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Effect of harmane, an endogenous β-carboline, on learning and memory in rats

Ipek Komsuoglu Celikyurt [a], Tijen Utkan [a,b], Semil Selcen Gocmez [a,c], Alan Hudson [d], Feyza Aricioglu [e,*]

[a] Kocaeli University, Faculty of Medicine, Department of Pharmacology, Umuttepe, Kocaeli, Turkey
[b] Kocaeli University, Experimental Medical Research Center, Umuttepe, Kocaeli, Turkey
[c] Namik Kemal University, Faculty of Medicine, Department of Pharmacology, Tekirdag, Turkey
[d] University of Alberta, Department of Pharmacology, 947 Medical Sciences Building, Edmonton, Canada T6G 2R3
[e] Marmara University, Faculty of Pharmacy, Department of Pharmacology and Psychopharmacology Research Unit, Haydarpaşa, Istanbul, Turkey

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ABSTRACT

Our aim was to investigate the effects of acute harmane administration upon learning and memory performance of rats using the three-panel runway paradigm and passive avoidance test. Male rats received harmane (2.5, 5, and 7.5 mg/kg, i.p.) or saline 30 min. before each session of experiments. In the three panel runway paradigm, harmane did not affect the number of errors and latency in reference memory. The effect of harmane on the errors of working memory was significantly higher following the doses of 5 mg/kg and 7.5 mg/kg. The latency was changed significantly at only 7.5 mg/kg in comparison to control group. Animals were given pre-training injection of harmane in the passive avoidance test in order to determine the learning function. Harmane treatment decreased the retention latency significantly and dose dependently, which indicates an impairment in learning. In this study, harmane impaired working memory in three panel runway test and learning in passive avoidance test. As an endogenous bioactive molecule, harmane might have a critical role in the modulation of learning and memory functions.

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1. Introduction

Harmane first isolated in *Peganum harmala*, and related alkaloids are distributed widely in medicinal plants and found endogenously in mammalian tissues, including the central nervous system, liver, platelets, in plasma and urine (Bidder et al., 1979; Airaksinen and Kari, 1981; Beck and Faul, 1986).

Radioligand binding and autoradiographical studies showed that [3H]-harmane was located in the central nervous system regions such as hypothalamus, hippocampus, cerebral cortex, striatum, cerebellum and spinal cord. Moreover the highest density of binding site was determined in the hypothalamus (Anderson et al., 2006; May et al., 1991). Functional characterization of harmane and related compounds has revealed a complex pharmacology following exogenous administration (Rommelspacher et al., 1994).

In particular, neurochemical and behavioral studies have shown that harmane is associated with the potentiation of monoaminergic pathways through monoamine oxidase (MAO) inhibition, blockade of reuptake sites and direct activation of monoamine receptors (Reniers et al., 2011; Herraiz, 2007; Cao et al., 2007). Functional studies have shown that harmane binds to imidazoline (I) I_1, I_2 and I_3 receptors with changes in blood pressure, monoamine turnover and insulin secretion following harmane administration (Musgrave and Badoer, 2000; Cooper et al., 2003).

Harmane has a number of effects on the central nervous system function pointing to its importance as a neuromodulator. Previous studies has been shown that treatment with harmane has a profound anti-allodynic effect in both mononeuropathic and acute pain; anxiolytic and antidepressant effect and it has been found to have a modulatory role on both in vivo and in vitro morphine withdrawal syndrome (Aricioglu et al., 2003; Aricioglu and Altunbas, 2003; Aricioglu and Utkan, 2003; Aricioglu-Kartal et al., 2003).

In general, memory function, as measured by changes in an animal's behavior is determined some time after learning, which reflects many processes, including the acquisition, consolidation and retrieval of memory (Abel and Lattal, 2001).

In this study, three panel runway paradigm and passive avoidance test were used to determine memory functions in rats. Three panel runway test allows one to analyze differences between working and reference memory. The passive avoidance test is a classic model behavioral test to determine learning and memory functions in rats and mice with a strong aversive component (Kameyama et al., 1986).

Previous studies have reported the involvement of dopamine D1/D2 receptors on harmane-induced amnesia in the step-down passive avoidance test (Nasehi et al., 2010) and the involvement of cholinergic system in the harmane-induced impairment of memory functions (Nasehi et al., 2012). It is already known that multiple neurotransmitter

* Corresponding author at: Marmara University, Faculty of Pharmacy, Department of Pharmacology and Psychopharmacology Research Unit, Haydarpaşa, 34668 Istanbul, Turkey. Tel.: +90 216 418 95 73; fax: +90 216 345 29 52.
E-mail address: feyza.aricioglu@gmail.com (F. Aricioglu).

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systems have been implicated in the formation of learning and memory but it is still unknown how the β-carboline harmane, may modulate learning and memory functions in naive animals.

The aim of the present study was to further evaluate the effect of acute harmane treatment on reference, working memory by the three panel runway paradigm and learning function by the passive avoidance test in rats.

2. Materials and methods

2.1. Animals

Male Wistar 139 rats weighing 200–250 g were obtained from Kocaeli University Experimental Research and Application Center (Kocaeli, Turkey) at 6 months of age and kept in an animal colony at a density of approximately 5–6 per cage for 2 weeks before the experiments. The experiments were conducted between 9:00 am and 12:00 pm under standard laboratory conditions which were maintained in a temperature (22±2 °C room temperature, 12-h light/dark cycle with lights on at 7:00 pm.). Rats were taken on a food deprivation schedule maintaining their weight at 80%–85% of the free-feeding level according to the method described in the literature, before the start of the three panel runway experiments (Ohno et al., 1992). All animals in this study were naive to the experimental tasks. Different rat groups were used in each experiments. All experiments were carried out according to the guidelines for the care of laboratory animals and ethical approval was granted by the Ethics Committee of Kocaeli University (Number: AEK 141/14, Kocaeli, Turkey).

2.2. Apparatus

2.2.1. Locomotor activity measurements

Since compounds altering locomotor activity may give false-positive/negative effects in these tests, an additional test was carried out with the specific aim of monitoring motor activity. The spontaneous locomotor activity of the animals was assessed by monitoring their activity in a locomotor activity cage. Locomotor activity was measured with a computerized system (40×40×35 cm box; May Commat, Ankara, Turkey). Total number of movements was measured for a 5 min period before the behavioral tests and is expressed as the sum of stereotypic, ambulatory and vertical activity.

2.2.2. Three-panel runway test

The three-panel runway test was used to assess reference and working memory performances of rats according to a previously described method (Furuya et al., 1988; Ohno et al., 1992). The three-panel runway apparatus (175×36×25 cm, length×width×height) is composed of a start box, a goal box, and 4 consecutive intervening choice points. Each choice point consists of a gate with 3 panels (12×25 cm, width×height). Rats are prevented from passing through 2 of the 3 panels in the gate by front stoppers. They are also prevented from returning either to the start box or to a previous choice point by rear stoppers affixed to each of the panels in all gates. When the rats reached the goal box, they received food pellets as positive reinforcement.

At the beginning of the test, all front stoppers were removed so that at each choice point a rat could pass through any 1 of the 3 panel gates. The rats were forced to run the task repeatedly until the time elapsed from leaving the start box to reaching the goal box fell consistently below 20 s. Once the rats reached this state, they were forced to run the task with the front stopper of only 1 of the 3 panel gates (the correct panel gate) removed at each choice point.

In the working memory task, 6 consecutive trials were performed each day at 2-min intervals (1 session); water was freely available between trials in the home cage. The locations of the correct panel gates were held constant within a session, but were changed from one session to the next. Thus, 12 different patterns of correct panel gate locations were used in this experiment, as described (Furuya et al., 1988). In the reference memory task, 6 consecutive trials were applied each day at 2-min intervals (1 session). The locations of the correct panel gates were held constant both within a session and in succeeding sessions.

The number of times an animal pushed an incorrect panel gate is defined as errors. The time required for the animal to take food pellets is defined as latency, were recorded for each rat in each trial of a session. The learning criterion was less than 6 and less than 12 errors summed across the 6 trials of a session in reference and working memory tasks, respectively. The criterion was defined according to the study reported by Ohno et al. (1992).

After the rats fulfilled this criterion throughout 3 consecutive sessions, they were divided into groups. Rats which failed to reach the learning criterion were discarded from the study.

Namely, for the reference memory task, the number of errors and the latency were summed across all 6 trials of a session because this task was taught in order to evaluate the ability of rats to retain the constant location of the correct panel gates. However, they were summed from the 2nd trial to the 6th trial of a session for the working memory since the 1st trial was given to present the correct panel gate location in each session and did not reflect any memory function. Thus, the number of errors and the latency summed from the 2nd trial to the 6th trial of a session was used to evaluate the ability of rats to remember new correct panel gate locations, and these data were presented separately from those recorded in the 1st trial.

2.2.3. Passive avoidance test

Animals were trained on a passive avoidance apparatus (Ugo Basile model 7551, Italy) for evaluating memory based on contextual fear conditioning and instrumental learning. In this task, the animal learns that a specific place should be avoided since it is associated with an aversive event. Decrease in stepthrough latency (STL, retention latency) indicates an impairment in memory in the PA task.

The training apparatus consisted of 2 compartments, each measuring 22×21×22 cm. The illuminated white chamber was connected to the dark chamber (i.e. conditioning chamber) which was equipped with an electric-cable grid floor and the shock was delivered to the animal's feet via a shock generator. The 2 chambers were separated by a flatbox partition, including an automatically operated sliding door at floor level.

The training trial was carried out as described by Hiramatsu et al. (1998) and Monleon et al. (2002). The animals received drugs prior to PA training. On the 1st day of training, rats were placed individually into the light compartment and allowed to explore the boxes for 5 min. After 30 s, the door between these 2 boxes was opened and the animal moved freely into the dark compartment (preacquisition trial).

The acquisition (training) trial was carried out 15 min after the preacquisition trial. Rats were again placed in the light compartment of the PA apparatus. After a 10-second adaptation period in the safe chamber, the door between the compartments was opened. Having entered the dark compartment, the sliding door was closed automatically and an electric foot shock (0.5 mA) of a 3-second duration was immediately delivered to the animal via the grid floor. The time taken to enter the dark compartment was recorded (training latency). Any animal failing to cross from the illuminated to the dark compartment within 300 s was excluded from the experiment. Animals were then removed from the dark chamber and returned to their home cages. Between each training session, both compartments of the chamber were cleaned to remove any confounding olfactory cues.

For the retention trial, recall of this inhibitory stimulus was evaluated 24 h after the training by returning the animals into the light compartment and recording their latency to enter the dark compartment (all 4 paws in). No foot shock was applied in this trial. If the animal had not entered the dark compartment within 300 s, it was returned to its cage and a maximum latency of 300 s was recorded. This latency

served as a measure of retention performance of the step-through avoidance response.

2.2.4. Drugs and treatment

Harmane HCl was obtained from Sigma Chemicals (Sigma, St. Louise, MO) and dissolved in saline and administered 2.5, 5, and 7.5 mg/kg intraperitoneally. Harmane was injected (i.p.) 30 min before behavioral tests. Harmane and saline was applied before the acquisition session (first day) of passive avoidance test. All drugs were prepared immediately prior to use and given intraperitoneally (i.p.) in a volume of 0.1 ml per 100 g body weight of rats. The drug doses and the administration time were selected according to the locomotor activity results of the animals and previous studies (Aricioglu et al., 2003; Aricioglu and Altunbas, 2003).

2.2.5. Statistical analysis

The number of errors and latency were summed across all six trials of a session for the reference memory task. They were summed from the first and second trials to the sixth trial of a session for the working memory. Group differences were tested by one way analysis of variance (ANOVA). When multiple comparisons were necessary after ANOVA, the Dunnett's test was applied. Locomotor activity and passive avoidance data were also normally distributed, hence the ANOVA was performed. Differences between means were consider significant if $p<0.05$.

3. Results

3.1. Locomotor activity performance

Increased locomotor activity may produce behavioral disinhibition and can affect learning and memory processes. To exclude this possibility, the locomotor activity of the animals was also assessed by measuring the number of movements over a 5 min period. Number of movements for control group ($n=16$) was 1019.4 ± 64.9. Statistical analysis of the data showed that harmane at 2.5 (1112.4 ± 69.6, $n=18$), 5 (1124.2 ± 55.8, $n=18$), and 7.5 (1006.6 ± 31.7, $n=22$) mg/kg doses did not modify the number of movements in the locomotor activity test significantly ($F(3,70)=1.264$, $p>0.05$) (Fig. 1).

3.2. Reference memory

The time of latency for the control group of animals was 24.72 ± 2.40 s ($n=8$). For animals treated with 2.5 mg/kg harmane the latency was 28.36 ± 2.92 s ($n=7$); for rats receiving 5 mg/kg harmane the latency was 26.76 ± 3.93 ($n=8$) and for rats receiving 7.5 mg/kg harmane the latency was 27.46 ± 2.59 s ($n=10$). In summary harmane treatment had no effect on the latency in reference memory trials $F(3,29)=0.2522$ $p=0.8522$ (Fig. 2).

Numbers of errors for the control group of animals were 1.38 ± 0.46 ($n=8$), for the 2.5 mg/kg harmane group they were 1.71 ± 0.68 ($n=7$), for the 5 mg/kg harmane group they were 2.13 ± 0.55 ($n=8$) and for the 7.5 mg/kg harmane group they were 2.70 ± 0.86 ($n=10$). Also harmane did not affect number of errors $F(3,29)=0.7352$, $p>0.05$ (Fig. 3).

3.3. Working memory

In trial 1, time of latency in control group was 8.58 ± 0.56 s ($n=8$), for rats receiving 2.5 mg/kg Harmane ($n=8$) the time of latency was 8.92 ± 0.77 s, for rats receiving 5 mg/kg Harmane the time of latency was 11.09 ± 0.61 s ($n=7$) and for rats receiving 7.5 mg/kg Harmane 9.14 ± 0.88 ($n=9$) (Fig. 4).

In trial 1, in control group, the number of errors was 2.9 ± 0.28 ($n=8$), in 2. 5 mg/kg harmane group they were 3.89 ± 0.59 ($n=8$), in 5 mg/kg harmane group they were 4.8 ± 0.49 ($n=7$) and in 7.5 mg/kg harmane group they were 5.3 ± 0.22 ($n=9$) (Fig. 5). In trial 1, harmane did not cause a significant increase in error response in any of the applied doses when compared to the saline group (Fig. 5).

In trials 2–6, the numbers of errors were 3.63 ± 0.65 ($n=8$) in the control group and 6.25 ± 1.41 ($n=8$) for the 2.5 mg/kg harmane group, 10.0 ± 1.33 ($n=7$) for the 5 mg/kg harmane group and 14.67 ± 2.45 in the 7.5 mg/kg harmane group ($n=9$). Harmane treatment of rats (5 and 7.5 mg/kg) significantly increased error responses in trials 2–6 whereas 2.5 mg/kg had no effect $F(3,28)=8529$, $p=0.0004$ (Fig. 6).

In trials (2–6) the latencies (sec) of working memory results were in control group 22.61 ± 0.96 ($n=8$), whereas for animals treated with 2.5 mg/kg harmane the mean latency was 22.0 ± 1.03 ($n=8$); for those receiving 5 mg/kg harmane the latency was 23.68 ± 1.47 ($n=7$) and for 7.5 mg/kg harmane the latency was 48 ± 1.78 ($n=9$). Harmane at 7.5 mg/kg also produced a significant increase in the total latency (sec) in both trials (2–6) $F(3,28)=6.679$ $p=0.0015$ (Fig. 7).

3.4. Passive avoidance performance

Step down latency (sec) in control group of animals was 251.43 ± 17.20 ($n=16$); whereas for rats treated with 2.5 mg/kg harmane the latency was 188.89 ± 31.62 ($n=18$); and latency was further reduced to 97.92 ± 24.74 ($n=18$) following 5 mg/kg treatment, and by 7.5 mg harmane 58.81 ± 17.68 ($n=22$). Harmane (2.5, 5, and 7.5 mg/kg, i.p.) treated rats showed a significant and dose-dependent effect on step down latency as compared to that of control rats. The retention time performed 24 h after the training test significantly decreased in harmane-treated rats $F(3,70)=13.779$, $P<0.0001$ (Fig. 8).

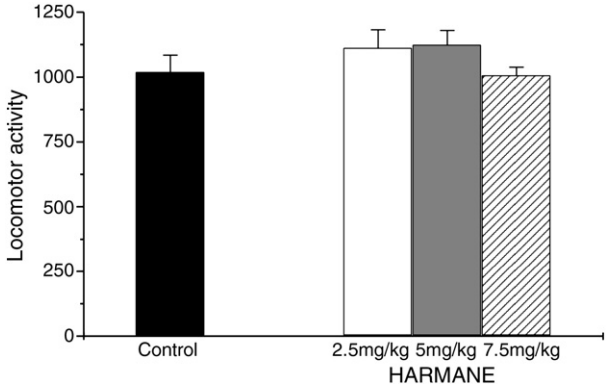

Fig. 1. Effects of harmane (2.5, 5 and 7.5 mg/kg, i.p.) on the locomotor activity of rats in the activity cage. The number of movements of rats as a total of horizontal, vertical and ambulatory activities in the activity cage is represented and expressed as mean ± SEM ($n=16$–22 rat/group).

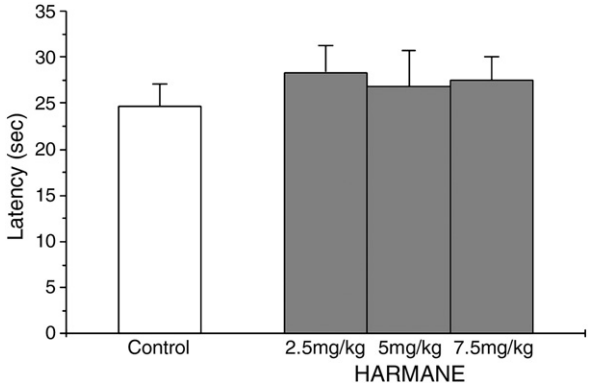

Fig. 2. Effects of harmane (2.5, 5 and 7.5 mg/kg, i.p.) on the latency (sec) in reference memory. Three-panel runway test. Each value represents the mean ± SEM of the parameters summed across all six trials of a session. ($n=7$–10 rat/group).

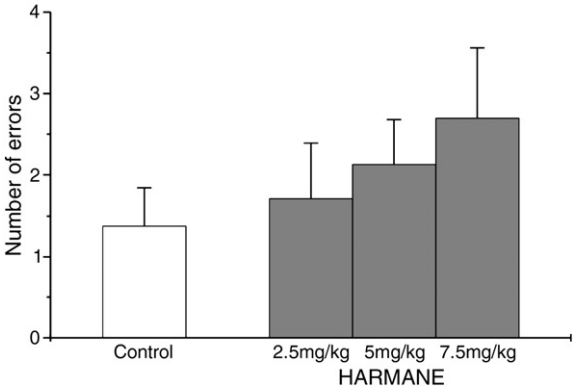

Fig. 3. Effects of harmane (2.5, 5 and 7.5 mg/kg, i.p.) on the number of errors in reference memory. Three-panel runway test. Each value represents the mean ± SEM of the parameters summed across all six trials of a session. (n = 7–10 rat/group).

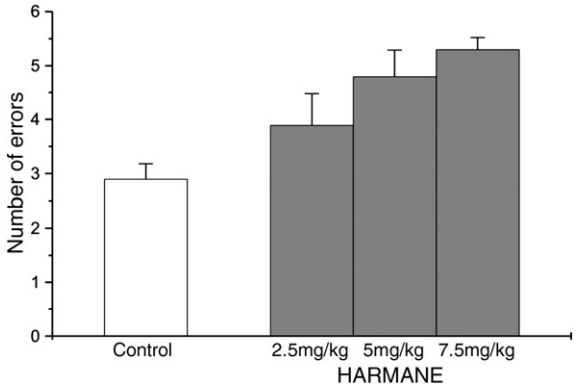

Fig. 5. Effects of harmane (2.5, 5 and 7.5 mg/kg, i.p.) on the number of errors on working memory (trial-1). Three panel runway test. Each value represents the mean ± SEM of the parameters recorded in the first trial. First trial which does not reflect any memory function was given to present the correct panel gate location. (n = 7–9 rat/group), *p<0.05, different from control group.

4. Discussion

This study demonstrates, for the first time, the effects of acute harmane administration on reference memory, working memory and learning function in rats. Our results showed that, harmane impaired working memory especially at higher doses in the three panel runway paradigm. It did not affect reference memory at any doses administered in this study. Harmane impaired learning function in the passive avoidance test.

The three panel runway paradigm distinguishes between reference and working memory. Working memory allows animals to remember information that is useful for a single session of an experiment but not for subsequent sessions, whereas reference memory is defined as the holding of an information that is continued to be of value across all sessions (Ohno et al., 1992). In this study, the effect of harmane on working memory in terms of errors was significant at 5 and 7.5 mg/kg whereas the latency performance of rats was only significant at 7.5 mg/kg of harmane. The worsening effect of harmane was significant in higher doses, therefore, harmane levels might be important in the modulation of working memory function and probably harmane may at least play a role in memory dysfunction. Factors which may modulate harmane's synthesis is still not clear, however, there might be several speculations explaining how harmane treatment might result in memory deficits in the three panel runway task or passive avoidance test.

It is well known that the cholinergic system plays a critical role in the modulation of neuronal activities that are related to different forms of learning and memory. The known mechanisms underlying working memory is that behavioral effects of drugs that block muscarinic acetylcholine receptors support a role for cholinergic modulation (Hasselmo and Stern, 2006). Besides, it has been shown in 1983 that acetylcholinesterase activity of homogenates from several brain structures were inhibited by beta-carbolines (Skup et al., 2006). The inhibition was of the noncompetitive type in the case of the enzyme. This effect was most strongly manifested by harmane whereas the activity of choline acetyltransferase was not altered. Among the many underlying reasons for the memory impairment, cholinergic system defects in the cortical and the hippocampal areas have been identified as one of the major causes of these abnormal behaviors (Ueki et al., 1994; Shinjo et al., 1998; Park et al., 2000).

Recently, it has been shown that the nicotinic cholinergic system involvement has a role in the harmane-induced impairment of memory formation in male NMRI mice which is consistent with our data in rats (Nasehi et al., 2012). In the same study, the pre-training administration of harmane decreased memory formation. Additionally, pre-test intra-CA1 injection of ineffective doses of nicotine fully reversed harmane-induced impairment of memory after pretraining injection of harmane; pretesting administration of mecamylamine fully reversed harmane-induced impairment of memory after pretraining injection of harmane (Nasehi et al., 2012). All these data may be a further evidence of the interactions between harmane and the cholinergic system. Recently, neurochemical and neuroendocrine effects of harmane in fear conditioned rats were investigated and it has been reported that harmane as a neuromodulator may have a role in altering behavior, brain chemistry and neuroendocrine function (Smith et al., 2012).

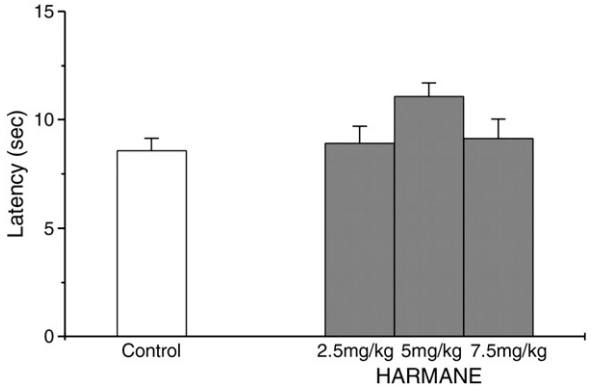

Fig. 4. Effects of harmane (2.5, 5 and 7.5 mg/kg, i.p.) on the working memory latency (sec) (trial-1). Three panel runway test. Each value represents the mean ± SEM of the parameters recorded in the first trial. First trial which does not reflect any memory function was given to present the correct panel gate location. (n = 7–9 rat/group), *p<0.05, different from control group.

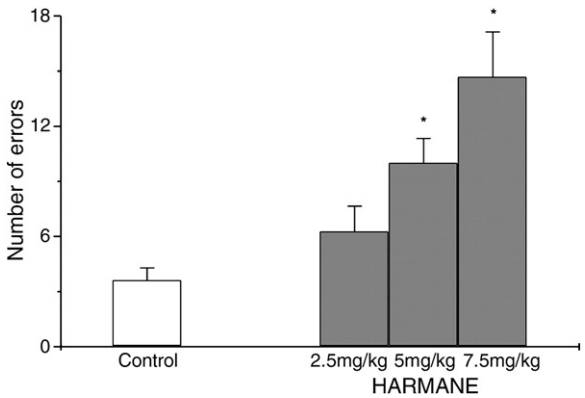

Fig. 6. Effects of harmane (2.5, 5 and 7.5 mg/kg, i.p.) on the number of errors on working memory (trials (2–6)). Three panel runway test. Each value represents the mean ± SEM of the parameters recorded those summed from the second to the sixth trials of a session. (n = 7–9 rat/group), *p<0.05, different from control group.

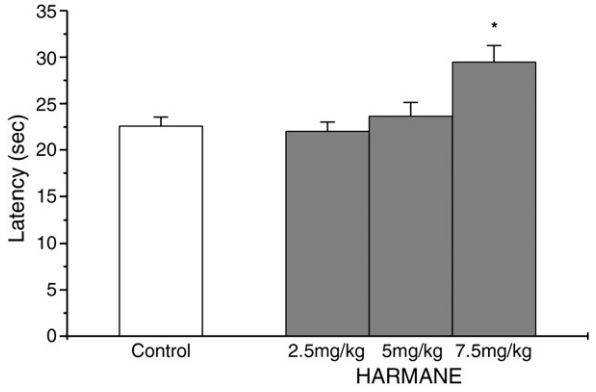

Fig. 7. Effects of harmane (2.5, 5 and 7.5 mg/kg, i.p.) on working memory latency (sec) (trials (2–6)). Three panel runway test. Each value represents the mean ± SEM of the parameters recorded those summed from the second to the sixth trials of a session. (n = 7–9 rat/group), *p<0.05, different from control group.

Another possibility is that harmane impaired memory via dopaminergic and/or serotonergic system. The neural mechanisms of dopamine and "working memory" in prefrontal cortex have been the subject of studies aimed to investigate the mechanisms of working memory (Surmeier, 2007). In agreement with our results, it has been shown that harmane impaired learning and memory, suggesting that this effect may be due to the elevated level of dopamine (Nasehi et al., 2010). Increasing evidence shows that 5-hydroxytryptamine (5-HT) also plays a modulatory role in memory functions (Meeter et al., 2006). Increases in 5-HT levels in the brain resulting from systemic administration of the 5-HT precursor 5-hydroxytryptophan or the 5-HT agonists fenfluramine and parachloramphetamine have been shown to stimulate 5-HT release and to worsen the acquisition and retention of conditioned responses. The changes in 5-HT activity in certain areas of the brain have been shown to be involved in the emotional mechanisms activating a memory trace. Furthermore, a memory retrieval deficit evoked by presentation of a habituated conditioned stimulus resulted in an enhanced 5-HT activity in those structures (Molodtsova, 2008). It has been shown that high doses of harmane increases both homovanilic acid and 5-hydroxyindoleacetic acid (5-HIAA) levels (Baum et al., 1996). In this line, we might, at least in part, speculated that harmane impaired working and learning process through above mentioned systems.

Our experimental study was planned to evaluate different stages of memory depending on the time course of the manipulations in harmane treatment. The animals received harmane prior to training for the passive avoidance test; consequently, the acquisition and memory formation were ameliorated in the passive avoidance test.

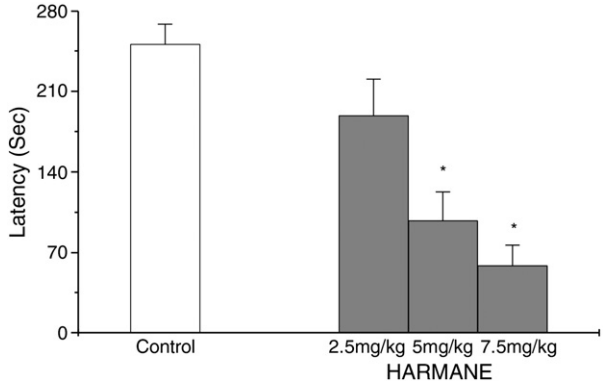

Fig. 8. Effects of harmane (2.5, 5 and 7.5 mg/kg, i.p.) on the retention latency (sec) of passive avoidance test. Each value represents the mean ± SEM of the latency to enter the dark compartment during one trial training and retention test of the step-down type of an inhibitory passive avoidance task. (n = 16–22 rat/group), *p<0.05, different from control group.

As the animal learned an association between a context and a shock memory acquisition processes occurs. During consolidation, formation of memory, which can last from minutes to days, this memory is moved from a labile to a more fixed state. During retrieval process, the animal is returned to the conditioning context, where memory for the context–shock association is assessed (Abel and Lattal, 2001). The passive avoidance test is based on instrumental learning and contextual fear conditioning that dependent on the hippocampus and amygdale (McGaugh et al., 1996). An explanation for the disturbing effect of harmane on avoidance learning function might be related to an action of harmane at imidazoline receptors. Harmane has been identified as the active component of the impure extract termed clonidine-displacing substance which previously shown to have affinity for imidazoline receptors (Parker et al., 2004). Drugs known as imidazoline ligands such as cirazoline, clonidine and moxonidine have also been shown to produce an impairment of memory scanning performance (Wesnes et al., 1997; Middleton et al., 1999; Arnsten and Jentsch, 1997).

Another possible mechanism for memory impairment might be due to the binding of harmane to benzodiazepine receptor complex. Thus, harmane is the most potent endogenously occurring inhibitor of benzodiazepine receptor binding known so far and suggested that it could function as the endogenous ligand of the benzodiazepine receptor (Mousah et al., 1986). Harmane binds to benzodiazepine receptor complex with a Ki value in the low micromolar range and could stimulate the benzodiazepine receptor in a inverse manner (Loew et al., 1985). It is already known that benzodiazepines are known to produce anterograde amnesia in humans (Lister, 1985) and laboratory animals (Jensen et al., 1979; Hodges and Gren, 1986; Nabeshima et al., 1990).

In conclusion, our study shows that harmane might have an important role in learning function and working memory modulation. It will be interesting to know whether endogenous production of harmane might increase in learning and memory deficits and/or the underlying mechanism. Further studies are required to determine the function of harmane's modulatory role in learning and memory which will also give us better understanding in cognitive pathologies.

Acknowledgments

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