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Original Article

# Interaction of Dorsal Hippocampal Nicotinic Receptors and Zinc Oxide Nanoparticles on Memory Consolidation

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## ABSTRACT

**Background:** Zinc oxide nanoparticles (ZnO NPs) are widely used in industry, medicine, and agriculture. Previous studies have shown that exposure to ZnO NPs can induce changes in learning and memory. Nicotinic receptors in the dorsal hippocampus are involved in drug-induced effects on memory formation.

**Objectives:** The present study investigates the possible role of dorsal hippocampal nicotinic receptors in the effects of ZnO NPs on memory consolidation.

**Methods:** Animals were bilaterally cannulated in the CA1 regions of the hippocampus. Saline, nicotine, and mecamylamine (a non-selective antagonist of nicotinic acetylcholine receptors) were administered immediately after training (intracerebral), and intraperitoneal injections of saline or ZnO NPs were given at 5-min intervals. Memory and motor activity were assessed using the passive avoidance test and the open field test, respectively.

**Results:** Post-training intra-CA1 microinjection of nicotine improved the amnesia induced by ZnO NPs. Mecamylamine potentiated the effects of an ineffective dose of ZnO NPs on memory consolidation.

**Conclusion:** ZnO NPs impair memory through interactions with nicotinic receptors in the dorsal hippocampus.

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## Introduction

Zinc oxide nanoparticles (ZnO NPs) are among the widely used metallic NPs in diverse fields of industry, medicine, and agriculture due to their exclusive physicochemical properties and also easy synthesis [1]. In biomedicine, ZnO NPs are added to oral care products as antibacterial and anti-inflammatory agents [2]. ZnO NPs have been integrated into sunscreen to protect against ultraviolet radiation [3]. In addition, ZnO NPs have been considered a therapeutic approach in cancer therapy because of their potential in drug delivery and ROS generation [4]. Moreover, ZnO NPs can increase the content and quality of crops [5]. ZnO NPs can enter the human body via skin penetration, inhalation, and digestion tract, and after distribution through circulating blood can cross the blood-brain-barrier and interact with neural cells [5]. Moreover, according to the literature, ZnO NPs can be transferred to the central nervous system via the olfactory [6] nerve translocation pathway. Thus, individuals are inevitably exposed to ZnO NPs.

One of the problems related to Zn ONPs use is their toxicity against brain tissue. According to one recent in vivo study [6], ZnO NPs induced vascular congestion in the brain of mice. In vitro studies revealed that treatment with ZnO induces oxidative stress, necrotic and apoptosis cell death, and also impaired mitochondrial function in neural cells [7-10]. In addition, in vivo studies have shown that Zno NPs induce alterations in memory and cognition functions. Xiaoli et al. (2017) also reported that prenatal exposure to ZnO NPs impairs memory in rat offspring [11]. In adult mice, ZnO NPs treatment impairs long-term and passive avoidance memory [12]. Moreover, induction of neuro-inflammation and altered synaptic plasticity by ZnO NPs have been reported [13, 14]. Although some mechanisms underlying the disturbing effects of ZnO nanoparticles on memory and cognition have been introduced [12, 13], the precise mechanism of ZnO NPs–induced memory deficit has not yet been thoroughly investigated.

The limbic system is a collection of structures that work in concert for memory and emotional behavioral processing [15]. The hippocampus is a vital structure of this system that plays an important role in different stages of memory processes, such as encoding, short-term and long-term consolidation, and retrieval [16-20]. There are several lines of evidence supporting that acetylcholine is a neuromodulator molecule that involved in hippocampal-dependent memory [21-27]. Acetylcholine exerts its biological effect via two main classes of cholinergic receptors, namely nicotinic receptors and muscarinic receptors [28]. Nicotinic receptors are ligand-gated ion channels; the activation of these receptors triggers different learning-associated cell-signaling cascades and modifies hippocampal synaptic plasticity [29]. Moreover, hippocampal nicotinic receptors are involved in the effects of drug exposure on learning and memory [30-32].

Some previous studies have shown that ZnO NPs affect brain neurotransmission, including the cholinergic system [6, 33-37] consequently modulates central nervous functions. Since the involvement of hippocampal nicotinic receptors in ZnO NPs induced effect on memory consolidation has not been studied, the present study evaluates the interaction between post-training injection of nicotinic receptor agents and ZnO NPs in passive avoidance tasks. In addition, this study maintains that the distribution and retention of ZnO NPs in the brain is time-dependent [38]; thus, the memory test is performed 1 and 3 days after training.

## Materials and Methods

### Study animals

In this study, male Wistar rats (a total of 126 animals; body weight=220-240 g) obtained from the Pasteur Institute of Iran (Tehran, Iran) were used. The rats were housed in clean transparent plexiglas cages (3–4 per cage) on a 12-h on/off lighting schedule with unrestricted access to food and water except during experiments. The rats were kept at a temperature maintained at 22±2 ˚C room. The lights were on between 7:00 PM and 7:00 AM. The rats were acclimatized to the laboratory condition for 7 days before surgery. To avoid experimental deviations due to diurnal variations, all behavioral tests were carried out between 9:00 AM and 2:00 PM. All procedures were approved by the local Ethics Committee and carried out following the ethical standards and principles of laboratory animal care (NIH publication) and laws of animal protection.

### Surgery procedure

One week after adaptation to laboratory condition, the rats were anesthetized with intraperitoneal administration of ketamine 10% (Alfasan, Woerden-Holand) and xylazine 2% (Alfasan, Woerden-Holand; 50 mg/kg and 5 mg/kg, respectively) cocktail. Two 22-gauge guide steel cannulas were implanted bilaterally in the dorsal hippocampus CA1 regions using the bregmatic and

lambdoid sutures of the skull as a reference point (coordinates according to the Atlas of Paxions and Watson [39]: -3 to -3.5 mm posterior to the bregma, ±1.8–2 mm lateral to midline, and -2.8 to -3 mm vertical to dura). The injection needle was 0.5 mm longer than the guide cannula. The animals were handled daily for 10 min during the recovery period. The microinjection procedure was started 7 days after implantation of guide cannulas.

## Drugs and microinjection procedure

ZnO NPs (20-30 nm, purity >99%) was purchased from Tecnan, Spain. Nicotine hydrogen tartrate and mecamylamine were purchased from Sigma Aldrich (St. Louis, MO purity >99%). Nicotine doses were calculated in their tartrate form and were dissolved in 0.9% saline and then the pH of the solution was adjusted to 7.2-7.4 with NaOH (0.1 normal solution). Mecamylamine was dissolved in sterile 0.9% saline. ZnO NPs were dissolved in 0.9% saline and were dispersed using the Ultrasonic Bath 2600S (Pars Nahand, Iran) for 20 min and before each injection, the compound was shaken for 1 min [40].

Immediately after training, rats were bilaterally (into the CA1) infused with 1 µL/rat of nicotine (0.3 and 0.4 µg/rat), mecamylamine (0.5 and 1 µg/rat), or vehicle. The infusion lasted 1 min (infusion rate: 0.5 µL/min, via 2 µL-Hamilton syringe) and the infusion cannula was carefully withdrawn 1 min after the end of the infusion. ZnO NPs prepared daily 30 min before the beginning of experiments and were given intraperitoneally at a dose of 0.5 mg/kg or 1 mg/kg (post-training, with 5 min intervals). The dose of drugs was selected based on our previous studies [41].

## Passive avoidance test

The inhibitory avoidance apparatus consists of two compartments of the same size: One light compartment and one dark compartment which is connected by a movable guillotine. The floor of the dark compartment consists of metal grid bars (placed 1 cm away from each other, 0.5 cm diameter) and connected to a shock generator, able to generate a shock (50 Hz, 3 s) with 1 mA intensity. All experiments were done in a testing room lit by a 60 W lamp placed above the center of the apparatus.

On the training day, 30 min before a training session, the rats were allowed to become familiar with the apparatus. After 30 min, an individual rat was kept in the light compartment for 10 s, after which the door was raised, and the time taken by the rat to move from the light compartment to the dark compartment was recorded. After 2 min, the rat was placed in the light compartment again, as soon as the rat completely entered into the dark compartment, the door was closed and an electrical foot shock was applied for 3 s. The rat was then removed from the apparatus. 2 min later, the procedure was repeated. Animals that did not enter the dark compartment even after 120 s were removed from the apparatus as successful training. All animals were trained with a maximum of 3 trials. Testing day was conducted after 24 h of acquisition trial, where no shock was applied when the rat came into the dark compartment, and retrieval latency was recorded (as step-through latency) by the time taken the rat to re-enter into the dark compartment (up to 300 s).

## Open field test

To assess the effects of the individual drug or drug combination on locomotor activity, the open field test was used [42]. On the first and third days after drug administration, all the individual rats were submitted to a 10-min open field test (Borj Sanat Co., Iran). The apparatus (40×40×40 cm) was equipped with 16 infrared photocells positioned every 2.5 cm. Immediately after testing on the passive avoidance apparatus, each rat was placed in the center of the box floor. The number of beam cuts was evaluated by the photocell monitoring system. After each test, the apparatus was carefully cleaned.

## Experimental design

The treatments were initiated after the recovery period (7 days after surgery).

### Experiment 1

In the first experiments, the rats in ZnO NPs groups after successful training were divided into 3 groups (n=7 rats per group). These rats received intra-CA1 microinjection of saline (1 µL/rat) and after 5 min, they were injected with saline (1 mg/kg, intraperitoneal) or ZnO NPs (0.5 or 1 mg/kg, intraperitoneal). Step-through latency and locomotor activity were measured on day 1 and day 3 after training.

### Experiment 2

To acquire a basic understanding of the effect of nicotine microinjection into the CA1 regions of the hippocampus, another 3 groups were used as nicotine/saline groups. The rats were microinjected either vehicle (1 µL/rat, intra-CA1) or nicotine (0.3 or 0.4 µL/rat) immediately after successful training. Saline (1 mL/kg) was administered 5 min after intra-CA1 injection via intra-

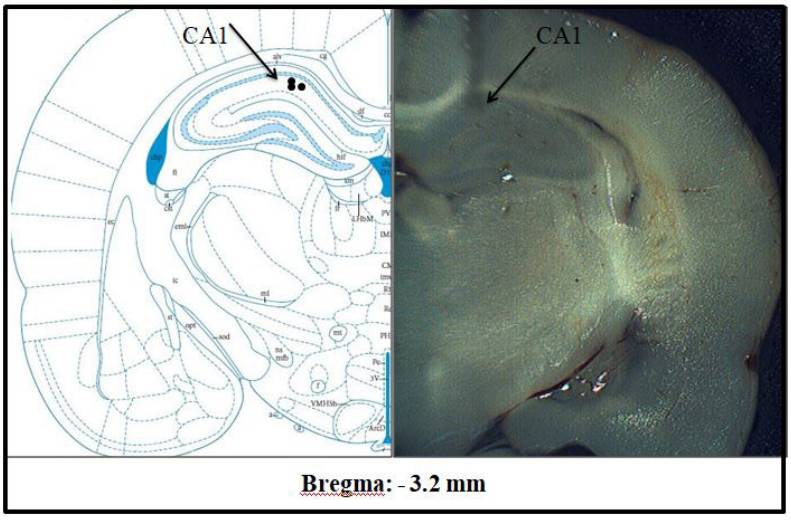




**Figure 1.** Photomicrograph showing the location of the injection into the Ca1 (right panel) and cross-sections from the Paxinos and Watson brain atlas (2007, left panel), showing the CA1

peritoneal injection. Step-through latency and locomotor activity were measured on day 1 and day 3 after training.

### Experiment 3

In experiment 3, four groups of animals were used as nicotine/ ZnO NPs groups. After successful training, the rats received vehicle (1 µL/rat, intra-CA1) or nicotine (0.3 or 0.4 µL/rat, intra-CA1), and after 5 min, they received saline (1 mg/kg, intraperitoneal) or ZnO NPs (1 mg/kg, intraperitoneal). Step-through latency and locomotor activity were measured on day 1 and day 3 after training.

### Experiment 4

After successful training, the rats in mecamylamine treatment groups received saline (1 µL/rat, intra-CA1) or mecamylamine (0.5 or 1 µL/rat, intra-CA1), and after 5 min, they received saline (1 mg/kg, intraperitoneal). Step-through latency and locomotor activity were measured on day 1 and day 3 after training.

### Experiment 5

Four groups of animals were used as mecamylamine / ZnO NPs groups. After successful training, the rats received saline (1 µL/rat, intra-CA1) or mecamylamine (0.5 µL/rat, intra-CA1), and after 5 min, they received saline (1 mg/kg, intraperitoneal) or ZnO NPs (0.5 mg/kg, intraperitoneal). Step-through latency and locomotor activity were measured on day 1 and day 3 after training.

## Histology

At the end of the experiments, the rats were all euthanized by decapitation under deep anesthesia with carbon dioxide and microinjection of 1 µL/rat of a 1% methylene-blue solution. After euthanizing, the brains were removed and fixed in the formaldehyde (10%), and stored at 4 ˚C until used for sectioning using a vibratome.

## Data analysis

Statistical analysis was performed using SPSS Software version, 22. Data are expressed as Mean±SE of the mean. Statistical significance was evaluated by one-way analysis of variance followed by the Tukey post hoc comparisons and defined as $P<0.05$.

# Results

## Histological verifications

Figure 1 shows the photomicrograph of the location of the injection into the CA1 (right panel) and cross-sections of the rat brain indicating injection locations according to the Paxinos and Watson atlas [39], left panel). Black circles show injection sites inside the CA1.

## Effect of post-training ZnO NPs administration on step-through latency

Figure 2 shows the effects of post-training administration of ZnO NPs on the latency of entrance into the dark compartment on day 1 (A) and day 3 (B) after training,

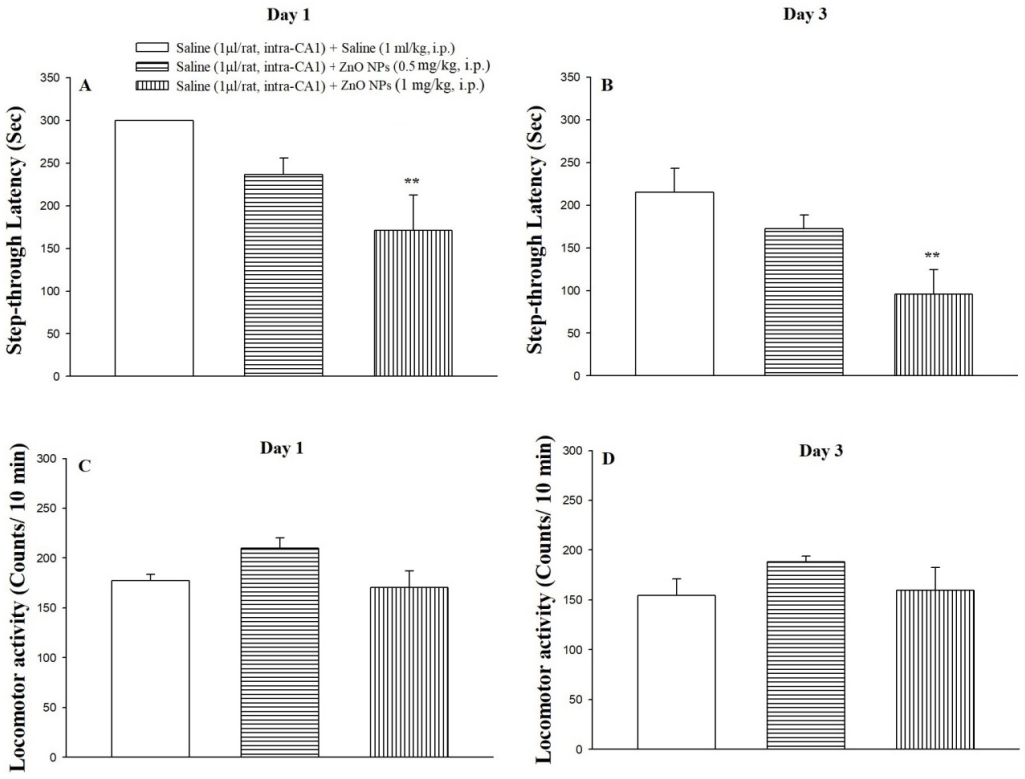


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**Figure 2.** The effects of post-training administration of ZnO NPs on the passive avoidance step-through latency

**P<0.01 as compared with saline (1 µL/rat, intra-CA1)/saline (1 mL/kg, intraperitoneal) control group.

Notes: Latency to cross into the dark compartment during day 1 (A) and day 3 (B) after training, and also locomotor activity during day 1 (C) and day 3 (D) after training. Data are presented as Mean±SE.

and also on locomotor activity (C and D). The one-way analysis of variance in day 1 ($F_{(2, 17)}$=6.06, P<0.01) and also day 3 ($F_{(2, 17)}$=5.95, P<0.01) after training showed a significant difference in step-through latency among the groups. Also, post hoc analysis revealed that the groups receiving 1 mg/kg of ZnO NPs (P<0.01) spent less time to re-enter the dark compartment as compared to the control saline group, indicating ZnO NPs-induced memory loss. Moreover, the one-way analysis of variance in Figures 2C and 2D indicates that the treatments on day 1 ($F_{(2, 17)}$=3.13, P>0.05) and also day 3 ($F_{(2, 17)}$=1.19, P>0.05) after training did not affect locomotor activity.

## Effect of post-training intra-CA1 nicotine microinjection on step-through latency

As shown in Figures 3A and 3B, post-training microinjection of nicotine (0.3 and 0.4 µg/rat, intra-CA1) had no significant effect on step-through latency at 1 ($F_{(2, 17)}$=2.083, P>0.05) and 3 ($F_{(2, 17)}$=3.27, P>0.05) days post-training, respectively, when compared to the control group. Figures 3C and 3D showed that the drug exposure did not alter the locomotor activity at 1 ($F_{(2, 17)}$=1.71, P>0.05) and 3 ($F_{(2, 17)}$=0.84, P>0.05) days post-training.

## The effect of the post-training intra-CA1 nicotinic acetylcholine receptors agonist (nicotine) on the impairment effect of ZnO NPs on the passive avoidance learning

Nicotine (intra-CA1) was microinjected 5 min before ZnO NPs administration to investigate the role of the CA1 nicotinic receptors in the impairment effect of ZnO NPs on the consolidation of passive avoidance learning. The results of the one-way analysis of variance are presented in Figure 4A, indicating a statistically significant difference in step-through latency score between the groups at 1 day ($F_{(3, 24)}$=12.93, P<0.001) post-training. Post-hoc analysis showed that 0.4 µg/rat of nicotine significantly increases the step-through latency (P<0.01) 1 day after training, while the group treated with 0.4 µg/rat of nicotine plus ZnO NPs showed no significant difference as compared to the control group 3 day after train-

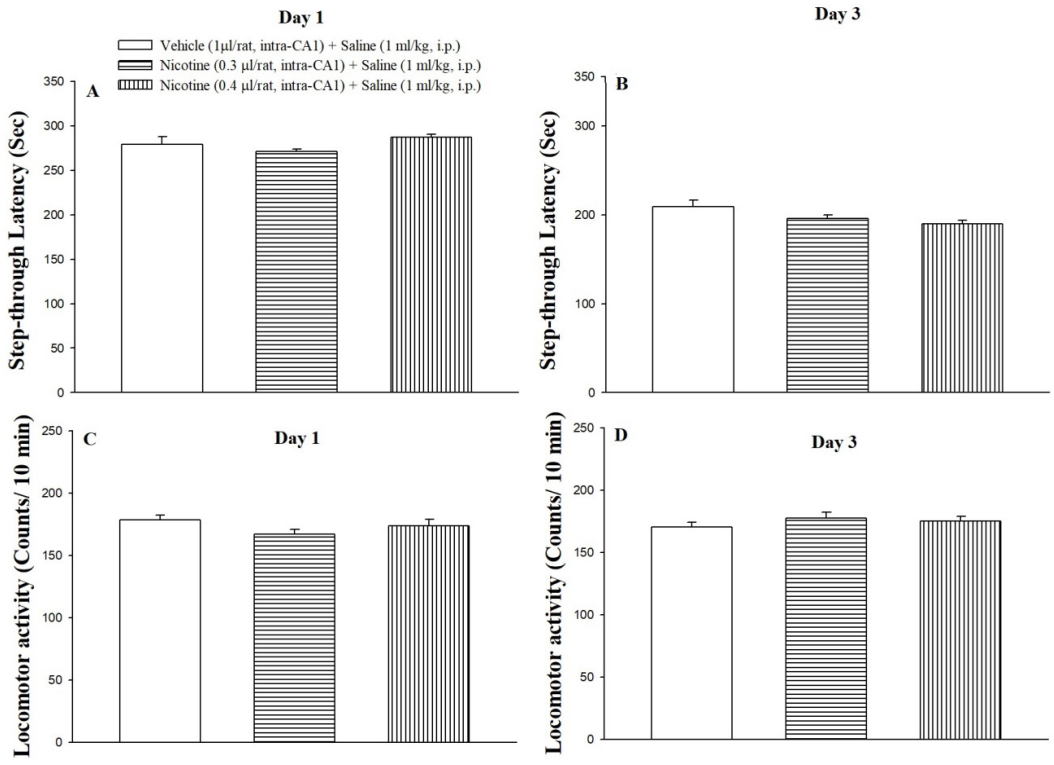




**Figure 3.** The effect of post-training intra-CA1 nicotine microinjection on step-through latency

Notes: Latency to cross into the dark compartment during day 1 (A) and day 3 (B) after training, and also locomotor activity during day 1 (C) and day 3 (D) after training. Data are presented as Mean±SE of the mean.

ing (Figure 4B, $F_{(3, 24)}$=10.46, P<0.001). Thus, post-training microinjection of nicotine could improve the ZnO NPs-induced reduction in the step-through latency (1 day after training). As shown in Figures 4C and 4D, the post-training microinjection of nicotine plus ZnO NPs did not affect the locomotor activity at 1 ($F_{(3, 24)}$=1.71, P>0.05) and 3 ($F_{(3, 24)}$=0.84, P>0.05) days post-training.

## Effect of post-training intra-CA1 mecamylamine microinjection on step-through latency

As shown in Figures 5A and 5B, post-training microinjection of mecamylamine (1 µg/rat, intra-CA1) had a significant effect on step-through latency at 1 ($F_{(2, 17)}$=4.14, P<0.05) and 3 ($F_{(2, 17)}$=3.90, P<0.05) days post-training, respectively, when compared to the control group and decreased step-through latency. Figures 5C and 5D showed that the drug exposure did not alter the locomotor activity at 1 ($F_{(2, 17)}$=1.23, P>0.05) and 3 ($F_{(2, 17)}$=1.89, P>0.05) days post-training.

## The effect of the post-training intra-CA1 microinjection of ineffective dose of mecamylamine with ineffective dose of ZnO NPs on the passive avoidance learning

In the last set of experiments, we determined whether the post-training intra-CA1 microinjection of an ineffective dose of mecamylamine enhances the influence of an ineffective dose of ZnO NPs on passive avoidance learning to induce memory loss. Toward this end, mecamylamine (0.5 µg/rat, intra-CA1) was microinjected 5 min before ZnO NPs (0.5 mg/kg, intraperitoneal) administration. The results of the one-way analysis of variance are presented in Figure 6A, indicating a statistical difference in step-through latency score between the groups on day 1 ($F_{(3, 24)}$=5.27, P<0.001) post-training. Post-hoc analysis showed that 0.5 µg/rat of mecamylamine significantly enhances the influence of ineffective dose of ZnO NPs on step-through latency (P<0.01) 1 day and also 3 days after training ($F_{(3, 24)}$=3.34, P<0.05; Figure 6B). Thus, post-training microinjection of mecamylamine could potentiate the ZnO NPs-induced reduction in the step-through latency. As shown in Figures 6C and

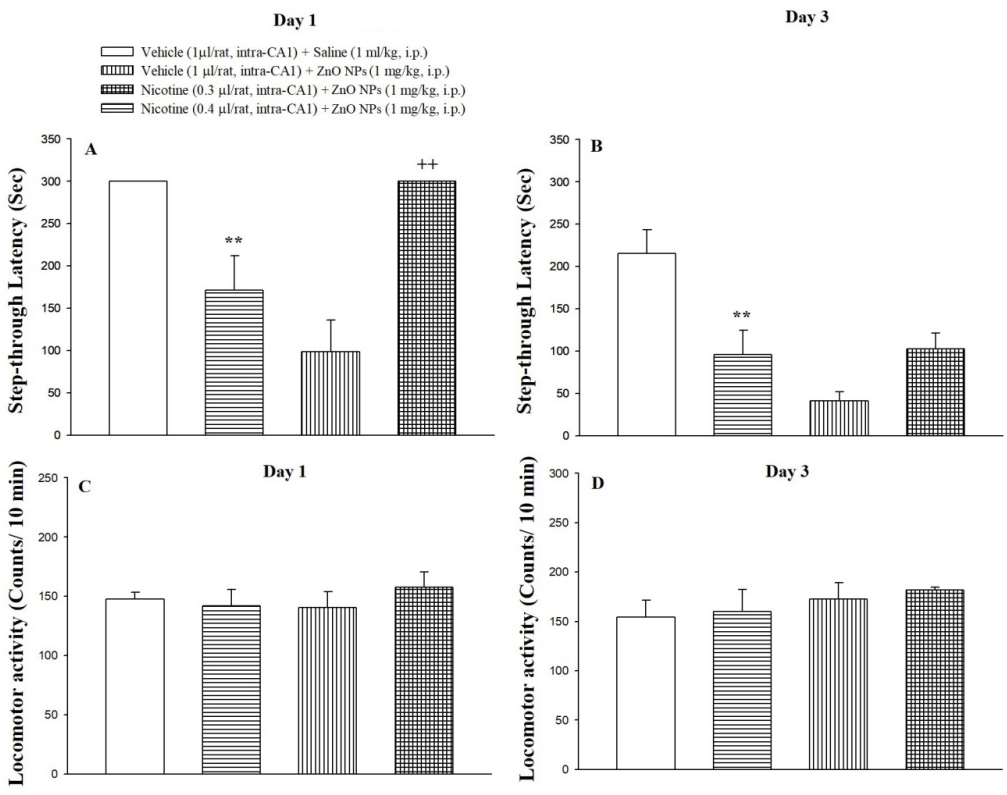


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**Figure 4.** The effects of the post-training intra-ca1 microinjection of nicotinic acetylcholine receptors agonist (nicotine) on the impairment effect of ZnO NPs on the retrieval of passive avoidance learning

**P<0.01 as compared with the vehicle (1 µL/rat, intra-CA1)/saline (1 mL/kg, intraperitoneal) control group, ++P<0.01 as compared with vehicle (1 µL/rat, intra-CA1)/ZnO NP (1 mg/kg, intraperitoneal).

Notes: Latency to cross into the dark compartment during day 1 (A) and day 3 (B) after training, and also locomotor activity during day 1 (C) and day 3 (D) after training. Data are presented as Mean±SE of the mean.

6D, the post-training microinjection of mecamylamine plus ZnO NPs did not affect the locomotor activity at 1 ($F_{(3, 24)}$=2.31, P>0.05) and 3 ($F_{(3, 24)}$=2.18, P>0.05) day post-training.

## Discussion

ZnO NPs have a broad range of applications. However, certain disadvantages and toxic side effects have been reported for biomedical applications of ZnO NPs. In recent years, the majority of prior studies have emphasized that ZnO NPs modulate learning and memory ability in animals [11-13, 43, 44]. Nevertheless, Amara et al. (2014) reported acute intravenous injection of ZnO-NPs did not affect working memory in exposed rats [45]. Kesmati et al. (2020) reported that nano-ZnO increased memory in novel object recognition test [43] and Xie et al. (2012) indicated that ZnO NPs could ameliorate the cognitive impairment in mice with depressive-like behaviors [46]. As shown in Figure 2, the present study showed that post-training intraperitoneal injection of ZnO NPs dose-dependently impaired passive avoidance memory retrieval at 1 and 3 days after training. In addition, ZnO NPs injection did not affect exploratory behavior in open field tests at 1 and 3 days after training. Our result is in line with studies that conducted by Tian et al. [12] and Han et al. [13].

The research on mechanisms by which ZnO NPs influence cognitive functions is being carried out. After bypassing the blood-brain barrier or direct entrance through the nose, ZnO NPs induce zinc deposition in functional brain regions including the hippocampus. Zinc is a neuromodulator that modulates synaptic transmission [47]. However, elevated concentrations result in functional and structural changes [8, 12, 34, 48]. Moreover, exposure to ZnO-NPs alters the homeostasis of Zn and results in cell apoptosis [6]. Liu et al. [8] and Attia et al. [10] observed the hippocampal accumulation of ZnO NPs following intravenous or oral administration

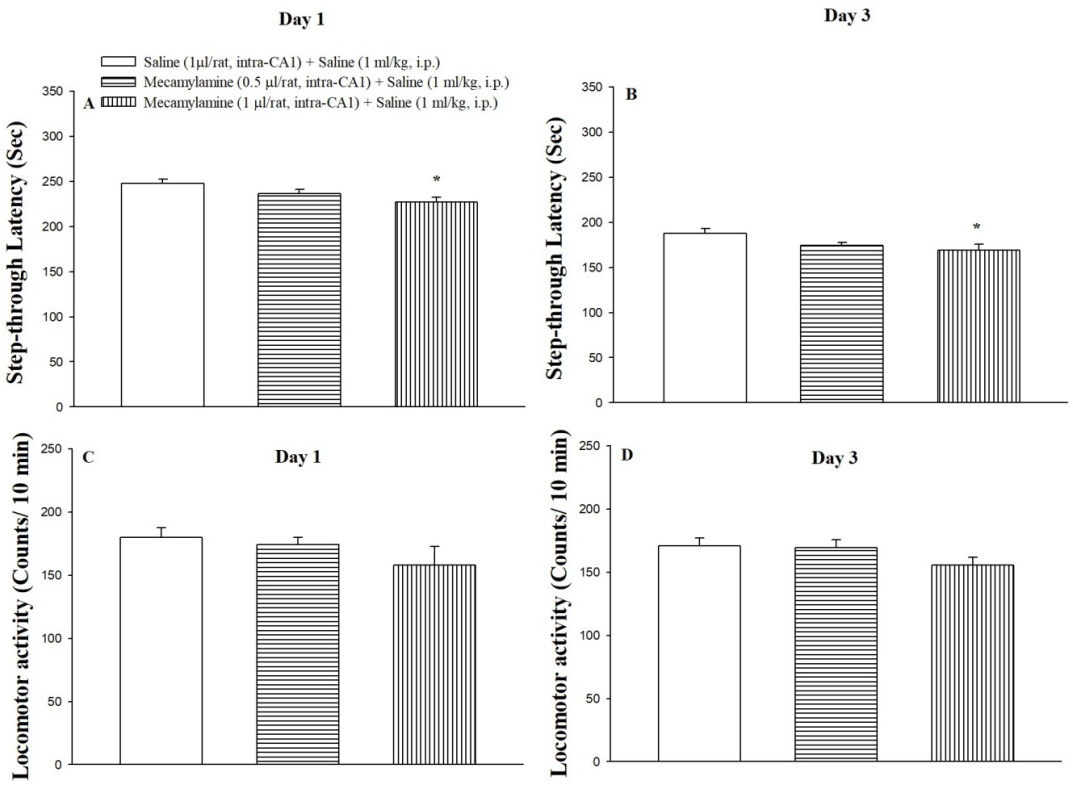


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**Figure 5.** The effects of post-training intra-CA1 nicotinic acetylcholine receptors antagonist (mecamylamine) on step-through latency

*P<0.05 as compared with saline (1 µL/rat, intra-CA1)/saline (1ml/kg, intraperitoneal) control group.

Notes: Latency to cross into the dark compartment during day 1 (A) and day 3 (B) after training, and also locomotor activity during day 1 (C) and day 3 (D) after training. Data are presented as Mean±SE of the mean.

led to oxidative damage, inflammatory responses, and histopathological damage in the hippocampus [8, 10]. Hippocampal oxidative stress and inflammation have been closely related to impairment in synaptic plasticity and memory [49-52]. Previous studies also have demonstrated that suppression of the hippocampal cAMP/CREB signaling pathway is involved in memory impairment following ZnO NPs administration [12]. Xie et al. (2012) using isolated rat hippocampal CA3 pyramidal neurons showed that ZnO NPs increase the current amplitude and excitability of neurons that may affect physiological functions of neurons [46]. It is also suggested that ZnO NPs disturb the metal ions homeostasis in the brain [6, 46, 53]. Homeostasis of metal ions is crucial for synaptic function [54]. Han et al. [13] reported that ZnO NPs disrupt the balance between long-term potentiation and depotentiation in the dentate gyrus of the hippocampus which leads to the alteration of synaptic plasticity and memory deficits [13]. In support of this effect, it has been shown that acute administration of ZnO NPs decreases glutamate levels in the hippocampus, a main excitatory neurotransmitter involved in long-term potentiation levels in the hippocampus [33].

In the present study, we showed that post-training intra-CA1 microinjection of nicotine dose-dependently could improve the ZnO NPs-induced reduction in the step-through latency in 1 day after training, but not after 3 days (Figure 4). Combined nicotine and ZnO NPs did not affect locomotion. These results support the involvement of hippocampal nicotinic receptors in ZnO NPs-induced deficit in passive avoidance memory. Moreover, post-training microinjection of nicotine alone had no impact by itself on step-through latency or locomotor activity in all days after training (Figure 3). Using omics technologies, Guo et al. [34] nominated cholinergic neurotransmission as the main biological process affected following ZnO NPs treatment [34]. Moreover, Hsiao et al. (2008, 2001) showed that zinc modulates nicotinic receptors in a subunit composition and dose-dependent manner [37, 55]. Yadava et al. [56] reported that ZnO NPs attenuated spatial learning and memory through an

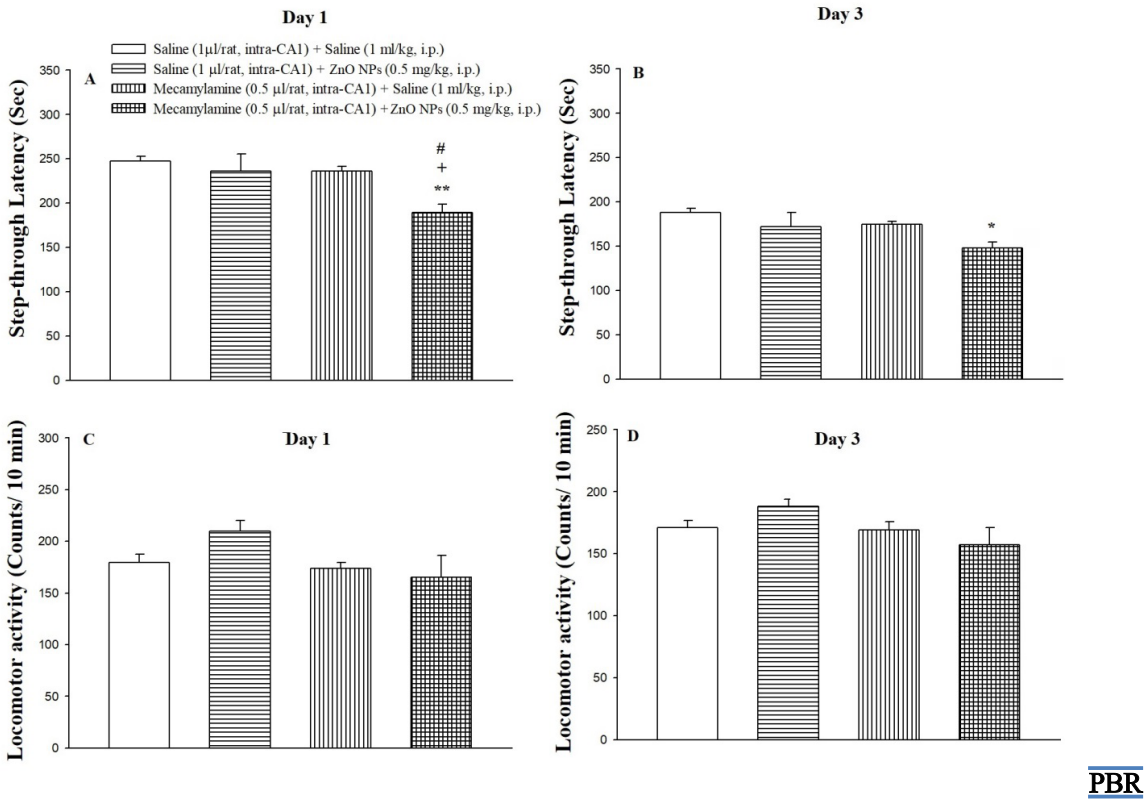


**Figure 6.** The effect of the post-training intra-CA1 microinjection of ineffective dose of mecamylamine with ineffective dose of ZnO NPs on the retrieval passive avoidance learning

*P<0.05 and **P<0.01 as compared with the saline (1 µL/rat, intra-CA1)/saline (1 mL/kg, intraperitoneal) control group, +P<0.05 as compared with saline (1 µL/rat, intra-CA1)/ZnO NP (0.5 mg/kg, intraperitoneal), #P<0.05 as compared with the mecamylamine (0.5 µL/rat, intra-CA1)/saline (1 mL/kg, intraperitoneal) control group.

Notes: Latency to cross into the dark compartment during day 1 (A) and day 3 (B) after training, and also locomotor activity during day 1 (C) and day 3 (D) after training. Data are presented as Mean±SE of the mean.

increase in oxidative stress and a decrease in acetylcholinesterase enzyme activity [56]. Taking these findings together, the results confirm the involvement of nicotinic receptor mechanisms in impairing the effect of ZnO NPs on memory formation. According to Figure 4, there is a difference in the rescuing effect of nicotine on ZnO NPs at 1 day (Figure 4A) but not at 3 days, which may be related to the time-dependent neurotoxicity of ZnO NPs in the brain [10, 38]. Chen et al. (2015) reported that as time increases Zn content increases in the brain, therefore, ZnO NPs accumulate in the brain in the long term [38].

In addition, to confirm the role of nicotinic acetylcholine receptors in the effect of ZnO NPs on memory, an ineffective dose of mecamylamine (0.5 µg/rat), a nicotinic acetylcholine receptor antagonist, which alone did not affect memory (Figure 5), injected into the hippocampus before the systemic administration of an ineffective dose of ZnO-NPs (0.5 mg/kg). The results showed that a low dose of a nicotinic acetylcholine receptor antagonist can synergize with a low dose of ZO NP to produce a memory impairment that may conform to the interaction between ZnO-NPs and nicotinic receptors in memory consolidation (Figure 6). Since ZnO NPs increase Zn deposition in the hippocampus [34] and considering to modulatory effect of ZnONPs on cholinergic nicotinic receptors [37, 55], it can be concluded that interaction between ZnONPs and nicotinic receptors is involved in ZnONPs –induced effect on memory.

On the other hand, nicotine treatment prevented memory deficits in brain disorders, exerted neuroprotective effects, upregulated cAMP/CREB signaling, and reduced reactive oxygen species generation through nAChR/Erk1/2 signaling pathway [57-59]. Considering these nicotine effects, an explanation for our obtained result can be that intra-CA1 administration of nicotine ameliorates the disturbing effects of ZnO NPs on the hippocampus via different mechanisms. However, more

studies are needed to precisely identify the mechanism of the modulatory effect of nicotinic receptors on ZnO NPs' effect on the memory process.

## Ethical Considerations

### Compliance with ethical guidelines

This study was approved by the Research and Ethics Committee of Arak University, Arak, Iran (Code: IR.ARAKMU.REC.1400.206).

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### Authors' contributions

Conceptualization, methodology, formal analysis and investigation: All authors; Writing the original draft: Ahmad Sadeghi, Farzaneh Nazari-Serenjeh and Zahra Ghasemzadeh; Project administration, supervision, review and editing: Niloufar Darbandi.

### Conflict of interest

The authors declared no conflict of interest.

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