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# A behavioral mechanistic investigation of the role of 5-HT$_{1A}$ receptors in the mediation of rat maternal behavior

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**Abstract**

Previous work suggests that 5-HT$_{1A}$ receptors play a special role in rodent maternal aggression, but not in other aspects of maternal care (e.g. pup retrieval and nest building). The present study re-assessed the basic effects of 5-HT$_{1A}$ activation or blockade on various maternal responses in postpartum female rats. We also examined the possible behavioral mechanisms underlying the maternal effects of 5-HT$_{1A}$. Sprague–Dawley mother rats were injected with a 5-HT$_{1A}$ agonist 8-OH-DPAT (0.1, 0.5 or 1.0 mg/kg, sc), a 5-HT$_{1A}$ antagonist WAY-101405 (0.1, 0.5 or 1.0 mg/kg, sc) or 0.9% saline solution on postpartum days 3, 5, and 7. Maternal behavior was tested

30 min before, 30 min, 120 min, and 240 min after the injection. Acute and repeated 8-OH-DPAT treatment significantly disrupted pup retrieval, pup licking, nursing, and nest building in a dose-dependent fashion, whereas WAY-101405 had no effect at the tested doses. The 5-$HT_{1A}$ receptor specificity of 8-OH-DPAT's action was confirmed as its maternal disruption effect was reversed by pretreatment of WAY-100635 (a highly selective 5-$HT_{1A}$ receptor antagonist). Subsequent pup preference test found that 8-OH-DPAT did not decrease the pup preference over a novel object, thus no inhibition on maternal motivation or maternal affect. The pup separation test and pup retrieval on an elevated plus maze test also failed to find any motivational and motor impairment effect with 8-OH-DPAT. However, 8-OH-DPAT at the maternal disruptive dose did disrupt the prepulse inhibition (a measure of attentional function) of acoustic startle response and enhanced the basal startle response. These findings suggest that stimulation of 5-$HT_{1A}$ receptors by 8-OH-DPAT impairs maternal care by partially interfering with the attentional processing or basal anxiety. More work is needed to further delineate the psychological and neuronal mechanisms underlying the maternal disruptive effect of 5-$HT_{1A}$ receptor activation.

## 1. Introduction

Serotonin 1A (5-$HT_{1A}$) receptor subtype is one of the most studied serotonin receptors in the neurobiological and psychopharmacological research due to its involvement in emotion regulation and motivation. It is present in high density in the mesencephalic raphe nuclei as well as in cortical and limbic areas (e.g. frontal cortex, entorhinal cortex, hippocampus, amygdala, septum) (Hensler et al., 1991; Kia et al., 1996) and is located on both presynaptic and postsynaptic membranes (Albert et al., 2014). In general, activation of presynaptic 5-$HT_{1A}$ receptors by a 5-$HT_{1A}$ agonist hyperpolarizes the cell membrane and results in a reduction of the firing rate of the serotonergic neuron in the raphe area, leading to a suppression of 5-HT synthesis, turnover and release. In contrast, activation of postsynaptic 5-$HT_{1A}$ receptors causes a decrease in the firing rate of the postsynaptic cell (Barnes and Sharp, 1999; Lesch and Gutknecht, 2004). Depending on the brain regions, this action may have different functional consequences.

The 5-$HT_{1A}$ receptors in various limbic and hypothalamic areas are important for a variety of motivated behaviors, such as eating, drinking, sexual behavior, aggression, and drug abuse and play a role in emotion regulation, motivation and cognition (Albert et al., 2014; Bendotti and Samanin, 1986; Burton et al., 2013; Cassaday et al., 2000; Clissold et al., 2013; Cools et al., 2008; Graeff et al., 1996; McBride et al., 1991; Snoeren et al., 2014). Maternal behavior is one of well-characterized motivated social behaviors, yet whether and how 5-$HT_{1A}$

is involved in the regulation of maternal behavior is still not clear. Several early studies have reported a special role of 5-$HT_{1A}$ receptors in maternal aggression (Ferreira et al., 2000), but not other aspects of maternal behavior (De Almeida and Lucion, 1994; Ferreira et al., 2000; Lonstein and Gammie, 2002; Veiga et al., 2007; Yoshimura and Ogawa, 1991). Yoshimura and Ogawa (1991) showed that acute and chronic treatment with SM-3997, a 5-$HT_{1A}$ receptor agonist, dose-dependently decreased maternal aggression in postpartum female rats. Olivier et al. (1995) reviewed the literature and concluded that although the 5-$HT_{1A}$ receptor agonists (e.g. busipirone, ipsapirone, and 8-OH-DPAT [(±)-8- Hydroxy-2-dipropylaminotetralin hydrobromide]) decrease maternal aggression, the effect is not specific because these drugs also simultaneously cause a marked decrease in social interest and activity. However, Ferreira et al. (2000) failed to find any effect of 8-OH-DPAT on various maternal responses, including pup retrieval and nest building. Centrally, De Almeida and Lucion (1994) reported that acute intracerebroventricular (ICV) injection of 8-OH-DPAT, a 5-$HT_{1A}$ receptor full agonist reduced maternal aggression, but did not affect maternal care and other non-aggressive social interaction with the intruder. Later, they showed that 8-OH-DPAT injected into the median raphe, dorsal periaqueductal gray and corticomedial amygdala nucleus also reduced maternal aggression (De Almeida and Lucion, 1997). However, when it was infused into the medial septum and dorsal raphe nuclei, 8- OH-DPAT actually increased the aggressive behavior of the lactating female rats (da Veiga et al., 2011; De Almeida and Lucion, 1997). These data suggest that the 5-$HT_{1A}$ receptors located in different brain regions and/or in different parts of the synapse (presynaptic versus postsynaptic membranes) may play different roles in maternal aggression. It is conceivable that activation of presynaptic 5-$HT_{1A}$ receptors may increase maternal aggression due to its suppression on serotonin synthesis and release, whereas activation of postsynaptic 5-$HT_{1A}$ receptor may decrease maternal aggression due to its enhancement effect on serotonin neurotransmission.

Another puzzling issue is why systemic treatment of 5-$HT_{1A}$ receptor agonists such as 8-OH-DPAT did not affect other maternal responses, especially the pup retrieval (Ferreira et al., 2000), if this effect is not aggression-specific, but rather on the general social behaviors. In the present study, we sought to re-evaluate the role of 5-$HT_{1A}$ receptors in maternal behavior in postpartum female rats. We treated mother rats with either 8-OH-DPAT, a 5-$HT_{1A}$ receptor full

agonist, or WAY-101405 [(R)-N-(2-methyl-(4-indolyl-1-piperazinyl) ethyl)-N-(2-pyridinyl)-cyclohexane carboxamide], a novel 5-$HT_{1A}$ receptor antagonist and tested their maternal responses in the home cage. We show that 8-OH-DPAT, but not WAY-101405, dose-dependently disrupted various maternal responses. Additionally, we used a highly selective 5-$HT_{1A}$ receptor antagonist WAY-100635 (Collinson and Dawson, 1997; Helsley et al., 1998) and determined the receptor specific effect of 8-OH-DPAT. To explore the possible behavioral mechanisms underlying the maternal disruptive effect of 8-OH-DPAT, we assessed how this drug affected the mother rats' maternal affect, maternal motivation, basic attentional ability, and motoric function in a pup preference test, a pup-modulated prepulse inhibition (PPI) of acoustic startle task, a pup separation and a pup retrieval on an elevated plus maze test. The pup preference test examines the relative strength of a mother's maternal motivation or affective response towards pups versus its motivation to seek novelty, thus it measures social motivation in general and maternal motivation/ affect in particular. The PPI test not only examines a mother rat's basal startle sensitivity (a putative indicator of the baseline level of stress sensitivity) and sensorimotor gating ability (an preattentive function) (Koch, 1999), but also examines how the presence of pups modulates these responses (see details in the Materials and methods), which allows us examine the specific effect of 8-OH-DPAT on pup-directed attention. Furthermore, we used the pup-separation technique (Hansen, 1994; Stamatakis et al., 2015; Wu et al., 2016; Zhao and Li, 2009b) and the pup retrieval on an elevated plus maze test (Pereira et al., 2005; Yang et al., 2015) to verify the retrieval-disruptive effect of 8-OH-DPAT and examined how much of this effect was attributable to the motivational impairment and how much to the motoric suppression.

## 2. Materials and methods

### *2.1. Animals*

Naive pregnant female Sprague-Dawley rats (gestational days 6 upon arrival to the animal facility) purchased from Charles River Inc., were used in this study except for Experiment 3, which used female rats

purchased from Chongqing Tengxin Biological Technology Co., Ltd., China and mated in our colony at Southwest University (China). All pregnant rats were housed individually in 48.3 cm×26.7 cm×20.3 cm transparent polycarbonate cages under 12-h light/dark conditions (6:30–18:30 or 8:00–20:00), and had access to standard laboratory rat chow and tap water ad libitum. Both colonies were maintained with a controlled temperature (22 ± 2 °C) and a relative humidity of 45–75%. Starting two or three days before the first possible expected parturition date, the subjects were monitored every day for signs of parturition. Once the female was found with pups in the morning, that day was designated as postpartum day 1 (PP1). If the pups were found in the afternoon, it was designated as PP0. Two shredded paper towels were provided to the dams as nesting materials. On PP2, each litter was culled to eight pups (4 males and 4 females with the most visible milk bands) and all subjects were changed to clean observation cages with their litters. All experiments were conducted during the light cycle. Animal use was reviewed and approved by the University of Nebraska Institutional Animal Care and Use Committee or by the local animal care and use committee at Southwest University, China, and was in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### *2.2. Drugs and choices of dosage*

8-OH-DPAT was obtained from Tocris Bioscience (Ellisville, MO, USA). WAY-101405 was the gift from the NIMH drug supply program. WAY-100635 was purchased from Tocris Bioscience (Ellisville, MO, USA). All drugs were dissolved in 0.9% saline (vehicle control) and administrated subcutaneously (sc) in a volume of 1.0 ml/kg. Three doses of each drug were chosen in the first two experiments based on our literature review: 8-OH-DPAT at 0.1, 0.5 and 1.0 mg/kg (Ferreira et al., 2000; Picazo et al., 2000; Stiedl et al., 2015), and WAY-101405 at 0.1, 0.5 and 1.0 mg/kg (Hirst et al., 2008; Rizzo et al., 2009). In the remaining experiments, we only tested 8-OH-DPAT at 0.5 or 1.0 mg/kg based on the results from Experiment 1. WAY-100635 at 0.5 mg/kg was used in Experiment 3 because at this dose, it shows a clear effect in counteracting the effects of 8-OH-DPAT (Collinson and Dawson, 1997; Helsley et al., 1998).

### *2.3. Experiment 1: basic effects of 5-HT$_{1A}$ activation by 8-OH-DPAT on maternal performance and pup preference*

In this experiment, we tested 24 postpartum rats and determined how 5-HT$_{1A}$ receptor activation affects maternal behavior and alters pup preference (a measure of maternal affect). Mother rats were randomly assigned to 1 of 4 groups (n = 6/group): VEH (saline) and treated with 1 of 3 doses of 8-OH-DPAT (0.1, 0.5 or 1.0 mg/kg, sc). On PP3, 5 and 7, maternal behavior in the home cage was observed at 4 time points, with the first one at 30 min before the drug injections (i.e., baseline), and the rest being carried out at 30 min, 120 min, and 240 min after the injection. Each test lasted for 10 min. This interval was chosen primarily based on the previous studies (Rizzo et al., 2009; Stiedl et al., 2015; Zhao and Li, 2009a).

The basic procedure of each maternal behavior test has been described previously (Chen et al., 2014; Wu et al., 2016; Zhao and Li, 2009b). Pups were first removed from their mother and the nest was destroyed. Ten seconds later, the pups were placed back in the cage at the corner diagonal to the original nest site or the corner where the dam stayed, and the dam's behavior in the home cage were video recorded and analyzed manually by an event-recording program (JWatcher). The raters were blind to each dam's treatment condition. The following behaviors were quantified: pup retrieval (a rat picking up a pup in her mouth and carrying it back to the nest site); hovering over pups (a mother rat stays above the pups in the nest area, occasionally nursing the pups); pup licking (a rat placing its tongue on the anogenital area and the rest of a pup's body); and nest building (a rat picking up nest material in her mouth and transporting it back to the nest site or pushing the material with her forepaws towards the nest site). The first pup retrieval latency was defined as the time elapsed from the first pup approach to the retrieval of the first pup into the nest. Six hundred seconds was assigned to non-responders. After each test, unretrieved pups were returned to the nest site. On PP2, to screen for baseline maternal performance and habituate dams to the testing procedure, we did a pup retrieval test (removing pups then return them 10 s later). Only those that retrieved all 8 pups were used in the subsequent tests.

One day after the last home-cage maternal behavior test (PP8), the pup preference test was performed in an open-field arena made of blue acrylic (76 cm×76 cm×52 cm). During the test, a cylinder-shape

box made of white acrylic top and bottom with stainless metal bars spaced in between (9.7 cm high×9.8 cm in diameter) was used to house four 8-day old pups (2 males and 2 females) of the subject rat. A glass bottle (17 cm high×8 cm in diameter) was used as a novel object. The cage and bottle were placed at the back corner of the arena. The subject dam could see, hear and smell the pups but not physically interact with them. Thirty minutes after the drug injection, the dam was placed midway at the front of the arena, and the total time spent exploring the two stimuli during the 10-min period was recorded using a digital video camera and analyzed using an automatic video-tracking software (TopScan, CleverSys Inc., Reston, VA). Exploration was defined as sniffing or touching the cage/bottle with the nose and/or forepaws. At the end of the test, the dam and pups were returned to their home cages. The apparatus and the isolation cages were cleaned and deodorized with a 70% ethanol solution after each test. The pup preference is calculated as the ratio of time spent exploring the pup cage over the total amount of time spent exploring both cage and bottle.

### *2.4. Experiment 2: basic effects of 5-HT$_{1A}$ blockade by WAY-101405 on maternal performance and pup preference*

In this experiment, a new batch of 24 postpartum rats was tested to determine whether 5-HT$_{1A}$ receptor inhibition affects rat maternal behavior. The basic procedure was identical to that of Experiment 1. Mother rats were randomly assigned to receive either VEH (saline), or one of the three doses of WAY-101405 (0.1, 0.5 or 1.0 mg/kg, sc) (n = 6/group).

### *2.5. Experiment 3: receptor specificity of 8-OH-DPAT's maternal disruptive effect*

This experiment was aimed to determine that 8-OH-DPAT disrupts maternal behavior by selectively stimulating 5-HT$_{1A}$ receptors. The highly selective 5-HT$_{1A}$ receptor antagonist WAY-100635 was used to counteract the effects of 8-OH-DPAT. Twenty-four postpartum rats were randomly assigned to 1 of 3 groups (n = 8/group) to receive either a concurrent double injection of saline (VEH+VEH), one injection of 8- OH-DPAT at 0.5 mg/kg and one injection of saline (8-OH-DPAT 0.5+VEH), or one injection of 8-OH-DPAT at 0.5 mg/kg and one injection of WAY-100635 at 0.5 mg/kg (8-OH-DPAT 0.5+WAY- 100635).

The maternal behavior testing procedure was identical to that of Experiment 1.

### *2.6. Experiment 4: effects of 4-h pup separation on 8-OH-DPAT-induced maternal disruption*

Our previous work has shown that 4-h pup separation before the maternal behavior test tends to enhance maternal performance, due to its enhancement effect on maternal motivation (Hansen, 1994; Stamatakis et al., 2015; Wu et al., 2016; Zhao and Li, 2009b). If 4-h pup separation were capable of reducing 8-OH-DPAT-induced maternal disruption, it would suggest that 8-OH-DPAT may disrupt maternal behavior partially via its action on maternal motivation. In this experiment, we tested a new batch of 22 postpartum rats under either 4-h pup separation or no pup separation condition. The basic procedure was identical to what has been described in our previous studies (Chen et al., 2014; Wu et al., 2016; Zhao and Li, 2009b). First, the mother rats were randomly assigned into four treatment groups: 8-OH-DPAT 1.0 mg/kg (n = 6) and VEH (n = 5) under the pup separation condition, 8-OH-DPAT 1.0 mg/kg (n = 6) and VEH (n = 5) under the no separation condition. On PP3, under the pup separation condition, pups were first removed from the dams and placed into a bowl with nesting materials (shredded paper towels) on a temperature-controlled heating pad (34 °C). Four hours later, they were returned to the dams and maternal behavior was recorded for 10 min. Two tests were scheduled at 30 min and 120 min after the injection. Under the no separation condition, no pups were removed before the test and the dams were similarly tested at 30 min and 120 min after the injection. On PP4, this processes was repeated.

### *2.7. Experiment 5: effects of 8-OH-DPAT on startle response, prepulse inhibition (PPI) and pup retrieval on an elevated plus maze*

Results from Experiment 4 showed that 4-h pup separation before the maternal behavior test failed to reduce the 8-OH-DPAT-induced maternal disruption. In this experiment, we used a modified acoustic startle and PPI test to further explore the behavioral mechanisms underlying the maternal effects of 8-OH-DPAT. We measured the effect of 8-OH-DPAT (1.0 mg/kg) on the baseline level of stress reactivity/sensitivity and sensorimotor gating ability/attention of dams

with or without the presence of pups following the 4-h pup separation tests. Specifically, on PP6, two days after the last pup separation test, all dams were first habituated to the startle chambers for 20 min with 70 dB background noise. The test apparatus (6 startle monitor systems, Kinder Scientific, Julian, CA) has been described in detail (Li et al., 2011a; Qiao et al., 2013; Zhang and Li, 2016). Then, on PP7 and PP8, mother rats were injected with vehicle or 8-OH-DPAT. Thirty minutes later, they were placed in the chambers and tested either with pups or without pups. Four groups were formed: VEH with pups (n = 6); VEH without pups (n = 4); 8-OH-DPAT with pups (n = 8); and 8-OH-DPAT without pups (n = 4). At the beginning of each test, mother rats were placed in a restrainer (35.56 cm wide×27.62 cm deep×49.53 cm high) with an adjustable ceiling positioned atop the box, providing only limited restraint while prohibiting ambulation. Under the pup presence condition, two pups of the subject dams were separately placed in a small container attached to each side of the restrainer. Each test started with 5 min period of acclimation with 70 dB background noise, followed by 4 different trial types: PULSE ALONE trials (105 dB white noise, 40 ms) and 3 types of PREPULSE+PULSE trials (a 20 ms 75, 78, or 82 dB prepulse, followed 100 ms later by a 105 dB pulse). Each trial type occurred 10 times, and a total of 40 trials were presented in a pseudo-random order. At the beginning and end of each session, four additional PULSE ALONE trials were added to examine the baseline startle response and they were not used in the calculation of PPI. The inter-trial interval ranges from 25 to 35 s (average 30 s). Startle magnitude is defined as the maximum force (Newtons) applied by the rat to the startle apparatus recorded over a period of 100 ms beginning at the onset of the PULSE stimulus. In this study, we averaged the startle magnitude of the 105 dB PULSE ALONE trials as the measurement of the baseline startle response. The percent PPI is defined as the percent reduction in startle from the PULSE-ALONE to the PREPULSE+PULSE trials and calculated as: %PPI = 100×[(averaged startle response to PULSE ALONE trials – averaged startle response to PREPULSE+PULSE trials) / averaged startle response to PULSE ALONE trials] (Li et al., 2011a, b).

Two days after the startle and PPI test, on PP10, dams were examined for their maternal motivation using the pup retrieval on an elevated plus maze (EPM) test. The same dams as used in the startle tests were tested. The VEH group included rats that were treated with vehicle in the pup separation and PPI test (n = 12), and the 8-OH-DPAT

group included those that were treated with 8-OH-DPAT (1.0 mg/kg). The basic procedure was identical to what has been described in our previous study (Yang et al., 2015). The EPM consisted of two open arms (50 cm×10 cm), two enclosed arms (50 cm×10 cm) and a central platform (10 cm×10 cm) made of black Plexiglas. Each arm was supported by a sturdy plastic leg and was elevated 50 cm above the floor. The two enclosed arms had high walls (38.5 cm in height), while the two open arms had raised edges (1.0 cm in height) along each side and end to decrease the possibility of falling during drug testing. Prior to the start of each test, EPM was cleaned by 70% ethanol and dried. Two pups were placed at the end of each open arm (4 pups total). Then, the mother rat was placed in the central square facing a closed arm and allowed to freely explore the maze for 10 min. All experimental sessions were recorded by a digital video camera and analyzed using a TopScan software (CleverSys, Inc. Reston, VA). The following parameters were obtained: (1) number of pups retrieved (the number of pups which were picked up and carried back from an open arm to a close arm); (2) % open arm entries (100%×number of entries into open arms/total number of entries); (3) % time in open arms (100%×time spent in the open arms/total time); (4) number of closed arm entries; (5) mean speed (the total distance travelled divided by 600 s). Because some pups fell from the open arm during the test, the data of 4 rats (2 rats in the 8- OT-DPAT group and 2 rats in the VEH group) were not included for analysis.

### *2.8. Statistical analysis*

Home cage maternal behavior on PP3, 5 and 7 were individually analyzed using a factorial repeated measures analysis of variance (ANOVA) with group (3 or 4 groups) as the between-subjects factor and testing time (3 time points) as the within-subjects factor by SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Group differences at different testing point were further examined using one-way ANOVA. The preference data were analyzed using a two-way ANOVA with group (4 groups) as the between-subjects factors and stimulus (pup and object) as the within-subjects factor. The exploration duration and pup preference ratio between groups was analyzed using one-way ANOVA. For simplicity, pup separation data from PP3 and PP4 were combined and analyzed using two-way ANOVA (drug and pup separation condition as the between-subjects factors). This was done also because

there was no difference on various maternal measures from both days. Averaged % PPI (the mean of the 75, 78, or 82 dB PPIs) and the startle response data were analyzed using the two-way ANOVA (drug and pup condition as the between-subjects factors), followed by post hoc Bonferroni test when necessary. Partial eta square ($\eta_{2p}$) and Cohen's d were used as measures of the effect size. Data in the EPM test were analyzed using the independent samples t-test for two-group comparison. All data are expressed as mean ± SEM. Statistical significance was accepted at $p < 0.05$, two-tailed.

## 3. Results

### *3.1. Experiment 1: basic effects of 5-HT$_{1A}$ activation by 8-OH-DPAT on maternal performance and pup preference*

Fig. 1A shows that acute 8-OH-DPAT treatment dose-dependently and time-dependently disrupted pup retrieval on all three test days (all $p < 0.05$). This disruption appeared to be transient, peaking at the 30 min post-injection and disappeared at 2 h point. Repeated measures ANOVA on the number of pups retrieved revealed a significant group× testing point interaction [PP3: $F(6, 40) = 321.000$, $p < 0.001$; PP5: $F(6, 40) = 10.641$, $p < 0.001$; PP7: $F(6, 40) = 9.291$, $p < 0.001$]. Other maternal responses, such as hovering over pups (Fig. 1B), pup licking (Fig. 1C) and nest building (Fig. 1D) were similarly disrupted by 8-OH-DPAT (all $p < 0.05$). Repeated measures ANOVA revealed a significant group×testing point interaction on hovering over pups [PP3: $F(6, 40) = 8.584$, $p < 0.001$; PP5: $F(6, 40) = 7.452$, $p < 0.001$; PP7: $F(6, 40) = 9.383$, $p < 0.001$], licking [PP3: $F(6, 40) = 5.467$, $p < 0.001$; PP5: $F(6, 40) = 5.297$, $p < 0.001$; PP7: $F(6, 40) = 6.941$, $p < 0.001$] and nest building [PP3: $F(6, 40) = 3.081$, $p = 0.014$; PP5: $F(6, 40) = 4.022$, $p = 0.003$; PP7: $F(6, 40) = 4.036$, $p = 0.003$].

In the pup preference test, mother rats spent more time exploring the box containing pups than a novel object (Fig. 3A, all $p < 0.001$). Two-way ANOVA revealed a main effect of stimulus [$F(1, 40) = 60.085$, $p < 0.001$]. 8-OH-DPAT reduced the object exploration [$F(3, 20) = 23.141$, $p < 0.001$], but did not affect the pup exploration, resulting in a relative increase in percentage time exploring the pups (i.e. an increase in pup preference ratio) [$F(3, 20) = 15.745$, $p < 0.001$] (Fig. 3B). The rats with 8-OH-DPAT showed even less object exploration and

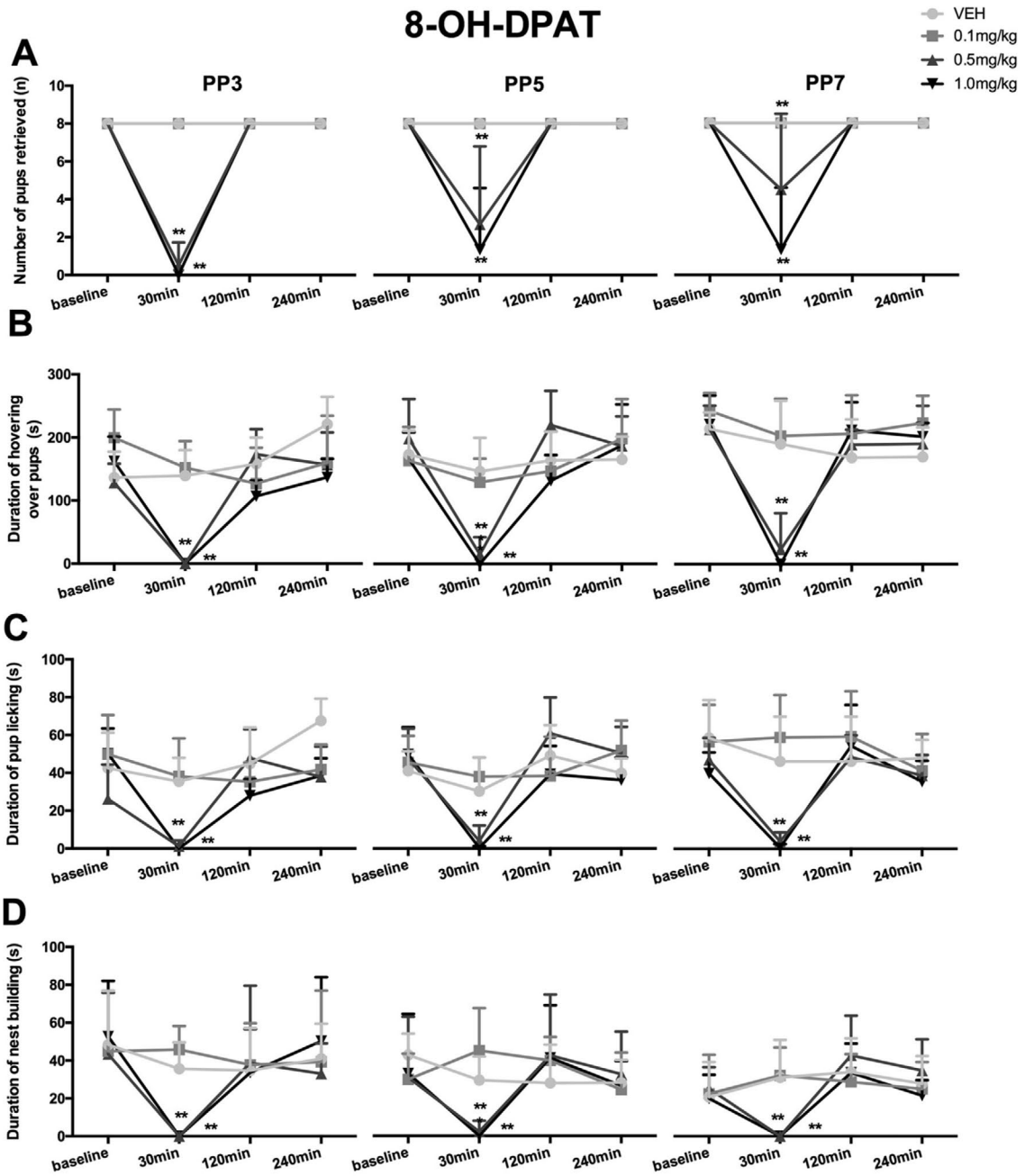


**Fig. 1.** Effects of systemic 8-OH-DPAT treatment (n = 6/group) on maternal behavior on the 3 test days (PP3, PP5 and PP7). On each test day, maternal behavior was observed for 10 min at 4 time points: 30 min before, 30, 120 and 240 min after the injection. Data are expressed as mean ± SEM. A, number of pups retrieved; B, duration of hovering over pups; C, duration of pup licking; D, duration of nest building. $^{**}p < 0.01$, a significant difference between the drug group and the vehicle group.

higher pup preference ratio than the vehicle (Fig. 3B) (all $p < 0.01$). This finding suggests that 8-OH-DPAT did not decrease a mother rat's maternal interest towards pups, thus its maternal disruption could not be due to a disruption of motivational or affective response towards pups (Pereira and Ferreira, 2016).

### *3.2. Experiment 2: basic effects of 5-HT$_{1A}$ blockade by WAY-101405 on maternal performance and pup preference*

Fig. 2 shows the results of acute and repeated WAY-101405 treatment on the number of pups retrieved (Fig. 2A), the duration of hovering over pups (Fig. 2B), pup licking (Fig. 2C), and nest building (Fig. 2D) at three testing time points on PP3, PP5 and PP7 (all $p > 0.05$). Repeated measures ANOVA revealed no significant main effect of group, time point, nor group×testing point interaction on any of these measures, suggesting that WAY-101405 had no effect on maternal behavior.

In the pup preference test, rats tested under WAY-101405 also spent more time exploring the pups than the object (all $p < 0.001$) (Fig. 3A). Two-way ANOVA also revealed a main effect of stimulus [$F(1, 40) = 54.839$, $p < 0.001$]. However, there was no significant difference between any of the WAY-101405 groups and the vehicle group on the pup exploration, object exploration and pup preference ratio (Fig. 3B).

### *3.3. Experiment 3: receptor specificity of 8-OH-DPAT's maternal disruptive effect*

Fig. 4A confirms that 8-OH-DPAT (0.5 mg/kg, sc) treatment disrupted pup retrieval on all three test days (all $p < 0.01$) at the 30 min post-injection point, but this effect was attenuated by WAY-100635 (0.5 mg/kg, sc). Repeated measures ANOVA revealed a significant group×testing point interaction [PP3: $F(4, 42) = 7.034$, $p < 0.001$; PP5: $F(4, 42) = 4.007$, $p < 0.01$; PP7: $F(4, 42) = 6.482$, $p < 0.001$] on the number of pups retrieved. Post hoc tests show that the 8-OHDPAT 0.5+VEH group retrieved fewer pups than VEH+VEH group (all $p < 0.01$) and 8-DPAT 0.5+WAY-100635 group (all $p < 0.05$), while the 8-OH-DPAT 0.5+WAY-100635 group retrieved more pups than the 8-OH-DPAT 0.5+VEH group (all $p < 0.01$).

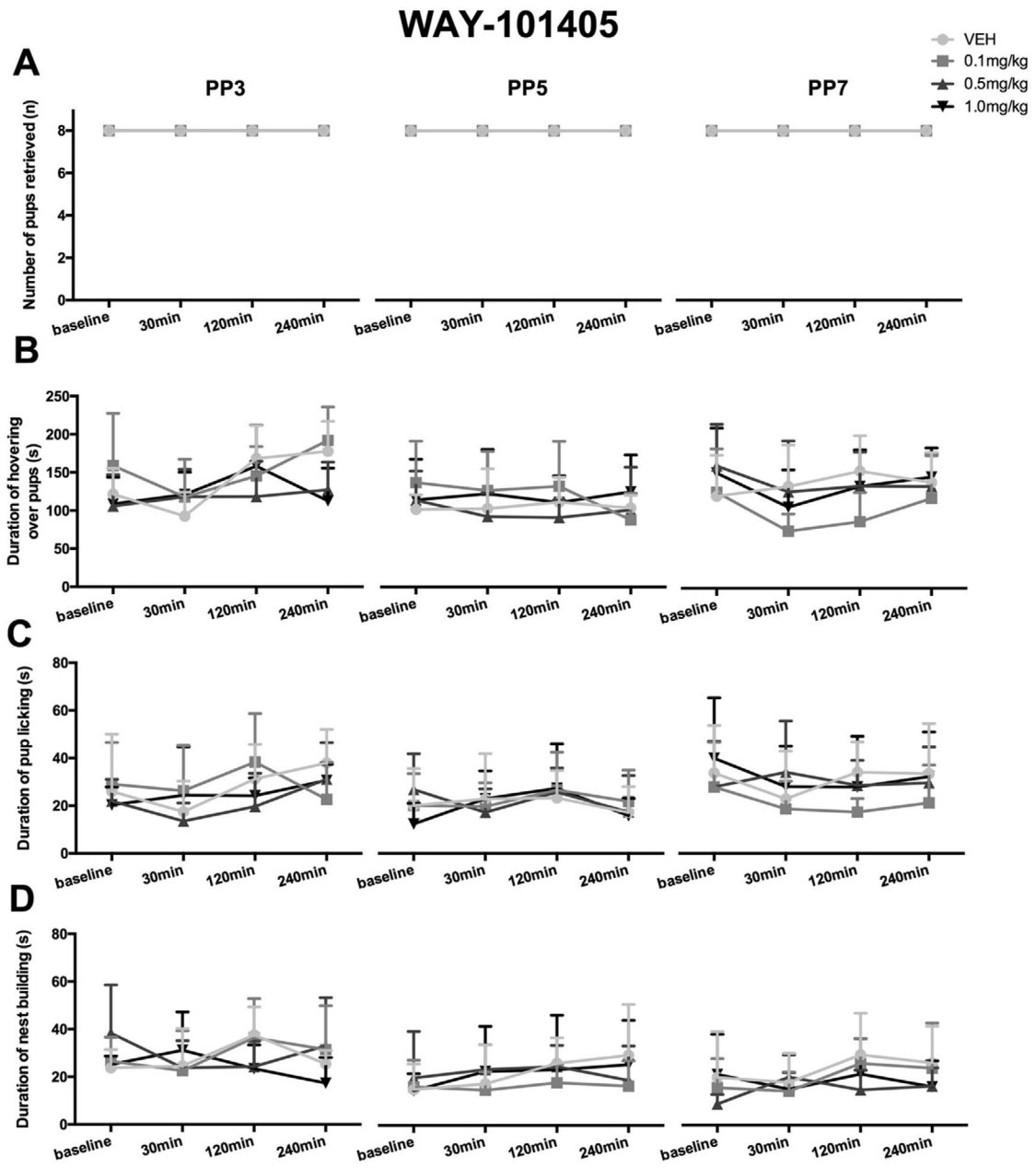


**Fig. 2.** Effects of systemic WAY-101405 treatment (n = 6/group) on maternal behavior on the 3 test days (PP3, PP5 and PP7). On each test day, maternal behavior was observed for 10 min at 4 time points: 30 min before, 30, 120 and 240 min after the injection. Data are expressed as mean ± SEM. A, number of pups retrieved; B, duration of hovering over pups; C, duration of pup licking; D, duration of nest building. $^{**}p < 0.01$, a significant difference between the drug group and the vehicle group.

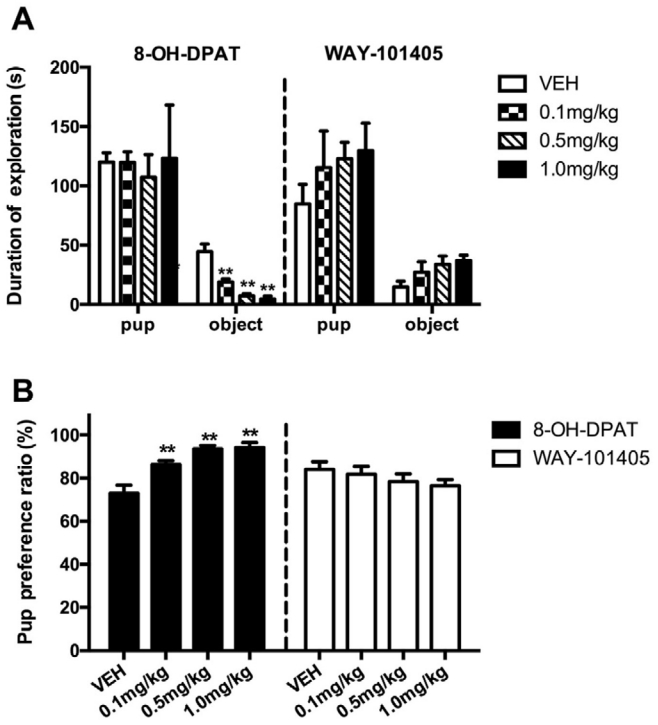


**Fig. 3.** Effects of 8-OH-DPAT or WAY-101405 on pup preference in the pup-object preference test (n = 6/group). Data are expressed as mean ± SEM. A, Duration of exploring for the two stimuli. B, Pup preference ratio. $^{**}p < 0.01$, a significant difference between the drug group and the vehicle group.

Other maternal responses, such as hovering over pups (Fig. 4B) on PP7, were also suppressed by 8-OH-DPAT, and its effect on hovering over pups was also attenuated by WAY-100635 ($p < 0.05$). Finally, the nest building activity on all test days (Fig. 4D) was disrupted by 8-OHDPAT (all $p < 0.01$) and reversed by WAY-100635 (all $p < 0.05$).

### *3.4. Experiment 4: effects of 4-h pup separation on 8-OH-DPAT-induced maternal disruption*

Consistent with the results from Experiment 1, acute 8-OH-DPAT injection (1.0 mg/kg) disrupted various active components of maternal behavior. Four-hour pup separation did not reduce this disruption. Specifically, dams treated with 8-OH-DPAT retrieved significantly fewer pups at the 30-min post injection under both the pup separation ($p < 0.01$) and no separation ($p < 0.01$) conditions (Fig. 5A). They also

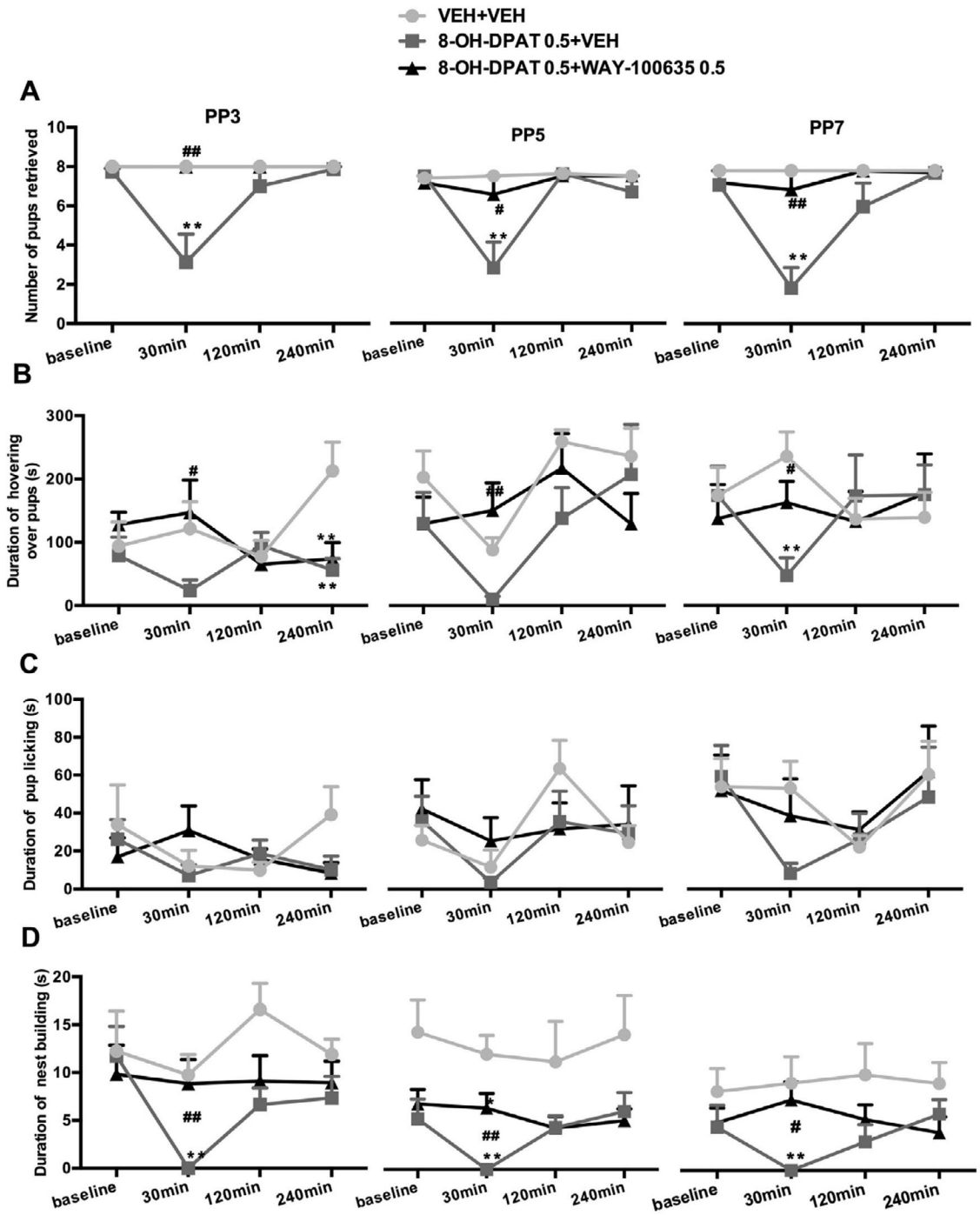


**Fig. 4.** Effects of systemic 8-OH-DPAT alone and in combination with WAY-100635 treatment (n = 8/group) on maternal behavior on the 3 test days (PP3, PP5 and PP7). On each test day, maternal behavior was observed for 10 min at 4 time points: 30 min before, 30, 120 and 240 min after the injection. Data are expressed as mean ± SEM. A, number of pups retrieved; B, duration of hovering over pups; C, duration of pup licking; D, duration of nest building. $^{**}p < 0.01$, a significant difference between the drug group and the VEH+VEH; $\#p < 0.05$, $\#\#p < 0.01$, a significant difference between the 8-OH-DPAT 0.5+WAY-100635 0.5 and 8-OH-DPAT 0.5+VEH.

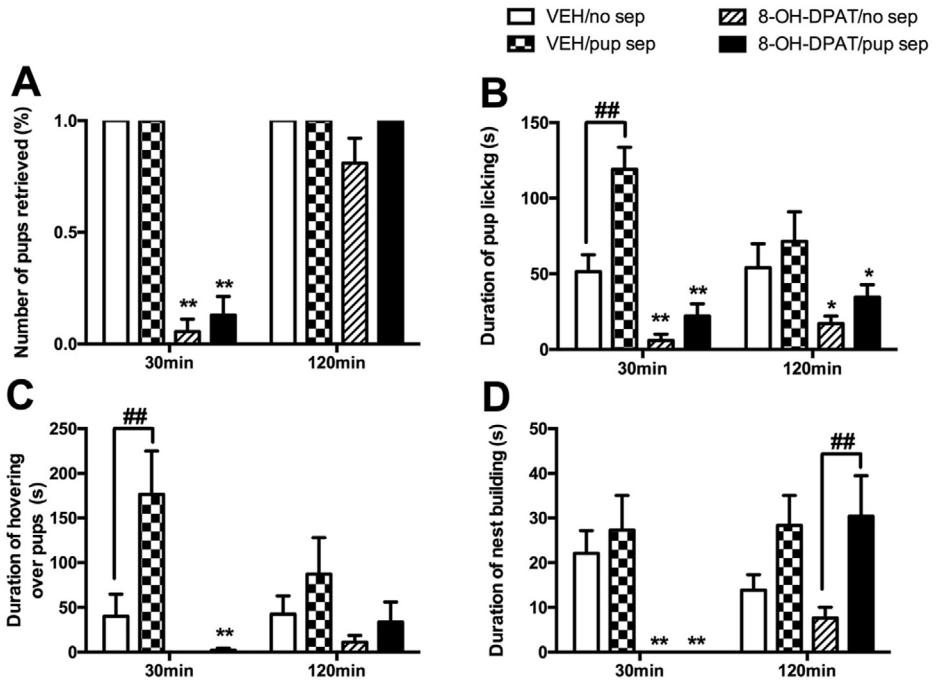


**Fig. 5.** Effects of systemic 8-OH-DPAT (1.0 mg/kg) treatment on maternal behavior under the 4-h pup separation (pup sep) and no separation (no sep) condition on postpartum day 3 and 4 (VEH/no sep: n = 5; VEH/pup sep: n = 5; 8-OH-DPAT/no sep: n = 6; 8-OH-DPAT/pup sep: n = 6). On each test day, maternal behavior was observed for 10 min at 2 time points: 30 and 120 min after the injection. Mother rats under the pup separation condition were injected with 1.0 mg/kg 8-OH-DPAT at 30 min before the end of pup separation. Data from the PP 3 and 4 under the same condition (pup sep or no sep) are combined and expressed as mean ± SEM. A, number of pups retrieved; B, duration of pup licking; C, duration of hovering over pups; D, duration of nest building. $^{**}p < 0.01$, $^{*}p < 0.05$, a significant difference between the different drug treatments (VEH vs. 8-OH-DPAT) within the same separation condition (pup sep or no sep). ## $p < 0.01$, a significant difference between the different separation conditions (pup sep or no sep) within the same drug treatment (VEH vs. 8-OH-DPAT).

spent less time on pup licking (all $p < 0.01$), hovering over pups (all $p < 0.01$) and nest building (all $p < 0.01$) (Fig. 5B, C and D). Pup separation did significantly increase the amount of time spent on licking and hovering but only in the dams treated with saline (all $p < 0.01$), confirming the effectiveness of this procedure to increase maternal performance (Fig. 5B and C). Analysis of the maternal behavior data at the 30 min post-injection point on pup retrieval [$F$ (1, 18) = 285.378, $p < 0.001$] and nest building [$F$ (1, 18) = 34.380, $p < 0.001$] showed a main effect of drug. Two-way ANOVA also revealed a significant

interaction between the two on licking [$F$ (1, 18) = 6.987, $p$ = 0.012] and hovering [$F$ (1, 18) = 7.326, $p$ = 0.01], suggesting that pup separation preferentially affected the control rats, not the drug-treated ones. At the 120 min testing point, the effect of 8- OH-DPAT on pup licking [$F$ (1, 18) = 8.698, $p$ = 0.005] was still significant, so was the separation effect on nest building [$F$ (1, 18) = 8.982, $p$ = 0.005]. Overall, 4-h pup separation enhanced maternal performance, but did not affect the maternal disruptive effect of 8-OH-DPAT, suggesting that 8-OH-DPAT disrupts maternal behavior not by decreasing maternal motivation.

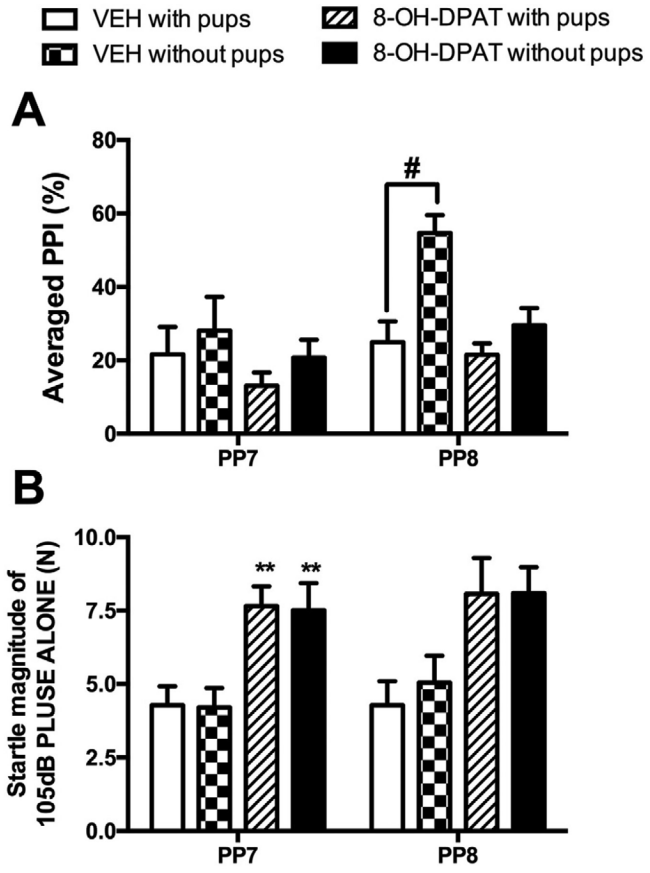


**Fig. 6.** Effect of 8-OH-DPAT on averaged prepulse inhibition (A) and startle response (B) on PP 7 and 8 (VEH with pups: n = 6; VEH without pups: n = 4; 8- OH-DPAT with pups: n = 8; 8-OH-DPAT without pups: n = 4). Mother rats were tested with pups or without pups 30 min after 8-OH-DPAT or saline injection. Data are presented as mean ± SEM. **$p$ < 0.01, *$p$ < 0.05, a significant difference between the different drug treatments (VEH vs. 8-OH-DPAT) within the same pup condition (pup presence or absence). ## $p$ < 0.01, a significant difference between the different pup conditions within the same drug treatment.

### *3.5. Experiment 5: effects of 8-OH-DPAT on startle response, pre-pulse inhibition (PPI) and pup retrieval on an elevated plus maze*

On the first day of startle and PPI testing (PP7), the averaged PPIs were low and there was no difference between groups. On PP8, there was a tendency that 8-OH-DPAT (1.0 mg/kg) treatment reduced the averaged PPI but only in mother rats tested under the no-pup condition. Interestingly, pup presence significantly reduced the averaged PPI preferentially in the vehicle control group ($p$ = 0.011, Cohen's $d$ = 2.918), suggesting that the presence of pups in the test chambers might have distracted the mothers' attention in this task (Fig. 6A). Two-way ANOVA revealed a main effect of drug [F (1, 18) = 6.246, $p$ = 0.022, $\eta^2_p$ = 0.247] and pup condition [F (1, 18) = 10.9, $p$ = 0.004, $\eta^2_p$ = 0.365]. On the startle response, 8-OH-DPAT enhanced startle response on PP7 (with pups, $p$ = 0.009, Cohen's $d$ = 2; without pups, $p$ = 0.009, Cohen's $d$ = 2.501), and with an enhancing tendency on PP8 (with pups, $p$ = 0.071, Cohen's $d$ = 1.753) (Fig. 6B), as there was a main effect of drug [PP7: F (1, 19) = 19.007, $p$ < 0.001, $\eta^2_p$ = 0.5; PP8: F (1, 18) = 8.707, $p$ = 0.008, $\eta^2_p$ = 0.316].

In comparison to the VEH treatment, 1.0 mg/kg 8-OH-DPAT decreased the number of pups retrieved on the elevated plus maze ($t$ = 8.919, $p$ < 0.001). The percentage of open arm entries ($t$ = 5.232, $p$ < 0.001) and time spent on the open arms ($t$ = 25.224, $p$ < 0.001) were also reduced. However, the number of closed arm entries and running speed were not affected by the drug (Fig. 7). These results suggested that although 8-OH-DPAT suppressed pup retrieval, it did not affect motor function, as there was no effect on the running speed or distance travelled on the maze (Yang et al., 2015).

## 4. Discussion

The present study demonstrated that acute and repeated treatment of the 5-$HT_{1A}$ receptor agonist 8-OH-DPAT has a disruptive effect on pup retrieval, pup licking, hovering over pups and nest building, four major components of rat maternal behavior. Rats treated with 8-OHDPAT had a longer pup retrieval latency, retrieved fewer pups into the nest, and spent less time on licking and hovering over pups, and on nest building. In contrast, the 5-$HT_{1A}$ receptor antagonist WAY-101405 had no effect on these maternal responses. The 5-$HT_{1A}$ receptor specificity

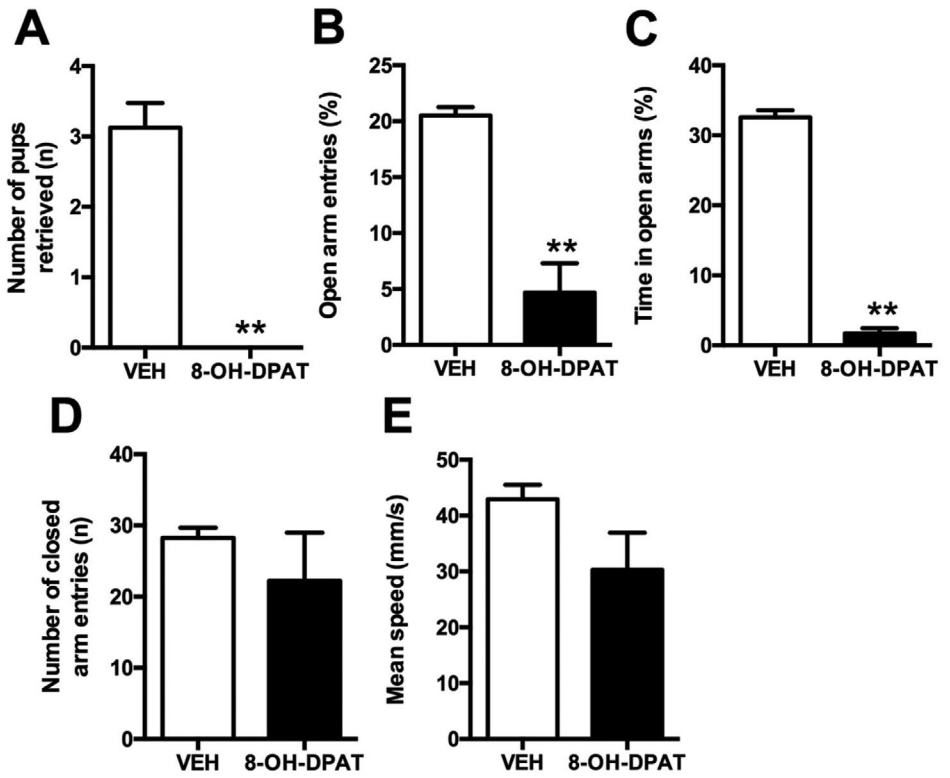


**Fig. 7.** Effects of 8-OH-DPAT on pup retrieval on the elevated plus maze on PP10 (VEH: n = 8; 8-OH-DPAT: n = 10). A, Number of pups retrieved; B, percentage of open arm entries; C, percentage of time in open arm; D, number of closed arm entries; E, running speed. Data are presented as mean ± SEM. $^{**}p < 0.01$, a significant difference between the drug group and the vehicle group.

of 8-OH-DPAT's action was confirmed, as its maternal disruption was reversed by pretreatment of WAY-100635 (a highly selective 5-$HT_{1A}$ receptor antagonist). These findings suggest that 5-$HT_{1A}$ receptors play an important role in several forms of maternal care, not just in maternal aggression. Importantly, 5-$HT_{1A}$ activation does not appear to have any effect on maternal affect, as mother rats treated with 8-OH-DPAT still preferred to interacting with the pups, as opposed to a novel object (Fig. 3). 5-$HT_{1A}$ activation also does not seem to have any effect on maternal motivation or motoric function either, as 4-h pup separation did not attenuate the 8-OH-DPAT's maternal disruption (Fig. 5). This finding is consistent with the results from the pup retrieval test on the elevated plus maze: mother rats under the 8-OH-DPAT treatment travelled a similar distance and had a similar moving speed as the controls, but they still failed to retrieve pups on the elevated plus maze

(Fig. 7). Activation of 5-$HT_{1A}$ receptors did disrupt the PPI (a measure of sensorimotor gating, an attentional function) and enhanced basal startle response (a putative measure of stress sensitivity) (Fig. 6). If these behavioral changes were related to the drug-induced maternal disruption, 8-OH-DPAT could potentially disrupt maternal behavior by interfering with the attentional processing and enhancing emotionality (De Almeida et al., 1998).

As mentioned in the Introduction, 8-OH-DPAT has been studied in rat maternal behavior. Olivier et al. (1995) shows that it reduces maternal aggression, whereas Ferreira et al. (2000) reports no effect on maternal aggression, and no effect on other maternal responses (e.g. pup retrieval and nest building). One possible explanation for the discrepancy is the methodological differences, such as the rat strain (Sprague-Dawley vs. Wister), route of drug administration (sc vs. ip), test time (light cycle vs. dark cycle) and duration (10 min vs. 5 min), etc. Because we observed the 8-OH-DPAT's maternal disruption in two different settings (home cage and the elevated plus maze), this effect appears to be robust. The more important question is how 8-OH-DPAT disrupts maternal behavior psychologically. We explored several possible mechanisms. First, we used the pup preference test and examined whether the dams under 8-OH-DPAT would show a reduction in time spent on exploring the pups. Because maternal behavior is largely regulated by the reinforcing value of the pups (Pereira and Ferreira, 2006), a reduction in pup exploration time presumably reflects a decrease in maternal interest or affect. In the laboratory, the rewarding and reinforcing effect of pups has been investigated using the conditioned place preference (CPP) paradigm in which mother rats show a clear preference to a pup-associated environment over a neutral one after allowing several days of pup-mother interactions in the conditioning box (Fleming et al., 1994). Unlike the conditioned place preference with pups, our pup preference test is a direct measure of emotional/social attachment towards pups because it does not require any prior conditioning. In this sense, it is more similar to the partner preference test commonly used in the study of pair bonding in monogamous prairie voles (Ahern et al., 2009; Curtis and Wang, 2005). Surprisingly, we did not find any decrease in maternal interest. It is possible that 5-$HT_{1A}$ receptor plays no role in the pup reward processing, as previous work suggests that it is mainly dopamine-dependent (Fleming et al., 1994; Lavi-Avnon et al., 2008). It would be interesting to further examine this issue using this paradigm, and explore

whether serotonin participates in the pup reward processing by influencing dopamine via other receptor systems, such as 5-$HT_{2A}$ or 5-$HT_{2C}$ receptors (Di Giovanni et al., 2000; Di Matteo et al., 2002; Ichikawa et al., 2001; Millan et al., 1998).

In the pup preference test, we found that 8-OH-DPAT reduced the object exploration, but did not affect the pup exploration, resulting in a relative increase in pup preference ratio. The inhibitory effect of 8-OHDPAT on novel object exploration has been demonstrated before (Carey et al., 2008). Therefore, the increase in pup preference ratio might not be a good indication of the increase in maternal affect. Nevertheless, the finding that 8-OH-DPAT did not reduce the pup exploration time suggests that it did not disrupt maternal behavior by inhibiting maternal affect, further suggesting that the 5-$HT_{1A}$ receptor is not likely involved in the processing of pup reward. This conclusion is consistent with other findings suggesting that 8-OH-DPAT completely lacks any emotional regulatory effect in lactating rats, despite its well-documented such an effect in male and nonlactating female animals (De Vry, 1995; Fernandez-Guasti et al., 1998; Picazo et al., 2000).

Next, we used the pup separation technique to assess whether 8-OHDPAT causes a disruption of maternal behavior by disrupting maternal motivation. Pup separation has been used to manipulate maternal motivation. Previous work shows that removing pups from dams for several hours (> 3 h) prior to maternal testing can significantly increase a mother rat's maternal motivation and restore pup retrieval deficits induced by dopamine depletion in the ventral striatum region in postpartum female rats (Hansen, 1994), and significantly attenuate drug-induced disruption in pup licking and nursing (Amitai et al., 2012; Wu et al., 2016; Zhao and Li, 2009b). In the present study, we also observed that pup separation significantly increased the amount of time spent by the mothers on hovering and licking, indicating the effectiveness of this procedure to increase maternal performance. However, this procedure did not alter the 8-OH-DPAT-induced disruption of pup retrieval, pup licking and nursing. These results imply that 8-OH-DPAT might not cause a disruption of maternal behavior by suppressing maternal motivation.

In light of the above findings, we decided to examine how 8-OHDPAT affects mother rat's attention towards pups by using a pup-based PPI test. The acoustic startle response is a protective response elicited by a sudden and intense acoustic stimulus, and is related to an organism's baseline level of stress reactivity. The magnitude of startle

response can be reduced by a low-intensity prepulse. This reduction is termed PPI and is thought to measures the sensorimotor gating ability, a pre-attentive information processing mechanism critically important for cognition (Swerdlow et al., 2008). Because PPI can be modulated by external factors, such as the emotional state of an animal and the salience of a stimulus (Du et al., 2010, 2011; Li et al., 2009; Zhang and Li, 2016), we also tested mother rats in the presence of pups to determine the specific effect of 8-OH-DPAT on pup-directed attention. We found that pup presence and 8-OH-DPAT reduced the PPI performance, and this drug effect was most conspicuous in the dams tested without pups, likely due to the floor effect in the pup presence condition (already too low to see even lower effect). These findings suggest that the presence of pups might have distracted the mothers' attention in this task and 8- OH-DPAT has a general disruptive effect on attentional processing, regardless of the testing conditions (with or without pups). In fact, the PPI-disruptive effect of 8-OH-DPAT has been well-documented in the literature (Gogos et al., 2005; Gogos and van den Buuse, 2007), so has its startle-enhancement effect (Conti, 2012; Nanry and Tilson, 1989; Svensson and Ahlenius, 1983), and its detrimental effect on impulsivity and executive control (Rogers et al., 2013; Winstanley et al., 2005). If the 8-OH-DPAT's disruption of PPI and enhancement of startle response are related to its maternal disruptive effect, we may speculate that it does so by interfering with the attentional processing and emotional level, possibly by diverting a mother rat's focused attention on pups towards other environmental cues, and make it more easily distracted and emotional disturbed by irrelevant environmental stimuli. This idea needs to be carefully examined in the future. We should also mention that other drugs such as apomorphine, cocaine and amphetamine that disrupts PPI, like 8-OH-DPAT does, also disrupt maternal behavior (Kinsley et al., 1994; Schiorring and Hecht, 1979; Stern and Protomastro, 2000). They might do so through a similar mechanism.

Furthermore, because the 8-OH-DPAT's disruption of PPI is shown to be predominantly due to the drug's activation of postsynaptic, rather than pre-synaptic 5-$HT_{1A}$ receptors (Gogos et al., 2005), we reasoned that 8-OH-DPAT's disruption of maternal behavior is also likely due to its agonist action on postsynaptic 5-$HT_{1A}$ receptors located in such areas as the prefrontal cortex, hippocampus, central amygdala, nucleus accumbens, and the medial preoptic area of the hypothalamus. These brain regions are part of the "maternal brain

circuitry" that are intimately involved in the control of maternal behavior in rats (Numan and Sheehan, 1997; Pereira and Ferreira, 2016). In fact, Stamatakis et al. (2015) recently showed that 5-$HT_{1A}$ receptors were reduced in these areas in mother rats that exhibited increased maternal responses. Thus, if reduced 5-$HT_{1A}$ receptors in the maternal neural network are associated with increased maternal behavior, activation of these receptors by 8-OH-DPAT is likely responsible for its maternal disruption. This finding also explains why the 5-$HT_{1A}$ antagonist WAY-101405 did not show any effect in lactating mothers as their level of maternal responding was already high; there was not much room for improvement.

The pup retrieval on an elevated plus maze test has been reported before (Yang et al., 2015). Yang et al. (2015) shows that in comparison to nulliparous rats, postpartum rats would retrieve pups placed at the end of two open arms into the closed arms. They also enter the open arms and closed arms more and have a higher overall running speed. They suggest that the running speed could be used as a valid index of maternal motivation. The present study also used this test and found that 8-OH-DPAT did not reduce the running speed (Fig. 7E). Thus it appears that this observation is in support of what we concluded from the pup separation test that 8-OH-DPAT did not appear to have reduced maternal motivation. However, as mentioned above, because 8-OHDPAT did reduce time spent in the open arms where pups were placed, this could mean that 8-OH-DPAT has an inhibitory effect on maternal motivation based on previous work showing that this is a sensitive measure of motivational aspect of maternal behavior (Pereira and Ferreira, 2006). Once again, we want to emphasize that this hypothesis needs to be further examined before we can completely rule out the motivational effect of 8-OH-DPAT.

Finally, we want to comment on the discrepancies in maternal behavior in the vehicle control groups between experiments. There were several possible reasons that may explain these discrepancies. First, the different sample sizes may be one factor: n = 6/group for Exp. 1 and 2, n = 8/group for Exp. 3. Second, different suppliers and housing conditions may be another. Pregnant rats from the Exp. 1 and 2 were purchased from Charles River Inc. and housed at the University of Nebraska-Lincoln, whereas female rats from the Exp. 3 were purchased from Chongqing Tengxin Biological technology Co., Ltd., China and were housed and mated in the colony at the Southwest University (China). Third, different experimenters (female in Exp. 1 and 2

and male in Exp. 3) were involved in these experiments, and they may have slightly different behavioral recording criteria and rats responded to them differently. Because of these differences, we were careful to always include a vehicle control group in each experiment, and compared the drug administration group with its baseline parameters and its corresponding vehicle group. Therefore, these discrepancies should not have altered the basic effects of 8-OH-DPAT, but should be formally checked in the future.

Taken together, the present study demonstrates that activation of 5- $HT_{1A}$ has a negative impact on rat maternal behavior. This finding, together with our recent work on 5-$HT_{2A}$ and 5-$HT_{2C}$ receptor (Chen et al., 2014; Wu et al., 2016; Zhao and Li, 2010), suggests that serotonin plays an important role in the regulation of maternal behavior. It may act through the 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors to affect maternal behavior by altering attention, emotionality, or executive control of the programmed sequential order of behavioral responses, whereas it may act on the 5-$HT_{2C}$ receptor to regulate maternal behavior by suppressing maternal motivation (Wu et al., 2016). Future work is needed to determine the specific neural circuitry through which 5-$HT_{1A}$ receptor produces its behavioral effect on maternal behavior in rats.

**Conflicts of interest —** The authors declare no conflicts of interests.

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