5-1-2020

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HHS Public Access

Author manuscript

Pharmacol Biochem Behav. Author manuscript; available in PMC 2020 May 01.

Published in final edited form as:

Pharmacol Biochem Behav. 2019 May ; 180: 32–43. doi:10.1016/j.pbb.2019.03.003.

5-HT_{2A} Receptors Modulate Dopamine D_2-mediated Maternal Effects

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Abstract

Serotonin 5-HT_{2A} receptors are expressed throughout the mesolimbic and mesocortical dopamine pathways, and manipulation of this receptor system has a profound impact on dopamine functions and dopamine-mediated behaviors. It is highly likely that 5-HT_{2A} receptors may also modulate the D_2-mediated maternal effects. The present study investigated this issue and also explored the possible behavioral mechanisms. We tested the effects of two D_2 drugs (an agonist quinpirole: 0.5, 1.0 mg/kg, and a potent D_2 antagonist haloperidol: 0.05, 0.10 mg/kg, sc) and their combinations with two 5-HT_{2A} drugs (a selective 5-HT_{2A} agonist TCB-2: 2.5 mg/kg, and 5-HT_{2A} antagonist MDL100907, 1.0 mg/kg, sc) on maternal behavior in Sprague-Dawley postpartum females. Individually, TCB-2 (2.5 mg/kg, sc) and quinpirole (0.5 and 1.0 mg/kg, sc) reduced pup preference and disrupted home-cage maternal behavior. In contrast, haloperidol (0.10 mg/kg, sc) only disrupted home-cage maternal behavior, but did not suppress pup preference. MDL100907 (1.0 mg/kg, sc) by itself had no effect on either pup preference or maternal behavior. When administered in combination, pretreatment of TCB-2 did not alter quinpirole's disruption of pup preference and home-cage maternal behavior (possibly due to the floor effect), however, it did enhance haloperidol's disruption of pup retrieval in the home cage. MDL100907 had no effect both quinpirole's and haloperidol's disruption of pup preference and home-cage maternal behavior. Interestingly, haloperidol attenuated TCB-2's disruptive effect on pup preference. These findings suggest that activation of 5-HT_{2A} receptors tends to enhance D_2-mediated maternal disruption, whereas blockade of 5-HT_{2A} receptors is less effective. They also suggest that 5-HT_{2A} receptors may have a direct effect on maternal behavior independent of their interaction with D_2

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Conflicts of Interest: The authors declare no conflict interests.

receptors. The possible behavioral and neural mechanisms by which 5-HT_{2A}-and D2-mediated maternal effects and their interaction are discussed.

Keywords

Serotonin 2A receptor; Dopamine D_2 receptor; Incentive motivation; Emotional processing; Pup preference; Maternal behavior

1. Introduction

Maternal behavior in rats involves dramatic changes in the perceptional, emotional and motivational responses of female rats toward infant rats (Numan and Young, 2016). Through mother-infant interaction, the enduring mother–infant bond (maternal bond) is formed, which drives a mother rat to display a strong preference to pups and pup-related cues over other stimuli. This maternal attraction towards pups is mediated in part by the mesolimbic and mesocortical dopamine pathways, the brain's reward system (Numan, 2007). Functional disturbances of these systems, such as lesions of nucleus accumbens (NAc), medial prefrontal cortex (mPFC) or ventral tegmental area (VTA) (Afonso et al., 2007; Hansen, 1994; Hansen et al., 1991; Li and Fleming, 2003; Pereira and Morrell, 2011), or disruption of connection between the medial preoptic area (MPOA) and VTA (Numan and Smith, 1984), or blockade of dopamine receptors in these regions (Keer and Stern, 1999; Numan et al., 2005), reduces active maternal responses (e.g., pup retrieval and pup licking in the home cage). Blockade of D_1 and D_2 receptors also prevents the development of pup-induced conditioned place preference (Fleming et al., 1994). On the other hand, stimulation of the dopamine systems by certain dopamine receptor agonists (e.g. apomorphine, quinpirole) is also disruptive (Stern and Protomastro, 2000; Zhao and Li, 2010). Thus, it appears that a balanced dopamine neurotransmission is critical for the normal expression of maternal behavior. Based on the detailed behavioral analysis of dopamine-induced alterations in maternal behavior, dopamine action on D_2 receptors (possibly D_1 too) exerts a regulatory control over a mother rat's (positively) emotional and motivation responses towards pups (Numan and Young, 2016; Zhao and Li, 2009c).

Because stimulation and blockade of dopamine D_2 receptor all cause a similar disruption of maternal behavior, one critical question is whether they do so through the same or different behavioral mechanisms. One idea is that stimulation or blockade of D_2 receptors *similarly* suppresses maternal motivation to achieve its maternal disruption, based on the observations that blockade of D_2 receptor specifically alters behavioral measures of maternal motivation (Stern and Keer, 1999; Yang et al., 2015; Zhao and Li, 2009c). An alternative hypothesis is that stimulation and blockade of D_2 receptors act through different behavioral processes to influence maternal behavior. For example, blockade of D_2 receptors may disrupt maternal behavior by suppressing maternal motivation; whereas stimulation of D_2 receptors may do so by certain aspects of executive functions (attention or working memory, behavioral switching) necessary for the normal expression of maternal behavior. There is an ample evidence in the literature that implicates D_2 activation in the regulation of the above mentioned behavioral functions, especially on the behavioral organization and emotion

regulation (Agnoli et al., 2013; Herold, 2010; Liu et al., 2008; Pezze et al., 2007). To identify the specific behavioral mechanisms underlying the D_2-mediated maternal effects, we have used various behavioral tests, such as the pup retrieval on the elevated plus maze (Yang et al., 2015), pup separation (Zhao and Li, 2009c), and home cage maternal observation (Zhao and Li, 2010).

Besides dopamine, serotonin is recently shown to modulate maternal behavior via its action on 5-HT_{2A} receptors (and also 5-HT_{2C} and 5-HT_{1A}) (Chen et al., 2014; Gao et al., 2018b; Li et al., 2018; Wu et al., 2016; Zhao and Li, 2010). We reported that activation of serotonin 5-HT_{2A} receptors disrupts maternal behavior in the home cage, whereas blockade of them has little effect (Chen et al., 2014; Gao et al., 2018b; Zhao and Li, 2009a, 2010). Central infusion of TCB-2 (a selective 5-HT_{2A} agonist) into the medial prefrontal cortex (mPFC), but not in the medial preoptic area (mPOA) also disrupts maternal behavior (Gao et al., 2018b). Behaviorally, we showed that activation of 5-HT_{2A} receptors does not seem to cause a disruption of maternal motivation. Two pieces of evidence seem consistent with this conclusion. The first one comes from the pup separation study (Gao et al., 2018b). Pup separation is a powerful way to enhance maternal performance by increasing maternal motivation (Hansen, 1994). Previous studies have shown that several hours of pup separation (3–6 h) can completely restore the pup retrieval deficit induced by 6-OHDA lesions in the ventral striatum (Hansen, 1994). The fact that pup separation fails to reduce TCB-2-induced maternal disruption suggests that TCB-2 is not likely to severely impair maternal motivation. If it does, TCB-2-treated dams should have performed better under the pup separation condition than under the no-separation condition. The second piece of evidence comes from a pup vs. male preference test (Wu et al., 2018). In this study, we found that TCB-2-treated dams increased time spent with pups and decreased time with male. If TCB-2 suppress maternal motivation, we should not expect to see an increase in pup exploration time. We speculate that activation of 5-HT_{2A} may disrupt some aspects of executive function by diverting a mother rat's focused attention on pups toward other environmental cues or by increasing behavioral fragmentation and premature or 'impulsive' responding. This hypothesis is consistent with extensive evidence showing the critical role of 5-HT_{2A} receptors in executive function and behavioral organization (Anastasio et al., 2015; Aznar and Hervig Mel, 2016).

Because 5-HT_{2A} receptors are expressed throughout the mesolimbic and corticostriatal circuits (Howell and Cunningham, 2015), and manipulation of this receptor system has a profound impact on dopamine release and dopamine-mediated behaviors (Ichikawa et al., 2001a; Ichikawa et al., 2001b; Ichikawa and Meltzer, 1995; Katsidoni et al., 2011; Kuroki et al., 2003; Zayara et al., 2011), and there exists 5-HT_{2A}-D_2 heteromer and crosstalk (Albizu et al., 2011), it is possible that 5-HT_{2A} and D_2 may interactively alter maternal responses via various behavioral mechanisms. The primary goal of the present study was to investigate how activation or blockade of 5-HT_{2A} receptors alter the D_2-mediated maternal effects and identify the possible behavioral mechanisms. Toward this end, we tested a D_2 agonist quinpirole, and a D_2 antagonist haloperidol, and their combination with a 5-HT_{2A} agonist TCB-2 and 5-HT_{2A} antagonist MDL100907 in a pup preference test and a home-cage maternal behavior test. The pup preference test mainly measures the emotional and attentive processing of pups, as well as the sociability of mother rats. It is assumed that if a mother rat

possesses a strong positive emotion and focused attention towards her offspring, she will spend more time in close proximity to the pups over other stimuli (Li et al., 2018; Wu et al., 2018). Thus, this paradigm could be used to measure executive function of mother rats. Following the pup preference test, we observed the home cage maternal behavior, focusing on the active responses such as pup retrieval and pup licking. Based on the evidence that blockade of 5-HT_{2A} receptors is able to inhibit the mesolimbic dopamine function, while activation of 5-HT_{2A} receptors enhance it (Celada et al., 2013; Hamon and Blier, 2013; Howell and Cunningham, 2015), we hypothesized that MDL100907 would reduce the quinpirole-induced maternal disruption, but enhance the haloperidol-induced one. Conversely, TCB-2 would enhance the quinpirole-induced maternal disruption, but reduce the haloperidol-induced one.

2. Materials and methods

2.1. Animals

Virgin female Sprague-Dawley rats weighing 220–260 g were purchased from Chongqing Tengxin Biological Technology Co., Ltd, China. They 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). Room temperature was maintained at 22±2°C with a relative humidity of 45–75%. The rats had free access to food and water in their home cages. One week after arrival, each female rat was placed into the cage of a proven stud male for 10 days to ensure pregnancy. Following the mating procedure, the pregnant females were singly housed until parturition after which they were housed together with their litters for the remainder of the experiment. Experiments were conducted during the light cycle. Starting 2 or 3 days prior to the expected parturition date, the subjects were monitored in the morning and afternoon for signs of parturition. Once the dam was found to be with pups in the morning (that day was designated as postpartum day 1, PPD 1) or in the afternoon (PPD 0), two shredded paper towels were provided as nesting materials. On PPD 2, each litter was culled to 8 pups (4 males and 4 females with the most visible milk bands). All animal procedures were approved by the animal care and use committee at Southwest University, China, and were carried out in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Dugs and choices of doses

MDL100907 [a selective 5-HT_{2A} receptor antagonist, (R)-(+)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemethanol] and quinpirole [a putative D_2 dopamine receptor agonist, (−)-Quinpirole hydrochloride] were purchased from Sigma-Aldrich (St. Louis, MO, USA). TCB-2 [a high-affinity 5-HT2A agonist, 4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl]methylamine hydrobromide] was purchased from Tocris Bioscience (Ellisville, MO, USA). Haloperidol (HAL, 5.0 mg/ml Ampoules) was purchased from Hunan Dongting Pharmaceutical Co., Ltd., Hunan, China. TCB-2, haloperidol and quinpirole were dissolved in 0.9% saline. MDL100907 (1.0 mg/kg, sc) was dissolved in 0.9% saline containing up to 1% glacial acetic acid (Wadenberg et al., 2001a). All drugs were administered subcutaneously (sc). The chosen dose of MDL100907 was based on our previous work showing it has no effect on maternal behavior at 1.0 mg/kg (Chen et al.,

2014). TCB-2 has a disruptive effect on maternal behavior and pup preference at 2.5 mg/kg (Gao et al., 2018b; Wu et al., 2018). Haloperidol at 0.1 and quinpirole at 1.0 mg/kg disrupt maternal behavior as observed in the home cage or on an elevated plus maze (Li et al., 2004; Yang et al., 2015; Zhao and Li, 2009b, 2010). Whether they would also affect pup preference have not been studied.

2.3. Experiment 1: comparison between mother rats and virgin females in the pup preference test

In our previous studies, we assumed that the pup preference test measures the rewarding effect of pups, as mother rats spend more time in close proximity to the pups over other stimuli (Li et al., 2018; Wu et al., 2018). However, we have not validated it. Thus, this first experiment was designed to determine the validity of this pup preference paradigm by comparing mother rats with virgin females tested with pups or without pups in an arena. Specifically, 18 postpartum rats and 16 nulliparous rats were assigned to one of the following four groups: mother tested with pups (n = 10) or without pups (n = 8), and virgin females tested with pups (n = 8) or without pups (n = 8).

Pup preference was tested for five consecutive days from PPD 4–8. One day before the 1st test (PPD 3), the subject rats were habituated to the testing room and arena for 30 min. They were brought into the testing room, removed from their home cages and placed into an open-field arena made of black acrylic (50 cm L × 50 cm W × 50 cm H). Inside the arena, there was a cylinder-shape cage made of transparent acrylic (top and bottom) with stainless metal bars spaced in between (8.0 cm H × 7.5 cm in diameter). This cage was used to confine 4 pups in the subsequent pup preference tests. On PPD 4–8, the subject rats were brought into the testing room. 30 min later, they were placed into the arena and their exploration time with one of two stimuli were recorded for 10 min. One stimulus was 4 pups confined into the small cage and another one was a cube (6.0 cm L × 6.0 cm W × 6.0 cm H) used as a novel object. The pup containing cage and object were placed at the center of two adjacent quadrants at the backend of the arena, while the subject rats were placed midway at the frontend. They could see, hear and smell the pups but not contact them physically. The total time spent exploring the two stimuli (in seconds), and the total distance travelled (in centimeters) during the 10-min period were recorded using a digital video camera and analyzed using the software of Noldus EthoVision XT 8.5 (Wageningen, The Netherlands). Exploration was defined as sniffing or touching the cage/object with the nose and/or forepaws. The arena and the cages were cleaned and deodorized with a 75% ethanol solution between subjects and after each test. Pup preference is calculated as the ratio of time spent exploring the pup cage over the total amount of time spent exploring the cage and object. When tested without pups, the subject rats were only exposed to the cage and cube, no pups were placed inside the cage.

2.4. Experiment 2: effect of activation or blockade of D_2 receptor on pup preference and maternal responses and its modulation by 5-HT_{2A} receptors

This experiment examined how acute and repeated treatment of quinpirole or haloperidol alters pup preference and maternal behavior in the home cage, and how activation or blockade of 5-HT_{2A} receptors by TCB-2 or MDL100907 might change the maternal effects

of quinpirole or haloperidol. We investigated the repeated drug effects despite the finding from Experiment 1 showing that mother rats did not differ from virgins on pup preference on PPD 5–8. The rationale was that the drug effects often undergo a sensitization (increase) or tolerance (decrease) process with repeated injection and testing (Li, 2016; Stewart and Badiani, 1993), thus we could potentially observe changes in drug effects from PPD 5 to PPD 8. Ninety-six postpartum rats were randomly assigned to 1 of the 11 groups. The first 7 groups examined the individual drug effect: VEH+VEH (n = 17), VEH+QUIN 0.5 (n = 8), VEH+QUIN 1.0 (n = 8), VEH+HAL 0.05 (n = 8), VEH+HAL 0.1 (n = 7), TCB-2 2.5+VEH (n = 9), MDL100907 1.0+VEH (n = 8). The interactive effects were examined by comparing the combined drug groups (the TCB-2 2.5+QUIN 1.0, n = 8, TCB-2 2.5+HAL 0.1, n = 9, MDL100907 1.0+QUIN 1.0, n = 7, or MDL100907 1.0+HAL 0.1, n = 7) with the individual drug and control groups (The VEH+VEH, VEH+QUIN 1.0, and VEH+HAL 0.1). On each test day, the mother rats received the first injection of either VEH, TCB-2, or MDL100907 30 min before receiving the second VEH, QUIN or HAL injection. One hour after the second injection, they were placed into the open-field arena and tested for pup preference on PPD 4–8.

On PPD 9, maternal behavior was tested in the home cages under the same drug treatment conditions. The basic procedure was identical to what has been described in our previous studies (Chen et al., 2014; Zhao and Li, 2009c). Pups were first removed from the dam 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 dam sleeping corner. Each test was recorded by a video camera for 10 min and analyzed manually using a computer with an event-recording program JWatcher (http://www.jwatcher.ucla.edu). The raters were blind to each dam's treatment condition. The following behaviors were recorded and analyzed: pup retrieval (a rat picking up a pup in her mouth and carrying it back to the nest site), crouching (a rat positioning herself over the pups with legs splayed to accommodate the pups, including hover, high and low crouching-over posture), pup licking (a rat placing its tongue on the anogenital area and the rest of a pup's body), 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, and 600s was assigned to non-responders who did not approach or retrieve the testing pups. After the test, unretrieved pups were returned to the nest site.

2.5. Statistical analysis

Statistical analyses were performed using SPSS 22 software (SPSS Inc., Chicago, IL, USA). Pup preference data on each test day (i.e. exploration time and preference ratio) were analyzed using a factorial repeated measures analysis of variance (ANOVA), with postpartum state (mother versus virgin females) and test condition (with or without pups) (Experiment 1), or treatment (Experiment 2) as the between-subjects factors, and object (pups vs. object) as the within-subjects factor. Data from each test day were analyzed separately by 2 (VEH vs. quinpirole or haloperidol) × 3 (pretreatment: VEH vs. TCB-2, and MDL100907) two-way ANOVA. Group differences of pup preference data at each test day and each stimulus were further investigated using one-way ANOVAs or independent t tests.

Specific group difference was detected using the LSD post hoc test following the identification of significant treatment effect or treatment by object interaction. The same method was used to analyze the home-cage maternal behavior data. In Experiment 2, the data of maternal behavior in home cage from one rat was missed because of video recording malfunction. All data are presented as mean ± SEM. Differences were considered statistically significant if $p < 0.05$.

3. Results

3.1. Experiment 1: comparison between mother rats and virgin females in pup preference

Both mother rats and virgin females spent more time exploring the cage containing pups than the object over the five test days, especially when they were tested with pups. Mothers tended to spend more time than virgin females on exploring the pup cage, but this enhanced exploration time was observed only on the 1st test day on PPD 4 (Fig. 1). On PPD 4, repeated measures ANOVA found a main effect of test condition (with or without pups) ($F(1,30) = 18.800$, $p < 0.001$), a main effect of object (pups vs. object) ($F(1,30) = 141.793$, $p < 0.001$). In addition, the maternal state × object, object × test condition (with or without pups), and object × maternal state × test condition were all significant (all $p < 0.024$), suggesting that mother rats differed from the virgins on the pup and object exploration time and this difference depended on the specific test condition (with or without pups). Specifically, when tested with pups on PPD 4 (Fig. 1A and B), mother rats spent more time exploring pups than virgins ($t(16) = 3.286$, $p = 0.005$), but their object exploration time was not significantly different from that of virgins ($t(16) = 1.870$, $p = 0.08$). When tested without pups (Fig. 1A), mother rats spent similar amount of time exploring the cage as virgins did ($t(14) = -0.565$, $p = 0.581$), but spent less time exploring the object ($t(14) = -2.806$, $p = 0.014$). With repeated testing on PND 5–8, mother rats did not differ from virgins on the exploration time with either pups or object (all $p > 0.05$). The only significant effect was the object (all $p < 0.003$), as both groups appeared to explore the pup cage more than the object, suggesting that the cage itself has a slightly higher intrinsic value than the object.

The enhanced pup preference found in mother rats under the pup presence condition was also apparent in the pup preference ratio. On PPD 4, mother rats tested with pups had a significantly higher pup preference ratio than mothers tested without pups ($t(16) = -5.554$, $p < 0.001$), and virgins tested with pups ($t(16) = -2.953$, $p = 0.009$) (Fig. 1C). Mother rats tested with pups appeared to be more active than those tested without pups ($t(16) = -3.071$, $p = 0.007$), who were also less active than the virgin females ($t(14) = -3.155$, $p = 0.007$) (Fig. 1d). Overall results suggest that the pup preference test is capable of detecting the emotional responses of mother rats towards pups and discriminating them from virgin females. With the current setup and chosen stimuli, only the first day appears to be valid in measuring the specific “maternal” responses. This work lays the foundation for the subsequent pharmacological work.

3.2. Experiment 2: effect of activation or blockade of D_2 receptor on pup preference and maternal responses and its modulation by 5-HT_{2A} receptors

3.2.1. Individual drug effects on pup preference: Contrasts between quinpirole and haloperidol, and between TCB-2 and MDL100907—

Fig. 2 shows the exploration time with the pup cage and object and pup preference ratio in mother rats treated with TCB-2 (2.5 mg/kg), MDL100907 (1.0 mg/kg), quinpirole (0.5 or 1.0 mg/kg), haloperidol (0.05 or 0.10 mg/kg) on PPD 4. TCB-2 and quinpirole decreased the pup exploration time and lowered pup preference ratio. Haloperidol increased the pup exploration time, but did not affect pup preference ratio. MDL100907 had no effect on either measure. Repeated measures ANOVA on the exploration time showed a main effect of object (a within-subjects factor; $F(1, 58) = 217.425$, $p < 0.001$), treatment (a between-subjects factor; $F(6, 58) = 10.537$, $p < 0.001$), and object × treatment interaction ($F(6, 58) = 15.625$, $p < 0.001$). One-way ANOVAs examining the specific group differences showed that TCB-2 and quinpirole decreased, while haloperidol (0.05 mg/kg) increased the pup exploration time (all $p < 0.005$). MDL100907 or haloperidol at 0.1 mg/kg had no effect on the pup exploration time (all $p > 0.411$). Only quinpirole at 1.0 mg/kg increased the object exploration time ($p < 0.001$). This resulted in a significant decrease in pup preference ratio in rats treated with TCB-2 and quinpirole (all $p < 0.005$, Fig. 2C). Furthermore, quinpirole and haloperidol at 0.1 mg/kg, but not TCB-2 and MDL100907, also significantly suppressed the movement, as measured by the distance travelled in the arena relative to the vehicle (Fig. 2D), suggesting that the differential effects among these four drugs on pup exploration time and pup preference are dissociated from their psychomotor effects.

3.2.2. Individual drug effects on home-cage maternal behavior—

Fig. 3 shows the home-cage maternal behavior of the mother rats treated with TCB-2 (2.5 mg/kg), MDL100907 (1.0 mg/kg), quinpirole (0.5 or 1.0 mg/kg), haloperidol (0.05 or 0.10 mg/kg) as tested on PPD 9. In agreement with our previous reports (Chen et al., 2014; Gao et al., 2018b; Li, 2015; Li et al., 2004; Zhao and Li, 2012), one single injection (after 5 injections during pup preference test) of TCB-2, quinpirole and haloperidol on the test day disrupted various components of maternal behavior, whereas MDL100907 had no effect. One-way ANOVA revealed a main effect of treatment in the number of pup retrieved ($F(6, 58) = 15.175$, $p < 0.001$), but not in the duration of pup crouching ($F(6, 58) = 1.778$, $p = 0.12$) and licking ($F(6, 58) = 1.6$, $p = 0.164$), nor nest building ($F(6, 58) = 1.108$, $p = 0.432$). Post hoc LSD tests indicated that rats treated with TCB-2, quinpirole, or haloperidol (0.1 mg/kg, not at 0.05 mg/kg) retrieved significantly fewer pups (all $p < 0.001$) than the VEH group. MDL100907 had no effect on the number of pups retrieved ($p = 0.683$) (Fig. 3A).

Taken together, results from the pup preference and home-cage maternal tests reveal individual drug profiles in maternal behavior: TCB-2 and quinpirole produced a consistent disruption of both pup preference and home cage maternal behavior. Haloperidol disrupted maternal behavior in the home cage, but did not disrupt pup preference. MDL100907 had no effect on either pup preference or maternal behavior in the home cage.

3.2.3. TCB-2 and MDL100907 pretreatment did not alter quinpirole's disruption of pup preference, but reduced its enhancement effect of object

exploration—To examine how activation and blockade of 5-HT_{2A} receptors by TCB-2 and MDL100907, respectively, modulates the effects of quinpirole, we conducted a 2 [VEH vs. QUIN] × 3 [VEH, TCB-2, and MDL100907] ANOVA on the pup preference PPD 4 data from mother rats pretreated with VEH (the VEH+VEH and VEH+QUIN groups) and those pretreated with either TCB-2 (the TCB-2+VEH and TCB-2+QUIN groups) or MDL100907 (the MDL100907+VEH and MDL100907+QUIN groups).

On the pup exploration time (Fig. 4A), pretreatment of TCB-2 but not MDL100907 had a stronger suppression in rats treated with VEH than those with quinpirole. Two-way ANOVA showed a main effect of pretreatment ($F(2, 51) = 19.234$, $p < 0.001$), quinpirole ($F(1, 51) = 12.386$, $p = 0.001$), and the interaction between the two ($F(2, 51) = 3.918$, $p = 0.026$). This interactive effect reflects the significantly suppressive effect of TCB-2 (not MDL100907) on the VEH-treated rats (VEH+VEH vs. TCB-2+VEH) and the lack of the effect on the quinpirole-treated ones (TCB-2+VEH vs. TCB-2+QUIN). Post hoc LSD tests confirmed that TCB-2 ($p < 0.001$) but not MDL100907 ($p = 0.412$) decreased the pup exploration time. One-way ANOVA followed by post hoc LSD tests further showed that the rats treated with either quinpirole, TCB-2 or their combination (i.e., the VEH+QUIN 1.0, TCB-2 2.5+VEH, TCB-2 2.5+QUIN 1.0 and MDL100907+QUIN 1.0) all had a significantly lower pup exploration time than the VEH+VEH group (all $p < 0.003$), reflecting the treatment effect of quinpirole and TCB-2. Pretreatment of TCB-2 or MDL100907 before quinpirole did not alter quinpirole's disruption of pup exploration (due to the floor effect).

Fig. 4B shows that the object exploration time was increased by quinpirole, however, pretreatment of TCB-2 or MDL100907 before quinpirole reduced the quinpirole's increasing effect on object exploration. Two-way ANOVA showed a significant interaction between pretreatment and quinpirole ($F(2, 51) = 4.251$, $p = 0.02$), but no main effect of pretreatment ($F(2, 51) = 1.499$, $p = 0.233$), or quinpirole ($F(1, 51) = 2.627$, $p = 0.111$). One-way ANOVA followed by post hoc LSD tests showed that only the VEH+QUIN 1.0 group had an increased object exploration time than the VEH+VEH group ($p = 0.001$). However, this treatment effect disappeared when TCB-2 or MDL100907 was added in the treatment (no significant group difference between the TCB-2 2.5+QUIN 1.0 or MDL100907 1.0+QUIN 1.0 and VEH+VEH group, $p = 0.437$ or 0.582, respectively). There was also a significant group difference between the TCB-2 2.5+QUIN 1.0 or MDL100907 1.0+QUIN 1.0 and the VEH+QUIN 1.0 group ($p = 0.025$ or 0.02, respectively), indicating the significant reduction by TCB-2 and MDL100907 on quinpirole's enhancing effect on object exploration. All these results suggest that TCB-2 or MDL100907 attenuated the quinpirole's increasing effect on object exploration.

Like its effect on the pup exploration time, TCB-2 or MDL100907 did not alter quinpirole's effect of pup preference ratio, but reduced the VEH-treated dams (Fig. 4C). Twoway ANOVA showed a main effect of pretreatment ($F(2, 51) = 21.629$, $p < 0.001$), but no main effect of quinpirole ($F(1, 51) = 2.852$, $p = 0.097$), or the interaction between the two ($F(2, 51) = 3.008$, $p = 0.058$). Post hoc LSD tests revealed that TCB-2, but not MDL100907 decreased the pup preference ratio ($p < 0.001$).

On the distance travelled (a measure of motor activity, Fig. 4D), two-way ANOVA showed a main effect of pretreatment ($F(2, 51) = 5.561$, $p = 0.007$), but no main effect of quinpirole ($F(1, 51) = 0$, $p = 0.995$), nor the interaction between them ($F(2, 51) = 2.361$, $p = 0.105$). Post hoc LSD tests revealed that TCB-2 ($p = 0.016$) but not MDL100907 ($p = 0.475$) increased the distance travelled in the arena.

Taken together, results from this analysis reveals that TCB-2 and MDL00907 did not alter quinpirole's suppressive effect on pup preference, mainly due to the floor effect. However, TCB-2 and MDL00907 individually did reduce quinpirole's increasing effect on object exploration. Thus, MDL100907 shares a similar effect with TCB-2 in reducing quinpirole's effect on object exploration.

3.2.4. Neither TCB-2 nor MDL100907 pretreatment altered the quinpirole's disruption of home-cage maternal behavior—Next, we examined the possible pretreatment effects of TCB-2 and MDL100907 on quinpirole-induced disruption of home-cage maternal behavior. Quinpirole and TCB-2, but not MDL100907 decreased various components of home-cage maternal behavior. Two-way ANOVA on the number of pups retrieved showed a main effect of pretreatment ($F(2, 51) = 17.014$, $p < 0.001$), quinpirole ($F(1, 51) = 36.658$, $p < 0.001$), and a significant interaction between the two ($F(2, 51) = 6.82$, $p = 0.002$). Post hoc LSD tests revealed that TCB-2 ($p < 0.001$) but not MDL100907 ($p = 0.628$) decreased the number of pups retrieved. In addition, quinpirole-treated mother rats also spent less time crouching over pups ($F(1, 51) = 6.424$, $p = 0.014$), licking pups ($F(1, 51) = 4.352$, $p = 0.042$), but not building nest ($F(1, 51) = 1.317$, $p = 0.257$). None of these effects of quinpirole was altered by 5-HT_{2A} pretreatment (TCB-2 or MDL100907), as its interaction with quinpirole was not significant (all $p > 0.248$). Therefore, neither TCB-2 nor MDL100907 altered the quinpirole's disruption of home-cage maternal behavior.

3.2.5. Neither TCB-2 nor MDL100907 pretreatment altered the haloperidol's lack effect on pup preference. However, haloperidol attenuated TCB-2's disruptive effect on pup preference—To examine how activation or blockade of 5-HT_{2A} receptors by TCB-2 or MDL100907, respectively modulates the haloperidol's effect on pup preference, the data from mother rats treated with VEH or haloperidol (0.1 mg/kg) on the 1st test day (PPD 4) were analyzed together with those from the dams pretreated with VEH, TCB-2 or MDL100907 (e.g., haloperidol together with VEH, TCB-2 at 2.5 mg/kg or MDL100907 at 1.0 mg/kg). As haloperidol by itself did not alter pup preference, while TCB-2 disrupted it, we also examined how haloperidol might alter TCB-2's effect. Fig. 5 shows the exploration time with the pup cage and object, pup preference ratio, and total distance moved in the six groups.

On the pup exploration time (Fig. 5A), two-way ANOVA showed a main effect of pretreatment ($F(2, 51) = 32.628$, $p < 0.001$), but no main effect of haloperidol ($F(1, 51) = 1.58$, $p = 0.215$), nor their interaction ($F(2, 51) = 0.238$, $p = 0.789$). Post hoc LSD tests revealed that TCB-2 ($p < 0.001$) but not MDL100907 ($p = 0.992$) decreased the pup exploration time.

TCB-2 decreased the object exploration time, especially in the haloperidol-treated dams. Two-way ANOVA showed a significant interaction between the 5-HT_{2A} pretreatment and haloperidol ($F(2, 51) = 6.392$, $p = 0.003$). One-way ANOVA followed by the post hoc tests showed that the TCB-2 2.5+HAL 0.1 group spent significantly less time on object exploration than the VEH+VEH group ($p = 0.039$), the VEH+HAL 0.1 and TCB-2 2.5+VEH groups ($p = 0.026$, 0.003, respectively). In contrast, the MDL100907 1.0+HAL 0.1 group spent significantly more time than the VEH+VEH group ($p = 0.046$) (Fig. 5B). More importantly, despite its lack of effect on pup preference, haloperidol reduced TCB-2-induced disruption of the pup preference ratio (Fig. 5C). Two-way ANOVA revealed a main effect of 5-HT_{2A} pretreatment ($F(2, 49) = 8.679$, $p = 0.001$), haloperidol ($F(1, 49) = 4.453$, $p = 0.04$), and the interaction between the two ($F(2, 49) = 5.44$, $p = 0.007$). Post hoc tests revealed that TCB-2 ($p < 0.001$) but not MDL100907 ($p = 0.554$) decreased the pup preference ratio of the VEH-treated group, as the TCB-2 2.5+VEH group had a significantly lower pup preference ratio than the VEH+VEH group ($p < 0.001$), but the TCB-2 2.5+HAL 0.1 group did not differ from the VEH+VEH group ($p = 0.407$). TCB-2 2.5+HAL 0.1 group actually had a higher pup preference ratio than the TCB-2 2.5+VEH group ($p < 0.001$), reflecting that haloperidol attenuated the TCB-2's disruptive effect on pup preference.

On the distance travelled (Fig. 5D), two-way ANOVA showed only a main effect of haloperidol ($F(1, 51) = 17.156$, $p < 0.001$), but no main effect of 5-HT_{2A} pretreatment ($F(2, 51) = 1.346$, $p = 0.269$), or the interaction between the two ($F(2, 51) = 1.088$, $p = 0.345$), a finding consistent with the motor suppressive profile of haloperidol.

3.2.6. TCB-2, but not MDL100907 pretreatment enhanced haloperidol's disruption of pup retrieval in the home cage—Fig. 6 shows the home-cage maternal behavior data from the mother rats treated with haloperidol (0.1 mg/kg), and haloperidol (0.1 mg/kg) together with TCB-2 (2.5 mg/kg) or MDL100907 (1.0 mg/kg). Both TCB-2 and haloperidol decreased the number of pups retrieved, and TCB-2 enhanced haloperidol's disruption effect. Two-way ANOVA showed a main effect of 5-HT_{2A} pretreatment ($F(2, 50) = 19.353$, $p < 0.001$), haloperidol ($F(1, 50) = 42.573$, $p < 0.001$), and the interaction between the two ($F(2, 50) = 6.87$, $p = 0.002$). Post hoc LSD tests revealed that TCB-2 ($p < 0.001$) and MDL100907 ($p = 0.005$) decreased the number of pups retrieved in the haloperidol-treatment groups. One-way ANOVA followed by post hoc LSD tests showed that the VEH +HAL 0.1, TCB-2 2.5+VEH, TCB-2 2.5+HAL 0.1, and MDL100907+HAL 0.1 groups all retrieved significantly fewer pups than the VEH+VEH group (all $p < 0.001$). Importantly, the TCB-2 2.5+HAL 0.1 group ($p = 0.027$) but not MDL100907+HAL 0.1 group ($p = 0.056$) retrieved even fewer pups than the VEH+HAL 0.1 group. On other maternal responses, the main effects of 5-HT_{2A} pretreatment and haloperidol, and their interaction all failed to reach a significant level (all $p > 0.067$), except that haloperidol significantly decreased the nest building duration ($F(1, 50) = 7.815$, $p = 0.007$).

Over all, these data indicate that TCB-2 enhanced haloperidol's disruption of pup retrieval in the home cage, and MDL100907 is ineffective to alter haloperidol's effect on maternal behavior. Haloperidol also reduced the pup preference disruption induced by TCB-2.

4. Discussion

The 5-HT_{2A} and D_2 receptors have a functional crosstalk (Albizu et al., 2011) and they are all richly expressed in the mesolimbic and mesocortical systems (Azmitia and Segal, 1978; McMahon et al., 2001), providing the neuroanatomical basis for their interaction. The present study examined to what extent acute and repeated activation or blockade of 5-HT_{2A} receptors modulates dopamine D_2-mediated effects on pup preference and home-cage maternal behavior. After we validated the pup preference paradigm, we found that acute administration of TCB-2 and quinpirole suppressed pup preference mainly by decreasing the pup exploration time. In contrast, acute haloperidol injection had no effect (1.0 mg/kg) or even an opposite effect (0.05 mg/kg) on pup exploration. At 0.05 mg/kg, haloperidol selectively increased the pup exploration time without affecting the object exploration. MDL100907 had no effect on the pup and object exploration time and pup preference ratio. In the subsequent home-cage maternal behavior test, TCB-2, quinpirole and haloperidol all caused a similar disruption of active maternal responses, especially the pup retrieval. Once again, MDL100907 failed to alter any maternal response in the home cage. When used together, TCB-2 was found to enhance haloperidol's disruption of home-cage pup retrieval, but did not alter quinpirole's pup preference and home-cage maternal disruption (due to the floor effect). MDL100907 did not change quinpirole's disruption of pup preference and home-cage maternal behavior, nor haloperidol's maternal effects. These findings suggest that activation of 5-HT_{2A} receptors (TCB-2) is capable of enhancing D_2-mediated disruption of maternal performance, whereas blockade of 5-HT_{2A} receptors (MDL100907) is less effective to alter D_2 mediated maternal effects. They also indicate that 5-HT_{2A} receptors may have a direct effect on maternal behavior independent of their interaction with D_2 receptors.

Naïve virgin female rats generally avoid infant rats, while mother rats are attracted to them and show a strong pup preference over other stimuli. Through maternal experience, mother rats develop strong social and emotional attachment towards the pups, as evidenced by the findings that they often increase their licking and nursing towards pups after a brief separation from their litters and exhibit depression-like behaviors if they experience a long period of repeated separation from their pups (Boccia et al., 2007). 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). In the present study, we used the pup preference test to assess this mother-infant bond (Olazabal et al., 2013). This test differs from the pup CPP, as it is a direct measure of emotional attachment towards pups, the so called "liking" aspect of maternal behavior (Berridge and Robinson, 2003) because it does not require any conditioning and any effort put forth by the mothers. We found that mother rats differed from virgins in their attractiveness to pups. Mothers tended to spend more time than virgin females on exploring the pup cage when they were tested with pups. When tested without pups, mother rats had similar amount of time exploring the cage as the virgins did. One drawback with the current setup is that the mother-virgin difference only appeared on the 1st test day and did not persist throughout the five-day testing period. This may be due to the

unexpected higher intrinsic rewarding value of the cage (i.e., ~60% preference ratio even for the empty cage). In the future work, we will use two identical copies of the cage to see if the mother-virgin difference will persist throughout the postpartum period.

Mother rats acutely treated with quinpirole (0.5 and 1.0 mg/kg) decreased the pup exploration time but increased the object exploration, so they showed a significantly lower pup preference ratio. They also showed impaired maternal performance in the home cage, consistent with our previous study (Zhao and Li, 2010). Behaviorally, it is possible that quinpirole may have decreased the emotional processing of the rewarding value of pups and this suppression could explain quinpirole's effects in both tests. In a food-induced conditioned place preference test, Liu et al. (2008) reported that microinjections of quinpirole into the posteromedial VTA led to conditioned place aversion. Quinpirole administered to this site also decreased food intake and basal dopamine concentration in the ventromedial striatum. Based on the finding that quinpirole microinjected into the posteromedial VTA reduces dopamine cell firing (Beckstead et al., 2004; Olijslagers et al., 2004), they concluded that inhibition of midbrain dopamine neurons causes a negative affective state and disrupts positive affective encoding of food reward. The same could be said regarding pup reward. Quinpirole may have made mother rats aversive to pups, leading them to reduce their pup exploration time. This idea could be tested in a pup place preference test to see if quinpirole causes mother rats to develop a conditioned place aversion to the pup associated environment.

Besides this possibility, quinpirole could also disrupt maternal behavior by impairing certain aspects of executive function, such as behavioral organization, attention and working memory, etc. Other studies have shown that D_2 activation often causes perseverative responding, deficit in attention and working memory (Agnoli et al., 2013; Bushnell and Levin, 1993; Herold, 2010; Liu et al., 2008; Pezze et al., 2007; Wang et al., 2004). Under the influence of quinpirole, a mother rat might lose its ability to select and maintain appropriate behavioral responses in the presence of pups. Collectively, quinpirole may disrupt maternal behavior through at least two possible behavioral mechanisms targeting two different brain areas. Quinpirole may cause a negative affect towards pups by acting on the D_2 autoreceptors in the VTA (Liu et al., 2008), while it may disrupt executive functions necessary for normal maternal behavior by targeting the postsynaptic D_2 receptors in the mPFC (Wang et al., 2004). Indeed, previous work does show that quinpirole significantly increases c-Fos expression in the mPFC, while it reduces c-Fos expression in the nucleus accumbens in postpartum female rats (Zhao and Li, 2010). Apparently, this intriguing idea needs to be rigorously tested in the future.

Although haloperidol, like quinpirole, disrupted home-cage maternal behavior, it did not suppress pup preference. Haloperidol is well known for its suppression on maternal motivation (Giordano et al., 1990; Li et al., 2004; Silva et al., 2001; Stern and Keer, 1999; Zhao and Li, 2010). This idea is supported by extensive evidence showing that haloperidol-induced maternal disruption can be reduced to some extent by 4-h pup separation (Zhao and Li, 2009c); and haloperidol selectively suppresses behavioral measures indicative of maternal motivation (Stern and Keer, 1999; Yang et al., 2015). The dissociation between haloperidol's lack of effect on pup preference and its strong disruption of home-cage

maternal behavior suggests that our pup preference test may not measure the motivational aspect of reward (pups) processing, especially the behavioral activation and effort-related aspects of maternal motivation (Salamone et al., 2016), but rather, measures the innate emotional aspect of reward processing and/or how a mother rat organizes its activities in response to two competing stimuli. These findings also suggest that a multi-test approach is often necessary to reveal the neurochemistry of specific psychological functions underlying maternal behavior. Although we were initially interested in how TCB-2 and MDL100907 alter the maternal effects of haloperidol, we also found that haloperidol reduced TCB-2-induced disruption of pup preference, indicating that blocking D_2 receptors can also reduce 5-HT_{2A} activation-induced maternal disruption. This action may be explained by haloperidol's inverse agonist action against 5-HT_{2A} receptors (Weiner et al., 2001).

Consistent with our previous study (Gao et al., 2018b; Wu et al., 2018), we found that TCB-2 disrupted both pup preference and home-cage maternal behavior. Based on the findings that 4-h pup separation (a technique presumably increases maternal motivation) failed to attenuate the TCB-2-induced maternal disruption and TCB-2-treated dams showed an increased pup preference in a pup-male preference test, we suggested that TCB-2 is unlikely to have a disruptive effect on motivational and emotional processing of the rewarding property of pups (TCB-2 may actually have an enhancement effect on pup reward). Rather, we proposed that TCB-2 could disrupt pup preference and maternal behavior by disrupting the executive function (Wu et al., 2018), giving its well-known hallucinatory effect and its disruption of impulsive response and behavioral organization (Koskinen et al., 2000a; Koskinen et al., 2000b; Winstanley et al., 2004). TCB-2 is *speculated* to disrupt maternal behavior and pup preference by either diverting a mother rat's focused attention on pups towards other environmental cues, and by making her more easily distracted by irrelevant environmental stimuli, or by increasing behavioral fragmentation and premature, or 'impulsive' responding. This hallucinogenic behavioral effect of TCB-2 in rat maternal behavior is also supported by the finding that activation of 5-HT_{2A} receptors in the mPFC is responsible for TCB-2's maternal effects (Gao et al., 2018b). This effect may be partially achieved through excessively stimulating dopamine release in the mesocortical and mesolimbic dopamine systems (Di Giovanni et al., 2000; Di Matteo et al., 2002), which in turn may cause a disruption of executive function. However, we do not have any *direct* evidence showing that activation of 5-HT_{2A} receptors causes disorganized maternal responses, which should be examined in the future.

Our hypothesis that both TCB-2 and quinpirole disrupt executive function of dams, whereas haloperidol mainly disrupts maternal motivation can be applied successfully to explain the findings that TCB-2 did not alter quinpirole's disruption of pup preference and home-cage maternal behavior, but enhanced haloperidol's disruption of pup retrieval in the home cage. In the case of quinpirole, because both TCB-2 and quinpirole act on the same psychological process (i.e., executive function), the lack of modulation by TCB-2 on quinpirole-induced home-cage maternal disruption may reflect the floor effect of both drugs on executive function. In the case of haloperidol, because TCB-2 and haloperidol disrupt executive function and maternal motivation separately, the enhancement effect of TCB-2 on haloperidol-induced disruption of home-cage maternal behavior may reflect the additive effect of TCB-2's disruption of executive function on top of haloperidol's disruption of

maternal motivation. Apparently, this hypothesis needs to be further tested before we can accept its validity.

MDL100907 at 1.0 mg/kg by itself did not alter pup preference or home-cage maternal behavior, a finding consistent with our previous report (Chen et al., 2014; Nie et al., 2018). It also did not change quinpirole's and haloperidol's effects in these two tests. Our finding on the MDL100907 and quinpirole interaction is consistent with a previous report showing that MDL100907 is ineffective in reducing contralateral rotational behavior induced quinpirole in adult rats with unilateral DA lesions (Taylor et al., 2006). The literature on the MDL100907 and haloperidol interaction is mixed, and three possible outcomes have been reported: a potentiation (Wadenberg et al., 2001b); a reduction (Benaliouad et al., 2007); and no effect (Creed-Carson et al., 2011; Gao et al., 2018a; Reavill et al., 1999). These findings, together with the present ones, suggest that the interactions between 5-HT_{2A} and D_2 receptors are much more complex than we originally thought, and may depend on specific D_2-mediated behaviors. Indeed, we recently used a pup retrieval on an elevated plus maze test (a test of maternal anxiety, maternal motivation, executive function and their interactions) and found that MDL100907 exacerbated the quinpirole-induced disruption of pup retrieval (Nie et al., 2018), supporting the notion that 5-HT_{2A} blockade's modulation on D_2-mediated maternal effects also depends on the specific behavioral tests, which rely on different psychological processes with different degrees of sensitivity to the D_2 × 5-HT_{2A} interactions.

In conclusion, the present study further demonstrates that the balanced dopamine D_2-mediated neurotransmission is critically important for the normal expression of maternal behavior. Stimulation or blockade this receptor by quinpirole and haloperidol, respectively, induces a change in pup preference and home-cage maternal behavior likely through different behavioral mechanisms. Quinpirole may disrupt maternal behavior by causing a negative maternal affect or disrupting executive function; whereas haloperidol may do so by suppressing maternal motivation. Importantly, we found an important modulation of 5-HT_{2A} on D_2-mediated maternal effects: activation of 5-HT_{2A} receptors by TCB-2 enhances D_2-mediated suppression of maternal motivation, whereas blockade of 5-HT_{2A} receptors by MDL100907 is less effective to alter D_2-mediated maternal effects. Future work will examine the neural basis of the interactive actions between D_2 and 5-HT_{2A} receptors and further elucidate the relevant behavioral mechanisms.

Acknowledgements:

This research was supported by a grant from the National Natural Science Foundation of China (NSFC) (No. 31500891) (J. G.), the Fundamental Research Funds for the Central Universities [(SWU1609131, SWU115030) (J. G.), (SWU116014) (M. L.)], the National Institute of Mental Health (R01MH097718) (M. L.), and Chongqing Collaborative Innovation Center for Brain Science, China (M. L.).

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Highlights

1. Activation of D_2 (Quinpirole) or 5-HT_{2A} (TCB-2) receptors suppressed pup preference
2. TCB-2, quinpirole, or haloperidol disrupted home-cage maternal behavior
3. TCB-2, but not MDL100907, enhanced haloperidol's disruption of pup retrieval
4. Haloperidol reduced TCB-2's disruptive effect on pup preference
5. TCB-2 or MDL100907 did not affect quinpirole's disruption of pup preference

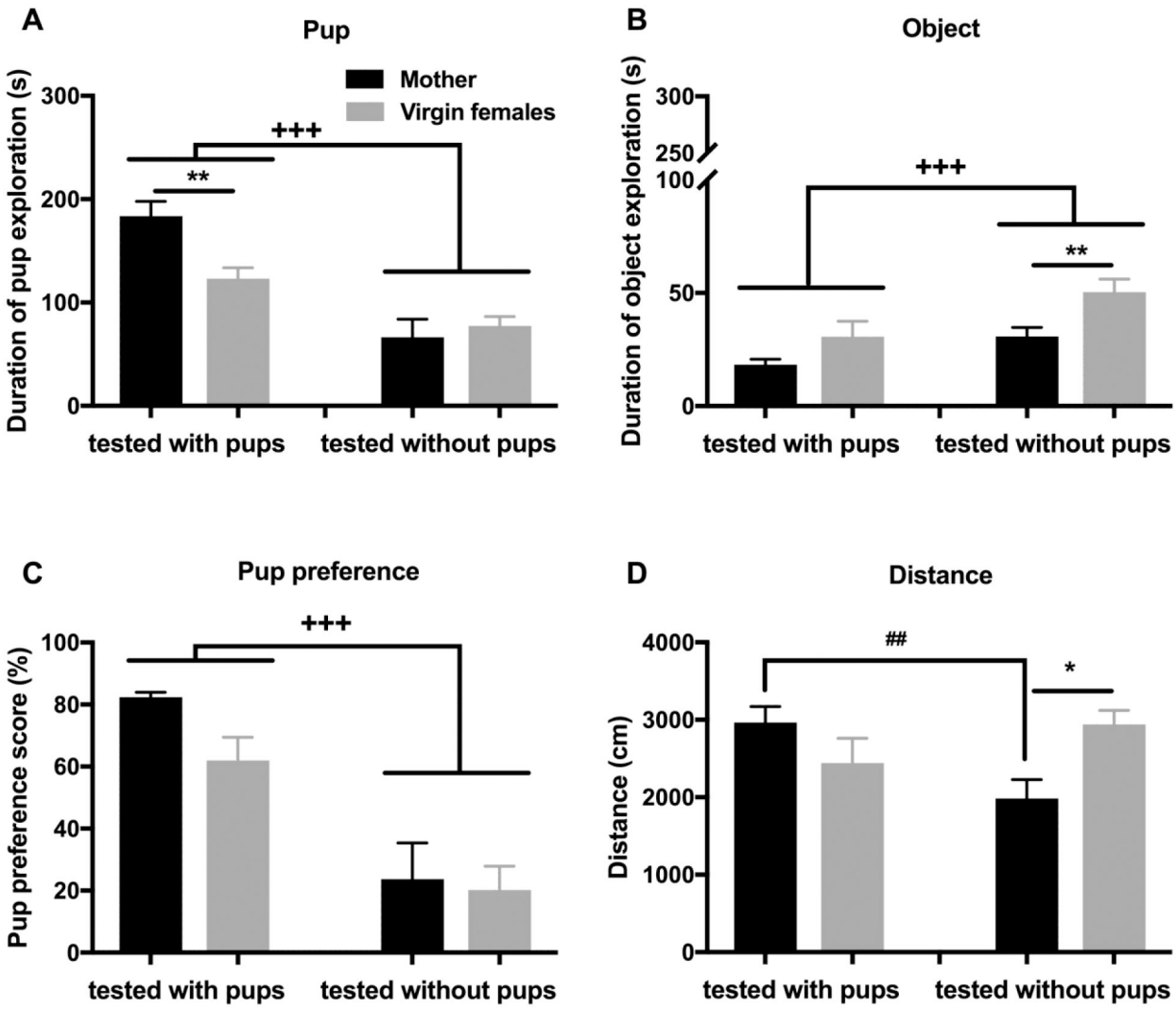

Fig. 1.

Comparison between mother rats and virgin females in the pup preference test on PPD 4. Duration of pup exploration time (A), object exploration time (B), pup preference ratio (C), and distance travelled (D) on PPD 4 are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$ significant difference between mother and virgin females; ##$p < 0.01$, +++$p < 0.001$ significant difference between two different test conditions (pup presence vs. pup absence).

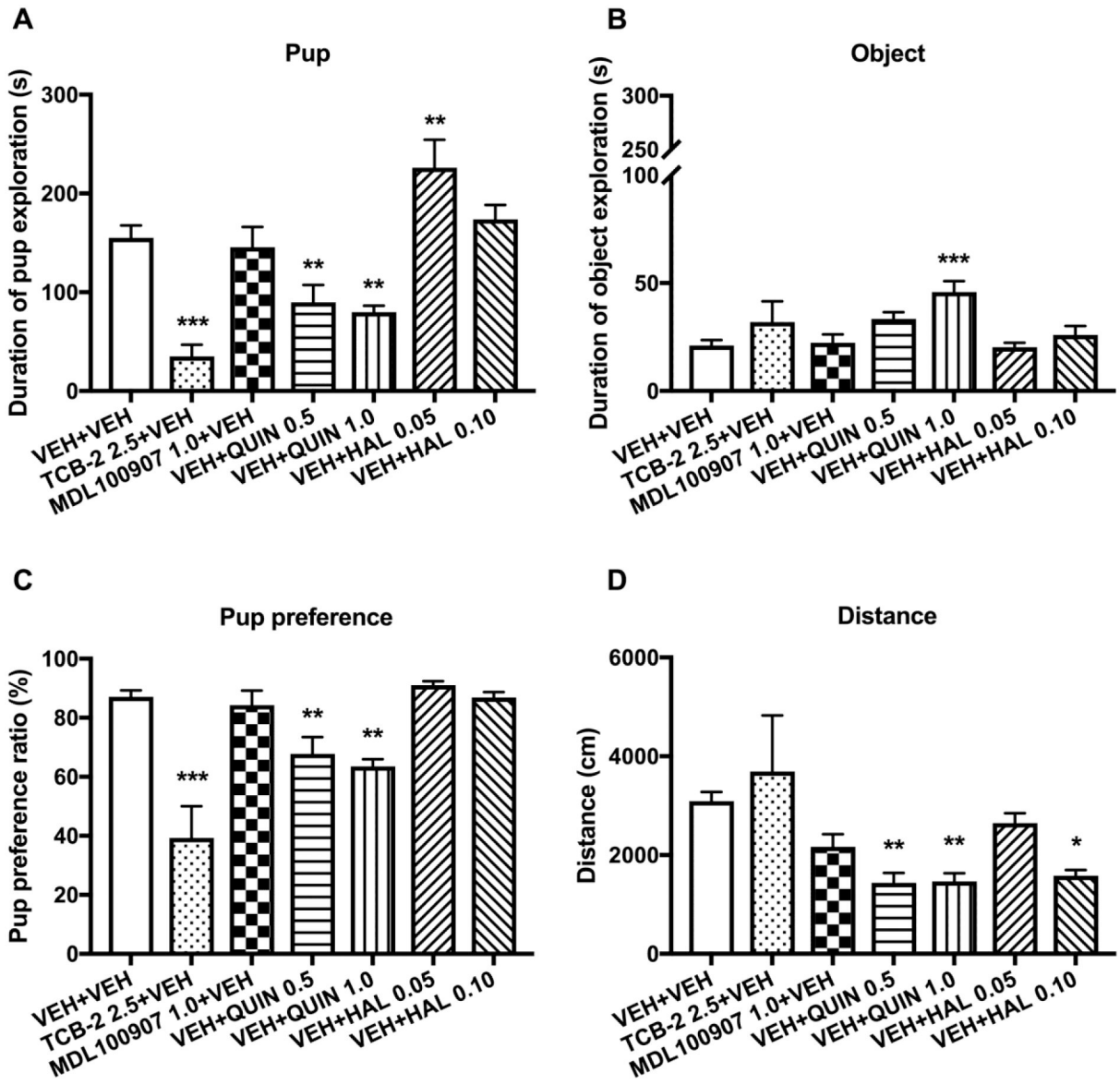

Fig. 2.

Effects of individual drugs that stimulate or block D_2 or 5-HT_{2A} receptors on pup preference. Duration of pup exploration time (A), object exploration time (B), pup preference ratio (C), and distance travelled (D) in mother rats treated with TCB-2 (2.5 mg/kg), MDL100907 (1.0 mg/kg), quinpirole (QUIN, 0.5 or 1.0 mg/kg), or haloperidol (HAL, 0.05 or 0.10 mg/kg) on PPD 4 are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ significant difference from VEH+VEH group.

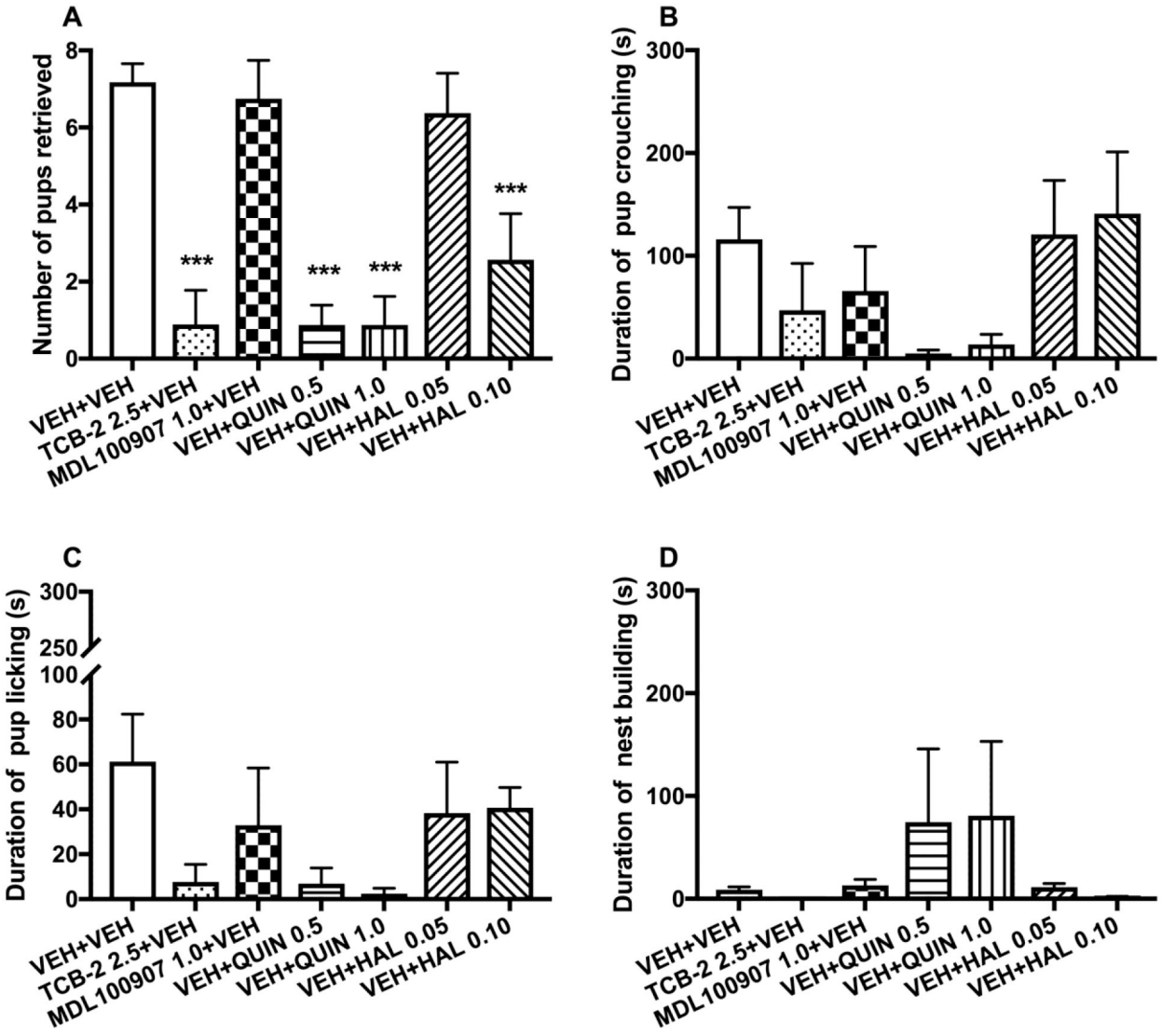

Fig. 3.

Effects of individual drugs that stimulate or block D_2 or 5-HT_{2A} receptors on home-cage maternal behavior on PPD 9. Number of pups retrieved (A), duration of pup crouching (B), duration of pup licking (C), and duration of nest building (D) in mother rats treated with TCB-2 (2.5 mg/kg), MDL100907 (1.0 mg/kg), quinpirole (QUIN, 0.5 or 1.0 mg/kg), or haloperidol (HAL, 0.05 or 0.10 mg/kg) and tested in the home cage are presented as mean ± SEM. ***$p < 0.001$ significant difference from VEH+VEH group.

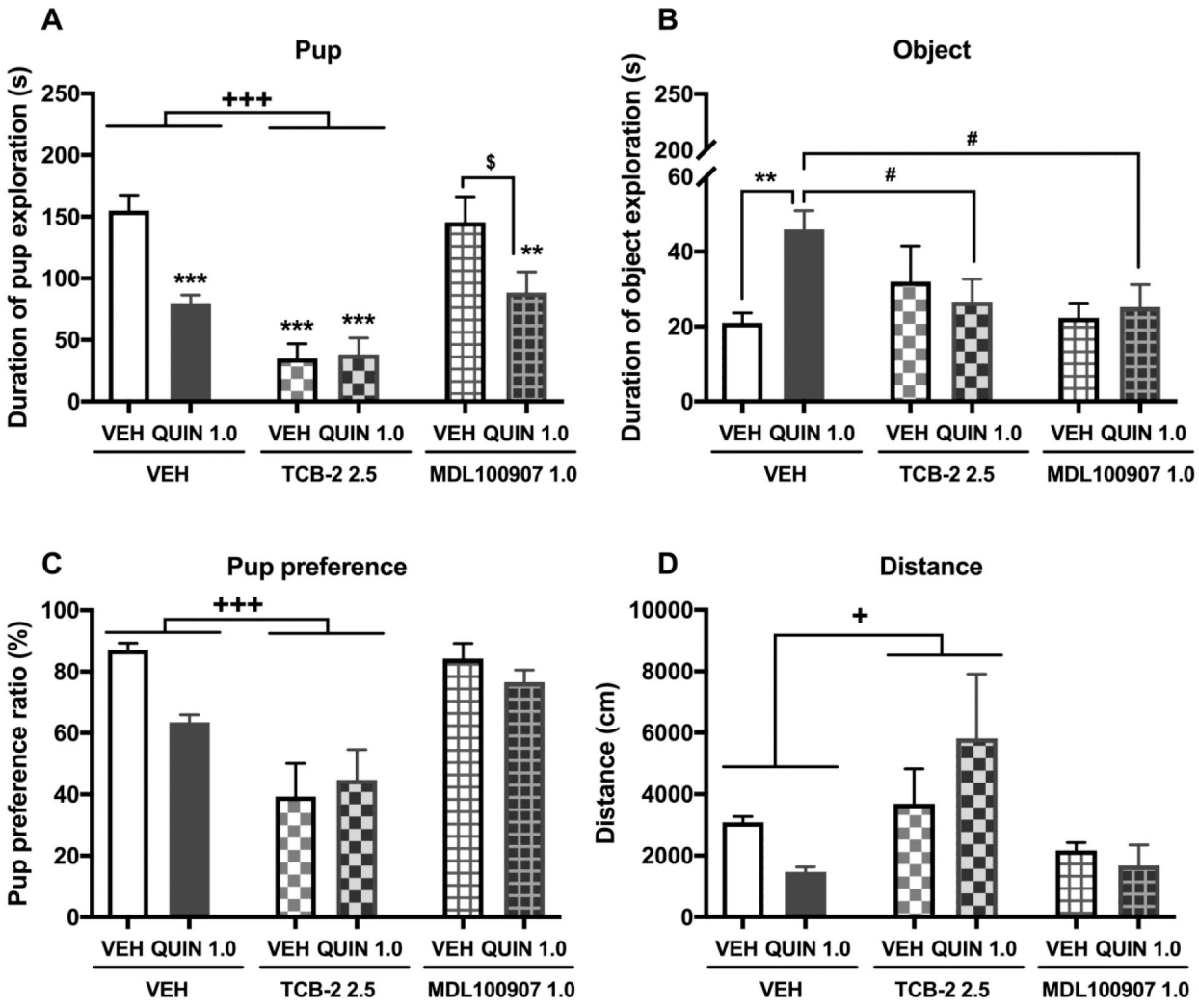

Fig. 4.

Modulation of activation or blockade of 5-HT_{2A} receptors on quinpirole's effect on pup preference. Duration of pup exploration time (A), object exploration time (B), pup preference ratio (C), and distance travelled (D) in mother rats treated with quinpirole (QUIN, 1.0 mg/kg), or quinpirole together with TCB-2 (2.5 mg/kg) or MDL100907 (1.0 mg/kg) are expressed as mean ± SEM. **$p < 0.01$, ***$p < 0.001$ significant difference from VEH+VEH group; #$p < 0.05$, ##$p < 0.01$ significant difference from VEH+QUIN 1.0 group; +++$p < 0.001$ significant difference between TCB-2 and VEH treatment.

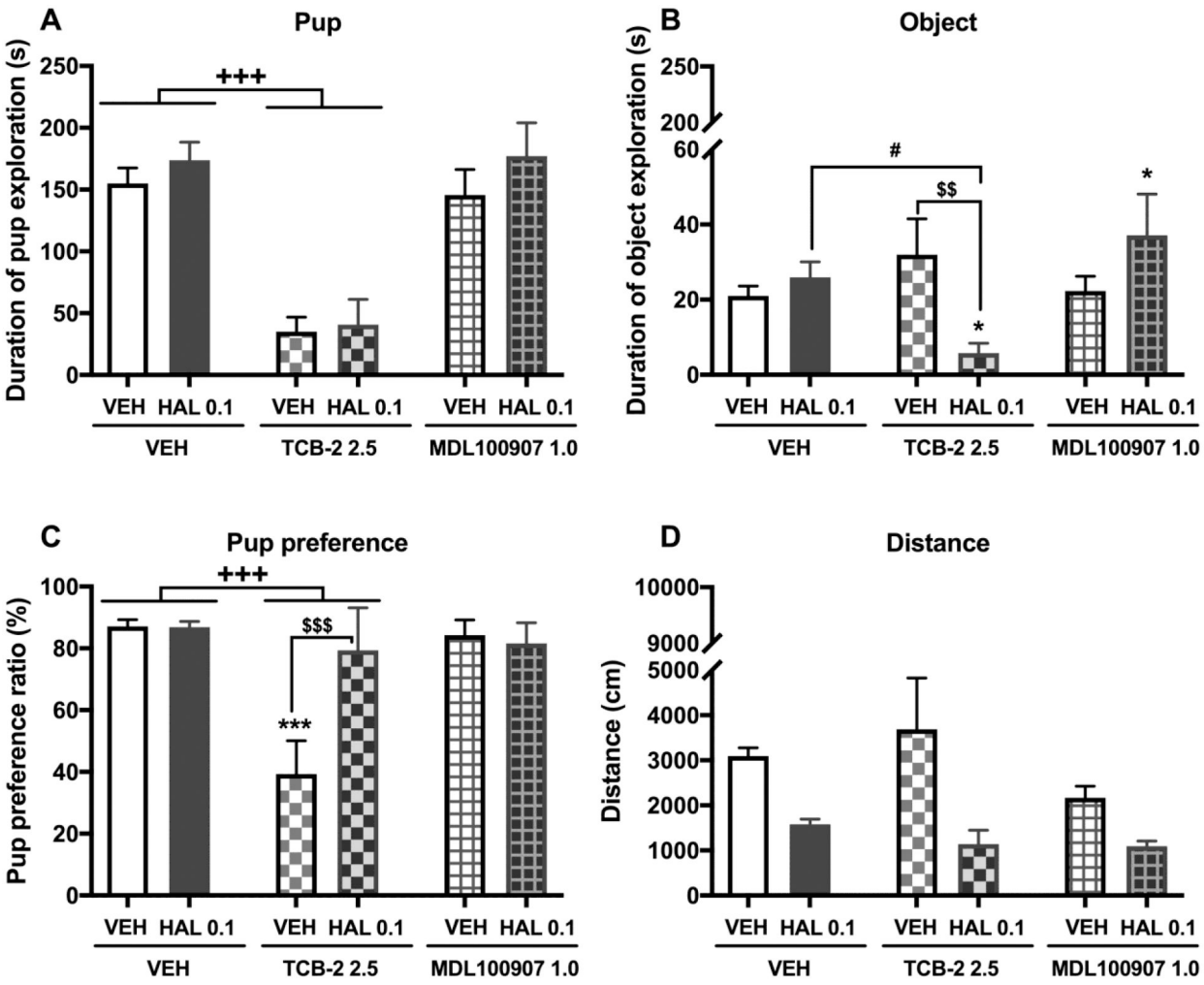

Fig. 5.

Modulation of activation or blockade of 5-HT_{2A} receptor on haloperidol's effect on pup preference. Duration of pup exploration time (A), object exploration time (B), pup preference ratio (C), and distance travelled (D) in mother rats treated with haloperidol (HAL, 0.1 mg/kg), or haloperidol together with TCB-2 (2.5 mg/kg) or MDL100907 (1.0 mg/kg) are expressed as mean ± SEM. *$p < 0.05$, ***$p < 0.001$ significant difference from VEH+VEH group; #$p < 0.05$ significant difference from VEH+HAL 0.1 group; $ $p < 0.01$, $ $ $p < 0.001$ significant difference from TCB-2 2.5+VEH group; +++$p < 0.001$ significant difference between TCB-2 and VEH treatment.

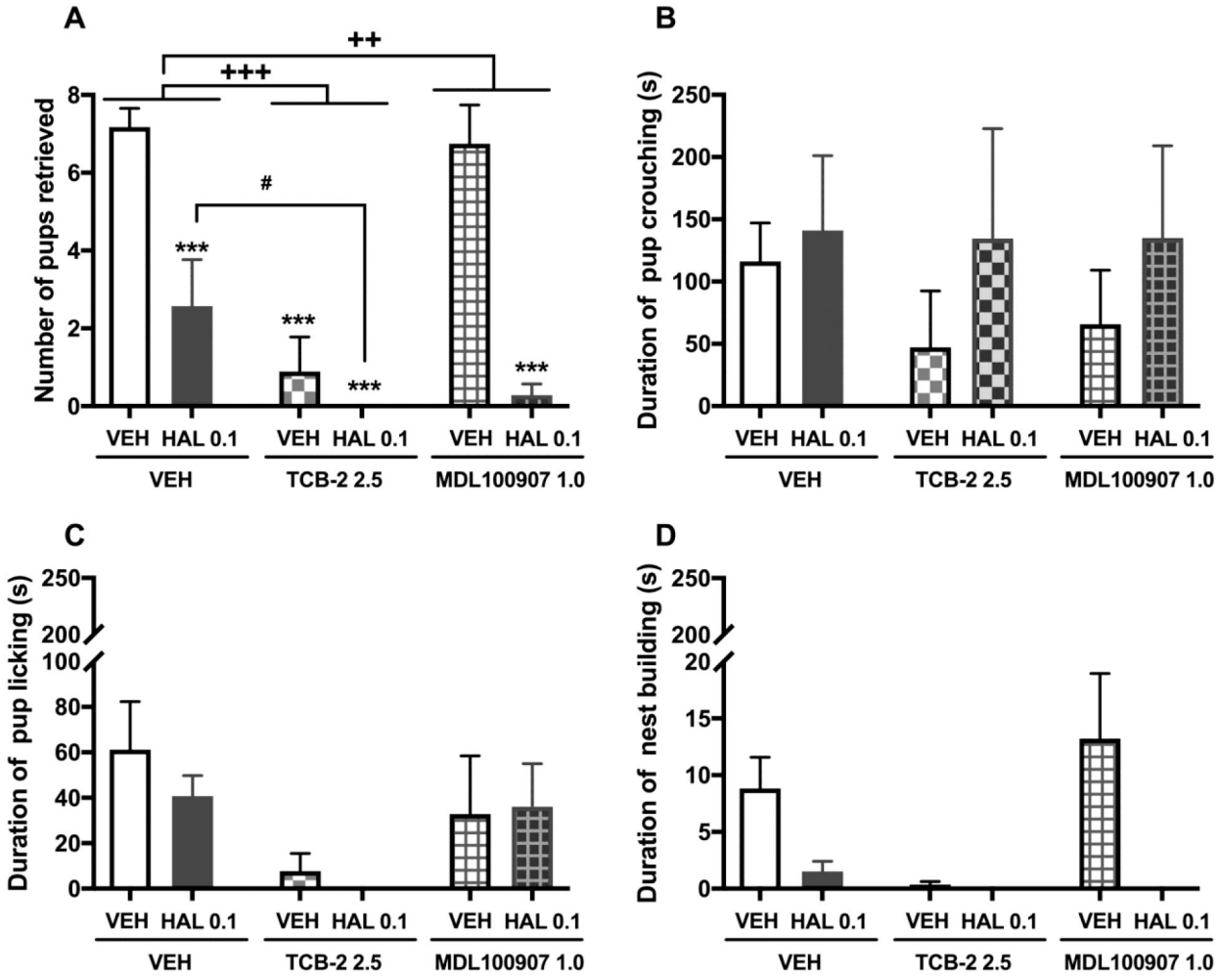

Fig. 6.

Modulation of activation or blockade of 5-HT_{2A} receptor on haloperidol's effect on home-cage maternal behavior. Number of pups retrieved (A), latency of first pup retrieval (B), latency of last pup retrieval (C), duration of pup crouching (D), duration of pup licking (E), and duration of nest building (F) in mother rats treated with haloperidol (0.1 mg/kg), or haloperidol together with TCB-2 (2.5 mg/kg) or MDL100907 (1.0 mg/kg) and tested in the home cage are presented as mean ± SEM. ***$p < 0.001$ significant difference from VEH +VEH group; #$p < 0.05$ significant difference from VEH+HAL 0.1 group; VEH+HAL 0.10 group. ++$p < 0.01$, +++$p < 0.001$ significant difference between TCB-2 or MDL100907 and VEH treatment.