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5-Hydroxytryptamine 2A receptor inverse agonist pimavanserin impairs maternal behavior in postpartum female rats

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ABSTRACT

Maternal behavior is a highly motivated and well-organized social behavior. Although previous studies have shown that 5-hydroxytryptamine 2A/2C (5-$HT_{2A/2C}$) receptors play an important role in mediating the expression of normal maternal behavior, the role of 5-HT_{2A} receptors in maternal behavior remains unclear. In the present study, the homecage maternal behavior test paradigm was used to investigate whether the low or constitutive (i.e., intrinsic or basal) activity of 5-HT_{2A} receptors influences the expression of normal maternal behavior. The inverse agonist pimavanserin (3, 6, and 12 mg/kg) and neural antagonist ketanserin (2.5 and 5 mg/kg) were used to induce the low or constitutive activity of 5-HT_{2A} receptors, respectively. The results showed that inverse agonism of 5-HT_{2A} receptors by pimavanserin slightly impaired maternal behavior in postpartum female rats, reflected by less time spent on nest building, and that ketanserin did not impair major components of maternal behavior. Furthermore, neither pimavanserin nor ketanserin impaired spontaneous locomotion. These data indicate that the reduced activity of 5-HT_{2A} receptors may impair the expression of normal maternal behavior and this effect is not due to a nonspecific sedative effect. Nevertheless, the constitutive activity of 5-HT_{2A} receptors in the absence of an endogenous agonist (i.e., serotonin) may be involved in the expression of normal maternal behavior.

1. Introduction

Maternal behavior in female rats is highly socially motivated and well-organized (Li, 2015). Female rats instinctively express maternal behavior at the birth of the first litter. Within hours of parturition, mother rats (dams) display the full repertoire of pup-directed behaviors such as building the nest for pups, putting them together in the nest site, hunching over them to permit suckling, and licking them with the mouth (Bridges, 2015; Li, 2015). This maternal behavior is observed in the subsequent three- to four-week period.

The role of serotonergic transmission in maternal behavior has been investigated in several studies, and evidence shows that 5-hydroxytryptamine 2A/2C (5-$HT_{2A/2C}$) receptors play a critical role in the expression of normal maternal behavior. Atypical antipsychotic drugs such as clozapine, olanzapine, risperidone, and quetiapine, as strong 5-$HT_{2A/2C}$ receptor antagonists, dose-dependently disrupt active components of maternal behavior (Li et al., 2004, 2005). A milestone in the findings of the roles of 5-$HT_{2A/2C}$ receptors in maternal behavior is a study indicating that clozapine disrupts maternal behavior primarily by the blockade of 5-$HT_{2A/2C}$ receptors and not dopamine D_2 receptors (Zhao and Li, 2009). In this study, pretreatment with 2,5-dimethoxy-4-iodo-amphetamine (DOI, a preferential 5-$HT_{2A/2C}$ receptor agonist), but not quinpirole (a selective dopamine D_2 receptor agonist), reversed the clozapine-induced disruption in maternal behavior. These data indicate that 5-$HT_{2A/2C}$ receptors play a critical role in the expression of normal maternal behavior.

As the 5-HT_{2A} receptor is an important target for antipsychotics, we should pay more attention to the role of 5-HT_{2A} receptors in maternal behavior. In general, the receptor has a constitutive (i.e., intrinsic or basal) level of activity in the absence of any ligand (e.g., serotonin). An agonist increases the activity of a receptor above its basal level, whereas an inverse agonist decreases the activity below the basal level. It remains unclear whether the reduced 5-HT_{2A} receptor activity disrupts maternal behavior. However, the prerequisite for inverse agonism is that the constitutive activity of 5-HT_{2A} receptors must contribute to maternal behavior (Kenakin, 2004). Furthermore, the role of constitutive 5-HT_{2A} receptor activity in the expression of normal maternal behavior is unclear. Our previous study showed that the selective 5-

Abbreviations: 5-HT, 5-hydroxytryptamine; PP, postpartum day; DOI, 2,5-dimethoxy-4-iodo-amphetamine

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HT_{2A} receptor antagonist MDL100907 did not significantly disrupt maternal behavior, although it showed a non-significant tendency to impair maternal behavior (all $P > 0.051$). As such, the present study involved more extensive research in order to investigate the role of 5-HT_{2A} receptors in the expression of normal maternal behavior, with a special focus on constitutive and reduced activities of 5-HT_{2A} receptors.

To address these issues, we used pharmacological methods to mediate activities of 5-HT_{2A} receptors and investigate the effect of constitutive or reduced activities of 5-HT_{2A} receptors on maternal behavior. The purpose of the current study was to test the following hypotheses: (1) Constitutive activity of 5-HT_{2A} receptors contributes to the expression of normal maternal behavior. Constitutive activity of 5-HT_{2A} receptors can be initiated by ketanserin, which is a neutral antagonist of 5-HT_{2A} receptors (Li et al., 2009). Therefore, it can antagonize endogenous serotonin and lack negative intrinsic efficacy. Therefore, the role of the constitutive activity of 5-HT_{2A} receptors in the maternal behavior can be tested when 5-HT_{2A} receptors are occupied by ketanserin. (2) Reduced activity of 5-HT_{2A} receptors may impair maternal behavior. Since overactivity of 5-HT_{2A} receptors has been proven to disrupt maternal behavior (Gao et al., 2018), we preferred to test the effect of low 5-HT_{2A} receptor activity on maternal behavior. A reduction in the activity of 5-HT_{2A} receptors can be induced by pimavanserin (ACP-103), a potent selective 5-HT_{2A} receptor inverse agonist that does not bind to D_2 receptors (Vanover et al., 2006). We hypothesized that the constitutive activity of 5-HT_{2A} receptors contributes to the expression of normal maternal behavior, and the reduced activity of 5-HT_{2A} receptors might disrupt that behavior.

2. Materials and methods

2.1. Animals

A total of 54 virgin, female Sprague-Dawley rats (60–80 days) were used as subjects in the present study. All animals were purchased from Experiment Animal Center, Daping Hospital & Research Institute of Surgery, Army Medical University, Chongqing, China. Animals were initially housed in pairs in transparent cages (47 cm L × 32 cm W × 21 cm H) with corn-cob granule for bedding in a colony on a 12-hour light/dark cycle (lights on at 08:00 h), and allowed ad libitum access to food and water. All experiments were conducted in strict accordance with the recommendations of “Regulations for the Administration of Affairs Concerning Experimental Animals,” the State Science and Technology Commission, China. All animal protocols were approved by the animal care and use committee at Southwest University, China.

2.2. Drugs

Pimavanserin tartrate and ketanserin tartrate were purchased from Dalian Meilun Biotechnology Corporate (Dalian, China). Pimavanserin tartrate and ketanserin tartrate were dissolved in 0.9% saline. Tested drugs were administered as salts (i.e., pimavanserin tartrate and ketanserin tartrate), and doses are expressed as free base. Doses of each drug were chosen based on a literature review and our pilot experiment: pimavanserin at 3, 6, and 12 mg/kg (Vanover et al., 2006), and ketanserin at 2.5 and 5.0 mg/kg (Bonilla-Jaime et al., 2015; Cui et al., 2018; Girish et al., 2012; Murotani et al., 2011).

2.3. Experiment 1: the effects of the 5-HT_{2A} receptor inverse agonist pimavanserin on maternal behavior

We measured the effects of pimavanserin on maternal behavior. The basic procedure was similar to what was described in our previous study (Chen et al., 2014). Briefly, 7 days after their arrival, each female rat was placed into the cage of a proven stud male for 10 days to ensure pregnancy. Then, pregnant females were housed singly until parturition after which they were housed together with their litters for the remainder of the experiment. Animals were observed every day for signs of parturition from two or three days before the first possible expected parturition date. The day that pups were found in cages at 10:00 a.m. was designated as Day 1 postpartum (PP1), and when the pups were found at 17:00 p.m., the day was designated as Day 0 postpartum (PP0). Once the dam was found with pups, two shredded paper towels were provided to dams as nesting materials. On Day 2 postpartum (PP2), we culled each litter to eight pups (4 males and 4 females with the most visible milk bands) and moved all dams to clean transparent cages with their pups. We started maternal behavior tests on PP3 (around 9:00 a.m. each day).

On PP3, dams were randomly assigned to one of four groups: saline group, and pimavanserin 3 mg/kg, 6 mg/kg, and 12 mg/kg groups. Maternal behavior was measured at six timepoints, and the first test was conducted 30 min prior to intraperitoneal drug injections (i.e., baseline level of maternal behavior). Saline group dams were injected with saline (0.9%). Dams in other groups were administered pimavanserin at 3, 6, or 12 mg/kg. The number of dams in each group was six. All drugs were administered intraperitoneally at a volume of 1.0 ml/kg. The other tests were conducted at 1, 3, 5, 24, and 48 h after administration. This timeframe covered the entire acute effect of the tested drugs (Bonilla-Jaime et al., 2015; Girish et al., 2012; Vanover et al., 2006). Each test consisted of two phases. In the first phase, maternal behavior was continuously observed for 10 min under the undisturbed condition. In the second phase, which immediately followed the first undisturbed phase, eight pups were taken away from the dams and the nest was destroyed. One minute later, the pups were placed back in the corner of the cage diagonal to the nest site and maternal behavior was continuously observed for 10 min. As such, pup retrieval behavior could be observed in the second phase. At the end of the 10-min period, we returned the unretrieved pups to the nest site. Both phases were recorded by video cameras and analyzed manually using a laptop computer with an event recording program (JWatcher, http://www.jwatcher.ucla.edu/). The analyzers were blind to each subject's drug condition. The following behaviors were recorded and analyzed as maternal behavior: pup retrieval (a dam picks up a pup outside of the nest in her mouth and carries it back to the nest site), pup nursing (a dam positions herself over the pups with legs splayed to accommodate the pups, providing the pups access to the dam's nipples), pup licking (a dam places its tongue on the anogenital area and the rest of a pup's body), nest building (a dam picks up nesting material in her mouth and transports it back to the nest site or pushes the material with her forepaws toward the nest site).

2.4. Experiment 2: the effects of the 5-HT_{2A} receptor neutral agonist ketanserin on maternal behavior

The aim of Experiment 2 was to investigate whether ketanserin disrupted maternal behavior. The procedure of Experiment 2 was similar to that in Experiment 1.

The dams were randomly assigned to one of three groups: saline group, ketanserin 2.5, or 5 mg/kg groups. Saline group animals were injected with saline (0.9%). Dams in other groups were administered ketanserin at 2.5 or 5 mg/kg. The number of dams in each group was six. All drugs were administered intraperitoneally at a volume of 1.0 ml/kg. The other tests were conducted at 1, 3, 5, 24, and 48 h after administration. This timeframe covered the entire acute effect of the tested drugs (Murotani et al., 2011). The observation procedure was similar to that in Experiment 1.

2.5. Experiment 3: to test whether ketanserin could block the inhibitory effect of pimavanserin

The procedure of Experiment 3 was similar to that of Experiment 1. Dams were randomly assigned to saline group and combined injection

group. Dams were administered saline or a combination of pimavanserin 3 mg/kg and ketanserin 5 mg/kg. The number of dams in each group was six. The observation procedure was similar to that in Experiment 1.

2.6. Experiment 4: effects of pimavanserin and ketanserin on locomotion activity

The aim of Experiment 4 was to determine whether test drugs destroyed maternal behavior by a nonspecific sedative effect or was more focused on maternal behavior. We investigated the effects of drugs at tested doses on spontaneous locomotor activity. The procedure of the spontaneous locomotor activity test conducted on PP7 has carefully been described by Seibenhener (Seibenhener and Wooten, 2015). The animals used to be tested in maternal behavior went through locomotion test on PP7 for the washout of injected drugs. Briefly, dams received the same drugs as they had received in the maternal behavior test and then dams were acclimatized to the experimental room for 1 h prior to starting the test. One hour after administration, a dam was placed in an open field maze (50 cm × 50 cm × 50 cm) for free and uninterrupted movement for 10 min. During the test, their pups were laid aside in their homecages. After the test, the dam was removed and transported back to the vivarium. All experiments were recorded by video cameras and the velocity during the test time for each animal was analyzed by a software called EthoVision 8.5 (Noldus Information Technology, Wageningen, the Netherlands).

2.7. Statistical analysis

Maternal behavior data for the 1st 10-min undisturbed test and 2nd 10-min pup retrieval test were similar, except for the nest building and pup retrieval behavior, because the nest was disrupted before the 2nd test and pup retrieval behavior only occurred during the 2nd test. To avoid redundancy, maternal behavior data for the 1st 10-min undisturbed test and 2nd 10-min pup retrieval test at each test time point were combined and were presented as mean ± SEM. Frequency and duration data (in seconds) on PP3 were analyzed separately using a factorial repeated measure analysis of variance (ANOVA) with the group as the between-subjects factor and test time point as the within-subjects factor. Group differences were further investigated using the least significant difference (LSD) post hoc test. Differences among the groups at specific time points were analyzed using one-way ANOVA, followed by post hoc LSD tests. Data on spontaneous locomotor activity on PP7 were analyzed by one-way ANOVA. SPSS 21 was used to analyze all data. Statistical significance was accepted at $P < 0.05$, two-tailed.

3. Results

3.1. Effects of pimavanserin on maternal behavior

3.1.1. Pimavanserin inhibited nest building behavior on PP3

Fig. 1A shows that pimavanserin significantly shortened nest building duration. Repeated measures ANOVA revealed a significant main effect of group [$F(3, 20) = 6.104$, $P = 0.004$], and a significant test time effect [$F(5, 100) = 8.674$, $P < 0.001$], but no significant interaction effect [$F(15, 100) = 1.487$, $P = 0.124$]. Post hoc LSD tests indicated that pimavanserin at 3, 6, and 12 mg/kg significantly impaired nest building behavior ($P = 0.032$, $P < 0.001$, and $P = 0.002$, respectively). One-way ANOVAs revealed that pimavanserin at 3 mg/kg significantly suppressed building at 1, 3, and 5 h after administration ($P = 0.007$, $P < 0.001$, and $P = 0.006$, respectively). Pimavanserin at 6 mg/kg significantly suppressed building at 1, 3, and 5 h after administration ($P = 0.002$, $P < 0.001$, and $P = 0.007$, respectively). Pimavanserin at 12 mg/kg significantly suppressed building at 1, 3, and 5 h after administration ($P = 0.034$, $P < 0.001$, and $P = 0.001$, respectively). Nest building behavior recovered to the control level at 24 and 48 h after administration (Fig. 1A).

3.1.2. Pimavanserin did not affect nursing and licking behavior and the number of pup retrievals on PP3

As shown in Fig. 1B–D, pimavanserin at 3, 6, and 12 mg/kg did not affect nursing and licking behavior and the number of pup retrievals at 1, 3, 5, 24, and 48 h after administration. Repeated measures ANOVA revealed that there was no significant effect of group (all $P > 0.413$) and no significant test time effect (all $P > 0.152$), or no interaction effect (all $P > 0.461$).

3.1.3. Pimavanserin slightly shortened the first pup retrieval latency, but did not affect the last pup retrieval latency on PP3

Fig. 1E shows the effects of pimavanserin on the first pup retrieval latency. Repeated measures ANOVA revealed there was no significant effect of group [$F(3, 20) = 1.428$, $P = 0.264$], but there was a significant test time effect [$F(5, 100) = 2.994$, $P = 0.015$] and interaction effect [$F(15, 100) = 2.188$, $P = 0.011$]. As such, pimavanserin 3 mg/kg at 24 and 48 h, 6 mg/kg at 24 and 48 h, as well as 12 mg/kg at 48 h after administration significantly shortened the first pup retrieval latency (all $P < 0.032$).

Fig. 1F shows that pimavanserin at 3, 6, and 12 mg/kg did not affect the last pup retrieval latency at 1, 3, 5, 24, and 48 h after administration. Repeated measures ANOVA revealed that there was no significant effect of group [$F(3, 20) = 0.405$, $P = 0.751$], a significant test time effect [$F(5, 100) = 2.548$, $P = 0.033$], but no interaction effect [$F(15, 100) = 1.018$, $P = 0.444$].

3.2. Ketanserin did not impair maternal behavior

3.2.1. Ketanserin did not affect nest building, nursing, licking behavior, and the number of pup retrievals on PP3

Fig. 2A–D shows that ketanserin at 2.5 and 5 mg/kg did not disrupt nursing behavior at 1, 3, 5, 24, and 48 h after administration. Repeated measures ANOVA revealed that there was no significant effect of group (all $P > 0.534$), no test time effect (all $P > 0.772$), and no interaction effect (all $P > 0.343$).

3.2.2. Ketanserin did not prolong the first and last pup retrieval latency on PP3

Fig. 2E and F show that ketanserin at 2.5 and 5 mg/kg did not prolong the first and last pup retrieval latency at 1, 3, 5, 24, and 48 h after administration. Repeated measures ANOVA revealed that there was no significant effect of group (both $P > 0.759$), no test time effect (both $P > 0.433$), and no interaction effect (both $P > 0.493$).

3.3. Ketanserin did not block the inhibitory effect of pimavanserin on maternal behavior

In theory, a neutral antagonist may block the effects of an inverse agonist (Nutt et al., 2017). As such to investigate whether the 5-HT_{2A} receptor neutral antagonist ketanserin could block the inhibitory effect of the 5-HT_{2A} receptor inverse agonist pimavanserin on maternal behavior, animals were injected with ketanserin 5 mg/kg and pimavanserin 3 mg/kg simultaneously at 1 h before the behavioral test. As shown in Fig. 3, combined injections of pimavanserin and ketanserin had no effects on nursing, licking, and pup retrieval behaviors (all $P > 0.145$, Fig. 3B–F). However, similar to the single injection of pimavanserin, combined injections still impaired nest building behavior (Fig. 3A). Repeated measures ANOVA showed a significant main effect of group [$F(1, 10) = 7.950$, $P = 0.018$], a significant test time effect [$(5, 50) = 4.212$, $P = 0.003$], and a significant interaction effect [$F(5, 50) = 6.024$, $P < 0.001$]. One-way ANOVA followed by post hoc LSD test revealed that pimavanserin at 3 mg/kg and ketanserin at 5 mg/kg suppressed building behavior at 1, 3, and 5 h after administration

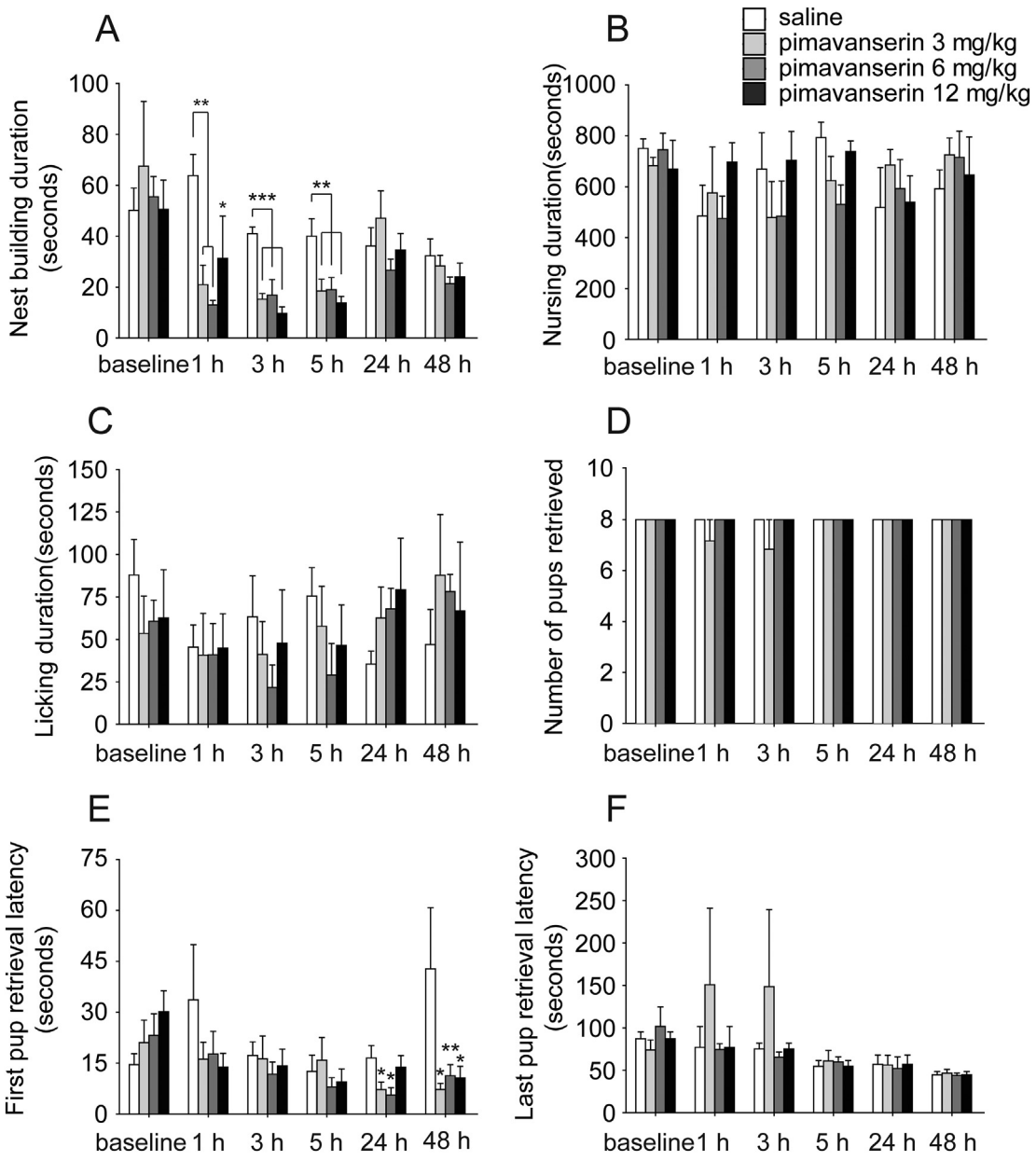

Fig. 1. Effects of pimavanserin on maternal behavior ($n = 6$). (A) Pimavanserin at 3, 6, and 12 mg/kg impaired nest building behavior. (B) Pimavanserin did not impair nursing behavior. (C) Pimavanserin did not impair licking behavior. (D) Pimavanserin did not reduce the number of pups retrieved. (E) Pimavanserin slightly shortened the first pup retrieval latency. (F) Pimavanserin did not prolong the last pup retrieval latency. The data on nursing, licking, nest building duration, number retrieved, first and last retrieval latencies are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus the saline-treated dams.

($P = 0.01$, $P = 0.001$, and $P = 0.002$, respectively).

3.4. Pimavanserin and ketanserin did not affect spontaneous locomotor activity

To determine whether test drugs destroyed maternal behavior by the nonspecific sedative effect or were more focused on maternal behavior, we investigated the effects of drugs at tested doses on the spontaneous locomotor activity on PP7. As shown in Fig. 4, pimavanserin at 3, 6, and 12 mg/kg, ketanserin at 2.5 and 5 mg/kg, as well as combined injections of pimavanserin (3 mg/kg) and ketanserin (5 mg/kg) did not affect spontaneous locomotor activity (cm/s) (all $P > 0.453$).

4. Discussion

The present study showed that the inverse agonism of 5-HT_{2A} receptors with pimavanserin slightly impaired maternal behavior in postpartum female rats, which was reflected by less time spent on nest building. This indicates that the reduced activity of 5-HT_{2A} receptors may impair the expression of normal maternal behavior. Nevertheless, ketanserin did not influence major components of maternal behavior, which indicates that constitutive (i.e., intrinsic or basal) activity of 5-HT_{2A} receptors in the absence of an endogenous agonist (i.e., serotonin) may be involved in the expression of normal maternal behavior. Furthermore, neither pimavanserin nor ketanserin impaired spontaneous locomotion. These data suggest that the maternal inhibitory effect induced by the reduction in the activity of 5-HT_{2A} receptors is not due to a nonspecific sedative effect.

In our study, pimavanserin slightly shortened the first pup retrieval

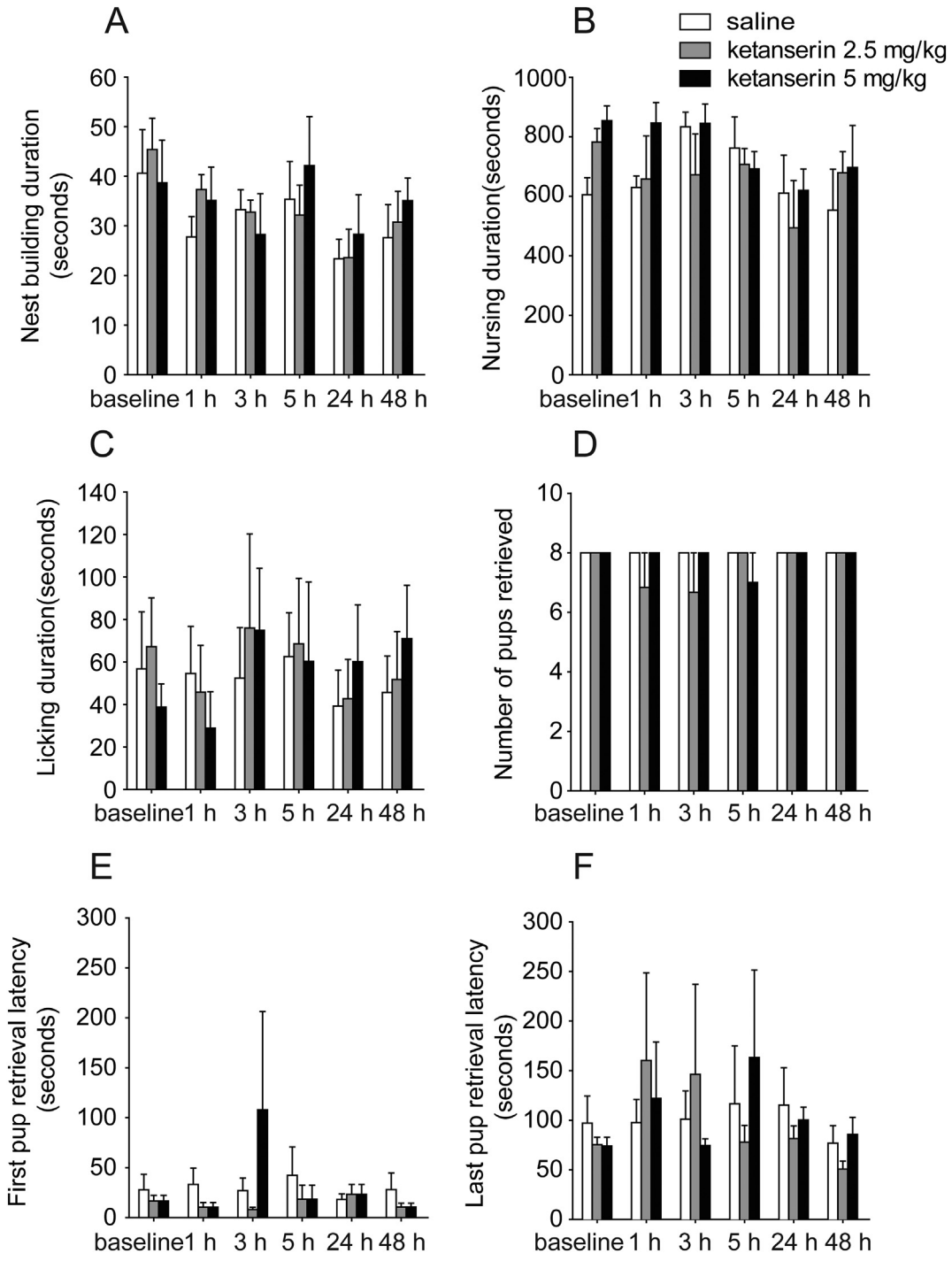

Fig. 2. Effects of ketanserin on maternal behavior ($n = 6$). Ketanserin did not impair maternal behavior, including nest building (A), nursing (B), licking (C), and pup retrieval behaviors (D-F). The data on nursing, licking, nest building duration, the number retrieved, and first and last retrieval latencies are expressed as mean ± SEM.

latency at 24 and 48 h after injection (Fig. 1E). However, this effect was too weak to demonstrate that the maternal motivation was enhanced, and it might be accidental, since the dams treated with combined pimavanserin and ketanserin did not show reduced first retrieval latency, but did show a reduced duration of nest building. Therefore, it is suggested that pimavanserin impairs maternal behavior.

Inverse agonism of 5-HT_{2A} receptor impaired maternal behavior may be due to the reduction in maternal motivation to care pups because 5-HT_{2A} receptor antagonists can reduce dopamine release in the nucleus accumbens (Ichikawa et al., 2001) and inhibit motivated behaviors (Popova and Amstislavskaya, 2002). As such the neural mechanism underlying the impaired maternal behavior induced by the 5-HT_{2A} receptor inverse agonist may be related with a reduction in dopamine release. In fact, the selective 5-HT_{2A} receptor antagonist MDL100907, which is also an inverse agonist for 5-HT_{2A} receptors (Vanover et al., 2006), did not significantly disrupt maternal behavior in our previous study, although it showed a non-significant trend in impairment of maternal behavior (all $P > 0.051$) (Chen et al., 2014). This may be due to the weaker inverse agonist activity of MDL100907 than that of pimavanserin, which needs to be demonstrated.

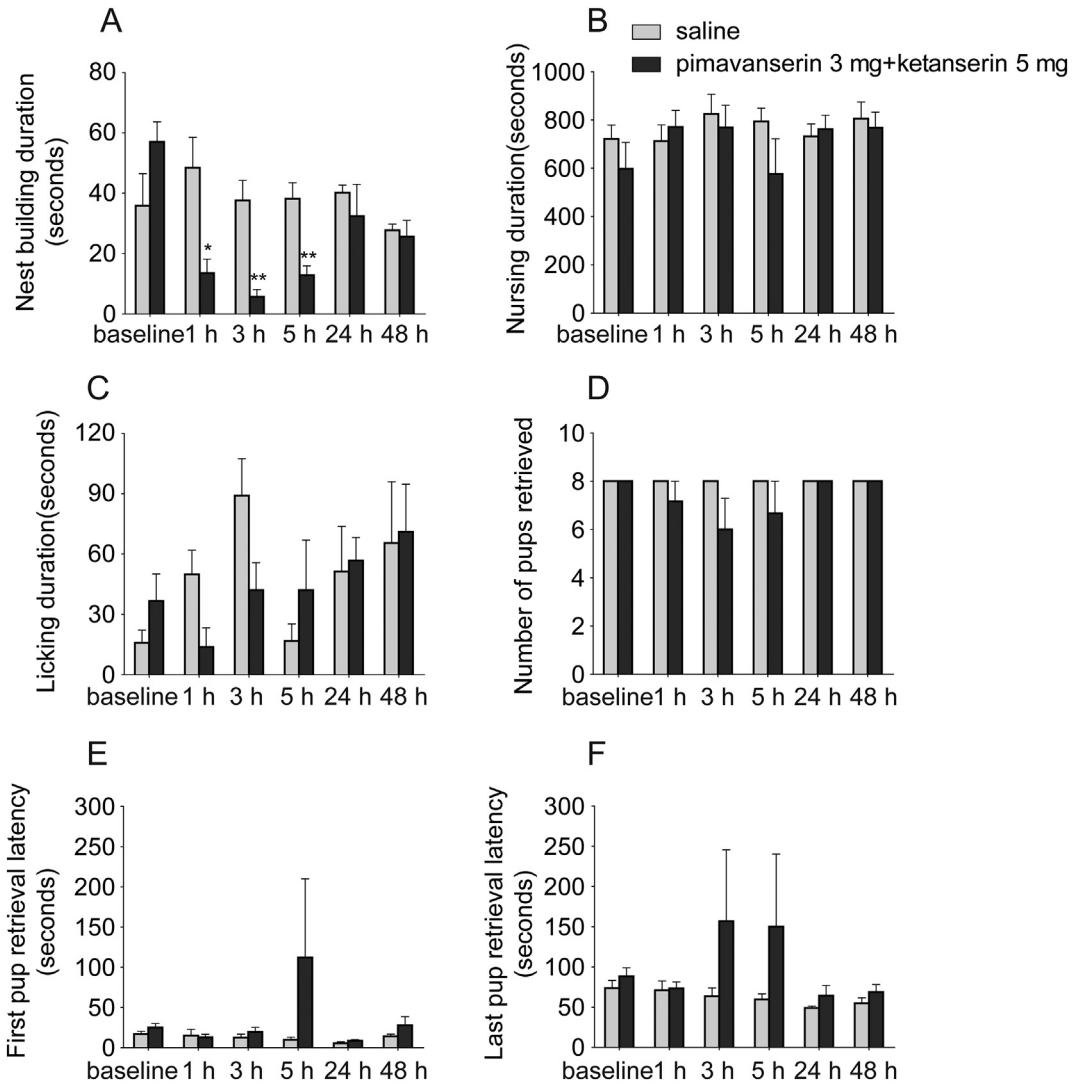

Fig. 3. Ketanserin did not block the inhibitory effect of pimavanserin on maternal behavior ($n = 6$). Combined injections of pimavanserin and ketanserin impaired nest building behavior (A), but had no effect on nursing (B), licking (C), and pup retrieval behaviors (D-F). The data on nursing, licking, nest building duration, the number retrieved, and first and last retrieval latencies are expressed as mean ± SEM. *$P < 0.05$, **$P < 0.01$ versus the saline group.

Although the previous study has reported that the excess activation of 5-HT_{2A} receptor disrupted maternal behavior (Gao et al., 2018), its mechanisms are completely different from 5-HT_{2A} receptor invers agonists. TCB-2, a selective 5-HT_{2A} receptor agonist (Fox et al., 2010), disrupted maternal behavior by the suppression of the behavioral organization aspect of the executive function (Gao et al., 2018) rather than inhibition of maternal motivation (Wu et al., 2018). For example, TCB-2 interrupted normal sequence of pup-directed responses, such as

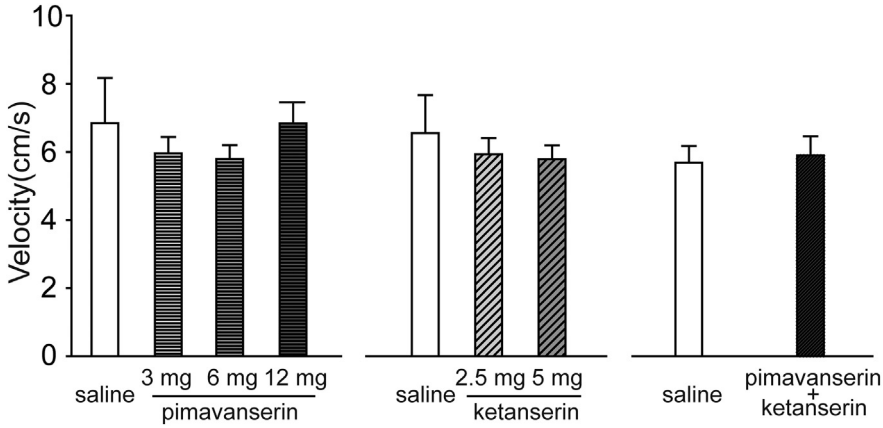

Fig. 4. Pimavanserin, ketanserin, as well as both injections of pimavanserin (3 mg/kg) and ketanserin (5 mg/kg) did not affect spontaneous locomotor activity on PP7. Velocity data are expressed as mean ± SEM.

pup retrieval and pup licking (Gao et al., 2018). However, TCB-2 did not attenuated maternal motivation, but even enhanced maternal motivation, for instance, TCB-2 treated dams displayed a stronger preference to pups over a male conspecific than vehicle-treated ones (Wu et al., 2018). Taken together, in combination with findings in the present study, too high or too low activities of 5-HT_{2A} receptors disrupts maternal behavior, but the mechanisms are different. The overactivity of 5-HT_{2A} receptors disrupt maternal behavior by interrupting normal behavioral organization aspect of the executive function, but the reduced activity of 5-HT_{2A} receptors may impair maternal behavior by reducing maternal motivation, which needs to be demonstrated.

Our previous study has shown that MK212, a 5-HT_{2C} receptor agonist, disrupted maternal behavior (Chen et al., 2014), which may be due to suppressing maternal motivation to take care of pups (Wu et al., 2016), as well as by the sedative effect or the motor suppression effect, as MK212 has been proved to induce hypolocomotion in rodents (Stiedl et al., 2007). 5-HT_{2A} and 5-HT_{2C} receptors play opposing roles in various brain functions and psychological processes related to motivation behavior. For example, agonism of 5-HT_{2C} receptors reduced dopamine release in the nucleus accumbens and cell firing in the ventral tegmental area, whereas agonism of 5-HT_{2A} receptors enhanced dopamine release (Di Matteo et al., 2002; Gobert and Millan, 1999). Similarly, the selective 5-HT_{2C} receptor antagonist SB242085 increased dopamine release in the frontal cortex (Millan et al., 1998), and 5-HT2A antagonist MDL100907 reduced dopamine release (Ichikawa et al., 2001). As such, although either agonism of 5-HT_{2A} receptors or 5-HT_{2C} receptors can disrupt maternal behavior, the mechanisms are completely different. Agonism of 5-HT_{2A} receptors disrupted maternal behavior by interrupting normal behavioral organization aspect of the executive function, but the maternal disruptive effect induced by the 5-TH_{2C} receptor agonist was due to the reduction in maternal motivation.

The most significant finding of the present study was the determination of the involvement of 5-HT_{2A} receptors in rat maternal behavior. We did not only investigate the effect of the neutral antagonist of 5-HT_{2A} receptors (i.e., ketanserin), but also the inverse agonist (i.e., pimavanserin) on maternal behavior. The results showed that pimavanserin specifically impaired maternal behavior, reflected by less time spent on nest building. However, ketanserin at tested doses did not affect the expression of normal maternal behavior. The present study also investigated whether ketanserin could block the inhibitory effect of pimavanserin on maternal behavior. It showed that ketanserin did not alleviate the inhibitory effect of pimavanserin, but the result is not surprising because the binding of $^{[3H]}$ketanserin to 5-HT_{2A} receptors can be completely blocked by pimavanserin in vitro (Vanover et al., 2006), which means that pimavanserin has higher affinity at 5-HT_{2A} receptors than ketanserin. Thus, the replacement of ketanserin with pimavanserin for the occupation of 5-HT_{2A} receptors impaired maternal behavior. The results further demonstrated that the reduced 5-HT_{2A} receptor activity, but not intrinsic activity, impairs maternal behavior. It is suggested that attenuated activity of 5-HT_{2A} receptors may inhibit maternal behavior, whereas medial activity induced by endogenous agonists (i.e., serotonin) and intrinsic activity are involved in the expression of normal maternal behavior.

The present study used a pharmacological manipulation to regulate the activity of 5-HT_{2A} receptor. The neutral activity of 5-HT_{2A} receptors was induced by ketanserin, which was regarded as a pure 5-HT_{2A} receptor antagonist in the previous study (Li et al., 2009). However, ketanserin is impossible to be a completely neutral antagonist. In addition, the reduced activity of 5-HT_{2A} receptors was induced by the inverse agonist pimavanserin. However, we cannot sure that pimavanserin completely eliminated the intrinsic activity of 5-HT_{2A} receptors. As such these are limitations in the pharmacological way to regulate the activity of receptors. It is important to use other ways to investigate the activities of receptors, for example, 5-HT_{2A} receptor knockout mice may be used to investigate the role of 5-HT_{2A} receptors on maternal behavior.

Although the present study shows that pimavanserin impairs maternal behavior in postpartum female rats, the psychological and behavioral mechanisms underlying the inhibitory effect remain unclear. As maternal behavior has motivational as well as motor components, the question of whether the disruptive effect of pimavanserin on maternal behavior is motivational or nonspecifically sedative arises. To rule out the possibility that pimavanserin impaired maternal behavior by a nonspecific sedation effect or motor disability, we conducted a spontaneous locomotor activity test on PP7. We found that the tested doses of pimavanserin did not significantly reduce movement velocity. These data indicate that pimavanserin did not disrupt maternal behavior through a sedative effect.

In conclusion, the present study showed that 5-HT_{2A} receptors are involved in regulating maternal behavior, and alterations in the 5-HT_{2A} receptor activity can lead to abnormal maternal behavior in postpartum female rats. First, the constitutive (intrinsic) activity of 5-HT_{2A} receptors induced by the neutral antagonist ketanserin may be involved in the expression of normal maternal behavior. Animals with constitutive 5-HT_{2A} receptor activity showed normal maternal behavior, similar to those with intact serotonergic transmission. Second, the reduction in the activity of 5-HT_{2A} receptors impairs maternal behavior. Lastly, pimavanserin at the doses that impair maternal behavior does not affect spontaneous locomotion, suggesting that pimavanserin impairs maternal behavior, and that this action is not mediated by nonspecific sedative effects. Taken together, our data show that the reduction in 5-HT_{2A} receptor activity may impair maternal behavior, whereas its constitutive activity is involved in the expression of normal maternal behavior.

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Conflict of interest statement

The authors have no conflict of interest.

References

Bonilla-Jaime, H., Guadarrama-Cruz, G., Alarcon-Aguilar, F.J., Limon-Morales, O., Vazquez-Palacios, G., 2015. Antidepressant-like activity of *Tagetes lucida* Cav. is mediated by 5-HT(1A) and 5-HT(2A) receptors. J. Nat. Med. 69 (4), 463–470.
Bridges, R.S., 2015. Neuroendocrine regulation of maternal behavior. Front. Neuroendocrinol. 36, 178–196.
Chen, W., Zhang, Q., Su, W., Zhang, H., Yang, Y., Qiao, J., Sui, N., Li, M., 2014. Effects of 5-hydroxytryptamine 2C receptor agonist MK212 and 2A receptor antagonist MDL100907 on maternal behavior in postpartum female rats. Pharmacol. Biochem. Behav. 117, 25–33.
Cui, R., Fan, J., Ge, T., Tang, L., Li, B., 2018. The mechanism of acute fasting-induced antidepressant-like effects in mice. J. Cell. Mol. Med. 22 (1), 223–229.
Di Matteo, V., Cacchio, M., Di Giulio, C., Esposito, E., 2002. Role of serotonin(2C) receptors in the control of brain dopaminergic function. Pharmacol. Biochem. Behav. 71 (4), 727–734.
Fox, M.A., French, H.T., LaPorte, J.L., Blackler, A.R., Murphy, D.L., 2010. The serotonin 5-HT(2A) receptor agonist TCB-2: a behavioral and neurophysiological analysis. Psychopharmacology 212 (1), 13–23.
Gao, J., Wu, R., Davis, C., Li, M., 2018. Activation of 5-HT2A receptor disrupts rat maternal behavior. Neuropharmacology 128, 96–105.
Girish, C., Raj, V., Arya, J., Balakrishnan, S., 2012. Evidence for the involvement of the monoaminergic system, but not the opioid system in the antidepressant-like activity of ellagic acid in mice. Eur. J. Pharmacol. 682 (1–3), 118–125.
Gobert, A., Millan, M.J., 1999. Serotonin (5-HT)2A receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. Neuropharmacology 38 (2), 315–317.
Ichikawa, J., Dai, J., Meltzer, H.Y., 2001. DOI, a 5-HT2A/2C receptor agonist, attenuates clozapine-induced cortical dopamine release. Brain Res. 907 (1–2), 151–155.
Kenakin, T., 2004. Principles: receptor theory in pharmacology. Trends Pharmacol. Sci. 25 (4), 186–192.
Li, M., 2015. Antipsychotic drugs on maternal behavior in rats. Behav. Pharmacol. 26 (6), 616–626.
Li, M., Davidson, P., Budin, R., Kapur, S., Fleming, A.S., 2004. Effects of typical and

atypical antipsychotic drugs on maternal behavior in postpartum female rats. Schizophr. Res. 70 (1), 69–80.
Li, M., Budin, R., Fleming, A.S., Kapur, S., 2005. Effects of chronic typical and atypical antipsychotic drug treatment on maternal behavior in rats. Schizophr. Res. 75 (2–3), 325–336.
Li, J.X., Unzeitig, A., Javors, M.A., Rice, K.C., Koek, W., France, C.P., 2009. Discriminative stimulus effects of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), ketanserin, and (R)-(+)-{alpha}-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidine-metha nol (MDL100907) in rats. J. Pharmacol. Exp. Ther. 331 (2), 671–679.
Millan, M.J., Dekeyne, A., Gobert, A., 1998. Serotonin (5-HT)2C receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT, release in the frontal cortex in vivo. Neuropharmacology 37 (7), 953–955.
Murotani, T., Ishizuka, T., Isogawa, Y., Karashima, M., Yamatodani, A., 2011. Possible involvement of serotonin 5-HT2 receptor in the regulation of feeding behavior through the histaminergic system. Neuropharmacology 61 (1–2), 228–233.
Nutt, D., Stahl, S., Blier, P., Drago, F., Zohar, J., Wilson, S., 2017. Inverse agonists - what do they mean for psychiatry? Eur. Neuropsychopharmacol. 27 (1), 87–90.
Popova, N.K., Amstislavskaya, T.G., 2002. 5-HT2A and 5-HT2C serotonin receptors differentially modulate mouse sexual arousal and the hypothalamo-pituitary-testicular response to the presence of a female. Neuroendocrinology 76 (1), 28–34.
Seibenhener, M.L., Wooten, M.C., 2015. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. J. Vis. Exp. 96, e52434.
Stiedl, O., Misane, I., Koch, M., Pattij, T., Meyer, M., Ogren, S.O., 2007. Activation of the brain 5-HT2C receptors causes hypolocomotion without anxiogenic-like cardiovascular adjustments in mice. Neuropharmacology 52 (3), 949–957.
Vanover, K.E., Weiner, D.M., Makhay, M., Veinbergs, I., Gardell, L.R., Lameh, J., Del Tredici, A.L., Piu, F., Schiffer, H.H., Ott, T.R., Burstein, E.S., Uldam, A.K., Thygesen, M.B., Schlienger, N., Andersson, C.M., Son, T.Y., Harvey, S.C., Powell, S.B., Geyer, M.A., Tolf, B.R., Brann, M.R., Davis, R.E., 2006. Pharmacological and behavioral profile of *N*-(4-fluorophenylmethyl)-*N*-(1-methylpiperidin-4-yl)-*N′*-(4-(2-methylpropyloxy) phen ylmethyl) carbamide (2R,3R)-dihydroxybutanedioate (2:1) (ACP-103), a novel 5-hydroxytryptamine(2A) receptor inverse agonist. J. Pharmacol. Exp. Ther. 317 (2), 910–918.
Wu, R., Gao, J., Chou, S., Davis, C., Li, M., 2016. Behavioral, pharmacological and neuroanatomical analysis of serotonin 2C receptor agonism on maternal behavior in rats. Psychoneuroendocrinology 73, 252–262.
Wu, R., Davis, C., Li, M., 2018. Behavioral mechanisms underlying the maternal disruptive effect of serotonin 5-HT2A receptor activation in Sprague-Dawley rats. J. Neural Transm. (Vienna) 125 (7), 1065–1075.
Zhao, C., Li, M., 2009. The receptor mechanisms underlying the disruptive effects of haloperidol and clozapine on rat maternal behavior: a double dissociation between dopamine D(2) and 5-HT(2A/2C) receptors. Pharmacol. Biochem. Behav. 93 (4), 433–442.