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Research Paper: Role of Hippocampal 5-HT6 Receptors in Glucocorticoid-Induced Enhancement of Memory Consolidation in Rats

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ABSTRACT

Introduction: of the study: Post-training administration of glucocorticoids enhance memory consolidation of inhibitory avoidance learning. Given the involvement of 5-HT6 receptors in memory processing and the interaction of glucocorticoids with the brain serotonergic system in modulating memory processing, we investigated whether the effect of glucocorticoids on the consolidation of emotionally arousing training depends on hippocampal 5-HT6 receptors.

Methods: Rats were trained in an inhibitory avoidance task and immediately received the systemic injections of corticosterone (CORT) as well as the intra-hippocampal injections of 5-HT receptors agonist or antagonist. The memory retention test was done 48 hours after training and immediately after the behavioral test, the animals were sacrificed and the hippocampi (left and right) rapidly dissected out for molecular studies.

Results: Post-training injections of different doses of CORT (1.25, 2.5, 5, and 10 mg/kg) enhanced memory retention in a dose-dependent manner. The CORT-induced enhancement of memory consolidation was blocked by bilateral intra-hippocampal injections of 5-HT6 receptor antagonist SB271046 (5 or 10 ng/per side), but not agonist EMD386088 (5 or 10 ng/per side). Furthermore, systemic CORT reduced 5-HT6 receptor mRNA and protein expression in the hippocampus. Both doses of 5-HT6 receptor agonist and antagonist significantly enhanced and reduced the expression of the 5-HT6 receptor, respectively, and both ligands at the higher dose (10 ng) enhanced memory consolidation. Moreover, CORT injection attenuated and enhanced, respectively, the effects of agonist and antagonist on 5-HT6 receptor expression.

Conclusion: These behavioral and molecular findings indicated an interaction between glucocorticoids and hippocampal 5-HT6 receptors in the consolidation of emotionally arousing experiences.

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Highlights

- Corticosterone enhanced memory consolidation of inhibitory avoidance training.
- Corticosterone reduced the expression of 5-HT6 receptor mRNA and protein.
- Intra-hippocampal infusions of 5-HT6 receptor antagonist SB271046 blocked the corticosterone effect.
- Intra-hippocampal infusions of 5-HT6 receptor agonist EMD386088 did not change the corticosterone effect.
- Glucocorticoid interacts with hippocampal 5-HT6 receptors to enhance memory consolidation.

Plain Language Summary

Previous studies have shown that glucocorticoids, which are released in stressful situations, enhance memory consolidation for emotional events. In the present study, we found that corticosterone, the main glucocorticoid in rodents, enhanced emotional memory consolidation in rats, and reduced the expression of 5-HT6 receptors in the hippocampus. The effect of corticosterone was blocked by the intra-hippocampal injections of the 5-HT receptor antagonist, but not agonist, suggesting an interaction between glucocorticoids and hippocampal serotonin system in the consolidation of emotionally arousing experiences.

1. Introduction

Glucocorticoid hormones (corticosterone (CORT) in rodents and cortisol in humans) are released from the adrenal cortex during stressful episodes and regulate a variety of physiological functions. It is well known that glucocorticoids enhance memory consolidation of emotionally arousing training experiences (Roozendaal, 2002). For example, systemic as well as intra-amygdala or intra-hippocampal injections of a specific glucocorticoid agonist enhance memory consolidation of inhibitory avoidance training (Cottrell & Nakajima, 1977; Roozendaal & McGaugh, 1997). Glucocorticoid Receptors (GRs) are widely expressed throughout the central nervous system, and particularly high amounts of GRs are expressed in the hippocampus (De Kloet, Vreugdenhil, Oitzl, & Joëls, 1998). The hippocampus is a major neural substrate in the learning of contextual information (Liao, Shi, Liu, & Zhao, 2013), and selectively is involved in memory consolidation for the training of the context component of inhibitory avoidance task (Malin & McGaugh, 2006).

A growing body of evidence indicates that the serotonergic system modulates therapeutic or pathophysiological aspects of learning and memory (Buhot, 1997; Garcia-Alloza et al., 2004; Lai et al., 2002). The serotonergic system with widespread distribution in the brain, especially in areas associated with learning and memory, such as the hippocampus, amygdala, and cerebral cortex plays a role in physiological functions, including mood, cognition, learning, and memory (Micheau & Marighetto, 2011; Sodhi & Sanders-Bush, 2004). Serotonin acts through several receptor types and subtypes. The 5-HT6 receptor is almost exclusively expressed within the central nervous system, with high abundance in areas associated with learning and memory, such as the hippocampus and cerebral cortex (Roberts et al., 2002). During the past two decades, among serotonin receptors, the 5HT6 receptor has received increasing attention and became a promising target for improving cognition (Mitchell, Hoplight, Lear, & Neumaier, 2006; Mitchell & Neumaier, 2005; Woolley, Marsden, & Fone, 2004). Previous studies have shown that both 5-HT6 receptor agonist and antagonist enhance learning and memory in animals' models. For example, it has been shown that the 5-HT6 receptor agonist WAY208466 administration following training enhanced memory consolidation of passive avoidance and increased c-fos and Arc expression in the hippocampus (Pereira, Martynhak, Andreatini, & Svenningsson, 2015). Also, 5-HT6 receptor antagonist SB271046 has been shown to reverse scopolamine-disrupted consolidation of a passive avoidance task and ameliorate spatial task deficits in old rats (Foley et al., 2004). There is growing evidence that the promoting effect of 5-HT6 receptor antagonist on memory is mediated, at least in part, by increased cholinergic activity (Marcos, Gil-Bea, Hirst, García-Alloza, & Ramírez, 2006; Riemer et al., 2003; Shirazi-Southall, Rodriguez, & Nomikos, 2002) as well as modulation of the glutamate (Dawson, Nguyen, & Li, 2000; Dawson, Nguyen, & Li, 2001).

Available evidence indicates that both glucocorticoids and serotonergic systems are involved in stress-related psychiatric disorders (Moore & Halaris, 1975). The reciprocal interaction between glucocorticoids and the 5-HT system is particularly controlled by the hippocampus (Lanfumey, Mongeau, Cohen-Salmon, & Hamon, 2008). The expression of the 5-HT6 receptor in the hippocampus is controlled by glucocorticoids as adrenalectomy increases 5-HT6 receptor mRNA expression in the hippocampus and this response reveres, in part, by CORT administration, suggesting that glucocorticoids modulate 5-HT6 receptor function (Yau, Noble, Widdowson, & Seckl, 1997). However, the interaction between glucocorticoids and 5-HT6 receptor in memory consolidation of inhibitory avoidance training has not yet been investigated. Thus, the aim of the present study was to examine the effect of glucocorticoid on memory consolidation in the presence or absence of 5-HT6 receptor agonist and antagonist. Moreover, the effect of glucocorticoid on the expression of 5-HT6 receptor mRNA and protein in the hippocampus was examined.

2. Materials and Methods

2.1. Animals

Adult male (three months, 250-300 g) Wistar rats obtained from the breeding colony of Shahid Beheshti University of Medical Sciences (Tehran, Iran) were used in the study. Rats were initially housed in large cages (50×26×25 cm), under 12-h light/dark cycles (6 am lights on–6 pm lights off). The cages were maintained in a temperature-controlled (22±2°C) room. Food and water were provided ad libitum. All behavioral experiments were conducted between 09:00 and 13:00 during the light cycle. All experimental procedures were approved by the Animal Ethics Committee of Semnan University of Medical Sciences, Semnan, Iran. All of the experimental trials were conducted in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

CORT (Sigma, England) was dissolved in dimethyl sulfoxide (DMSO) followed by dilution in saline to reach the appropriate concentration and was injected intraperitoneally (1.25, 2.5, 5, or 10 mg/kg) at a volume of 1 ml/kg. The 5-HT6 receptor agonist EMD386088 and antagonist SB271046 were purchased from Tocris Chemical Company. Both drugs were dissolved in DMSO followed by dilution in physiological saline to reach the appropriate concentration and administered by intra-hippocampal injection at the doses of 5 or 10 ng/µl to each hemisphere. The final concentration of DMSO for CORT and 5-HT6 receptor ligands dissolution was 2%. The drug doses were mainly derived from pilot studies.

2.3. Surgery and cannulae implantation

At least one week before behavioral training, the rats were anesthetized with ketamine hydrochloride (70 mg/kg) and xylazine (10 mg/kg) intraperitoneally and placed in a stereotaxic instrument, and two temporary stainless steel guide cannulae (8 mm lo ng) of 23-gauge were implanted toward the dorsal hippocampus (-4.2 mm Anterior-Posterior, ±4.1 mm lateral, and 2 mm ventral) according to the atlas of rat brain (Paxinos & Watson, 2009) and fixed to the skull with dental cement. Steel stylets were inserted into the guide cannulae to maintain patency until the rats received the infusions.

2.4. Intra-hippocampal administration

For bilateral intra-hippocampal injections of vehicle or drugs, the stylets were removed immediately before 30-gauge needles, 17 mm extending 2 mm beyond the tips of the cannulae, were inserted. Each injection needle was connected to a 10- ml syringe via polyethylene tubing and 1 µl of the vehicle or drug dissolved in the vehicle was infused for 60 s while the animal was restrained by hand. The needle was left in place for another 120 s before it was slowly withdrawn.

2.5. Passive avoidance training

The apparatus used for inhibitory avoidance (IA) training consisted of a two-compartment box (Mokhtari-Zaer et al., 2014). The lit chamber (22cm × 21cm × 23 (h) cm) made from transparent plastic was connected by a guillotine door to the dark compartment of the same dimensions with black opaque walls and ceiling. Stainless steel bares (3 mm in diameter and 1cm apart) made the floor of the dark chamber, through which a current pulse could be delivered from a source of constant current to the plantar surface of the paw. All experimental animals were habituated to the equipment at first. The animal was placed in the light chamber and 5 s later, the guillotine door was opened. After entering the rat into the dark chamber, the door was closed, and then the animal was returned into the home cage and 30 min later, the habituation trial was repeated and followed 30 min later again by the acquisition trial, during which after closing the guillotine door, a 50-Hz, 0.5 mA constant current shock within 1.5 s was applied immediately after the rat had entered into the dark chamber. At the end of the acquisition trial, the ani-

mal was returned from the dark chamber to the home cage. Then, 48 h after training, the rat was placed in the light chamber and after 5 s, the guillotine door was opened and the latency of entering into the dark chamber (step-through latency, STL) was recorded. This latency time was considered as the measure of memory retention. Nine minutes was considered as a cut-off point to enter into the dark chamber and was recorded if occurred.

2.6. Real-time reverse transcription-quantitative Polymerase Chain Reaction (PCR)

Immediately after the end of the behavioral test in experiment 2, half of the animals from each group were randomly sacrificed by decapitation and the hippocampi (left and right) rapidly dissected out the brain. Total RNA of the left hippocampus was extracted using the Trizol reagent (PR037A, TakaRa Japan) extraction method. RNA was ReR verse Transcribed (RT) into cDNA through a commercial cDNA synthesis kit (Bioneer Inc., Seoul, South Korea) and stored at –20°C until further use. Gene amplification was done in a 20-µL final volume reaction mixture containing 0.4 µL of each target-specific primer, 10 µL of 1×SYBR Green PCR master mix (Bioneer Inc., Seoul, South Korea), and 2 µL of cDNA. PCR thermal profile included the initial denaturation at 95°C for 2 min, followed by 45 cycles of denaturation at 94°C for 30 s, target gene-specific annealing for 30 s, and extension at 72°C for 45 s. The amplification was performed on Bio-Rad iQ5 Multicolor RT PCR (Bio-Rad Laboratories, Hercules, CA, USA). Gene expression was normalized to the levels of GAPDH as a reference gene in all reactions and the 2-ΔΔCt method was served for the calculation of gene expression. All primers (Table 1) were designed using AlleleID® version 7.5 Primer Select program (PREMIER Biosoft, USA) and synthesized by Bioneer Inc. (Seoul, South Korea). The accuracy of the primers was confirmed by direct sequencing of target sequences. All tests were performed in duplicate for each sample

2.7. Western blotting

Rats were sacrificed, and the right hippocampal tissues were collected, and then immediately frozen at −80°C. Hippocampal samples were homogenized and prepared in lysis buffer (137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP-40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, 1 µg/ml leupeptin, and 0.5 mM sodium vanadate). Tissue extracts were centrifuged to remove insoluble materials (12,500 × g for 20 min at 4°C) and total protein concentration was determined according to the Micro BCA procedure (Pierce, Rockford, IL, USA). Equal amounts (25 µg) of protein from each sample were loaded on 15% polyacrylamide gels and separated by a standard SDS-PAGE. Protein bands were transferred to polyvinylil dene fluoride (PVDF) membranes and then blocked using 5% skim milk and 0.1% Tween-20 in Tris-buffered saline. Membranes were incubated with primary antibodies overa night at 4°C [anti-5-HT6 receptor (ab93055); and anti-Gapdh; (ab181602)] followed by a secondary antibody [(goat anti-rabbit IgG horseradish peroxidase-conjugated antibody, (ab-7090)] for 1 h at room temperature. Images were acquired by DAB colorimetric detection kit according to the manufacturer's instructions. The result for the 5-HT6 protein receptor was quantified by densitometric analysis (using Gel-Pro analyzer imaging software).

2.8. Experimental design

2.8.1. Experiment 1

This experiment examined the systemic injection effects of different doses of CORT (1.25, 2.5, 5, or 10 mg/kg) on long-term memory consolidation. Rats were randomly divided into 5 groups (n=10 in each group). Immediately after training, the animals received vehicle or different doses of CORT (1.25, 2.5, 5, and 10 mg/kg). After 48 h, the animals were re-exposed to the same context and their step-through latencies and time spent in the lit side during 540 s were recorded. In this experiment, the most effective dose of CORT (5 mg/kg) was obtained for the next experiment.

2.8.2. Experiment 2

This experiment examined the effect of systemic administration of CORT (5 mg/kg) on memory consolidation in the presence or absence of the 5-HT6 receptor agonist EMD386088 (EMD) and antagonist SB-271046 (SB). Rats were randomly divided into 10 groups (n=10 in each

Table 1. Primer sequences used in quantitative reverse transcriptase-polymerase chain reaction

Primer Name	Sequence
5-HT 6 receptor	Forward, 5'-TGAGAATAGGAGGTGGTAGGT'-3' Reverse, 5'-ATGAGAGAAAGGGATGAGGA-3'
GAPDAH	Forward, 5'-AAGTTCAACGGCACAGTCAAGG-3' Reverse, (5'- CATACTCAGCACCAGCATCACC-3'

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group) and trained under shock according to described procedures. Immediately after training, the animals received bilateral intra-hippocampal injections of EMD or SB, followed by systemic CORT administration. The 10 experimental groups were: VEH+VEH, VEH+CORT, EMD (5 ng/1µl)+VEH, EMD (10 ng/1µl) + VEH, SB (5 ng/1µl) + VEH, SB (10 ng/1µl)+VEH, EMD (5 ng/1µl)+CORT, EMD (10 ng/1µl)+CORT, SB (5 ng/1µl)+CORT, and SB (10 ng/1µl)+CORT.

Moreover, at the conclusion of behavioral tests, 5-HT6 receptor mRNA, or protein expression was examined in half of the animals randomly selected from each group.

2.9. Statistical analysis

The inhibitory avoidance retention data are expressed as Mean±SEM. The one-way Analysis of Variance (ANOVA) was used to analyze data from experiment 1. Data from experiment 2 were analyzed by two - way ANOVA followed by the Tukey post hoc test using CORT and vehicle treatments both as between-subjects variables. In all comparisons, $P<0.05$ was considered to indicate statistical significance.

3. Results

3.1. Experiment 1: Effects of different doses of CORT on memory consolidation

A one-way ANOVA on step-through latency data (Figure 1) collected during the retention test revealed significant differences between the latencies of the groups ($F_{4,44}=4.266$, P=0.005). The post - hoc comparison indicated that CORT at the doses of 2.5 and 5mg/kg significantly enhanced memory consolidation compared with the control group (P=0.031 and P=0.003, respectively).

3.2. Experiment 2

3.2.1. Effect of systemic injection of CORT on memory consolidation in the presence or absence of 5-HT6 receptor agonist and antagonist

A tow-way ANOVA on step-through latencies revealed the significant effect of group ($F_{4,90}=3.325$, P=0.002), no significant effect of treatment ($F_{1,90}=1.254$, P=0.267), and a significant interaction between two factors ($F_{4,90}=3.914$, P=0.006). Post hoc comparisons by Turkey's test showed that step-through latencies of animals receiving VEH+CORT were significantly longer than the VEH+VEH group (P=0.009) (Figure 2). The step-through latencies of animals receiving VEH+CORT were significantly shorter than the SB (5 ng)+CORT (P=0.003), and SB (10 ng)+CORT (P=0.004) groups. The differences between the EMD (10 ng)+VEH and SB (10 ng)+VEH groups were significant compared with the VEH+VEH group (P=0.025 and P=0.017, respectively).

3.2.2. Effect of systemic injection of CORT on the expression of 5HT6 receptor protein in the hippocampus using Western blot test

A two-way ANOVA of 5-HT6 receptor protein expression (Figures 3A and B) revealed significant effects of treatment ($F_{1,39}=51.842$, P=0.0001), groups ($F_{4,39}=62.391$, P=0.0001), and the interaction between both factors ($F_{4,39}=8.347$, P=0.0001). 5-HT6 receptor protein expression reduced in the VEH+COT group than the VEH+VEH group (P=0.017). Also, 5-HT6 receptor mRNA protein expression significantly increased in the EMD (5 ng)+VEH and EMD (10 ng)+VEH groups than the VEH+VEH group (P=0.002 and P=0.001, respectively), while it significantly decreased in the SB (5 ng)+VEH and SB (10 ng)+VEH groups than the VEH+VEH group (P=0.01 and P=0.004, respectively). The difference between the VEH+CORT group and the EMD (5 ng)+VEH (P=0.02), SB (5 ng)+CORT (P=0.013), and SB (10 ng)+CORT (P=0.00) groups was significant.

3.2.3. Effect of systemic injection of CORT on the expression of 5HT6 receptor mRNA expression in the hippocampus using Real-time PCR

A two-way ANOVA of 5-HT6 receptor mRNA expression (Figures 3C and D) revealed significant effects of treatment ($F_{1,39}=193.429$, P=0.0001), groups ($F_{4,39}=94.474$, P=0.0001), and the interaction between both factors ($F_{4,39}=35.978$, P=0.0001). Also, 5-HT6 receptor mRNA expression reduced in the VEH+COT group than the VEH+VEH group (P=0.047). In addition, 5-HT6 receptor mRNA expression significantly increased in the EMD (5 ng)+VEH and EMD (10 ng)+VEH groups than the VEH+VEH group (both, P=0.001), while it significantly decreased in the SB (5 ng)+VEH and SB (10 ng)+VEH groups than the VEH+VEH group (P=0.009 and P=0.0001, respectively). The difference between the VEH+CORT group and the EM (5 ng)+CORT) (P=0.02), EM (10 ng)+CORT (P=0.03), and SB (10 ng)+CORT (P=0.003) was significant.

4. Discussion

The findings of the present study indicated that post-training administration of CORT enhanced memory consolidation of inhibitory avoidance training in a dose-dependent manner. This enhancing effect can be blocked by intra-hippocampal injections of 5-HT6 receptor antagonist, but not agonist. Moreover, systemic administrated CORT decreased the ex-

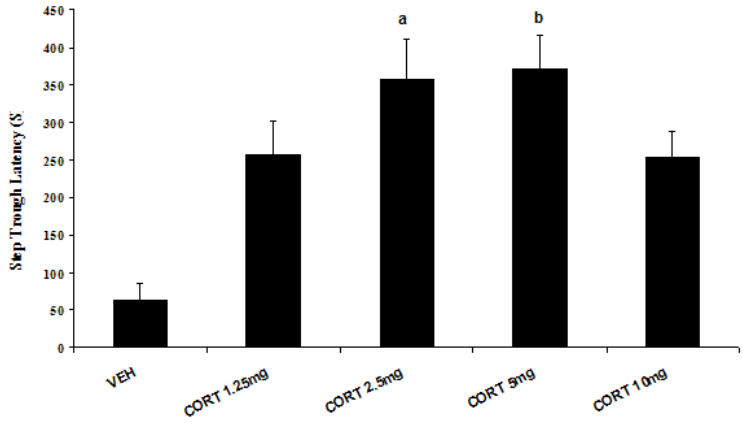

Figure 1. Effects of systemic injections of corticosterone on memory consolidation of inhibitory avoidance training.

[a] P=0.03; and [b] P=0.003 significant different than the control group; VEH: Vehicle; CORT: Corticosterone

pression of 5-HT6 receptor mRNA and protein as measured 48 h after behavioral training and CORT injection. These behavioral and molecular findings indicated an interaction between glucocorticoids and hippocampal 5-HT6 receptor in the consolidation of emotionally arousing experiences.

The first experiment showed that post-training administrations of CORT enhanced memory consolidation in an inverted U-shape. Our findings are in line with reports showing an improving effect of glucocorticoids on memory consolidation for inhibitory avoidance training (Roozendaal, 2000). Glucocorticoid enhancement of memory consolidation is mediated, at least in part, via glucocorticoid receptors located in the hippocampus as well as the amygdala (Roozendaal, 2000).

The second experiment examined whether glucocorticoid interacts with the hippocampal 5-HT6 receptor in influencing memory consolidation for inhibitory avoidance training. Similar to the results of the first experiment, we found post-training administration of CORT at a dose of 5 mg/kg to animals receiving bilateral intra-hippocam-

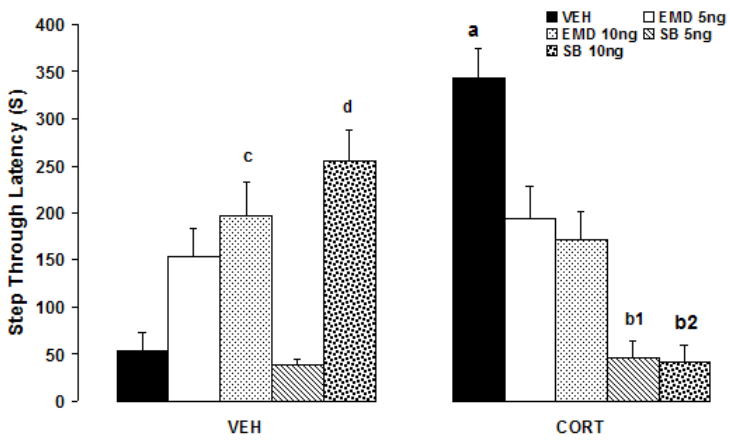

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Figure 2. Effect of systemic corticosterone and intra-hippocampal injections of 5-HT6 receptor agonist and antagonist on memory consolidation of inhibitory avoidance

[a] P= 0.009 significant different than the VEH-VEH group; [b1]P= 0.003; and [b2] P=0.004 significant different than the VEH-CORT group; [c] P=0.025; [d] P=0.017 significant different than the VEH-VEH group; VEH: Vehicle; CORT: Corticosterone; SB: SB271046 (5-HT6 receptor antagonist); EB: EMD386088 (5-HT receptor agonist)

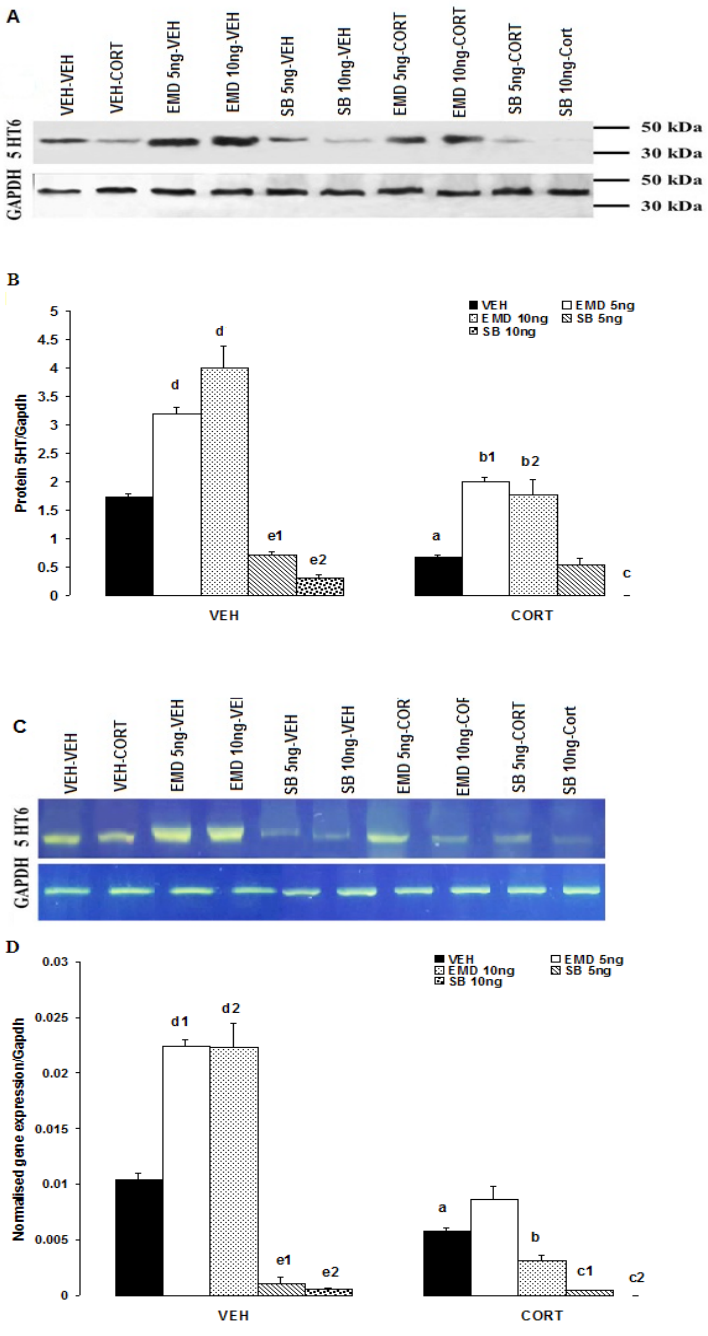

Figure 3. Effects of systemic corticosterone and intra-hippocampal injections

A & B: 5-HT6 receptor agonist and antagonist 5-HT6 receptor protein; and C and D: mRNA expression in the hippocampus.

A: Representative immunoblotting micrographs;

B: [a] P=0.017 significant different than the VEH-VEH group; [b] P=0.02; [c1] P=0.013; [c2] P=0.001 significant different than the VEH-CORT group; [d1] P=0.002; [d2] P=0.001; [e1] P=0.001; and [e2]P=0.004 significant different than the VEH-VEH group;

C: Representative of gene expression micrographs;

D: [a]P=0.047 significant different than the VEH-VEH group; [b1] P=0.02; [b2]P=0.03; and [c] P=0.003 significant different than the VEH-CORT group; [d] P=0.001; [e1] P=0.009; and [e2] P=0.0001 significant different than the VEH-VEH group; mRNA: Messenger RNA; 5-HT6: 5 hyroxythryptamine receptor 6; VEH: Vehicle, CORT: Corticosterone; SB: SB271046 (5-HT6 receptor antagonist); and EB: EMD386088 (5-HT receptor agonist).

pal injections of the vehicle to enhance memory retention when tested 48 h after training. Moreover, systemic CORT reduced the expression of 5-HT6 receptor density in the dorsal hippocampus, which seems to be related to the enhancing effect of CORT on memory consolidation. This finding is in a line of previous studies showing that pharmacological adrenalectomy increases 5-HT6 mRNA expression in the CA1 area of the hippocampus, and this effect is partly reversed by CORT replacement (Yau et al., 1997). The detailed functional significance of this effect and its correlation with CORT-induced memory enhancement is unclear and needs further studies.

The memory-enhancing effect of CORT was blocked by intra-hippocampal injections of the 5-HT6 receptor antagonist SB271046, suggesting the existence of an interaction between the 5-HT6 receptor and glucocorticoids effects on memory processing. A similar functional interaction between 5-HT6 receptors and the Hypothalamic-Pituitary-Adrenal axis (HPA) was reported in a study showing that 5-HT6 receptors blockade by SB271046 could reverse memory disturbances associated with increased HPA axis activity (Marcos, Aisa, & Ramírez, 2008). Further studies are needed to determine how the blockade of hippocampal 5-HT6 receptors could prevent the memory-enhancing effects of systemic CORT. The present study also showed that both 5-HT6 receptor agonist and antagonist at the higher dose enhanced memory consolidation. The finding that 5-HT6 agonism in the hippocampus enhances emotional memory is in line with a recent study demonstrating that systemic administration of the potent and selective 5-HT6 agonist WAY208466 facilitated cognitive processing in the passive avoidance test in mice by promoting the neuronal plasticity in caudate-putamen, hippocampus, and prefrontal cortex (Pereira et al., 2015).

Previous studies also have shown that systemic administrations of 5-HT6 receptor antagonists enhance cognitive performance in different learning and memory tasks in rodents (Foley et al., 2004; Woods, Clarke, Layfield, & Fone, 2012). These findings are confirmed by the observed enhancing effect of intra-hippocampal administration of the 5-HT6 antagonist SB271046 in the current study, which also indicated that the memory-improving effects of systemic 5-HT6 antagonists, at least in part, mediated via 5-HT6 receptors located in the hippocampus.

A recent study also has shown that both the 5-HT6 receptor agonist and antagonist reversed cholinergic- and glutamatergic-induced deficits in associative learning (Woods et al., 2012), but the underlying mechanisms of these similar effects are unclear. In the hippocampus, biochemical pathways associated with pERK1/2 expression seem to be related to the cognitive-enhancing properties of 5-HT6 receptor antagonists (Marcos, Cabero, Solas, Aisa, & Ramirez, 2010). Acetylcholine and glutamate, the two important neurotransmitters involved in learning and memory, seem to be involved in the effects of 5-HT receptor antagonist on cognitive functions (Riemer et al., 2003; Woods et al., 2012)

The present study demonstrated that both doses of 5-HT6 receptor agonist and antagonist significantly enhanced and reduced the expression of the 5-HT6 receptor, respectively. Similarly, it has been reported that systemic administration of the 5-HT6 receptor antagonist SB-399885 significantly reduced the 5-HT6 receptor density in the CA1, CA2, and CA3 hippocampal area, which seems to be associated with SB-399885-related improved memory consolidation (Meneses, Manuel-Apolinar, Castillo, & Castillo, 2007). Moreover, CORT injection attenuated and enhanced, respectively, the effects of agonist and antagonist on 5-HT6 receptor expression. This response was expected since CORT per se reduced the expression of the 5-HT6 receptor. The functional significance of these responses and its relation with CORT and 5-HT6 receptor ligands on memory awaits further experimentation.

The underlying mechanisms of interaction between glucocorticoids and 5-HT5 receptors in memory consolidation are not known. Acute stress increases the release of serotonin in the amygdala and hippocampus, two critical brain regions involved in emotional memory (Chaouloff, 2000). Since these structures play an important role in mediating the effects of glucocorticoids on memory consolidation (Roozendaal, 2000), based on the present data, it is logical to assume that the enhanced 5-HT neurotransmission following glucocorticoid administration may mediate or modulate, at least in part, the effects of glucocorticoids on memory consolidation. However, the nature of functional interaction between glucocorticoids and 5-HT system is presently unclear.

In summary, we showed that systemic CORT enhanced memory consolidation and reduced the 5-HT6 receptor expression in the hippocampus. Intra-hippocampal injection of SB271046, a selective 5-HT6 receptor antagonist, but not EM386088, a selective 5-HT6 receptor agonist, is able to prevent the memory-enhancing effect of CORT. Moreover, both 5-HT6 receptor agonist and antagonist enhanced and reduced per se, respectively, the hippocampal 5-HT6 receptor expression, and both ligands at the higher dose enhanced memory consolidation. These findings provide important evidence regard-

ing the functional interaction between glucocorticoids and the 5-HT6 receptor in cognitive functions.

Ethical Considerations

Compliance with ethical guidelines

All experimental procedures were approved by the Animal Ethics Committee of Semnan University of Medical Sciences, Semnan, Iran. All of the experimental trials were conducted in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animal experiments reported here are in accordance with the advance principles of the 3R guidelines; particularly every effort was made to reduce the number of animals and minimize any distress experienced by animals.

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Authors' contributions

Conceptualization, supervision: Ali Rahidy-Pour, Abdolhossien Shirravi; Methodology: Abbas Ali Vafaei, Rajab Mohammad Rezaei, Seyed Ali Seyedinia, Nasroallah Moradi Kor; Investigation, writing, review and editing: Rajab Mohammad Rezaei, Seyed Ali Seyedinia, Nasroallah Moradi-Kor; Writing original draft: Rajab Mohammad Rezae, Funding acquisition and resources: Ali Rahidy-Pour.

Conflict of interest

The authors declared no conflict of interest.

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