

Modification of spatial recognition memory and object discrimination after chronic administration of haloperidol, amitriptyline, sodium valproate or olanzapine in normal and anhedonic rats



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Abstract

In the present study we have investigated the effects of a chronic administration of olanzapine (Ola) on visual and spatial memory in normal and anhedonic rats. The effects of Ola have been compared to those of the typical antipsychotic Hal, the tricyclic antidepressant amitriptyline (Ami), and the mood stabilizer VPA. Anhedonia (assessed by reduction of sucrose preference) was induced by administration of a chronic mild stress (CMS) protocol, in which rats were exposed sequentially, over a period of 4 wk, to a variety of unpredictable mild stressors. The spatial memory was evaluated by testing the ability of the rats to discriminate a familiar vs. a novel environment, while the visual memory was assessed by testing the ability of the rats to discriminate familiar vs. novel objects. In CMS-free rats, VPA (5 or 30 mg/kg . d), Ola (0.02 or 0.1 mg/kg . d), Ami (2 mg/kg . d) and Hal (0.2 mg/kg . d) caused no detectable modifications of visual memory, whereas VPA (5 mg/kg . d), Ami (2 mg/kg . d) and Ola (0.02 mg/kg . d) did not modify spatial memory performance. In our experimental conditions, the administration of the CMS protocol caused an impairment of both visual and spatial memory. The chronic treatment of anhedonic rats with Ola (0.02 mg/kg . d) or Ami (2 mg/kg . d) prevented, at least in part, the stress-induced impairment of visuospatial performance. In conclusion, the results of the present preclinical study seem to indicate that the chronic administration of low doses of Ola or Ami has the potential to lead to substantial cognitive benefits in depressed patients.

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Introduction

Several studies have reported findings indicating that cognitive dysfunction is characteristic of psychiatric diseases like schizophrenia and affective disorders (Hoff et al., 1990; Silverstein et al., 1988, 1990). Schizophrenia is characterized by profound disruption in cognition and emotion, affecting the most fundamental human attributes: language, thought,

perception, affect, and sense of self. As cognitive deficits may prevent a schizophrenic patient from retaining or relearning skills that are necessary for community functioning and reintegration, the improvement of these deficits is hypothesized to lead to improved illness outcome (Bilder, 1997; Green, 1996). Patients with major depression were found to be impaired across a range of cognitive domains, including attention/executive function and visuospatial learning and memory (Porter et al., 2003). The cognitive impairment is related to the acute state of illness; however, some neuropsychological deficits in depressed patients may persist after symptom remission (Trichard et al., 1995). Similarly, deficits in executive functions, psychomotor skills, and memory have been

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reported in bipolar patients (van Gorp et al., 1998). Clinical studies suggest that it is often difficult to attribute cognitive dysfunction to illness-specific vs. treatment-related factors. In this respect, evidence exists that acute administration of tricyclic antidepressants with marked sedative or anticholinergic effects may cause cognitive deficits. In depressed patients, however, long-term administration of tricyclics usually causes a normalization of cognitive function that parallels mood improvement (Amado-Boccaro et al., 1995). Although clinical data on cognitive effects of selective serotonin reuptake inhibitors are incomplete, these drugs may produce smaller decrements in performance than tricyclics (Stein and Strickland, 1998). In bipolar patients, older mood stabilizers (such as lithium) have been historically associated with a high potential to interfere with attention, memory as well as motor speed and coordination. Among mood stabilizers, the anti-convulsant sodium valproate (VPA) is considered to have fewer cognitive side-effects than lithium. Stoll et al. (1996) reported that in bipolar patients lithium-associated cognitive deficits were reduced by switching to VPA. However, data from epilepsy trials (Gallassi et al., 1990) suggest that some adverse effects were noted among patients taking VPA with regard to attention, visuomotor processing and global level of performance. Haloperidol (Hal), like other typical antipsychotics, is associated with relief of positive symptoms but relatively little additional therapeutic benefit. In particular, classical antipsychotics do not remediate cognitive dysfunction (King, 1990; Verdoux et al., 1995). Atypical antipsychotic compounds introduce a pharmacological diversity that may, in turn, differentially impact schizophrenic signs and symptoms, including anxiety, depression and cognitive impairment. A meta-analysis of 15 studies that examined the effects of a variety of atypical antipsychotics on cognitive deficits in schizophrenic patients (Keefe et al., 1999) indicated that these drugs, when compared to typical antipsychotics, improved cognitive functions in patients with schizophrenia. Among atypical antipsychotics, olanzapine (Ola) seems to have a favourable cognitive profile. In patients suffering from chronic schizophrenia, a 6-month period of treatment with Ola produced a significantly greater improvement in verbal memory than risperidone or typical antipsychotics (Cuesta et al., 2001). Other reports seem to be in line with these observations (Rybakowski and Borkowska, 2001; Smith et al., 2001). Moreover, recent studies suggest that, in the rat, Ola enhances cholinergic function in both the medial prefrontal cortex (Ichikawa et al.,

2002) and hippocampus (Shirazi-Southall et al., 2002) and this may mediate its ability to improve cognition. In the present study we have investigated the effects of the chronic administration of Ola on visual and spatial memory in normal and anhedonic rats. The effects of Ola have been compared to those of Hal, the tricyclic antidepressant amitriptyline (Ami) and the mood stabilizer VPA. Anhedonia, defined as the loss of the capacity to feel pleasure, constitutes a core symptom of both depression and schizophrenia. In our experimental conditions it has been induced by administration of a chronic mild stress (CMS) protocol, a naturalistic paradigm of a hostile environment that has proven to be especially successful in the functional identification of antidepressant drugs and, therefore, has a high degree of predictive validity (Ferretti et al., 1995; Ghi et al., 1995; Katz, 1982; Papp et al., 1996; Willner et al., 1987).

Methods

Animals

Male albino rats (Charles River Laboratories Italia, Lecco, Italy) of the Wistar strain were used. Prior to start the experiments, the rats were housed in a temperature-controlled colony room (22 ± 2 °C) with free access to food and water, were maintained four per cage under standard laboratory conditions and were submitted to daily handling for at least 2 wk. The weight of the animals at the beginning of the experiments was 180–200 g. Drug- and saline-treated rats were weighed once a week in order to verify their weight gain. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 and D.L. of 27 January 1992 no. 116 (86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Sucrose preference training and testing

Animals were housed individually with two 50-ml graduate tubes containing either 1% sucrose solution or tap water with standard laboratory chow continuously available. They were allowed 24 h to adapt to these two bottles and, within this period, the sucrose consumption was evaluated every 2 h from the beginning of the experiment. Both the water and sucrose intakes were measured by weighing the pre-weighed bottles containing the respective solutions. For each animal, the basal sucrose consumption was evaluated after the conclusion of the training procedure (24–26 h period). Subsequently, sucrose intake was measured

under similar conditions (2-h periods), 3 and 4 wk after the beginning of the treatments both in CMS and CMS-free rats.

CMS protocol

The procedure previously described was followed (Orsetti et al., 2005). The CMS protocol was designed to maximize the unpredictable nature of the stressors. One of the following stressors was administered daily (in random order) over a period of 4 wk: crowding, by placing eight animals in standard individual cages for 24 h, food deprivation for 24 h, 45° cage tilt for 5 h, shaker stress for 10 min, soiled cage for 5 h, intermittent overnight illumination (light on and off every 3 h for 24 h), light on overnight, tail pinch for 2 min, swimming in cold water (16 °C) for 5 min. In developing our CMS protocol, we have made changes to the procedure previously described by Katz (1982), since the severity of the stressors employed was greatly reduced. Indeed, the individual stressors we have used do not include elements like intense foot shock, restraint stress or 48 h water/food deprivation. In this respect, our CMS protocol follows the procedure of CMS adopted by Willner et al. (1987). Immediately after the conclusion of each stress session, the animals were returned to the colony room and maintained in standard conditions until the next stress session of the CMS regime. Non-stressed control animals were housed in a separate room and had no contact with the stressed animals. Sucrose consumption tests were never performed the day after the food deprivation. In drug experiments, stress was continued throughout the treatment period and sucrose preference tests were carried out 24 h after the drug administration.

Evaluation of visuospatial memory

Place recognition test

The apparatus used was a Y-maze made of opaque Plexiglas. Each arm was 50 cm long, 18 cm wide and 35 cm high. The maze was placed in a sound-isolated room equipped with a constant illumination (a 60-W lamp located 150 cm above the centre of the maze). Several visual cues were placed in the testing room near the maze and were kept constant during all the experiments. As previously described (Dellu et al., 1992; Orsetti et al., 2001), the place recognition test consisted of two trials, separated by different retention intervals. In the first trial, one arm of the maze was closed with a guillotine door and rats were allowed to visit the other two open arms for 10 min.

During the second trial, rats had free access to the three arms and were allowed to explore the maze for 5 min.

At the beginning of both trials, each rat was placed in the same arm and was oriented in the opposite direction to the centre of the maze. In our experiments the entry arm was changed randomly to reduce the influence of external cues on animal performance. Similarly, the position of the novel arm (the arm closed by the guillotine door in the training trial) was kept in a random order at the left for half the rats and at the right of the entry arm for the other half. After each trial, the maze was carefully cleaned to eliminate olfactory stimuli. The dependent variables measured in the testing trial were the number of entries made into each arm to determine spatial memory and the total entries into each arm to determine locomotion. A visit was recorded only when the rat entered with its four paws the rectangular space (18 cm × 25 cm) representing the distal half of the arm.

Object discrimination test

A two-arm maze of opaque Plexiglas was placed in a sound-isolated room equipped with constant illumination (a 60-W lamp located 150 cm above the centre of the maze). As previously described by Dellu et al. (1992) and Ghi et al. (1999), the object recognition test consisted of two trials separated by different retention intervals. The objects to be discriminated were glass bottles and metal boxes that were too heavy to be displaced by a rat. In the first trial, two identical objects were placed at the ends of the two arms and the rats were placed in the middle of the maze, their heads oriented in the opposite direction to the objects. They were allowed to explore the maze for 12 min. During the second trial, both objects were replaced by a new and novel (markedly different in material, shape and brightness) and by a new and familiar (identical to the one before). The animals were allowed to explore the maze for 8 min under the same conditions as in the first trial. To determine visual memory, the dependent variable measured in the second trial was the duration of exploration of each object. Exploration was defined as time spent with the head oriented towards and within 2–3 cm of the object. From rat to rat, the nature of the two objects used during the test, whether familiar or novel, was randomized and counterbalanced. The position of the novel stimulus was at the left of the starting place for half the rats and at the right for the other half. The objects and the maze were cleaned, after testing each animal, to eliminate olfactory stimuli.

Drug administration

Separate groups of control (CMS-free) and stressed animals (CMS rats) ($n = 15$ rats per group) were treated intraperitoneally with saline, 2-propyl-pentanoic acid sodium salt (5 or 30 mg/kg.d; sodium valproate, Sigma-Aldrich Co., Milano, Italy), haloperidol hydrochloride (0.2 mg/kg.d; Tocris Cookson Ltd, Bristol, UK), amitriptyline hydrochloride (2 or 5 mg/kg.d; Sigma-Aldrich Co.) and olanzapine (0.02–0.1 or 0.5 mg/kg.d; synthesized at Eli Lilly and Co., Indianapolis, IN, USA). In CMS rats, saline or drug administration was carried out every day (from 15:00 to 16:00 hours, at the end of each stress session) for 4 wk during the administration of CMS protocol.

Statistical analysis

Sucrose preference

Sucrose consumption data were analysed by a mixed designed analysis of variance (ANOVA) using stress and treatment as between factors and testing day as repeated measures factor. Post-hoc comparisons were performed by least significant difference (LSD) test, when appropriate.

Place recognition

The entries made into each arm during the second trial were converted into percentages of total entries made into all three arms. To assess spatial memory within a given condition, a Wilcoxon non-parametric matched paired test was used to compare entries into the novel and other arms. Rats showing intact spatial memory will enter the novel arm more than the other arm, whereas rats with impaired spatial memory will enter the novel and other arms similarly. Differences in spatial memory across groups were evaluated by ANOVA using retention interval (in control experiments) or stress and treatment (in drug experiments) as the independent variables. In all conditions, the dependent variable was a difference score (% entries into the novel arm – % entries into the familiar arm). Total entries (sum of entries into all three arms) were also used to determine whether motivation to explore the maze differed among the groups. LSD post-hoc comparisons were performed when ANOVA reached significance. In all cases a value of $p < 0.05$ was considered to be significant.

Object discrimination

For each experiment, novel object preference was expressed as a preference ratio, which was calculated by

dividing the amount of exploration of the novel object by the total amount of object exploration during the test session. Therefore, a preference ratio above 0.5 would indicate novel object preference, below 0.5 familiar object preference, or equal to 0.5 no preference. Differences in visual memory across groups were evaluated by ANOVA using retention interval (in control experiments) or stress and treatment (in drug experiments) as the independent variables. The dependent variable was the preference ratio. LSD post-hoc comparisons were performed when ANOVA reached significance. In all cases a value of $p < 0.05$ was considered to be significant.

Results

Effect of CMS protocol, saline and drug treatments on rat sucrose preference

The sucrose preference of CMS-free rats and rats subjected to the CMS protocol is reported in Table 1. In our experimental conditions, 4 wk after the beginning of the saline treatment, in the group of CMS rats the sucrose intake fell to 47.3% of basal value (5.82 ± 0.66 g), whereas the intake remained at the same level in the group of CMS-free rats. As shown in Table 1, at the end of the chronic treatment with 2 mg/kg.d Ami, 5 mg/kg.d Ami or 0.02 mg/kg.d Ola, the sucrose preference of CMS rats was not significantly different from the basal sucrose preference of CMS-free rats treated with saline (LSD test: $p = 0.51$, $p = 0.60$ and $p = 0.77$ respectively). These results clearly indicate the efficacy of Ami and the lowest dose of Ola in preventing the CMS-induced anhedonia. In contrast, the administration of 0.2 mg/kg.d Hal, 5 or 30 mg/kg.d VPA, and 0.1 or 0.5 mg/kg.d Ola in CMS rats had no effect (LSD test: respectively $p = 0.83$, $p = 0.25$, $p = 0.48$, $p = 0.23$ and $p = 0.46$ vs. CMS rats treated with saline). Similarly, all drugs tested had no significant effects on sucrose intake of rats not subjected to the CMS protocol.

Visuospatial memory in CMS-free and anhedonic rats

In preliminary experiments, control rats exhibited better spatial memory performance at 60-, 120- and 240-min retention intervals, as indicated by ANOVA [$F(3, 56) = 5.02$, $p = 0.0037$] and subsequent LSD post-hoc test (Figure 1a). As shown in Figure 1b, Wilcoxon tests supported these results with rats tested at 60-, 120- and 240-min retention intervals entering the novel arm more than the familiar arm. Conversely, the group of rats tested at 270-min retention interval

Table 1. Modifications of sucrose intake caused by chronic mild stress (CMS), saline and drug administration. The basal sucrose consumption was measured 24 h before the beginning of CMS protocol or chronic saline/drug administrations. Both CMS and chronic treatments lasted 4 wk and cognitive tests (place recognition and object discrimination) were performed 24 h after the 4th-week sucrose intake evaluation. (Data are shown as means \pm S.E.M.)

	Sucrose intake (g)		
	Basal	3rd week	4th week
CMS-free rats			
Saline	13.1 \pm 0.9	13.3 \pm 0.78	12.88 \pm 0.7
Ami (2 mg/kg . d)	12.4 \pm 1.1	12.8 \pm 0.65	13.02 \pm 0.77
Ami (5 mg/kg . d)	12.8 \pm 0.68	13.2 \pm 0.7	13.4 \pm 0.82
Hal (0.2 mg/kg . d)	12.6 \pm 0.9	12.6 \pm 1.1	12.9 \pm 0.7
VPA (5 mg/kg . d)	13.3 \pm 1.2	12.7 \pm 0.92	12.8 \pm 1.2
VPA (30 mg/kg . d)	12.5 \pm 0.85	12.4 \pm 0.57	13.0 \pm 0.9
Ola (0.02 mg/kg . d)	13.1 \pm 0.52	13.4 \pm 1.3	12.6 \pm 0.83
Ola (0.1 mg/kg . d)	12.3 \pm 0.88	12.9 \pm 1.2	13.2 \pm 0.74
Ola (0.5 mg/kg . d)	13.5 \pm 1.3	12.5 \pm 0.9	12.9 \pm 0.69
CMS rats			
Saline	12.3 \pm 0.81	6.02 \pm 0.48 ^a	5.82 \pm 0.66 ^a
Ami (2 mg/kg . d)	12.9 \pm 0.66	11.5 \pm 1.2 ^b	13.1 \pm 0.56 ^b
Ami (5 mg/kg . d)	13.3 \pm 0.87	11.9 \pm 1.4 ^b	12.6 \pm 0.62 ^b
Hal (0.2 mg/kg . d)	12.3 \pm 0.76	6.33 \pm 0.45 ^a	6.14 \pm 0.8 ^a
VPA (5 mg/kg . d)	13.2 \pm 0.64	7.3 \pm 0.6 ^a	6.9 \pm 0.65 ^a
VPA (30 mg/kg . d)	12.4 \pm 0.7	6.8 \pm 0.82 ^a	7.1 \pm 0.64 ^a
Ola (0.02 mg/kg . d)	12.9 \pm 0.82	13.5 \pm 0.7 ^b	13.7 \pm 0.55 ^b
Ola (0.1 mg/kg . d)	13.4 \pm 0.63	7.2 \pm 0.68 ^a	6.7 \pm 0.8 ^a
Ola (0.5 mg/kg . d)	12.5 \pm 0.84	6.2 \pm 0.61 ^a	6.02 \pm 0.5 ^a

A mixed designed two-way ANOVA applied to these data indicated significant main effects of stress [$F(1, 252) = 84.5$, $p < 0.001$], treatment [$F(8, 252) = 5.58$, $p < 0.001$] and testing day [$F(2, 504) = 64.5$, $p < 0.001$] on sucrose preference and significant stress \times treatment [$F(8, 252) = 5.1$, $p < 0.001$], stress \times testing day [$F(2, 504) = 70$, $p < 0.001$], treatment \times testing day [$F(16, 504) = 4.33$, $p < 0.001$] and stress \times treatment \times testing day [$F(16, 504) = 3.62$, $p < 0.001$] interactions.

^a $p < 0.05$ vs. basal sucrose intake; ^b $p < 0.05$ vs. saline-treated CMS group (LSD post-hoc test).

entered the novel and familiar arms similarly. Therefore, in our experimental conditions the memory for the environment lasted > 240 min but < 270 min. According to these results, in chronic administration experiments both saline- and drug-treated rats, CMS-free or subjected to the CMS protocol, were tested at the 240-min retention interval.

In the object discrimination task the visual memory lasts > 60 min but < 75 min, since in preliminary

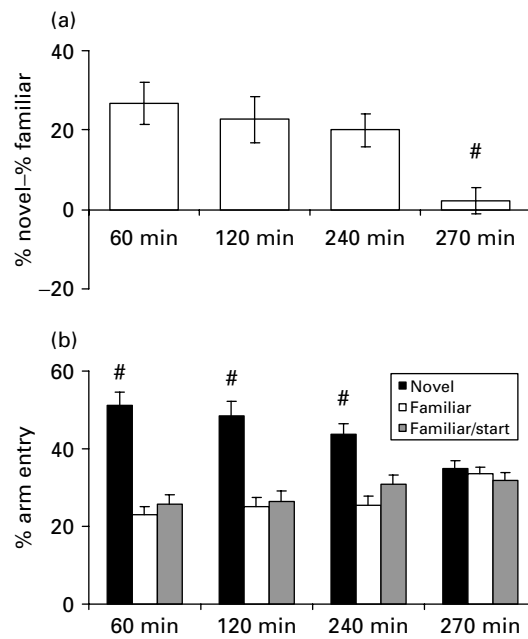


Figure 1. Effect of different retention intervals on spatial memory performance of CMS-free rats (place recognition task). (a) The difference score between the novel and familiar arm was greater for 60-, 120- and 240-min groups compared to 270-min group ([#] $p < 0.05$ vs. other retention intervals, LSD test). (b) Rats tested at 60-, 120- and 240-min retention intervals entered the novel arm more than the other arms, while rats tested at 270 min entered the novel and other arms similarly ([#] $p < 0.05$ vs. other arms, Wilcoxon test).

experiments control rats exhibited novel object preference in the second trial at 15-min ($n = 10$), 30-min ($n = 10$) and 60-min ($n = 12$) retention intervals as indicated by ANOVA [$F(3, 40) = 6.74$, $p < 0.001$] and subsequent LSD post-hoc test. In contrast, after a 75-min interval ($n = 12$), rats exhibited no difference between the exploration times of novel and familiar objects, as measured by the preference ratio (Figure 6a). According to these results, in chronic administration experiments both saline- and drug-treated rats, CMS-free or subjected to the CMS protocol, were tested at 60-min retention interval.

Effects of chronic Ami administration

A two-way ANOVA showed a significant main effect of stress on the difference score for entries [$F(1, 84) = 9.93$, $p = 0.002$] and a significant interaction between stress and treatment [$F(2, 84) = 3.48$, $p = 0.035$]. The main effect for treatment was not significant [$F(2, 84) = 1.99$, $p = 0.14$]. Saline-treated rats and rats treated with 2 mg/kg.d Ami showed

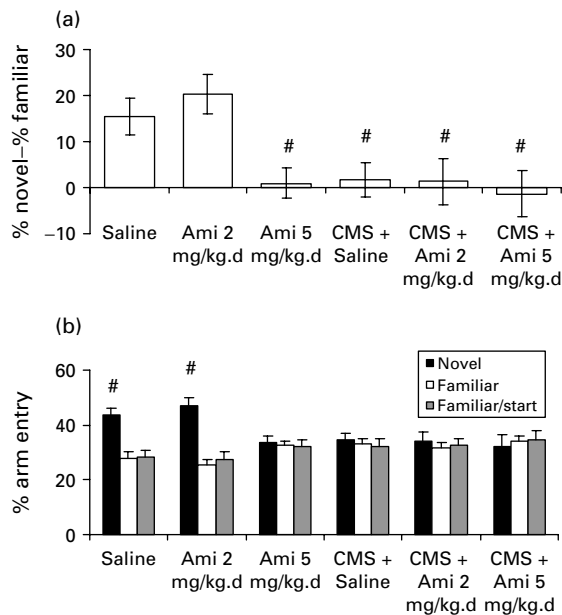


Figure 2. Effect of chronic administration of Ami on spatial memory performance (place recognition task, 240-min retention interval) of CMS-free and anhedonic rats. Both CMS-free rats and rats subjected to CMS protocol made a similar number of total entries into the three arms, suggesting that they had similar motor activity and motivation to explore the Y-maze [two-way ANOVA, stress: $F(1, 84) = 0.13, p = 0.71$; treatment: $F(2, 84) = 0.8, p = 0.45$; interaction: $F(2, 84) = 0.24, p = 0.78$]. (a) Two-way ANOVA showed a significant main effect of stress on the difference score for entries [$F(1, 84) = 9.93, p = 0.002$] and a significant interaction between stress and treatment [$F(2, 84) = 3.48, p = 0.035$]. The main effect for treatment was not significant [$F(2, 84) = 1.99, p = 0.14$]. The difference score between the novel and familiar arm was greater for groups treated with saline and 2 mg/kg.d Ami compared to other groups ($\# p < 0.05$ vs. saline, LSD test). (b) Rats treated with saline and 2 mg/kg.d Ami entered the novel arm more than the other arms, while CMS-free rats treated with 5 mg/kg.d Ami and CMS rats treated with saline or both doses of Ami entered the novel and other arms similarly ($\# p < 0.05$ vs. other arms, Wilcoxon test).

preference for the novel arm whereas the other groups (5 mg/kg.d Ami, CMS+saline, CMS+2 mg/kg.d Ami, CMS+5 mg/kg.d Ami) had values of difference score near zero, suggesting no arm preference (Figure 2a). Wilcoxon tests supported these observations with saline-treated rats and rats treated with 2 mg/kg.d Ami entering the novel arm more than the other arm and the other groups entering the novel and the other arms equally (Figure 2b). Overall, the results indicated that: (i) the CMS protocol caused an impairment of spatial memory in saline-treated rats (ii)

the administration of 5 mg/kg.d Ami caused a memory impairment in CMS-free rats (iii) both doses of Ami are ineffective in reverting the memory damage caused by CMS.

In the object discrimination task, saline-treated rats and, regardless of stress history, rats treated with 2 mg/kg.d Ami, exhibited novel object preference in the second trial. Conversely, CMS+saline rats and CMS or CMS-free rats treated with 5 mg/kg.d Ami showed no difference between the exploration times of novel and familiar objects (Figure 6b). Thus, our data indicated that (i) the CMS protocol caused an impairment of visual memory in saline-treated rats and (ii) the administration of 5 mg/kg.d Ami caused a memory impairment in CMS-free rats (iii) the administration of 2 mg/kg.d Ami was effective to prevent the damage of visual memory caused by CMS.

Effects of chronic VPA administration

Saline-treated rats and rats treated with 5 mg/kg.d VPA showed preference for the novel arm whereas the other groups (30 mg/kg.d VPA, CMS+saline, CMS+5 mg/kg.d VPA, CMS+30 mg/kg.d VPA) had values of difference score near zero, suggesting no arm preference (Figure 3a). Wilcoxon tests supported these observations with saline-treated rats and rats treated with 5 mg/kg.d VPA entering the novel arm more than the other arm and the other groups entering the novel and the other arms equally (Figure 3b). These results indicated that (i) both doses of VPA did not revert the CMS-induced deficit of spatial memory, (ii) the repeated administration of 30 mg/kg.d VPA caused an impairment of the spatial performance in CMS-free rats.

Object discrimination data indicated that saline-treated rats and rats treated with both doses of VPA exhibited novel object preference in the second trial. Conversely, CMS+saline rats and CMS rats treated with 5 or 30 mg/kg.d VPA showed no difference between the exploration times of novel and familiar objects (Figure 6c). Therefore, in our experiments the chronic administration of 5 or 30 mg/kg.d VPA had no effects in CMS-free rats and did not prevent the impairment of visual memory caused by CMS.

Effects of chronic Hal administration

Saline-treated rats showed preference for the novel arm whereas the other groups (0.2 mg/kg.d Hal, CMS+saline, CMS+0.2 mg/kg.d Hal) had values of difference score near zero, suggesting no arm preference (Figure 4a). Wilcoxon tests supported these observations with saline-treated rats entering the

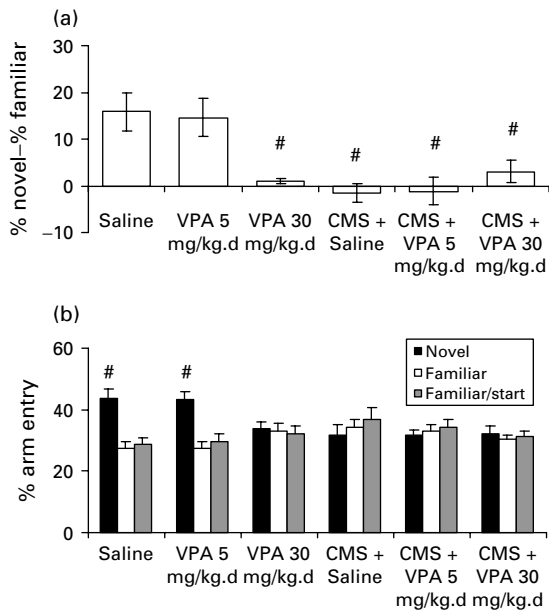


Figure 3. Effect of chronic administration of VPA on spatial memory performance (place recognition task, 240-min retention interval) of CMS-free and anhedonic rats. Both CMS-free rats and rats subjected to CMS protocol made a similar number of total entries into the three arms, suggesting that they had similar motor activity and motivation to explore the Y-maze [two-way ANOVA, stress: $F(1, 84) = 0.09, p = 0.77$; treatment: $F(2, 84) = 0.81, p = 0.45$; interaction: $F(2, 84) = 0.84, p = 0.43$]. (a) Two-way ANOVA yielded a significant effect of stress on the difference score for entries [$F(1, 84) = 19.6, p < 0.001$] and a significant interaction between stress and treatment [$F(2, 84) = 8.1, p < 0.001$]. The main effect for treatment was not significant [$F(2, 84) = 1.43, p = 0.24$]. The difference score between the novel and familiar arm was greater for groups treated with saline and 5 mg/kg . d VPA compared to other groups ($\# p < 0.05$ vs. saline, LSD test). (b) Rats treated with saline and 5 mg/kg . d VPA entered the novel arm more than the other arms, while CMS-free rats treated with 30 mg/kg . d VPA and CMS rats treated with saline or both doses of VPA entered the novel and other arms similarly ($\# p < 0.05$ vs. other arms, Wilcoxon test).

novel arm more than the other arm and the other groups entering the novel and the other arms equally (Figure 4b).

In the object discrimination task, saline-treated rats and rats treated with 0.2 mg/kg . d Hal exhibited novel object preference in the second trial. Conversely, CMS+saline rats and CMS rats treated with 0.2 mg/kg . d Hal showed no difference between the exploration times of novel and familiar objects (Figure 6d). Overall, the data indicated that (i) CMS-free rats treated with 0.2 mg/kg . d Hal showed spatial memory impairment and no modifications of behavioural

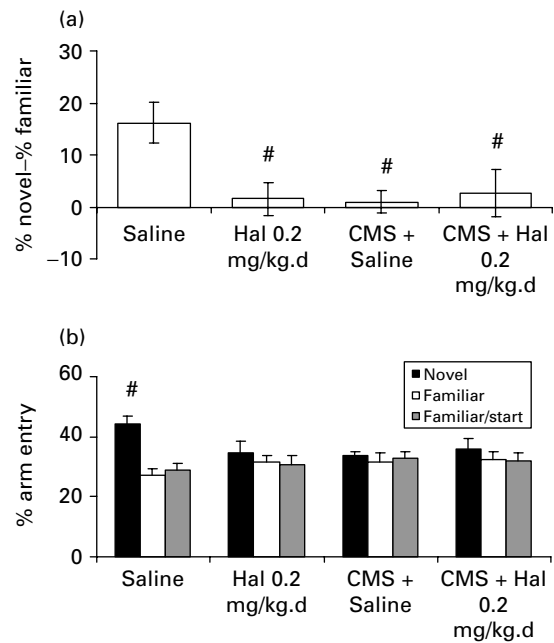


Figure 4. Effect of chronic administration of Hal on spatial memory performance (place recognition task, 240-min retention interval) of CMS-free and anhedonic rats. Both CMS-free rats and rats subjected to CMS protocol made a similar number of total entries into the three arms, suggesting that they had similar motor activity and motivation to explore the Y-maze [two-way ANOVA, stress: $F(1, 56) = 0.24, p = 0.62$; treatment: $F(1, 56) = 3.8, p = 0.056$; interaction: $F(1, 56) = 3.08, p = 0.084$]. (a) Two-way ANOVA for entry difference scores showed a significant effect of stress [$F(1, 56) = 4.47, p = 0.039$] and a significant stress \times treatment interaction [$F(1, 56) = 8.31, p = 0.005$] but no significant main effect of treatment [$F(1, 56) = 3.2, p = 0.078$]. The difference score between the novel and familiar arm was greater for the group treated with saline compared to other groups ($\# p < 0.05$ vs. saline, LSD test). (b) Rats treated with saline entered the novel arm more than the other arms, while CMS-free rats treated with Hal 0.2 mg/kg . d and CMS rats treated with saline or Hal entered the novel and other arms similarly ($\# p < 0.05$ vs. other arms, Wilcoxon test).

performance in the object discrimination paradigm, (ii) the drug did not prevent the deficits of visual or spatial memory caused by the CMS protocol.

Effects of chronic Ola administration

Saline-treated rats and, regardless of stress history, rats treated with 0.02 mg/kg . d Ola, showed preference for the novel arm whereas the other groups (0.1 mg/kg . d Ola, 0.5 mg/kg . d Ola, CMS+saline, CMS+0.1 mg/kg . d Ola, CMS+0.5 mg/kg . d Ola) had values of difference score near zero, suggesting

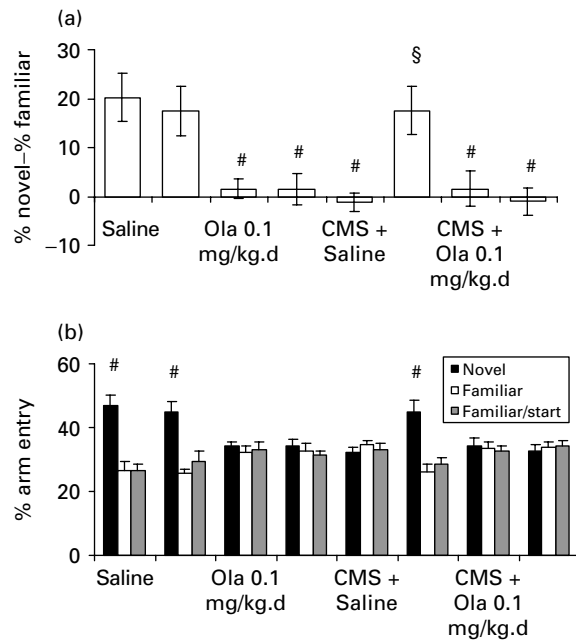


Figure 5. Effect of chronic administration of Ola on spatial memory performance (place recognition task, 240 min retention interval) of CMS-free and anhedonic rats. Both CMS-free rats and rats subjected to CMS protocol made a similar number of total entries into the three arms, suggesting that they had similar motor activity and motivation to explore the Y-maze [two-way ANOVA, stress: $F(1, 112) = 0.99$, $p = 0.32$; treatment: $F(3, 112) = 1.88$, $p = 0.13$; interaction: $F(3, 112) = 0.47$, $p = 0.70$]. (a) Two-way ANOVA for entry difference scores revealed significant effects of stress [$F(1, 112) = 4.67$, $p = 0.032$] and treatment [$F(3, 112) = 8.77$, $p < 0.001$] and a significant interaction between the two factors [$F(3, 112) = 3.89$, $p = 0.01$]. The difference score between the novel and familiar arm was greater for CMS-free rats treated with saline or 0.02 mg/kg . d Ola and CMS rats treated with 0.02 mg/kg . d Ola compared to other groups ($\# p < 0.05$ vs. saline, $\$ p < 0.05$ vs. CMS + saline, LSD test). (b) CMS-free rats treated with saline or 0.02 mg/kg . d Ola and CMS rats treated with 0.02 mg/kg . d Ola entered the novel arm more than the other arms, while CMS rats treated with saline and all groups treated with 0.1 or 0.5 mg/kg . d Ola entered the novel and other arms similarly ($\# p < 0.05$ vs. other arms, Wilcoxon test).

no arm preference (Figure 5a). Wilcoxon tests supported these observations with saline-treated rats and both CMS and CMS-free rats treated with 0.02 mg/kg . d Ola entering the novel arm more than the other arm and the other groups entering the novel and the other arms equally (Figure 5b). Overall, the results indicated that (i) the administration of 0.1 or 0.5 mg/kg . d Ola in CMS-free rats caused an impairment of spatial memory, (ii) the same drug doses did not

prevent the damage of spatial memory induced by CMS protocol, (iii) the lower dose of Ola was effective in preventing the stress-induced impairment of spatial memory without modifying memory performance in CMS-free rats.

Object discrimination data indicated that saline-treated rats and rats treated with 0.02 or 0.1 mg/kg . d Ola exhibited novel object preference in the second trial. Conversely, rats treated with 0.5 mg/kg . d Ola and CMS rats treated with saline or 0.02, 0.1 or 0.5 mg/kg . d Ola showed no difference between the exploration times of novel and familiar objects (Figure 6e). Therefore, our results indicated that (i) in CMS-free rats the administration of 0.02 or 0.1 mg/kg . d Ola did not cause any modification of memory performance whereas 0.5 mg/kg . d Ola caused an impairment of visual memory, (ii) the administration of 0.02 or 0.1 mg/kg . d Ola was ineffective to prevent the stress-induced impairment of visual memory.

Discussion

Several reports indicate that exposure to chronic stress results in cognitive impairments. Six hours per day of chronic restraint stress for 21 d cause a deficit of spatial memory performance on the Y-maze (Conrad et al., 1996) or a reversible, temporally limited impairment of spatial memory by using the eight-arm radial maze (Luine et al., 1994). Administration of chronic restraint stress causes not only a damage of spatial memory but also a decrease of visual memory performance in the object discrimination test (Beck and Luine, 1999). In addition, Park et al. (2001) reported an evident impairment of spatial learning and memory in rats subjected to a protocol of chronic psychosocial stress (exposure to a cat for 5 wk). In our experimental conditions, rats submitted to unpredictable mild stressors over weeks showed a general decrease in responsiveness to reward, as revealed by decreased sucrose preference. Administration of the CMS protocol also caused an evident impairment of spatial memory, since anhedonic rats performed poorly in the place recognition test in comparison to CMS-free rats. Indeed, at the 240-min retention interval, CMS-free rats treated with saline showed greater novel arm preference whereas saline-treated rats subjected to CMS protocol did not enter the novel arm more often than both of the two remaining arms. However, during the second trial total entries were similar among groups, indicating that stressed, CMS-free, drug- or saline-treated rats had similar motor activity and motivation (see Table 2). An evident impairment of visual memory was also observed in the

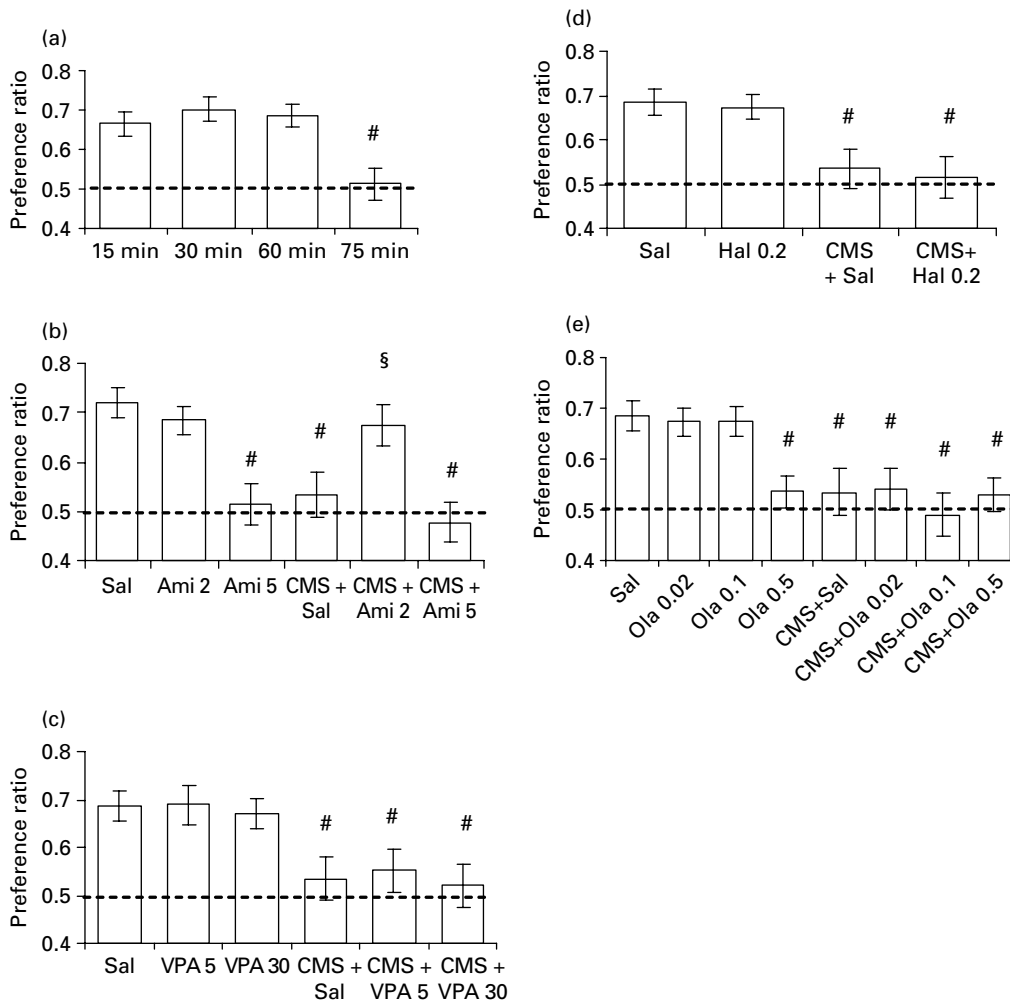


Figure 6. Effect of retention interval or chronic administration of saline, Ami, VPA, Hal and Ola on visual memory performance (object discrimination task) of CMS-free and anhedonic rats. Drug experiments were performed by using a 60-min retention interval. (a) Rats tested at 15-, 30- and 60-min retention intervals exhibit novel object preference as measured by dividing the amount of exploration of the novel object by the total amount of object exploration during the second trial. After a 75-min interval, rats show no preference for the novel object ($^{\#}p < 0.05$ vs. other retention intervals, LSD test). (b) Significant effects of stress [$F(1, 84) = 7.32, p = 0.0082$] and treatment [$F(2, 84) = 11.48, p < 0.001$] and a significant interaction between the two factors [$F(2, 84) = 12.3, p < 0.001$] on preference ratio have been obtained. The chronic administration of 5 mg/kg . d Ami in CMS-free rats impairs visual memory whereas chronic Ami 2 mg/kg . d prevents the CMS-induced memory damage ($^{\#}p < 0.05$ vs. saline group; $^{\S}p < 0.05$ vs. CMS+saline group; LSD test). (c) Two-way ANOVA showed a significant main effect for stress [$F(1, 84) = 20.16, p < 0.001$]. The effect for treatment [$F(2, 84) = 0.15, p = 0.85$] and the stress \times treatment interaction [$F(2, 84) = 0.58, p = 0.56$] were not significant. The chronic administration of VPA has no effect in CMS-free rats and does not prevent the CMS-induced deficit of visual memory ($^{\#}p < 0.05$ vs. saline group, LSD test). (d) Two-way ANOVA showed a significant main effect for stress [$F(1, 56) = 19.7, p < 0.001$]. The effect for treatment [$F(1, 56) = 1.38, p = 0.24$] and the stress \times treatment interaction [$F(1, 56) = 0.02, p = 0.88$] were not significant. The chronic administration of Hal has no effect in CMS-free rats and does not prevent the CMS-induced deficit of visual memory ($^{\#}p < 0.05$ vs. saline group, LSD test). (e) Significant effects of stress [$F(1, 112) = 24.05, p < 0.001$] and treatment [$F(3, 112) = 3.95, p = 0.01$] and a significant interaction between the two factors [$F(3, 112) = 2.73, p = 0.047$] on preference ratio have been obtained. The chronic administration of Ola does not prevent the CMS-induced memory damage whereas chronic 0.5 mg/kg . d Ola impairs visual memory in CMS-free rats ($^{\#}p < 0.05$ vs. saline group, LSD test).

object discrimination task in rats previously subjected to the CMS protocol. The present findings confirm and extend those of Vasconcellos et al. (2003), who

reported that a 40-d variable stressor paradigm causes a marked decrease in reference memory in the Morris water maze task. In our experiments, the treatment

Table 2. Total entries into the three arms of the Y-maze during the second trial of the place recognition test (time of exploration: 5 min). (Data are shown as means \pm S.E.M.)

CMS-free rats	
Saline	9.8 \pm 0.85
Ami (2 mg/kg . d)	8.86 \pm 0.84
Ami (5 mg/kg . d)	8.5 \pm 0.74
Hal (0.2 mg/kg . d)	7.9 \pm 1.4
VPA (5 mg/kg . d)	9.7 \pm 0.67
VPA (30 mg/kg . d)	8.2 \pm 0.96
Ola (0.02 mg/kg . d)	8.9 \pm 0.83
Ola (0.1 mg/kg . d)	8.8 \pm 0.78
Ola (0.5 mg/kg . d)	9.2 \pm 0.95
CMS rats	
Saline	9.1 \pm 0.9
Ami (2 mg/kg . d)	9.13 \pm 0.94
Ami (5 mg/kg . d)	9.3 \pm 0.95
Hal (0.2 mg/kg . d)	8.3 \pm 0.9
VPA (5 mg/kg . d)	9.46 \pm 0.8
VPA (30 mg/kg . d)	9.11 \pm 0.82
Ola (0.02 mg/kg . d)	8.5 \pm 0.88
Ola (0.1 mg/kg . d)	9.4 \pm 0.89
Ola (0.5 mg/kg . d)	9.0 \pm 0.76

CMS, Chronic mild stress.

A two-way ANOVA applied to these data (stress and treatment as the independent variables) indicated no significant main effects of stress [$F(1, 252) = 0.12, p = 0.72$], treatment [$F(8, 252) = 1.37, p = 0.21$] and no significant stress \times treatment interaction [$F(8, 252) = 0.32, p = 0.95$].

with 0.02 mg/kg . d Ola, or 2 or 5 mg/kg . d Ami was effective in preventing the onset of anhedonia in rats subjected to the CMS protocol. In addition, 0.02 mg/kg . d Ola and 2 mg/kg . d Ami did not modify the cognitive performance of CMS-free rats in both place recognition and object discrimination tasks but the two drugs showed significant effects on the CMS-induced impairments of visual (2 mg/kg . d Ami) or spatial (0.02 mg/kg . d Ola) memory. These data confirm the antidepressant effect of low-dose Ola previously reported (Orsetti et al., 2005) and seem to indicate a favourable profile of Ola on spatial short-term memory within the same dose range.

It is well known that normal dopamine function is necessary for acquisition and retention of spatial information. In this respect, the infusion into hippocampal CA1 area of SKF 38393, a D_1 agonist, 3 or 6 h after training, enhances memory while infusion of SCH 23390, a D_1 receptor blocker, has amnesic effects (Izquierdo and Medina, 1997). On the other hand, the blockade of dopamine D_2 receptors impairs spatial memory (Skarsfeldt, 1996). Overall, these findings indicate that D_1 - D_2 receptor antagonism has deleterious

effects on cognition and this may be a partial explanation for the negative action of Hal on memory observed in the present study. It has also been established that central serotonergic pathways play a role in learning and memory (Meneses and Hong, 1997). In particular, blockade of central 5-HT_{2A} receptors may have beneficial cognitive effects (Altman and Normile, 1988; Nabeshima et al., 1989), whereas their activation may rather impair cognitive functions (Lawlor et al., 1989; Noda et al., 1991). Our data concerning the effects of a chronic low-dose Ola treatment may be explained, at least in part, on the basis of these considerations. As reported by Kapur et al. (1998), at low doses (5–10 mg/d) Ola occupies over 90% of 5-HT₂ sites and ~50% of D_2 receptors. By increasing the doses, Ola loses the atypical profile, achieving a receptor blockade of up to 80% of D_2 sites and no further antagonism at 5-HT₂ receptors. Therefore, the positive effects on visuospatial memory observed in our study after repeated administration of 0.02 mg/kg . d Ola might be ascribed to the high ratio of 5-HT₂/ D_2 antagonism that the drug exhibits at low doses. In this respect, two recent papers (Ichikawa et al., 2002; Shirazi-Southall et al., 2002) have indicated that potent 5-HT_{2A} relative to weak D_2 receptor antagonism may contribute to the ability of Ola and other atypical antipsychotics to increase acetylcholine release in the prefrontal cortex and hippocampus, two brain areas that play a central role in memory processes. Less favourable effects on cognition have been observed after chronic administration of greater doses of Ola. The 4-wk treatment with 0.1 or 0.5 mg/kg . d Ola did not revert the spatial memory deficit in rats subjected to CMS protocol and impaired the spatial performance in CMS-free rats. Overall, these findings seem to indicate that Ola, within the range of therapeutic doses, can cause different, opposite effects on visuospatial memory. Preclinical studies on acute administration of Ola provide further support to our results. Impairment of memory in young adult rats in the Morris water maze after administration of high doses of the drug (2 or 8 mg/kg) has been observed by Skarsfeldt (1996). By contrast, low doses of Ola, comparable to those employed in the current study (0.063 or 0.125 mg/kg), reversed the dizocilpine-induced memory impairment in both the active avoidance paradigm and the elevated plus maze (Ninan and Kulkarni, 1999). In our experiments, the positive effects on visuospatial memory observed after chronic treatment with 2 mg/kg . d Ami disappeared after increasing the dose. This may be explained, at least in part, by the strong anti-muscarinic activity that Ami and other tricyclic antidepressants exhibit at high

doses. Indeed it has been reported that 5 mg/kg Ami causes an impairment of avoidance learning in mice, an effect reversed by the acetylcholinesterase inhibitor tacrine (Pavone et al., 1997). Numerous studies have assessed the cognitive impact of tricyclic compounds on depressed patients (Amado-Boccaro et al., 1995). In general, the same anticholinergic compounds that produce adverse attention and memory effects in healthy adults, show the same pattern of adverse effects in clinical populations. Under chronic administration, patients may take longer to show normalization of cognitive tests, but these adverse effects may be partially offset by the potential benefits of successfully treating depression. In spite of these clinical data, our results seem to indicate that the beneficial effects on memory are not related to the recovery from anhedonia, as the group of CMS rats treated with 5 mg/kg.d Ami showed normal sucrose preference but performed poorly in both memory tasks. At present, the reasons for such a discrepancy are not known. In general, our results concerning chronic administration of VPA indicate a favourable profile on visuospatial memory in CMS-free rats, in line with previous studies (Stoll et al., 1996). The drug, however, was unable to prevent CMS-induced anhedonia and had no effect on the cognitive impairment caused by the CMS protocol. Therefore, the chronic administration of low-dose Ola seems to be more effective than administration of VPA or Hal to prevent anhedonia and the CMS-related damage of visual and spatial short-term memories. In this respect, the 4-wk treatments with 0.02 mg/kg.d Ola or 2 mg/kg.d Ami are fully comparable.

In conclusion, the main findings of the present study can be summarized as follows: (i) chronic administration of low doses of Ola prevents anhedonia and has a better profile on visuospatial memory performance in comparison to VPA and Hal, (ii) the cognitive effects of Ola are comparable to that of low doses of Ami (2 mg/kg.d), (iii) the pro-cognitive effects of Ola and Ami are dose-dependent, since 4-wk treatments with 0.1–0.5 mg/kg.d Ola or 5 mg/kg.d Ami did not revert the memory deficit in rats subjected to the CMS protocol and impaired spatial performance in CMS-free rats. Taken together the results of the present preclinical study seem to indicate that Ola has the potential to lead to substantial cognitive benefits in depressed patients. Therefore, the superiority of Ola effects on cognitive functioning over Hal, a conventional antipsychotic, or VPA, a mood stabilizer, should be considered in a clinical context to improve the quality of life of schizophrenic or bipolar patients during long-term pharmacological treatment.

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Statement of Interest

None.

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