observed in multiple disorders, including addictions, as well as certain disorders of pathological eating, where maladaptive behavior perseverates in spite of medical, psychological, emotional, and social impairment (APA, 2013; Gearhardt *et al*, 2014). In our conditions, feeding is suppressed when the control rats face the aversive bright compartment of the light/dark box, whereas bingeing rats compulsively consume the palatable diet in face of the potentially risky circumstances (Cottone *et al*, 2012; Dore *et al*, 2014; Velazquez-Sanchez *et al*, 2014). TAAR1 activation fully blocked compulsive eating of palatable food, reducing intake in the bright compartment by $75.8 \pm 8.2\%$ ($M \pm$ SEM) as compared with vehicle-treated rats in the same group. Importantly, the alternative interpretation that this effect was induced by a potential anxiogenic profile of drug treatment can be confidently excluded as RO5256390 exerted no effect on either anxiety- or depressive-like behavior.

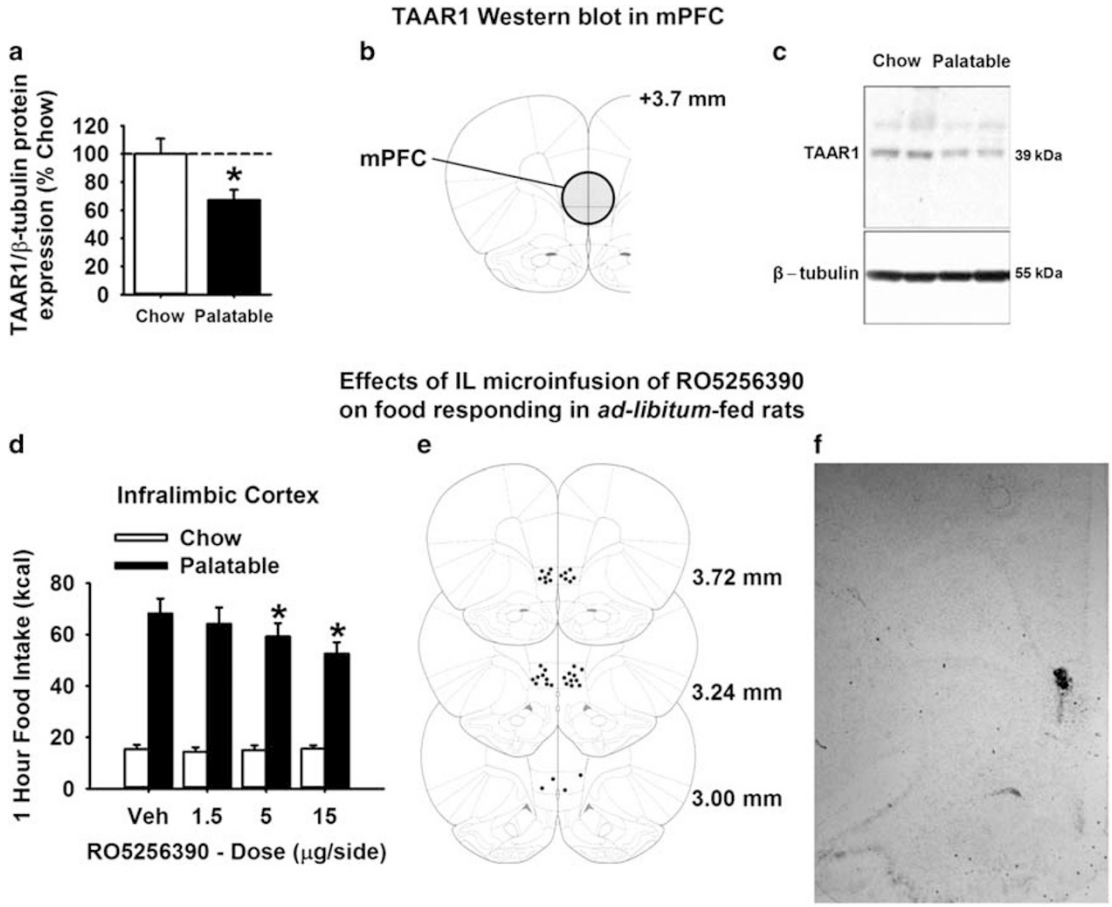

Figure 5 Limited access to palatable food decreases TAAR1 protein levels in the prefrontal cortex; infralimbic cortex (IL) activation of TAAR1 reduces binge-like eating. (a) TAAR1 protein levels in the mPFC of male Wistar rats self-administering 1 h per day either a standard chow or a highly palatable diet ($n = 10–12$ per group). (b) Drawing of rat brain slices used for western blotting studies; circle shows punch of mPFC. (c) Representative western blot of TAAR1 and β-tubulin. (d) Effects of microinfusion of the TAAR1 agonist RO5256390 (0, 1.5, 5, 15 μg per side) into the infralimbic cortex (IL) subregion of the mPFC on 1 h food self-administration of either a standard chow or a highly palatable diet ($n = 8–10$ per group). (e) Drawing of coronal rats' brain slices; dots represent the injection sites in the IL included in the data analysis. Panels represent $M \pm$ SEM. (f) Photomicrograph shows a coronal section of the brain of a rat with representative injection sites in the IL. Panels represent $M \pm$ SEM. $^*p \leqslant 0.05$ *vs* Chow.

RO5256390 Blocks the Strength of Palatable-food-associated Stimuli

In this study, RO5256390 blocked the strength of palatable-food associated stimuli in driving behavior, as observed in two different tasks: the CPP test, which measures food reward, and the second-order schedule of reinforcement, which measures food-seeking behavior. Evolutionarily, food, and in particular energy-dense highly palatable food, has the ability to increase the salience of associated contextual stimuli. Through this mechanism, contextual stimuli can, therefore, exert a strong control over behavior even in absence of food *per se*, and in pathological conditions they have the power to initiate and sustain binge eating (Giuliano and Cottone, 2015). Our results show that tactile and visual cues associated with the highly palatable food were able to induce a strong place preference, unlike stimuli associated to the standard chow diet. We observed that RO5256390 treatment fully and selectively blocked the expression of CPP in rats of the Palatable food group. Furthermore, a palatable food-associated stimulus light was able to induce a vigorous food-seeking behavior in a second-order schedule of reinforcement (Everitt and Robbins, 2000), which was fully and selectively blocked by RO5256390 treatment. Importantly, the drug effect on food seeking was observed during the first interval, and, therefore, before food ingestion occurred, suggesting an effect on incentive value of palatable food. Once again, we can confidently exclude that this effect was induced by an overall behavioral deficit induced by the drug as inactive lever responding was unaffected. In addition, responding for the cue associated with the standard chow diet was not affected by drug treatment.

Role of mPFC TAAR1 in Binge-like Eating of Palatable Food

In this study, we found that binge-like eating of palatable food decreased TAAR1 protein levels in the mPFC. The mPFC is a key area involved in inhibitory control and decision making, and dysfunctions of this region are thought to contribute to compulsive, binge-like eating behavior (Blasio *et al*, 2014; Calu *et al*, 2013; Cottone *et al*, 2012; Mena *et al*, 2013). TAAR1 KO mice have been shown to display behaviors consistent with cortical dysfunctions, such

as perseverative behavior and impulsivity; consistently, TAAR1 agonism improves these behaviors in wild-type animals (Espinoza *et al*, 2015), suggesting that a deficiency in TAAR1, like the one we observed in the mPFC of rats in the Palatable food group, may result in deficits in cognitive function and behavioral flexibility. Therefore, it can be hypothesized that TAAR1 agonism may reduce compulsive, binge eating of palatable food by normalizing the palatable-induced impairment in TAAR1 signaling in the mPFC. In support of this hypothesis, as well as of a functional role of the TAAR1 protein reduction observed in mPFC, is our finding that intra-mPFC administration of the TAAR1 agonist decreased binge-like eating in rats from the Palatable food group, without affecting the intake of regular chow. Interestingly, only intra-IL (and not intra-PrL) administration of TAAR1 reduced intake in binge eating rats, supporting the widely accepted notion that these two subregions have separable roles in behavior due to their very distinct connectivity. It should be acknowledged that, although unlikely, a potential explanation for the differential effect of RO5256390 microinfusion within either the IL or the PrL cortices may be the large variability of operant responding under vehicle conditions in the two cohorts of rats. Of relevance in this context are the observations that the IL has been shown to have elevated basal dopamine levels as compared with PrL, potentially consistent with higher dopaminergic innervation, and that the IL subregion is crucial for the development and expression of inflexible reward seeking, as well as habitual behavior (Barker *et al*, 2014). To be noted is that the degree of reduction observed following intra-IL RO5256390 microinfusion was lower than that observed following intraperitoneal administration of the drug, suggesting that other TAAR1-expressing brain areas in addition to the IL may be contributing to the systemic drug effects (eg ventral tegmental area, dorsal raphe, and so on).

CONCLUSIONS

The TAAR1 system has been reported to regulate the behavioral actions of drugs of abuse. Indeed, consistent with our results, TAAR1 agonists were shown to be able to block cocaine seeking (Pei *et al*, 2014), suppress the reinforcing and rewarding effects of cocaine (Pei *et al*, 2015), attenuate cocaine behavioral sensitization, and reinstatement of cocaine seeking, as well as the expression of cocaine-induced CPP (Liu *et al*, 2016; Thorn *et al*, 2014). In line with the results from the pharmacological studies, TAAR1 KO mice display heightened locomotor response and context-dependent locomotor sensitization to amphetamine, and increased sensitivity to reinstatement of amphetamine-induced CPP, and they consume more alcohol and methamphetamine, compared with wild-type mice (Harkness *et al*, 2015; Lindemann *et al*, 2008; Lynch *et al*, 2013; Sukhanov *et al*, 2016; Wolinsky *et al*, 2007). It is hypothesized that TAAR1 involvement on drug-related behaviors may occur through the modulation of dopaminergic transmission. Indeed, TAAR1 knockout mice display a spontaneously elevated firing rate of dopaminergic neurons in the ventral tegmental area (Lindemann *et al*, 2008), and TAAR1 agonists have been shown to suppress the firing of dopamine neurons (Bradaia *et al*, 2009; Lindemann *et al*, 2008; Revel *et al*, 2011), suggesting that endogenous TAAR1 activation may dampen dopaminergic neuronal firing.

Therefore, here it can be speculated that the effects of TAAR1 agonism on food reward may occur through modulation of the dopaminergic terminals in the mPFC. Palatable food consumption activates the reward circuitry and releases dopamine in the mPFC (Babbs *et al*, 2013; Volkow *et al*, 2008). In addition, limited access to palatable food results in neuroadaptations in the reward circuitries, which contribute to compulsive overeating (Volkow *et al*, 2013). Moreover, obese and binge eating individuals display sensitized responses to conditioned food cues, such as increased prefrontal activation as well as higher dopamine release when exposed to food cues (Dimitropoulos *et al*, 2012). Hence, the observed effects of TAAR1 activation on food reward may be due to the ability of RO5256390 treatment to restore an impaired prefrontocortical dopaminergic transmission induced by excessive consumption of highly palatable food, although additional studies will be needed to confirm this hypothesis.

In summary, our results substantiate the potential for RO5256390 as a pharmacological treatment for disorders characterized by compulsive, binge eating.

FUNDING AND DISCLOSURE

This publication was made possible by grant numbers DA030425, MH091945, and MH093650 from the National Institute on Drug Abuse (NIDA), and the National Institute of Mental Health (NIMH), by the Peter Paul Career Development Professorship (PC), the McManus Charitable Trust (VS), and Boston University's Undergraduate Research Opportunities Program (UROP). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health. MCH is an employee of F. Hoffmann-La Roche. The remaining authors declare no potential conflict of interest.

ACKNOWLEDGMENTS

We thank Clara Velázquez-Sánchez, Lillian Huang, Shilpa Prasad, Jesse Mendoza, and Rachel Leavitt for technical and editorial assistance. We also thank Dr Roger Norcross for the synthesis of the TAAR1 agonist RO5256390.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)