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Effects of 5-hydroxytryptamine 2C receptor agonist MK212 and 2A receptor antagonist MDL100907 on maternal behavior in postpartum female rats

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Abstract

Maternal behavior in rats is a highly motivated and well-organized social behavior. Given the known roles of serotonin (5-HT) in emotion, motivation, social behavior, and major depression - and its known interaction with dopamine - it is likely that serotonin also plays a crucial role in this behavior. So far, there are surprisingly few studies focusing on 5-HT in maternal behavior, except for maternal aggression. In the present study, we examined the effects of 5-HT_{2C} receptor agonism and 5-HT_{2A} receptor antagonism on maternal behavior in postpartum female rats. We hypothesized that activation of 5-HT_{2C} receptors and blockade of 5-HT_{2A} receptors would produce a functionally equivalent disruption of maternal behavior because these two receptor subtypes often exert opposite effects on various brain functions and psychological processes relevant to rat maternal behavior. On postpartum Days 5, 7, and 9, Sprague-Dawley mother rats were given a single injection of 0.9% NaCl solution, the 5-HT_{2C} agonist MK212 (0.5, 1.0 or 2.0 mg/kg, ip), or the 5-HT_{2A} antagonist MDL100907 (0.05, 0.5 or 2.0 mg/kg, ip). Maternal behavior was tested 30 min before and 30 min, 120 min, 240 min after injection. Acute injection of MK212 significantly disrupted pup retrieval, pup licking, pup nursing, and nest building in a dose-dependent fashion. At the tested doses, MDL100907 had little effect on various components of rat maternal behavior. Across the 3 days of testing, no apparent sensitization or tolerance associated with repeated administration of MK212 and MDL100907 was found. We concluded that rat maternal performance is critically dependent on 5-HT_{2C} receptors, while the role of 5-HT_{2A}

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receptors is still inconclusive. Possible behavioral mechanisms of actions of 5-HT_{2C} receptor in maternal behavior are discussed.

Keywords

5-hydroxytryptamine 2C receptor; 5-hydroxytryptamine 2A receptor; maternal behavior; MK212; MDL100907

Introduction

Maternal behavior in rats is often used to model human mothering behaviors and to investigate the neurobiological mechanisms of mothering (Fleming and Corter, 1988; Fleming and Li, 2003; Rosenblatt, 1989). The full repertoire of rat maternal behavior includes pup-licking, pup retrieval, nest-building, and nursing, as well as maternal aggression (Galef, 1981; Rosenblatt and Lehrman, 1963). Extensive research has delineated the core neural circuits that mediate the expression of maternal behavior, including the medial preoptic area (MPOA), ventral bed nucleus of the stria terminalis (vBNST), medial amygdala and ventromedial hypothalamus (VMH) (Numan, 2007; Numan and Insel, 2003). Recent work shows that the mesolimbic and mesocortical dopamine (DA) systems also play a role in maternal behavior, especially in the appetitive aspect of this behavior (e.g. pup retrieval) (Afonso et al., 2007; Febo et al., 2010; Hansen et al., 1991; Keer and Stern, 1999; Li, 2002; Li and Fleming, 2003; Numan, 2007; Numan et al., 2005). The current view is that the mesolimbic and mesocortical DA systems are part of a nonspecific or general motivational system which serves to increase an organism's responsiveness to a wide variety of biologically significant stimuli, including pups.

Serotonin is an important neurotransmitter implicated in many psychological processes relevant to maternal behavior. Surprisingly, *there are few studies focusing on 5-HT in maternal behavior*, except for maternal aggression (De Almeida and Lucion, 1994; Veiga et al., 2007). Earlier studies using lesion and pharmacological tools suggest that disruption of 5-HT neurotransmission only causes a transient and nonspecific deficit (Barofsky et al., 1983). Recent evidence from studies on mutant mice suggests that 5-HT may be involved in the regulation of maternal behavior (Alenina et al., 2009; Lerch-Haner et al., 2008). Because there are at least 14 known 5-HT receptors in the brain (excluding splice variants and edited isoforms), the specific 5-HT receptor subtype involved is unclear. Our previous work did suggest that 5-HT_{2A} and/or 5-HT_{2C} receptors are two likely targets. *First*, acute administration of 2,5-dimethoxy-4-iodo-amphetamine (DOI, a selective 5-HT_{2A/2C} agonist) disrupts maternal performance (Zhao and Li, 2010). *Second*, atypical antipsychotic drugs such as clozapine, olanzapine, risperidone, and quetiapine - strong antagonists against 5-HT_{2A/2C} receptors - also disrupt active components of maternal behavior in a dose-dependent fashion (Li et al., 2005a; Li et al., 2004). Since both atypical antipsychotics and DOI are nonselective for 5-HT_{2A} versus 5-HT_{2C} receptors, their relative contributions in regulating maternal behavior is still unclear.

Accumulating evidence suggests that 5-HT_{2A} and 5-HT_{2C} receptors play opposing roles in various brain functions and psychological processes relevant to rat maternal behavior. For

example, activation of 5-HT_{2C} receptors decreases dopamine (DA) release in the nucleus accumbens and cell firing in the ventral tegmental area, whereas activation of 5-HT_{2A} receptors enhances these measures (Di Giovanni et al., 2000; Di Matteo et al., 2002). Conversely, blockade of 5-HT_{2C} receptors increases DA release, whereas blockade of 5-HT_{2A} receptors decreases it (Ichikawa et al., 2001; Millan et al., 1998). In several motivated behaviors, 5-HT_{2A} and 5-HT_{2C} receptors also exert opposing effects. For example, Popova and Amstislavskaya reported that administration of 5-HT_{2A} receptor antagonist ketanserin diminished male sexual motivation and behavioral responses, whereas administration of the selective 5-HT_{2C} antagonist RS 102221 increased sexual motivation (Popova and Amstislavskaya, 2002). Also, impulsive action induced by amphetamine, cocaine and MK801 is reduced by 5-HT_{2C} receptor agonists and 5-HT_{2A} receptor antagonists (Fletcher et al., 2011). Opposing effects also exist for 5-HT_{2A} and 5-HT_{2C} receptors in modulating hyperlocomotion induced by MK801 (Martin et al., 1997), in head-twitch response (Vickers et al., 2001), and in the differential reinforcement of low-rate 72-s task, a behavioral screening model for antidepressant agents (Marek et al., 1988; O'Donnell et al., 2005). It appears that activation of 5-HT_{2C} receptors or inhibition of 5-HT_{2A} receptors may decrease incentive motivation by decreasing dopamine neurotransmission, which could contribute to their potential maternal-disruptive effects. Therefore, elucidating the precise behavioral functions of these receptors in maternal behavior is critically important for enhancing our understanding of the neurochemistry of maternal behavior.

The primary goal of the present study was to determine the respective roles of 5-HT_{2A} and 5-HT_{2C} receptors in maternal behavior. Specifically, we were interested in whether both 5-HT_{2C} receptor agonism and 5-HT_{2A} receptor antagonism would produce a similar disruption of maternal performance in postpartum female rats. We took a pharmacological approach by using a highly selective 5-HT_{2A} antagonist MDL100907 and a highly selective 5-HT_{2C} agonist MK212.

Materials and Methods

Subjects and housing

Virgin female Sprague-Dawley rats (60–80 days) weighing 200–250 g were purchased from Experiment Animal Center, Chongqing Medical University, 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). One week after arrival, each female rat was placed into the cage of a proven stud male for ten days to ensure pregnancy. Following the mating procedure, pregnant females were singly housed until parturition after which they were housed together with their litters for the remainder of the experiment. The rats had free access to food and water in their home cages. All animal procedures were approved by the animal care and use committee at Southwest University, China.

Groups and choices of drug doses

The tested drugs were selected on the basis of our extensive literature research that suggested these drugs represented the best available drugs to test our hypothesis. MK212 (6-

Chloro-2-(1-piperazinyl)pyrazine hydrochloride) shows an agonist-preferential action at the 5-HT_{2C} receptor (Sanchez and Arnt, 2000). M100907 (R-(1)-a-(2,3-dimethoxyphenyl)-21-[2-(4-fluorophenylethyl)]24-piperidine-methanol) has been reported to have a 100- to 300-fold greater selectivity for 5-HT_{2A} receptors than for 5-HT_{2C} receptors (Johnson et al., 1996; Kehne et al., 1996; Palfreyman et al., 1993). Three doses of each drug were chosen based on our literature review: MK212 (Tocris: 5-HT_{2C} agonist) at 0.5, 1.0 and 2.0 mg/kg (Batman et al., 2005; Filip et al., 2004), and MDL100907 (National Institutes of Health: 5-HT_{2A} antagonist) at 0.05, 0.5 and 2.0 mg/kg (Wadenberg et al., 2001). At the chosen dose ranges, MK212 and MDL100907 are behaviorally effective. For example, MK212 was shown to reduce acute cocaine-evoked hyperactivity at 2.0 mg/kg, but to increase cocaine-evoked hyperactivity at 0.3 mg/kg (Filip et al., 2004). Similarly, MDL100907 was found to impair reversal learning at 0.03 and 0.1 mg/kg (Boulougouris et al., 2008). MK212 was dissolved in 0.9% saline. MDL100907 was first dissolved in saline with pH 3.5 before the pH was adjusted to 6.25 using 0.1 M NaOH and 0.1 M HCl. Finally, it was diluted to desired concentrations with 0.9% saline. All drugs were administered intraperitoneally (ip) in a volume of 1.0 ml/kg 30 min prior to the start of behavioral tests. This interval was chosen primarily based on the previous studies regarding MDL100907 (Ferguson et al., 2010; Fletcher et al., 2012; Griebel et al. 1997; Sorensen et al. 1993) and MK212 (de Mello Cruz et al., 2005; Miranda et al., 2001; Van de Kar et al., 1992). 0.9% saline was used as vehicle.

Procedure

The basic procedure was similar to what has been described in Zhao and Li (Zhao and Li, 2009a). Briefly, starting two or three days before the first possible expected parturition date, the subjects were monitored every day for signs of parturition. The day on which the dam was found with pups at 10:00 a.m. was designated as Day 1 postpartum, and at 17:00 p.m. as Day 0 postpartum. Once the dam was found with pups, two shredded paper towels were also provided as nesting materials. On Day 3 postpartum, each litter was culled to eight pups (4 males and 4 females with the most visible milk bands) and all subjects were changed (moved) to clean observation cages with their litters. Maternal behavior tests were conducted on Days 5, 7 and 9 postpartum (PP5, PP7, PP9) at the same time of day (around 9:00 am each day). On each test day, maternal behavior was observed at 4 time points, with the first one at 0.5 h before the drug injections (i.e., baseline), and the rest being carried out at 0.5, 2, and 4 h after the injections. This time-frame covered the entire acute effect of the tested drugs. Each test consisted of two phases. The first phase lasted 10 min which consisted of continuous observations of maternal behavior under the undisturbed condition. The second phase was a 10-min pup retrieval test starting immediately after the first undisturbed test. This phase was initiated by taking the 8 pups away from the mother and destroying the nest. One minute later, the pups were placed in the corner of the cage diagonal to the nest site. At the end of the 10-min period, unretrieved pups were returned to the nest site. Both phases were recorded by video cameras and analyzed manually using a laptop computer with an event recording program (JWatcher, <http://www.jwatcher.ucla.edu/>). The raters were blind to each subject's drug 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), *pup nursing* (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). The first and last pup retrieval latencies were defined as the time elapsed from the first pup approach to the retrieval of the first and eighth pup into the nest, respectively. 600 s was assigned to non-responders who did not approach or retrieve the testing pups. The number of animals in vehicle and MK212-2.0 mg/kg groups was seven, and in other groups was six.

Statistical Analysis

Maternal behavior data from the 1st 10 min undisturbed observation period and 2nd 10 min pup retrieval test period at each test time point were combined and were presented as mean \pm SEM, except for the latency data which were displayed as median \pm interquartile range. Frequency and duration (in second) data on PP5, PP7 and PP9 were analyzed separately using a factorial repeated measures analysis of variance ANOVA) with group (4 levels) as the between-subjects factor and test time point (3 levels) as the within-subjects factor (a 4×3 ANOVA). Group differences were further investigated using simple main effect tests (one-way ANOVA) followed by LSD post hoc tests. Because the latency data were not normally distributed (e.g. the cut-off time set at 600 s), they were analyzed using nonparametric Kruskal-Wallis test. Once the overall significant effects were determined, two-group comparisons between the drug and vehicle treatment were performed using Mann-Whitney *U* test. Statistical significance was accepted at $p < 0.05$, two-tailed.

Results

MK212 dose-dependently prolonged pup retrieval latency, while MDL100907 had little effect

Table 1 illustrates the effects of the 3 doses of MK212 and MDL100907 on the first and last pup retrieval latency at 30 min before and 30 min after injection on PP5, PP7 and PP9 (the latency data at other test time points are not shown). No significant group difference was found at 30 min before injection. At 30 min after MK212 administration, Kruskal-Wallis test revealed a significant overall drug treatment effect on the first and last pup retrieval latency on PP5, PP7 and PP9 (all $ps < 0.001$). Mann-Whitney *U* test showed that rats treated with MK212 2.0 mg/kg took significantly longer to retrieve their pups to the nest in comparison to the vehicle treatment (all $ps < 0.004$), while rats treated with MK212 1.0 mg/kg and 0.5 mg/kg took longer than the vehicle treatment only at some post-injection test time points (see Table 1). MDL100907 had no effect on these measures in comparison to the vehicle (all $ps > 0.058$) (Table 1).

MK212 dose-dependently reduced number of pups retrieved, while MDL100907 had little effect

Figure 1 shows the results of MK212 and MDL100907 treatment on pup retrieval at each test time on PP5, PP7 and PP9 in comparison to the vehicle group. Pup retrieval was significantly impaired in animals treated with MK212 on PP5 (Figure 1A). Repeated measures ANOVA revealed a main effect of group [$F(3, 22) = 7.305, p = 0.001$], a main effect of test time [$F(2, 44) = 7.647, p = 0.001$], and a significant group \times test time

interaction [$F(6,44) = 4.583, p = 0.001$] on PP5. Post hoc LSD tests indicated that MK212 2.0 mg/kg group and 1.0 mg/kg group retrieved fewer pups than the vehicle group ($p=0.001$ and $p=0.006$, respectively). To identify the time point(s) at which MK212-treated rats differed from the vehicle-treated rats, one-way ANOVAs followed by post hoc LSD tests were used. Results showed that dams injected with MK212 2.0 mg/kg retrieved fewer pups at 30 min and 120 min after drug injection ($p = 0.000$ and $p = 0.031$, respectively). Dams treated with MK212 1.0 mg/kg retrieved fewer pups at 30 min and 240 min after drug injection ($p = 0.003$ and $p = 0.029$, respectively).

The disruptive effect of MK212 was also found on PP7 (Figure 1B). The main effect of group [$F(3, 22) = 5.218, p = 0.007$], test time [$F(2, 44) = 9.104, p = 0.001$], and drug \times test time interaction [$F(6, 44) = 5.218, p = 0.001$] were significant. Post hoc tests indicated that MK212 2.0 mg/kg group retrieved fewer pups than the vehicle group ($p = 0.002$). One-way ANOVAs on specific test time points showed that dams injected with 2.0 mg/kg of MK212 retrieved fewer pups at 30 min after drug injection ($p = 0.002$). On PP9, pup retrieval was still significantly impaired in dams treated with MK212 (Figure 1C). Once again, the main effect of drug [$F(3, 22)=6.537, p = 0.003$], test time [$F(2, 44) = 14.951, p = 0.000$], and drug \times test time interaction [$F(6, 44) = 6.161, p = 0.000$] were all significant. Dams injected with 2.0 mg/kg of MK212 retrieved fewer pups at 30 min after drug injection ($p = 0.000$).

Figure 1 (D, E, F) shows the effects of MDL100907 on pup retrieval on the three test days. There was no significant effect of group, test time or their interaction on any of the test days (all $ps > 0.225$), suggesting that MDL100907 at the tested doses did not affect pup retrieval.

MK212, but not MDL100907 dose-dependently impaired pup nursing

Effects of MK212 and MDL100907 on nursing behavior at each test time on PP5, PP7 and PP9 are shown in Figure 2. On PP5, nursing duration was significantly shortened in mother rats treated with MK212 (Figure 2A). Repeated measures ANOVA revealed a main effect of group [$F(3, 22) = 6.292, p = 0.003$], a main effect of test time [$F(2, 44) = 20.717, p = 0.000$], and a significant group \times time interaction [$F(6, 44) = 4.031, p = 0.003$]. Post hoc tests indicated that the MK212 2.0 mg/kg group and 1.0 mg/kg group were significantly different from the vehicle group ($p = 0.001$ and $p = 0.005$, respectively). One way ANOVAs followed by post hoc LSD test revealed that MK212 2.0 mg/kg suppressed nursing at 30 min after injection on PP5 ($p = 0.000$), but not at any other test time points. MK212 1.0 mg/kg significantly inhibited nursing at 30 min and 120 min on PP5 ($p = 0.003$ and $p = 0.008$, respectively). Nursing behavior recovered to the vehicle level at 240 min test time point.

The disruptive effect of MK212 on nursing behavior seems to be still present on PP7 (Figure 2B). However, repeated measures ANOVA only revealed a main effect of test time [$F(2, 44) = 12.724, p = 0.000$], but no main effect of group [$F(3, 22) = 2.377, p = 0.097$] and no significant group \times time interaction [$F(6, 44)=1.893, p=0.103$]. On PP9 (Figure 2C), the effects of test time [$F(2, 44) = 15.865, p = 0.000$] and group \times time interaction [$F(6, 44) = 2.636, p = 0.029$] were significant. One way ANOVAs found that MK212 2.0 mg/kg and 1.0 mg/kg suppressed nursing at the 30 min test time ($p = 0.012$ and $p = 0.001$, respectively).

Figure 2 (D, E, F) shows the effects of MDL100907 on nursing behavior on the 3 test days. Repeated measures ANOVA did not find a main effect of group, nor group \times time interaction on any of the 3 test days (all $ps > 0.051$), suggesting that MDL100907 at the tested doses did not affect pup nursing.

MK212, but not MDL100907 dose-dependently reduced pup licking

Figure 3 shows the effects of MK212 and MDL100907 on pup licking at each test time on PP5, PP7 and PP9. On PP5, repeated measures ANOVAs revealed a main effect of group [$F(3, 22) = 4.890, p = 0.009$], a main effect of test time [$F(2, 44) = 11.621, p = 0.001$], but no significant group \times time interaction [$F(6, 44) = 1.097, p = 0.379$] in animals treated with MK212 (Figure 3A). Post hoc tests indicated that MK212 2.0 mg/kg group was significantly different from the vehicle group ($p = 0.001$). Dams injected with MK212 2.0 mg/kg had significantly lower licking activity at 30 min and 240 min after injection ($p = 0.019$ and $p = 0.014$, respectively), but no significant at 120 min after injection ($p = 0.084$).

On PP7, the disruptive effect of MK212 on pup licking was present, but the magnitude did not reach the significant level (Figure 3B). Repeated measure ANOVAs revealed no main effect of group [$F(3, 22) = 2.987, p = 0.053$], test time [$F(2, 44) = 2.670, p = 0.080$], and no significant group \times test time interaction [$F(6, 44) = 1.115, p = 0.369$].

On PP9 (Figure 3C), the disruptive effect of MK212 was still present. Repeated measures ANOVA revealed a main effect of group [$F(3, 22) = 3.771, p = 0.025$], but no main effect of test time [$F(2, 44) = 0.922, p = 0.405$], nor significant group \times test time interaction [$F(6, 44) = 1.234, p = 0.307$]. One way ANOVAs followed by post hoc LSD tests showed that dams injected with MK212 at 2.0 and 1.0 mg/kg had a much lower level of licking activity at 30 min ($p=0.034$ and $p=0.009$, respectively).

Figure 3 (D, E, F) shows the effects of MDL100907 on pup licking on the three test days. There was no significant effect of group, or time and group interaction on any of the test days (all $ps > 0.062$), suggesting that MDL100907 at the tested doses did not affect pup licking.

MK212, but not MDL100907 dose-dependently suppressed nest building

Figure 4 (A, B, C) shows the effects of MK212 and MDL100907 treatment on nest building at each test time on PP5, PP7 and PP9. In comparison to the vehicle group, nest building activity was severely impaired by MK212 on PP5. Repeated measures ANOVA revealed a main effect of group [$F(3, 22) = 3.827, p = 0.024$], but no main effect of test time [$F(2, 44) = 0.179, p = 0.836$], no significant group \times test time interaction [$F(6, 44) = 0.240, p = 0.961$]. Post hoc tests indicated that the MK212 2.0 mg/kg and 1.0 mg/kg groups had significant lower nest building activity than the vehicle group ($p = 0.005$ and $p = 0.018$, respectively). One way ANOVAs followed by post hoc LSD tests showed that dams injected with MK212 2.0 mg/kg showed a suppressed nest building activity at 30 min and 240 min after injection ($p = 0.022$ and $p = 0.027$, respectively). MK212 1.0 mg/kg disrupted nest building at 240 min point ($p = 0.022$) (Figure 4A).

The disruptive effect of MK212 on nest building was also found on PP7 (Figure 4B). There was a main effect of group [$F(3, 22) = 3.956, p = 0.021$], test time [$F(2,44) = 4.250, p = 0.021$], but no significant group \times test time interaction [$F(6, 44) = 1.197, p = 0.326$]. Post hoc tests indicated that all three MK212 groups were significantly different from the vehicle group ($p = 0.006$ for MK212 2.0 mg/kg; $p = 0.011$ for MK212 1.0 mg/kg; $p = 0.032$ for MK212 0.5 mg/kg). One way ANOVAs followed by post hoc LSD tests showed that MK212 at 2.0 mg/kg disrupted nest building activity at 30 min and 120 min after injection ($p = 0.006$ and $p = 0.026$, respectively) and 1.0 mg/kg did so at 240 min ($p = 0.036$).

On PP9, the main effect of group was significant [$F(3, 22) = 3.715, p = 0.027$], but the effects of test time [$F(2,44) = 0.389, p = 0.680$] and group \times test time interaction were not [$F(6, 44) = 0.899, p = 0.504$]. Post hoc tests indicated that MK212 2.0mg/kg and 1.0 mg/kg groups were significantly different from the vehicle group ($p=0.010$ and $p=0.009$, respectively). Dams injected with MK212 at 2.0 and 1.0 mg/kg spent significantly less time on nest activity at 30 min after injection ($p=0.005$ and $p=0.010$, respectively).

Figure 4 (D, E, F) shows the effects of MDL100907 on nest building on the three test days. There was no significant effect of group, test time or their interaction on any of the test days (all $ps > 0.083$), suggesting that MDL100907 at the tested doses did not affect nest building.

Time course of MK212 and MDL100907 effects across the three test days

To examine how the effects of MK212 and MDL100907 on maternal behavior were changed over the three days of testing and with repeated drug administration, we selected the 30 min post-injection time point and compared the drug effects across the three test days (PP5, PP7 and PP9) (Figure 5 and 6). Overall, there did not appear to be any consistent pattern. No apparent sensitization (i.e. increase in the disruptive effect of MK212) or tolerance (i.e. decrease in the disruptive effect of MK212) was observed. Statistical analysis also did not find any significant difference between PP5 and PP9 (all $ps > 0.05$).

Discussion

The present study demonstrated that the 5-HT_{2C} receptor agonist MK212 had a disruptive effect on pup retrieval, pup licking, pup nursing, and nest building, four major components of rat maternal behavior. Rats treated with MK212 had a longer pup retrieval latency, retrieved fewer pups into the nest, spent less time on licking and nursing pups and on building the disturbed nest. In contrast, the 5-HT_{2A} receptor antagonist MDL100907 had little effect on these maternal responses. These findings suggest that 5-HT_{2C} receptors play an important role in the regulation of maternal behavior, and activation of this receptor system could lead to a disruption of this behavior. In contrast, the role of 5-HT_{2A} receptors is still not clear, as MDL100907 at the tested doses did not cause any change in maternal behavior, a negative finding precluding any firm conclusion on the role of 5-HT_{2A} in maternal behavior.

The most significant finding of the present study was the determination of the involvement of 5-HT_{2C} receptor in rat maternal behavior. As mentioned in the Introduction, there is a general lack of research on the role of serotonin in maternal behavior (De Almeida and

Lucion, 1994; Veiga et al., 2007). Earlier studies using lesion and pharmacological tools suggest that disruption of 5-HT neurotransmission only causes a transient and nonspecific deficit (Barofsky *et al.*, 1983). Recent evidence from studies on mutant mice suggests that 5-HT may be involved (Alenina et al., 2009; Lerch-Haner et al., 2008). Lerch-Haner et al. reported that *Pet-1*^{-/-} mouse dams (*Pet-1* also known as *Fev*, is an ETS transcription factor restrictedly expressed in 5-HT neurons) showed reduced expression of the serotonergic gene and 5-HT synthesis (Lerch-Haner *et al.*, 2008). Mouse dams also displayed impaired pup retrieval, nursing and nest building. Alenina et al. reported that the tryptophan hydroxylase 2-deficient dams failed to retrieve their pups into the nest site and nurse them (Alenina *et al.*, 2009). Because these genes are critical for proper brain maturation and homeostatic modulation of neural circuits, lack of these genes throughout the lifetime may disrupt the development of the neural circuits governing maternal behavior. Thus, it is still not clear whether the maternal deficits seen in these mutant dams are caused by altered 5-HT neurotransmission (a primary effect) or by changed brain structures (a secondary effect). Also, none of the previous studies have examined the specific types of 5-HT receptors in maternal behavior. Previously, we demonstrated that atypical drugs (e.g. clozapine) disrupt active components of maternal behavior in a dose-dependent fashion (Li et al., 2005a; Li et al., 2004). Because these drugs possess potent antagonist actions against 5-HT_{2A/2C} receptors, in addition to their antagonist action on dopamine D₂ receptor, we hypothesized that blockade of 5-HT_{2A/2C}-mediated neurotransmission contributed to their maternal disruptive effects, which was validated in our published studies. Specifically, we found that pretreatment of DOI (1.0 and 2.5 mg/kg), a selective 5-HT_{2A/2C} serotonergic receptor agonist, dose-dependently reversed the clozapine-induced disruption of maternal behavior (e.g. pup retrieval). Interestingly, rats treated with DOI itself also showed deficits in maternal behavior. Because both 5-HT_{2A/2C} receptor **antagonist** clozapine, and **agonist** DOI disrupt maternal behavior, these findings suggest that *balanced* 5-HT_{2A} and/or 5-HT_{2C} receptor mediated neurotransmission is critical for the normal expression of maternal behavior. Too little or too much 5-HT signaling via 5-HT_{2A/2C} receptors could lead to maternal disruptions.

Despite these findings, we are still not clear about the relative roles of each 5-HT₂ subtype (5-HT_{2A} or 5-HT_{2C}) in the maternal behavior. This is because clozapine has dual action on both 5-HT_{2A} and 5-HT_{2C} receptors, and DOI is nonselective for 5-HT_{2A} versus 5-HT_{2C} receptors. The present study was designed to further tease this out by using a highly selective 5-HT_{2C} receptor agonist MK212 and 5-HT_{2A} antagonist MDL100907. We decided to first test the effects of 5-HT_{2C} receptor agonism and 5-HT_{2A} receptor antagonism on maternal behavior because 5-HT_{2A} and 5-HT_{2C} receptors often play opposing roles in various brain functions and psychological processes. We hypothesized that MK212 and MDL100907 would produce a functionally equivalent disruption of maternal behavior. Our result that MK212 disrupted various components of maternal behavior supports our hypothesis that 5-HT_{2C} receptor is critically involved in the normal expression of maternal behavior. However, the lack of MDL100907 effect was surprising, both inconsistent with our hypothesis and the ample evidence suggesting the functional equivalency between 5-HT_{2A} antagonism and 5-HT_{2C} agonism (see introduction). The lack of any effect with MDL100907 was also unexpected given that DOI's behavioral effects (Halberstadt et al.,

2009; Schreiber et al., 1995; Sipes and Geyer, 1995, 1997; Smith et al., 2003), as well as those of clozapine's (Kuroki et al., 2003; Li et al., 2010; Meltzer, 2002) are shown to be primarily mediated by their antagonist action on 5-HT_{2A}. Since the chosen doses of MDL100907 are behaviorally active, shown to decrease premature (or 'impulsive') responding on the five-choice serial reaction time task (Fletcher et al., 2007; Winstanley et al., 2004), impair reversal learning (Boulougouris et al., 2008), reduce reinstatement of nicotine self-administration (Fletcher et al., 2012), and attenuate cocaine-induced locomotion (Burton et al., 2013), it is difficult to argue that 5-HT_{2A} receptors were not altered by MDL100907. Nevertheless, the involvement of 5-HT_{2A} receptors in maternal behavior needs to be further investigated, either by expanding the MDL100907 dose range, or by using other selective 5-HT_{2A} receptor antagonists or agonists. Notably, the contrasting effect of 5-HT_{2C} agonism and 5-HT_{2A} antagonism found in the present study is similar to that reported by Wolf (Wolf et al., 1999), who showed that in a test of female sexual behavior, SB206553 (5-HT_{2C} antagonist) was more effective than MDL100907 in reducing lordosis behavior and in attenuating the ability of DOI to facilitate the behavior. They concluded that 5-HT_{2C}, rather than 5-HT_{2A}, receptors are primarily responsible for the effects of 5-HT₂ receptor-active drugs on lordosis behavior. These differential effects of 5-HT_{2C} agonism and 5-HT_{2A} antagonism on maternal behavior is also consistent with that of earlier investigations in other paradigms. For example, the treatment of MDL100907 alone did not produce a potent effect on conditioned avoidance response (Wadenberg et al., 1998), but 5-HT_{2C} agonist WAY-163909 reduced avoidance response (Marquis et al., 2007). Fletcher showed that the 5-HT_{2C} receptor agonist Ro60-0175 reduced nicotine self-administration responding in both a FR5 and a progressive ratio schedule, and also reduced responding for food reinforcement; whereas MDL100907 did not alter responding for nicotine, or food, on either schedule (Fletcher *et al.*, 2011).

MK212 may disrupt maternal behavior via several possible mechanisms. First, it may directly activate the 5-HT_{2C} receptors localized in the brain regions important for maternal behavior, such as the nucleus accumbens, ventral hypothalamus, ventral tegmental area, medial prefrontal cortex and ventral bed nucleus of stria terminalis, etc. There is considerable evidence demonstrating that 5-HT_{2C} receptors are localized in these brain regions (Bubar et al., 2011; Di Giovanni et al., 2006; Millan et al., 1998; Pompeiano et al., 1994). Second, MK212 may cause maternal behavior deficits through its indirect action on the dopaminergic systems, which play an important role in the regulation of maternal behavior. It is well known that activation of 5-HT_{2C} receptors can decrease dopamine release in the nucleus accumbens and cell firing in the ventral tegmental area (Di Giovanni et al., 2000; Di Matteo et al., 2002), and this decrease of dopamine neurotransmission is capable of causing maternal disruption, as both systemic (e.g., haloperidol, a D₂ antagonist) and central injection of dopamine receptor antagonists such as SCH-23390 (a D₁ antagonist), pimozide (a D₂ antagonist) and cis-flupenthixol (a mixed D₁/D₂ antagonist) into the specific brain regions implicated in maternal behavior (e.g., medial preoptic area, nucleus accumbens) disrupt various active maternal behaviors (Giordano et al., 1990; Keer and Stern, 1999; Li et al., 2005b; Miller and Lonstein, 2005; Numan et al., 2005; Zhao and Li, 2012, 2009b). Given the well-known role of dopamine in incentive motivation (Berridge and Robinson, 1998), and evidence showing that 5-HT_{2C} receptor agonism tends to decrease

various motivated behaviors (Grauer et al., 2009; Wadenberg and Hicks, 1999), a 5-HT_{2C} agonist such as MK212 may achieve its maternal disruption by *decreasing* maternal (incentive) motivation. Future research should directly address the neuronal pathways that mediate the modulatory effects of 5-HT_{2C} receptors on the mesolimbic dopamine system and its role in maternal behavior.

Taken together, in this study, we demonstrated that MK212 dose-dependently disrupts major components of maternal behavior. In contrast, MDL100907 did not show any effect. Therefore, 5-HT_{2C} receptors (not 5-HT_{2A}) may be the predominant 5-HT₂ receptor in the relevant brain areas (the mesolimbic and mesocortical dopamine systems and the hypothalamic regions, etc.) that are involved in the modulation of maternal behavior in rats. In other words, serotonin may exert its regulatory effects on rat maternal behavior primarily through 5-HT_{2C} receptor rather than 5-HT_{2A} receptor. Further work is needed to determine the involvement of 5-HT_{2A} receptors in maternal behavior.

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Abbreviations

5-HT	5-hydroxytryptamine
DA	dopamine
PP5	Day 5 postpartum
PP7	Day 7 postpartum
PP9	Day 9 postpartum

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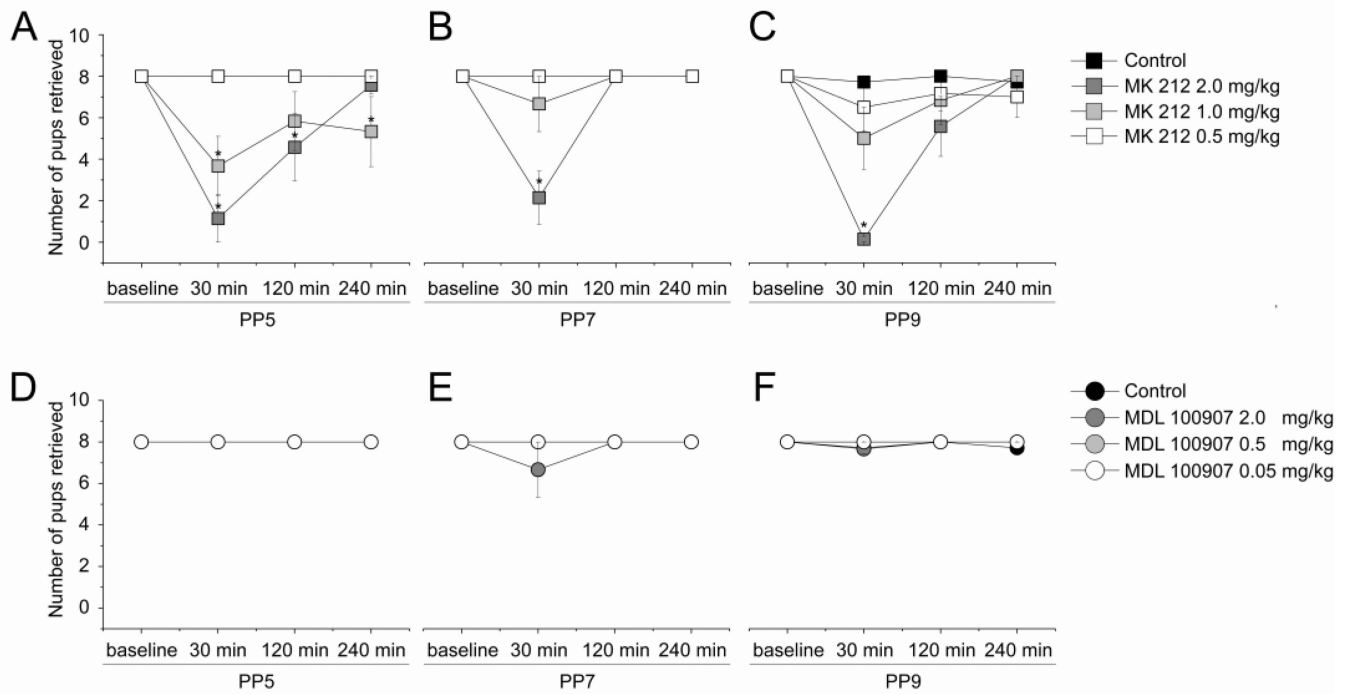
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Highlights

- ◆ MK212 (a 5-HT_{2C} agonist) disrupted pup retrieval, pup licking, pup nursing, and nest building.
- ◆ MDL100907 (a 5-HT_{2A} antagonist) had little effect on various components of rat maternal behavior.
- ◆ Across the 3 days of testing, no apparent sensitization or tolerance was found.
- ◆ Serotonin 5-HT_{2C} receptor mediates maternal behavior in rats

**Figure 1.**

Effects of MK212 (A, B, C) and MDL100907 (E, F, G) treatment on pup retrieval throughout the three test days (PP5, PP7 and PP9). Number of pups retrieved at each test time point is expressed as mean \pm SEM. The test lasted 10 min. * $p < 0.05$, compared to vehicle.

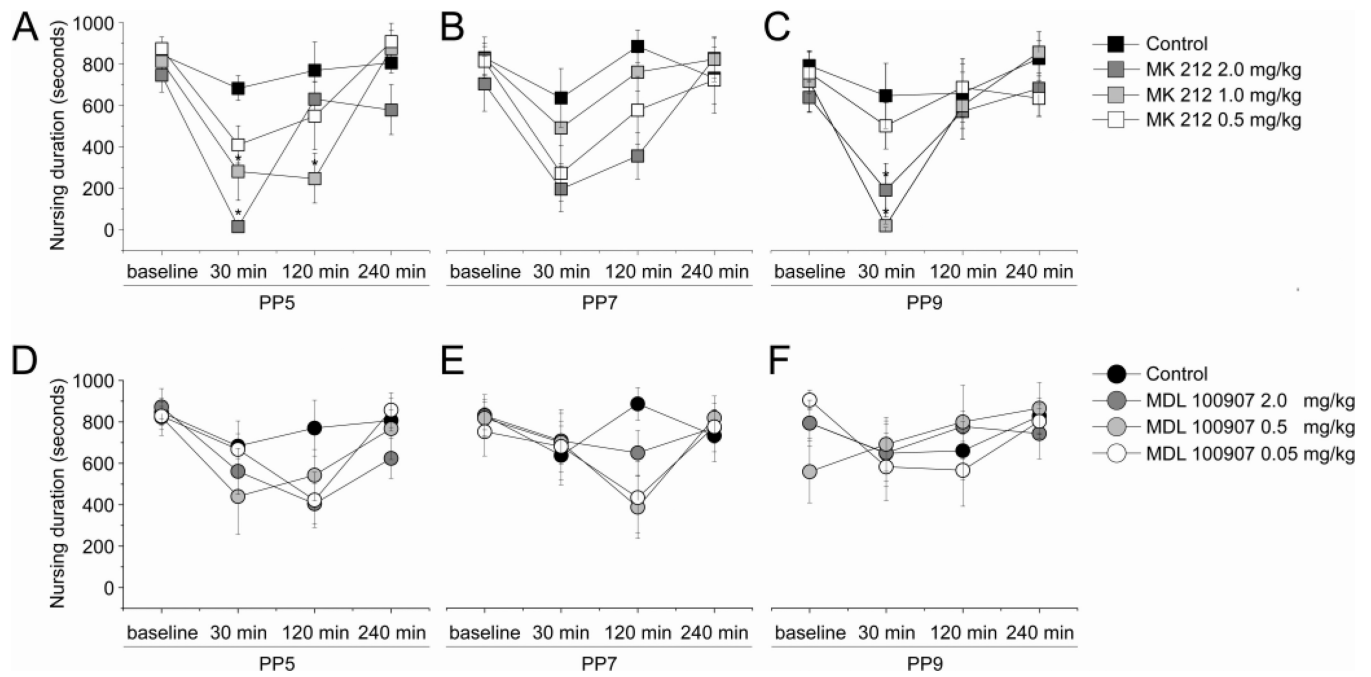
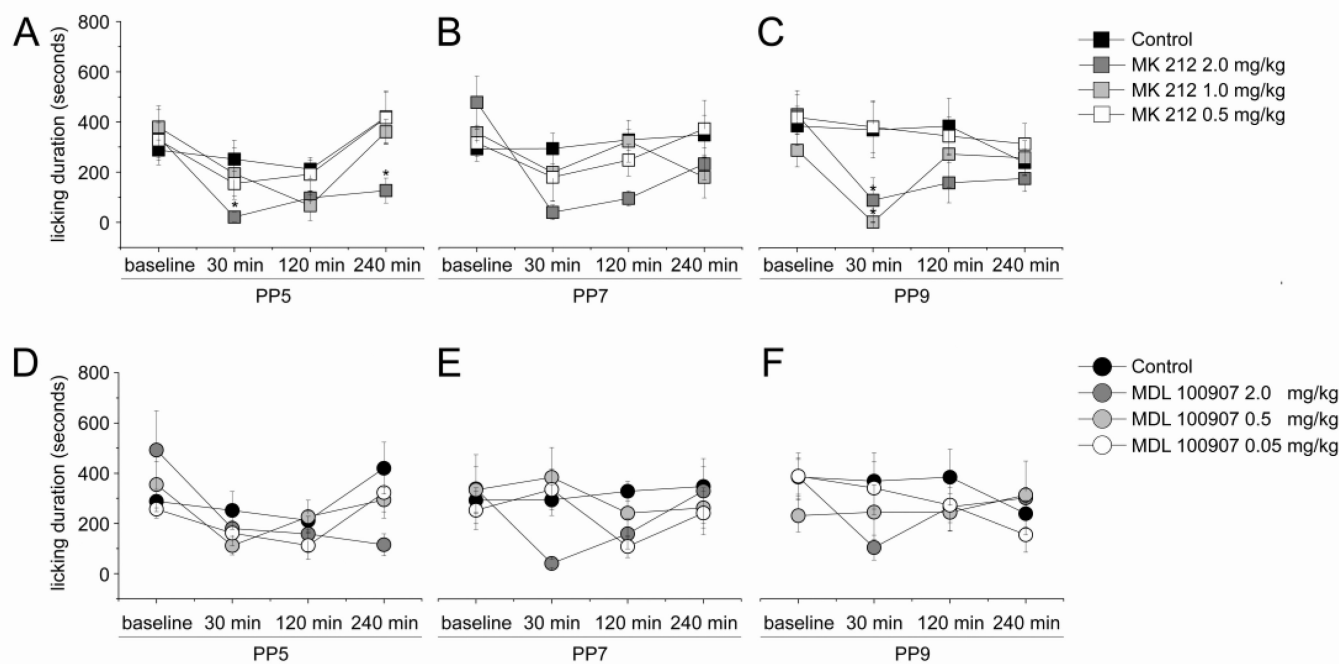


Figure 2.

Effects of MK212 (A, B, C) and MDL100907 (E, F, G) treatment on pup nursing throughout the three test days (PP5, PP7 and PP9). Nursing duration at each test time point is expressed as mean ± SEM. * $p < 0.05$ versus the vehicle group.

**Figure 3.**

Effects of MK212 (A, B, C) and MDL100907 (E, F, G) treatment on pup licking throughout the three test days (PP5, PP7 and PP9). Licking duration at each test time point is expressed as mean \pm SEM. * $p < 0.05$ versus the vehicle group.

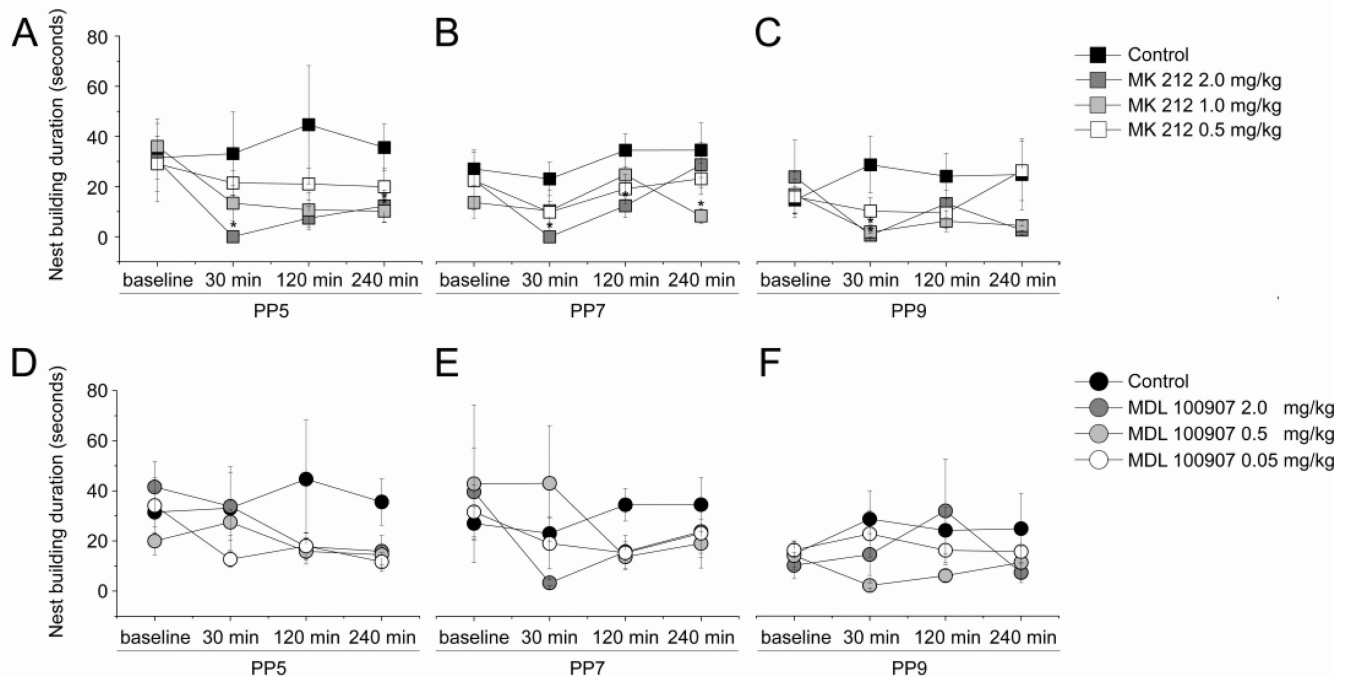


Figure 4.

Effects of MK212 (A, B, C) and MDL100907 (E, F, G) treatment on nest building throughout the three test days (PP5, PP7 and PP9). Time spent on building the nest at each test time point is expressed as mean \pm SEM. * $p < 0.05$ versus the vehicle group.

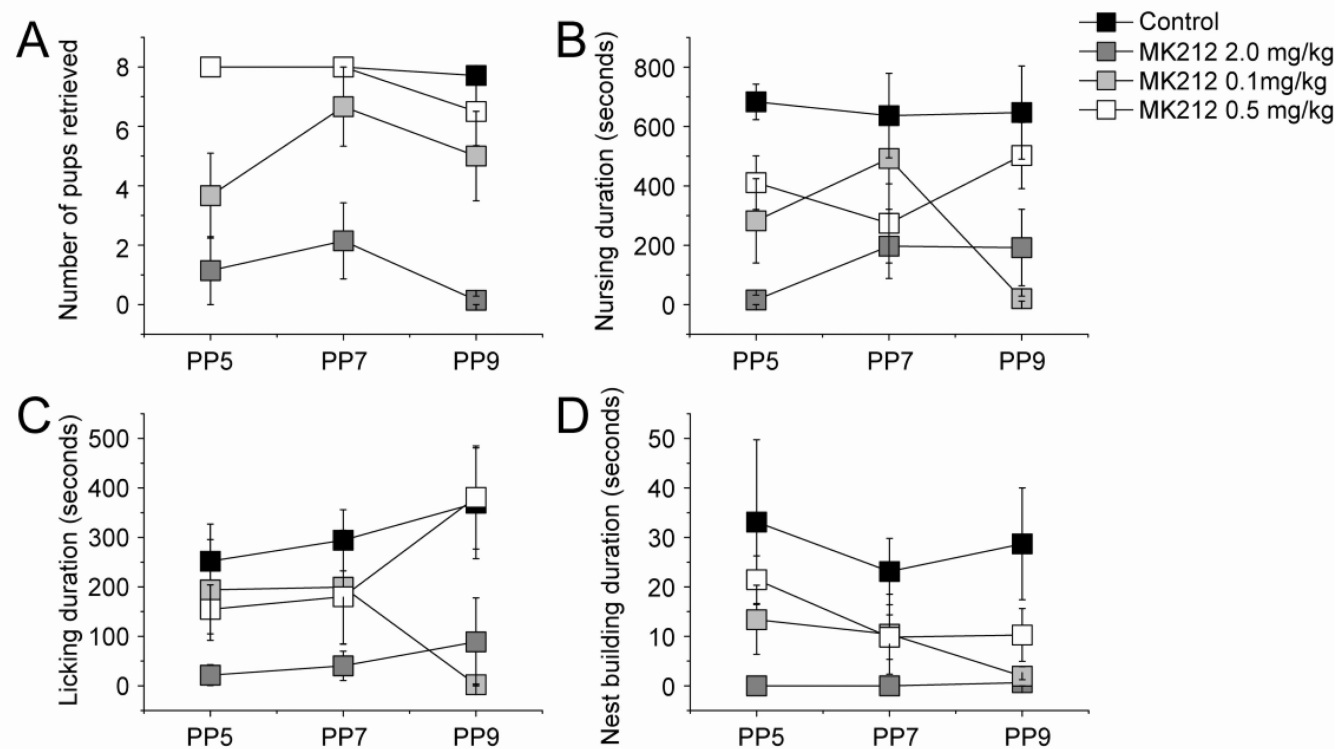
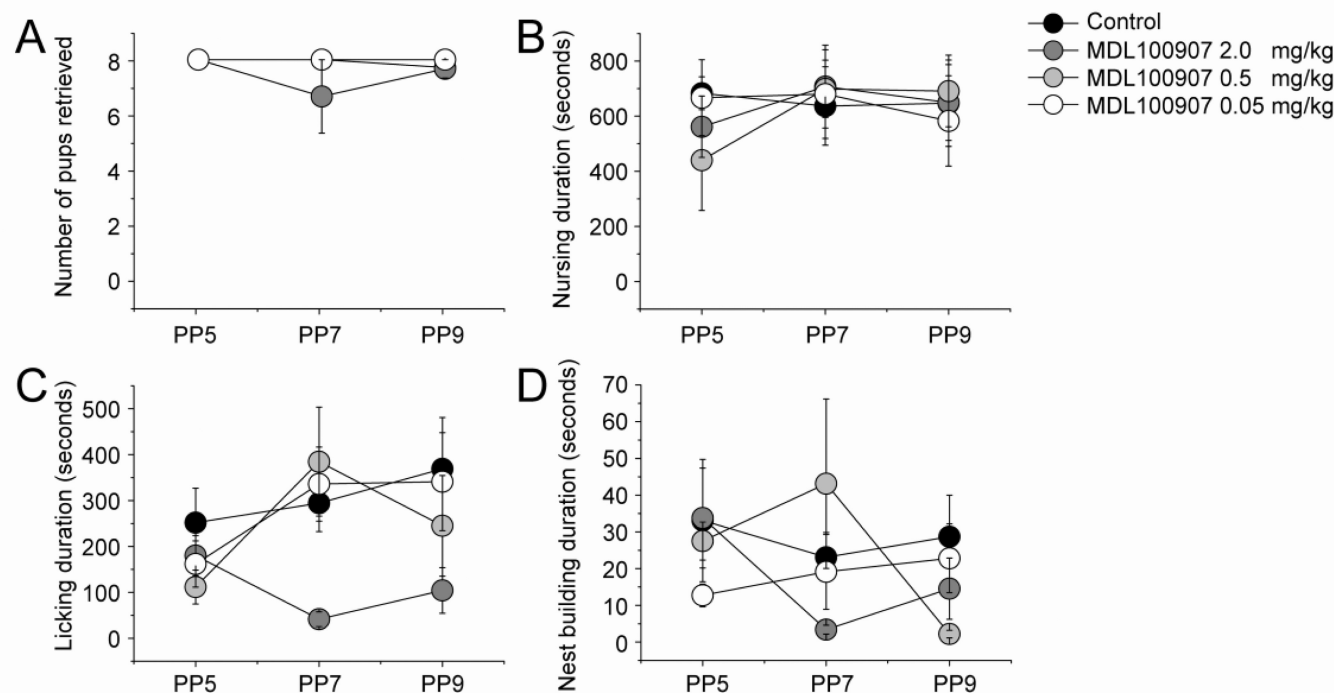


Figure 5.

Time course of effects of MK212 on pup retrieval (A), pup nursing (B), pup licking (C), and nest building (D) at the 30 min time point after injection on each of the 3 test days.

**Figure 6.**

Time course of effects of MDL100907 on pup retrieval (A), pup nursing (B), pup licking (C), and nest building (D) at the 30 min time point after injection on each of the 3 test days.

Table 1

Pup retrieval latency in postpartum female rats treated with MK212 and MDL100907

Group	N	PP5						PP7						PP9					
		baseline			30 min after injection			baseline			30 min after injection			baseline			30 min after injection		
		First pup retrieval latency (s)	Last pup retrieval latency (s)	Latency (s)	First pup retrieval latency (s)	Last pup retrieval latency (s)	Latency (s)	First pup retrieval latency (s)	Last pup retrieval latency (s)	Latency (s)	First pup retrieval latency (s)	Last pup retrieval latency (s)	Latency (s)	First pup retrieval latency (s)	Last pup retrieval latency (s)	Latency (s)	First pup retrieval latency (s)	Last pup retrieval latency (s)	Latency (s)
Vehicle	7	5.2 (5.5)	37.5 (24.2)	4.9 (4.0)	32.8 (31.9)	6.2 (11.0)	31.9 (44.5)	2.3 (3.2)	27.0 (18.0)	2.3 (2.6)	31.4 (28.4)	3.6 (6.9)	38.0 (117.5)	2.3 (2.6)	31.4 (28.4)	3.6 (6.9)	38.0 (117.5)	2.3 (2.6)	31.4 (28.4)
MK212-2.0 mg/kg	7	5.0 (2.0)	42.9 (16.6)	600.0 (0)	600.0 (0)	10.2 (26.8)	53.2 (45.4)	481.7 (555.3)	600.0 (216.6)	9.6 (42.8)	50.2 (84.8)	600.0 (0)	600.0 (0)	9.6 (42.8)	50.2 (84.8)	600.0 (0)	600.0 (0)	9.6 (42.8)	50.2 (84.8)
MK212-1.0 mg/kg	6	42.5 (9.3)	42.0 (19.3)	11.9 (11.5)	600.0 (489.0)	4.5 (2.4)	41.5 (17.6)	16.4 (595.9)	58.2 (554.9)	5.4 (26.9)	34.4 (78.0)	96.8 (332.8)	372.7 (533.8)	5.4 (26.9)	34.4 (78.0)	96.8 (332.8)	372.7 (533.8)	5.4 (26.9)	34.4 (78.0)
MK212-0.5 mg/kg	6	5.6 (4.5)	36.9 (23.0)	7.1 (11.1)	55.2 (35.4)	3.5 (0.5)	46.7 (64.6)	5.3 (10.9)	42.7 (15.1)	5.5 (2.2)	32.0 (14.8)	6.1 (20.2)	77.8 (91.9)	5.5 (2.2)	32.0 (14.8)	6.1 (20.2)	77.8 (91.9)	5.5 (2.2)	32.0 (14.8)
MDL100907-2.0 mg/kg	6	5.3 (5.8)	35.5 (15.0)	4.8 (6.28)	39.7 (17.6)	7.8 (26.7)	36.0 (101.6)	6.5 (7.0)	42.8 (41.7)	14.0 (20.2)	43.0 (46.9)	44.8 (209.8)	189.7 (531.3)	14.0 (20.2)	43.0 (46.9)	44.8 (209.8)	189.7 (531.3)	14.0 (20.2)	43.0 (46.9)
MDL100907-0.5 mg/kg	6	6.1 (7.0)	37.4 (9.0)	8.7 (7.2)	43.15 (25.7)	8.4 (12.6)	46.5 (45.4)	5.6 (10.7)	36.4 (20.0)	5.9 (11.4)	40.4 (39.8)	4.9 (1.3)	34.7 (13.4)	5.9 (11.4)	40.4 (39.8)	4.9 (1.3)	34.7 (13.4)	5.9 (11.4)	40.4 (39.8)
MDL100907-0.05 mg/kg	6	3.4 (5.6)	37.7 (2.0)	4.35 (1.3)	25.9 (17.9)	7.6 (13.5)	44.3 (60.8)	2.8 (6.2)	31.8 (35.0)	6.6 (24.9)	47.3 (49.3)	3.0 (13.6)	59.0 (65.7)	6.6 (24.9)	47.3 (49.3)	3.0 (13.6)	59.0 (65.7)	6.6 (24.9)	47.3 (49.3)

Data are expressed as median±interquartile range.

* $p<0.05$.

** $p<0.01$ significantly different compared to the vehicle group using Mann-Whitney U test.