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Differently lasting effects of prenatal and postnatal chronic clozapine/haloperidol on activity and memory in mouse offspring

Jian Hong Wang [a,b], Jian Zhen Yang [a], Fraser A.W. Wilson [a], Yuan Ye Ma [a,*]

[a] *Key Laboratory of Animal Models and Human Disease Mechanisms and Section of Cognitive Brain Research, Kunming Institute of Zoology, Chinese Academy of Sciences, #32 Jiao Chang Dong Lu, Kunming, Yunnan, 650223, PR China*
[b] *Graduate School of Chinese Academy of Sciences, Beijing, PR China*

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Abstract

We evaluated the behavioral effects of chronic haloperidol (HAL) and clozapine (CLO) during gestation and CNS development, compared with transient treatments that stopped 1–3 weeks before the test. *Results*: 1) Chronic HAL (6 mg/l in drinking water) but not HAL-withdrawal caused hypo-activity in open-field test on postnatal days (PNDs) 35, 42 and 56. However, hyper-activity was found in both CLO (90 mg/l) and CLO-withdrawal pups. 2) In the step-through test, retention performance was significantly higher in HAL-treated mice than in the controls on PND 42, as well as in withdrawal mice on PNDs 35 and 42. However, both chronic CLO (90 mg/l) exposure and CLO-withdrawal tended to improve the acquisition of memory. Furthermore, chronic CLO (180 mg/l) ameliorated scopolamine (3 mg/kg)-induced impairment of memory on PND 56. 3) In the water-maze test, both chronic HAL and HAL-withdrawal treatments significantly impaired performance on the 4th training day at PND 35, but not PNDs 42 and 56. Neither chronic CLO exposure nor CLO-withdrawal affected spatial memory. 4) Body weight following HAL/CLO administration decreased when compared with the controls during PND 21–35, but approached control levels at PND 40. *Conclusion*: HAL doesn't elicit permanent behavioral changes in offspring. By contrast, CLO had longer-lasting effects than HAL. The pups at age before PND 35 seem more sensitive to HAL/CLO than elder pups.

Keywords: Haloperidol; Clozapine; Offspring; Open-field; Step-through; Water-maze

1. Introduction

Haloperidol, a typical antipsychotic agent of the butyrophenone group, is an antagonist of dopamine (DA) receptors in general and D2 subtypes in particular (Seeman, 1987). It may cause extrapyramidal symptoms (EPS) (Tandon and Jibson, 2002) and a sedating effect, which may lead to a decreased speed on cognitive tasks involving motor output. Therefore, it has been reported that haloperidol is more potent in blocking motility, but at the risk of impaired cognitive function (Gallhofer et al., 1996).

Pre- and postnatal administration of haloperidol can influence the development of the DA system including the nigrostriatal pathways in the offspring. Backhouse et al., 1982 found that prenatal exposure to haloperidol reduced the cell proliferation in the brain, decreased D1 and D2 receptor densities in the caudate and nucleus accumbens (Scalzo et al., 1989), increased the striatal D2 receptor binding (Fox et al., 1994), attenuated DA autoreceptor function (Scalzo and Spear, 1985), reduced offspring brain weight (Williams et al., 1992). Moreover, haloperidol can alter the developmental accumulation of central catecholaminergic neurotransmitters (Hill and Engblom, 1984). However, whether chronic haloperidol exposure during gestation and postnatal (PN) development temporarily or permanently affect the behavior in the offspring is still unclear.

On the other hand, clozapine, an atypical antipsychotic agent, has selectivity but is a less potent dopamine antagonist than haloperidol by showing a lower affinity for D2 receptors, a little higher affinity (10 times as strongly as for binding D2 receptors)

* Corresponding author. Tel.: +86 871 5193083; fax: +86 871 5191823.
E-mail address: yuanma0716@vip.sina.com (Y.Y. Ma).

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for D4 receptors in contrast to haloperidol (Brunello et al., 1995; Schotte et al., 1996; Arnt and Skarsfeldt, 1998). By definition, clozapine has high affinity for many of the receptors including acetylcholinergic, serotoninergic (5-HT6, 5-HT2A), the histamine H1 and α-adrenergic receptors (Meltzer et al., 1989; Leysen et al., 1993; Brunello et al., 1995).

Unlike haloperidol, repeated clozapine influence the developing brain through decreasing D1 receptors in dorsolateral frontal and medial prefrontal cortex (MPC) of juvenile rats (Moran-Gates et al., 2006).

Additionally, it has been known that dopaminergic activity is involved in memory function. However, conventional antipsychotics, which primarily block dopamine D2 receptors, do not favorably affect cognitive function in schizophrenia (Hagger et al., 1993; Sharma, 1999), whereas the atypical neuroleptic drugs might have a beneficial effect on cognitive impairment in schizophrenia (Meltzer and McGurk, 1999).

Similarly, in rodents, both acute haloperidol and clozapine failed to impair the learning and memory in passive avoidance and in an eight-arm radial maze (Baratti et al., 1983; Ichihara et al., 1988; Wolff and Leander, 2003). However, chronic exposure to haloperidol and clozapine impaired learning performance in the water-maze and eight-arm radial maze (Terry et al., 2002; Rosengarten and Quartermain, 2002).

It's important for us to know whether prenatal chronic administrations of antipsychotics permanently or temporally influence the behaviour in offspring, particularly compared with drug withdrawal. In our present study, we evaluated the behavioral effects of chronic haloperidol and clozapine during gestation and postnatal (PN) development in mouse offspring at different ages, compared with transient treatments that stopped 1–3 weeks before the test.

Considering there are critical periods during rat development and these periods appear to be particularly sensitive to pharmacological treatments, which may lead to permanent disturbances in adulthood. DA system undergoes developmental changes until as long as 2 months postnatally in both biochemical and behavioral studies (Shalaby and Spear, 1980b; Hedner and Lundborg, 1985). Dopaminergic nerve terminal growth into the developing rat striatum found changes in DA neurons as late as postnatal week 8(Le et al., 1992). Behavioral studies have demonstrated that the D2 receptor is functional at 21 days of age but not at 10 days of age in rat (Lin and Walters, 1994). In addition, D2 receptors are functionally coupled to second messenger system by postnatal week 2 (De Vries et al., 1992). Therefore, in order to know the effects of antipsychotics on behavior in offspring mice at different developmental periods, we chose mouse offspring at postnatal day (PND) 35 (as pre-matured mice), PND 42 (as sexually matured mice) and PND 56 (as physically matured mice) to conduct the behavioral tests.

Behavioral tests included an open-field test to measure locomotor activity in mice offspring. A one trial passive avoidance (PA) paradigm and a water-maze were used to access memory in the offspring. The PA task is an electric shock-punished non-spatial task, whereas the water-maze task asks the animals to escape from the water and requires learning about spatial cues.

Additionally, scopolamine, an M cholinergic receptor antagonist, was used to identify the possible mechanism of clozapine as a partial agonist at the M cholinergic receptor in the passive avoidance paradigm.

Since psychotropic medications are commonly prescribed for women of childbearing age, the identification of an antipsychotic with minimal effects on cognition is of particular importance besides some primary effects such as teratogenicity, neonatal toxicity, and postnatal behavioral sequelae in young children. Moreover, it's still ambiguous with regards to how long the effects of the antipsychotics will last in the offspring, particularly on cognitive function. In the present study, two antipsychotic agents, a typical (haloperidol) and an atypical (clozapine) antipsychotic agents were used to take an insight into the clinical treatment of antipsychotics in breeding mother and newborn children.

2. Materials and methods

2.1. Animals

2.1.1. Treatment, reproductive parameters and maternal data

Male and female adult outbred albino Kunming strain mice (30–34 g body weight, 9 weeks of age, originally introduced from Swiss mice at the Hoffkine Institute, India in 1944.) from breeding colonies at the Kunming Institute of Zoology were mated. Cages containing two female mice and one male each were placed in standard environment (a 12-hr light/dark cycle with light on from 07:00 to 19:00 hr, temperature was: 21±2 °C) with food and vehicle. Male and female mice were bred together for 3–4 days. Male mice were removed after mating. The pregnant mice, 1 or 2 in each cage, were housed under standard conditions with food and normal vehicle or vehicle containing 6 mg/l of haloperidol (Product of Shanghai Medical Company), 90 mg/l or 180 mg/l of clozapine (Product of Shanghai Medical Company). The water intake was not restricted but recorded at different ages of mice offspring.

2.2. Offspring studies

All the pregnant rats were allowed to give birth and nurture their offspring normally. At PND 21, the pups were weaned and separated by gender with 8–10 pups keeping in each cage and housing under standard conditions. Pups were weighted each week since they were weaned. We averagely allotted the litters with the gender to different groups for behavioral testing: groups for continuously drinking vehicle containing haloperidol/clozapine until behavioral tests at the ages of PND 35, 42 and 56. Other separated groups were respectively stopped the drug drinking and replaced with the normal water 1, 2 and 3 weeks before the behavioral tests at the ages of PND 35, 42 and 56. Control groups drank normal water.

The offspring mice were exposed to the experimenter and the testing environment for 1 week before the tests started. They performed the behavioral testing on PND 35, 42 and 56 respectively. Testing procedures were conducted between 08:30 a.m. and 13:00 p.m. Male and female pups were equally distributed within the total number of tested animals. The expeiments were conducted

according to the guidelines for the national Care and Use of Animals approved by the National Animal Research Authority.

2.3. Drug treatment

Drugs were dissolved in drinking water and administered daily to the pregnant mice. During the pregnant period, the dose of haloperidol (HAL) for mice was 1 mg/kg (Alberch et al., 1991; Lang et al., 1992), doses of clozapine (CLO) were 15 mg/kg and 30 mg/kg (Lang et al., 1992). We measured the average daily water consumption in the pregnant female mice and counted the doses of 1 mg/kg per day as 6 mg/l in drinking water for haloperidol, 15 mg/kg and 30 mg/kg per day as 90 mg/l and 180 mg/l in drinking water respectively for clozapine. Pups continued drinking HAL (6 mg/l), CLO (90, 180 mg/l) water until PND 35, 42 and 56 of age or started drinking normal water 1, 2 and 3 weeks before the behavioral tests. Control pups were drinking normal water all the time during the experiment.

The doses of haloperidol and clozapine in pups may be different during development since the average daily water consumption might change as the offspring grew and change with time as animals become habituated to the taste of the drugs. Thus, we use the doses of drugs in volume instead of in weight.

Scopolamine (Sigma Company, USA.; Scop.) 3 mg/kg was intraperitoneally (i.p.) administered in the volume of 0.2 ml per mouse 15 min before the passive avoidance training, saline (0.2 ml each mouse) was i.p. injected to the controls mice at the same time.

2.4. Behavioral apparatus and method

2.4.1. Open-field behaviour

The open-field test measures the activity and habituation response of animals on placement in a novel environment. This test can effectively detect behavioral changes resulting from prenatal exposure to drug administration, and other factors in rodents (Archer, 1973).

Two grey iron boxes were 40.0 cm × 40.0 cm × 17.0 cm (width/length/height), the floor consisting of a white plastic pad painted with black grids dividing the field into 25 (5 × 5) equal squares. Locomotor activity in male and female mice was measured in separate boxes. The open-field behaviors of male and female offspring were measured at PND 35, PND 42 and PND 56. Each mouse was placed in a certain corner of the box with its head toward the wall, and allowed to explore the box for 3 min. The number of open-field ambulations (floor units mouse entered with both feet), rearings (the animal stood on its hind legs) and faecal droppings were recorded. The device was washed with clean water after the tests in order to obviate possible biasing effects due to odor clues left by previous mice.

2.5. Passive avoidance response

Experimental sessions were conducted using two sets of GEMINI Active and Passive Avoidance System (San Diego Instruments, USA.) connected to a computer. Each system has a bright and a dark compartment with a computer-controlled door between them. Each animal was familiarized with the behavioral apparatus for 2–3 min the day before the training session. Female and male mice were tested in different GEMINI systems to avoid disturbance from the smell of different gender. The delivering of electric shocks and the raising and lowering of the door were controlled by the computer. The latencies at which the animals stepped into the dark from the bright compartment were recorded by the computer.

A one trial step-through procedure was used in this experiment. On the training day (day 1), the mouse was placed into the bright compartment with its head toward the wall. The animal was given a foot-shock (0.34 mA, 3 s) whenever it entered the dark compartment. After training, the mice were immediately returned to their cages.

All animals were tested for retention latencies 24 h after the training. Electric shocks were not applied when the mice entered the dark compartment during the retention test. The latencies to enter the dark compartment were recorded by the computer.

2.6. Water-maze test

A rectangular maze (62 cm × 37 cm × 20 cm, width/length/height) was made of grey Plexiglass and consisted of vertical panels inside. There is one safe area with a stairway under the water, three start areas (S1, S2, S3) with movable guillotine doors, and three error areas (E) defined as corners deviated from the correct pathway which are dead ends in the maze. (Wang et al., 1997) (Fig. 1) The maze was filled with 10 cm deep water (temperature was 23 ± 1 °C) and located in a corner of the room with some cues on the wall. There was a light hung 2 m above the centre of the maze.

On day 1, the guillotine door of S1 was closed, the mouse was individually placed into the start area S1 with its head toward the wall and allowed to acclimatize to the safe area with the underwater stairway three times. The maximal time for each trial was 2 min. They were returned to their cages after the acclimation.

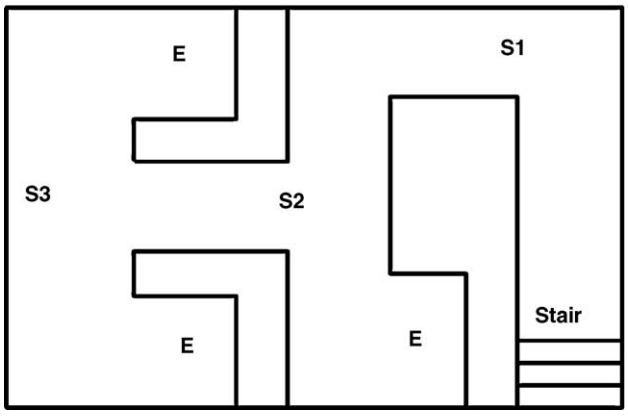
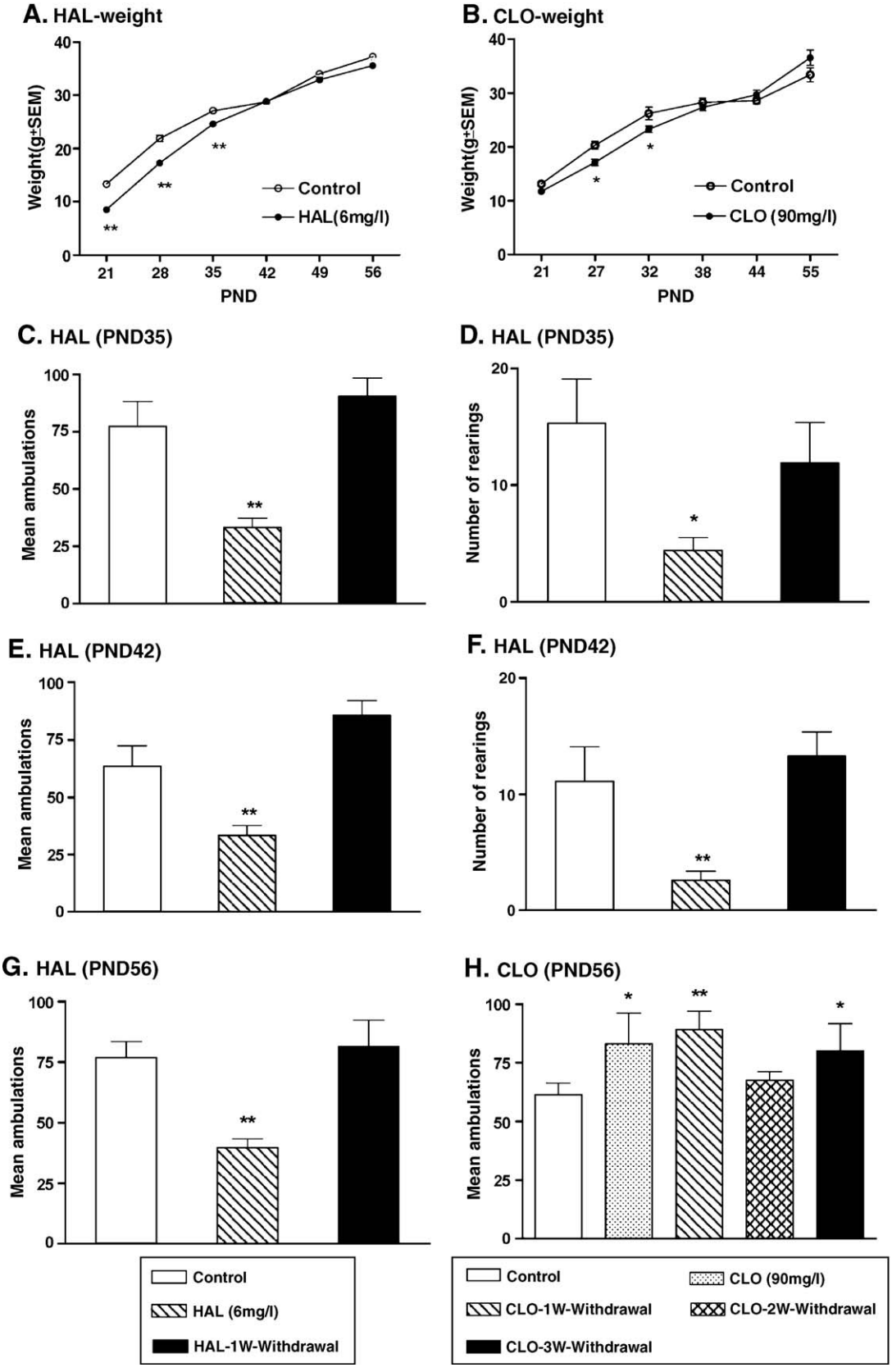


Fig. 1. The figure of the water-maze. S1, S2 and S3: start areas 1–3. Mice were individually placed in the start area with the head toward the wall during training. They started training from S1 on day 1, from S2 on day 2, from S3 on day 3–5. E: error area. There are 3 error areas which are dead ends in the maze. Stair: the underwater stairway as a safe area where the mice could climb out from the water. The maze is filled with water (23±1 °C) 10 cm in depth.


A. HAL-weight
Weight(g±SEM)
PND
Control
HAL(6mg/l)
B. CLO-weight
Weight(g±SEM)
PND
Control
CLO (90mg/l)
C. HAL (PND35)
Mean ambulations
D. HAL (PND35)
Number of rearings
E. HAL (PND42)
Mean ambulations
F. HAL (PND42)
Number of rearings
G. HAL (PND56)
Mean ambulations
H. CLO (PND56)
Mean ambulations
Control
HAL (6mg/l)
HAL-1W-Withdrawal
Control
CLO (90mg/l)
CLO-1W-Withdrawal
CLO-2W-Withdrawal
CLO-3W-Withdrawal

On day 2, the animal was individually placed into the S2 with its head toward the wall. The guillotine door of S2 was closed, while the door of S1 was open. Mice were taken to their cages after they arrived to the safe area. Mice were removed to the cages if they couldn't find the safe area within 2 min.

On day 3–5, each mouse was placed into the S3 with its head toward the wall. The guillotine doors of S1 and S2 were raised. The training session ended after the mice reached the safe area and climbed out from the water. Latencies for mice arrived at the safe area and the number of errors were recorded.

In the training sessions, the mice were removed from the maze into their cages by the experimenter if they failed to reach the safe area within 2 min.

Male mice were trained after the female mice finished training. The maze was cleaned and water was refreshed every day after the experiment.

The sequence of behavioral tasks for each mouse in different groups was first the step-through test, second the open-field test and last the water-maze test.

2.7. Analysis of data

All data are expressed as the mean ± standard error of the mean (SEM). The statistical package SPSS 11.0 was used. Statistically significant differences between treatments were assessed by the analysis of variance (ANOVA) with repeated measures where appropriate. Between-group comparisons were completed with Post Hoc Comparisons Fisher's Least-Significant-Difference test (LSD). Differences were considered significant if $P<0.05$.

3. Results

3.1. Gestation, body weight of the offspring and general observation

Female mice treated with HAL showed lower birth rate than CLO-treated and control female mice.

As shown in Fig. 2A–B, there were significant differences in the average body weight between the groups during the developmental period (PND 21–35). Pups with the HAL/CLO drink increased in body weight more slowly than the control pups at the beginning of the development (HAL-treated offspring: effect of treatment $F_{(1,70)}=140.72$, $P<0.001$, day × treatment $F_{(2,140)}=4.39$, $P=0.014$. CLO-treated offspring: effect of treatment $F_{(1,45)}=9.84$, $P=0.003$).

However, on PND 42 and 38, neither the haloperidol nor clozapine treated mice showed difference in body weight, their body weights were very close to the controls. This situation lasted for the following days.

Additionally, mice that stopped HAL/CLO administrations showed no significance in body weight when compared with the HAL/CLO treated groups.

Unlike the healthy situation observed in the control pups, poor and few hairs, sedation and transient extrapyramidal symptoms (EPS), such as catalepsy was generally found in pups, particularly in pre-weaning pups, drinking the haloperidol water during the developmental period.

3.2. Open-field behaviour

3.2.1. HAL reduced locomotor activity in mice at PNDs 35, 42 and 56

Haloperidol treatment significantly reduced locomotor activity on PND 35, 42 and 56 in offspring mouse in comparison to the controls. (Fig. 2C–G) Ambulations (number of floor units mice moved) in 3 min test: $F_{(2,27)}=13.7$, $P<0.001$ on PND 35, $F_{(2,32)}=14.9$, $P<0.001$ on PND 42, and $F_{(2,30)}=10.7$, $P<0.001$ on PND 56. Number of rearing (the animal stood on its hind legs) in 3 min: $F_{(2,27)}=3.3$, $P=0.05$ on PND 35, $F_{(2,32)}=7.0$ and $P=0.003$ on PND 42. The number of faecal dropping in 3 min: $F_{(2,32)}=5.5$, $P=0.009$ on PND 42 and $F_{(2,30)}=11.3$, $P<0.001$ on PND 56.

3.2.2. HAL-withdrawal had no effect on locomotor activity

The offspring mice that stopped HAL drinking for 1 week showed no impairment in locomotor activities on PND 35 and 56, when compared with the controls. Furthermore, HAL-1 week-withdrawal treatment increased the activities in pups at the age of PND 42 (Ambulations: $P=0.027$ *vs.* controls). (Fig. 2C–G).

3.2.3. CLO and CLO-withdrawal increased locomotor activity

Increased locomotor activities were found in either mice administered with clozapine or mice with clozapine-withdrawal for 1 week (LSD: $P=0.022$ compared with controls), 2 weeks (LSD: $P=0.004$), and 3 weeks (LSD: $P=0.04$). Main effect of treatment on the number of ambulations $F_{(4,57)}=3.0$, $P=0.025$ (Fig. 2H).

3.3. Passive avoidance response

3.3.1. Effect of HAL/HAL-withdrawal on training latencies in offspring

There were no significant differences in the latencies among all groups on the training day, except HAL-treated mice had a significantly increased latency compared with the control group on PND 42 ($F_{(2,52)}=6.4$, $P=0.003$; LSD: $P=0.01$) (Fig. 3A–C).

Fig. 2. Effect of prenatal and postnatal exposure to haloperidol (6 mg/l) and clozapine (90 mg/l) on mice weight and locomotor activity in the open-field test. Panel A shows chronic haloperidol (HAL) 6 mg/l decreased the weight in young offspring at the age of PND 21 to 35, while haloperidol had no effect on the weight in elder offspring at the age of PND 42 to 56. Panel B shows chronic clozapine (CLO) 90 mg/l decreased the weight in young offspring at the age of PND 21 to 32, while clozapine had no effect on the weight in elder offspring at the age of PND 38–55. Panel C–G show haloperidol (HAL) 6 mg/l significantly decreased the locomotor activity while HAL-1 week-withdrawal didn't alter the locomotor activity when compared with the controls in mice at the age of PND 35(Panel C–D), PND 42(Panel E–F) and PND 56 (Panel G). Panel H shows clozapine (CLO) 90 mg/l and clozapine-withdrawal for 1 week and 3 weeks increased the locomotor activity when compared with the controls in mice at the age of PND 56. Panel A–B: Data are expressed as mean weight (gram) ± SEM. (**$P<0.01$, *$P<0.05$ for the difference in the weight of chronic haloperidol/clozapine treated animals *vs.* controls). Panel C–H: Data are expressed as the mean number of ambulations and rearings respectively. (**$P<0.01$, *$P<0.05$ for the difference in the number of ambulations and rearings in HAL/CLO-treated and CLO-withdrawn mice *vs.* controls).

3.3.2. Effect of HAL on retention latencies in offspring

Prenatal and postnatal exposure to haloperidol didn't reduce the retention latencies on PND 35 ($F_{(1,18)}$=0.004, P=0.95) and PND 56 ($F_{(1,21)}$=0.59, P=0.45) when tested 24 h after the training in comparison to the controls. Moreover, chronic HAL increased the retention latency on PND 42 ($F_{(1,35)}$=7.3, P=0.01). (Fig. 3A–C).

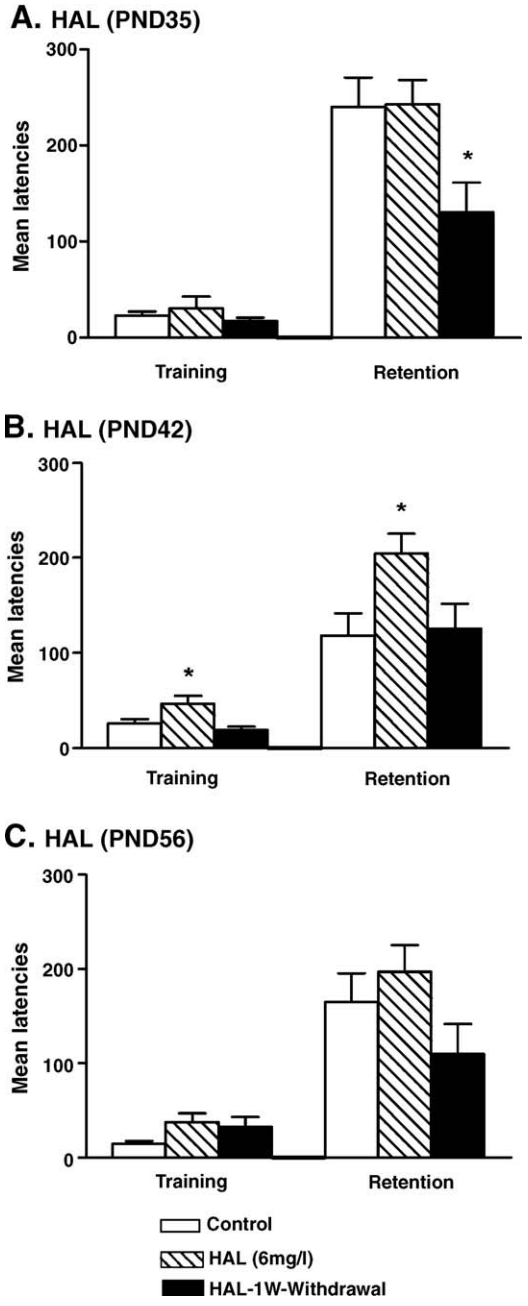


Fig. 3. The effect of prenatal and postnatal exposure to haloperidol (6 mg/l) and haloperidol-withdrawal on training and retention latencies in the step-through paradigm. Panel A shows haloperidol (HAL) 6 mg/l has no effect on training and retention latencies in mice at the age of PND 35 while the HAL-1 week-withdrawal decrease the retention latency when compared with the control animals. Panel B shows haloperidol (HAL) 6 mg/l increase the training and retention latencies when mice were at the age of PND 42. Panel C shows haloperidol (HAL) 6 mg/l had no effect on the latencies when mice were at the age of PND 56, while the haloperidol-1 week-withdrawal tended to decrease the latency when compared with the control animals. Data are expressed as mean latency for mice entered the dark compartment±SEM. (*P<0.05 for difference in latencies of HAL/HAL-withdrawal mice *vs.* control on the training day and retention day respectively.)

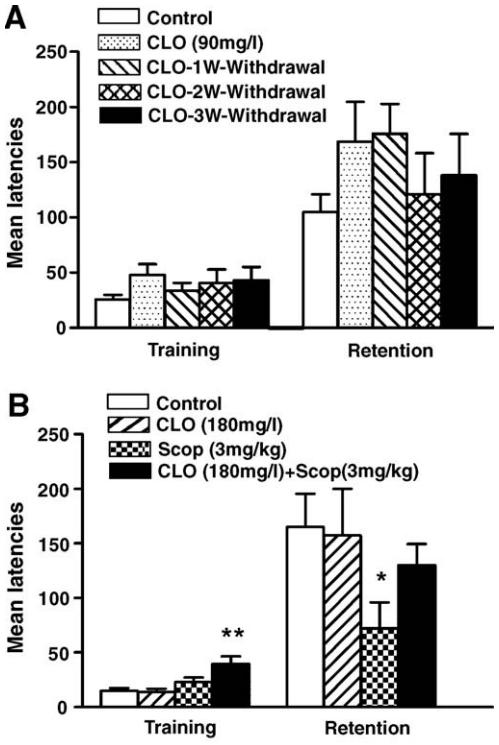


Fig. 4. The effect of prenatal and early postnatal exposure to clozapine, clozapine-withdrawal and scopolamine on retention latencies in the step-through paradigm. Panel A shows prenatal and early postnatal exposure to clozapine (CLO) 90 mg/l and clozapine-withdrawal-1 week, 2 weeks and 3 weeks had no impairment on the training and retention latencies. Panel B shows clozapine (CLO, 180 mg/l) had no effect on the latency in step-through test. However, pre-training injection of scopolamine (Scop., 3 mg/kg) significantly impaired the acquisition of memory in normal mice while scopolamine (3 mg/kg) had no impairment on retention latency in clozapine treated mice. Clozapine(180 mg/l)+scopolamine(3 mg/kg) increased the latency on the training day. Data are expressed as mean latency for mice entered the dark compartment±SEM in the retention test. (*P<0.05, **P<0.01 for difference in performance of scopolamine treated mice *vs.* control on the retention day, and CLO+Scop mice *vs.* control on the training day).

Additionally, HAL-treated animals showed an improved retention performance among three groups (main effect of treatment $F_{(2,51)}$=4.0, P=0.024. LSD: P=0.012 compared with controls, P=0.026 compared with HAL-withdrawn mice) (Fig. 3B).

3.3.3. Effect of HAL-withdrawal on retention latencies in offspring

On the contrary, performance for the retention test was disrupted by HAL-1 week-withdrawal on PND 35 (main effect of treatment on the retention latency $F_{(2,27)}$=4.9, P=0.015. LSD: P=0.012 *vs.* controls, P=0.011 *vs.* HAL groups (Fig. 3A).

On PND 42, HAL-1 week-withdrawal significantly decreased the retention latency when compared with HAL treatment ($F_{(1,32)}$=5.5, P=0.025) (Fig. 3B).

The offspring mice at the age of 56 days with haloperidol withdrawal for 1 week also showed lower retention latencies than the controls and HAL groups but didn't obtain a significant difference ($F_{(1,19)}$=1.6, P=0.23) (Fig. 3C).

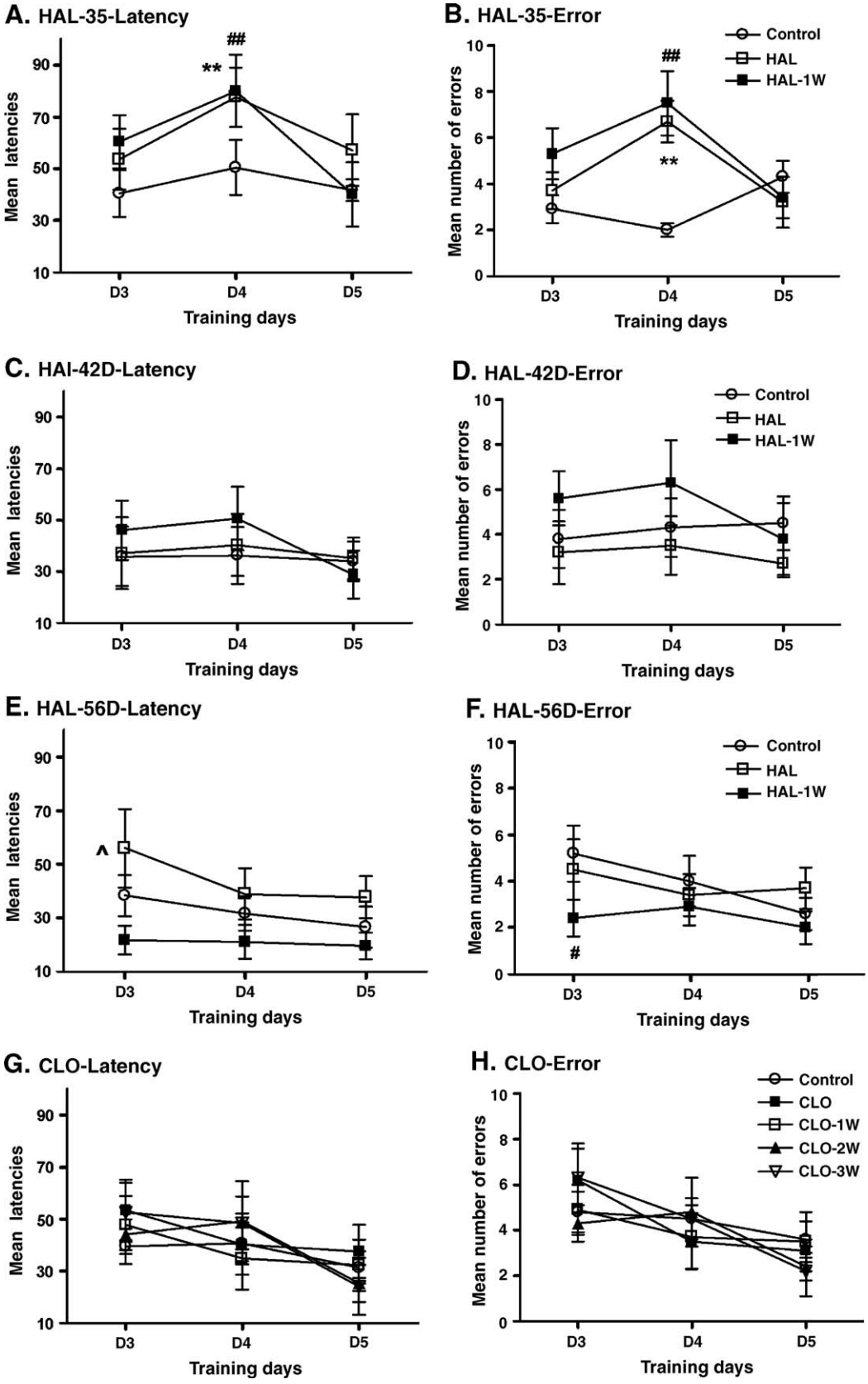


Fig. 5. The effects of prenatal and early postnatal exposure to haloperidol (HAL 6 mg/l)/clozapine (CLO 90 mg/l) and haloperidol/clozapine-withdrawal on performance in the water-maze task. Data were expressed as mean latency for mice arriving at the safe stair or mean number of errors for mice swimming ± S.E.M. on the training days. Panels A and B: haloperidol (HAL) 6 mg/l treated and haloperidol-1W-withdrawn (HAL-1W) mice at age of PND 35 showed increased latencies (A) and number of errors (B) on the 4th day in the water-maze task. Panels C–F: no impairments were shown in HAL and HAL-1W mice at age of PND 42 (C-D) and 56 (E-F) in the water-maze task. Panels G and H: mice treated with CLO 90 mg/l and CLO-withdrawal for 1, 2 and 3 weeks did not show impaired learning and memory in the water maze at PND 56. (**, $^{\#\#}P<0.01$ for difference in performance of HAL treated and HAL-1W-withdrawn mice vs control on the 4th training day).

Thus, HAL-1 week-withdrawal caused a reduction in retention latencies, but HAL treatment did not.

3.3.4. Effect of CLO/CLO-withdrawal on training and retention latencies in mice at PND 56

Neither CLO (90 mg/l) treatment nor CLO (90 mg/l)-withdrawal for 1, 2 and 3 weeks caused significant alternation in training and retention (Fig. 4A) latencies when compared with controls on PND 56 in step-through test ($F_{(4,70)}=1.5$, $P=0.23$).

However, CLO and CLO-1 week-withdrawal tended to improve the memory (Fig. 4A).

No significance was found in the retention latencies between CLO(180 mg/l) treated and control mice (Fig 4B) on PND 56 ($F_{(1,17)}=0.02$, $P=0.88$), whereas scopolamine significantly impaired the acquisition of memory when injected 15 min before the training ($F_{(1,19)}=5.5$, $P=0.029$). However, scopolamine (3 mg/kg) failed to disrupt the retention latency in CLO (180 mg/l) treated mice ($F_{(1,18)}=0.84$, $P=0.37$).

On the training day, CLO(180 mg/l) combined with scopolamine (3 mg/kg) increased training latency when compared with the control. ($F_{(3,34)}=6.9$, $P=0.001$; LSD: $P<0.001$) (Fig. 4B).

There were no significant differences in training and retention performance between the male and female offspring mice (data not shown).

3.4. Water-maze test

3.4.1. Effect of HAL/HAL-withdrawal on mice at PND 35

Since latencies would be confounded with movement effects, thus we suggested that the number of errors should be a potent measurement in this study.

Both HAL and HAL-withdrawal treatment significantly impaired the performance when mice were at the age of 35 days in the water-maze. (Main effect of treatment: latency, $F_{(2,25)}=6.49$, $P=0.005$; the number of errors, $F_{(2,25)}=5.05$, $P=0.014$.)

On the 4th training day, HAL and HAL-withdrawal showed poor scores in the latencies ($F_{(2,25)}=10.94$, $P<0.001$. LSD: $P<0.001$, HAL/ HAL-withdrawal *vs.* control) and in the number of errors ($F_{(2,25)}=7.61$, $P=0.003$. LSD: $P=0.002$, HAL/HAL-withdrawal *vs.* control) (Fig. 5A–B).

On the 3rd training day, low performance was also found in HAL and HAL-withdrawal treated mice but didn't obtain a significant difference in latencies and the number of errors.

However on the 5th day of the training process, all groups had similar latencies and number of errors.

3.4.2. Effect of HAL/HAL-withdrawal on mice at PND 42

When mice were tested on PND 42, HAL-1 week-withdrawal tended to increase in both latency and number of errors on the 3rd and the 4th day of the training but didn't obtain a significant difference in comparison with the controls. All groups had similar latencies and number of errors on the 5th day (Fig. 5C–D).

3.4.3. Effect of HAL/HAL-withdrawal on mice at PND 56

Moreover, when mice were tested at the age of 56 days, haloperidol pups showed poor performance (main effect of treatment: $F_{(2,30)}=5.98$, $P=0.007$).

Higher latencies were found in HAL-treated mice when compared with the HAL-withdrawal mice on the 3rd day of the training (LSD: $P=0.031$) (Fig. 5E–F). But no difference was found in the number of errors.

3.4.4. Effect of CLO/CLO-withdrawal on mice at PND 56

In both clozapine (90 mg/l) treated pups and CLO-withdrawal for 1, 2 and 3 weeks pups, no significant differences in latency and the number of errors were found during the training processes in the water-maze task (Fig. 5G–H).

4. Discussion

In this study, we compared the behavioral effects of prenatal haloperidol and clozapine exposure with postnatal withdrawal at different stages in mouse offspring. The behavioral tests include the non-spatial (Passive Avoidance Response; PAR), spatial (Water-maze) memory paradigms and locomotor activity (Open-field) tests. Haloperidol and clozapine were administered by oral water since the female mice were pregnant and lasted to the postnatal period in offspring. Our main finding was that prenatal and postnatal exposure to haloperidol did not produce permanent alterations on the cognitive function and locomotor activity in the offspring at the ages of postnatal 35 days, 42 days and 56 days. In contrast, clozapine had a longer lasting effect than haloperidol.

4.1. Comparing effects of haloperidol and haloperidol-withdrawal on behaviour

4.1.1. PA and water-maze tasks

Prenatal and postnatal exposure to haloperidol (HAL) improved the acquisition of non-spatial memory in step-through test in mouse offspring at postnatal 42 days of age (sexually mature, PND 42), which is in line with recent research in humans that low doses of haloperidol has a beneficial effect on neurocognitive function in patients, with very little difference from atypical medications (Keefe et al., 2004).

However, such effects were not found in mice at pre-mature (PND 35) and body-mature (PND 56) which is consistent with the general agreement in schizophrenia (Sharma et al., 2003) and in rats (Gemperle et al., 2003; Abdul-Monim et al., 2003) that typical neuroleptics don't improve cognition like atypical neuroleptics usually do.

It is worth noting that the control group had a longer latency on PND 35 than in the elder offspring (PND 42 and 56) demonstrating that the retention memory may attenuate as they age. However, the latencies in haloperidol treated mice were stable (above or around 200 s) during PND 35–56.

In contrast to the haloperidol treatment, 1 week-withdrawal of haloperidol caused deterioration in memory among mice at the ages of PND 35, 42 and 56. It is partly consistent with Gilbertson and van Kammen, 1997 finding in schizophrenic patients that withdrawal of haloperidol for 3 weeks produced significant decreases in recent verbal memory, with significant increases in remote verbal memory compared with haloperidol-maintained patients.

Andersen and Gazzara, 1996 found D2 antagonist sulpiride increased dopamine release in the neostriatum in rats at 5, 10 and 15 days of age. Additionally, chronic administration of haloperidol or a couple of weeks after haloperidol exposure during lactation leads to an increase in striatal D2 receptor binding (Fox et al., 1994). Whereas evidence of increased dopamine-receptor sensitivity was observed in the pups if haloperidol was administered to their mothers postpartum during nursing (Rosengarten and Friedhoff, 1979). Altered D2 gene activity may contribute to increased striatal D2 density after haloperidol treatment, but the evidence is equivocal (Rogue et al., 1991; Xu et al., 1992; Fox et al., 1994).

Dopamine D2 receptors exert a tonic inhibitory control on acetylcholine release since long-term blockade of D2 receptor with haloperidol increased striatal acetylcholine release to a maximum of 80% (Imperato et al., 1995).

Considering critical involvement of the striatum in passive avoidance learning and memory (Prado-Alcala et al., 1975), the striatal D2 super-sensitivity and an increased acetylcholine release induced by chronic haloperidol may be involved in the improvement on acquisition of memory in PA task.

Withdrawal of haloperidol may cause dopamine receptor down-regulation. Additionally, transient withdrawal from haloperidol may interfere with the striatal cholinergic system as well.

In the passive avoidance paradigm, both antipsychotics treated and withdrawn mice entered the dark compartment on the training day (day 1) in less than 50 s, suggesting that the longer retention latencies in haloperidol treated mice were due to improved memory but not the reductions in locomotor activity.

In the water-maze, both haloperidol-treated and-withdrawn mice showed decreases in spatial learning (on the 4th day) at the age of postnatal 35 days which was partly in line with previous findings in Morris water-maze (Ploeger et al., 1992; Skarsfeldt, 1996).

Since Eastwood et al., 1997 found that 16 weeks haloperidol administration lead to an increase in synaptophysin mRNA in the striatum and frontoparietal cortex but not in the hippocampus, it seemed that the effect of chronic haloperidol on the striatum is more flexible than on the hippocampus. Haloperidol treatment caused inversed effect in striatum therefore led an improvement of memory in step-through paradigm, and decrease in memory after the withdrawal. However, the effect of haloperidol on the hippocampus was more stable during the young period, which resulted into amnesia in hippocampus-dependent water-maze task both in haloperidol treated and withdrawn offspring.

Mice at elder ages (PND 42 and 56) failed to show any impairment in the spatial memory in water-maze task, suggesting that the hippocampus of mice at young age (before mature) was more sensitive to haloperidol than at post-mature ages.

4.1.2. Locomotor activity and body weight

We found that prenatal and postnatal haloperidol treatment caused sedation which lead to a hypo-activity in mice offspring. However, a transient withdrawal from haloperidol increased the locomotor activity to normal level, suggesting that haloperidol did not cause a permanent change in motor activity after the drug withdrawal. It may be partly explained by what Scalzo and Eastwood have found that prenatal haloperidol exposure did not produce permanent alterations in presynaptic DA autoreceptor function (Scalzo et al., 1989) and a sustained alteration of neuronal plasticity (Eastwood et al., 1997).

Findings in offspring body weight following haloperidol administration during critical developmental stages are conflicting including reduced body weight (Scalzo et al., 1989), no alterations (Rosengarten and Friedhoff, 1979) or increased body weight (Shalaby and Spear, 1980a). However, in our study, decreased fetal body weight at the early age during PND 21–35 was found, whereas fetal body weight approached control levels after PND 40 when haloperidol was prenatally and postnatally administrated, suggesting mice at pre-mature ages were more sensitive than mice at elder ages.

4.2. Comparing effects of clozapine and clozapine-withdrawal on behaviour

Unlike haloperidol, long-lasting (at least 3 weeks) effects of clozapine on passive avoidance paradigm and locomotor activity were found in our experiment.

4.2.1. PA and water-maze tasks

Chronic clozapine treatment and transient withdrawal caused no impairment in the acquisition of memory in the step-through paradigm. Moreover, either chronic clozapine or clozapine withdrawal for 1 and 3 weeks tended to improve memory.

Clozapine has a broader receptor binding profile and more complex pharmacology than haloperidol has. As an atypical antipsychotic agent, clozapine shows improvement in cognition may rely on its ability to increase dopaminergic and cholinergic activity in the prefrontal cortex (PFC) (Ichikawa et al., 2002) and hippocampus (Chung et al., 2004; Johnson et al., 2005) which are vital to cognitive function, antagonism at the 5-HT2a, 5-HT1a, 6, and 7 sites (Stip et al., 2005), and blockade of neurotoxic effects of glutamate (Olney and Farber, 1995). Adrenergic receptors might be also involved in cognitive effects of clozapine as well. Additionally, longer half-life of clozapine than other typical antipsychotics including haloperidol may contribute to this improved memory either (Baldessarini et al., 1993).

Pre- and postnatal clozapine ameliorated the scopolamine-induced memory impairment in step-through test was consistent with the prior reports (Ninan and Kulkarni, 1996). Since clozapine can enhance the release of acetylcholine in the prefrontal cortex and hippocampus via blockade of terminal muscarinic M2 autoreceptors (Ichikawa et al., 2002; Johnson et al., 2005), thus, it can reduce the scopolamine-induced dysfunction in memory.

Neither chronic clozapine nor clozapine-withdrawal treatment altered the spatial memory in water-maze tests which agreed with the general opinion that atypical antipsychotic drugs like clozapine would yield greater cognitive benefits than would haloperidol.

4.2.2. Locomotor activity and body weight

Sedate effects were not found in clozapine-treated pups. However, clozapine administration and clozapine-withdrawal for 1–3 weeks caused hyper-activity when compared with the control mice. According to Bilder, clozapine had a significant beneficial impact on motor performance. This might be explained by clozapine's relative low affinity for the dopamine D2 receptor or

its unique regional distribution of effects within the basal ganglia (Bilder et al., 2002).

Additionally, in our study transient EPS and poor hairs were observed only in HAL-treated pups, particularly in pre-weaning offspring. By contrast, similar situation was not found in CLO-treated mouse offspring.

Typical antipsychotics block postsynaptic dopamine D2 receptors (Farde et al., 1986). When more than 80% blockade occurs, extrapyramidal symptoms (EPS) appear (Farde and Nordstrom, 1992). However, atypical antipsychotics have weaker effect on the dopamine D2 receptors resulted in less EPS than conventional agents. According to Meltzer, 1990, atypical antipsychotics' weak propensity for EPS may be due to their greater affinity to serotonin 5-HT2 receptors than for D2 receptors.

Clinically, this EPS advantage of atypical antipsychotics translates into several important benefits, including less impaired cognition, better negative symptom efficacy, less dysphoria and better overall outcome (Tandon and Jibson, 2002).

Similar to chronic haloperidol, pre- and postnatal clozapine treatment decreased the average body weight in mice offspring at early ages (before sexual and physical mature) but not at elder age, suggesting that there exited a sensitive developmental state to antipsychotic in mouse offspring.

In summary, we may state that administration of clozapine during sensitive periods of rat brain development can have longer prolonged effects on cognitive function and locomotor activity than haloperidol does.

Prenatal and postnatal exposure to haloperidol and clozapine improved or tented to improve acquisition of non-spatial memory in mice offspring. Haloperidol impaired the spatial memory in young offspring before they were sexually matured.

Haloperidol caused hypo-locomotor which could diminish quickly after the medication withdrawal. In contrast, clozapine-induced hyper-locomotor activity and this effect lasted more than at least 3 weeks after clozapine was stopped.

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