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Involvement of TRPV1 in the Olfactory Bulb in Rimonabant-Induced Olfactory Discrimination Deficit

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Abstract

Rimonabant is well recognized as a cannabinoid CB_1 receptor antagonist/inverse agonist. Rimonabant not only antagonizes the effects induced by exogenous cannabinoids and endocannabinoids at CB_1 receptors, it also exerts several pharmacological and behavioral effects independent of CB_1 receptor inactivation. For example, rimonabant can function as a low-potency mixed agonist/antagonist of the transient receptor potential vanilloid receptor 1 (TRPV1). Hence, it is important to explain the underlying mechanisms of the diverse physiological effects induced by rimonabant with caution. Interestingly, CB_1 receptor has recently been suggested to play a role in olfactory functions. Olfaction not only is involved in food intake, visual perception and social interaction, but also is proposed as a putative marker for schizophrenia and autism. Therefore, the present study aimed to investigate whether CB_1 receptor and TRPV1 played a role in olfactory functions. We first used the genetic disruption approach to examine the role of CB_1 receptor in olfactory functions and found that CB_1 knockout mice exhibited olfactory discrimination deficit. However, it is important to point out that these CB_1 knockout mice, despite their normal locomotivity, displayed deficiencies in the olfactory foraging and novel object exploration tasks. These results imply that general exploratory behaviors toward odorant and odorless objects are compromised in CB_1 knockout mice. We next turned to the pharmacological approach to examine the role of CB_1 receptor and TRPV1 in olfactory functions. We found that the short-term administration of rimonabant, injected systemically or directly into the olfactory bulb (OB), impaired olfactory discrimination that was rescued by the TRPV1 antagonist capsazepine (CPZ), *via* the same route of rimonabant, in wild-type mice. These results suggest that TRPV1 in the OB is involved in rimonabant-induced olfactory discrimination deficit. However, the rimonabant and/or CPZ treatments neither affected locomotivity nor general exploratory behaviors in wild-type mice. Finally, the acute systemic administration of rimonabant, unlike the short-term administration regimen, did not affect olfactory discrimination. Taken together, this study not only is the first one, to the best of our knowledge, suggests that the olfactory TRPV1 plays a role in olfactory functions, but also provides a possible mechanism for the olfactory discrimination deficit induced by rimonabant.

Key Words: cannabinoids, capsazepine, CB_1 knockout mice, CB_1 receptors, olfactory bulb, olfactory functions, TRPV1

Introduction

Rimonabant, also known as SR141716A or trade name Acomplia, is best recognized as a cannabinoid CB_1 receptor antagonist/inverse agonist. Rimonabant binds to CB_1 receptors with a K_i in the nanomolar range and prevents those effects induced by exogenously applied cannabinoids and endocannabinoids at this

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receptor (39, 43, 45). Moreover, rimonabant *per se* elicits a number of biochemical and behavioral effects. For example, at normal pharmacological doses (0.3-5 mg/kg), rimonabant reduces food intake (8, 60), suppresses diabetes-induced mechanical allodynia (10) and alleviates neuropathic pain (12), facilitates extinction and modulates consolidation of cocaine-induced conditioned place preference memory (23), as well as increases intestinal propulsion and transit (9, 25) in rodents. Moreover, rimonabant increases locomotor activity (3) and induces paw tremors, head shakes and scratching (11) at much higher doses (10-30 mg/kg) in mice. The mechanisms by which the sole rimonabant causes the above effects have not yet been fully explored, however, the potential mechanisms could be *via* [1] antagonism at CB_1 receptors of endogenously released endocannabinoids, [2] inverse agonism that negatively modulates the constitutive activity of CB_1 receptors and [3] CB_1 receptor-independent mechanisms (44).

As for the CB_1-independent mechanisms, rimonabant can bind to several other targets, including the transient receptor potential vanilloid receptor 1 (TRPV1) (46), at concentrations typically encountered in the experimental studies of CB_1 receptor-mediated functions. TRPV1 is initially identified as the receptor for capsaicin, the pungent ingredient in hot chili peppers (7). TRPV1 acts as a nonselective cation channel with significant permeability to calcium, protons and large polyvalent cations, and can be activated by noxious heat (> 43°C) (7, 30, 56, 61). TRPV1 is well characterized at the terminals of sensory neurons in the pain and inflammation pathway (34, 49, 63). Moreover, TRPV1 is highly expressed in the central nervous system, including the hippocampus, the spinal cord and the olfactory bulb (OB) (14, 20, 58). While the hippocampal TRPV1 is implicated in learning and memory (20), the function of TRPV1 in the OB remains elusive. On the other hand, the endocannabinoids/endovanilloids anandamide and *N*-arachidonoyl dopamine activate both TRPV1 and CB_1 receptors (24, 59, 62). Importantly, rimonabant has been shown to act as a low-potency TRPV1 mixed agonist/antagonist (46). For example, rimonabant induces neuroprotective effects, which is reversed by the TRPV1 antagonist capsazepine (CPZ), on global cerebral ischemia in gerbils (42). Rimonabant also stimulates neurogenesis in the subventricular zone (SVZ) that is abolished in TRPV1 knockout mice (29). In addition, several *in vitro* studies show that rimonabant blocks TRPV1 at low micromolar concentrations (*e.g.*, 2.5-30 μM) (13, 20, 47, 66), whereas activates TRPV1 at concentration of 50 μM (47).

Interestingly, a recent study shows that the cannabinoid Δ^9-tetrahydrocannabinol (THC) impairs olfactory habituation, promotes odor detection and increases food intake in fasted mice (54). These effects of THC are suggested to be mediated by CB_1 receptor since they are prevented by a CB_1 receptor antagonist AM251, which is structurally similar to rimonabant, and are absent in the conditional CB_1 knockout mice (54). On the other hand, despite being an anti-obesity agent, the sole effect of rimonabant on olfactory functions has not yet been elucidated. Normal olfactory functions facilitate food intake (54) and olfactory dysfunctions are implicated in food intake disorders (1, 6). Olfaction also affects visual perception and social interaction in humans (26, 64). Moreover, olfactory dysfunctions are proposed as a biomarker for the early symptoms of several psychiatric disorders such as schizophrenia (2, 31, 40, 50, 51) and autism (5, 17). Therefore, the objectives of this study were to examine whether CB_1 receptor and TRPV1 played a role in olfactory functions by using the genetic disruption (CB_1 knockout mice) and the pharmacological inactivation (rimonabant and CPZ) approaches. We first used the genetic disruption approach to test whether CB_1 knockout mice, when compared to wild-type mice, exhibited olfactory deficiency (Experiment 1). Next, we switched to the pharmacological inactivation approach to investigate the role of CB_1 receptor and TRPV1 in olfactory functions. We examined whether the short-term *vs.* the acute administration regimen of rimonabant, injected systemically or directly into the OB, affected olfactory discrimination and whether the TRPV1 antagonist CPZ could rescue rimonabant's effect in wild-type mice (Experiment 2). As stated above, rimonabant activates TRPV1 at the concentration of 50 μM (47), which equals to 0.023 mg/ml (the molecular weight of rimonabant = 463.79). Therefore, I hypothesized that the dosage of rimonabant used in this study (3 mg/kg for the systemic injection = 0.3 mg/ml rimonabant in a volume of 10 ml/kg or 1.5 μg/μl = 1.5 mg/ml per side for the intra-OB injection) might activate TRPV1. Therefore, I examined whether the effect of rimonabant on olfactory functions could be antagonized by the TRPV1 antagonist CPZ, administered *via* the same route of rimonabant. Finally, in an attempt to rule out the possibility that CB_1 knockout mice and drug-treated wild-type mice had defective exploratory behaviors and locomotor activities, these mice were tested on the olfactory foraging, the novel object exploration and the locomotor activity tasks sequentially.

Materials and Methods

Animals

Male C57BL/6J wild-type mice were obtained from the National Laboratory Animal Center (NLAC), Taipei, Taiwan. The CB_1 receptor knockout homoge-

neous mice crossed for more than 10 generations on a C57BL/6J background were kindly provided by Dr. Andreas Zimmer (65) *via* Dr. Ming-Shiu Hung at National Health Research Institutes in Miaoli, Taiwan and used to breed the mice for this study. Lack of the CB_1 mRNA in CB_1 knockout mice was verified by genotyping and reverse transcription-polymerase chain reaction. After weaning, mice were group housed in plastic cages (5 per cage) in a temperature- and humidity-controlled colony room on a 12-hour light/dark cycle with light on at 7:00 AM. The behavioral experiments started when mice reached 10 weeks old. Mice had access to food (Purina Mouse Chow, Richmond, IN, USA) and tap water *ad libitum*. All procedures used in this study were approved by the Institutional Animal Care Committee of the National Cheng Kung University College of Medicine and conformed to the Guidelines of the National Institutes of Health on the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996.

Drug Treatments

Rimonabant (also known as SR144716A or SR1; trade name Acomplia) was purchased from APIChem Technology (Hangzhou, Zhejiang, PRC). The TRPV1 antagonist CPZ were obtained from Alfa Aesar [Ward Hill (Haverhill), MA, USA). Rimonabant (3 mg/kg) was dissolved in the ethanol/cremophor/saline (1:1:18; v/v/v) vehicle for intraperitoneal (i.p.) injection, while CPZ (1 mg/kg) was dissolved in the ethanol/Tween-80/saline (1:1:8; v/v/v) vehicle for subcutaneous (s.c.) injection, both in a volume of 10 ml/kg. The different vehicle solutions used to dissolve rimonabant (ethanol/cremophor/saline, 1:1:18) and CPZ (ethanol/Tween-80/saline, 1:1:8) were adopted from an *in vivo* study (42). Both vehicles are commonly used in the cannabinoid research field and do not have any reported detrimental effects on behaviors thus far. Moreover, cremophor, emulphor and Tween-80 are sometimes used exchangeably to dissolve cannabinoid-related compounds including rimonabant and CPZ (33, 55). On the other hand, both rimonabant (1.5 μg) and CPZ (1 μg) were dissolved in 100% DMSO vehicle for the bilateral OB injections. Several labs (18, 22) have previously used DMSO as a diluent for intracerebroventricular (i.c.v.) or intracerebral (i.c.) studies without any adverse effects. All drugs were freshly prepared before use.

Stereotaxic Surgery, Guide Cannula Implantation and Histology

In order to examine the role of the olfactory TRPV1 in rimonabant-induced olfactory discrimination deficit, a cohort of the wild-type mice were implanted with bilateral 26-gauge guide cannula (Coordinates: A.P., +4.2 mm; M.L., ±1.0 mm; D.V., –2.0 mm) (41, 54) under sodium pentobarbital anesthesia (40 mg/kg, i.p.) 1 week before the beginning of the behavioral experiment. Bregma and the skull surface served as the stereotaxic zero point. Clearance through the guide cannula was maintained with dummy cannula. The infusion cannula, a 33-gauge dental needle, was inserted into the guide cannula and was lowered 0.5 mm below the guide cannula. A 0.5 μl of rimonabant (1.5 μg/μl per side) or CPZ (1 μg/μl per side) was infused bilaterally with a Hamilton 10 μl microsyringe driven by a microdialysis pump (CMA 400/Microdialysis, Solna, Sweden) at a rate of 0.1 μl/min. After injection, the infusion cannulas were left for an additional 5 min before withdrawal to avoid reflux of the infused drug solution.

In the end of each experiment, all mice were euthanized with pentobarbital overdose. The mice underwent the guide cannula implantation were deeply anesthetized and perfused with phosphate-buffered saline (PBS) and 4% paraformaldehyde. The OBs were postfixed with 4% paraformaldehyde overnight, cryoprotected in 30% sucrose/PBS and frozenly cut into 40 μm coronal sections with a Cryostat (Leica, CM1850, Wetzlar, Germany) and Nissl stained with cresyl violet. A light microscope (Olympus Microscope Cooperation, Tokyo, Japan) was used to visualize the injection sites and sizes in the OB, which were then verified according to a mouse brain atlas (41). Only mice (n = 39) with both cannulas correctly placed in the OB were included in the final data analysis, and a total of 9 mice were excluded for misplacement.

Olfactory Discrimination Task

This task was modified from a previous study (38). In order to examine whether chronic genetic disruption of CB_1 receptor affected olfactory functions, CB_1 knockout mice *vs.* wild-type mice, both free of drug treatment, were individually placed in a plastic cage (21 cm H × 26 cm W × 47 cm L) with an empty tea ball hanging on the microisolator top in a ventilation hood for 15 min per day for 3 consecutive days to ensure their acclimation (Days 1-3). On Day 4, these mice first underwent the olfactory foraging task (see below) and were then tested on the olfactory discrimination task consisting of six successive trials. For each trial, the cage was opened and a tea ball containing an odorant solution soaked in cotton was hung within the cage and each mouse was allowed to explore odor for 3 min with 5-min inter-trial interval. Water was used as the first blank trial (T1). Orange extract (10% of 1 ml solution soaked in cotton) was used as the first novel odor and presented for the next four trials to examine olfactory

habituation process (T2 to T5). Vanilla extract (10%) was used for the last trial to examine olfactory discrimination ability (T6). Time spent on olfactory investigation for each trial, defined as direct nasal contact or sniffing close to (≤ 1 cm) the tea ball was recorded for further analysis (Experiment 1).

Next, the pharmacological inactivation approach was used to examine whether CB_1 receptor or TRPV1 was involved in olfactory functions. Different groups of wild-type mice were treated with rimonabant using the short-term *vs*. the acute administration regimen. For the short-term administration regimen, mice were injected with rimonabant or vehicle systemically (one rimonabant injection per day) immediately before the 15-min acclimation period for 3 days. Next day, these drug-free mice underwent the olfactory foraging and olfactory discrimination tasks. For the acute administration regimen, the drug-free mice underwent the 15-min acclimation period for 3 days first. Next day, these mice were injected with rimonabant approximately 15 min before the olfactory foraging and olfactory discrimination tasks. Since we found that only the short-term, but not the acute, systemic administration of rimonabant impaired olfactory discrimination, we next examined whether the intra-OB infusion of rimonabant affected olfactory functions using the short-term administration regimen. Finally, to examine whether TRPV1 mediated rimonabant-induced olfactory deficit, the TRPV1 antagonist CPZ was administered approximately 30 min before each rimonabant injection *via* the same route before the 15-min acclimation period for 3 days. Next day, these mice underwent the olfactory behavioral tasks as described above (Experiment 2).

Olfactory Foraging Task

This task was revised from a previous study (16) and used to test whether the olfactory detection and exploration remained intact in CB_1 knockout mice or drug-treated wild-type mice. Before the olfactory discrimination task, a small piece of chocolate chip was dropped at the farthest diagonal corner from the back of a mouse without letting him notice the action. The latency for each mouse to locate the chip was recorded.

Novel Object Exploration Task and Locomotor Activity

In an attempt to rule out the possibility that CB_1 knockout mice or drug-treated wild-type mice had defective exploratory behaviors, the novel object exploration task modified from previous studies was performed (31, 57). Briefly, CB_1 knockout mice or wild-type mice treated with drug were individually placed in a plastic cage in a ventilation hood during the 15-min acclimation period for 3 days. A day later, each mouse was placed in the cage again and allowed to explore two novel objects for 10 min. Accumulated time spent on exploring those two novel objects was analyzed.

Finally, to rule out the possibility that CB_1 knockout mice or drug-treated wild-type mice had defective levels of activity, mice were monitored for their locomotor activity approximately one day after the last injection of drug. Locomotor activity was monitored in a custom-made transparent Plexiglas chamber (41 cm H × 41 cm W × 30 cm L) inside the Optovarimax (Columbus Instrument, Columbus, OH, USA). Mice were individually placed in the center of the chamber and allowed free navigation for 15 min. Locomotor activity is defined as the IR break count number, which is a combination of the vertical rearing infrared beam break and the horizontal distance traveled in the chamber.

Experimental Groupings

In Experiment 1, 12 wild-type mice and 12 CB_1 knockout mice, free of drug treatment, were acclimatized to the behavioral cages for 3 days first. Next day, they were tested on the olfactory foraging task (Fig. 1A), followed by the olfactory discrimination task (Fig. 1B). Approximately one week later, the same 12 mice in each group were tested on the novel object exploration task (Fig. 1C), followed by the locomotor activity task (Fig. 1D).

In Experiment 2, 18 and 19 wild-type mice were treated with systemic vehicle and rimonabant respectively for 3 days. A day later, they were tested on the olfactory foraging task (Fig. 3A), followed by the olfactory discrimination task (Fig. 2A). Approximately one week later, 15 mice out of each group used above (vehicle *vs*. rimonabant), were tested on the novel object exploration task (Fig. 3B). Next, only 10 mice out of each group used above (vehicle *vs*. rimonabant) were tested for their locomotor activity levels owing to the limited time availability of the Optovarimax (Fig. 3C). A new cohort of the wild-type mice, 11 for vehicle and 11 for rimonabant, underwent the stereotaxic surgery first. One week later, they were injected with the corresponding drugs into the OB for 3 days and tested on the olfactory foraging and the olfactory discrimination tasks on next day (Fig. 2B). Moreover, to examine whether the TRPV1 antagonist CPZ could rescue rimonabant-induced olfactory deficit, 15 and 16 wild-type mice were assigned to the CPZ plus vehicle and the CPZ plus rimonabant groups and injected systemically with the corresponding drugs for 3 days. Next day, they were tested on the olfactory foraging task (Fig. 3A), followed by the olfactory discrimination task (Fig. 2A). Approximately one

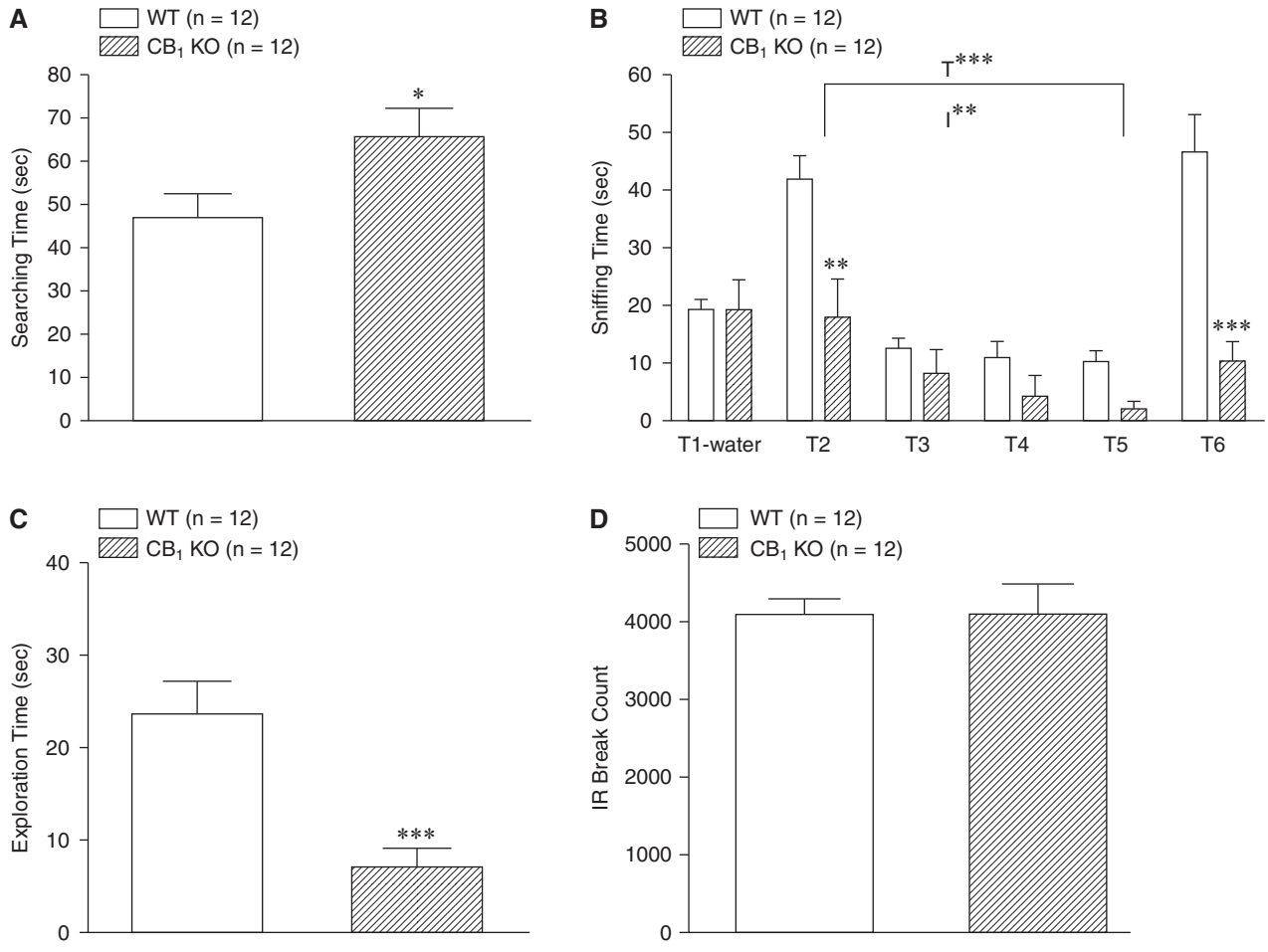

Fig. 1. CB_1 receptor knockout (KO) mice *per se* show deficiencies in olfactory discrimination, olfactory foraging and novel object exploration abilities. (A) It took longer time for CB_1 knockout mice to find the chocolate chip in the olfactory foraging task. (B) In the olfactory discrimination task, time sniffing the water in T1 was not affected in CB_1 knockout mice. Both CB_1 knockout mice and wild-type mice spent less time sniffing the same odor from T2 to T5, although in a different rate. T*** denotes significant main effect of time; I** denotes significant interaction effect of CB_1 × time. Moreover, CB_1 knockout mice spent less time sniffing the novel odors (T2 and T6). (C) CB_1 knockout mice spent less time exploring the novel objects. (D) CB_1 knockout mice displayed normal levels of the locomotor activity. Data are presented as mean ± SEM. *** denotes $P < 0.001$; ** denotes $P < 0.01$; * denotes $P < 0.05$.

week later, both groups (CPZ plus vehicle *vs.* CPZ plus rimonabant) were tested on the novel object exploration (Fig. 3B) and the locomotor activity tasks (Fig. 3C). Another cohort of wild-type mice, 8 for the CPZ plus vehicle group and 9 for the CPZ plus rimonabant group, underwent the stereotaxic surgery first. One week later, they were injected with the corresponding drugs into the OB before the acclimation period for 3 days and tested on the olfactory foraging and the olfactory discrimination tasks on next day (Fig. 2B). Finally, for the acute administration regimen, another cohort of wild-type mice was acclimatized to the behavioral cage for 3 days first. Next day, 14 and 15 mice were treated with vehicle and rimonabant respectively at 15 min before the olfactory foraging and the olfactory discrimination tasks (Fig. 3D).

Statistical Analysis

All data were indicated as the mean ± standard error of mean (SEM). Two-group comparisons were analyzed by two-tailed Student's *t*-test. A three-way (CPZ × SR1 × time) repeated measure ANOVA with the times of trials (time) as a repeated measure variable was used to examine the effects of CPZ and rimonabant on sniffing time for the same odor after successive trials (T2 to T5). Two-way (CPZ × SR1) ANOVAs were used to examine the effects of CPZ and rimonabant on differences of time spending in the olfactory foraging, the novel object exploration and the locomotor activity tasks, as well as in different trials of the discrimination task (T1, T2 and T6). The levels of statistical significance were set at $P < 0.05$.

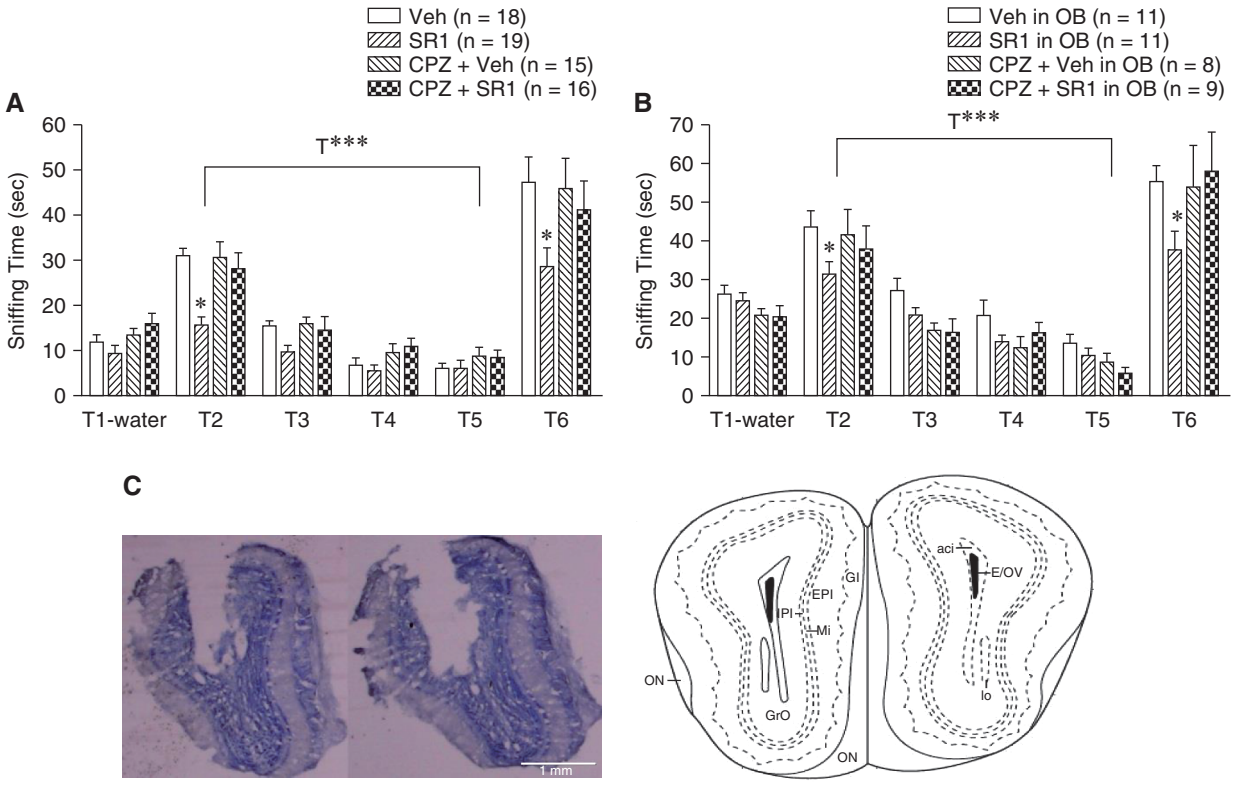

Fig. 2. The olfactory TRPV1 is involved in rimonabant (SR1)-induced olfactory discrimination deficit in wild-type mice. (A) Systemic administration of the TRPV1 antagonist CPZ prevented the systemic rimonabant-induced olfactory discrimination deficit. All mice in the four systemic drug treatment groups (vehicle (Veh), rimonabant, CPZ plus vehicle and CPZ plus rimonabant) habituated to the same odor from T2 to T5. T*** denotes significant main effect of time. In addition, mice treated with rimonabant systemically spent less time sniffing novel odors presented at T2 and T6. (B) The intra-OB administration of the TRPV1 antagonist CPZ prevented the intra-OB rimonabant-induced olfactory discrimination deficit. All mice in the four intra-OB drug treatment groups (vehicle, rimonabant, CPZ plus vehicle and CPZ plus rimonabant) habituated to the same odor from T2 to T5. T*** denotes significant main effect of time. Moreover, mice treated with rimonabant into the OB spent less time sniffing novel odors presented at T2 and T6. (C) Representative photograph of correct cannula location in the OB on the cresyl violet-stained section and the corresponding OB in the mouse brain atlas. Scale bar = 1 mm. Data are presented as mean ± SEM. *** denotes $P < 0.001$; * denotes $P < 0.05$.

Results

CB_1 Knockout Mice Showed Deficiencies in Olfactory Discrimination, Olfactory Foraging and Novel Object Exploration, but not in Locomotor Activity

In Experiment 1, we examined whether chronic genetic disruption of CB_1 receptor affected olfactory functions using CB_1 knockout mice *vs.* wild-type mice. Surprisingly, we found that it took longer time for CB_1 knockout mice to find the chocolate chip in the olfactory foraging task when compared to wild-type mice [$t(22) = 2.169$, $P < 0.05$] (Fig. 1A). For the olfactory discrimination task, CB_1 knockout and wild-type mice displayed similar sniffing time for water in T1 [$t(22) = 0.537$, $P > 0.05$] (Fig. 1B). During the next four trials, both groups spent less time sniffing the same odor from T2 to T5 [main effect of time: $F(3, 66) = 29.216$, $P < 0.001$], despite the rate of odor habituation seemed to be different [CB_1 x time interaction effect: $F(3, 66) = 4.68$, $P < 0.01$] (Fig. 1B). Finally, CB_1 knockout mice spent less time sniffing novel odors presented at T2 and T6 [T2: $t(22) = 3.085$, $P < 0.01$; T6: $t(22) = 5.048$, $P < 0.001$]. To further rule out the possibility that CB_1 knockout mice *per se* had deficiencies in exploratory behaviors or locomotivity, exploratory time on novel odorless objects or locomotor activity was measured. Intriguingly, CB_1 knockout mice spent less time exploring novel objects when compared to wild-type mice [$t(22) = 4.127$, $P < 0.001$] (Fig. 1C), despite they showed normal levels of locomotor activity [$t(22) = 0.024$, $P > 0.05$] (Fig. 1D). Together, these results suggest that CB_1 knockout mice not only habituate to the same odor in a different rate, but also exhibit deficiencies in general exploratory behaviors, including searching chocolate chips in the olfactory

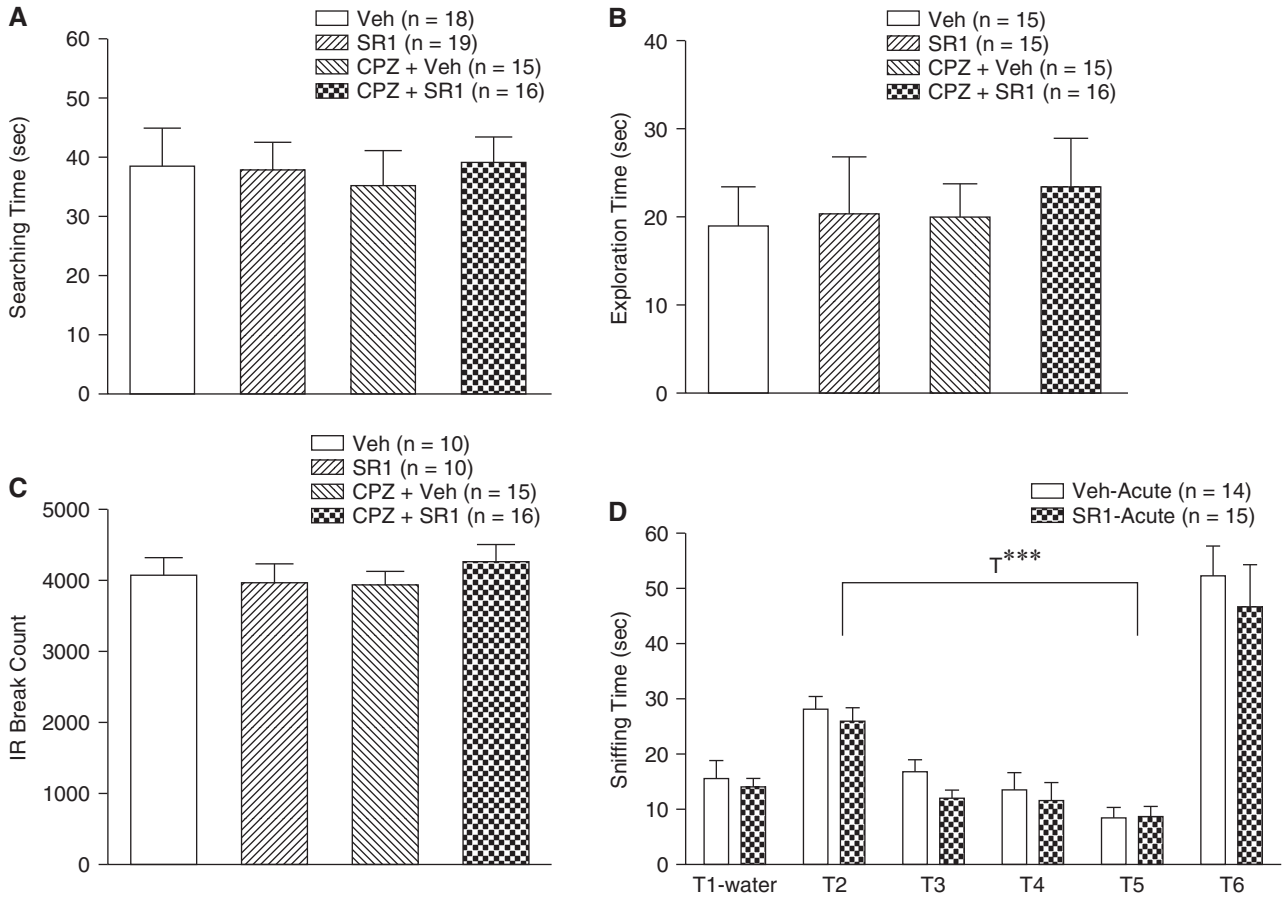

Fig. 3. The short-term systemic administration of rimonabant (SR1) does not affect olfactory foraging, novel object exploration and locomotivity, whereas the acute systemic administration regimen does not affect olfactory discrimination in wild-type mice. (A) Time spent on foraging chocolate chip was not influenced by the short-term systemic rimonabant treatment. (B) The same short-term administration regimen did not affect novel object exploration. (C) The same short-term administration regimen did not affect the level of locomotor activity. (D) The acute systemic administration of rimonabant did not affect olfactory discrimination since both groups spent comparable time sniffing novel odors presented at T2 and T6. Both groups of mice habituated to the same odor from T2 to T5. T*** denotes significant main effect of time. Data are presented as mean ± SEM. *** denotes $P < 0.001$.

foraging task, identifying novel odors in the olfactory discrimination task and exploring novel objects in the novel object exploration task.

Short-Term but not Acute Administration of Rimonabant, Injected Systemically or into the OB, Impaired Olfactory Discrimination that Was Rescued by CPZ in Wild-Type Mice

In Experiment 2, we used the pharmacological inactivation approach to examine whether CB_1 receptor or TRPV1 was involved in olfactory functions. We first tested whether the short-term systemic injection of rimonabant for 3 days affected olfactory discrimination and whether the TRPV1 antagonist CPZ, at 30 min before rimonabant, could prevent rimonabant's effect in wild-type mice. Mice were randomly divided into four systemic drug treatment groups (vehicle, rimonabant, CPZ plus vehicle, CPZ plus rimonabant) and then underwent the olfactory discrimination task. First, no differences were observed in sniffing time for water in the blank trial (T1) among these four groups (all $P > 0.05$). All mice spent less time sniffing the same odor after successive trials (T2 to T5) [main effect of time: $F(3, 192) = 88.344$, $P < 0.001$]. Importantly, the rimonabant group, but not the vehicle, the CPZ plus vehicle or the CPZ plus rimonabant group, spent less time sniffing novel odors presented at T2 and T6 [CPZ × SR1 interaction effect for T2: $F(1, 64) = 6.016$, $P < 0.05$; for T6: $F(1, 64) = 4.525$, $P < 0.05$] (Fig. 2A). These results suggest that systemic administration of CPZ prevents the olfactory discrimination deficit induced by systemic rimonabant in wild-type mice.

Next, we examined whether the short-term administration of rimonabant directly into the OB for

3 days affected olfactory discrimination and whether this effect could be rescued by intra-OB infusion of CPZ in wild-type mice. Similarly, mice were randomly divided into four intra-OB drug treatment groups (vehicle, rimonabant, CPZ plus vehicle, CPZ plus rimonabant) and underwent the olfactory discrimination task. No differences were observed in sniffing time for water in the blank trial (T1) among these four groups (all $P > 0.05$). Moreover, all mice spent less time sniffing the same odor after successive trials (T2 to T5) [main effect of time: $F(3, 105) = 88.141$, $P < 0.001$]. Importantly, mice infused with rimonabant into the OB spent less time sniffing novel odors presented at T2 and T6 when compared to the remaining three groups [CPZ × SR1 interaction effect for T2: $F(1, 35) = 4.618$, $P < 0.05$; for T6: $F(1, 35) = 4.96$, $P < 0.05$] (Fig. 2B). These results suggest that intra-OB administration of CPZ prevents the olfactory discrimination deficit induced by intra-OB infusion of rimonabant in wild-type mice. Finally, only mice with both cannulas correctly placed in the OB were included for final data analysis. It is important to point out that the coordinates of the guide cannula implantation in the OB were adopted from an *in vivo* study, which verified the restriction of drug diffusion to the granule cell layer in the OB by trypan blue (54). Inspection of the OB tissue revealed evidence of a small lesion and gliosis at the site of injection, while the surrounding tissue was generally intact. An example of the correct localization of cannulas in the OB and the corresponding OB in the mouse brain atlas was shown in Fig. 2C. Together, these results indicate that systemic or intra-OB CPZ treatment prevents the olfactory discrimination deficit induced by rimonabant, *via* the same route of CPZ, in wild-type mice.

Finally, in an attempt to rule out the possibility that drug-treated wild-type mice had defective exploratory behaviors or defective locomotor activities, the same four groups of mice (vehicle, rimonabant, CPZ plus vehicle and CPZ plus rimonabant) underwent the olfactory foraging, the novel object exploration and the locomotor activity tasks. No differences were observed in the olfactory foraging task (all $P > 0.05$, Fig. 3A), the novel object exploration task (all $P > 0.05$, Fig. 3B) and the locomotor activity task (all $P > 0.05$, Fig. 3C) among these four groups, suggesting that the drug treatments affect neither locomotivity nor general exploratory behaviors toward odorant and odorless objects. On the other hand, to test the acute effect of rimonabant on olfactory functions, after the acclimation period for 3 days, rimonabant or vehicle was injected systemically at 15 min before the olfactory discrimination task. We found that both groups spent less time sniffing the same odor from T2 to T5 [main effect of time: $F(3, 81) = 29.872$, $P < 0.001$; no SR1 × time interaction effect, $P > 0.05$], suggesting that they both habituate to odor normally. Moreover, the acute administration regimen neither affected olfactory foraging nor olfactory discrimination in wild-type mice (foraging, T1, T2 and T6: all $P > 0.05$, Fig. 3D). These results suggest that the acute systemic administration of rimonabant, unlike the short-term administration regimen, does not affect olfactory discrimination in wild-type mice.

Discussion

This study examined whether the cannabinoid CB_1 receptor or the vanilloid receptor TRPV1 played a role in olfactory functions using the genetic disruption and the pharmacological inactivation approaches. We first demonstrated that CB_1 knockout mice exhibited olfactory discrimination deficit and habituated differentially when compared to wild-type mice. However, despite their normal locomotivity, CB_1 knockout mice showed deficiencies in general exploratory behaviors toward the odorant and odorless objects, which might be confounded with their olfactory discrimination deficit. We next turned to the pharmacological inactivation approach and showed that short-term systemic or intra-OB administration of rimonabant, but not the acute systemic administration regimen, impaired olfactory discrimination in wild-type mice. Importantly, rimonabant-induced olfactory discrimination deficit was rescued by the TRPV1 antagonist CPZ, administered *via* the same route of rimonabant. However, rimonabant and CPZ treatments neither affected locomotivity nor general exploratory behaviors in wild-type mice. These results suggest that TRPV1 in the OB is involved in rimonabant-induced olfactory discrimination deficit, whereas CB_1 knockout mice display deficiencies in olfactory discrimination as well as in general exploratory behaviors toward the odorant and odorless objects. To the best of our knowledge, this is the first study suggests that the olfactory TRPV1 plays a role in olfactory functions and also offers a possible mechanism for the olfactory discrimination deficit induced by rimonabant.

The most intriguing finding in the present study is involvement of the olfactory TRPV1 in the defective odor discrimination induced by rimonabant. We demonstrated that systemic or intra-OB administration of the TRPV1 antagonist CPZ, given 30 min before the daily rimonabant injection for 3 days, *via* the same route of rimonabant, prevented rimonabant-induced olfactory discrimination deficit in wild-type mice (Fig. 2). However, it is important to emphasize that rimonabant and CPZ treatments do not affect locomotivity or general exploratory behaviors in wild-type mice (Fig. 3). TRPV1, the capsaicin receptor, is a non-selective cation channel expressed throughout the CNS, including the cerebral cortex, hippocampus,

hypothalamus, olfactory nuclei and spinal cord (14, 48, 58). Moreover, TRPV1 is robustly expressed in the sensory neurons of the dorsal root ganglion, where it is well characterized to mediate nociception and inflammation (7, 28, 53). In addition to its central role in pain perception, TRPV1 also modulates other sensory modalities such as thermosensation, mechanotransduction, vision (7, 52) as well as synaptic plasticity (20). More importantly, TRPV1 has been shown to mediate several physiological effects of rimonabant. For instance, CPZ reverses the rimonabant-induced neuroprotective effects against the electroencephalographic flattening, memory impairment and hippocampal cornu ammonis 1 (CA1) neuronal loss caused by cerebral ischemia in gerbils (42). Moreover, unlike the reduction of neurogenesis in the SVZ observed in CB_1 knockout mice, rimonabant paradoxically increases neurogenesis in the SVZ that is abolished only in TRPV1 knockout mice (29). Similarly, our results suggest that the olfactory TRPV1 is involved in rimonabant-induced odor discrimination deficit. To the best of our knowledge, this is the first study reporting that TRPV1 in the OB mediates rimonabant-induced olfactory discrimination deficit, a sensory modality that has not yet been reported for the involvement of TRPV1. However, it is important to point out that wild-type mice treated with CPZ alone, either systemically (1 mg/kg, s.c.) or directly into the OB (1 μg), for 3 days did not exhibit olfactory discrimination deficit. Therefore, it is worth further examining the exact role of TRPV1 in olfactory functions. For example, it is of great interest to establish a dose-dependent curve for the effects of CPZ on olfactory discrimination. Moreover, whether the TRPV1 agonist capsaicin affects olfactory discrimination similar to rimonabant or whether TRPV1 knockout mice *per se* display deficiencies in olfactory foraging, olfactory habituation and olfactory discrimination deserves further investigation. Finally, since rimonabant-induced olfactory discrimination deficit is prevented by CPZ, it is of interest to examine whether this deficit could be rescued when rimonabant is administered to TRPV1 knockout mice in the future.

In addition, we employed the genetic disruption approach to examine the role of the cannabinoid CB_1 receptor in olfactory functions and found that CB_1 knockout mice *per se* displayed deficiencies in general exploratory behaviors (Fig. 1). Although CB_1 knockout mice showed normal levels of locomotor activity, it took longer for them to locate the chocolate chip, when compared to wild-type mice, in the olfactory foraging task. Furthermore, CB_1 knockout mice spent less time exploring the novel objects in the novel object exploration task. Hence, the olfactory discrimination deficit observed in CB_1 knockout mice is confounded with their deficiencies in general exploratory behaviors toward the odorant and odorless objects. Likewise, previous studies showed that transgenic mice lacking CB_1 receptor expression ubiquitously (CB_1 knockout mice) or specifically in cortical glutamatergic neurons (Glu-CB_1 knockout mice) display deficits in object exploration, object recognition and social interaction (21, 27), which is in accordance with the defective exploratory abilities of CB_1 knockout mice observed in the present study. In contrast, mice lacking CB_1 receptors in γ-aminobutyric acid-ergic (GABAergic) neurons (GABA-CB_1 knockout mice) show increased exploratory drive (21). It is therefore suggested that exploratory behaviors are balanced by the endocannabinoid system *via* the CB_1 receptor activation on the two opposing neuronal subpopulations. Because the olfactory discrimination ability is defined as exploratory behaviors toward the novel odor and is confounded with general exploratory deficiencies observed in CB_1 knockout mice *per se*, whether or not CB_1 receptor is involved in rimonabant-induced olfactory deficiency remains inconclusive. However, since the cannabinoid THC promotes olfactory detection and impairs olfactory habituation *via* CB_1 activation in fasted mice (54), it is speculative that rimonabant-induced olfactory discrimination deficit is mediated both by TRPV1 and by CB_1 receptor. The putative synergistic effect of TRPV1 and CB_1 receptor on rimonabant-induced olfactory discrimination deficit may be one of the reasons for the phenotype discrepancy between rimonabant-treated wild-type mice (olfactory discrimination deficit only) and CB_1 knockout mice (general exploratory behavior deficiencies). Moreover, chronic genetic disruption of CB_1 receptor in CB_1 knockout mice is likely to cause several developmental alterations and compensatory changes, which are super-imposed on the true effects of CB_1 inactivation/removal. Therefore, short-term pharmacological inactivation *vs.* chronic genetic disruption of CB_1 receptor may affect general exploratory behaviors differentially.

We also found that only the short-term administration of rimonabant, but not the acute administration regimen, impaired olfactory discrimination in wild-type mice (Fig. 3). The lack of rimonabant's effect on the acute administration regimen was not due to difference in rimonabant dosages since our preliminary data showed that 3 consecutive injections of rimonabant, like the single rimonabant injection, at approximately 15 min before the olfactory behavioral tasks did not affect olfactory discrimination (data not shown). These findings suggest that it takes 3 days for rimonabant to exert its detrimental effect on olfactory discrimination, which is likely to be mediated by other neuronal processes. For example, defective neurogenesis in the SVZ-OB pathway has been suggested to mediate odor discrimination deficit (4, 19, 31).

However, previous research indicates that rimonabant, injected at a daily dose (1 mg/kg, i.p.) for 3 days, increases neurogenesis in the SVZ at one week after the last drug administration (29). The neurogenesis-promoting effect of rimonabant in the SVZ is mediated by TRPV1 since it persists in CB_1 knockout mice, but is abolished in TRPV1 knockout mice (29). Although the neurogenesis-promoting effect of rimonabant in the SVZ observed in the previous study seems to contradict with rimonabant-induced olfactory discrimination deficit observed in the present one, it is of interest to speculate several possibilities. First, despite a correlation between diminished neurogenesis in the OB and olfactory discrimination deficit has been suggested (15, 19), it is important to emphasize that the neurogenesis-promoting effect of rimonabant in the SVZ is observed on the seventh day after the last drug treatment (29). It usually takes 7 to 14 days for the neuroblasts in the SVZ to migrate tangentially along the rostral migratory stream to the OB where they differentiate into interneurons (32, 35, 36). Therefore, the defective odor discrimination caused by rimonabant on one day after drug treatment may affect different subpopulations of newly migrated neurons in the OB directly, thus may have nothing to do with the increased neurogenesis in the SVZ on the seventh day. Interestingly, olfactory learning reduces survival of the newborn granule cells in the OB when dissimilar odorants are used (37). Hence, it is possible that the neurogenesis-promoting effect observed on the seventh day serves as a compensatory mechanism for the olfactory discrimination deficit caused by rimonabant. Since it is unknown whether rimonabant increases or decreases neurogenesis in the SVZ on one day after the last drug treatment, the relationship between rimonabant-induced odor deficiency and rimonabant-affected neurogenesis in the SVZ remains to be determined. Moreover, a high dose of rimonabant (3 mg/kg) is used to impair odor discrimination in the present study, whereas a low dose of rimonabant (1 mg/kg) exerts the neurogenesis-promoting effect in the SVZ. As discussed above, rimonabant seems to exert a biphasic effect at TRPV1 by blocking the channel at low concentrations whereas activating it at high concentrations (13, 20, 47, 66). Therefore, it is likely that different dosages of rimonabant exert the opposite effects on neurogenesis in the SVZ as well as olfactory discrimination *via* different mechanisms. Finally, it is possible that the rimonabant-induced and TRPV1-involved odor discrimination deficit is mediated by neuronal mechanisms other than neurogenesis in the SVZ-OB pathway, which awaits further investigation. Taken together, the short-term *vs*. the acute administration of rimonabant affects olfactory discrimination ability differentially.

Olfactory processes in human are often neglected by bias because most odor information is processed subconsciously when compared to other sensory modalities. However, partner selection and visual perception in human are influenced by unconscious olfactory cues (26, 64). Moreover, olfactory dysfunctions are implicated in food intake disorders (1, 6), schizophrenia (2, 40, 50, 51) and autism patients (5, 17). Therefore, olfactory discrimination deficit may serve as a biomarker for the early symptoms of several psychiatric disorders. Importantly, rimonabant-induced olfactory discrimination deficit observed in this study may be related to its mood and anxiety side effects when it is used as an anti-obesity agent in several clinical trials by the US Food and Drug Administration*, which deserves further elucidation. In conclusion, our findings offer a possible mechanism for the defective odor discrimination induced by rimonabant and shed light on a potential role of the olfactory TRPV1 in olfactory functions.

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