Intra-hippocampal injection of abscisic acid attenuates learning and memory deficits, and changes oxidative stress indices in REM sleep deprived rats

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Abstract

This study evaluated if intra hippocampal administration of ABA is able to modulate learning and memory performance and oxidative stress biomarkers activities in cerebral cortex of rats exposed to rapid eye movement (REM) sleep deprivation. Adult male Wistar rats were cannulated in CA1 area of hippocampus. After recovery, the rats were subjected to REM sleep deprivation for 4 days. Then, the groups of REM sleep deprived (SD) rats were treated with ABA (5, 10, and 15 µg) and ABA (10 µg) + bicuculline (Bic), a competitive GABAA receptor antagonist. Memory and learning were evaluated with Morris water maze (MWM) and shuttle box tests. Moreover, alterations in the levels of catalase as antioxidant enzyme, and MDA and H2O2 as oxidants biomarkers were determined in rat brain cortex. REM SD rats indicated noteworthy learning and memory deficits in both MWM and shuttle box tests when compared to control rats. However, intra-CA1 injection of ABA (10 µg) decreased cognitive impairment in REM SD rats. Bic (1 µg/rat) was not able to change ABA (10 µg) effects. In addition, an increase in catalase activity, and decrease in MDA and H2O2 were indicated in the brain cortex of ABA (10 µg) and ABA+ Bic treated groups. Overall, the data showed ABA aptitude to attenuate REM sleep deprivation-induced learning and memory disruption and oxidative demages in rats. Manipulation of GABAA receptor failed to inhibit ABA effects in REM SD rats.

Abbreviations

CAT: Catalase

REM: Rapid eye movement

STZ: Streptozotocin

Bic: Bicuculline

MWM: Morris water maze

MDA: Malondialdehyde

H2O2: Hydrogen peroxide

SD: Sleep deprivation

PPARs : Peroxisome proliferator-activated receptors

ABA: Abscisic acid

ROS : Reactive oxygen species

CGRP : Calcitonin gene-related peptide

Introduction

Sleep deprivation (SD), is a condition of inadequate sleep , that can be considered a physiological disorder or as a result of people's inappropriate lifestyle [1, 2]. Sleep quality has a significant impact on the regulation of other physiological processes including learning and memory [3, 4]. It has been shown that SD disrupt memory retrieval and consolidation through changing in hippocampus structural constancy [5]. The patterns of rhythmic brain wave in non-rapid eye movement sleep also show relationship with hippocampal activities[6][6]. Hippocampal mediated learning and memory as well as neurotransmitters are affected by sleep quality [7, 8]. REM sleep deprivation could decline motor and sensory learning experience in animals [9, 10].

Abscisic acid (ABA) is produced in the all parts of plants, and plays notable roles in their physiological functions specially regulation of stress responses [11, 12]. ABA is synthesized from pro-vitamin A carotenoids [13], which is found in high concentrations in plants [12]. Moreover, in animal , ABA is founded in various brain areas including hippocampus, cerebral

cortex and cerebellum [14, 15]. ABA receptors are peroxisome proliferator-activated receptors (PPARs) and lanthionine synthetase C-like protein 2 [16, 17]. ABA signalling shows variation, but changes in calcium concentration and activation of cyclic ADP-ribose are the most mutual pathways [18-20].

ABA exerts modulatory effects on a variety of physiological functions including nociception, anxiety and depression like behavior, sleep and learning and memory performances in rats [15, 21]. Central administration of ABA exhibited analgesic effect which is facilitated by the PPAR β/δ and opioid signalling [22]. Moreover, ABA meaningfully improved the pentobarbital-related sub hypnotic effects and also endorsed sleep induction. Such effects showed dependency with GABAA receptors and PPARβ/ PPARγ signalling [23].

The main goal of present study was to evaluate if intra-hippocampal treatment of ABA is able to alter learning and memory performance in rats exposed to REM-SD. Moreover, bicuculline was used to evaluate the possible association of ABA with GABA A receptor. In a pervious study, pretreatment with bicuculline was found to block ABA's ability to extend sleep duration in a rat model of pentobarbital-induced sleep. Bicuculline is a competitive GABAA receptor antagonist that blocks GABA's inhibitory effects by preventing chloride ion influx, leading to increased neuronal excitability and potential seizure activity[24]. The alteration of pro-oxidant/antioxidant biomarkers were also assessed in cerebral cortex of SD rats.

Results

PA test

The SD group showed an increase in the number of acquisition trials when compared with control group ($p<.001$) (Fig. 1A). However, the number of acquisition trials was significantly decreased in SD groups post-treated with ABA (10 µg and 15 µg) ($p<0.001$). No major

alteration in acquisition trials was found in SD rats post-treated with Bic +ABA (10 µg) as compared to SD+ABA (10 µg) group. In addition, an increase in the step-through latency and decline in time spent in dark cavity were determined in SD group ($p<.001$). ABA (10 µg) was able to increase the step-through latency and decrease time spent in dark chamber in SD rats ($p<0.001$). In addition, no significant alteration was founded in SD rats response infused with Bic + ABA (10 µg) as compared with the ABA (10 µg) group (Fig. 1B and 1C).

MWM test

In acquisition trials, the latency time to catch the concealed platform was pointedly increased in SD group in comparison to the control group ($p<.001$). Intra-hippocampal infusion of ABA (10 µg /rat) expressively decreased the latency time to catch the concealed stage in SD rats ($p<0.01$) (Fig. 2A). Moreover, SD+Bic+ABA (10 µg /rat) and SD+ ABA (10 µg /rat) treated groups show no change the latency to discover the hidden platform. Moreover, the groups showed major differences in space moved to touch the concealed platform on the acquisition test. As shown in Fig. 2B, the distance trekked to touch the hidden stage was meaningfully increased in SD group ($p<0.001$). Besides, SD group treated with ABA (10 µg/rat) travelled lower distance to reach the hidden platform as compared to SD group ($p<0.001$). In SD group injected with Bic+ ABA (10 µg/rat) the distance traveled to find the platform showed no difference as compared to the ABA (10 µg/rat) group (Fig. 2B).

Fig.3 indicates the results of probe trial. The figure indicated that time spent and the traveled distance in the object zone significantly decreased in SD group than control group ($P<0.001$) (Fig. 3A). Moreover, ABA weakened the effects of SD on the time spent in the object area ($P<0.05$) (Fig. 3A). Further, ABA meaningfully improved distance traveled in the object area in SD-treated rats ($P<0.01$) (Fig. 3B). As notated in Fig. 3, SD rats infused with Bic + ABA

show no significant difference in spent time and distance travelled in the target quadrat in comparison to ABA (10 µg/rat) group ($P<0.05$).

Biochemical assay

The activity of antioxidant enzyme CAT was significantly decreased in SD group when compared with control rats. As shown in Fig. 4A, ABA at 10 µg/rat and Bic+ABA (10 µg/rat) were able to increase CAT activity in SD group. Moreover, there were significant increases in the activity of pro-oxidant biomarker H2O2 and MDA concentration in the cerebral cortex of SD group as compared to control. However, post-treatment of SD rats with ABA (10 µg/rat) or Bic+ABA significantly attenuated H2O2 activity and MDA level in the cerebral cortex (Fig. 4B and 4C).

Discussions

The present study showed the deteriorate effects on REM sleep deprivation on memory and learning performance of rats assessed in the MWM and shuttle box tests. However, intra- CA1 microinjection of ABA decreased SD- induced learning and memory deficiency in rats. Moreover, the sleep deprived rats indicated a disruption in oxidant/antioxidant biomarkers verified by decrease in CAT activity, and increases in lipid peroxidation and H2O2 production in the cerebral cortex which was prevented by ABA (10 µg) treatment. The ABA effects in behavioral and biochemical experiments did not diminish with GABA receptors antagonist bicuculline.

The importance of sleep quality on cognitive performance, especially hippocampal depended learning and memory has been strongly supported with evidences from clinical and experimental studies [25]. In this study, the rats learning and memory performances were assessed after a continuous 72 h period of REM SD. The 72-hour REM sleep deprivation period in rats reflects severe sleep loss but is not directly equivalent to 72 hours in humans due to differences in metabolism and sleep architecture. In humans, this timeframe would likely

correspond to several days of significant sleep restriction or chronic sleep disruption rather than total sleep deprivation. Rodent models typically involve more intense and compressed sleep deprivation protocols compared to human studies. To bridge the gap between rodent and human studies, future research could explore the effects of varying durations of REM sleep deprivation in animal models and attempt to correlate these findings with human studies involving partial sleep restriction or chronic sleep fragmentation. Learning and memory changes following SD are highly dependent on the lasting duration of the SD. In line with our result, most studies showed the highest detrimental effects of SD on memory performance when lasted for 72 h. Nevertheless, in some cases, shorter term of SD lasting for 24 or 48 h have been associated with no alteration or even increases in hippocampal synaptic plasticity and memory impairment [26-28]. The mechanism(s) underling different effects of SD lasting on learning and memory function are complex and still not well understood.

This study for first time shows ABA ability to increase learning and memory performance in SD rats. ABA interventions on sleep and learning and memory have been supported by individual previous studies on rodents. It has been indicated that ABA decreases onset time and prolongs sleep duration in a rat model of pentobarbital-induced sleep [23]. Moreover, ABA treatment decreased learning and memory deficit in rat's model of STZ-induced Alzheimer's disease [29]. In addition, ABA infusion decreased learning and memory deficit both in MWM and shuttle box tasks in STZ diabetic rats[30]. The mechanism(s) of ABA involvement to attenuate sleep deprivation weaken effects on learning and memory is not understood. It is postulated the effects might be intended by manipulation of related neurotransmitters and distinct neural networks within brain.

The data showed pharmacological blockage of GABAA receptor with bicuculline did not inhibit ABA efficiency on learning and memory performances in sleep deprived rats. In a related study, pretreatment with bicuculline could obstruct ABA impending to prolong sleep

duration in a rat model of pentobarbital-induced sleep [23]. This duality suggests that ABA could engage different pathways—supporting both sleep recovery and neurocognitive resilience—depending on the physiological or experimental conditions.

GABAergic synapses are profoundly founded on hippocampus CA1 pyramidal neurons [31, 32]. While the baseline GABA levels in the hippocampus improves learning and memory performance, increase in $GABA_A$ receptor activity has been shown to decline network excitability and reduces synaptic plasticity in the CA1 area [33, 34]. Indeed, memory retrieval has impeded by the glutamate and GABA concentration balance in brain[35]. In the rats subjected to SD impairment of memory performance has been associated with imbalances in Glu/GABA ratio[36]. Although, this study did not find ABA interfering on GABAA receptor, however, more data are still required to describe the details of ABA impact on GABAergic system to modulate learning and memory of SD exposed rats.

In the present study, REM sleep deprivation increased oxidative stress damages defined with increases in lipid peroxidation and H2O2 levels, and a decrease in CAT activity in cerebral cortex of rats. However, post treatment with ABA (10 µg/rat), which was the most effective dose to increase learning and memory behaviors, could inhibit oxidative stress imbalances in SD rats. This data is supported by many of previous studies display ABA antioxidant capacity in rodents. Oral treatment with ABA in drinking water increased antioxidant defence systems indices and decreased MDA levels in many tissues of rats [37]. Moreover, intra-lateral ventricles infusion of ABA increased feeding behavior and increased the antioxidant enzymes activity, while attenuated stress oxidative enzymes[38]. In a mouse model of thioacetamide-induced hepatic fibrosis ABA treatment decreased oxidative stress enlargements and inflammation by induction of NF-κB signaling path [39]. Indeed, this study data support an association between ABA antioxidant properties and reduction of REM-SD induced learning and memory deficits.

It has been shown that ABA as lipidgenic compound binds to PPARs and activates a number of intracellular signaling molecules essential in regulation of learning and memory performance[16]. Pretreatment with PPAR β/δ antagonist was able to suppress ABA anti-nociceptive effects in rats[16]. Moreover, ABA decreased diabetes-induced learning and memory deficit in rats via intonation of PPARγ receptors [30]. In addition, PPARγ receptors antagonist prevented ABA ability to increase sleep duration in a rat model of pentobarbital-induced sleep[23]. On the other hands, motivation of PPARγ receptors with ABA modifies calcium channel activity and induces PI3K/PKC pathway in rat's brain to modulate learning and memory and anxiety like behavior [40]. Totally, it is possible that ABA efficiency on learning and memory responses in SD rats at least partially mediated by manipulations of PPARs system and induction the downstream signaling molecules involved in learning and memory performance.

Our study primarily focused on learning and memory performance using specific behavioral tests (e.g., acquisition trials). While these tests provide valuable insights, they may not fully capture the broader spectrum of cognitive functions affected by sleep deprivation or ABA treatment. While oxidative stress biomarkers (catalase, MDA, H2O2) were evaluated, other potential mechanisms (e.g., neuroinflammation, synaptic plasticity) were not explored, leaving gaps in understanding ABA's comprehensive effects.

Conclusions

Overall, the data of this study showed the potential of intra-hippocampal administration of ABA to increase antioxidant indices in brain and attenuation learning and memory deficit in RAM-SD rats. Pretreatment infusion with GABAA receptors antagonist did not change ABA induced responses.

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Material and methods

2.1. Animals

Adult male Wistar rats (2 months) weighing 230–270 grams were used in this study. The animals were contained four per cage in a room with a temperature of 23 ± 2 °C under a 12-h light/dark cycle with limitless entrance to food and water. All trial procedures were permitted by the Animal Research Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran.

2.2 Surgery and microinjection

Rats were profoundly anesthetized with a mixture of ketamine (100 mg)-xylazine (5 mg) and placed in a stereotaxic apparatus (Estoelting CO, USA). Guide cannulae were bilaterally inserted in the CA1 region (3.8 mm posterior to the bregma, 2.2 mm lateral from the midline and 3.2 mm depth to the cortical surface). Afterward, rats were kept separately and endorsed 1 week to recover from surgery prior to treatments [41]. The drugs (1 µL each side) were delivered using a 27-gauge stainless steel needle devoted to a Hamilton micro-syringe.

2.3 Experimental design

The animals were randomly alienated into six experimental groups (n=7) as follows: control (untreated rats); sleep deprivation (SD): which located in small platform; SD +ABA groups: treated intra-CA1 with ABA (5, 10, and 15 µg/ rat) and then located on small platform; SD + ABA (10 µg/ rat) + bicuculline (1 µg/rat): treated intra-CA1 with ABA and bicuculline and then located on small platform. The groups were exposed to SD procedures for 72 h and then injected with specific treatments. Ten minutes after intra-hippocampal injection, the rats were verified in MWM and Shuttle box test, respectively.

2.4. Sleep deprivation (SD)

In first tests, the single small platform method of SD was used. Animals were sited on a single stage in the central of a water cistern. The water reached up to 2 cm under the shallow of the stage. Based on the multiple small platform's method, five stages (each 5 cm diameter) were used. In this method, the stages were spread out (8–10 cm apart) so that animals were able to simply travel amongst them but could not lie through any two. The control group was tested using single large stage method, where the size of the stage was enlarged to 15.2 cm to admire sleep to arise. All the treatments lasted 72 h [42].

2.5. Learning and memory assessment

2.5.1 MWM

In this study, all the experimental groups were subjected to four days training trials in the MWM as defined previously [43]. A video camera was attached straight overhead the water maze pool and the tracking system of Any maze was provided to assess the time to reach the concealed platform (the escape latency) and the length of swim (traveled distance) of each rat in training time. Twenty-four hour later, the rats were evaluated in the probe trial in which the escape platform was detached from the pool and the animal permitted to swim for 60 sec. The total time spent, and the numbers of visit across the past position of the platform were measured to appraise spatial memory.

2.5.2. Shuttle box test

The apparatus encompassed of identical sized light and dark partitions that were separated by a sliding guillotine door. Floor of the dark and light partitions consisted of a stainless-steel shock grid. This test was divided into training and memory stages. In the instruction phase, each animal was positioned in the lightened partition and after 5 s, the gate was unlocked and the rats were indorsed to transfer freely into the dark space. Upon entry into the dark chamber, the door was barred and the rat was assumed 1 mA electrical shock in 1 second. The instruction

trial was completed when the rat endured in the light hall for 5 continuous min. Twenty-four hour later, in stage 2 (retrieval session), each rat was positioned in the light side of box. Ensuing 30 s acclimatization, the door was raised. The number of electrical amazements trial, latency to enter the dark chamber initial time spent to wholly enter the dark room (STL) , as well as whole time consumed in the dark box were measured an important in passive avoidance test [44].

2.6. Biochemical assay

The rats were euthanized with CO2 and the brains were detached and the separated brain regions, hippocampus and prefrontal lobe, were kept in liquid nitrogen for assessment of biochemical parameters. Brain malondialdehyde (MDA) and hydrogen peroxide (H2O2) assay were evaluated as lipid peroxidation product and oxidative stress index [26, 45]. Moreover, the measurement of catalase enzyme activity was done as an index of antioxidant activity [46].

2.7. Statistical analysis

The results are expressed as mean ± SEM. The statistical analyses were prepared using SPSS (version 22) software. One way analyze of variance (ANOVA) was used to evaluation of significant change amongst groups. Tukey Post hoc assessment was achieved to explore differences between each group. Variances were measured significance at level of $P<0.05$.

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Conflict of interest

No competing interests to declare.

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Figure legends

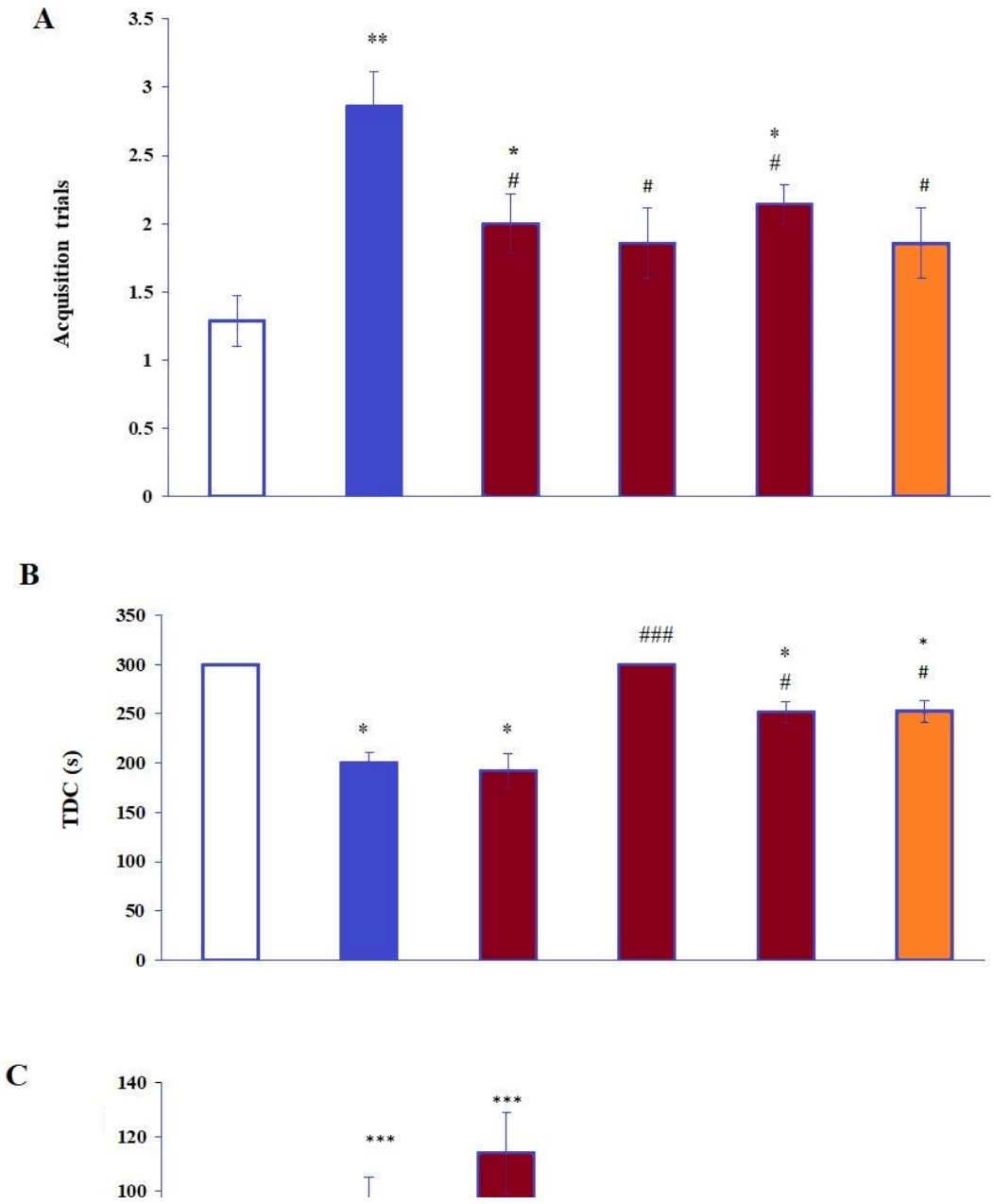

Fig.1.The effect of intra- hippocampal administration of ABA (5, 10 and 15 µg/rat) or Bic+ABA (10 µg/rat) on the number of acquisition trials (A), step through latency (B) and time spent in dark chamber (C) in passive avoidance test in SD rats. Values are expressed as mean ± SEM. * $p<0.05$ ** $p<0.01$ and *** $p<.001$ versus control groups, $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$, $^{\#\#\#}$ $p<0.001$ versus SD group

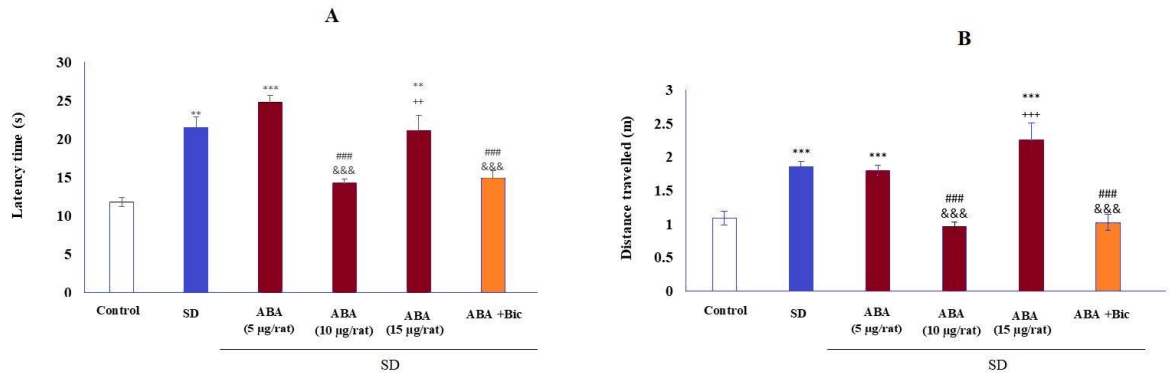

Fig. 2 The effect of intra- hippocampal administration of ABA (5, 10 and 15 µg/rat) or Bic+ABA (10 µg/rat) on the escape latency time (A), and distance travelled to find the hidden platform in MWM test in SD rats. Values are expressed as mean ± SEM. ** $p<0.01$ and *** $p<.001$ versus control groups, ### $p<0.001$ versus SD group, &&& $p<0.001$ versus SD + ABA (5 µg/rat) group, ++ $p<0.01$, +++ $p<0.001$ versus SD + ABA (10 µg/rat) group

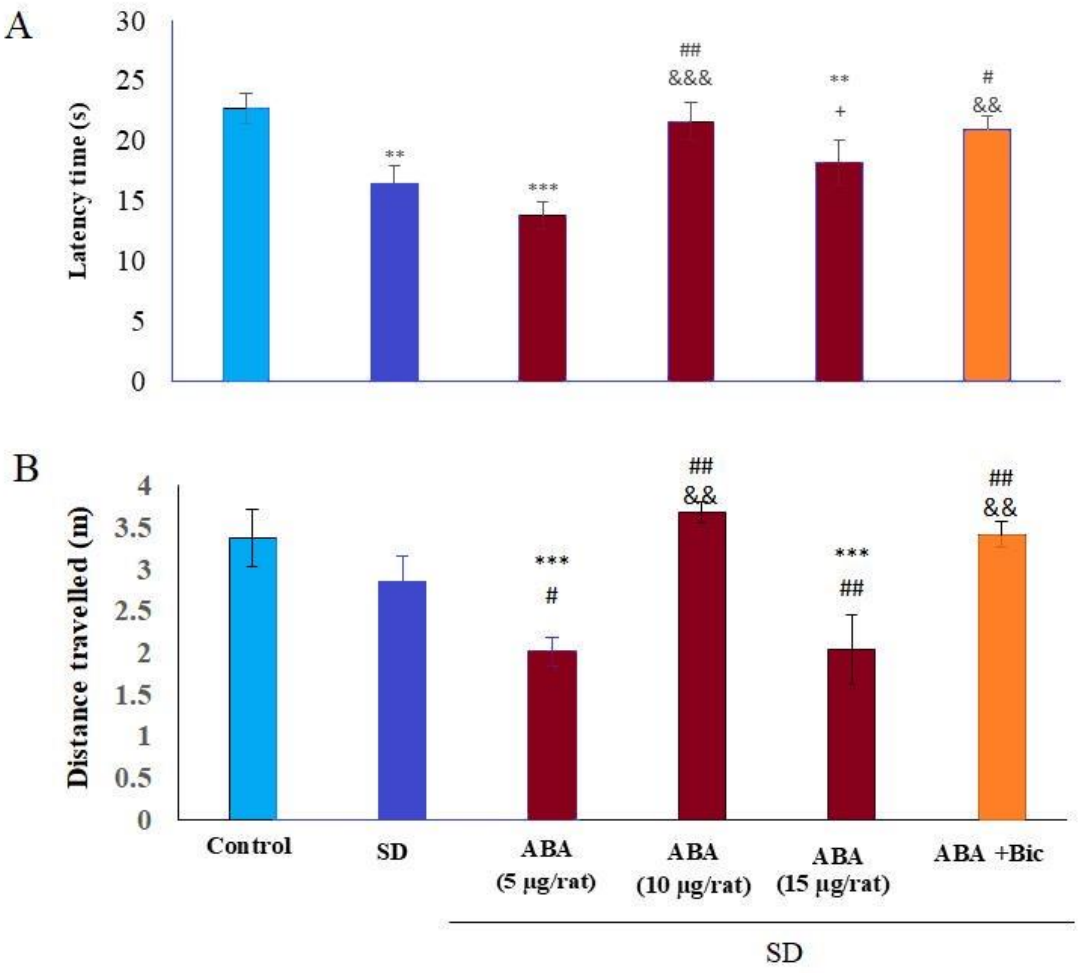

Fig. 3 The effect of intra- hippocampal administration of ABA (5, 10 and 15 µg/rat) or Bic+ABA (10 µg/rat) on the duration time (A), and distance travelled in target zone in SD rats in probe trial of MWM test. Values are expressed as mean ± SEM. ** $p<0.01$ and *** $p<.001$ versus control groups, $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$ versus SD group, $^{\&\&}$ $p<0.05$, $^{\&\&\&}$ $p<0.001$ versus SD + ABA (5 µg/rat) group, + $p<0.05$ versus SD + ABA (10 µg/rat) group

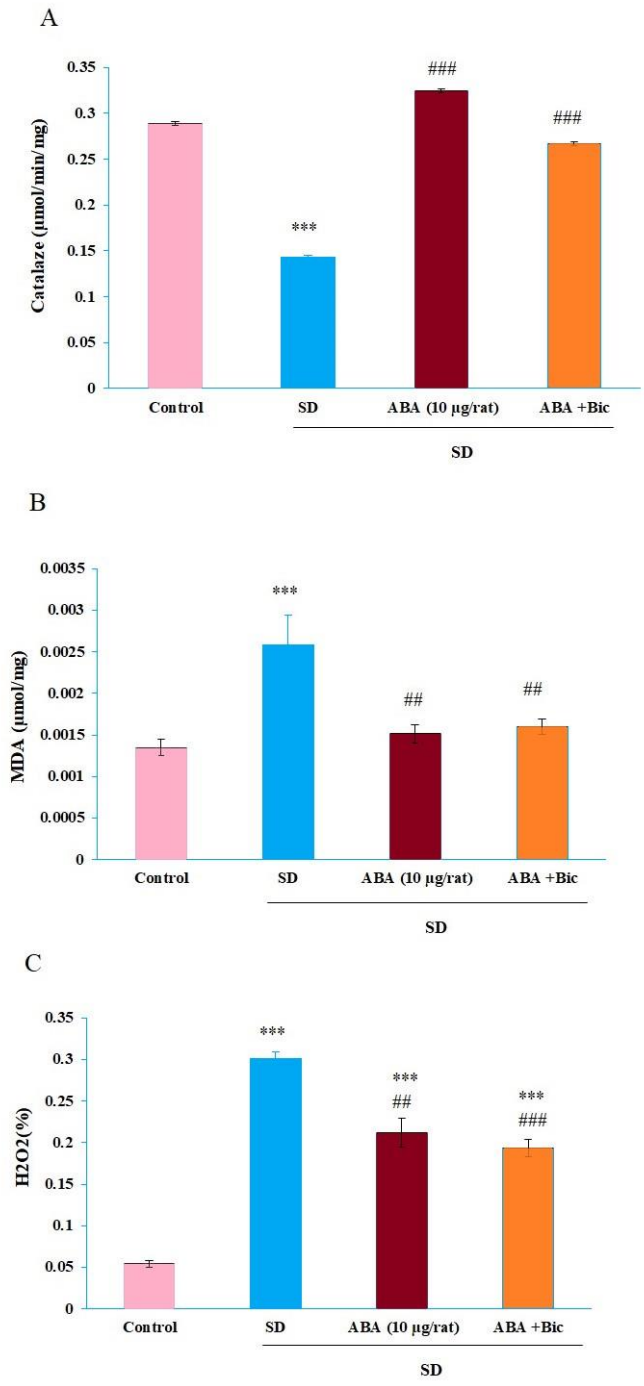

Fig.4. The effect of intra- hippocampal administration of ABA (10 µg/rat) or Bic+ABA (10 µg/rat) on the activity of CAT enzyme (A), MDA concentration (B) and H2O2 activity in the cerebral cortex of rats. Values are expressed as mean ± SEM. ** $p<0.01$ and *** $p<.001$ versus control groups, $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$ versus SD group, $^{\&\&}$ $p<0.05$, $^{\&\&\&}$ $p<0.001$ versus SD + ABA (5 µg/rat) group, + $p<0.05$ versus SD + ABA (10 µg/rat) group

تزریق داخل هیپوکمپی آبسیزیک اسید اختلال حافظه و یادگیری و تغییر در عوامل استرس اکسیداتیو را در موش‌های صحرایی محروم از خواب REM کاهش می‌دهد

چکیده

در این مطالعه اثرات تزریق داخل هیپوکامپی آبسیزیک اسید (ABA) بر تعدیل عملکرد یادگیری و حافظه و تغییر عوامل استرس اکسیداتیو در قشر مغز موش‌هایی صحرایی محروم از خواب با حرکت سریع چشم (REM) مورد ارزیابی قرار گرفت. موش های صحرایی نر بالغ نژاد ویستار در ناحیه CA1 هیپوکامپ کانول‌گذاری شدند. پس از بهبودی، موش‌ها به مدت 4 روز در معرض محرومیت از خواب REM قرار گرفتند. سپس گروه‌های حیوانات با ABA در دوزهای 10و 15 میکروگرم و ABA + بیکوکولین تحت درمان قرار گرفتند. یادگیری اجتنابی غیرفعال و حافظه فضایی به ترتیب با آزمون‌های شاتل باکس و ماز آبی موریس (MWM) ارزیابی شد. علاوه بر این، تغییرات در سطوح کاتالاز به عنوان آنزیم آنتی اکسیدان و MDA و H2O2 به عنوان بیومارکرهای استرس اکسیداتیو در قشر مغز موش‌های صحرایی تعین شد. نتایج نشان داد در حیوانات محروم از خواب REM در مقایسه با کنترل، اختلال قابل‌توجهی در یادگیری و حافظه در آزمون‌های MWM و شاتل باکس ایجاد شده است. تزریق داخل هیپوکمپی ABA در دوز 10 میکروگرم باعث کاهش اختلال حافظه و یادگیری در موش‌های محروم از خواب REM گردید. پیش تیمار حیوانات با بیکوکولین قادر به تغییر اثرات ناشی از ABA بر حافظه و یادگیری نبود. علاوه بر این، افزایش فعالیت کاتالاز و کاهش MDA و H2O2 در قشر مغز گروه‌های تحت درمان با ABA در دوز 10 میکروگرم و ABA+ Bic مشاهده شد. به طور کلی، داده‌ها نشان دهنده توانایی ABA برای کاهش اختلال یادگیری و حافظه و آسیب‌های اکسیداتیو ناشی از محرومیت از خواب REM در موش‌های صحرایی بود. بلوک کردن گیرنده GABA نتوانست اثرات ABA را مهار کند.

کلمات کلیدی: محرومیت از خواب، آبسیزیک اسید، بیکوکولین، حافظه و یادگیری، موش صحرایی