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The Role of Hippocampal 5HT3 Receptors in Harmaline-Induced Memory Deficit

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ABSTRACT

Introduction: Review literature shows that serotonergic system is involved in cognitive and non-cognitive behaviors induced by β-carbolines such as harmaline. Therefore we investigated the effect of activation and deactivation of hippocampal 5-HT3 receptors on the impairment of memory acquisition induced by harmaline.

Methods: Adult NMRI mice were tested with single-trial step-down passive avoidance, open field, and tail flick tasks for memory assessment, locomotor activity, and pain behaviors, respectively. Harmaline was injected peritoneally; 5-HT3 receptor agonist (M-CHL) and 5-HT3 receptor antagonist (Y-25130) were injected intra-hippocampal.

Results: Pre-training administration of the highest dose of harmaline (1 mg/kg), M-CHL (0.5 ng/mouse), and Y-25130 (5 ng/mouse) impaired memory acquisition, per se. In addition, both activation and deactivation of CA1 5-HT3 receptors by the subthreshold dose of M-CHL (0.005 ng/mouse) and Y-25130 (0.5 ng/mouse), respectively, prevented harmaline-induced memory acquisition deficit. Further studies about locomotor activity and pain threshold indicated that the interventions were ineffective on behaviors.

Discussion: Our data showed that hippocampal 5-HT3 receptor play a critical role and a modulatory effect on harmaline induced amnesia. However, further studies are needed to reveal the actual mechanism of action of CA1 5-HT3 receptors.

Key Words:
Memory reconsolidation,
Glucocorticoids,
Cholinergic agents

1. Introduction

One of the most important compound in the brain is serotonin (5-hydroxytryptamine; 5-HT), which influences learning and memory formation (Cammarota, Bevilaqua, Medina, & Izquierdo, 2008; Huerta-Rivas, Perez-Garcia, Gonzalez-Espinosa, & Meneses, 2010), In mammals, serotonin pathways originate from raphe nuclei, and ascending 5-HT fibers innervate brain regions (e.g. cortex, hippocampus), which make it well placed in mediating normal and dysfunctional learning and memory (Gonzalez-Burgos & Feria-Velasco, 2008; Hensler, 2006). 5-HT mediates its action by 14 different receptor subtypes with different functional properties (Perez-Garcia & Meneses, 2008a), which have been classified into families according to their pharmacological, molecular, and second messenger systems profiles (Meneses, 2003; Walstab, Rappold, & Niesler, 2010). 5-HT3 receptor is a ligand-gated ion channel, that induced rapid actions for hippocampal LTP modulation (Berumen, Rodriguez, Miledi, & Garcia-Alcocer, 2012). Other 5-HT receptors belong to the G-protein coupled receptor superfamily (Charnay & Leger, 2010). It seems that 5-HT receptors have critical role in regulation of behaviors induced by hippocampus, including learning and memory process (Berumen et al., 2012).

β-carbolines are a class of alkaloids which have elicited considerable research interest (Moura et al., 2006;

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Nasehi, Mashaghi, Khakpai, & Zarrindast, 2013). These alkaloids consist of an indole nucleus and a pyridine ring (Moura et al., 2006; Nasehi, Sharifi, & Zarrindast, 2012). β-carbolines can be divided into three structural groups, depending upon their degree of ring saturation: (a) harmane; (b) harmalane; and (c) the harmaline (Moura et al., 2006). These compounds, also known as harmala alkaloids, have been isolated from Peganumharmala (Rook et al., 2010). Though, harmala alkaloids are also said to occur endogenously in normal body constituents, such as heart, liver, kidney, blood plasma central nervous system et al., 2005; Zheng, et al., 2000). β-carbolines by inhibition of MAO_A or MAO_B increase the extracellular norepinephrine, dopamine and 5-HT levels in several brain regions (Herraiz & Chaparro, 2005). According previous data: 1-β-carboline could enhance 5-HT levels in several brain area through inhibition of MAO enzyme and 2-the role of β-carbolines (Venault & Chapouthier, 2007), 5-HT receptors (Cammarota et al., 2008; Liy-Salmeron & Meneses, 2007), and hippocampus (Burgess, Maguire, & O'Keefe, 2002) in memory process, the aim of this study was to investigate the involvement of harmaline on memory acquisition/exploratory behaviors/pain response with regards to 5-HT3 receptors on these phenomenon in the step-down passive avoidance, open field and tail flick tests in mice.

2. Methods

2.1. Animals

Male NMRI mice weighing 25–30 g obtained from the institute for cognitive sciences (ICSS) were used. Animals maintained at a controlled temperature (22±2°C) with 12/12-h light/dark cycle, which that behavioral tests were done during the light phase. The mice freely received food and water except during the limited times of experiments. In each group, ten animals were used and each mouse was used once only. All treatments have been designed in accordance with institutional guidelines for animal care and use that was approved by the Ethics Committee of the Faculty of Science of the University of Tehran.

2.2. Stereotaxic surgery

The animals were anaesthetized by xylazine (5 mg/kg) and ketamine hydrochloride (50 mg/kg) solution and then placed in a stereotaxic apparatus (Stoelting Co, Illinois, USA). Two stainless-steel guide cannulae (8 mm length, 22 gauge) were placed 1 mm above of hippocampus in accordance with atlas of Paxinos and Franklin (2 mm posterior of bregma, 1.6 from the sagital suture and 1.5 mm blow from the skull surface) (Paxinos & Franklin, 2001). The dental acrylic cement was used for cannulae fixation to the bone. For preventing possible obstruction, a stylet was presented into the guide cannula. The animals were rested in the home cage during 5-7 days for clearing the effect of the anesthetic drugs and recovery (Yousefi, Farjad, Nasehi, & Zarrindast, 2013).

2.3. Memory assessment

The passive avoidance task comprised of a plastic box (30×30×40 cm^3). There are parallel stainless steel rods (0.3 cm in diameter, spaced 1 cm apart) in the floor of task with a wooden platform (4×4×4 cm^3) in the center. An isolated stimulator (Grass S44, Quincy, MA, USA) delivered an electric shocks (1 Hz, 0.5 s and 50VDC) to the grid floor (Nasehi, Jamshidi-Mehr, Khakpai, & Zarrindast, 2014).

The training and retrieval protocols were carried out among 9:00 a.m. and 2:00 p.m. The animals were gently placed on the wooden platform and when the animal stepped down from the platform to the grid floor, intermittent electric shocks were delivered for 15 s continuously. 24 hours later, each animal was located on the platform again, and the step-down latency was measured as passive avoidance behavior. An upper cut-off time of 300 s was set (Nasehi, Amin Yavari, & Zarrindast, 2013; Nasehi et al., 2014; Nasehi, Piri, Abbolhasani, & Zarrindast, 2013).

2.4. Measurement of locomotor activity

The locomotion task (BorjSanat Co, Tehran, Iran) comprised of perspex container box (30 cm×30 cm×40 cm high). The task has a gray perspex panel (30 cm×30 cm×2.2 cm thick) with 16 photocells which separated the box to 16 equal-sized squares. Locomotor activity was recorded as the number of crossings from one square to another during 5 min (Khakpai, Nasehi, Haeri-Rohani, Eidi, & Zarrindast, 2012; Nasehi et al., 2014; Yousefi, Nasehi, Khakpai, & Zarrindast, 2012).

2.5. Tail flick test

The tail flick task had been used for animal pain response assessment (Nasehi et al., 2014; Tabatabai, Zarrindast, Lashkari, & Shafiee, 1999; Zarrindast, Dinkoub, Homayoun, Bakhtiarian, & Khavandgar, 2002). In this method, latency of flicks of animal's tail following a light beam that focused on the animal's tail was assessment. This behavior testing was done 5 min after training.

2.6. Drugs

Harmaline (1-methyl-7-methoxy-3, 4-dihydro-bcarboline) from Sigma (St. Louis, MO), 5-HT3 receptor agonist (M-CHL) and 5-HT3 receptor antagonist (Y-25130) from (Tocris Bioscience United Kingdom) were used in this study. The time of administration and doses of drugs were chosen according to pilot studies and published work in scientific literature (Nasehi, Amin Yavari, et al., 2013; Nasehi, Mashaghi, et al., 2013; Nasehi, Piri, Abdollahian, & Zarrindast, 2013; Nasehi et al., 2012). The drugs were tested at doses: harmaline: 0.25, 0.5 and 1 mg/kg, M-CHL: 0.005, 0.05 and 0.5 ng/mouse and Y-25130: 0.05, 0.5 and 5 ng/mouse. Harmaline was dissolved in sterile 0.9% saline solution and the compound was stirred for 1h before obtaining the final solution; other drugs were dissolved in 0.9% saline, just before the experiments.

2.7. Drug microinjections

After restrained gently animals by hand, during a 60 s period, the serotonergic agents were injected manually, via 27-gauge needles (1 mm below the tip of the guide cannulae) in a total volume of 1 µl/mouse (0.5 µl in each side). For facilitating the drug diffusion, the injection needles were left in place for an extra 60 s (Nasehi, Piri, Abdollahian, et al., 2013; Zarrindast, Hoseindoost, & Nasehi, 2012).

2.8. Statistical analysis

Due to the presence of individual variations in step-down apparatus data, we chose to analyze data using the Kruskal-Wallis non-parametric one-way analysis of variance (ANOVA) followed by a two-tailed Mann-Whitney's U-test. Holmes Sequential Bonferroni Correction Test was used for paired comparisons when appropriate. The median as well as interquartile ranges of the step-down latencies were recorded for ten mice in each experimental group. One/two way ANOVA followed by post-hoc test was used for statistical evaluation in the tail flick and open filed tasks. In all evaluations $P<0.05$ was considered statistically significant.

2.8.1. Experiment 1: Effects of pre-training 5-HT3 drugs administration on memory acquisition

In this experiment, eight groups of mice were used. Four groups of animals received saline (1 µl/mouse) or three doses of M-CHL (0.005, 0.05 and 0.5 ng/mouse) 5 min prior training. The other four groups received saline (1 µl/mouse) or three doses of Y-25130 (0.05, 0.5 and 5 ng/mouse) 5 min before training.

2.8.2. Experiment 2: Effects of pre-training 5-HT3 receptor drugs administration on memory acquisition under the disruptive effect of harmaline

In this experiment, 12 groups (three arms) of animals were used. The mice received saline (1µl/mouse) or various doses of harmaline (0.25, 0.5 and 1 mg/kg; i.p.) 15 min before training. These animals received intra-hippocampal pre-training saline (1 µl/mouse, for groups), subthreshold does of M-CHL (0.005 ng/mouse, for groups) or Y-25130 (0.05 ng/mouse, for groups) 5 min earlier training.

2.9. Histology

After behavioral testing, the animals were decapitated and the results were plotted on representative sections taken from the mice brain atlas of Paxinos and Franklin (Paxinos & Franklin, 2001). 200 from 221 mice with correct cannulae into the hippocampus regions were included in statistical analyses.

3. Results

3.1. Effects of pre-training intra-hippocampal administration of 5-HT3 drugs on memory acquisition, locomotor activity and tail flick

Kruskal-Wallis and Mann-Whitney results shows that the administration of M-CHL [$H(3)=9.30$, $P<0.05$; 0.5 ng/mouse, figure 1A; left panel] and Y-25130 [$H(3)=8.26$, $P<0.05$; 5 ng/mouse, figure 1A; right panel], 5 min before training, decreased memory acquisition. Furthermore, one-way ANOVA postulates that all interventions did not alter locomotor activity [$F(3, 36)=1.99$, $P>0.05$ for M-CHL (figure 1B; left panel) and $F(3, 36)=1.06$, $P>0.05$ for Y-25130 (figure 1B; right panel)] and tail flick [$F(3, 36)=1.96$, $P>0.05$ for M-CHL (figure 1B; left panel) and $F(3, 36)=0.46$, $P>0.05$ for Y-25130 (figure 1C; right panel)].

3.2. Effects of pre-training 5-HT3 receptor drugs administration on memory acquisition, locomotor activity and tail flick under the amnesia induced by harmaline

The data show that intraperitoneal injection of harmaline (1 mg/kg, i.p.) 15 min before training, decreased memory acquisition [$H(3)=19.03$, $P<0.001$, Figure 2A; left panel, using Kruskal-Wallis and Mann-Whitney], but did not alter locomotor activity [$F(3, 36)=2.3$,

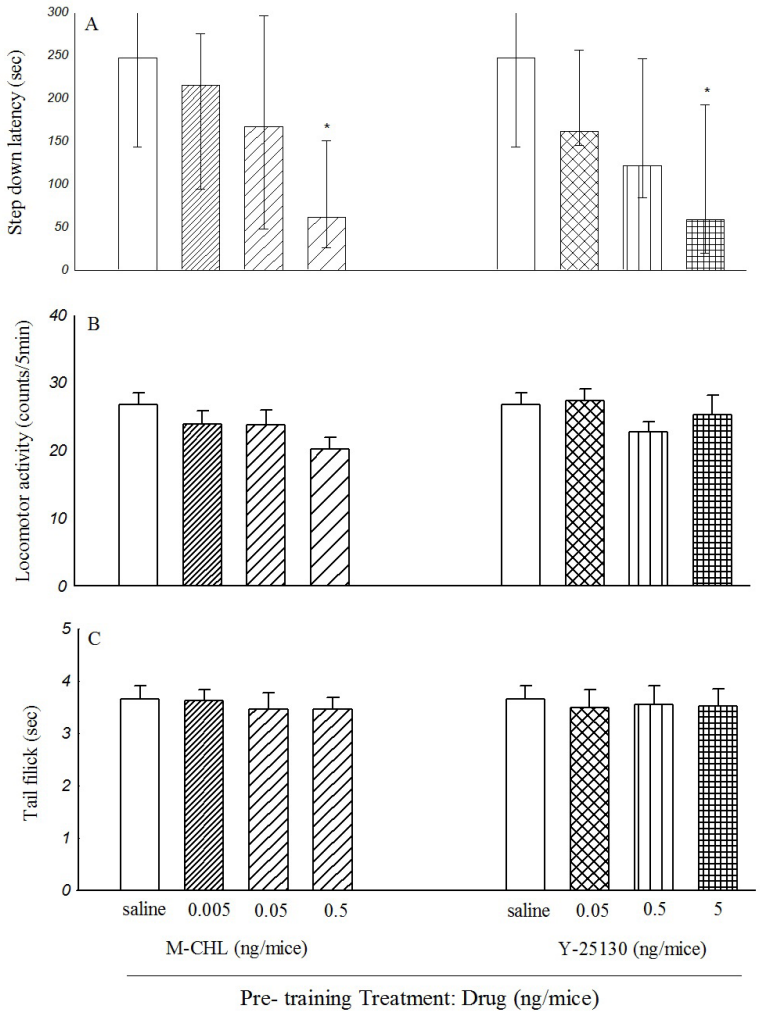

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Figure 1. The effects of pre-training intra-hippocampal administration of saline, M-CHL and Y-25130 on memory acquisition, locomotor activity and tail flick.

Left and right panels indicate the effects of pre-training administration of M-CHL (0.005, 0.05 and 0.5 ng/mouse) or Y-25130 (0.05, 0.5 and 5 ng/mouse) on memory acquisition, respectively (panel A). Step-down latencies are expressed as median and quartile for 10 animals.

Additionally, locomotor activity in panel B was examined 5 min after memory testing and tail flick in panel C was tested 5 min after training. Each bar is mean±SEM. *P<0.05 when compared to saline group.

P>0.05; figure 2B, left panel] and tail flick behaviors [F(3, 36)=1.05, P>0.05; figure 2C, left panel], following one-way ANOVA testing.

Kruskal-Wallis and Mann-Whitney results in according to harmaline-treated groups show that a subthreshold dose of M-CHL [H(3)=12.48, P<0.001; 0.005 ng/mouse, figure 2A, middle panel] or Y-25130 [H(3)=18.88, P<0.001; 0.05 ng/mouse, figure 2A, right panel] revers harmaline-induced amnesia.

Additionally, two-way ANOVA postulates that locomotor activity-induced by harmaline did not alter following M-CHL {figure 2B; middle panel [Dose Effect F(3, 72)=1.43, P>0.05; Treatment Effect F(1,72)=1.28, P>0.05 and Dose-Treatment interaction Effect F(3, 72)=0.83, P>0.05]} or Y-25130 {figure 2B; right panel [Dose Effect F(3, 72)=1.62, P>0.05; Treatment Effect F(1,72)=0.6, P>0.05 and Dose-Treatment interaction Effect F(3, 72)=0.63, P>0.05]} injections.

Similar analysis showed that tail flick-induced by harmaline did not alter following M-CHL {figure 2C; middle panel [Dose Effect F(3, 72)=2.29, P>0.05; Treatment Effect F(1, 72)=0.39, P>0.05 and Dose-Treatment interaction Effect F(3, 72)=0.61, P>0.05]} or Y-25130{figure 2B; right panel [Dose Effect F(3, 72)=1.26, P>0.05; Treatment Effect F(1, 72)=1.84, P>0.05 and Dose-Treatment interaction Effect F(3, 72)=1.56, P>0.05]} injections.

4. Discussion

The obtained results showed that pre-training administration of 5-HT3 receptor agonist (M-CHL) and 5-HT3 receptor antagonist (Y-25130) impaired memory acquisition, by itself. In all experimental groups, these interventions did not effect on locomotor activity and tail flick. Direct participation of 5-HT in learning and memory (Gonzalez, Chavez-Pascacio, & Meneses, 2013; Meneses & Perez-Garcia, 2007), has been demonstrated by decreasing 5-HT brain levels using acute 5-HT depletion, which impaired memory formation (Meneses, 2007). In contrast, enhancing brain serotonin activity by means of its precursor (i.e. tryptophan) improved memory (Meneses & Perez-Garcia, 2007). 5-HT3 receptors are found in the brain in presynaptic and postsynaptic areas associated with axons and nerve terminals (Faer-

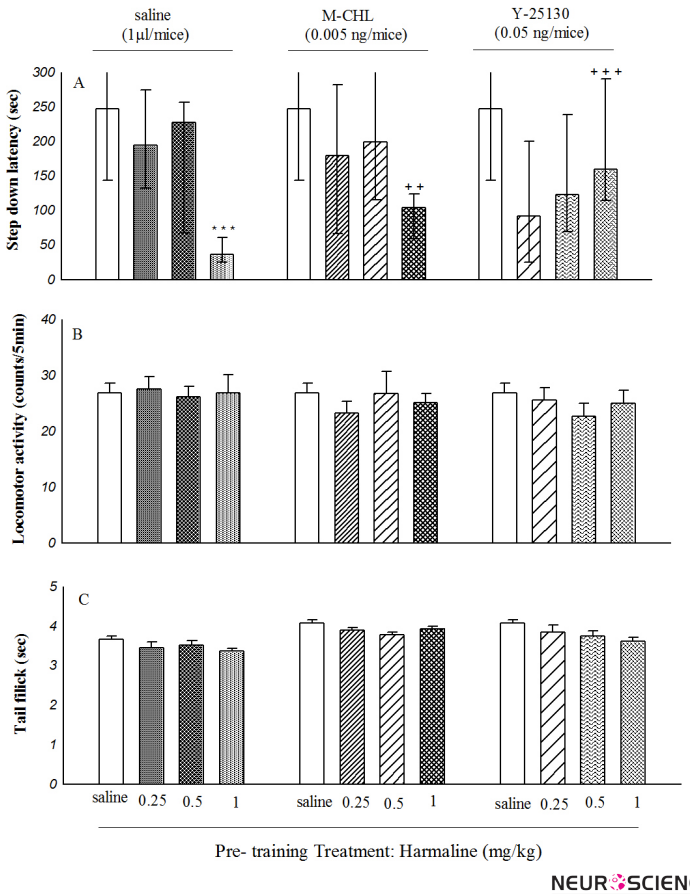

Figure 2. The effects of harmaline on memory acquisition, locomotor activity and tail flick in the present and absence 5-HT3 receptor agents.

Figure 2A describes the effects of pre-training administration of harmaline (0.25, 0.5 and 1 mg/kg, i.p) on animals which were trained under the influence of saline (1 µl/mouse; intra-hippocampus; left panel), M-CHL (0.005 ng/mouse, intra-hippocampus; middle panel) or Y-25130 (0.05 ng/mouse, intra-hippocampus; right panel). Test session step-down latencies are expressed as median and quartile for 10 animals.

Also, locomotor activity in figure 2 B was tested 5 min after memory testing and tail flick in figure 2 C was recorded 5 min after training. Each bar is mean±SEM. ***P<0.001 when compared with saline/saline group. ++P<0.01 and +++P<0.001 when compared with harmaline (1 mg/kg)/saline group.

ber, Drechsler, Ladenburger, Gschaidmeier, & Fischer, 2007). Thus 5-HT3 receptor agonist (M-CHL) through acting on presynaptic receptors impair memory (Meneses, 2007). Since, 5-HT serves as a link between synaptic plasticity at the receptor and post receptor level (i.e. signal transduction pathways) during learning and memory formation (Meneses, 2003), we propose that 5-HT3 receptor antagonist (Y-25130) decrease memory formation via operating on postsynaptic receptors. Some expected agonists showed rather silent or antagonistic effects depending on the level of ligand independent activity (Pytliak, Vargova, Mechirova, & Felsoci, 2011).

In consistent with our data, some evidences revealed that 5-HT3 receptor agonists (Meneses, 2007) and 5-HT3 receptor antagonist (Modica et al., 2000) impaired memory. On the other hand, some studies reported that 5-HT3 receptor antagonists improve learning and memory (Fakhfouri, Rahimian, Ghia, Khan, & Dehpour, 2012), or did not affect memory. Hence, the specific role of 5-HT in learning and memory function has remained unclear, partly due to many contradictory findings (Ogren et al., 2008). For instance, 5-HT antagonists have been reported to impair memory, to facilitate memory, or have no effect on memory. In the case of 5-HT agonists, there are like conflicting results with reports of impaired or facilitated, or unaffected memory (Volpe, Hendrix, Park, Towle, & Davis, 1992).

As well, it is reported that the destruction or pharmacological blockade of 5HTergic pathways impair, have no effect, or even could facilitate the performance of rats in different spatial memory tasks (Gutierrez-Guzman et al., 2011; Ogren et al., 2008). Certainly, the role of 5-HT receptors may varies according to many aspects of memory tasks, including the nature and degree of difficulty of behavioral tasks used, brain areas involved, training time (e.g. number of trials), site (systemic or central) of administration and specific drugs (Manuel-Apolinar et al., 2005; Meneses, 2004; Perez-Garcia & Meneses, 2008b; Petkov, Belcheva, Konstantinova, & Kehayov, 1995).

Additionally, our data exhibits that pre-training administration of harmaline declined memory acquisition, while did not alter tail flick and locomotor activity. There are several evidence that harmaline has a number of diverse effects such as excitation, euphoria (Rommelspacher, Strauss, & Lindemann, 1980), motor tremor, alteration in associative and motor learning and calcium channel opening, with a resultant rise in neuronal excitability (Moura et al., 2006). According to our results, it has been reported that harmaline impair different memories (Moura et al., 2006; Nasehi et al., 2010; Nasehi et al., 2012). In contrast, some evidence indicated that the dihydro-β-carbolineharmaline mostly improved long term memory (Moura et al., 2006). The controversial reports may be due to methods, route of infusion and/or the doses of drugs used (Nasehi et al., 2010).

In the next section of this paper, we investigate the involvement of 5-HT3 receptors on impairment of avoidance memory induced by harmaline. Our results displayed that in mice trained under harmaline injection, pre-training intra-hippocampal administration of M-CHL and Y-25130 restored harmaline-induced amnesia. β-carboline alkaloids bind to some CNS receptors such as 5-HT and imidazoline receptors (Glennon et al., 2000). Furthermore, β-carbolines enhance the extracellular 5-HT levels in some brain regions through inhibition of MAO_A (Venault & Chapouthier, 2007). Harmaline has a partial aromaticity structure containing methoxyllig and at C-7 position and a methy l group at C-1 (Jimenez, Riveron-Negrete, Abdullaev, Espinosa-Aguirre, & Rodriguez-Arnaiz, 2008). Methoxyl group in C-7 position favors the inhibition of MAO_A. This notion explains the inhibition of MAO_A, heighten extracellular serotonin levels (Herraiz & Chaparro, 2005; Moura et al., 2006; Robinson, Platt, & Riedel, 2011), so in mice that were trained under harmaline infusion, pre-training intra- hippocampal injection of M-CHL and Y-25130 cause enhancement of memory acquisition.

In conclusion, the data showed that a modulatory effect for hippocampal 5HT4 receptor on Harmaline induced amnesia.

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