2009

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Published in *Pharmacology, Biochemistry and Behavior* 93 (2009): 433-442. Copyright 2009, Elsevier. Used by permission. doi:10.1016/j.pbb.2009.06.005.

The Receptor Mechanisms Underlying the Disruptive Effects of Haloperidol and Clozapine on Rat Maternal Behavior: A Double Dissociation between Dopamine D_2 and 5-$HT_{2A/2C}$ Receptors

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Abstract

Many antipsychotic drugs disrupt active components of maternal behavior such as pup approach, pup retrieval and nest building at clinically relevant doses in postpartum female rats. However, the neurochemical mechanisms underlying such a disruptive effect remain to be determined. This study examined the neurochemical mechanisms that mediate the disruptive effects of haloperidol (a typical antipsychotic) and clozapine (an atypical antipsychotic) on rat maternal behavior. Postpartum rats were administered with haloperidol (0.2 mg/kg, sc) or clozapine (10.0 mg/kg, sc) together with either vehicle (saline or water), quinpirole (a selective dopamine D_2/D_3 agonist, 0.5 or 1.0 mg/kg, sc), or 2,5-dimethoxy-4-iodo-amphetamine (DOI, a selective 5-$HT_{2A/2C}$ agonist, 1.0 or 2.5 mg/kg, sc), and their maternal behaviors were tested at different time points before and after drug administration. Haloperidol and clozapine treatment disrupted pup approach, pup retrieval, pup licking and nest building. Pretreatment of quinpirole, but not DOI, dose-dependently reversed the haloperidol-induced disruptions. In contrast, pretreatment of DOI, but not quinpirole, dose-dependently reversed the clozapine-induced disruptions. Quinpirole pretreatment even exacerbated the clozapine-induced disruption of pup retrieval and nest building. These findings suggest a double dissociation mechanism underlying the disruption of haloperidol and clozapine on rat maternal behavior. Specifically, haloperidol disrupts maternal behavior primarily by blocking dopamine D_2 receptors, whereas clozapine exerts its disruptive effect primarily by blocking the 5-$HT_{2A/2C}$ receptors. Our findings also suggest that 5-HT receptors are involved in the mediation of rat maternal behavior.

Received 6 April 6, 2009; received in revised form June 3, 2009; accepted June 10, 2009; available online June 17, 2009.

Keywords: Haloperidol; Clozapine; Dopamine D_2/D_3 receptor; Quinpirole; 5-$HT_{2A/2C}$ receptor; DOI; Rat maternal behavior.

1. Introduction

Maternal behavior in rats is a complex behavior that shares many features with human mothering behavior (Fleming and Corter, 1988). It is naturally expressed for the first time with the birth of the first litter. Within hours of parturition, the mother rat reconstructs the nest, retrieves the displaced pups, gathers them together in the nest site and adopts a nursing posture over the pups to permit suckling (Dollinger *et al.*, 1980; Rosenblatt and Lehrman, 1963). The mother rat (dam) continues to exhibit maternal behavior (pup licking, pup retrieval, nest building and nursing) over the subsequent 3-week period, although as the pups mature, the intensity and quality of her behavior changes (Galef, 1981; Rosenblatt and Lehrman, 1963).

In recent years, we have used the rat maternal behavior as an ecologically relevant model to investigate the behavioral mechanisms of action of antipsychotic drugs and attempted to capture the possible side effects of antipsychotic medications on human parental behaviors. We and others have shown that clinically comparable doses of haloperidol (HAL), clozapine (CLZ), risperidone and quetiapine (~50%–70%, dopamine D_2 occupancy) disrupt active components of rat maternal behavior, such as pup retrieval, pup licking and nest building (Li *et al.*, 2004; Stern and Taylor, 1991; Stern and Keer, 1999; Zhao and Li, 2009). HAL causes a prolonged disruption (up to 6 h), whereas CLZ and other atypical antipsychotics induce an early onset but transient disruption (less than 6 h). Novel antipsychotics such as amisulpride and aripiprazole also exhibit a certain degree of inhibition on active maternal responses in a dose-dependent fashion (Li *et al.*, 2005a). Chronic treatment with HAL or olanzapine via minipumps or repeated daily injections significantly inhibits rat active maternal responses as well (Li *et al.*, 2005b). It seems that antipsychoticinduced inhibition on pup retrieval, pup licking and nest building may be an inherent feature of all currently available antipsychotics (Li *et al.*, 2004, 2005a; Silva *et al.*, 2001; Stern and Taylor, 1991; Stern and Keer, 1999; Zhao and Li, 2009). Our recent work further identifies that sedation and suppression of maternal motivation are two important behavioral mechanisms underlying the disruptive effects of antipsychotic treatment on maternal behavior (Zhao and Li, 2009).

The present study investigated the neurochemical basis of antipsychotic-induced disruptive effects on rat maternal behavior. For typical antipsychotics, it is generally assumed that they disrupt maternal behavior by blocking dopamine D_2 receptors because they are pri-

marily dopamine D_2 antagonists (Dragunow *et al.*, 1990; Seeman *et al.*, 1976), and because apomorphine, a dopamine receptor agonist, can reverse the inhibitory effects of haloperidol (Giordano *et al.*, 1990). However, solid evidence demonstrating that typical antipsychotics do act through this specific D_2 mechanism is still lacking. For atypical antipsychotics (*e.g.*, clozapine, olanzapine), because they generally have multiple-receptor binding profiles (Meltzer, 1989; Miyamoto *et al.*, 2005), it is hard to pinpoint their exact neurochemical mechanisms. Most atypical antipsychotics possess a much more potent antagonism on the 5-HT_2 receptor in addition to relatively weak antagonism on D_2 receptor (Meltzer *et al.*, 2003). It is thus possible that the disruptive effects of atypical antipsychotics on maternal behavior could be attributed to their action on D_2 receptor alone (Kapur and Seeman, 2001) or to its dual action on both 5-HT_2 and D_2 receptors (Meltzer *et al.*, 1989a,b) or on other receptors.

In the present study, we administered quinpirole, a selective D_2/D_3 dopaminergic receptor agonist or 2,5-dimethoxy-4-iodo-amphetamine (DOI), a selective 5-$HT_{2A/2C}$ serotonergic receptor agonist together with HAL (0.2mg/kg) or CLZ (10.0mg/kg) to postpartum-rats.We examined which of these two agonists was able to reverse the disruptive effects induced by HAL or CLZ. We hypothesized that quinpirole, but not DOI, would be able to reverse the HAL-induced disruption on maternal behavior and may also be effective in alleviating the CLZ-induced disruption to some extent. We had no prior conviction regarding the effect of DOI due to lack of sufficient evidence in the literature on the role of serotonin in maternal behavior (Numan and Insel, 2003).

2. Materials and methods

2.1. Subjects

Animals were naive pregnant female Sprague–Dawley rats (9–12 weeks old at the start of pregnancy) purchased from Charles River Inc. (Portage, MI). Upon arrival to our animal facility, the rats were between gestational days 13–15. Each subject was housed individually in 48.3 cm × 26.7 cm × 20.3 cm transparent polycarbonate cage lined with aspen shavings in a colony on a 12-h light/dark cycle (lights on at 6:30 AM). All behavioral tests were performed in the light phase between 9:00 AM and 4:00 PM. The colony was maintained under standard vivarium conditions with an ambient temperature of 23°C, relative humidity of 55–60%. Standard laboratory rat chow and water were available *ad libitum*. All animal procedures were approved by the University of Nebraska Institutional Animal Care and Use Committee.

2.2. Drugs and choices of doses

HAL (5.0 mg/ml ampoules, Sicor Pharmaceuticals, Inc, Irvine, CA) was diluted with sterile water. CLZ (a gift from NIMH drug supply program) was dissolved in 1.0% glacial acetic acid in distilled water. Quinpirole (QUI) and DOI (RBI-Sigma, Natick, MA) were dissolved in 0.9% saline. HAL (0.2 mg/kg), CLZ (10.0mg/kg), QUI (0.5 or 1.0 mg/kg), DOI (1.0 or 2.5 mg/kg) and vehicle (water or saline) were all administered subcutaneously in a volume of 1.0 ml/kg body weight. Choices of drug doses for HAL and CLZ were based on our previous studies showing that, at these doses, HAL and CLZ effectively disrupt active maternal behaviors in rats (Li *et al.*, 2004; Zhao and Li, 2009). The doses of QUI were chosen based on our preliminary study showing that 1.0mg/kg of QUI improved the HAL's disruption on pup retrieval. DOI doses were chosen on the basis of reports showing that DOI at these doses potentiates the effects of amphetamine on dopamine release in the striatum, medial prefrontal cortex and nucleus accumbens (Ichikawa and-Meltzer, 1995; Kuroki *et al.*, 2003) and blocks the CLZ-induced dopamine release in the medial prefrontal cortex (Ichikawa *et al.*, 2001).

2.3. Basic experimental procedure

Starting 2 or 3 days prior to the first possible expected parturition date, the subjects were monitored every morning for signs of parturition. Once the dam was found with pups in the morning (that day was designated as Day 1 postpartum), the mother was transferred into a clean cage with wood shavings for bedding. Two shredded paper towels were also provided for nesting material. The litter was culled to 8 pups (4 males and 4 females with the most visible milk bands). Maternal behavior tests were conducted on one day between Days 6 and 8 postpartum.

2.4. Maternal behavior test

The basic procedure was similar to what has been described in Zhao and Li (2009). Using a laptop computer with an event recording program (JWatcher, http://www.jwatcher.ucla.edu/), we recorded various components of maternal behavior in an 8-min testing session at different time points before and after drug administration. Each test started by removing the 8 pups from the dam and destroying the nest. Ten seconds later, the pups were returned to the corner of the cage diagonal to the nest site or dam sleeping corner. When the subject picked up a pup in her mouth and carried it back to the nest site, it was referred to as a successful pup retrieval. The total number of pups retrieved was recorded. Approach latency was defined as the time taken for mother rats to approach and sniff the pups from the reunion. First and last pup retrieval latency was defined as the time elapsed from the first pup approach to the retrieval of the first and eighth pup into the nest, respectively. A score of 480 s was assigned to nonresponders who did not approach or retrieve the testing pups at all.

The occurrence of other behaviors was also recorded, including pup nursing behavior (a rat positioning herself over the pups with legs splayed to accommodate the pups, including hover, high and low crouching over postures), pup licking (a female rat placing its tongue on the anogenital area and the rest of a pup's body), nest building (a rat picking up nesting material in her mouth and transporting it back to the nest site or pushing the material with her forepaws toward the nest site). At the conclusion of the test, any unretrieved pups were returned to the nest site.

2.4.1. Experiment 1. Effects of pretreatment with quinpirole or DOI on haloperidol-induced maternal behavior deficits in rats

Forty-four postpartum rats were randomly assigned to one of the following six groups: VEH+VEH (n=4, sterile water or saline), VEH+HAL (n = 8), QUI-0.5+HAL (n = 8), QUI-1.0+HAL (n = 8), DOI-1.0+HAL (n = 8), and DOI-2.5+HAL (n = 8). On the drug test day (1 day on either Postpartum Day 6, 7, or 8), all subjects were tested 5 times at different time points before and after drug administrations, with the first maternal behavior test starting at 30 min prior to the HAL or vehicle injection (i.e., baseline) and the other tests occurring at 30, 60, 120 and 240min after HAL or vehicle injection. QUI, DOI or vehicle was injected s.c. twice with the first injection at 10 min before and the second at 50 min after the HAL or vehicle injection.We adopted such an injection strategy because our preliminary data showed that injection of QUI at 10 min prior to the administration of HAL could optimally reverse its disruption on pup retrieval. Also, agonists were injected twice separately on the basis of their relatively short duration of action and rapid peak plasma concentrations (Deutch and Duman, 1996; Whitaker and Lindstrom, 1987).

2.4.2. Experiment 2. Effects of pretreatment with quinpirole or DOI on clozapine-induced maternal behavior deficits in rats

The basic procedure was identical to that of Experiment 1 with the exception that CLZ was tested in this experiment instead of haloperidol. Forty-four postpartum rats were randomly assigned to one of the following six groups and their maternal behaviors were tested: VEH+VEH (n = 4, sterile water or saline), VEH+CLZ (n = 8), QUI-0.5+

CLZ (n = 8), QUI-1.0+CLZ (n = 8), DOI-1.0+CLZ (n = 8), and DOI-2.5+CLZ (n = 8).

2.5. Data analysis

With the exception of latency data, various measurements of maternal behavior were expressed as mean ± SEM. To increase statistical power, we combined the two vehicle control groups from Experiments 1 and 2 into a single group since they did not differ significantly on any measure (Independent-Samples T test or Mann–Whitney U test, all ps > 0.10). Data were analyzed using a factorial repeated measures analysis of variance (ANOVA) with the between-subjects factor being the treatment groups (*e.g.* saline, HAL) and the within-subjects factor being test time points (baseline, 30, 60, 120 and 240 min post-injection). Group differences at different time points were further analyzed using simple main effect test (one-way ANOVA) followed by Turkey's HSD post hoc tests where appropriate.

For the latency data, because they were not normally distributed (*e.g.* the cut-off time assigned 480 s for the latency data), these data were expressed as median ± interquartile range. Nonparametric Kruskal–Wallis test was used for analyzing the differences among the treatment groups. Once the overall significant effects were determined, two-group comparisons were performed using Mann–Whitney U test. A conventional two-tailed level of significance at the 0.05 level was required.

3. Results

3.1. Experiment 1. Effects of pretreatment with QUI or DOI on haloperidol-induced maternal behavior deficits

3.1.1. Haloperidol treatment disrupted various components of maternal behavior

Consistent with our previous findings (Li *et al.*, 2004; Zhao and Li, 2009), a single injection of HAL significantly disrupted various active components of maternal behavior (pup retrieval, pup licking, nest building, but not pup nursing). Rats treated with HAL took a much longer time to initiate contact with pups and retrieve their pups into the nest sites (Table 1) and retrieved fewer pups at the 30, 60, 120 and 240 min test points (Figures 1, 2A) (0.001 < all ps < 0.05) compared to the vehicle-treated rats. They also spent less time licking their pups at the 30, 60 and 120 min test points and rebuilding the nest at the 30, 60, 120 and 240 min test points (Figures 1, 2B, C) (0.001 < all ps < 0.05). However, HAL treatment had no significant effect on nursing activity (Figures 1, 2D).

3.1.2. QUI pretreatment dose-dependently improved the haloperidol-induced maternal behavior deficits

As can be seen in Table 1 and Figure1A–C, both doses of QUI improved the HAL-induced deficits in pup approach, pup retrieval, pup licking and nest building, as described below.

3.1.2.1. Latency to approach and retrieve pups. QUI shortened the HAL-induced increase in pup approach latency and the first and last pup retrieval latencies (Table 1). Both 0.5 and 1.0 mg/kg doses of QUI were effective in facilitating the HAL-treated dams to initiate contact with their pups and completely restored such behavior to the normal level (*e.g.*, VEH+VEH group) across the testing period. For the pup retrieval latency, it appeared that both doses of QUI shortened the time the HAL-treated rats took to retrieve their first and last pups to the nest site and reinstated the first pup retrieval to the normal level at the 30, 120 and 240 min test points post-injection.

3.1.2.2. Number of pups retrieved. Both 0.5 and 1.0 mg/kg of QUI increased the number of pups retrieved in the HAL-treated rats and restored it to the normal level at certain time points of testing. Repeated measures ANOVA revealed that there was a significant interaction between the treatment groups and time points [$F_{(20, 168)}$ = 5.97, p < 0.001], a significant main effect of treatment groups [$F_{(5, 42)}$ = 26.30, p < 0.001], and a significant effect of time points [$F_{(4, 168)}$ = 35.22, p < 0.001]. One-way ANOVA and post hoc tests showed that the QUI-0.5+HAL group was significantly different from the VEH+HAL group at the 30 and 120 min time points (Figure 1A, p < 0.001 for 30 min, p = 0.022 for 120 min), whereas the QUI-1.0+HAL group differed from the VEH+HAL group at the 30, 120 and 240 min test points (Figure 1A, p < 0.001 for 30 and 120 min, p = 0.014 for 240 min). Further analysis showed that the reversing effect of 0.5mg/kg of QUI reached the control level at the 30 and 120 min test points while the 1.0 mg/kg of QUI restored the CLZ-induced disruption to the normal level at the 30, 120 and 240 min test pointswhen the two QUI+HAL groupswere compared with the VEH+VEH control group (Figure 1A, all ps > 0.10). It appears that the strongest reversal effect of QUI occurred 120 min after HAL administration (Figure 1A).

3.1.2.3. Pup licking. QUI produced a reversing effect on HAL-induced pup licking deficit. Repeated measures ANOVA revealed a main effect of treatment groups [$F_{(5, 42)}$ = 4.56, p = 0.002], a significant effect of test time points [$F_{(4, 168)}$ = 7.71, p < 0.001], but no significant interaction between the two factors. One-way ANOVA and post hoc tests showed that both QUI groups differed significantly fromthe VEH+HAL group at the 120 min test point (Figure 1B, p = 0.045 for QUI-0.5+HAL group, p = 0.042 for QUI-1.0+HAL group). Further

Table 1
Effects of pretreatment with quinpirole or DOI on pup approach latency and pup retrieval latency in postpartum female rats treated with haloperidol or vehicle.

Groups	N	Approach latency (s)					First pup retrieval latency (s)					Last pup retrieval latency (s)				
		Baseline	30 min	60 min	120 min	240 min	Baseline	30 min	60 min	120 min	240 min	Baseline	30 min	60 min	120 min	240 min
VEH+VEH	8	2.1 (2.0)	1.2 (0.7)	1.5 (1.2)	1.5 (0.8)	1.9 (4.6)	2.8 (87.0)	2.0 (0.9)	2.6 (1.4)	2.1 (0.7)	1.9 (0.5)	38.3 (104.0)	22.6 (9.7)	23.9 (7.7)	21.7 (9.8)	24.0 (14.4)
VEH+HAL	8	1.8 (0.9)	15.6[a] (106.9)	87.5[a] (311.7)	45.4[a] (224.6)	23.2[a] (135.1)	11.7 (100.8)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)	245.6[a] (476.1)	112.4 (382.9)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (272.7)
QUI-0.5+HAL	8	1.4 (0.8)	2.2[b] (15.1)	3.4[b] (26.9)	2.6[b] (5.6)	8.8[b] (41.6)	47.4 (89.6)	4.0[b] (35.7)	480.0[a] (357.8)	3.3[b] (358.2)	3.3[b] (358.4)	141.0 (289.1)	75.9[a,b] (55.9)	480.0[a] (241.2)	285.4[a] (443.6)	74.0[a,b] (352.6)
QUI-1.0+HAL	8	2.8 (4.1)	2.8[b] (7.4)	12.5[b] (78.1)	7.7[b] (24.5)	5.6[b] (14.2)	30.7 (84.1)	4.3[b] (12.0)	247.1[a] (474.9)	3.1[b] (2.0)	2.4[b] (0.7)	152.3 (260.0)	105.7[a,b] (417.8)	480.0[a] (284.9)	145.9[a,b] (104.0)	67.1[a,b] (45.5)
DOI-1.0+HAL	8	1.3 (0.7)	13.1[a] (29.5)	115.7[a] (122.6)	43.6[a] (52.2)	30.6[a] (361.4)	2.8 (189.40	293.6[a] (441.1)	480.0[a] (0.0)	480.0[a] (0.0)	65.2[a] (476.8)	179.7 (450.6)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (356.1)
DOI-2.5+HAL	8	2.0 (5.9)	49.3[a] (107.8)	480.0[a,b] (169.4)	278.6[a] (446.2)	81.9[a] (216.1)	3.1 (15.3)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)	69.6 (59.2)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)

Data are represented as median ± interquartile range.
[a] p<0.05 relative to the VEH+VEH group.
[b] p<0.05 relative to the VEH+HAL group.

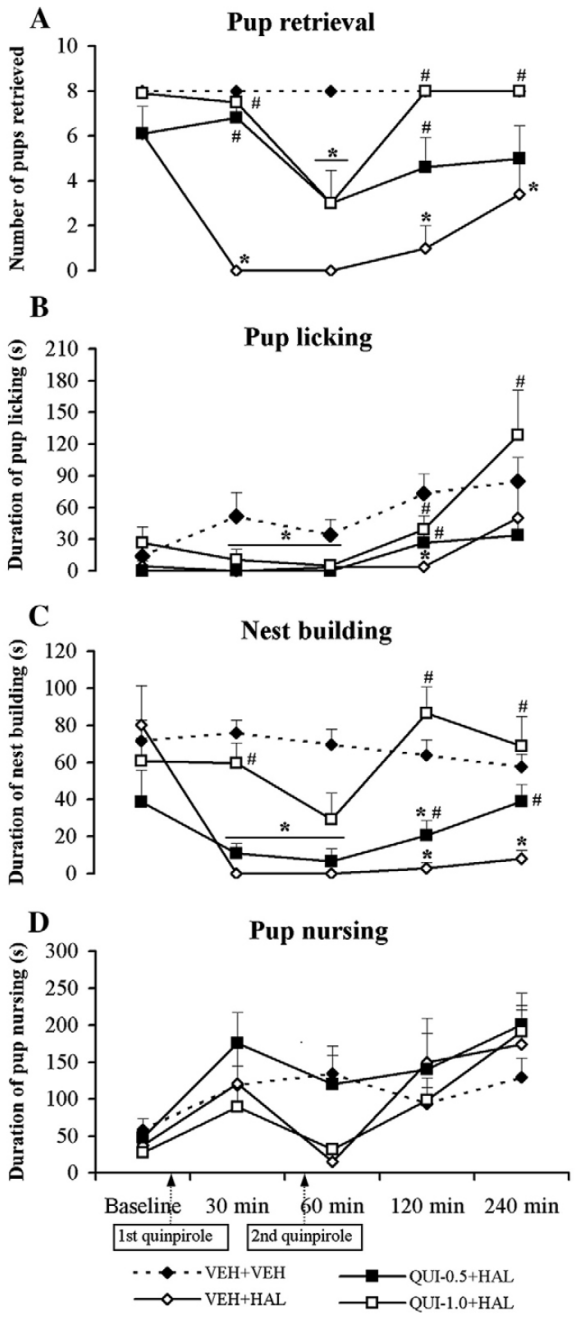

Fig. 1. The time course of effects of the dopamine D_2/D_3 receptor agonist quinpirole on various HAL-induced maternal behavior deficits in postpartum female rats. Pup retrieval (A), pup licking (B), nest building (C) and pup nursing (D) were tested at baseline, 30, 60, 120 and 240 min after injection of HAL or vehicle. HAL disrupted all the active maternal behaviors tested (A–C), but had no significant effect on pup nursing (D). Both 0.5 and 1.0 mg/kg of quinpirole improved the maternal behavior deficits induced by HAL in a dose-dependent fashion (A–C). Data were represented as mean + SEM. $^{*}p<0.05$ *versus* VEH+VEH control; $^{\#}p<0.05$ *versus* VEH+HAL group.

analysis showed that the reversing effect reached the normal level when the two QUI+HAL groups were compared to the VEH+VEH control group (Figure1B, $p = 0.85$ for QUI-0.5+HAL group, $p = 0.95$ for QUI-1.0+HAL group).

3.1.2.4. Nest building. Similar to the effect on pup retrieval and pup licking, both doses of QUI significantly improved the HAL-induced disruption of nest building activity. Repeatedmeasures ANOVA revealed a significant interaction between the two factors [$F_{(20, 168)} = 6.18$, $p < 0.001$], a significant main effect of treatment groups [$F_{(5, 42)} = 17.52$, $p < 0.001$], and a significant effect of testing time [$F_{(4, 168)} = 35.95$, $p < 0.001$]. One-way ANOVA and post hoc tests showed that the QUI-0.5+HAL group was significantly different from the VEH+HAL group at the 120 and 240 min test points (both ps = 0.021), whereas the QUI-1.0+HAL group differed significantly from the VEH+HAL group at the 30, 120 and 240 min test points post-injection (Figure 1C, all ps < 0.001). In particular, although both doses of QUI reversed

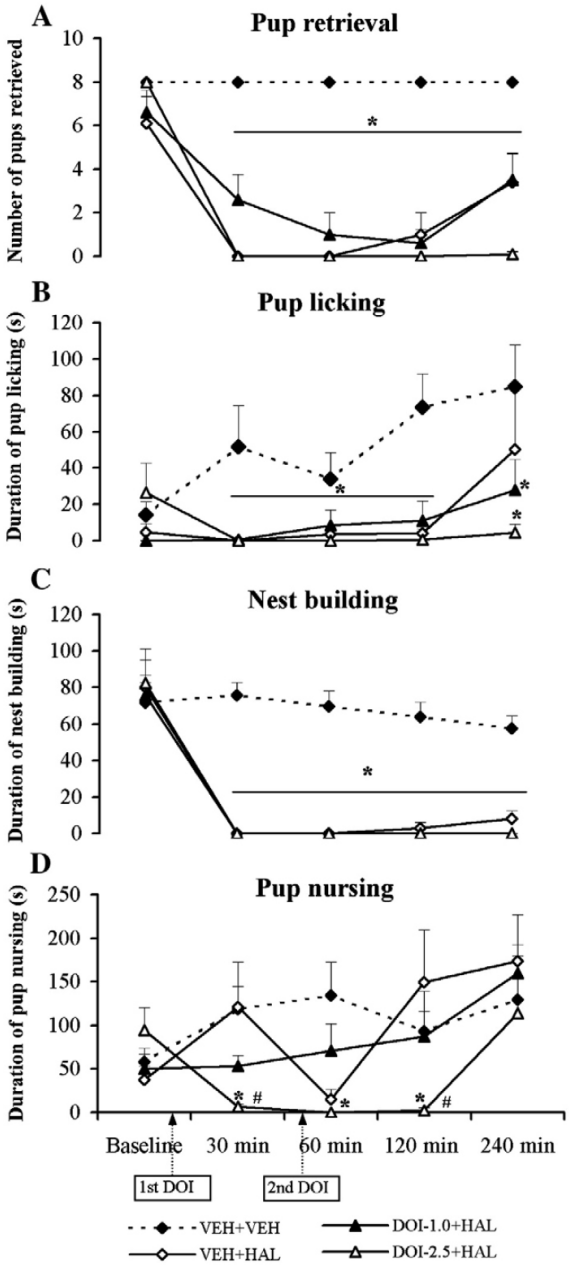

Fig. 2. The time course of effects of the 5-$HT_{2A/2C}$ receptor agonist DOI on various HAL-induced maternal behavior deficits in postpartum female rats. Pup retrieval (A), pup licking (B), nest building (C) and pup nursing (D) were tested at baseline, 30, 60, 120 and 240 min after injection of HAL or vehicle. HAL disrupted all the active maternal behaviors tested (A–C), but had no significant effect on pup nursing (D). Neither 1.0 nor 2.5 mg/kg of DOI rescued the HAL-induced deficits on active maternal behaviors (A–C), but 2.5 mg/kg of DOI further suppressed nursing activity in HAL-treated dams (D). Data were represented as mean + SEM. $^{*}p<0.05$ *versus* VEH+VEH control; $^{\#}p<0.05$ *versus* VEH+HAL group.

the disruptive effect of HAL at the 120 min test point, only 1.0 mg/kg of QUI was capable of improving it to the normal level when compared to the VEH+VEH group (Figure 1C, $p = 0.34$).

3.1.2.5. Pup nursing. As can be seen in Figure1D,QUI pretreatment had no significant effect on pup nursing. Repeatedmeasures ANOVA revealed a significant interaction between the two factors [$F_{(20, 168)} = 2.14$, $p = 0.005$], a significant main effect of treatment groups [$F_{(5, 42)} = 4.66$, $p = 0.002$], and a significant effect of testing time [$F_{(4, 168)} = 10.53$, $p < 0.001$]. Post hoc tests showed that both QUI groups were not significantly different from the VEH+HAL group (both ps > 0.10).

3.1.3. DOI pretreatment failed to improve the haloperidol-induced maternal behavior deficits

Both 1.0 and 2.5 mg/kg of DOI failed to improve the HAL-induced disruption on pup approach and pup retrieval, as evidenced by no significant improvement in the approach latency nor in first and last pup retrieval latency (Table 1) when the two DOI+HAL groups were compared to the VEH+HAL group. Rather, 2.5 mg/kg of DOI further exacerbated the HAL-induced disruption on the approach latency at the 60 min test point (Table 1, $p = 0.005$). Post hoc tests on the number of pups retrieved, pup licking and nest building revealed that there was no significant difference between the two DOI+HAL groups and the VEH+HAL group. For pup nursing, one-way ANOVA and post hoc tests indicated that the DOI-2.5+HAL group showed an even more suppressed nursing duration at the 30 and 120 min test points compared to the VEH+HAL group ($p = 0.048$ for 30 min, $p = 0.041$ for 120 min) (Figure 2D). The DOI-1.0+HAL group did not differ significantly from the VEH+HAL group across the testing period.

3.2. Experiment 2. Effects of pretreatment with QUI or DOI on clozapine-induced maternal behavior deficits

3.2.1. Clozapine treatment impaired various components of maternal behavior

Consistent with our previous findings (Li *et al.*, 2004; Zhao and Li, 2009), the present study showed that a single injection of CLZ significantly disrupted various active components of maternal behavior and maintained such a disruptive effect for about 2 h. In comparison with the vehicle-treated ones, rats treated with CLZ took a much longer time to approach and retrieve their first pups into the nest sites (Table 2) at the 30, 60 and 120 min test points (0.001 < all ps < 0.01) and took longer to bring the last pups into the nest with less pups retrieved across the testing period (Table 2, Figures 3, 4A) (0.001 < all ps < 0.05). They also spent less time on pup licking, nest building and pup nursing at the 30, 60 and 120 min test points (Figures 3, 4B, C, D) (all ps < 0.05).

3.2.2. QUI pretreatment failed to improve the clozapine-induced maternal behavior deficits

In contrast to its effects on HAL-induced maternal behavior deficits, both doses of QUI had little effect on the CLZ-induced disruptions of pup approach, pup retrieval latency (Table 2), the number of pups retrieved and nest building (Figure 3A,C). Contrarily, QUI further potentiated the disruptive effect on these behaviors (Table 2, Figure 3A,C). Repeated measures ANOVA on the number of pups retrieved and nest building revealed a significant interaction between the treatment groups and test time points [the number of pups retrieved: $F_{(20, 168)} = 9.56$, $p < 0.001$; nest building: $F_{(20, 168)} = 2.77$, $p < 0.001$], a significant main effect of treatment groups [the number of pups retrieved: $F_{(5, 42)} = 20.41$, $p < 0.001$; nest building: $F_{(5, 42)} = 13.61$, $p < 0.001$], and a significant effect of test time points [the number of pups retrieved: $F_{(4, 168)} = 61.43$, $p < 0.001$; nest building: $F_{(4, 168)} = 38.33$, $p < 0.001$]. One-way ANOVA and post hoc tests showed that the QUI-1.0+CLZ group differed significantly from the VEH+CLZ group in the number of pups retrieved and the duration of nest building at the 240 min test point (Figure 3A, C, $p = 0.001$ for the number of pups retrieved, $p = 0.007$ for the nest building), while the QUI-0.5+CLZ group differed from the VEH+CLZ group in nest building at the 240 min test point (Figure 3C, $p = 0.026$). QUI pretreatment also did not improve the CLZ-induced deficits in pup licking and pup nursing activities (Figure 3B,D) at any test points. Repeated measures ANOVA on pup licking and pup nursing revealed a significant interaction between the two factors [pup licking: $F_{(20, 168)} = 1.68$, $p = 0.04$; pup nursing: $F_{(20, 168)} = 2.05$, $p = 0.007$], a significant main effect of treatment groups [pup licking: $F_{(5, 42)} = 4.47$, $p = 0.002$; pup nursing: $F_{(5, 42)} = 5.52$, $p = 0.001$], and a significant effect of test time points [pup licking: $F_{(4, 168)} = 8.02$, $p < 0.001$; pup nursing: $F_{(4, 168)} = 6.72$, $p < 0.001$]. Post hoc tests on pup licking and pup nursing revealed that there was no significant difference between the two QUI+CLZ groups and the VEH+CLZ group.

3.2.3. DOI pretreatment dose-dependently reversed the clozapine-induced maternal behavior deficits

As shown in Table 2 and Figure 4A,B, pretreatment of both doses of DOI (1.0 and 2.5 mg/kg) significantly improved the CLZ-induced disruption on pup approach, pup retrieval, and pup licking. However, the reversing effect of DOI on CLZ-induced disruption of nest building and pup nursing was not apparent (Figure 4C, D).

3.2.3.1. Latency to approach and retrieve pups. As shown in Table 2, both 1.0 and 2.5 mg/kg of DOI shortened the prolonged approach latency induced by CLZ when the two DOI+CLZ groups were compared to the VEH+CLZ group and restored it to the normal level compared to the VEH+VEH group at the 30, 60 and 120 min test points (0.001 < all ps < 0.01). It also reduced the CLZ-induced deficits of the first pup

Table 2
Effects of pretreatment with quinpirole or DOI on pup approach latency and pup retrieval latency in postpartum female rats treated with clozapine or vehicle.

Groups	N	Approach latency (s)					First pup retrieval latency (s)					Last pup retrieval latency (s)				
		Baseline	30 min	60 min	120 min	240 min	Baseline	30 min	60 min	120 min	240 min	Baseline	30 min	60 min	120 min	240 min
VEH+VEH	8	2.1 (2.0)	1.2 (0.7)	1.5 (1.2)	1.5 (0.8)	1.9 (4.6)	2.8 (87.0)	2.0 (0.9)	2.6 (1.4)	2.1 (0.7)	1.9 (0.5)	38.3 (104.0)	22.6 (9.7)	23.9 (7.7)	21.7 (9.8)	24.0 (14.4)
VEH+CLZ	8	1.8 (1.7)	68.5[a] (357.3)	290.8[a] (474.6)	21.8[a] (477.2)	10.2 (43.7)	5.9 (10.4)	480.0[a] (357.1)	480.0[a] (0.0)	480.0[a] (346.9)	13.0 (154.8)	40.8 (130.8)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)	115.4[a] (420.6)
QUI-0.5+CLZ	8	8.7 (16.2)	177.2[a] (473.2)	302.9[a] (330.3)	268.2[a] (432.7)	3.7 (7.6)	14.3 (20.6)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a,b] (339.1)	65.7 (146.3)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] $(0.0)	480.0[a] (0.0)
QUI-1.0+CLZ	8	5.3 (9.1)	159.9[a] (460.3)	129.0[a] (240.4)	319.3[a] (464.0)	401.4[a,b] (442.5)	10.4 (75.1)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a,b] (0.0)	143.4 (408.2)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)	480.0[a] (0.0)
DOI-1.0+CLZ	8	2.2 (1.1)	6.9[b] (38.4)	1.8[b] (24.5)	1.7[b] (1.2)	2.2 (5.3)	7.7 (168.0)	8.0[a] (363.1)	2.8[b] (358.7)	3.4 (441.4)	3.2 (360.7)	107.3 (442.8)	391.4[a] (440.3)	58.3[b] (453.3)	214.7[b] (452.0)	44.8 (456.8)
DOI-2.5+CLZ	8	1.6 (2.6)	1.8[b] (0.4)	1.3[b] (7.2)	1.3[b] (34.6)	1.8 (4.9)	2.7 (1.7)	21.8[a] (371.5)	2.5[b] (63.4)	2.4[b] (34.8)	2.2 (3.8)	37.13 (32.0)	480.0[a] (396.9)	56.93[b] (372.2)	45.13[b] (104.7)	34.4[b] (54.7)

Data are represented as median ± interquartile range.
[a] $p<0.05$ relative to the VEH+VEH group.
[b] $p<0.05$ relative to the VEH+CLZ group.

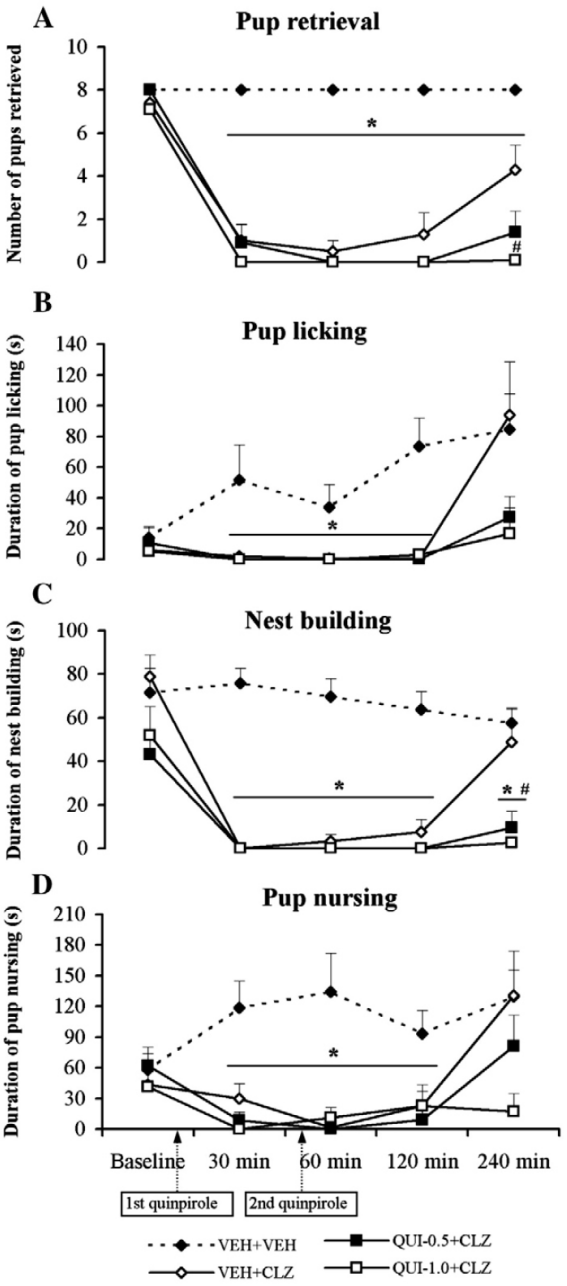

Fig. 3. The time course of effects of the dopamine D_2/D_3 receptor agonist quinpirole on various CLZ-induced maternal behavior deficits in postpartum female rats. Pup retrieval (A), pup licking (B), nest building (C) and pup nursing (D) were tested at baseline, 30, 60, 120 and 240 min after injection of CLZ or vehicle. CLZ disrupted all maternal behaviors tested (A–D). Both doses of quinpirole (0.5 and 1.0 mg/kg) failed to improve the maternal behavior deficits induced by CLZ (A–D), but rather worsened pup retrieval (A) and nest building (C) deficits. Data were represented as mean + SEM. $^{*}p<0.05$ *versus* VEH+VEH control; $^{\#}p<0.05$ *versus* VEH+CLZ group.

retrieval and restored it to the normal level at the 60 min test point ($p = 0.035$) at a dose of 1.0 mg/kg as well as at the 60 and 120 min test points (both ps < 0.01) at a dose of 2.5 mg/kg. For the last pup retrieval latency, both doses of DOI completely rescued the disruption at the 60 and 120 min (0.001 < all ps < 0.01) test points. Furthermore, 2.5 mg/kg of DOI shortened the last pup retrieval latency at the 240 min test point ($p = 0.001$).

3.2.3.2. Number of pups retrieved. Both doses of DOI increased the number of pups retrieved in the CLZ-treated rats. One-way ANOVA and post hoc tests showed that there was a significant difference between the DOI-1.0+CLZ group and the VEH+CLZ group at the 60 and 120 min test points ($p = 0.006$ for 60 min, $p = 0.008$ for 120 min), whereas

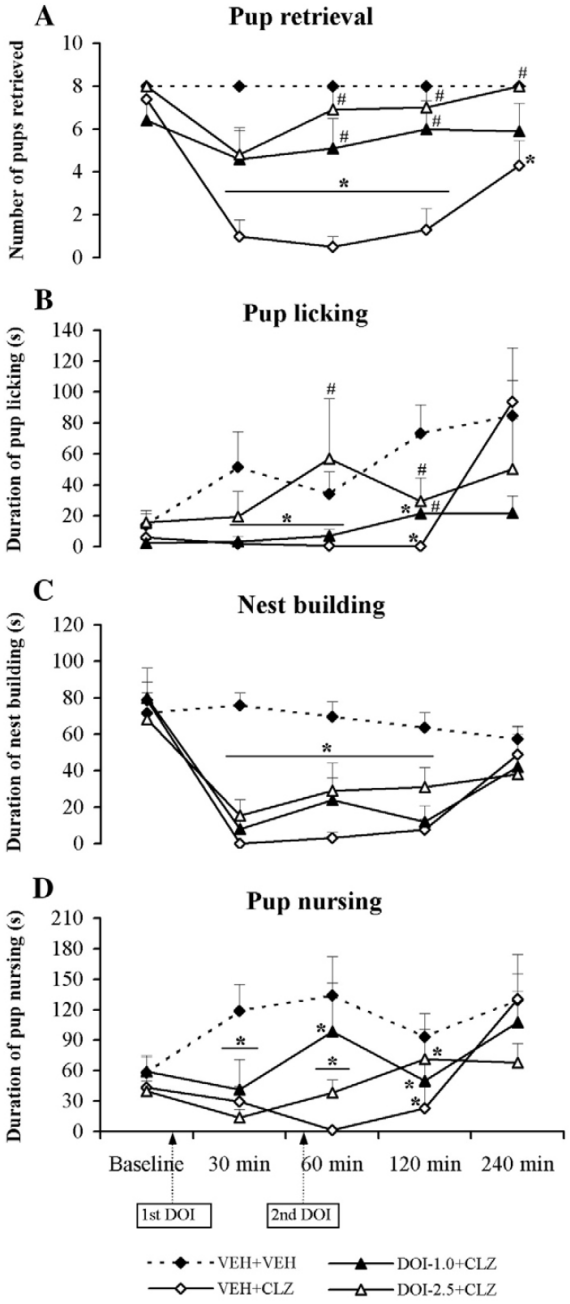

Fig. 4. The time course of effects of the 5-$HT_{2A/2C}$ receptor agonist DOI on various CLZ-induced maternal behavior deficits in postpartum female rats. Pup retrieval (A), pup licking (B), nest building (C) and pup nursing (D) were tested at baseline, 30, 60, 120 and 240 min after injection of CLZ or vehicle. CLZ disrupted all maternal behaviors tested (A–D). Both doses of DOI (1.0 and 2.5 mg/kg) significantly improved the pup retrieval and pup licking deficits induced by CLZ (A, B). However, DOI did not improve the CLZ-induced disruption on nest building and pup nursing (C, D). Data were represented as mean + SEM. $^{*}p<0.05$ *versus* VEH+VEH control; $^{\#}p<0.05$ *versus* VEH+CLZ group.

the DOI-2.5+CLZ group differed significantly from the VEH+CLZ group even at the 240 min test point (Figure 4A, 0.001 < all *p*s < 0.05). Further analysis showed that the reversing effect of 1.0 mg/kg of DOI reached the normal level at the 60 and 120 min test points while the 2.5 mg/kg of DOI restored the CLZ-induced disruption to the normal level at 60, 120 and 240 min test points (Figure 4A, all *p*s > 0.05). It appears that the strongest reversal effect of DOI occurred 120 min after CLZ administration (Figure 4A).

3.2.3.3. Pup licking. Both doses of DOI alleviated the CLZ-induced disruption on pup licking. One-way ANOVA and post hoc tests showed that there was a significant difference between the DOI-1.0+CLZ group and the VEH+CLZ group at the 120 min test point ($p = 0.049$), while the DOI-2.5+CLZ group differed significantly from the VEH+CLZ group at the 60 and 120 min test points (Figure 4B, $p = 0.034$ for 60 min, $p = 0.015$ for 60 min). Further analysis showed that only 2.5 mg/kg of DOI restored this behavior to the normal level at the 60 and 120 min test points when the two DOI+CLZ groups were compared with the VEH+VEH control group (Figure 4B, both *p*s > 0.05).3.2.3.4. Nest building and pup nursing. Compared to the reversing effects on pup retrieval and pup licking, DOI at the currently tested doses appeared to be ineffective in reversing the CLZ-induced deficits on nest building and pup nursing. Post hoc tests on nest building and pup nursing revealed that therewas no significant difference between the two DOI+CLZ groups and the VEH+CLZ group (Figure 4C, D, all *p*s > 0.05).

4. Discussion

The present study demonstrates an interesting double dissociation between dopamine and serotonin receptor mechanisms in the mediation of HAL- and CLZ-induced maternal behavior deficits in postpartum rats. Acute treatment of HAL and CLZ disrupted various components of rat maternal behavior. Pretreatment of QUI, a selective D_2/D_3 dopaminergic receptor agonist, but not DOI, a selective 5-$HT_{2A/2C}$ serotonergic receptor agonist, dose-dependently improved HAL-induced disruption of pup approach, pup retrieval, pup licking and nest building, whereas pretreatment of DOI, but not QUI, dose-dependently improved CLZ-induced disruptionof pup approach, pup retrieval and pup licking. These data strongly suggest that the HAL-induced maternal deficits are primarily mediated by its blockade of D_2 dopamine receptors, whereas the CLZ-induced maternal deficits are primarily mediated by the blockade of 5-$HT_{2A/2C}$ receptors.

The dopamine systems have been well documented to play an important role in the regulation of maternal behavior. For example, systemic (*e.g.*, haloperidol, a D_2 antagonist) or microinfusion of dopamine receptor antagonists such as SCH-23390 (a D_1 antagonist), pimozide (a D_2 antagonist) and cis-flupenthixol (a mixed D_1/D_2 antagonist) into the specific brain regions implicated in maternal behavior (*e.g.*, medial preoptic area, nucleus accumbens) disrupts various active maternal behaviors (Byrnes *et al.*, 2002; Giordano *et al.*, 1990; Keer and Stern, 1999; Li *et al.*, 2004; Miller and Lonstein, 2005; Numan *et al.*, 2005; Numan and Stolzenberg, 2009; Silva *et al.*, 2001, 2003; Stern and Taylor, 1991). 6-OHDA lesion of the ventral striatum and ventral tegmental area disrupts pup retrieval (Hansen, 1994; Hansen *et al.*, 1991a,b). Additionally, mother–pup separation, which putatively enhances maternal motivation (Hansen, 1994), stimulates dopamine release in the ventral striatum of maternal rats (Hansen *et al.*, 1993). Because HAL is primarily a D_2 receptor antagonist, we hypothesized that it disrupts maternal behavior by blocking D_2 dopamine receptors. We tested this hypothesis by co-administrating QUI, a selective D_2/D_3 agonist together with HAL and examined whether QUI could reverse the effects of HAL. As expected, QUI did significantly improve the HAL-induced maternal behavior deficits. HAL rats pretreated with QUI showed shortened pup approach and retrieval latency than those pretreated with vehicle. This result is in general agreement with a previous study showing that apomorphine, a mixed D_1/D_2 receptor agonist with a preferential affinity and potency for the D_2 over D_1 receptor subtype (Kebabian and Calne, 1979), reversed HAL-induced pup retrieval deficits in postpartum rats (Giordano *et al.*, 1990). We extended this line of research by showing that HAL specifically acts on D_2 receptors to achieve its disruptive effect. Of note, this finding does not necessarily rule out the involvement of the D_1 receptor mechanism because HAL does have a weak D_1 antagonism (Miyamoto *et al.*, 2005), and other studies also find that D_1 receptors are involved in the regulation of maternal behavior (Miller and Lonstein, 2005; Numan *et al.*, 2005; Numan and Stolzenberg, 2009).

The lack of the effect of DOI treatment on HAL-induced maternal behavior deficits is not surprising given the fact that DOI is a selective 5-$HT_{2A/2C}$ agonist and HAL lacks an action on these serotonergic receptor systems. Interestingly, DOI pretreatment further decreased the duration of pup nursing in the HAL-treated rats (see Figure 2D). Informal observations indicate that after the pups were returned to the cage, the HAL rats pretreated with 2.5 mg/kg of DOI did not approach and retrieve their pups, but stayed in the corner of the cage with no apparent movement. This may explain the reduction of pup nursing by DOI. In light that DOI itself reduces locomotor activity (Hameleers *et al.*, 2007; Krebs-Thomson and Geyer, 1996; Mittman and Geyer, 1991; Wing *et al.*, 1990), it is possible that DOI-induced hypolocomotor activity may have contributed to its disruptive effect on pup nursing.

Our original hypothesis that QUI may also attenuate the CLZ-induced disruption to some extent, as CLZ also possesses a D_2 antagonism, was not supported by the present findings. In contrast, QUI actually worsened some maternal behavior deficits, such as on pup retrieval and nest building. These findings indicate that CLZ-induced maternal deficits are not primarily mediated by its weak antagonism of D_2 receptor. Our preliminary work (data not shown) finds that QUI at a dose of 1.0 mg/kg by itself disrupts pup retrieval and nest building. Therefore, it is possible that a combination of clozapine and QUI may provide an additive disruption on the CLZ-induced maternal deficits.

The most striking finding from the present study was that DOI pretreatment rescued most, if not all, maternal behavior deficits induced by CLZ. We found that CLZ rats pretreated with DOI took a much shorter time to initiate contact with pups and retrieve their pups into the nest sites (Table 2), retrieved more pups (Figure 4A) and spent more time licking their pups than CLZ rats pretreated with vehicle (Figure 4B). CLZ has a unique and multifaceted pharmacological, behavioral and clinical profile in comparison with other antipsychotic drugs (Matsubara *et al.*, 1993; Meltzer, 1989; Safferman *et al.*, 1991; Schmauss *et al.*, 1989) due to its multiple-receptor action, including a relatively high affinity for D_4, histaminergic (H_1), muscarinic, serotoninergic (5-HT_2) and alpha-1-adrenergic receptors (Baldessarini *et al.*, 1992; Lane *et al.*, 1988; Meltzer, 1989; Meltzer *et al.*, 1989a; Miller and Hiley, 1974; Seeman, 1992; Zavitsanou *et al.*, 2007), moderate affinity for D_1, D_2, D_5, alpha-2-adrenergic and 5-HT3 receptors (Ellenbroek *et al.*, 1991; Matsubara *et al.*, 1993; Miyamoto *et al.*, 2005; Murray and Waddington, 1990; Perry *et al.*, 1983; Remington and Kapur, 2000). The unique antipsychotic property of CLZ (*e.g.*, low EPS and less prolactin elevation) has been correlated with its lowaffinity and fast dissociation at the D_2 receptor (Kapur and Remington, 2001; Kapur and Seeman, 2001; Seeman, 2002). This profile makes it difficult to pinpoint the exact mechanisms mediating the antipsychotic and behavioral effects of CLZ. Our finding that DOI dose-dependently improved the CLZ-induced maternal behavior deficits suggests that CLZ exerts its disruptive effect on maternal behavior through a 5-$HT_{2A/2C}$ receptor-mediated mechanism, and that this effect thus might not be directly related to its antipsychotic efficacy, which is mediated by blockade of D_2 receptors (Seeman, 2002).

There are several possible mechanisms underlying the reversing effect of DOI on CLZ-induced maternal behavior deficits. First, DOI may directly activate the 5-$HT_{2A/2C}$ receptors localized in the brain regions important for maternal behavior, such as the nucleus accumbens (NA) and the ventral tegmental area (VTA), etc. There is considerable evidence demonstrating that moderate to high levels of 5-$HT_{2A/2C}$ receptor immunoreactivity (Bubar *et al.*, 2005; Clemett *et al.*, 2000; Cornea-Hébert *et al.*, 1999; Jakab and Goldman-Rakic, 1998) and mRNAs (Eberle-Wang *et al.*, 1997; Pompeiano *et al.*, 1994; Wright *et al.*, 1995) are expressed in the NA and VTA, thus, DOI may antagonize CLZ's blockade on 5-$HT_{2A/2C}$ receptors through competitive binding to 5-HT receptors in these areas to alleviate the CLZ-induced maternal behavior deficits.

Second, DOI may improve CLZ-induced maternal deficits through its indirect action on the dopaminergic systems. The mesolimbic dopamine system, originating in the VTA and terminating in the NA has been implicated in the appetitive aspects of maternal behavior (Hansen, 1994; Hansen *et al.*, 1991a,b, 1993; Numan, 2007; Numan *et al.*, 2005). Recently, 5-$HT_{2A/2C}$ receptors were found to be localized on the dopamine neurons in the VTA (Bubar and Cunningham, 2007; Doherty and Pickel, 2000; Ji *et al.*, 2006; Nocjar *et al.*, 2002). This finding provides evidence in support of the possible interaction between the serotonin and dopamine systems in the VTA (Alex and Pehek, 2007; Di Matteo *et al.*, 2001). More importantly, a growing body of evidence shows that 5-HT_2 receptors play a prominent role in the modulation of mesolimbic dopamine-mediated function. For example, in the NA, activation of 5-HT_2 receptor appears to facilitate dopamine release that can be blocked by local injection of a 5-HT_2 receptor antagonist (Parsons and Justice, 1993). DOI, a mixed 5-$HT_{2A/2C}$ agonist, has been shown to increase extracellular levels of dopamine in the posterior NA (Bowers *et al.*, 2000). Thus, it is possible that DOI may reverse the effect of CLZ by regulating the DA release in the NA and thus indirectly regulatematernal behavior via the DA system. Future research should directly address the neural pathways that mediate the modulatory effects of 5-$HT_{2A/2C}$ receptors on themesolimbic dopamine systems.

Third, at the behavioral level, the diminished sedation could be a contributing factor in reversing CLZ-induced maternal deficits by DOI. In the present study, we noted that DOI treatment significantly alleviated CLZ-induced sedative effect as those rats showed increased motor activity, no sign of closed eyes or bowed head. It has been reported that acute and repeated injection of CLZ at a dose of 10.0 mg/kg produces substantial sedation in animals (Chesler and Salamone, 1996). The CLZ-induced sedative effect has also been reported in the clinic (Burke and Sebastian, 1993; Safferman *et al.*, 1991). Moreover, recent work from our laboratory shows that the sedative effect of CLZ contributes to its disruption on maternal behavior (Zhao and Li, 2009). At this point, it is still premature to suggest that DOI reverses CLZ's disruption on maternal behavior by alleviating the sedative effect of CLZ, because CLZ is not known to cause sedation by blocking 5-$HT_{2A/2C}$ receptors, but rather by blocking alpha-adrenergic and histamine receptors (Lieberman *et al.*, 1989; Safferman *et al.*, 1991). More research is needed to examine whether CLZ can cause sedation via action on 5-$HT_{2A/2C}$ receptors.

Because improvement of CLZ-induced disruption of nest rebuilding was not apparently observed after DOI pretreatment, it is possible that other neurotransmitter systems may be involved in CLZ-induced nest building deficit. One such system could involve the dopamine D_2/D_3 receptors, as our results show that HAL-induced disruption of nest building was reversed and restored to the normal level by QUI, a selective D_2/D_3 receptor agonist. Other systems may include dopamine D_1, D_4 and D_5 receptors. CLZ has high affinity for D_4 and moderate affinity for D_1 and D_5 receptors. Consistent with this hypothesis, previous studies have shown that D_1 receptor activity is critical for the regulation of maternal behavior. Microinjection of a dopamine D_1 receptor antagonist SCH-23390 into the medial preoptic area (MPOA) or nucleus accumbens (NA) greatly impairs pup retrieval in postpartum rats (Miller and Lonstein, 2005; Numan *et al.*, 2005). Microinfusion of a dopamine D_1 receptor agonist SKF-38393 into either the MPOA or the NA promotes the onset of maternal behavior in pregnancy-terminated rats (Stolzenberg *et al.*, 2007). However, it is unknown whether the D_4 and/or D_5 receptors are involved in the regulation of maternal behavior due to lack of evidence. Alternatively, nest building activity may still be mediated by 5-$HT_{2A/2C}$ receptors, but is more sensitive and vulnerable to pharmacological intervention (Li *et al.*, 2004; Silva *et al.*, 2001; Zhao and Li, 2009). Future work should address this issue in detail.

The present study provides strong evidence supporting the involvement of 5-$HT_{2A/2C}$ receptors in the mediation of rat maternal behavior. Previous work on this issue is inadequate and inconsistent. For example, Brunner *et al.* (1999) reported that 5-HT_{1B} receptor knockout mother mice did not display pup retrieval deficits. More recently, Lerch-Haner *et al.* (2008) reported that transgenic mouse dams with a specific disruption in serotonin neuron development displayed profound maternal deficits (*e.g.*, pup retrieval, pup nursing, nest building). This study, however, did not answer which subtypes of 5-HT receptor are implicated in maternal activity. Other studies have found that 5-HT_{1B} and 5-$HT_{2A/2C}$ receptors are involved in maternal aggressive behavior (De Almeida *et al.*, 2005, 2006a,b; Olivier *et al.*, 1995; Veiga *et al.*, 2007). For example, intracerebroventricular injections of DOI, a 5-$HT_{2A/2C}$ agonist, inhibited maternal aggression in postpartum female rats (De Almeida and Lucion, 1994). To the best of our knowledge, the present study was the first to demonstrate that 5-$HT_{2A/2C}$ receptors may be important in regulating rat maternal behavior.

Consistent with our previous work (Li *et al.*, 2004), we found that HAL and CLZ had different time courses of action. HAL tended to produce a prolonged disruption, whereas CLZ produced a transient one. For instance, the HAL-induced disruption of nest building was still apparent even at the 240 min test point (4 h post-injection), whereas the effect of CLZ was diminished (Figures 1, 3, C). It appears that the reversal effect of QUI on HAL-induced maternal deficits also differed in regard to time course of action from that of DOI on CLZ-induced deficits. For example, the reversal effect of DOI on CLZ-induced disruption of pup retrieval was somewhat delayed in onset when compared to that of quinpirole on HAL-induced impairment (Figures 1, 4A), which may be attributable to the rapid peak plasma concentration of quinpirole (Whitaker and Lindstrom, 1987). Taken together, the present study demonstrates an interesting double dissociation receptor mechanism underlying the HAL and CLZ-induced maternal behavior deficits. Our data suggest that the HAL-induced maternal deficits are primarily mediated by the blockade of D_2 dopamine receptors, whereas the CLZ-induced maternal deficits are primarily mediated by the blockade of 5-$HT_{2A/2C}$ receptors. Because not all components of CLZ-induced maternal behavior deficits were reversed by DOI, other receptor mechanisms such as the D_1 receptor may also be involved, prompting the need for further investigation.

Acknowledgements

This research was supported by a grant from the National Institute of Mental Health (5R03MH080822-02). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Mental Health or the National Institutes of Health. We would like to thank Wei He for his administrative and technical help with this work.

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