ORIGINAL ARTICLE

The Effect of Nitric Oxide Precursor (L-Arginine) and Inhibitor (L-NAME) in Nucleus Accumbens on Learning and Memory in Stressed Rats

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Abstract

OBJECTIVES: This study investigates the effect of nucleus accumbens nitrergic system manipulation on learning and memory under stress conditions, using precursor (L-Arginine) and inhibitor (L-NAME) of Nitric Oxide.
METHODS: Cannulation was performed in the brain nucleus accumbens shell of 120 Wistar rats. Saline and various dosages of L-Arg and N(ω)-Nitro-L-Arginine Methyl Ester (L-NAME) at 1, 5, and 10 μg/rat dose administered in the nucleus accumbens according to grouping. After five minutes, the stress groups received an electric foot shock for four consecutive days (according to the protocol), but the non-stressed groups did not receive any shock. Corticosterone levels were measured on the first and fourth days. The Barnes maze test measured learning and memory in the following.
RESULTS: Stress raised corticosterone substantially, whereas NO modulation lowered it at 10 μg/rat dosage. Both the stress group (positive control) and the groups with injection of NO modulation without stress circumstances showed a decrease in errors during the learning phase. Long-term memory was also improved by stress and L-Arg at a dose of 5 μg/rat. The effects of L-NAME on memory were attenuated at a dosage of 5 μg/rat. The L-Arg-treated rats continued using serial or direct movement strategies well into the long-term memory phase. The beneficial effects of 1 μg/rat of L-Arg on long-term memory were attenuated by stress, whereas the adverse effects of 5 μg/rat of L-NAME were amplified.
CONCLUSION: Due to its effectiveness in lowering mistake rates, the stress we used in this investigation may be eustress. L-Arg enhances cognitive performance and memory retention whereas L-NAME has the opposite effect. Additionally, modest doses of L-Arg enhanced stress' beneficial impact on memory, but L-NAME reduced it.

Introduction

Stress is defined as any environmental or mental factor that complicates the survival of living organisms and affects physiological homeostasis (Hosseini & Chahardehi 2022; Ostovar *et al.* 2022; Tilbrook *et al.* 2000). The paraventricular nucleus (PVN) of the hypothalamus is the pinnacle point of the HPA stress response (Chaves *et al.* 2021; Miller *et al.* 2016). Parvocellular neurons produce corticotropin-releasing hormone (CRH) in response to stress, and also the PVN nitric oxidergic system activates and releases nitric oxide (NO) (Miller *et al.* 2016). Adrenocorticotropic hormone (ACTH) is secreted into the bloodstream when CRH stimulates the anterior pituitary (Hosseini 2023). Corticosterone sensitizes and release of adrenal gland (Chaves *et al.* 2021). Stress stimulate ACTH release through an NO dependent mechanism that enhance NO production in brain areas (amygdala, PVN, etc.) (Hosseini *et al.* 2023; Miller *et al.* 2016). These areas are important in controlling the HPA axis (Ostovar *et al.* 2022). In addition, NO has an inhibitory effect on release of CRH, ACTH and corticosterone (Di Chiara *et al.* 1999). Glucocorticoids can help maintain memory at normal performance (Kalivas & Duffy 1995). The stress-responsive systems exhibit interconnectivity in specific brain regions, including the hippocampus and amygdala (Chahardehi *et al.* 2023). However, the accumulation of corticosterone (CS) in the hippocampus due to chronic stress has been observed to cause impairment of memory (Handra *et al.* 2019).

The Nucleus Accumbens (NAc) is a crucial component of the anterior brain and ventral striatum, comprising two distinct subregions, namely the shell and core. The NAc's shell is known as the extended amygdala, which, in junction with the hippocampus and central amygdala (Chiara *et al.* 1999), is critical for stress and brain plasticity responses related to learning (Bradley & Steinert 2016; Handra *et al.* 2019; Kalivas & Duffy 1995) by modification of nitric oxide (NO) (Calabrese *et al.* 2007). Any manipulation in the shell of the NAc can affect the "expanded amygdala" and its activities (Groenewegen *et al.* 1999; Jackson & Moghaddam 2001). NO is a critical mediator activated when the NMDA glutamate receptors are triggered (Calabrese *et al.* 2007; Philippu 2016). Although manipulating the nitrergic system in the left NAc shell alters metabolic activity in response to stress (Husseini *et al.* 2019), inactivating the left NAc has no substantial effect on recalling new learning material (Vafaei *et al.* 2002). NO enhances long-term synaptic potential (LTP), synaptic structural changes, memory, and learning modifications (Bradley & Steinert 2016).

Although the number of anatomical and electrophysiological studies on the NAc has increased in recent years, there have been relatively few investigations into the NAc's role in stress (Lupien *et al.* 2007) and its link to memory and learning (Rinaldi *et al.* 2012). Therefore, the specific brain circuits involved in stress remain unknown (Ekstrom 2020). Spatial learning refers to a type of memory responsible for an individual's spatial orientation (Shelton & Gabrieli 2004). The brain's hippocampus, amygdala, striatum, mammillary bodies, and Broadman region of the frontal cortex are thought to have a role in preserving spatial memory after a stressful event (Murray *et al.* 2017). Several tests, for instance, the Barnes test, are utilized to assess specific memory. In comparison to other assessments, this experiment aims to investigate the potential of reducing stress levels in animals (Harrison *et al.* 2009). This study assessed learning and memory in response to stress and non-stress conditions according stimulating or inhibiting NO production in the left NAc cortex. To evaluate the effects of NO modulation, L-Arg (NO precursor) and N(ω)-Nitro-L-Arginine Methyl Ester (L-NAME) as a NO synthesis inhibitor was injected into the left NAc in different doses and examine learning and memory in stressed and non-stressed male rats. The study hypothesized that the L-Arg and L-NAME had different interaction effects with stress on corticosterone level, learning and memory.

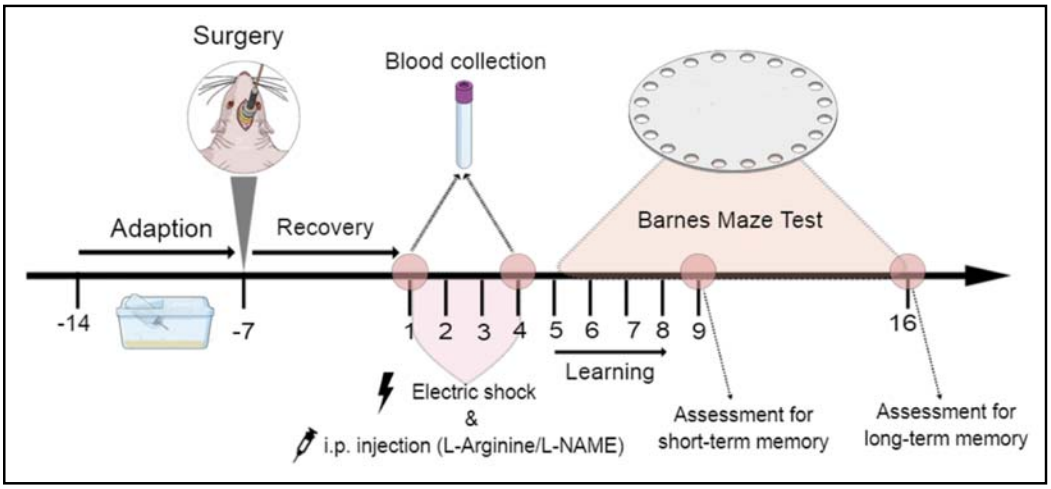

Fig. 1. The timetable of study

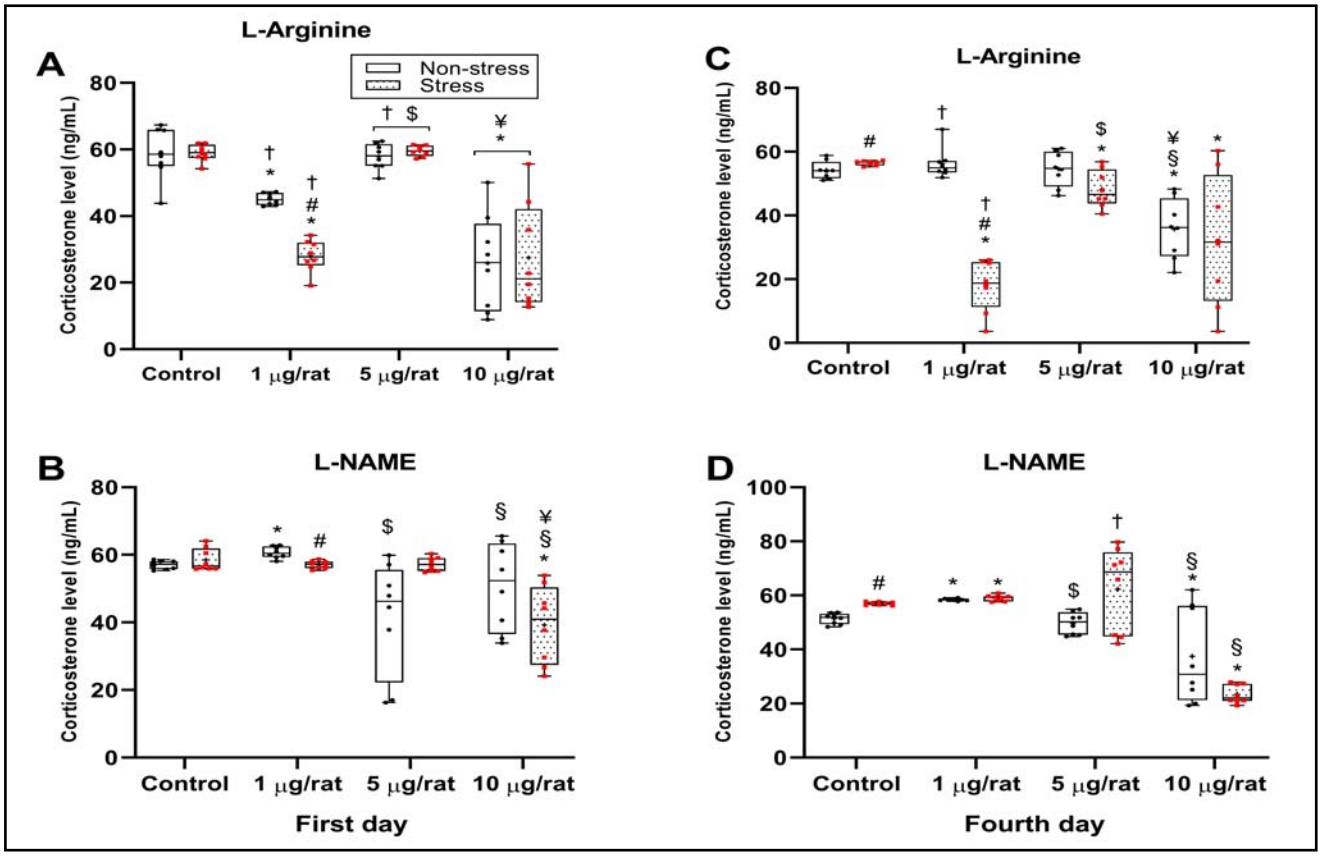

Fig. 2. Comparison of serum corticosterone levels in rats under non-stress and stress groups. **A)** L-Arg was tested in the first day, **B)** L-NAME was tested in the first day, **C)** L-Arg was tested in the fourth day, and **D)** L-NAME was tested in the fourth day. *: significance of stress versus non-stress condition in forth day. #: significance between control and each drug in non-stress condition, $: significance between 1 μg/rat and 10 μg/rat of L-Arg in stress condition. §: significance between L-NAME 5 μg/rat with other groups in stress condition, ¥: significance between L-NAME 5 μg/rat and 1 μg/rat in stress condition, †: significance between L-NAME 10 μg/rat and 1 μg/rat in non-stress condition (n = 8, p < 0.05) (derived from previous study).

Materials and methods

Animal

In this study employed a sample of 112 male Wistar rats of adult age, with a weight range of 170-180 g. The rats were housed under standard laboratory conditions, which included a controlled temperature range of 21-23°C, a humidity level of 55%, and a 12-hour light/dark cycle. The rats were kept in cages measuring 49 x 27 x 18 cm^3, with a density of 4-5 rats per cage. All the animals were provided unrestricted access to both food and water. The rats were habituated to the lab for a full week before they were handled (Sadeghi *et al.* 2023). All experiments were performed following guidelines established by the AJA University Ethics Committee and the National Institutes of Health guide for the care and use of laboratory animals. The ethics number was registered by the Council of Laboratory Animals of AJA University of Medical Sciences, Tehran, Iran (Approval No. IR.AJAUMS.REC.1400.214 in November 2021).

Grouping

The animals were classified into 14 groups (n = 8), including the following: two control groups (stressed or positive control group, and non-stressed or negative control group), three stressed L-Arg-administered groups, three non-stressed L-Arg-administered groups, three stressed L-NAME-administered groups, and three non-stressed L-Arg-administered groups. Drug grouping for each drug was done based on 1, 5, and 10 μg/rat doses.

Experimental Design

Figure 1 depicts the chronological sequence of events in the experiment, including the stages of adaptation, surgery, electric shock induction, drug injection, learning, short-term memory, and long-term memory.

Surgery

The rats were administered anesthesia using ketamine (60 mg/kg, i.p. route) and xylazine (10 mg/kg, i.p. route) (Geiger *et al.* 2008). The animals' heads were shaved from the auricular region to the interorbital area. Subsequently, the animals were immobilized within a stereotaxic apparatus, and thermal homeostasis was maintained via a heating pad that was regulated by a thermostat (ALA Instruments, Inc.).

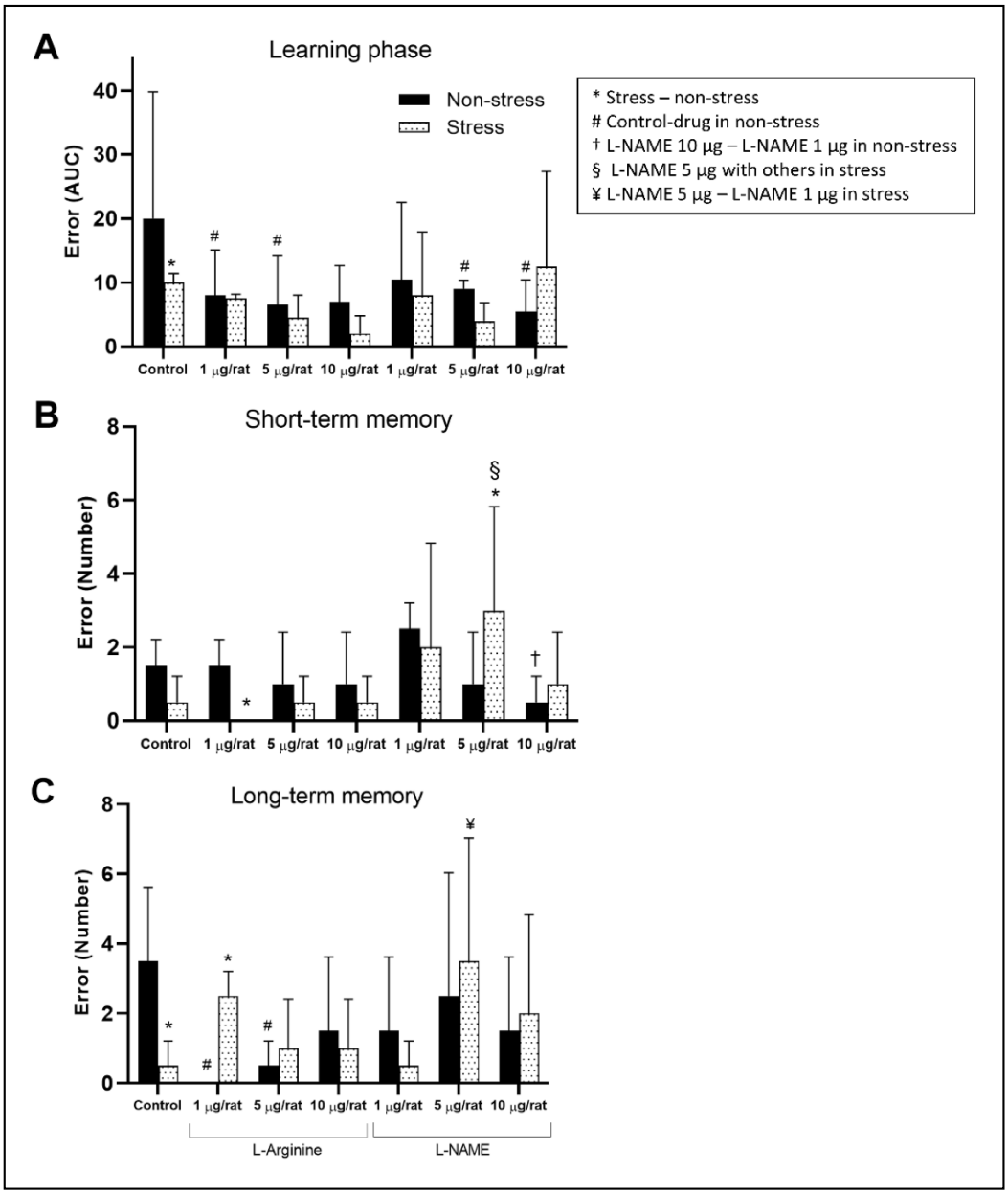

Fig. 3. The graphs represent the mean and 95% confidence intervals for error in control groups, L-Arg, and L-NAME at 1, 5, and 10 µg/rat concentrations. Plot **(A)** depicts the AUC of error for the first four days of the learning phase. Plot **(B)** shows the number of errors in the short-term memory phase. Plot **(C)** shows the number of errors in the long-term memory phase, *: significance of stress versus non-stress condition, #: significance between control and each drug, $: significance between L-Arg 1 µg/rat and others in the non-stress condition, †: significance between L-NAME 5 µg/rat with L-NAME 1 µg/rat, ¥: significance between L-NAME 5 µg/rat with others ($n = 8$, $p < 0.05$).

Surgical procedure involved the creation of a 2-cm-long incision in the skin, followed by the removal of soft tissue from the skull. According to Paxinos atlas, the guiding cannula was inserted into the left NAc shell (from Bregma 1.6 mm anterior, 0.6 mm lateral, and 7 mm deep) (Manago *et al.* 2009; Schwarz *et al.* 2006) and fixed with two screws for glasses and dental cement. The experiments began one week after the recovery.

Drug injection into the nucleus accumbens shell

Normal saline was used to dissolve the drugs. Then, using a Hamilton syringe, 0.5 Landa of drugs at doses of 1, 5, and 10 µg/rat were progressively injected into the shell of the NAc for 1 minute (Husseini *et al.* 2021). The animals were allowed unrestricted movement during the injection procedure.

A method of inducing acute and repetitive stress

In this study, electric shocks with a voltage of 40 mV and a power frequency of 10 Hz were applied to the soles of the animals' feet for 60 seconds on four consecutive days. Before the shock treatment, the animals spent 30 minutes in a stress box to become used to the environment. Furthermore, the shock was sustained for an additional duration of 30 minutes to facilitate recovery. L-NAME (a nitric oxide synthase inhibitor) and L-Arg (a nitric oxide precursor) were injected into the NAc shell five minutes before to each session at 1, 5, and 10 g/rat. Prior to each session, the floor and walls of the stress box were thoroughly sanitized with alcohol.

The stress box consisted of nine similar parts measuring 16 × 16 × 54 cm (length × width × height), made of plexiglass. Each part was connected via tiny holes that allowed the animals to interact visually. Its

floor was composed of multiple 4 mm steel bars spaced 1.3 mm apart. This set was linked to the electric shock generator (Husseini *et al.* 2021).

Measurement of blood CS level

On the first and fourth days, blood samples were obtained from the caudal vein. Plasma was extracted after the blood was centrifuged at 3000 RPM for 6 minutes. The ELISA technique measured plasma CS concentration using a Zellbio CS kit (Germany).

Measurement of spatial memory and learning

Pre-training: On day zero, the rat was placed in the center of the Barnes maze table and given one hour to move freely.

Training sessions (4 trials per day for 4 days): In each trial, the rat was first placed in the escape box in the middle of the Barnes maze table for 90 seconds. Second, the escape box was removed and the rat was given 90 s to find the target chamber. Third, if the rat found the chamber, remained in it for 90 s, but if did not, it was manually guided into the chamber and rested for 90 s. Finally, the rat was returned to its cage for 90 seconds to recuperate and prepare for the next training session (Asalgoo *et al.* 2018). Learning behavior was evaluated based on the rats' replies. Five minutes prior to each stress session, three doses of L-NAME or L-Arg (1, 5, 10 μg/rat) were injected into the shell of the NAc, and the rats were evaluated during the four days. Short-term memory was evaluated once on the fifth day of the experiment. In addition, a memory test was conducted one week later to assess long-term memory (day 16). After each test, the table was cleaned with a saline and alcohol solution. Errors, distance traveled, navigational method (random, serial, and direct), and time to reach the target chamber were all measured and analyzed. Motion tracking was recorded with the AutoVision camera and its software (Barns maze software).

Statistical analysis

Data were expressed as a mean and 95% confidence interval. Interventions included stress induction, L-Arg, and L-NAME injections. The distribution of variables was normal. A two-way univariate ANOVA was run to analyze the interaction effect of conditions (stress and non-stress), groups (control, L-Arg, and L-NAME at 1, 5, and 10 μg/rat) on the plasma CS levels on the first and fourth day. The statistical test was employed for the computation of the area under the curve (AUC) pertaining to the error, time, and distance variables of the Barnes maze test. It was determined by examining the learning index over four days, and the 9th day and 16th-day tests were considered short-term and long-term memory indices, respectively. The Bonferroni test was used for pairwise comparisons of variables in the Barnes maze test, and the Mann-Whitney test was used for pairwise comparisons in the corticosterone. A chi-squared test evaluated motor strategies. $p < 0.05$ was considered statistically significant.

Results

The CS levels in groups

After four days, two-way ANOVA showed a significant interaction effect of conditions (stress and non-stress), groups (control, L-Arg, and L-NAME and their doses of 1, 5, and 10 μg/rat) with F = 3.92 and $p = 0.014$, and then the Mann-Whitney test was used to compare each factor separately. The positive control group's CS level increased significantly more than the negative control group's (Mann-Whitney U = 0.000, $p = 0.029$). The injection of both NO modulators decreased the level of CS compared to the control group at 10 μg/rat in both conditions (Mann-Whitney U = 0.000, $p = 0.029$). In the stress condition, L-Arg 1 and 5 μg/rat groups decreased the level of CS compared to the control group (Mann-Whitney U = 0.000, $p = 0.029$). In both stress and non-stress conditions, the L-NAME 1 μg/rat increased the level of CS compared to the control group ($p < 0.05$), as did the same dose of L-Arg (Mann-Whitney U = 0.000, $p = 0.029$; Fig. 2). These findings were previously discussed in our published publication (Husseini *et al.* 2021).

Effects of stress, L-Arg, and L-NAME on the number of errors

When the number of errors made during the learning phase in the Barnes maze reduces, learning and memory improve (Asalgoo *et al.* 2018). The factor of condition and group was significant (F = 47, $p = 0.0004$ for the learning phase; F = 2.23, $p = 0.05$ for short-term memory; F = 2.87, $p = 0.015$ for long-term memory). Stress boosted the CS level considerably and decreased the errors during the learning and long-term memory phases (Adjustment for multiple comparisons: Bonferroni = 0.00001 for learning, $p = 0.018$ for long-term memory). Under non-stress conditions, injections of L-Arg and L-NAME at all doses decreased errors in the learning phase. However, stress had no significant interaction effect on the effectiveness of drugs. In short-term memory, the L-Arg groups demonstrated a significant decrease in errors at 1 μg/rat the following stress compared to non-stressed conditions (Adjustment for multiple comparisons: Bonferroni: $p = 0.008$; Fig. 3).

Nevertheless, L-NAME at 5 μg/rat dose increased the errors significantly after stressful conditions compared to the other drugs and non-stress conditions in the short-term memory phase ($p < 0.05$). In contrast, L-Arg at 1 and 5 μg/rat in long-term memory significantly reduced the errors compared to the control group in the non-stress condition (Adjustment for multiple comparisons: Bonferroni: $p = 0.006$ for L-Arg at 1 μg/rat and $p = 0.017$ for L-Arg at 5 μg/rat; Fig. 3). The CS levels and error rate were decreased significantly after injection of L-Arg at 1 μg/rat in stress conditions in the short-term

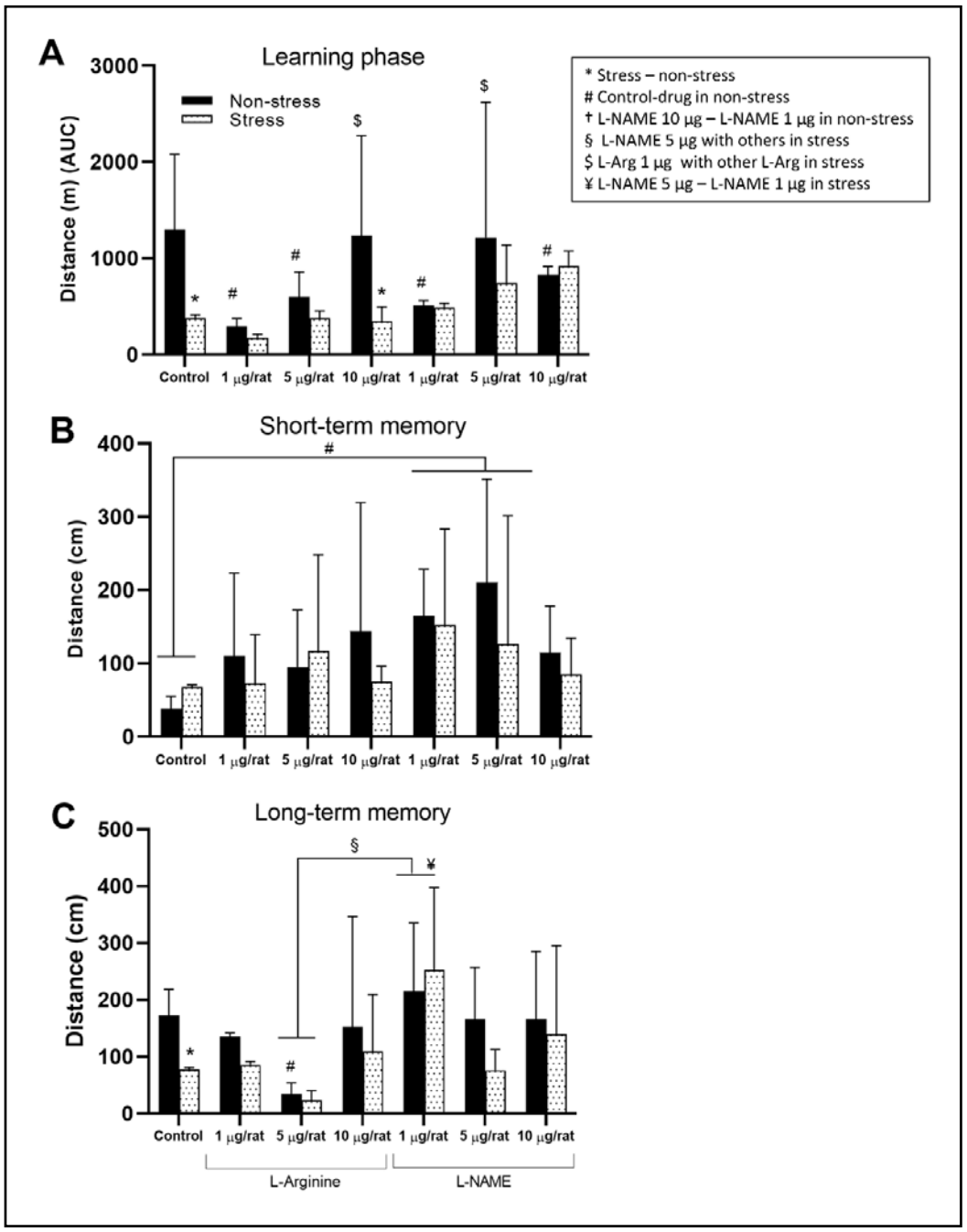

Fig. 4. The graphs represent the mean and 95% confidence intervals for the distance traveled in control groups, L-Arg, and L-NAME at concentrations of 1, 5, and 10 µg/rat. Plot **(A)** shows the AUC of the distance between four days of the learning phase. Plot **(B)** shows the number of errors in the short-term memory phase. Plot **(C)** shows the distance in the long-term memory phase, *: significance of stress versus non-stress condition. #: significance between control and each drug in non-stress condition. $: significance between L-Arg 1 µg/rat and other drugs in non-stress conditions, §: significance between L-Arg 1 µg/rat and L-NAME 1 µg/rat in both conditions, ¥: significance between control and L-NAME 1 µg/rat in non-stress condition, †: significance between L-NAME 5 µg/rat and 1 µg/rat in non-stress condition ($n = 8$, $p < 0.05$).

memory compared to non-stressed conditions (Adjustment for multiple comparisons: Bonferroni: $p = 0.008$). This finding was observed for comparing the L-Arg at 10 µg/rat in stress conditions in the learning phase compared to non-stressed conditions (Adjustment for multiple comparisons: Bonferroni: $p = 0.038$). However, in the long-term memory phase, the errors in the L-Arg at 1 µg/rat after stress were significantly higher (Adjustment for multiple comparisons: Bonferroni: $p = 0.005$) than in the non-stressed condition (Fig. 3).

Blood CS levels increased in non-stress conditions after 5 µg/rat injection of L-NAME. However, they decreased at the 5 and 10 µg/rat doses, which was statistically significant at the 10 µg/rat dose (Mann-Whitney U = 0.000, $p = 0.029$; Fig. 2). Under stress conditions, L-NAME injection, especially at 5 µg/rat enhanced errors in the short-term memory test compared to the non-stress condition (Adjustment for multiple comparisons: Bonferroni: $p = 0.03$). In the long-term memory tests, the errors increased with the L-NAME at 5 µg compared to the L-NAME at 1 µg/rat in the stress condition (Adjustment for multiple comparisons: Bonferroni: $p = 0.007$; Fig. 3B). However, after injection of the L-NAME at 10 µg/rat, the errors in the short-term memory test were decreased in the non-stress condition compared with L-NAME at 1 µg/rat (Adjustment for multiple comparisons: Bonferroni: $p = 0.013$; Fig. 3C).

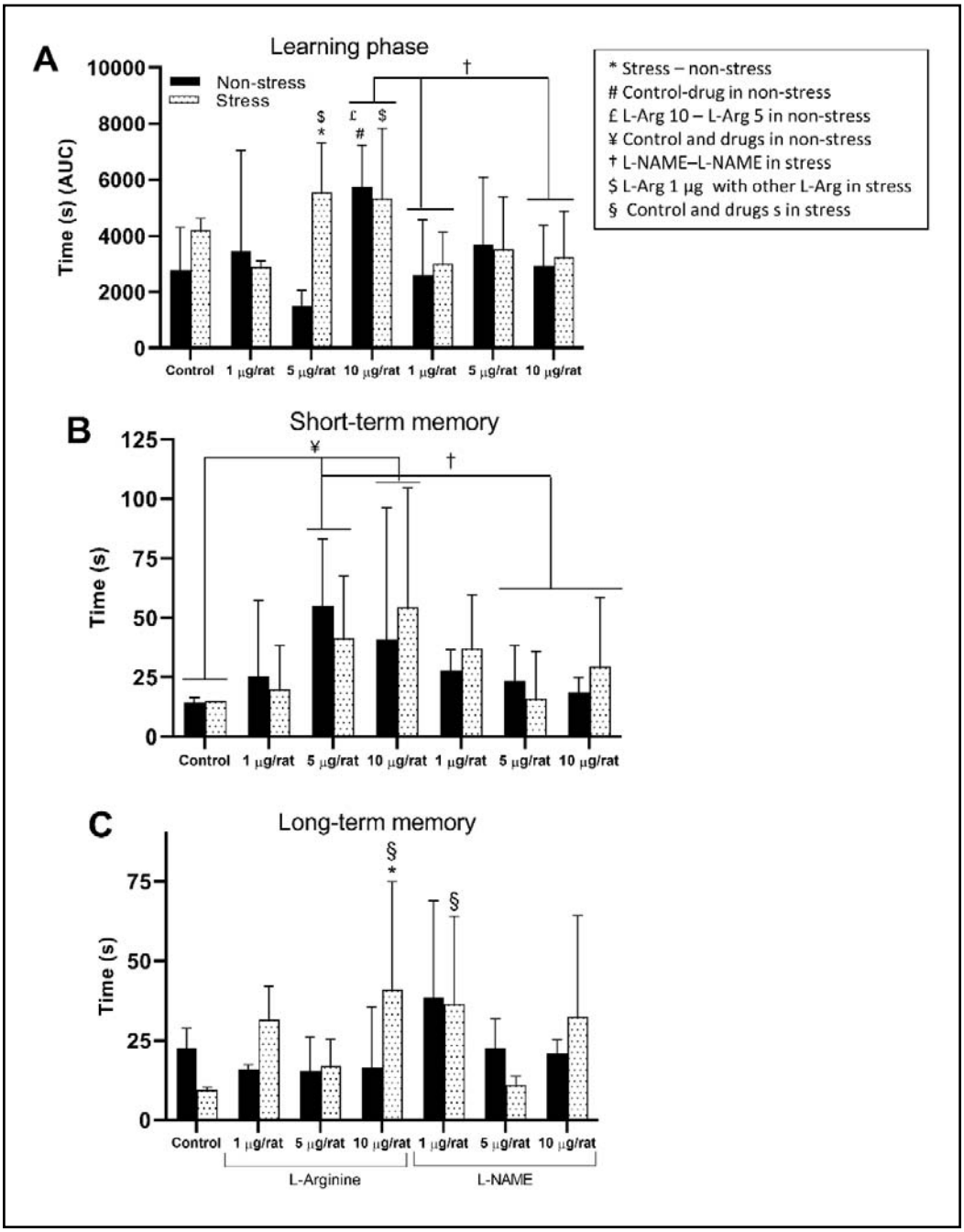

Fig. 5. The plots show the mean and 95% CI of time in groups of control, L-Arg and L-NAME at the 1, 5 and 10 µg/rat. Plot **(A)** shows the AUC of four days of the learning phase. Plot **(B)** depicts the time spent in the short-term memory phase. Plot **(C)** shows the time in the long-term memory phase, *: significance of stress versus non-stress condition, #: significance between control and each drug in non-stress condition. $: significance between L-Arg 1 µg/rat with 10 and 5 µg/rat in stress conditions, §: significance between control and drug in stress condition, ¥: significance between control and drug in both conditions, †: significance between L-NAME and L-Arg in both conditions, and £: significance between L-Arg 1and 5 µg/rat in non-stress condition ($n = 8$, $p < 0.05$).

Effects of stress, L-Arg, and L-NAME on the distance

Two-way ANOVA analysis showed that the group (control, L-Arg, and L-NAME, each in three doses) and condition (stress and non-stress) had a significant interaction effect on the distance to reach the target chamber in the learning phase, as determined by the AUC index ($F = 4.09$ $p = 0.001$). A Bonferroni pairwise comparison showed that the NO modulation drugs (L-Arg at 1 and 5 µg/rat and L-NAME at 1 and 10 µg/rat) considerably decreased the distance as compared to the control group in the non-stress condition ($p < 0.05$). The stress caused a significant decrease in the distance in control and L-Arg 10 µg/rat to compare non-stress conditions ($p < 0.001$; Fig.4A). In the short-term memory test, the group effect was significant on distance ($F = 4.68$, $p = 0.0003$). The L-NAME at 1 and 5 µg/rat significantly increased distance in both conditions compared to the control group (Fig.4B). In the long-term memory test, the significant effect of the group on the distance ($F = 8.36$, $p < 0.001$). In both conditions, L-NAME 1 µg/rat significantly increased distance compared to the control and L-Arg 5 µg/rat ($p = 0.004$; Fig.4C).

Effects of stress, L-Arg, and L-NAME on the Time

A two-way ANOVA test showed that the group (control, L-Arg, and L-NAME, each one in three doses) and condition (stress and non-stress) had a significant interaction effect on the time for reaching the target chamber in the

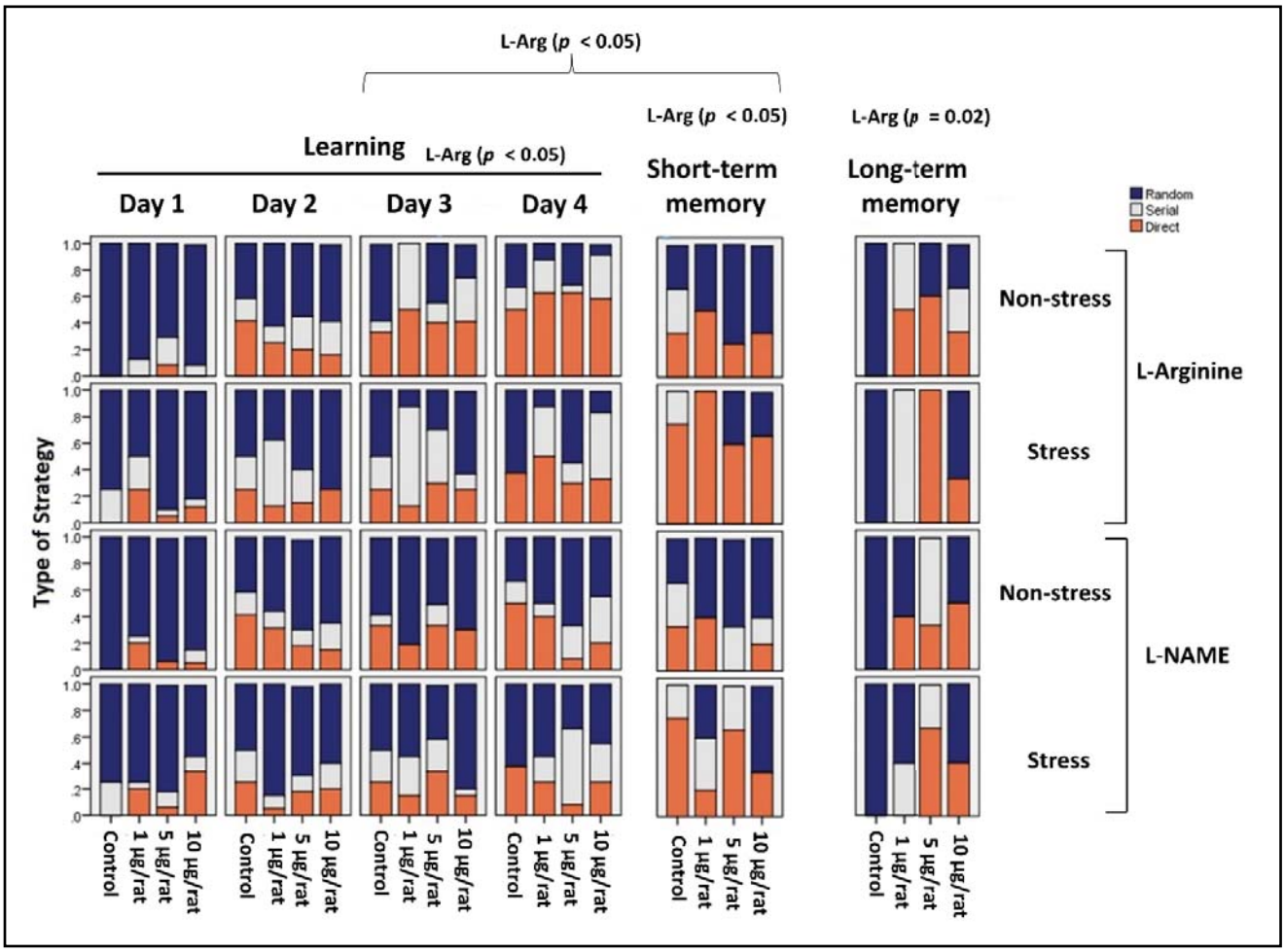

Fig. 6. The prevalence ratio of different strategies on the whole in the three phases of learning, short-term memory, and long-term memory. The Chi-Square qualitative test results showed that the L-Arg 1 µg/rat group was more changed than the rest in the learning phase on the third day. Learning in the presence of L-NAME was weakened. L-Arg produced a significantly more direct strategy than L-NAME, especially in stress conditions in short-term memory. In contrast, L-Arg 1 µg/rat and then 5 µg/rat was a more repetitive and direct strategy to compare L-NAME in the long-term memory phase ($n = 8$, $p < 0.05$).

learning phase. This was measured as an AUC index ($F = 5.08$, $p = 0.0001$). The following Bonferroni pairwise comparison showed that time in the L-Arg 5 µg/rat group in the stress condition was more than non-stress condition. In non-stress conditions, the time was significantly increased in the L-Arg 10 µg/rat compared to the control, L-Arg 1 and 5 µg/rat, and L-NAME 1 and 10 µg/rat groups ($p < 0.007$; Fig. 5A).

In the short-term memory test, the group effect was significant on time ($F = 6.53$, $p = 0.000007$). The time was increased significantly in the L-Arg 5 µg/rat to compare the control and L-NAME 5 and 10 µg/rat groups and in the L-Arg 10 µg/rat to compare the control group in both conditions ($p < 0.005$; Fig. 5B).

In the long-term memory test, the interaction effect of condition and groups was significant ($F = 2.33$, $p = 0.037$; Fig. 5C). The following Bonferroni pairwise comparison showed that the time was significantly increased in the stress condition compared to the non-stress condition in the L-Arg 10 µg/rat group ($p = 0.012$). However, the time in the L-Arg 10 µg/rat and L-NAME 1 µg/rat groups was significantly more than the control group in the stress condition ($p < 0.05$).

Effects of stress, L-Arg, and L-NAME on the type of navigation strategy

There is considerable stress throughout each day under stressful conditions compared to the non-stressed conditions during the learning and short-term phases. According to a chi-square test, during the short-term memory phase, the random strategy was considerably reduced in the positive control group, whereas it was shown to increase in both groups during the long-term memory phase. When comparing the L-Arg groups, it was found that navigation throughout the learning phase was less random and more direct in the non-stress condition and more repetitious in the stress condition ($p < 0.05$). The rats given L-Arg 1 µg/rat with stress opted for a direct strategy in the short-term and a repetitive strategy in the long-term ($p < 0.05$).

In the long-term phase, rats received L-Arg 5 µg/rat, and stress chose the direct strategy solely. However, the

navigation strategy was more random in the L-NAME groups, particularly in the non-stress condition, despite the stress condition being more repetitive and direct, especially in the L-NAME 5 μg/rat group ($p < 0.05$; Fig. 6).

Discussion

The effect of stress on learning, memory and NOS

Previous research was used to determine the optimal dosages and design the experimental protocol for this investigation (Husseini *et al.* 2021). The findings indicate a notable increase in CS levels in the positive control group compared to the negative control group after four days. Additionally, the control group exhibited a decrease in stress levels, which in turn reduced the distance traveled and the number of errors made during the learning phase of the Barnes maze test. However, time and distance were unaffected by short- or long-term stress. The random navigation strategies were erased due to stress in the short-term memory phase. Although stress still significantly impacted the error and distance to find the target chamber, it did not change the navigation strategies in the long-term phase compared to the baseline test.

Long-term stress is associated with memory loss and behavioral abnormalities (Yamamoto *et al.* 2009). Stress can activate the neurological and endocrine systems and the brain's behavioral reactions to maintain a stable state of equilibrium (McCarty 2016; Modarresi Chahardehi & Hosseini 2022). Many neurotransmitter pathways in the brain, including dopaminergic, glutamatergic, and cholinergic, are activated by stress (Mora *et al.* 2012). The stress used in this research was eustress due to the positive effects seen in decreased mistake rates, shorter walking distances, and enhanced navigational strategies. Hence, enhanced awareness was found to improve motor learning and memory.

Multiple inflammatory mediators, including nitric oxide (NO), prostanoids, cytokines and transcription factors, are activated in the brain and other systems in response to acute and chronic stress. (Gądek-Michalska *et al.* 2013). Acute stress reduces the efficiency of the brain's NOS/NO and COX/PG systems. The prefrontal cortex, hypothalamus, hippocampus, all show modifications to their constitutive (COX-1) and inducible (COX-2) cyclooxygenase responses to stress due to chronic stress. This modulation involves both NO and PG produced in the PVN (Gądek-Michalska *et al.* 2013). The NOS family includes; endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) (Costa *et al.* 2016). The presence of stress hormones within the nervous system has been observed to result in a reduction of eNOS synthase levels in the blood vessels of the hypophysis (Lopez-Figueroa *et al.*1998). eNOS is an endogenously produced enzyme triggered by shear stress from the blood flow or agonists like acetylcholine and bradykinin (Li *et al.* 2014). Despite some conflicting findings, acute stress has been found to impact endothelial function negatively. chronic stress consistently impairs endothelial function (Toda & Nakanishi-Toda 2011). Inositol phosphates (iNOS) have been observed to modulate glucose and lipid metabolism to survive and adapt to stress conditions (Anavi & Tirosh 2020). Under normal conditions, iNOS synthase is not detectable, whereas it is induced in response to stress and inflammatory cytokines (Gądek-Michalska *et al.* 2013). The isoenzyme of NOS, neuronal nitric oxide synthase (nNOS), is produced throughout the nervous system (Gądek-Michalska *et al.* 2013). The phenomenon under consideration pertains to the modulation of learning and memory (Dagdeviren 2017).

The effect of NO modulating drugs on learning and spatial memory

The administration of L-Arg and L-NAME at a dosage of 10 μg/rat significantly reduced the blood CS levels in both scenarios. Additionally, under stress conditions, a dosage of 1 μg/rat of L-Arg also led to a noteworthy decrease in blood CS levels. The L-Arg and L-NAME injections at all dosages reduced errors during the learning phase in the non-stress condition; however, the interaction of stress and the NO modulator injection altered the drug's efficacy in various ways. After a stressful event, the injection of 5 μg/rat of L-Arg and L-NAME had the opposite impact on errors in the short-term memory and long-term memory tests. It means the errors in long-term memory decreased dramatically following the injection of 1 and 5 μg/rat of L-Arg. However, the error rate increased after the injection of L-NAME at a dose of 5 μg/rat, especially after stress. The time to reach the target chamber during learning and short-term memory stages, was significantly longer in L-Arg doses (5 or 10 g/mice) compared to control and L-NAME groups. Even in the long-term memory phase, the navigation approach was less random and more direct and repetitive in the L-Arg groups. However, the random strategy remained in the L-NAME groups, especially the non-stress groups.

Despite this, NO modulators continued to influence navigational tactics for almost two weeks after the final injection. Compared to L-NAME, L-Arg injection in the shell of NAc reduced errors and improved navigation strategy. The L-NAME injection impaired short-term memory and resulted in a more random strategy. In a study by Ebadi *et al.* (2010), it was demonstrated that L-NAME induced the destruction of brain tissue under non-stress situations and that the inhibition of nitric oxide improved memory performance in stressed rats (Ebadi *et al.* 2010). Hence, changes in NO probably affect memory and learning under stress (Paul & Ekambaram 2011). According to Boultadakis *et al.* (2010), L-NAME, a NO-generation blocker that interferes with the NMDA receptor's excitation, may impair learning (Boultadakis *et al.* 2010).

Our study showed that L-Arg, as a NO precursor in the nucleus accumbens, causes a significant improvement in spatial learning and memory. On the other hand, L-NAME enhanced learning while impairing memory. NO may have an impact on synaptic plasticity (Korshunova & Balaban 2014). A synaptic plasticity mechanism of NO-induced spatial memory is the stimulation and re-uptake of dopamine and glutamate released from surrounding neurons and developing a close loop to maintain the interaction between presynaptic and post-synaptic neurons (Najafi *et al.* 2013). In addition, NO production alters AMPAR subunit composition and surface expression via a series of metabolic pathways (Ivanova *et al.* 2020) in NAc (Boudreau & Wolf 2005). Then, inhibiting NO generation slows the learning process by reducing AMPA channels.

It appears that the stress utilized in this study was either beneficial or that L-Arg, especially at 1 μg/rat boosted the eustress's impact on memory. At the same time, L-NAME attenuated its effect on memory. Stress and L-Arg, which both promote NO's impact on the hippocampus, are most likely to cause a rise in NO and a toxic state in this circumstance (Hosseini *et al.* 2010). NO production may act as an oxidative stressor, resulting in learning and memory impairment (Hosseini *et al.* 2010) or deficiency of energy and oxygen, activation of glutamate receptors for NMDA (Sadeghi *et al.* 2022), mitochondrial dysfunction, and cell death, all contributing to the destruction of learning and memory (Najafi *et al.* 2013). Our results supported this finding because stress reversed the positive impact of L-Arg at 1 μg/rat, and L-Arg doses of 5 and 10 μg/rat failed to produce any detectable effects on learning and memory under stress. A study by Bannerman *et al.* showed that L-NAME disrupted learning and spatial memory in the Morris water maze test and reduced mobility (Bannerman *et al.* 1994). In stress situations, dendritic atrophy (Wolf 2006) and L-NAME disrupt the NMDA receptor (Boultadakis *et al.* 2010). Our result showed that under stressful conditions in rats, an injection of L-NAME into the NAc diminished short-term memory but not learning. Our results may suggest a synergistic impact of NMDA receptors and glucocorticoids in modulating stress-induced effects of by L-NAME and L-Arg (Gawali *et al.* 2017).

Conclusion

In conclusion, this study demonstrated that changing the nitric oxide system via nucleus accumbens function adjustment likely impacts its interactions with stress-related areas and their outputs. The stress we utilized in this study may be classified as eustress because it reduced errors during the learning and long-term memory phases. It was also shown that L-Arg in low doses enhanced the effect of positive stress on memory, whereas L-NAME weakened it. L-Arg improves learning in short-term and long-term memory, whereas L-NAME decreases learning memory function. According to the dose-dependent effects of NO modulators, future studies should use L-Arg doses of less than 1 μg/rat to improve learning and memory. Our findings revealed that, compared to other indices of the Barnes maze test, the evaluation of movement strategy more clearly showed the influence of stress and NO changes on spatial learning and memory.

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