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Neuroanatomical substrates of the disruptive effect of olanzapine on rat maternal behavior as revealed by c-Fos immunoreactivity

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Abstract

Olanzapine is one of the most widely prescribed atypical antipsychotic drugs in the treatment of schizophrenia. Besides its well-known side effect on weight gain, it may also impair human parental behavior. In this study, we took a preclinical approach to examine the behavioral effects of olanzapine on rat maternal behavior and investigated the associated neural basis using the c-Fos immunohistochemistry. On postpartum Days 6–8, Sprague-Dawley mother rats were given a single injection of sterile water or olanzapine (1.0, 3.0 or 5.0 mg/kg, sc). Maternal behavior was tested 2 h later, after which rats were sacrificed and brain tissues were collected. Ten brain regions that were either implicated in the action of antipsychotic drugs and/or in the regulation of maternal behavior were examined for c-Fos immunoreactivity. Acute olanzapine treatment dose-dependently disrupted various components of maternal behavior (e.g., pup retrieval, pup licking, nest building, crouching) and increased c-Fos immunoreactivity in the medial prefrontal cortex (mPFC), nucleus accumbens shell and core (NAs and NAc), dorsolateral striatum (DLSt), ventral lateral septum (LSv), central amygdala (CeA) and ventral tegmental area (VTA), important brain areas generally implicated in the incentive motivation and reward processing. In contrast, olanzapine treatment did not alter c-Fos in the medial preoptic nucleus (MPN), ventral bed nucleus of the stria terminalis (vBST) and medial amygdala (MeA), the core brain areas directly involved in the mediation of rat maternal behavior. These findings suggest that olanzapine disrupts rat maternal behavior primarily by suppressing incentive motivation and reward processing via its action on the mesocorticolimbic dopamine systems, other limbic and striatal areas, but not by disrupting the core processes involved in the mediation of maternal behavior in particular.

Keywords

c-Fos; olanzapine; antipsychotic drugs; maternal behavior; rat

1. Introduction

Several studies have found that over half of the women with schizophrenia are also mothers, a rate that is comparable with the general population (Seeman, 2004). Like mothers with

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other mental illnesses, most mothers with schizophrenia raise their own children (Abel et al., 2005), feel the pride of looking after a child, and many demonstrate a desire to take responsibility despite their mental illness and often adverse circumstances (Mowbray et al., 1995). Studies on the mother-child relationship reveal that the quality of maternal care from schizophrenic mothers is generally inferior to that from healthy mothers (Bosanac et al., 2003, Wan et al., 2008). One contributing factor recognized by clinicians and patients is the antipsychotic medications. Mother patients are aware of the problems of taking antipsychotic drugs, and some mothers are reported purposely missing their medications in order to stay alert and focused on their child (Seeman, 2004).

In recent years, we have utilized the rat maternal behavior model to investigate the behavioral and neurobiological mechanisms of antipsychotic action in maternal behavior (Zhao and Li, 2009a, b, 2010). Rat maternal behavior is a natural and complex behavior system that cuts across mammalian species and shares many direct features with human mothering behaviors (Fleming and Corter, 1988, Rosenblatt, 1989). In addition, the neural (e.g., the mesolimbic DA system, extended amygdala, etc.) and neurochemical substrates (e.g., dopamine, estrogen, etc.) of maternal behavior have also been implicated in schizophrenia and are important for the therapeutic effects of antipsychotics (Carlsson, 1978, Kulkarni et al., 2001, Meltzer et al., 1989, Seeman, 1987). In the early studies, we showed that a variety of antipsychotic drugs (e.g., haloperidol, clozapine, olanzapine, etc) possess a common disruptive effect on active maternal responses (e.g., pup retrieval, pup licking, nest building) in postpartum rats. In addition, different antipsychotic drugs display different behavioral profiles. For example, acute haloperidol treatment produces a prolonged disruption (>6 h), whereas acute clozapine produces a transient disruption (<6 h) (Li et al., 2004a). Both drugs disrupt active maternal responses primarily by suppressing maternal motivation, as mother-pup separation, a technique known to increase maternal motivation, is able to attenuate the maternal disruptive effect of these drugs. Clozapine-induced sedation also contributes to its disruption (Zhao and Li, 2009b). Finally, different antipsychotic drugs disrupt maternal behavior through different neurochemical and neuroanatomical mechanisms. For instance, haloperidol appears to work primarily by blocking dopamine D₂ receptors in the nucleus accumbens shell, whereas clozapine works primarily by blocking 5-HT_{2A/2C} receptors in the nucleus accumbens shell and possibly 5-HT_{2A/2C} receptors in the prefrontal cortex and lateral septum (Zhao and Li, 2009a, 2010).

The present study extended this line of research and investigated the behavioral effect and associated neural basis of olanzapine in rat maternal behavior. Olanzapine is one of most widely prescribed atypical antipsychotic drugs with a high antagonist action against serotonin 5-HT_{2A/2C} receptors, in addition to its action on dopamine D₂ receptors (Bymaster et al., 1999a, b). Mechanistically, it shares the D₂ antagonism with haloperidol and clozapine, and 5-HT_{2A/2C} antagonism with clozapine. Thus, it resides in the pharmacological space in between (or combined) haloperidol and clozapine in terms of D₂ occupancy coupled with 5-HT_{2A/2C} and other actions. Our previous work shows that both acute and chronic olanzapine treatments disrupt active components of maternal behavior (e.g., pup retrieval, pup licking and nest building) (Li et al., 2005). However, in that study, a relatively high dose of olanzapine (7.5 mg/kg, sc) was used, thus it is still not clear whether olanzapine at much lower doses that are commonly used in the behavioral studies of antipsychotic drugs (1.0, 3.0 or 5.0 mg/kg (Kapur et al., 2003; Li et al., 2010; Moy et al., 2001) would also disrupt maternal behavior. Additionally, little is known about the neural basis of this effect of olanzapine. The purpose of the present study was to establish a dose-dependent function of the maternal disruptive effect of olanzapine and to further delineate the neural basis of its action.

2. Materials and methods

2.1. Animals

Experimental naïve pregnant female Sprague–Dawley rats (gestational days 13–15 upon arrival) purchased from Charles River Inc. (Portage, MI) were used in this study. All rats were housed individually in 48.3 cm×26.7 cm×20.3 cm transparent polycarbonate cages under 12-h light/dark conditions (lights on between 6:30 am and 6:30 pm), and had free access to standard laboratory rat chow and tap water in their home cages. The colony was maintained with a controlled temperature (21 ± 1 °C) and a relative humidity of 45–60%. Experiments were conducted during the light cycle. All animal manipulations followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Animal Care and Use Committee at the University of Nebraska-Lincoln.

2.2. Olanzapine doses

The injection solution of olanzapine (a gift from NIMH drug supply program) was obtained by mixing the drug with 1.0% glacial acetic acid in distilled water and administered subcutaneously. We chose olanzapine (OLZ) at doses of 1.0, 3.0 and 5.0 mg/kg because these are the commonly used doses in many behavioral studies of this drug (Kapur et al., 2003; Li et al., 2010; Moy 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 postpartum Day 1), 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). Maternal behavior tests were conducted on postpartum Days 6–8.

2.4. Maternal behavior test

A total of 32 postpartum Sprague–Dawley rats were randomly assigned to four groups ($n=8$ /group): vehicle (VEH, sterile water), OLZ-1.0 mg/kg, OLZ-3.0 mg/kg and OLZ-5.0 mg/kg. The basic procedure for maternal behavior test was identical to that described by Zhao and Li (2009b) with slight modifications. On postpartum Days 6–8, maternal behavior was tested twice in the home cages, with the first test starting at 30 min prior to the drug (three doses of olanzapine) or vehicle injection (i.e., baseline) and the second test occurring at 120 min after drug or vehicle injection. Each test session lasted 20 min and was initiated by taking the 8 pups away from the mother and destroying the nest. Ten seconds later, the pups were placed in the corner of the cage diagonal to the nest site or dam sleeping corner. Pup retrieval (the subject picking up a pup in her mouth and carried it back to the nest site), pup licking (a female rat placing its tongue on the anogenital area and the rest of a pups 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) and crouching (a rat positioning herself over pups with legs splayed to accommodate the pups, including hover, high and low crouching over pups) were recorded by an observer unaware of the drug condition of each subject using a Jwatcher program (<http://www.jwatcher.ucla.edu/>). Approach latency was defined as the time elapsed from the return of the pups till mother rats approaching the pups within 1 cm. 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 1200 s was assigned to non-responders who did not approach or retrieve the testing pups.

2.5. c-Fos immunohistochemistry

Immediately after the second maternal behavior test, four out of eight rats in each group were randomly chosen to be perfused for c-Fos immunohistochemical assay as described in our previous work (Zhao and Li, 2010, Zhao et al., 2012). Briefly, forty-micrometer-thick coronal sections were incubated with a rabbit polyclonal anti-c-Fos antibody (Ab-5, PC38, 1:20000, Calbiochem, CA, USA) for 48 h at 4°C. Sections were then incubated with a biotinylated goat anti-rabbit secondary antibody (1:200, Vector Laboratories, Burlingame, CA, USA) for 2 h at room temperature. They were processed with avidin-biotin horseradish peroxidase complex (1:200, Vectastain Elite ABC Kit, Vector Laboratories). The immunoreaction was visualized with peroxidase substrate (DAB Substrate Kit for Peroxidase, Vector Laboratories). After staining, sections were mounted on gelatin-coated slides, air-dried, dehydrated and coverslipped. As a control, the primary antibody was substituted with normal rabbit serum. No corresponding nucleus or cytoplasm was immunostained in the control.

2.6. Estimate of Fos-immunoreactive (Fos-I) labeling

Photomicrographs were captured with a digital camera (INFINITY lite, Canada) equipped with an Olympus CX41RF microscope (Japan) using $\times 10$ objective lens. Fos-I cells characterized by clearly stained nuclei was counted bilaterally in one section with comparable anatomical levels across the treatment groups. The brain regions analyzed included the neural sites that were either implicated in the action of antipsychotic drugs [e.g., the medial prefrontal cortex (mPFC), nucleus accumbence shell (NAs), nucleus accumbence core (NAc), dorsolateral striatum (DLSt), ventral part of lateral septal nucleus (LSv)] (Robertson and Fibiger, 1992, 1996, Robertson et al., 1994), and/or in the regulation of maternal behavior [e.g., medial preoptic nucleus (MPN), ventral bed nucleus of the stria terminalis (vBST), medial amygdaloid nucleus (MeA), central amygdaloid nucleus (CeA), ventral tegmental area (VTA) and nucleus accumbens shell and core] (Li and Fleming, 2003a, b, Numan and Insel, 2003, Numan et al., 2005). The levels of brain slices examined were presented in Fig. 1. The number of Fos-I cells in a given brain region was counted within a $680 \times 510 \mu\text{m}^2$ unit area using ImageJ software by an observer blind to the experimental condition. In a given area from different groups, the images were first thresholded to the same value by means of eliminating background and noise staining to ensure that all cells containing any Fos-I labeling were selected, and then analyzed. The number of Fos-I nuclei of a given brain region from bilateral sites per rat was averaged. The values from four rats of each treatment group were averaged to obtain the final group mean \pm SEM.

2.7. Statistical analysis

Statistical analyses were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Data for maternal behavior except for latency data and the number of Fos-I cells were expressed as mean \pm SEM and analyzed using a one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) post hoc comparisons. For the latency data, because they were not normally distributed (e.g. the cut-off time set at 1200 s), data for latency were displayed as median \pm interquartile range and nonparametric Kruskal–Wallis test was used for analyzing the difference between the drug treatment groups. Once the overall significant effects were determined, two-group comparisons between the drug and vehicle treatment 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. Olanzapine disrupted various components of rat maternal behavior in a dose-dependent fashion

A single injection of olanzapine dose-dependently disrupted various components of maternal behavior. At 2 h after olanzapine administration, Kruskal–Wallis test revealed a significant overall drug treatment effect on pup approach latency (Chi-square = 19.73, $p < 0.001$), the first (Chi-square = 18.43, $p < 0.001$) and last (Chi-square = 15.45, $p = 0.001$) pup retrieval latency. Mann–Whitney U test showed that rats treated with olanzapine 3.0 and 5.0 mg/kg took significantly longer time to approach and retrieve their pups to the nest in comparison to the vehicle treatment (all $ps < 0.01$), while olanzapine 1.0 mg/kg had no effect on these measures (Table 1). One-way ANOVA revealed a significant drug treatment effect on the number of pups retrieved [$F(3, 28) = 9.70$, $p < 0.001$], pup licking [$F(3, 28) = 13.16$, $p < 0.001$], nest building [$F(3, 28) = 22.90$, $p < 0.001$], and crouching [$F(3, 28) = 10.90$, $p < 0.001$]. Post hoc analysis indicated that rats treated with all three doses of olanzapine spent less time licking and nursing their pups, and building the nest in comparison to the vehicle-treated ones (all $ps < 0.001$; Fig. 2B,C,D). Olanzapine 3.0 and 5.0 mg/kg (both $ps < 0.001$), but not 1.0 mg/kg, also reduced the number of pups retrieved (Fig. 2A). Olanzapine at 3.0 and 5.0 mg/kg significantly reduced the duration of nest building to a greater extent than olanzapine at 1.0 mg/kg (both $ps < 0.05$; Fig. 2C).

3.2. Olanzapine dose-dependently increased c-Fos immunoreactivity in distinct brain regions

Acute olanzapine treatment dose-dependently increased c-Fos immunoreactivity in various brain regions of maternally behaving rats. Of the ten brain regions examined, one-way ANOVA revealed a main effect of the drug treatment on c-Fos immunoreactivity in seven areas (mPFC, NAs, NAc, DLSt, LSv, CeA and VTA) [mPFC: $F(3, 12) = 5.74$, $p = 0.011$; NAs: $F(3, 12) = 63.06$, $p < 0.001$; NAc: $F(3, 12) = 15.15$, $p < 0.001$; DLSt: $F(3, 12) = 171.20$, $p < 0.001$; LSv: $F(3, 12) = 65.78$, $p < 0.001$; CeA: $F(3, 12) = 123.82$, $p < 0.001$; VTA: $F(3, 12) = 158.68$, $p < 0.001$]. Olanzapine at both 3.0 and 5.0 mg/kg significantly increased c-Fos immunoreactivity in all seven regions (all $ps < 0.001$; Figs. 3,4), with a greater effect in NAs and LSv (Fig. 3). Olanzapine 1.0 mg/kg also increased c-Fos immunoreactivity but only in the NAs, DLSt and LSv ($ps < 0.05$; Figs. 3,4). Interestingly, olanzapine at all three tested doses did not have an effect in the vBST, MPN and MeA, three areas known to be critically involved in the regulation of rat maternal behavior (Fig. 3).

4. Discussion

The present study demonstrated that olanzapine exerts a dose-dependent disruptive effect on rat maternal behavior. More importantly, using c-Fos immunoreactivity, we identified the possible brain regions that olanzapine may act on to achieve this disruptive effect. Here we found that olanzapine dose-dependently increased c-Fos immunoreactivity in the mPFC, NAs, NAc, DLSt, LSv, CeA and VTA, but did not alter c-Fos in the MPN, vBST and MeA. These findings suggest that olanzapine may disrupt rat maternal behavior by acting on the mesocorticolimbic, other limbic and striatal areas, but not on the sites involved in the mediation of maternal behavior per se (e.g. MPN, vBST and MeA).

Consistent with our previous study (Li et al., 2005), the present study found that olanzapine at much lower doses still suppressed various components of maternal behavior. The behavioral mechanisms underlying such a disruptive effect are unknown and have not been systematically investigated. Since maternal behavior has motivational as well as motor components, and given that antipsychotics are known to produce motivational and motoric impairments (Ikemoto and Panksepp, 1999; Li et al., 2004b, 2007b, 2009; Salamone and

Correa, 2002; Zhang et al., 2011), it raises an important question as to whether this disruptive effect is motivational or simply motoric. Our findings appear to suggest that olanzapine-induced maternal disruption is not a simple motor suppression, as olanzapine at 1.0 mg/kg had little effect on pup retrieval, an active form of motor responses, although it is sufficient to suppress several motoric responses, such as conditioned avoidance response (Li et al., 2007, 2009, 2012), level pressing (Trevitt et al., 1999). If this effect of olanzapine was a simple motor suppression, we would expect that all maternal responses should have been suppressed. On the basis of this finding, we postulate that the disruptive influence of olanzapine may arise from its effect on maternal motivation. First, olanzapine antagonizes dopamine D₂ receptors in the striatum and it has been shown that clinical doses of olanzapine are best predicted by its D₂ binding affinity (rather than 5-HT₂ or any other receptor activity) (Kapur and Seeman, 2001). Second, olanzapine increased c-Fos expression in the mPFC, VTA and NAs and NAc (Fig. 3), and most studies find that dopamine deficiencies induced by either 6-OHDA lesions or antagonists in these regions give rise to deficits in maternal motivation, but not in maternal performance (Afonso et al., 2007; Hansen, 1994; Stern and Keer, 1999). Behaviorally, many of these brain regions have been implicated in incentive motivation and reward processing (Ahn and Phillips, 2002, Ikemoto and Panksepp, 1999, Taylor and Robbins, 1986; Tzschentke, 2000). They also play an important role in maternal behavior, especially in the appetitive aspect of this behavior (e.g., pup retrieval) (Numan, 2007). For example, lesions of the NA, or mPFC disrupt maternal behavior (Afonso et al., 2007; Hansen et al., 1991a; Li and Fleming, 2003b). Central infusion of DA D₁ or D₂ receptor antagonists into the NAs or infusion of tetrodotoxin or GABA agonists into the mPFC also disrupts maternal behavior (Febo et al., 2010; Keer and Stern, 1999; Numan et al., 2005). Therefore, the c-Fos action of olanzapine in these areas implies that it has a suppressive action on the motivational system. Because 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 (Numan, 2007), we think that it is more likely that olanzapine suppresses the function of the mesocortical and mesolimbic dopamine systems which leads to a general disruption of the translation of motivation-into-action (Mogenson et al. 1980). Nevertheless, because many maternal responses were significantly reduced by olanzapine treatment, with the highest dose producing near-total elimination of behavioral response, it suggests that suppression of motor functions may also contribute to its effects. The finding that olanzapine also significantly increased c-Fos immunoreactivity in the DLSt (a critical brain area involved in motor functions) also supports this idea. We should point out that it is somewhat difficult to completely separate the motivational effect of olanzapine from its motor function because both components overlap considerably and have the common neurochemical and neuroanatomical bases (Salamone, 1987, 1988, 1991, 1992; Salamone et al., 1989). In addition, because olanzapine also gives rise to sedation due to its actions on histamine H₁ receptors and/or adrenergic receptors (Fleischhacker et al., 1994), and sedative effect induced by atypical antipsychotics also contributes to maternal disruption (Zhao and Li, 2009b), olanzapine-induced sedation could also explain part of its disruption. Future work using various behavioral techniques (e.g., pup separation, repeated drug testing regimen, etc) would help reveal the exact behavioral mechanisms underlying the maternal disruptive effect of olanzapine (Zhao and Li, 2009b).

Our c-Fos findings are consistent with many previous studies using the immunohistochemistry and in situ hybridization techniques which show that olanzapine induces an increase in c-Fos protein and c-fos mRNA expression in various limbic and striatal regions, including mPFC, NAs, NAc, DLSt, LSv, CeA, the hypothalamic paraventricular nucleus and locus coeruleus (Kiss et al., 2010, Ohashi et al., 2000, Oka et al., 2004, Robertson and Fibiger, 1996, Sebens et al., 1998, Seillier et al., 2003, Verma et al., 2006). These brain regions have been suggested to mediate olanzapine's antipsychotic

action and its clinical effects. In addition, we also found increased c-Fos immunoreactivity in the VTA, a neural site that has previously been linked to the display of maternal behavior (Hansen et al., 1991b, Numan et al., 2009).

Extensive research has delineated the core neural circuits that mediate the expression of maternal behavior, including the medial preoptic area (MPOA), vBST, MeA and ventromedial of hypothalamus (VMH) (Numan, 2007, Numan and Insel, 2003). The lack of olanzapine effect in the MPN, vBST and MeA is also consistent with our recent work showing that haloperidol (a typical antipsychotic) and clozapine (an atypical antipsychotic) also fail to induce an increase in c-Fos expression in these regions (Zhao and Li, 2010). One possible explanation is the ceiling effect (i.e. c-Fos immunoreactivity was already at a high level in maternally behaving rats and there was no room for further increase) because maternal behavior itself also causes a significant increase in c-Fos expression in these regions (Lonstein and De Vries, 2000, Lonstein et al., 1998, Numan and Numan, 1994, 1995). Therefore, there is a possibility that olanzapine may still act on these regions to achieve its maternal disruptive effect. Testing postpartum female rats in the absence of pups (i.e. not allowing maternal behavior to occur) may help resolve this issue.

The neurochemical basis of the maternal disruptive effect of olanzapine remains to be clarified. Previous work from our laboratory shows that typical antipsychotic haloperidol disrupts active maternal behavior primarily by blocking dopamine D₂ receptors, whereas atypical clozapine achieves its maternal disruptive effect primarily by blocking 5-HT_{2A/2C} receptors (Zhao and Li, 2009a). We found that pretreatment of quinpirole (0.5 or 1.0 mg/kg, sc), a selective D₂/D₃ dopaminergic receptor agonist, but not 2,5-dimethoxy-4-iodoamphetamine (DOI, 1.0 or 2.5 mg/kg, sc), a selective 5-HT_{2A/2C} serotonergic receptor agonist, dose-dependently improved the HAL-induced disruption of pup approach, pup retrieval, pup licking and nest building, whereas pretreatment of DOI, but not QUI, dose-dependently improved the CLZ-induced disruption of pup approach, pup retrieval and pup licking. Olanzapine, as a widely prescribed atypical antipsychotic, exhibits a broad receptor binding profile. On the one hand, it resembles clozapine in its dual antagonistic action on D₂ and 5-HT_{2A/2C} receptors (Bymaster et al., 1999a, b), thus, it may disrupt maternal behavior through similar mechanisms as those of clozapine. On the other hand, because olanzapine also resembles haloperidol with its high affinities for D₁ and D₂ receptors but a weaker affinity for adrenergic α_1 receptor (Bymaster et al., 1999a, b), it thus may achieve its disruptive effect via similar mechanisms as those of haloperidol.

A number of factors are known to cause an increase in c-Fos immunoreactivity, including stress, pharmacological manipulations, sensory stimulations from pups and exhibition of various behaviors (e.g., maternal care and maternal aggression). Given that our subjects were under the influence of many of these factors when they were sacrificed, the observed changes in c-Fos immunoreactivity are likely to reflect the combined impacts of these factors, as opposed to the pharmacological effect of olanzapine alone. It is conceivable that drug-induced alterations in maternal responses might also have changed Fos immunoreactivity, as maternal behavior itself could activate Fos expression in various examined brain areas (Fleming et al., 1994; Kalinichev et al., 2000; Lonstein et al., 1998; Numan and Numan, 1994, 1995). Also, c-Fos immunoreactivity was assessed at 140 min after the drug administration, by this time, the impact of olanzapine on c-Fos immunoreactivity may have somewhat waned while the impact of altered maternal behavior might have increased. This is because Fos activation typically peaks 1.5–2.0 h after pharmacological manipulations. When interpreting our c-Fos data, it is important to keep this caveat in mind. These data alone should be regarded as preliminary in delineating the neuroanatomical basis of olanzapine effect in maternal behavior. It still remains to be determined the behavioral significance of olanzapine-induced c-Fos immunoreactivity in

each of these regions. Future work using a microinjection technique (i.e. directly infusion of olanzapine into these brain regions) may help address this issue.

Taken together, the present study demonstrated that olanzapine dose-dependently disrupted major components of maternal behavior. It may affect the neuronal functions in the mPFC, nucleus accumbens, DLSt, LSv, CeA and VTA to achieve its maternal disruptive effect.

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List of Abbreviations

CeA	central amygdaloid nucleus
DLSt	dorsolateral striatum
LSv	ventral part of lateral septal nucleus
MeA	medial amygdaloid nucleus
mPFC	medial prefrontal cortex
MPN	medial preoptic nucleus
NAc	nucleus accumbens core
NAs	nucleus accumbens shell
OLZ	olanzapine
vBST	ventral bed nucleus of the stria terminalis
VTA	ventral tegmental area

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Highlights

- Acute olanzapine treatment dose-dependently disrupted maternal behavior.
- Acute olanzapine increased c-Fos in the mPFC, NAs, NAc, DLSt, LSv, CeA and VTA.
- Acute olanzapine treatment did not alter c-Fos expression in the MPN, vBST and MeA.

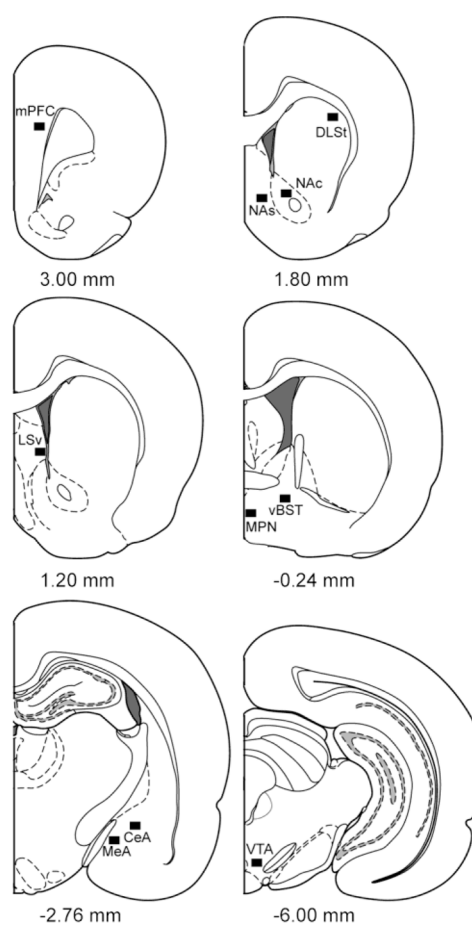


Fig. 1.

Schematic representation of the brain regions (black boxes) in which the c-Fos immunoreactive neurons were counted. Distance from Bregma in the rostrocaudal planes is indicated. Drawings were modified from the atlas of Paxinos and Watson (2007).

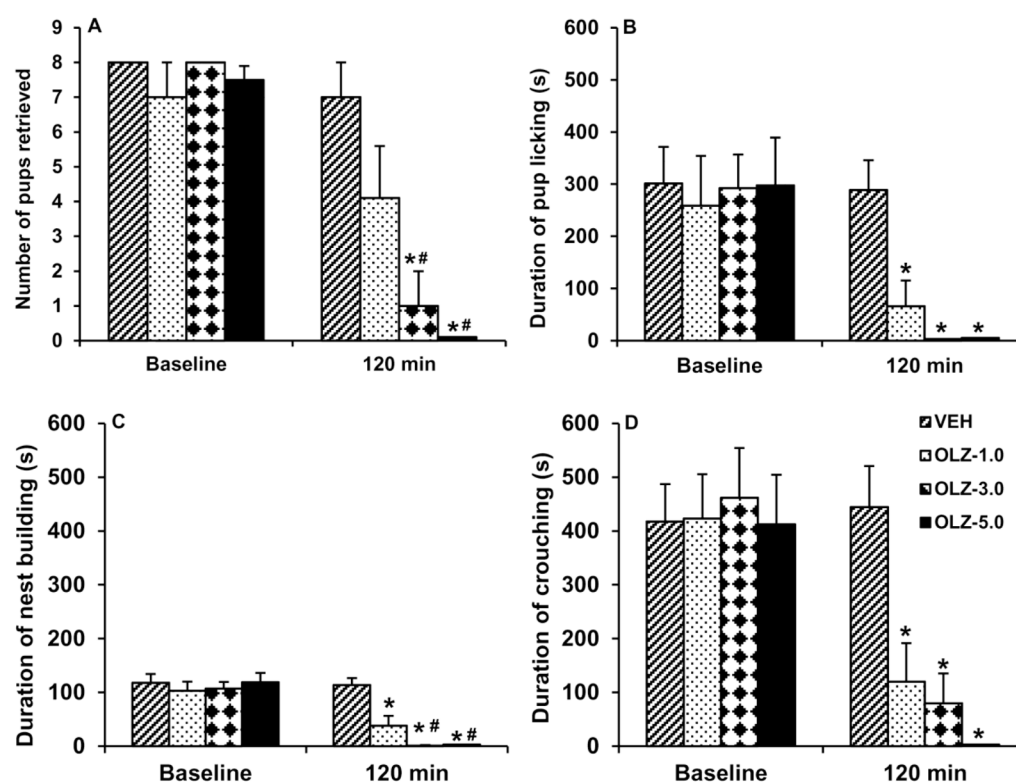


Fig. 2.

Effects of olanzapine treatment on maternal behavior in postpartum female rats. Pup retrieval (A), pup licking (B), nest building (C) and crouching (D) were tested at baseline and 120 min after injection of olanzapine or vehicle. Olanzapine dose-dependently disrupted all components of maternal behaviors. Each bar represents the mean + SEM of duplicate determinations from eight rats. * $P < 0.05$ versus VEH control; # $p < 0.05$ versus OLZ-1.0 group.

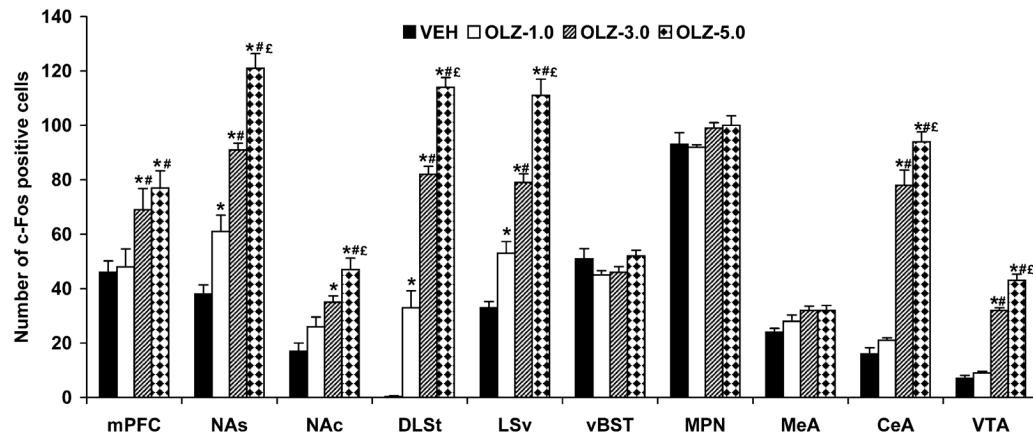


Fig. 3.

Effects of olanzapine treatment on c-Fos immunoreactivity in various brain regions. Olanzapine dose-dependently increased c-Fos immunoreactivity in the mPFC, NAs, NAc, DLSt, LSv, CeA and VTA, whereas was without effect in the vBST, MPN and MeA. Each bar represents the mean + SEM of duplicate determinations from four rats. * $P < 0.05$ versus VEH control; # $p < 0.05$ versus OLZ-1.0 group, £ $P < 0.05$ versus OLZ-3.0 group.

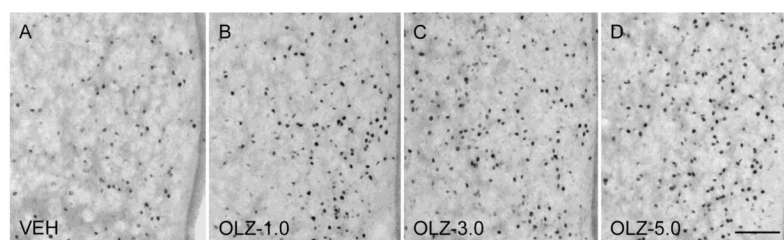


Fig. 4.

Sample c-Fos staining photomicrographs showing the effects of olanzapine treatment on c-Fos immunoreactivity in the ventral part of lateral septal nucleus (LSv). Note that in comparison to the vehicle treatment (A), olanzapine dose-dependently increased c-Fos immunoreactivity in the LSv (B,C,D). Scale bar = 100 μ m.

Table 1

Pup approach latency and pup retrieval latency in postpartum female rats treated with vehicle and olanzapine

Groups	Approach latency (s)		First pup retrieval latency (s)		Last pup retrieval latency (s)	
	Baseline	120 min	Baseline	120 min	Baseline	120 min
VEH	3.0 (3.8)	3.5 (3.8)	8.5 (75.3)	10.5 (15.5)	55.0 (644.3)	63.5 (190.3)
OLZ-1.0	3.5 (2.6)	7.0 (50.5)	11.5 (276.0)	11.0 (256.8)	112.0 (483.3)	892.0 (1139.5)
OLZ-3.0	5.0 (13.0)	37.0 * (188.0)	40.0 (464.5)	1200.0 * (849.0)	82.5 (603.0)	1200.0 * (0.0)
OLZ-5.0	2.5 (2.5)	1200.0 * (0.0)	5.0 (4.5)	1200.0 * (0.0)	52.0 (156.3)	1200.0 * (0.0)

Data are expressed as median \pm interquartile range.

* $P < 0.05$ indicates a significant difference between the vehicle and olanzapine treatment.