



## Memantine is a useful drug to prevent the spatial and non-spatial memory deficits induced by methamphetamine in rats

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### ARTICLE INFO

#### Article history:

Received 14 April 2010

Received in revised form 19 May 2010

Accepted 20 May 2010

#### Keywords:

Memantine  
Methamphetamine  
Learning  
Memory  
Object recognition  
Morris water maze

### ABSTRACT

Methamphetamine (METH) is a street drug that is abused by young people. In previous studies, we demonstrated the effectiveness of alpha-7 nicotinic receptor antagonists in preventing the neurotoxicity induced by this amphetamine derivative. The present study seeks to determine whether pre-treatment with memantine (MEM) (an antagonist of both NMDA and alpha-7 nicotinic receptors) counteracts the memory impairment induced by METH administration in male Long Evans rats.

Non-spatial memory was tested in the object recognition test and spatial learning memory was tested in the Morris water maze. In our experimental conditions, rats that received the MEM (5 mg/kg, intraperitoneally) pre-treatment recovered the ability to discriminate between a familiar and a novel object. This ability had been abolished by METH (10 mg/kg, subcutaneously) at 72 h and 1 week after treatment. Moreover, MEM pre-treatment also inhibited the thigmotaxis behaviour induced by METH.

Rats treated with METH showed impaired learning in the Morris water maze. The results of the probe trial demonstrated that METH-treated rats did not remember the location of the platform, but this memory impairment was also prevented by MEM pre-treatment. Moreover, MEM by itself improved the learning of the task. Finally, MEM significantly improved the learning and memory impairment induced by METH.

Therefore, MEM constitutes the first successful approach to prevent the cognitive deficits induced by amphetamine derivatives which are frequently abused in western countries.

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### 1. Introduction

Methamphetamine (METH) is a highly addictive drug of abuse and addiction to this drug has increased to epidemic proportions worldwide [1]. In humans, METH abuse is associated with neurotoxicity to frontostriatal brain regions [2] and also cognitive deficits have been reported [3–5].

In rodents, METH decreases multiple indices of dopamine terminal integrity, especially in the striatum. The striatal changes produced by extended exposure of rats to METH include long-lasting decreases in dopamine content [6], dopamine metabolites [7], tyrosine hydroxylase activity [8] and loss of dopamine transporters [9]. Also, these METH-induced reductions in specific biochemical parameters are accompanied by reactive gliosis, a marker of neuronal injury [10,11].

The development of animal models will be essential for characterizing the cognitive deficits induced by amphetamines and

for understanding the neurobiological changes responsible for amphetamine-induced alterations in cognition. Although there are few papers about an animal model that reflects METH-induced alterations of memory processes [12,13], some beneficial effects were reported using baclofen or quetiapine in rats and mice [14,15].

Memantine (MEM) is a non-competitive antagonist of the NMDA receptor and a clinically useful drug to treat moderate to severe Alzheimer's disease. Moreover, Aracava et al. [16] demonstrated that MEM, at clinically relevant concentrations, blocks alpha-7 nicotinic acetylcholine receptors in a non-competitive manner, and more effectively that it block NMDA receptors. Previous results from our group demonstrated the effectiveness of alpha-7 nicotinic acetylcholine receptors antagonists preventing amphetamine-induced neurotoxicity [17,18]. When neuroprotection was assayed with MEM, an effective improvement in the cognitive deficits was obtained also ahead of serotonergic injury induced by another frequently abused amphetamine derivative, such as MDMA, in rats [18,19].

The aim of this paper is to study the utility of MEM in preventing the learning and memory deficits induced by the administration of METH to rats. These memory deficits were studied by examining the effects of METH and MEM on non-spatial and spatial learning. The

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relevance of positive results in this study points to MEM as the first useful drug to prevent cognitive deficits induced by amphetamine derivatives, which are commonly abused.

## 2. Materials and methods

### 2.1. Drugs

Methamphetamine hydrochloride was purchased by Sigma Chem. Co. (St. Louis, MO, USA). Memantine was generously supplied by Lundbeck laboratories (Copenhagen, Denmark). All drugs were dissolved in saline (NaCl 0.9%).

### 2.2. Animals and treatment

Male Long Evans rats (8-week old, 125–150 g) (Janvier, France) were used. The animals were housed two per cage in a regulated environment ( $21 \pm 1^\circ\text{C}$ ; 12 h light/dark cycle, lights on at 08:00 h) with free access to food and water. Experiments took place between 09:00 and 15:00 h. Experimental protocols for the use of animals in this study were approved by the Animal Ethics Committee of the University of Barcelona under the supervision of the Autonomous Government of Catalonia, and following the guidelines of the European Communities Council (86/609/EEC). Efforts were made to minimize suffering and reduce the number of animals used.

Animals were divided into four groups of treatment: saline group (5 ml/kg, s.c.); METH group (5 or 10 mg/kg, s.c.); MEM group (5 mg/kg, i.p.), and MEM + METH group. In this last group animals received MEM (5 mg/kg, i.p.) 20 min prior to METH (10 mg/kg, s.c.). We administered four doses (separated by 2 h) on 1 day for each group of treatment in their home cage.

### 2.3. Body temperature

Body temperature was measured immediately after each dose of treatment schedule and using a lubricated, flexible rectal probe inserted 2 cm into the rectum (for 40 s) and attached to a digital thermometer (LE0331 Panlab SL, Barcelona, Spain).

### 2.4. Object recognition task

The object recognition test measures non-spatial memory in the rat. The advantage of this test is that it does not require punishment or reward and is quick and simple to perform [20]. The test was performed according to the original description of Ennaceur and Delacour with slight modifications [21]. Briefly, the test room was isolated from sounds and fully lit with overhead light (38.5 lux). In the week preceding testing, the animals were handled twice daily. Rats received drug treatment as previously described and were tested 72 h and 1 week after treatment. The testing arena was a circular piece of plastic measuring 100 cm diameter and 45 cm in high. In the first trial (familiarization), rats were exposed to two identical objects (gum eggs:  $A_1$  and  $A_2$ ), with sufficient space (39 cm) around for the rats to survey each side of the object. Objects were affixed to the arena using Velcro. Rats were allowed to explore the identical objects for 5 min, after which they were removed and returned to their cages for 2 h until the second trial (testing) was performed. Before each phase of the test, objects and the arena were rinsed with a 10% ethanol solution to remove any olfactory cue. In the testing trial, one of the objects was replaced by a new, novel object (a gum mouse). The position of the novel object (left or right placement of the object) was selected randomly using a Gellerman schedule. The nature and the spatial position of the objects were counterbalanced within each group in order to avoid any bias due to a preference that rats may have for a given object or its position in the arena. In the testing trial, rats were allowed to explore the novel and familiar

objects for 5 min. Exploration of the objects was defined as sniffing, touching and having moving vibrissae whilst directing the nose towards the object at a distance of less than 1 cm. The observer was blind to the treatment group. Cumulative time spent by the rats at each of the objects was recorded by measuring the time in seconds that each rat spent investigating each object.

Additionally we calculated the “Discrimination Index” which represents the ability to discriminate the novel from the familiar object:  $(\text{novel object (s)} - \text{familiar object (s)}) / (\text{novel object (s)} + \text{familiar object (s)})$ .

### 2.5. Open field behaviour

In order to test for possible sensorimotor effects of the different treatments, rats were monitored for 5 min during the familiarization trials, described previously, by a video surveillance camera mounted on the ceiling and connected to the corresponding software (Smart Junior, Panlab SL, Barcelona, Spain). The floor of the circular arena was divided virtually into two zones, centre and periphery, of 70 and 100 cm in diameter, respectively. The distance travelled, the speed, the time spent, and the number of entries in these two defined zones was recorded.

### 2.6. Morris water maze

Non-spatial (cued) and spatial learning and memory were assessed in a Morris water maze 2 weeks after treatment. Male Long Evans rats were trained in the water maze which consisted of a circular pool (160 cm in diameter and 45 cm in high) that was filled with water ( $22 \pm 1^\circ\text{C}$ ) to a depth of 25 cm and rendered opaque by the addition of a non-toxic latex solution. The pool was in an isolated room and black curtains were closed around it to minimize static room cues. Four positions around the edge of the pool were arbitrarily designated north (N), south (S), east (E), and west (W); this provided four alternative start positions and also defined the division of the pool into four quadrants: NE, SE, SW, and NW. A Plexiglas escape platform (11 cm diameter) was submerged to a depth of 1 cm from the water surface and was not visible at water level.

In the cued learning task, rats were first trained to locate a platform clearly marked by a beacon (8 cm diameter) and painted in white and blue. The cue was affixed to the platform on a 20 cm rod held in position by being fitted into a small hole in the platform. The distance between the surface of the water and the bottom edge of the cue was 19 cm. Rats were trained on two consecutive days (four trials per day). Trial time limit was 2 min, and animals spent 30 s on the platform. Start and platform positions were varied randomly on every trial. The escape latency or total time needed by the rats to find the platform (in seconds) was measured using computer software (Smart Junior, Panlab SL, Barcelona, Spain).

For the spatial learning (acquisition and probe trials) task, inside the black enclosure, a number of objects were placed. They were suspended from the false ceiling 0.23 m above the surface of the water and with the midline directly above the wall of the pool. These objects, or landmarks, were placed around the circumference of the pool and defined the location of the platform. The landmarks were A: a 40-W fixed light placed inside a white plastic; B: a plastic beach ball 25 cm in diameter with mixed colours; C: a vertical white plastic structure with three truncated cones of decreasing size (22, 17 and 12 cm of diameter at the top and 16, 12 and 8 cm at the base); and D: a figure formed by three plastic bottles attached together and individually covered with silver foil, 29 cm high and 7 cm diameter at the base. The platform was always between A and B landmarks. To ensure that the rats used these landmarks rather than any inadvertently remaining static room cues to locate the platform, the landmarks and platform were semi-randomly rotated with respect

to the room (90°, 180°, 270°, or 360°), with the restriction that all the orientations were used equally throughout the experiment and their arrangement remained topological constant. In this situation, rats tend to solve spatial tasks using configurations of landmarks, rather than by learning elementally, about individual landmarks [22,23].

Rats received one training session, consisting of eight trials per day (the average inter-trial interval was 40 min), and were tested on three consecutive days. A trial was started by placing the animal so that it faced the wall of the circular pool in one of four quadrants delineated by marks at the four cardinal directions. Rats were allowed to swim to the hidden platform, and the escape latency was determined. If an animal did not escape within 60 s, it was gently guided manually to the platform by the experimenter. Rats were allowed to rest for 30 s on the platform (even those that failed to locate it) and were then removed from the pool until the next trial. This procedure was repeated with each rat starting in each of the four quadrants.

The rats were subjected to a single test session (probe trial) after the last training session (free swimming without platform) to assess their memory of the platform location for 1 min. Different parameters of the rat's performance were analyzed: the total time spent swimming in the target quadrant (where it should be located the platform) and the opposite quadrant, the relative distance travelled, the number of entries in both quadrants and the swim speed were measured.

## 2.7. Data analysis

Results are given as the mean  $\pm$  S.E.M. (standard error of the mean). One-way ANOVA, followed by Tukey's test, was used to verify the significance between means. *P* values less than 0.05 were considered significant. Results from object recognition task experiments were analyzed using the paired Student's *t*-test. Linear regression was analyzed using Graph Pad Prism (GraphPad Software, San Diego, CA, USA).

## 3. Results

### 3.1. Body temperature

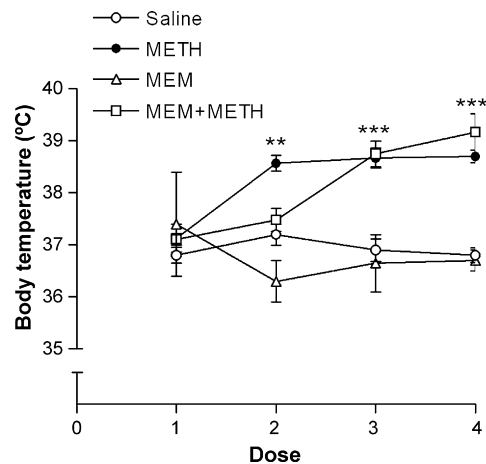
As can be seen in Fig. 1, in an ambient temperature of  $21 \pm 1$  °C, METH, at a dose of 10 mg/kg, exerted a hyperthermic effect in male Long Evans rats ( $38.70 \pm 0.12$  °C, after the fourth dose). Measuring body temperature after each dose of METH, the hyperthermic response elicited by the amphetamine derivative was not antagonized by the prior treatment with MEM ( $39.17 \pm 0.35$  °C, after the fourth dose). Moreover, MEM alone did not induce any significant change in the body temperature.

**Table 1**

Time spent by rats (in seconds) in the object recognition task performed at 72 h and 1 week after drug treatment. Familiarization trial is denoted by the time spent by animals exploring two identical objects ( $A_1$  and  $A_2$ ) and testing trial is denoted by the time spent by rats exploring the old (A) and the new (B) object. Discrimination Index (DI) was calculated according to the following expression:  $(B - A)/(B + A)$ . Results are expressed as means  $\pm$  S.E.M.,  $n = 8-10$ .

Drug	72 h after treatment					1 week after treatment				
	$A_1$	$A_2$	A	B	DI	$A_1$	$A_2$	A	B	DI
Saline	$3.51 \pm 0.06$	$3.63 \pm 0.40$	$1.65 \pm 0.25$	$8.37 \pm 1.39^{***}$	$0.67 \pm 0.06$	$2.35 \pm 0.24$	$2.28 \pm 0.21$	$1.37 \pm 0.28$	$6.45 \pm 0.89^{***}$	$0.70 \pm 0.04$
METH 5	$2.66 \pm 0.44$	$2.28 \pm 0.29$	$2.64 \pm 0.25$	$3.93 \pm 1.06$	$0.50 \pm 0.10$	$2.09 \pm 0.28$	$2.24 \pm 0.20$	$1.58 \pm 0.54$	$5.12 \pm 1.34^*$	$0.41 \pm 0.20$
METH 10	$2.90 \pm 0.27$	$2.73 \pm 0.55$	$2.09 \pm 0.74$	$2.12 \pm 0.73$	$0.01 \pm 0.04^{###}$	$1.86 \pm 0.20$	$2.13 \pm 1.26$	$2.04 \pm 0.62$	$3.89 \pm 0.98$	$0.31 \pm 0.08^{##}$
MEM	$3.86 \pm 0.61$	$3.96 \pm 0.47$	$4.75 \pm 1.80$	$13.87 \pm 2.72^{***}$	$0.52 \pm 0.07$	$3.17 \pm 0.88$	$3.54 \pm 0.76$	$2.40 \pm 0.39$	$9.88 \pm 1.76^{***}$	$0.63 \pm 0.08$
MEM + METH 10	$3.83 \pm 0.66$	$3.87 \pm 0.68$	$1.55 \pm 0.46$	$5.63 \pm 1.29^{**}$	$0.57 \pm 0.06$	$3.76 \pm 0.58$	$4.12 \pm 0.65$	$1.91 \pm 0.42$	$6.56 \pm 1.01^{***}$	$0.55 \pm 0.07$

\* $P < 0.05$ ; \*\* $P < 0.01$  and \*\*\* $P < 0.001$  indicate the significant difference between the old (A) vs. the novel (B) object. Paired *t*-tests were performed within each group. ## $P < 0.01$  and ### $P < 0.001$  vs. saline group. One-way ANOVA, followed by Tukey's test were performed between groups.



**Fig. 1.** Evolution of body temperature of male Long Evans rats treated with saline, METH (10 mg/kg, s.c.), MEM, (5 mg/kg, i.p.) and their association (MEM 30 min before METH), respectively. Body temperature was measured using a rectal probe after each dose of the treatment regimen. \*\* $P < 0.01$  and \*\*\* $P < 0.001$  vs. rats treated with saline. Data are mean  $\pm$  S.E.M.;  $n = 8-10$ .

### 3.2. Object recognition task

Present studies demonstrate that METH at doses of 5 and 10 mg/kg induced an impairment of recognition memory at 72 h post-treatment. Only at the highest dose of METH, this impairment was still present at 1 week post-treatment. For this reason the effect of MEM was only tested against the dose of 10 mg/kg of METH.

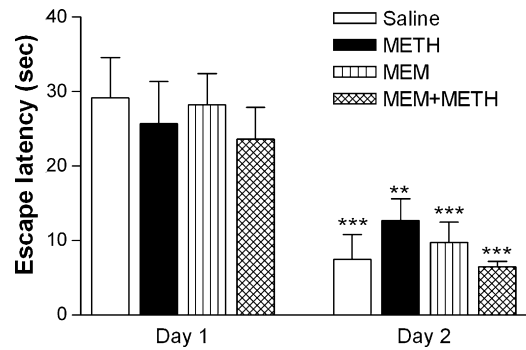
In the familiarization experiment, rats spent a similar period of time exploring the two identical objects in the first trial (see Table 1). Statistical analysis showed no significant difference in the exploration of the identical objects ( $A_1$  and  $A_2$ ) during this phase in any treatment group. In the testing experiment, statistical analysis demonstrated that saline-treated animals spent significantly longer exploring the novel object compared with the familiar object ( $P < 0.001$ ). This ability to discriminate the familiar and the novel object was abolished following METH treatment, whereby there was no significant difference in exploration time of the novel and familiar object.

Rats receiving MEM treatment, spent significantly ( $P < 0.001$ ) longer exploring the novel object. Furthermore pre-treatment with MEM abolished the lack of discrimination that appeared in the METH-treated animals. The time (in seconds) spent in exploring the novel object by the animals treated with MEM + METH did not differ significantly from that of the saline group. Accordingly, the value of the Discrimination Index calculated for each treatment group reflected the impairment of the task in METH-treated animals.

**Table 2** Effect of saline, methamphetamine (METH) and memantine (MEM), alone or in combination, in the rat open field behaviour. Data were collected during the familiarization trial session of the object recognition task performed after 72 h and 1 week of treatment. Data are expressed as means ± S.E.M., n = 8–10.

Rat behaviour	Drug treatment			
	Saline	METH (10 mg/kg)	MEM (5 mg/kg)	MEM (5 mg/kg) + METH (10 mg/kg)
	72 h	72 h	72 h	72 h
	1 week	1 week	1 week	1 week
<b>Time (s)</b>				
Centre	24.43 ± 1.78	14.75 ± 2.59*	93.36 ± 4.80***	19.94 ± 1.42
Periphery	275.57 ± 1.78	285.24 ± 2.20*	206.58 ± 4.75***	279.92 ± 1.04
Ratio C/P	0.089 ± 0.004	0.052 ± 0.007*	0.452 ± 0.009***	0.071 ± 0.005
<b>Distance (cm)</b>				
Centre	384.87 ± 43.21	200.93 ± 21.11	719.78 ± 141.75**	238.40 ± 36.62
Periphery	2807.84 ± 84.98	1913.41 ± 143.95	1093.78 ± 119.11**	1594.49 ± 201.01**
Ratio C/P	0.137 ± 0.005	0.165 ± 0.018	0.120 ± 0.011*	0.150 ± 0.021

\*P < 0.05; \*\*P < 0.01 and \*\*\*P < 0.001 vs. the corresponding value of saline-treated animals.



**Fig. 2.** Effect of treatment with saline, METH (10 mg/kg, s.c.), MEM (5 mg/kg, i.p.) and their association (MEM + METH) in the cued learning (visible platform version of the Morris water maze task) phase developed over 2 days. \*\*P < 0.01 and \*\*\*P < 0.001 vs. day 1. The escape latency (the time required for rats to locate the platform) is expressed as mean ± S.E.M. of 8–10 animals.

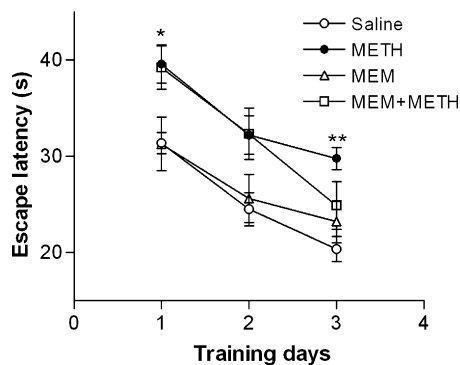
### 3.3. Open field behaviour

Preliminary studies demonstrated that METH (5 and 10 mg/kg) induced dose-dependent alterations of rat behaviour in the open field test (data not shown). We measured parameters of exploratory behaviour (total distance travelled and time spent in the centre and the periphery). The results in Table 2 show that METH treatment induced thigmotaxis evidenced by a significant decrease in time spent in the centre zone ( $F_{3,16} = 3.952, P < 0.05$ ) accompanied by a more significant increase in time spent in the periphery ( $F_{3,16} = 5.699, P < 0.01$ ). On the contrary, MEM-treated rats spent longer in the centre ( $F_{3,16} = 78.108, P < 0.001$ ) and remained a shorter period of time in the periphery ( $F_{3,16} = 75.973, P < 0.001$ ). Moreover, MEM inhibited the alterations induced by METH, and MEM pre-treatment fully reversed the behaviour profile induced by METH (rats spent in the centre or in the periphery the same time than saline-treated animals). These results were confirmed when comparing the obtained values of the corresponding ratios between centre and periphery data. The results obtained in the two sessions displayed a similar profile. The altered behaviour induced by METH and its prevention by MEM were evidenced at 72 h and 1 week after treatment. Although a similar pattern was observed as regards the distance travelled by the animals, this parameter displayed a great variability within the different group of treatment. In any case, the effect of MEM preventing the alterations induced by METH was more evident in the centre zone. When studying the speed of animals in the different sessions, significant differences due to drug treatment were not detected.

### 3.4. Morris water maze

**Cued learning:** the visible platform version of the Morris water maze task was used to test non-spatial learning and to assess whether spatial learning deficits are a result of deficient escape motivation or impairment of visual and/or motor performance. As illustrated in Fig. 2, the significant difference in escape latency on days 1 and 2 demonstrates that all groups learned the task. Furthermore, no significant differences between the different treatment groups were obtained when the distance travelled or the mean swim speed were analyzed.

**Spatial learning:** there was an appropriate learning of the task as can be deduced from the statistical analysis, demonstrating a significant difference among the escape latencies for finding the platform in some groups of treatment. The escape latency time was significantly shorter in animals treated with saline ( $F_{2,189} = 4.941, P < 0.01$ ), MEM ( $F_{2,189} = 4.776, P < 0.01$ ), or MEM + METH ( $F_{2,189} = 7.263, P < 0.001$ ), as the trials progressed



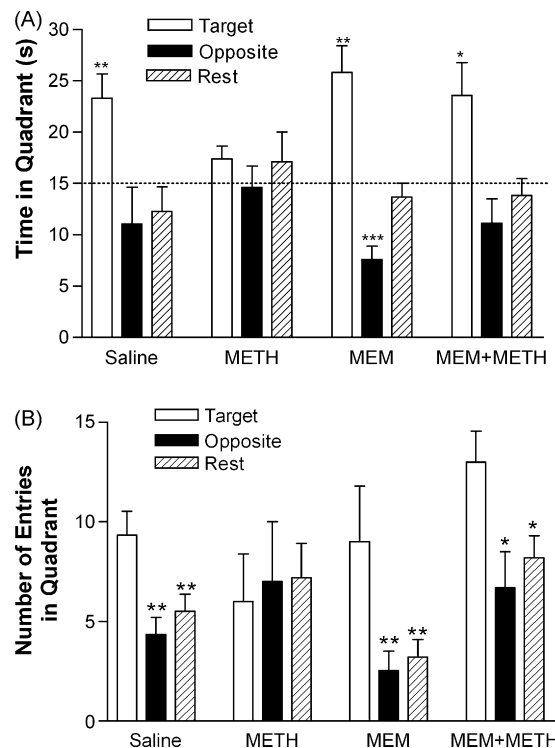
**Fig. 3.** Effect of treatment with saline, METH (10 mg/kg, s.c.), MEM (5 mg/kg, i.p.) and their association (MEM + METH) in the acquisition phase of spatial learning in the Morris water maze. The escape latency (the time required for rats to locate the platform) for the three different days is expressed as mean  $\pm$  S.E.M. of 8–10 animals. \* $P < 0.05$  and \*\* $P < 0.01$  vs. saline.

(from days 1 to 3) and was indicative of learning. On the contrary, METH-treated rats showed significant ( $P < 0.05$ ) greater escape latency than saline-treated animals on day 1, and this difference was also observed on day 3 (Fig. 3). Rats treated with METH also showed impaired learning evidenced by the non-significant difference in the escape latency time in the trials performed on days 2 and 3. Moreover, the slope values from the linear regression performed on days 1–3, also reflects this impairment and the effectiveness of MEM treatment. The slope value of METH group was  $-4.90 \pm 1.44$ , ( $F_{1,1} = 11.62$ ,  $P = 0.182$ , no different from zero); saline group:  $-6.12 \pm 0.66$ , ( $F_{1,1} = 189$ ,  $P < 0.01$  vs. zero); MEM group:  $-6.55 \pm 0.08$ , ( $F_{1,1} = 1334$ ,  $P < 0.001$ ); MEM + METH group:  $-6.67 \pm 0.12$ , ( $F_{1,1} = 1896$ ,  $P < 0.001$ ).

The probe trial demonstrated that animals treated with saline and MEM spent significantly longer ( $P < 0.01$  vs. the random time of 15.00 s) in the target quadrant (that in which the platform should be found) than in the opposite, or in the rest of quadrants (Fig. 4A). Moreover, MEM alone induced an improvement of the learning task as evidenced by a significant increase in the time spent in the target quadrant ( $25.82 \pm 2.44$  s,  $P < 0.01$ ), this being accompanied by a significant reduction in the time spent in the opposite quadrant ( $7.6 \pm 1.30$  s,  $P < 0.001$ ). METH-treated rats did not remember well the location of the platform, since the time spent in the target ( $17.40 \pm 1.23$  s), the opposite ( $14.62 \pm 2.10$  s) or the rest of quadrants ( $17.11 \pm 2.91$  s) was not significantly different from the random time. This memory impairment was also prevented by the MEM pre-treatment ( $23.58 \pm 3.22$  s,  $P < 0.05$  target;  $11.14 \pm 2.35$  s, opposite;  $13.83 \pm 1.66$  s, rest of quadrants). Moreover, the time spent in the target zone was not significantly different from that of saline-treated group. A similar behavioural profile of memory performance was evidenced when we analyzed the number of entries in the different quadrants of the maze (Fig. 4B).

The relative distance travelled in the target quadrant with respect to the distance travelled in the whole maze revealed a significant difference between the groups ( $F_{5,22} = 9.507$ ,  $P < 0.001$ ) and a similar pattern of learning was obtained. Saline: target:  $34.55 \pm 4.69\%$ ,  $P < 0.05$  vs. 25%, opposite:  $17.24 \pm 1.53\%$ ,  $P < 0.05$ ; MEM: target:  $31.62 \pm 2.74\%$ ,  $P < 0.05$ , opposite:  $8.54 \pm 1.75\%$ ,  $P < 0.001$ ; METH: target:  $20.74 \pm 5.15\%$ , n.s., opposite:  $16.20 \pm 3.99\%$ , n.s.; and MEM + METH: target:  $37.37 \pm 3.78\%$ ,  $P < 0.05$ , opposite:  $12.62 \pm 2.30\%$ ,  $P < 0.05$ .

Additionally, the resting time (in seconds) spent by each group in the target or the opposite quadrant was not significantly different (saline group, target:  $5.47 \pm 1.10$ , opposite:  $3.22 \pm 0.85$ ; MEM group, target:  $4.77 \pm 2.37$ , opposite:  $2.97 \pm 0.94$ ; METH group, target:  $6.09 \pm 0.64$ , opposite:  $7.00 \pm 3.00$  and MEM + METH group, target:  $6.38 \pm 1.02$ , opposite:  $3.10 \pm 0.55$ ). Similarly, the



**Fig. 4.** Impairment of Morris water maze learning induced by METH and its prevention by memantine. Results of the probe trial in the hidden-platform version of the water maze. Panel A shows the time spent by the animals in the target quadrant (where the platform should supposedly be found), the opposite and the rest of quadrants. Dotted line denotes the random level. Panel B shows the number of entries in the same quadrants. Data represent the mean  $\pm$  S.E.M. of 8–10 animals. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  vs. random level in panel A, or the difference between the number of entries in the target vs. the other quadrants in panel B.

analysis of the swimming speed denoted a no differences between groups. Moreover, the time spent by different groups at the border of the water tank was not significantly different (Saline:  $1.10 \pm 0.22$  s; MEM:  $1.27 \pm 0.38$  s; METH:  $1.44 \pm 0.27$  and MEM + METH:  $1.84 \pm 0.28$ ).

#### 4. Discussion

In the present study we have investigated the efficacy of MEM to prevent the spatial and non-spatial cognitive deficits induced by a dose regimen of METH, that has been previously described as neurotoxic [9,11,24], in male Long Evans rats.

The highest dose of METH used in this study (10 mg/kg) induced consistent cognitive impairment in rats. Using the interspecies scaling formula (Dose Human = Dose Animal (weight Human/weight Animal)<sup>0.7</sup>), this dose in a 150-g rat is equivalent to a dose of 11 mg in a 70 kg human [25], which may actually be normally taken by chronic abusers.

METH-induced thigmotaxis behaviour in the open field that was evidenced by a decrease in the time spent by animals in the central area that was accompanied by an augmentation of the time spent in the peripheral zone. Although more parameters must be taken into account, METH-induced thigmotaxis can be indicative of an anxiety-like state, as already has been described [26]. In our experimental conditions, MEM inhibited this altered behaviour and, in agreement with previous observations [27], MEM pre-treatment fully reversed the anxiety behaviour profile induced by METH. Present results demonstrate that this effect of MEM was evident at both 72 h and 1 week after treatment.

It has been described that MEM, at high doses (about 20 mg/kg), impaired aversive memory; while at low doses (5 mg/kg, the same used in this study) it had no effect on memory performance in a two-choice avoidance task [28]. Conversely, other authors have demonstrated the beneficial effect of MEM in rats displaying memory deficits [29] or in chicks [30,31].

The present study employed two different task models to show that multiple administration of METH impaired memory function in the object recognition test and the Morris water maze.

The object recognition task is based on the spontaneous tendency of rodents to explore a novel object. In agreement with previous papers [32], present results demonstrate an effect of METH treatment on the object recognition test in rats. With the effects on this task reported here, this effect is now the most widely replicated cognitive effect arising from exposure to a neurotoxic dosing regimen of METH [14,33]. Although the same two objects were repeatedly used across the two object memory tests (performed at 72 h and 1 week following treatment), and potentially confounded the second test when both objects had been explored before, METH, at doses of 5 and 10 mg/kg, induced an impairment of recognition memory at 72 h post-treatment. Only at the highest dose of METH, this impairment was still present at 1 week post-treatment. Animals treated with MEM spent longer time exploring the novel object and the pre-treatment with this drug abolished the lack of discrimination that appeared in the METH-treated animals. The Discrimination Index value corroborates the impairment of the task in METH-treated animals and also the memory recovery in the MEM + METH-treated group.

In this regard, NMDA receptor activation is obligatory for some forms of cognition, including recognition memory [34]. The finding that NMDA receptor activation is an important component of the induction of memory formation appears to contradict the observation that MEM, a non-competitive NMDA receptor antagonist, can be effective in memory disorders. In fact, MEM, as a low-affinity, non-competitive open-channel blocker with a relatively fast dissociation from the channel [35], can decrease pathological activation of NMDA receptors without affecting physiological NMDA receptor activity [36]. Furthermore, the antagonism of  $\alpha$ -7 nicotinic receptor by MEM can be of undoubted importance [18,37].

During the sessions with the visible platform, all treatment groups of rats performed equally well in the time required to reach the platform (latency). This provides no evidence that spatial learning deficits observed in this task are due to deficient escape motivation or impairment of visual and/or motor performance.

In the acquisition phase trials, animals treated with METH showed impaired learning evidenced by a significant difference between the escape latency times for the trials performed on the third day and a slope value no significantly different from zero. This impairment was abolished by the pre-treatment with MEM. Here we demonstrate that MEM treatment resulted in a significant improvement in water maze acquisition in male Long Evans rats. One study in Fisher rats also reported improved water maze learning with MEM but at a high dose (30 mg/kg/day for 8 weeks) [38].

Despite the demanding task used in this study (about 40-min inter-trial) that will presumably involve intra-session consolidation-like processes, we found a decreased latency in finding the platform across the training sessions performed in days 1 and 3 and slope values were different from zero in either saline, MEM or MEM + METH-treated groups, thus indicating that these groups learned the task adequately.

Characteristic effects of NMDA receptor antagonists such as MK-801 in rodents are dose-dependent hyperactivity [39,40], impairment in water maze performance, and increased thigmotaxis [41]. This raises the question as to whether the beneficial effect of MEM in the water maze task can be explained, at least in part, by

reduced thigmotaxis. It is unlikely because the proportion of time spent by different groups in the border of the water tank were not significantly different.

The probe trial was designed to examine the extent of spatial discrimination learning (spatial bias) following the last session of hidden platform training. Results of this trial demonstrate that METH-treated rats failed to show preferential searching in the target quadrant during the probe trial compared to all other groups. In contrast to METH-treated animals, saline or MEM-treated groups spent more time in the target quadrant than in any other quadrant indicating spatial memory retention. Moreover, MEM pre-treatment also abolished the deficiency in the task evidenced in the METH group and corroborates the effect of this drug and represents an improvement in animals' spatial learning and memory.

These obvious impairments in memory function induced by METH were not due to the possible impairment of motor function because the measurement of speed in the open field test or in the Morris water maze remained unchanged in all groups of treatment.

The beneficial effect of MEM on learning and memory described here was present at low doses of MEM, indicating a specific profile, since it has been described that the beneficial effect of MEM at high doses (100 mg/kg) in the water maze test may come, at least partially, from its potential anxiolytic effects [42].

It is important to denote that METH impairs the thermoregulatory response in rodents [18]. As can be deduced from present results, METH induced a significant increase in body temperature lasting for at least 6 h. Hypothermia is neuroprotective against amphetamine derivatives-induced neurotoxicity [43]. Present results demonstrate that MEM given alone did not induce any change in body temperature. Furthermore, when MEM was administered previously to METH, the hyperthermic response to this amphetamine derivative was not modified. As a consequence, a neuroprotective effect based on an antihyperthermic mechanism of MEM must be ruled out.

In conclusion we have demonstrated that repeated METH treatment induced an impairment of spatial and non-spatial memory in male Long Evans rats that persisted for at least 14 days after the withdrawal of METH. These alterations in the memory were prevented by MEM pre-treatment. Taking together these results with those previously published [19], suggest that MEM constitutes the first useful approach to prevent the cognitive deficits induced by amphetamine derivatives, which are frequently abused in western countries.

## Conflict of interests

The authors declare no conflicts of interest.

## Acknowledgments

We are grateful to the Language Advisory Service of the University of Barcelona for revising the language of the manuscript. This work was supported by a grant from the Plan Nacional sobre Drogas (Ministerio de Sanidad y Política Social) (2008/003) and Generalitat de Catalunya (2009SGR 977).

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