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Effects of olanzapine, sertindole and clozapine on MK-801 induced visual memory deficits in mice

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ABSTRACT

We investigated the effects of the second generation antipsychotics olanzapine, sertindole and clozapine on visual recognition memory using the novel object recognition (NOR) test in naive and MK-801-treated animals. The effects of drug treatment on locomotion and anxiety were also determined using the open field test.
Male Balb-c mice were treated with olanzapine (0.2, 0.4 and 0.6 mg/kg; i.p.), sertindole (0.63, 1.3 and 2.5 mg/kg; s.c.) or clozapine (0.5 and 1 mg/kg; i.p.), and cognitive deficits were induced by MK-801 (0.2 mg/kg; i.p.) administration. Olanzapine treatment decreased the ratio index in the NOR test, whereas sertindole and clozapine had no effect in naive mice. MK-801-induced cognitive impairment was reversed by treatment with olanzapine, sertindole or clozapine. While olanzapine, sertindole and clozapine had no effect on the anxiety of naive mice as determined by the open field test, MK-801 significantly increased the total distance traveled, time spent in the center zone and the velocity of the animals. MK-801-induced effects on locomotion and anxiety in the open field test were reversed by olanzapine, sertindole or clozapine treatment. The results of the present study demonstrated that olanzapine, sertindole and clozapine improved cognition in MK-801 treated mice, and indicate that these drugs have a potential to improve cognition in schizophrenia.

1. Introduction

The effects of antipsychotics on cognition are controversial, and classical antipsychotics have been postulated to cause more cognitive deterioration than atypical antipsychotics (Purdon et al., 2001; Gallhofer, 1999). In clinical studies, the second generation atypical antipsychotic agents, like clozapine, ziprasidone, quetiapine and olanzapine, improved cognitive impairment, whereas typical agents, such as haloperidol, had no effect (Harvey and Keefe, 2001; Purdon et al., 2001). In preclinical cognitive tests, varying results on normal cognitive functions have been observed with atypical antipsychotics (Didriksen, 1995; Skarsfeldt, 1996; Didriksen et al., 2006).

Clozapine is considered the "prototypical" atypical antipsychotic. It has proven particularly efficacious in instances in which other antipsychotics fail. Although it is very effective, clozapine is not considered a first-line agent because it can lead to potentially life-threatening side effects (Alvir et al., 1993). Olanzapine is a $5HT_{2A}/D_2$ antagonist that possesses a chemical structure similar to that of clozapine. Even at high doses, it induces only mild extrapyramidal side effects, and it is commonly used in the clinic. Olanzapine has M_1, H_1 and alpha$_1$ antagonistic properties, which all cause sedation (Bymaster et al., 1996). Sertindole is a new atypical antipsychotic with $5HT_{2A}/D_2$ antagonism. In previous studies, it has been shown to have a unique pharmacological profile. Sertindole has been proven to have beneficial effects in treatment-resistant patients; however, it also has cardiovascular side effects (Arnt and Skarsfeldt, 1998). The effects of sertindole on cognitive function in humans have not been extensively investigated.

The N-methyl-D-aspartate (NMDA) receptor is a subclass of ionotropic glutamate receptors. Antagonists of the NMDA receptor block hippocampal long-term potentiation and impair hippocampal-dependent behavior (e.g., spatial memory tasks) (Bischoff and Tiedtke, 1992). Non-competitive antagonists of the NMDA receptor, such as ketamine or phencyclidine (PCP), have strong psychotomimetic effects in humans (Javitt and Zukin, 1991). MK-801 is a non-competitive antagonist that binds to the PCP binding site within the NMDA receptor-ion complex (Wong and Nielsen, 1989). It impairs animal performance in various learning and memory paradigms (Castellano et al., 2001; Riedel et al., 2003). MK-801 also produces various effects on rodent behavior, including deficits in sensory processing (Al-Amin and Schwarzkopf, 1996), hypermotility (Carlsson, 1993), stereotypy and ataxia (Tricklebank et al., 1989).

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The novel object recognition (NOR) task is based on the natural preference of mice to explore novel objects (Ennaceur and Delacour, 1988). It is a relevant non-rewarded test for studying visual learning and memory deficits in schizophrenia. The aim of this study was to investigate the effects of the second generation antipsychotics olanzapine, sertindole and clozapine on the visual recognition memory of naive and MK-801-treated mice.

2. Materials and methods

2.1. Animals

Male inbred BALB/c ByJ mice (MAM TUBİTAK, Gebze, Kocaeli, Turkey) that were 7 weeks old at the time of arrival to the laboratory were used in this study. The animals (4–5 per cage) were housed at 21 ± 1.5 °C under a 12 h light/dark cycle (lights on at 8:00 p.m.). Tap water and food pellets were available ad libitum. All procedures were in compliance with the European Community Council Directive of 24 November 1986, and ethical approval was granted by the Kocaeli University Ethics Committee (Number: AEK 1/2, Kocaeli, Turkey). All animals used were naive to the experimental apparatus. Experiments were conducted between 9:00 and 12:00 in a semi-soundproof and semi-dark room. Different mice were used for each experiment.

2.2. Drugs

MK-801 and clozapine were purchased from Sigma (St. Louis, USA). Olanzapine was a gift from Biofarma (İstanbul, Turkey), and sertindole was a gift from John Arth (Lundbeck Company, Denmark). Olanzapine and clozapine were dissolved in saline supplemented with 0.1 M hydrochloric acid. Sertindole was dissolved in distilled water supplemented with 0.1 M hydrochloric acid. MK-801 was dissolved in saline. All drugs were freshly prepared and given in a volume of 0.1 ml per 10 g of mouse body weight. Control groups received the same volume of vehicle. Drug doses were selected according to behavioral and neurochemical studies, showing that the drugs have the intended effect (Skarsfeldt, 1996; Didriksen et al., 2006; Didriksen et al., 2007).

2.3. Experimental design

Olanzapine (0.2, 0.4 and 0.6 mg/kg), clozapine (0.5 and 1 mg/kg) and MK-801 (0.2 mg/kg) were administered intraperitoneally (i.p.) 60, 30 and 30 min, respectively, before the retention trial of the NOR test or before the open field test; sertindole (0.63, 1.3 and 2.5 mg/kg) was injected subcutaneously (s.c.) 60 min before the test. The number of animals per group ranged from 6 to 10 (Table 1). Different animals were used for each test.

2.4. Novel object recognition test

We used a novel object recognition (NOR) test protocol according to Ennaceur and Delacour (1988) with slight modification. The apparatus consisted of a circular open field (40-cm diameter and 30-cm height) made of PVC with a black-and-white striped cardboard pattern (30 × 20 cm) nailed to one of the walls. The floor was divided into six peripheral sections and one central section of the same dimension. A light bulb above the central section provided constant illumination of approximately 100 lx. The NOR task procedure consisted of three trials: habituation, training and retention. Each mouse was individually habituated to the apparatus for 5 min in the absence of objects (habituation trial). Thirty minutes after the habituation trial, the mouse was placed in the apparatus for the training trial, and two identical objects (moon or butterfly) were hung in a symmetrical position 10 cm above the side wall. The order of objects used per subject per trial was determined randomly. The total time spent exploring the two objects was recorded for 5 min by the experimenter. Exploration of an object was defined as directing the nose toward the object and/or touching it with the nose. After a predetermined retention interval of 1 h, the mouse was placed back into the apparatus for the retention trial; however, during this trial, two dissimilar objects were presented, a familiar one and a new one. The object not used in the training trial was used as the novel object in the retention trial. The animals were then allowed to explore freely for 5 min, and the time spent exploring each object was recorded. If recognition memory was intact, the mouse is expected to spend more time exploring the novel object (Ennaceur and Delacour, 1988). An index of discrimination (DI) was calculated as the time spent exploring the new object (N) divided by the total time exploring both objects (N + R) multiplied by 100. A higher index of discrimination was considered to reflect greater memory retention.

2.5. Open field test

Treatment effects on animal locomotor activity were measured using the open field test. This test is also used to examine anxiety-like behaviors and is used to evaluate anxiolytic treatment (Prut and Belzung, 2003). This experiment was performed as previously described (Belzung, 1999). Briefly, the testing apparatus consisted of a wooden box (33 cm × 33 cm × 30 cm) with an indirect red light. An animal was placed in the center of the test box, and the total distance moved throughout the area, the velocity of locomotion and the time spent in the center zone were recorded using the Ethovision-XT (Noldus) for 5 min. Center zone of the open field was a circle that had a 12-cm diameter.

2.6. Statistics

The index of discrimination (DI), total duration of exploration times of the animals in the NOR test, total distance moved, velocity

Table 1
Name of the groups and n numbers in each group in seperate set of experiments.

Test	Groups (mg/kg) (n)							
NOR test (Fig. 1a)	Control (8)	Ola 0.2 (10)	Ola 0.4 (10)	Ola 0.6 (10)	MK-801 0.2 (8)	0la 0.2 + MK-801 (8)	Ola 0.4 + MK-801 (7)	Ola 0.6 + MK-801 (8)
NOR test (Fig. 1b)	Control (8)	Sert 0.63 (8)	Sert 1.3 (8)	Sert 2.5 (7)	MK-801 0.2 (7)	Sert 0.63 + MK-801 (8)	Sert 1.3 + MK-801 (10)	Sert 2.5 + MK-801 (8)
NOR test (Fig. 1c)	Control (8)	Clo 0.5 (9)	Clo1 (7)	MK-801 0.2 (10)	Clo 0.5 + MK-801 (7)	Clo 1 + MK-801 (8)		
Open Field Test (Fig. 2)	Control (8)	Ola 0.2 (8)	Ola 0.4 (8)	Ola 0.6 (8)	Ola 5 (6)	MK-801 0.2 (6)	Ola 0.2 + MK-801 (6)	Ola 5 + MK-801 (6)
Open Field Test (Fig. 3)	Control (8)	Sert 0.63 (6)	Sert 2.5 (6)	MK-801 0.2 (6)	Sert 0.63 + MK-801 (6)	Sert 2.5 + MK-801 (6)		
Open Field Test (Fig. 4)	Control (8)	Clo 0.5 (6)	Clo1 (6)	MK-801 0.2 (6)	Clo 0.5 + MK-801 (6)	Clo 1 + MK-801 (6)		

and time spent in the center zone in the open field test were analyzed using a two-way ANOVA followed by the Dunnett post-hoc test. Data are expressed as the mean value ± SEM. Differences were considered to be statistically significant when p value was equal or less than 0.05.

3. Results

3.1. Effects of olanzapine, sertindole and clozapine on visual memory in the novel object recognition test

Overall ANOVA demonstrated significant differences between saline-treated and dizocilpine (MK-801)-treated mice which received a single injection of either olanzapine (0.2, 0.4 and 0.6 mg/kg, $F7,61 = 6.73$; $p<0.001$), sertindole (0.63, 1.3 and 2.5 mg/kg, $F7,56 = 2.79$; $p<0.002$) or clozapine (0.5 and 1 mg/kg, $F5,43 = 3.81$; $p<0.007$). Post-hoc comparisons showed that olanzapine significantly decreased the index of discrimination (DI) at 0.4 and 0.6 mg/kg doses ($p<0.01$), while it had no effect at 0.2 mg/kg ($p>0.05$) compared to saline group. MK-801 also reduced the DI in this test ($p<0.01$). Olanzapine at 0.4 and 0.6 mg/kg significantly increased the DI in MK-801-treated mice ($p<0.01$ and $p<0.05$, respectively) (Fig. 1a). Sertindole did not affect DI at the doses tested compared to saline group, but it reversed the MK-801-induced decrease in the DI index with 0.63 ($p<0.05$) and 1.3 mg/kg doses ($p<0.01$) (Fig. 1b). Clozapine at 0.5 and 1 mg/kg had no effect on the DI of naive mice, while it

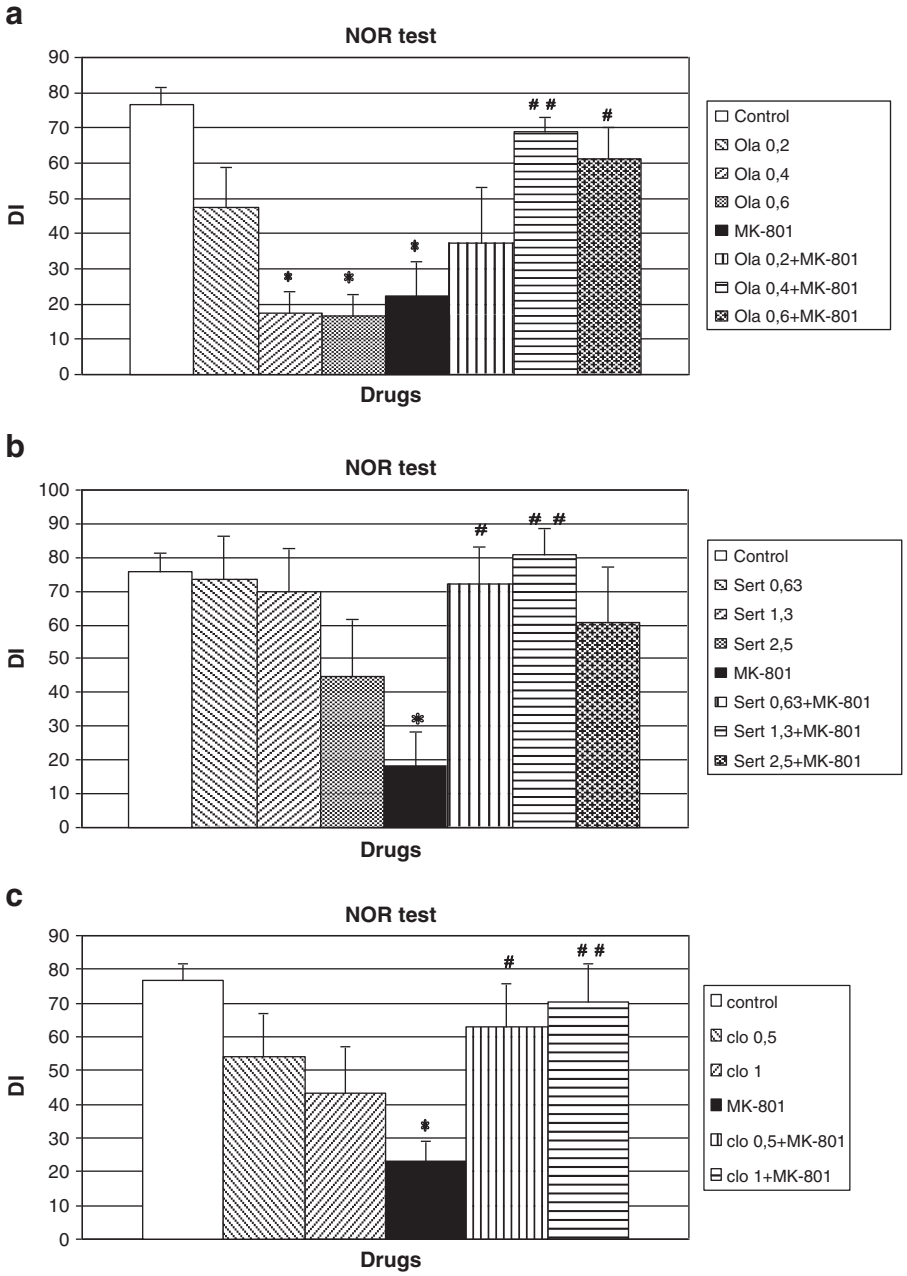

Fig. 1. (Results 3.1.). Effects of drugs on index of discrimination (DI) in the novel object recognition test. Results are expressed as mean ± SEM. a: olanzapine (0.2, 0.4, 0.6 mg/kg), MK-801 (0.2 mg/kg) or olanzapine + MK-801. Drugs were injected 60 and 30 min respectively, prior to testing. * $p<0.01$ vs.control, # $p<0.05$, ## $p<0.01$ vs. MK-801 treated group; b: sertindole (0.63, 1.3, 2.5 mg/kg), MK-801 (0.2 mg/kg) or sertindole + MK-801. Drugs were injected 60 and 30 min respectively, prior to testing. * $p<0.01$ vs. control, # $p<0.05$, ## $p<0.01$ vs. MK-801 treated group; c: clozapine (0.5, 1 mg/kg), MK-801 (0.2 mg/kg) or clozapine + MK-801. Drugs were injected 30 min prior to testing. * $p<0.01$ vs.control, # $p<0.05$, ## $p<0.01$ vs. MK-801 treated group.

Table 2
(Results 3.2.) Effects of drugs on total duration of exploration times for both trials in the novel object recognition test. Results are expressed as mean ± SEM. a: olanzapine (0.2, 0.4, 0.6 mg/kg), MK-801 (0.2 mg/kg) or olanzapine + MK-801. Drugs were injected 60 and 30 min respectively, prior to testing. * $p<0.05$, ** $p<0.001$ vs. control b: sertindole (0.63, 1.3, 2.5 mg/kg), MK-801 (0.2 mg/kg) or sertindole + MK-801. Drugs were injected 60 and 30 min respectively, prior to testing. * $p<0.001$ vs. control c: clozapine (0.5, 1 mg/kg), MK-801 (0.2 mg/kg) or clozapine + MK-801. Drugs were injected 30 min prior to testing. * $p<0.01$ vs. control.

a

Total duration of exploration times	Control	Ola 0.2	Ola 0.4	Ola 0.6	MK-801 (0,2)	0la 0.2 + MK-801	Ola 0.4 + MK-801	Ola 0.6 + MK-801
1st Trial	9.98 ± 2.6	12.65 ± 2.4	8.56 ± 1.8	14.12 ± 2.4	18.07 ± 2.3	8.42 ± 1.8	17.82 ± 3.9	21.30 ± 7.7
2nd Trial	10.87 ± 2.7	9.06 ± 2.6	9.40 ± 2.8	9.54 ± 1.8	41.70 ± 2.6**	35.39 ± 9.1*	30.09 ± 5.4	26.36 ± 6.8

b

Total duration of exploration times	Control	Ser 0.63	Ser 1.3	Ser 2.5	MK-801 (0,2)	Ser 0.63 + MK-801	Ser 1.3 + MK-801	Ser 2.5 + MK-801
1st Trial	15.53 ± 2.8	19.06 ± 6	24.01 ± 4.6	21.61 ± 3.4	17.23 ± 2.5	16.51 ± 4.5	9.98 ± 2.6	15.81 ± 5.6
2nd Trial	11.33 ± 2.5	5.25 ± 1.6	3.75 ± 1	3.16 ± 0.5	37.78 ± 3.3*	27.76 ± 4.5	25.79 ± 5	25.85 ± 5.8

c

Total duration of exploration times	Control	Clo 0.5	Clo 1	MK-801 (0,2)	Clo 0.5 + MK-801	Clo 1 + MK-801
1st Trial	12.67 ± 2.9	19.68 ± 4.4	24.71 ± 4.5	17.04 ± 1.9	11.41 ± 1.7	19.26 ± 4.7
2nd Trial	11.02 ± 2.6	8.99 ± 4.4	23.2 ± 11	41.78 ± 3.8*	18.29 ± 6.4	22.01 ± 7.1

significantly reversed the MK-80-induced reduction in DI ($p<0.05$ and $p<0.01$, respectively) (Fig. 1c).

3.2. Effects of olanzapine, sertindole and clozapine on total duration of exploration times in the novel object recognition test

Overall ANOVA revealed no significant difference between groups in the total time spent exploring the objects in the sample phase in mice treated with olanzapine ($F7,61=1.87$; $p=0.09$), sertindole ($F7,56=1.09$; $p>0.10$) and clozapine ($F5,43=1.69$; $p>0.10$) [Table 2a, b, c]. However, there were significant difference between groups in the total time spent exploring the objects in the choice phase in mice treated with olanzapine (0.2, 0.4 and 0.6 mg/kg, $F7,61=8.47$; $p<0.0001$), sertindole (0.63, 1.3 and 2.5 mg/kg, $F7,56=11.71$; $p<0.0001$) and clozapine (0.5 and 1 mg/kg, $F5,43=4.34$; $p<0.003$). Post-hoc comparisons showed that MK-801 treatment compared to control significantly increased the total time spent exploring the objects in the choice phase ($p<0.001$; $p<0.001$; $p<0.01$, respectively, Table 2a, b, c), however this effect was not reversed by olanzapine, sertindole and clozapine.

3.3. Effects of olanzapine on locomotion and anxiety in the open field test

Overall ANOVA revealed significant difference between groups ($F7, 48=7.86$; $p<0.001$) in the total distance travelled in naive and MK-801-treated mice when challenged with a single injection of olanzapine (0.2, 0.4, 0.6 and 5 mg/kg). Post-hoc comparisons showed that the total distance travelled was significantly increased by MK-801 and decreased by olanzapine (at 5 mg/kg only) compared to control ($p<0.05$). MK-801-induced increase in total distance travelled was prevented by 5 mg/kg olanzapine treatment ($p<0.01$) (Fig. 2a).

The time spent in the center zone of the open-field was a significantly different between groups ($F7,48=19.36$; $p<0.001$). It was significantly increased with MK-801 ($p<0.01$) and unaffected with olanzapine. However, MK-801 increase of centre entries was significantly reversed with olanzapine (0.2 and 5 mg/kg) ($p<0.01$) (Fig. 2b).

A significant difference between groups was also observed on velocity ($F7,48=6.82$; $p<0.001$). It was significantly increased with MK-801 and decreased with 5 mg/kg olanzapine ($p<0.05$). The effect of MK-801 was reversed with olanzapine at 0.2 ($p<0.05$) and 5 mg/kg ($p<0.01$) (Fig. 2c).

3.4. Effects of sertindole on locomotion and anxiety in the open field test

Sertindole treatment (0.63 and 2.5 mg/kg) affected significantly the total distance travelled in naive and MK-801-treated mice in the open field test ($F5,32=5.11$; $p<0.002$). However, post-hoc comparisons showed that sertindole had no effect on the total distance traveled when given alone ($p>0.05$), but it significantly decreased MK-801-induced locomotion ($p<0.01$) at the 0.63 ($p<0.01$) and 2.5 mg/kg doses ($p<0.05$) (Fig. 3a).

A significant difference between the groups was observed on the time spent in the center zone and on animal velocity in the open field ($F5,32=5.42$; $p<0.02$; $F5,32=3.09$; $p<0.03$, respectively). Sertindole treatment did not affect the time spent in the center zone (Fig. 3b) or animal velocity (Fig. 3c). In addition, MK-801-induced increase of the time spent in the center zone (compared to control, $p<0.05$) was not reversed by sertindole. However, sertindole (2.5 mg/kg) treatment significantly reversed MK-801-induced increase of animal velocity ($p<0.05$).

3.5. Effects of clozapine on locomotion and anxiety in the open field test

Significant effects of clozapine (0.5 and 1 mg/kg) treatment on the total distance traveled in naive and MK-801-treated mice were observed in the open field ($F5,32=4.17$; $p<0.006$). Post-hoc comparisons showed that clozapine did not affect the total distance travelled ($p>0.05$), while MK-801 treatment significantly increased this parameter ($p<0.01$). The effect of MK-801 was reversed with clozapine (1 mg/kg; $p<0.05$) (Fig. 4a).

A significant difference between the groups was also observed on the time spent in the center zone and on animal velocity in the open field ($F5,32=10.36$; $p<0.001$; $F5,32=3.97$; $p<0.008$, respectively). Clozapine treatment did not affect the time spent in the center zone (Fig. 4b) or animal velocity (Fig. 4c). The MK-801-induced increase in time spent in the center zone and animal velocity (compared to control, $p<0.01$) were significantly prevented with clozapine (1 mg/kg) treatment ($p<0.01$ and $p<0.05$, respectively).

4. Discussion

This study revealed that visual memory was impaired by olanzapine (0.4 and 0.6 mg/kg) treatment in naive mice, while treatment with sertindole and clozapine had no effect. Each antipsychotic that was tested reversed the visual memory impairment induced by MK-801. In the open field test, only olanzapine at 5 mg/kg disturbed the locomotion of naive mice, while the other treatments did not affect locomotion or anxiety. Each drug treatment reversed MK-801-induced effects on locomotion and anxiety at the higher doses tested.

The NOR test for rodents was formulated by Ennaceur and Delacour (1988), in which the spontaneous exploratory activity toward a novel object and a familiar object is measured. This test does

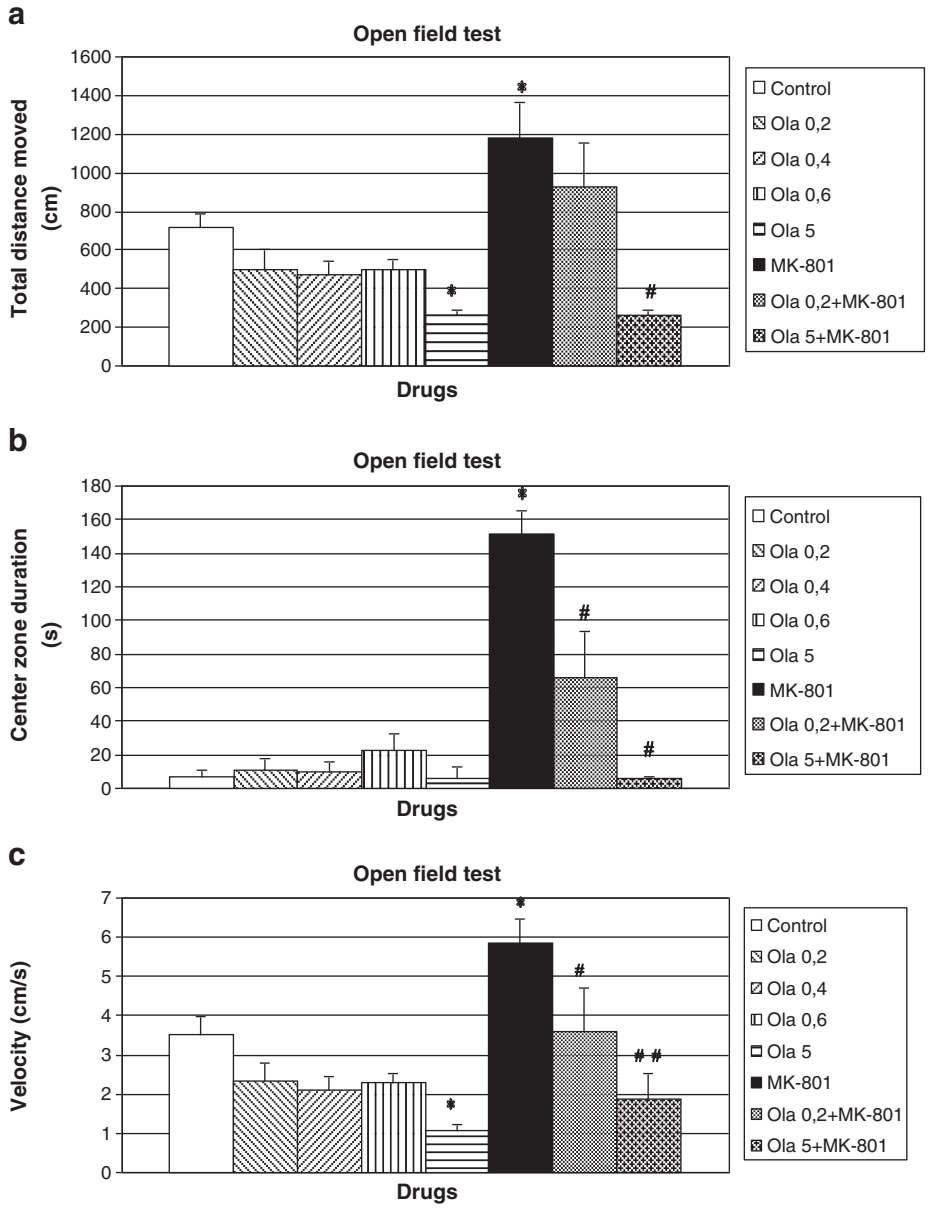

Fig. 2. (Results 3.3.). Effects of olanzapine (0.2, 0.4, 0.6, 5 mg/kg), MK-801 (0.2 mg/kg) or olanzapine + MK-801 administration in open field test. Drugs were injected 60 and 30 min, respectively, prior to testing Results are expressed as mean ± SEM. a: on total distance moved. * $p<0.05$ vs.control, # $p<0.01$ vs. MK-801 treated group; b: on center zone duration. * $p<0.01$ vs. control, # $p<0.01$ vs. MK-801 treated group; c: on velocity * $p<0.05$ vs. control, # $p<0.05$, ## $p<0.01$ vs. MK-801 treated group.

not involve rule learning or reinforcement and is thought to evaluate working and visual memory. In addition, schizophrenic patients demonstrate impaired recognition of visually-presented objects (Calkins et al., 2005). This test has many useful applications to study the neurobiological mechanisms of learning and memory.

Systemic administration of MK-801 impaired both the acquisition and retention of object recognition memory in rats (de Lima et al., 2005), suggesting that NMDA receptor blockade may impair recognition memory. Thus, NMDA antagonist-impaired preference for novel objects in the NOR test is a model to evaluate the effects of drug treatment on cognitive deficits in schizophrenia.

Hall (1934) originally described the open field test for the study of rat emotion. The procedure consists of placing an animal in an unknown environment from which escape is prevented by the surrounding walls (Walsh and Cummins, 1976). The open field test is a very common procedure used in animal psychology (see Belzung, 1999 for a review). In this situation, rodents naturally prefer the periphery of the apparatus to the middle area of the open field. Indeed, mice and rats walk close to the walls, a behavior called thigmotaxis. Treatments that increase the time spent in the central area without impairment of locomotion and vertical exploration are deemed anxiolytic-like, while treatments that decrease these variables produce anxiogenic effects.

Both typical and atypical antipsychotic drugs sometimes display anxiolytic properties. Behavioral studies have reported anxiolytic-like and anxiogenic-like effects and a lack of effect after treatment with typical or atypical antipsychotic drugs in a broad range of animal models of fear or anxiety (Ishida-Tokuda et al., 1996; Timmerman et al., 1990). In recent studies, clozapine, olanzapine and chlordiazepoxide treatment induced anxiolytic-like effects when fear/anxiety was measured (Marx et al., 2006a; Marx et al., 2006b). However, in our study, olanzapine, sertindole and clozapine had no effect on anxiety in naive mice. This disagreement may be due to aspects of methodology, route of administration or dose tested.

In contrast to classical antipsychotics that disturb memory, the effects of atypical antipsychotics on cognition are controversial.

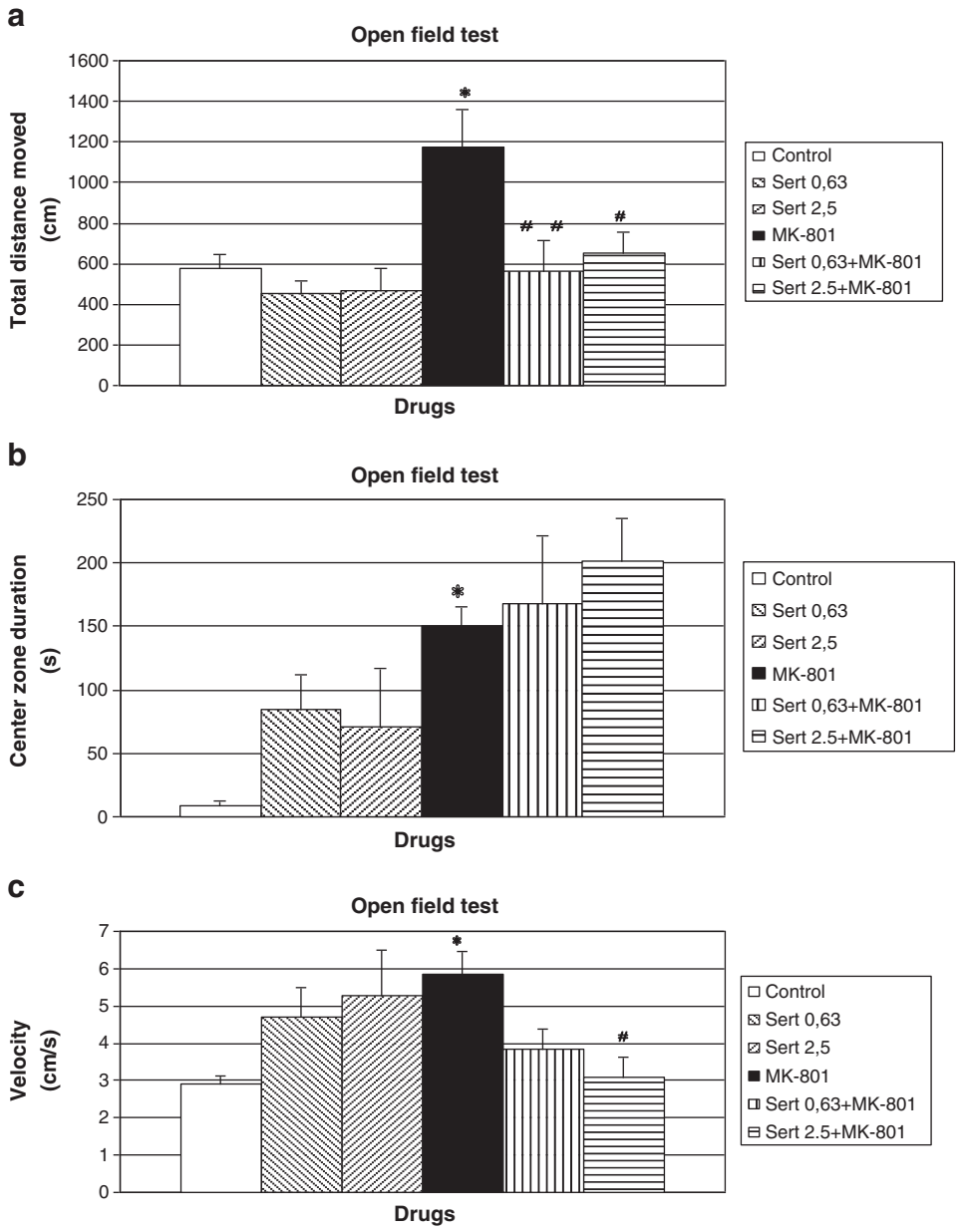

Fig. 3. (Results 3.4.). Effects of sertindole (0.63, 2.5 mg/kg), MK-801 (0.2 mg/kg) or sertindole + MK-801 administration in open field test. Drugs were injected 60 and 30 min, respectively, prior to testing Results are expressed as mean ± SEM. a: on total distance moved. * $p<0.01$ vs. control, # $p<0.05$, ## $p<0.01$ vs. MK-801 treated group; b: on center zone duration. * $p<0.05$ vs. control; c: on velocity * $p<0.05$ vs. control, # $p<0.05$ vs. MK-801 treated group.

Treatment with the dopamine D2 receptor antagonist haloperidol disrupts water maze performance (Skarsfeldt, 1996) and delayed-non-match to position performance (Didriksen, 1995) in rats and further potentiates the disruption after chronic treatment (Didriksen and Sams-Dodd, 1997). Wolff and Leander (2003) concluded that olanzapine has detrimental effects on learning but enhances memory consolidation and/or retention in the delayed radial arm maze test. Haloperidol and risperidone treatment show marked cognitive side effects at doses that are active in animal psychosis models, while clozapine and sertindole were active in psychosis models but did not produce a detrimental effect on cognition (Skarsfeldt, 1996; Arnt and Skarsfeldt, 1998; Didriksen et al., 2006). In our previous study, olanzapine disturbed water maze performance, but sertindole and clozapine induced no cognitive impairment in naive animals; MK-801-induced cognitive impairment was reversed by the second generation antipsychotics olanzapine, sertindole and clozapine in the Morris water maze test (Mutlu et al., 2011).

In our study, each of the second generation antipsychotics investigated reversed MK-801-induced memory impairment in the NOR test, which is similar to our previous study (Mutlu et al., 2011). Olanzapine has numerous properties that may contribute to the improvement of cognition. For instance, it robustly increases acetylcholine release in the hippocampus (Shirazi-Southall et al., 2002) and increases extracellular acetylcholine concentrations in the rat medial prefrontal cortex (Ichikawa et al., 2002). Olanzapine treatment also causes a dose-dependent increase in the extracellular concentration of dopamine in the rat prefrontal cortex (Xi-Ming et al., 1998), which may improve cognitive function. Clozapine has a very broad profile, including significant activity at dopamine receptors (D_1, D_3, D_4), serotonin receptors (5-HT_{2A}, 5-HT_{1A}, 5- $HT_{3,6,7}$) and alpha-1 adrenergic receptors (Arnt and Skarsfeldt, 1998; Leysen 2000), making the evaluation of the mechanism of action very complex. Therefore, to summarize, many variables determine the effects of clozapine on cognition. In a recent study, Hashimoto et al. (2005) showed that clozapine (5 mg/kg), but not

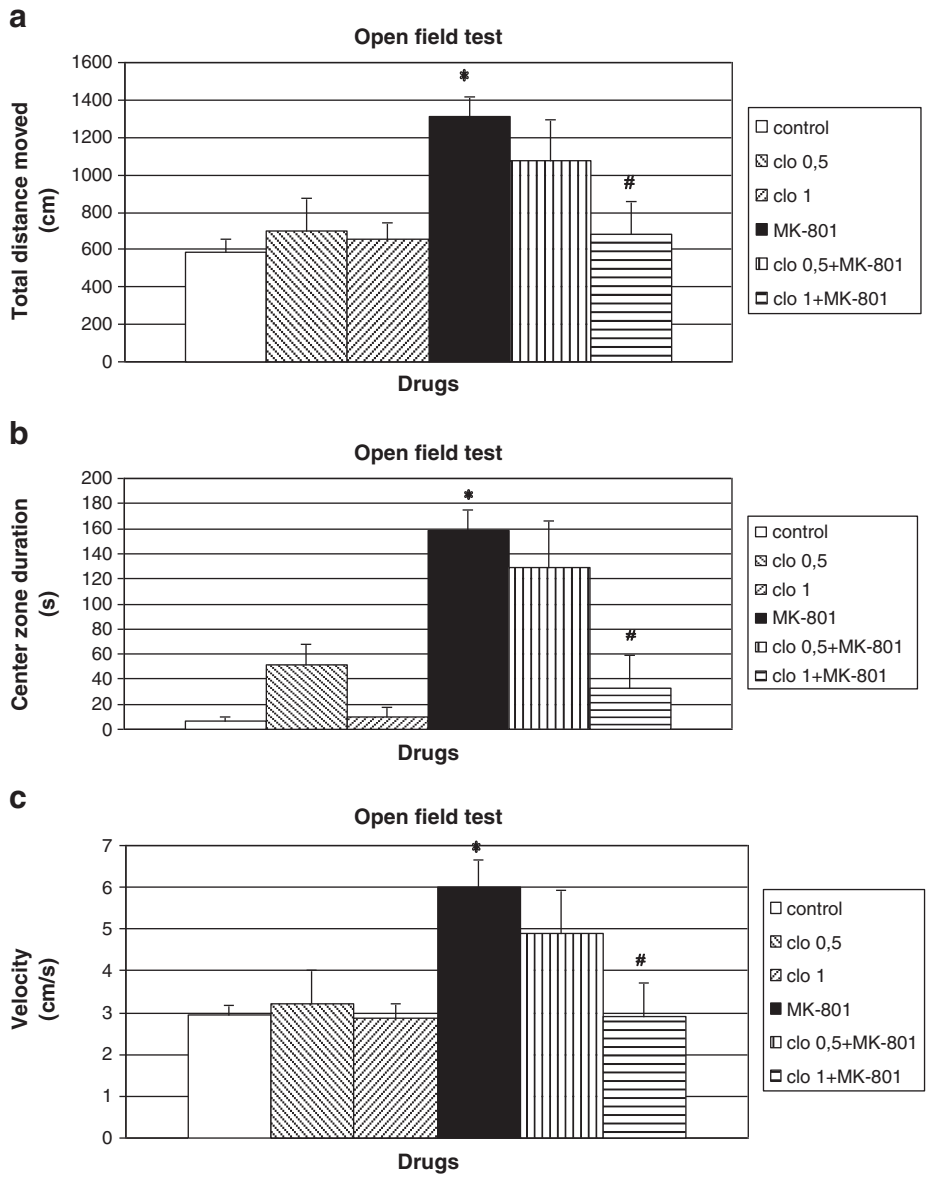

Fig. 4. (Results 3.5.). Effects of clozapine (0.5 and 1 mg/kg), MK-801 (0.2 mg/kg) or clozapine + MK-801 administration in open field test. Drugs were injected 30 min prior to testing Results are expressed as mean ± SEM. a: on total distance moved. * $p<0.01$ vs. control, # $p<0.05$ vs. MK-801 treated group; b: on center zone duration. * $p<0.01$ vs. control, # $p<0.01$ vs. MK-801 treated group; c: on velocity * $p<0.01$ vs. control, # $p<0.05$ vs. MK-801 treated group.

haloperidol (0.1 mg/kg), attenuated the sub-chronic PCP-induced deficit in object recognition in mice. The ability of clozapine to reverse cognitive deficits in the NOR task may be attributed to its high affinity for 5-HT_{2A} receptors (Bymaster et al., 1996). Sertindole has an equal preference for the 5-HT_{2A} and dopamine D_2 receptors in vitro, whereas it exhibits minimal dopamine D_2 receptor blockade in vivo (Arnt and Skarsfeldt, 1998). In contrast to clozapine and olanzapine, sertindole has very high affinity for 5-HT_6 receptors but fails to affect α_2-adrenergic, histaminergic and muscarinic receptors (Arnt and Skarsfeldt, 1998). 5-HT_6 receptor activation enhances cognition, and this receptor is highly expressed in the hippocampus and cortex. In addition, the 5-HT_6 receptor interacts with the cholinergic and glutamatergic systems. Blockade of the 5-HT_6 receptor has pro-cognitive effects and increases dopamine, glutamate, and acetylcholine concentrations in the frontal cortex (Hirst et al., 2006). Therefore, the marked efficacy of sertindole on cognition in both naive mice and MK-801-treated mice may be attributed to the antagonism of 5-HT_6 receptors.

In addition to possessing antagonist activity at the 5-HT_{2A} and dopamine D_2 receptors, clozapine and olanzapine, in contrast to sertindole, block muscarinic and histaminergic H_1 receptors (Zhang and Bymaster, 1999; Schotte et al., 1996). It is well known that inhibition of muscarinic cholinergic transmission impairs cognitive function, as evaluated in the water maze test (Riekkinen et al., 1990). In our study, clozapine had no effect on cognition in naive mice, and it improved MK-801-induced cognitive impairment. This finding may result from agonistic activity of clozapine and desmethyl-clozapine, its primary metabolite, on some muscarinic receptor subtypes (Weiner et al., 2004). In contrast, olanzapine, a muscarinic antagonist, elicits the opposite effect (Bymaster et al., 1996). Muscarinic M_1 agonism may improve cognition, whereas antimuscarinic activity potentially worsens cognitive function. Therefore, the effects of clozapine depend on a balance between the plasma and brain concentration of the compound (Weiner et al., 2004; Didriksen et al., 2007). The antihistaminergic effects of olanzapine (Arnt and Skarsfeldt, 1998; Schotte et al., 1996) may also influence cognitive performance. In human studies that combine functional neuroimaging with cognitive task evaluation, a strong correlation between increased histamine H_1 occupancy and decreased cognitive performance was found (Okamura et al., 2000; Tashiro et al.,

2002). Therefore, the deleterious effects of olanzapine on cognition in naive mice in the NOR test may be associated with its anticholinergic and antihistaminergic properties.

The behavioral syndrome induced by PCP and MK-801 treatment has been suggested to be an animal model of cognitive deficits in schizophrenia (Bardgett et al., 2003). NMDA receptor antagonists produce various dose-dependent motor dysfunctions in rats, which are characterized by locomotor hyperactivity at lower doses and behavioral stereotypes and ataxia at higher doses (Koek et al., 1988). All second generation antipsychotics share potent antagonistic effects at the 5-HT_{2A}, 5-HT_{2C} and α_1-adrenergic receptors. Selective ligands that affect these receptors [e.g., M100907 (5-HT_{2A}) and prazosin (α_1)] inhibit locomotor hyperactivity induced by PCP or by the prototypical NMDA antagonist MK-801 (Gleason and Shannon, 1997). In addition, most typical and atypical antipsychotics reduce hyperactivity and many other behavioral abnormalities produced by noncompetitive NMDA receptor antagonist treatment (Cartmell et al., 2000; Abdul-Monim et al., 2003). Antipsychotics with potent 5-HT_{2A} and α_1-adrenergic antagonistic activity also readily block PCP- or MK-801-induced hyperactivity (Gleason and Shannon, 1997). The ability of antipsychotics to reverse the effects of MK-801 treatment can be explained by a combination of these mechanisms.

MK-801 is known to cause hyperactivity, stereotypies, ataxia and anxiolytic effects in previous studies (Sharma and Kulkarni 1991; Xie and Commissaris 1992); therefore, MK-801 can produce some nonspecific effects on cognition. In our study, MK-801 seemed to produce neophobia for the novel object, and this could be the reason why MK-801-treated mice showed a strong preference for the familiar object even more than expected, because usually in this model, animals showing impaired memory spend approximately equal time exploring the two objects. The reversal of MK-801-induced memory impairment by olanzapine, sertindole and clozapine could also be affected by some nonspecific effects, because except for the clozapine experiment, the doses that are required to restore object recognition memory in MK-801-treated mice are of the same magnitude as those needed to reduce the MK-801-induced hyperactivity.

In conclusion, the present study demonstrates that MK-801 treatment in the NOR test may be a useful model for detecting compounds with therapeutic potential for the treatment of cognitive dysfunction associated with schizophrenia. Future studies are required to elucidate the mechanisms underlying MK-801 effects in this test and the ability of the atypical antipsychotics olanzapine, sertindole and clozapine to reverse the deficits induced by MK-801 administration. An improvement in cognitive function should be used as a target effect in the development of drugs for the treatment of schizophrenia.

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