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School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Effect of acute and chronic stress on memory impairment and hippocampal oxidative stress following global cerebral ischemia in adult male rats

Nafiseh Forghani[1], Sara Hosseinian[2,3], Zahra Akhoond-Ali[4,5], Arman Abroumand Gholami[5,6], Reza Assaran-Darban[1,*], and Farzaneh Vafaee[4,5,*]

[1]Department of Biology, Faculty of Sciences, Mashhad Branch, Islamic Azad University, Mashhad, Iran.
[2]Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[3]Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
[4]Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
[5]Department of Neuroscience, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[6]Department of Cellular Biology and Anatomical Sciences, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Abstract

Background and purpose: Stress, especially immobility stress, is quite common and one of the most important and influential risk factors in neurological disorders. This study aimed to investigate the effect of acute and chronic immobility stress on the level of cortical and hippocampal oxidative stress indicators and memory impairment following global cerebral ischemia.
Experimental approach: In this study, 48 male Wistar rats were randomly divided into 6 groups: 1, sham (S); 2, sham-acute stress (SSA); 3, sham-chronic stress (SSC); 4, ischemia (IS); 5, ischemia-acute stress (ISA); 6, ischemia-chronic stress (ISC). The Morris water maze (MWM) test was performed 14 days after surgery, and cortisol levels and oxidative stress factors such as malondialdehyde MDA and total thiol were measured.
Findings/Results: In the MWM test, the time to find the platform (latency time) in the ISC and IS groups significantly increased compared to the S group. The time spent in the target quarter in these two groups was significantly reduced compared to the S group on the day of the probe. The results showed a significant increase in cortisol levels and malondialdehyde concentration in the ISA, ISC, and IS groups compared to the S group, but there was no significant difference in total thiol concentration. No significant difference was observed in the level of oxidative stress factors in the cortex.
Conclusion and implication: Chronic immobility stress could reduce antioxidant factors in the hippocampus and exacerbate memory impairment caused by global ischemia.

Keywords: Acute and chronic stress; Global ischemia; Learning and memory; Oxidative Stress; Time latency

INTRODUCTION

Anxiety and stress cause several serious psychosocial and cognitive diseases through the release of corticosteroid hormones from the adrenal gland (1). Stress affects memory neural networks and disrupts memory consolidation. However, the effects of stressful experiences on cognitive functions, including memory, are not uniform (2). Studies have shown that moderate stress can facilitate information storage, while memory is greatly reduced following severe chronic stress (3). In addition, chronic stress accelerates the aging process and is an important risk factor in cerebral dementia (4).

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*Corresponding authors:
F. Vafaee, Tel: +98-5138002474, Fax: +98-5138002477
Email: Vafaeebf@mums.ac.ir, Farzanehvafaee@yahoo.com
R. Assaran-Darban, Tel & Fax: +98-5138435000
Email: Mrassaran78@gmail.com

The effects of stress on spatial memory have been observed in Morris water maze (MVM) experiments. Under stress, rats showed a significant increase in the time to find the platform (latency time) in the MVM test compared to the sham group (5). Moreover, chronic stress also leads to morphological and biochemical changes in the brain and reduced nerve flexibility and density of dendritic spines, especially in the hippocampus (6). It also suppresses neurogenesis in dentate gyrus granular neurons (7). Stress, especially when it is long-term and chronic, disrupts the balance between oxidative and antioxidant factors in the brain, which is involved in the pathogenesis of many cognitive disorders (8,9). Long-term exposure to stress increases inflammation and oxidant factors which reduce the volume of the hippocampus and cause dementia (10).

The hippocampus is the organ that contributes to the formation of explicit memory and is very vulnerable to the lack of oxygen and glucose; thus, when the hippocampal blood flow decreases, *e.g*. in global ischemia, it can lead to various types of memory disorders (11,12). After cerebral ischemia, oxygen radicals initiate cell death signaling pathways. In addition, damage to unsaturated fatty acids in the membrane due to increased lipid peroxidation and oxygen free radicals in cerebral ischemia causes hippocampal neuronal death (13,14). The hippocampus is very sensitive to oxidative stress and has a limited capacity for antioxidants. Increased oxidative stress in the hippocampus rapidly causes neuronal dysfunction and eventually apoptosis (15,16). The genetic manipulation of intrinsic antioxidants has provided invaluable insights into the role of oxygen radicals in ischemic brain injury (11,17,18). However, it seems that some risk factors such as anxiety and stress reduce the number of natural antioxidants in the body and play a role in disease exacerbation. Although studies show an association between oxidative stress and cognitive disorders, the underlying relationship between oxidative stress and neurological and cognitive diseases is not yet fully understood. Understanding the relationship between stress, oxidative stress, and neurological diseases, including brain injuries and strokes, a topic that has not been studied so far, is imperative. Therefore, this study aimed to investigate the effect of acute and chronic stress as one of the main risk factors for stroke in oxidative stress and spatial memory after the induction of global ischemia.

MATERIALS AND METHODS

Animals

The study was performed on 48 male Wistar rats weighing 240-280 g. The animals were kept in standard conditions of water, food, and light (12/12-h light/dark cycle) at an almost constant temperature (22 ± 2 ℃). The animals were randomly divided into 6 groups of 8 each as follows: (1) sham (S), they underwent surgery but global ischemia was not induced; (2) sham-acute stress (SSA), they were under acute stress the day before the surgery; (3) sham-chronic stress (SSC), they underwent stress (2 h per day) for 10 consecutive days before the surgery; (4) ischemia (IS), common carotid arteries were blocked for 30 min to induce global ischemia; (5) ischemia-acute stress (ISA), they were under acute stress the day before the induction of global ischemia; (6) ischemia-chronic stress (ISC), they were under stress (2 h per day) for 10 consecutive days before the induction of global ischemia.

Induction of acute and chronic immobility stress

In this method, the animals must remain immobile inside the animal restraint apparatus for a certain period. For this purpose, we placed the animals inside the device at specific times such that their noses protruded from the end of the device and they were allowed to breathe without restriction. The rear end of the device was closed with guillotine doors based on the size of the animal so that the animal remained immobile inside the device. To induce chronic stress, the animals were placed in the restraint apparatus for 10 consecutive days (2 h per day). To induce acute stress, the animals were placed in the device for 2 h only the day before the surgery (19,20).

Induction of global ischemia

Global ischemia was induced using a two-vessel occlusion model. After 12 h of fasting, the rats were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine. The two

common carotid arteries were occluded using small arterial clips for 30 min after vagal nerve separation. After 30 min, the arterial clips were removed, blood flow returned to normal, and the skin of the neck area was sutured. After recovery, the animals were returned to the cage (21).

Evaluation of neurological severity score

The animals' neurological severity score was assessed as follows: no neurological disorder, 0; hump state, 1; ptosis, 2; bypass behavior, 3; rear limbs, 4; epileptic seizures, 5. High scores indicate more serious injury. Neurological severity scores were measured on postoperative days 1, 3, 7, 14, and 21 (22).

Locomotor activity assessment with wire-hanging test

This test included a wire with a diameter of 2 mm and a length of 60 cm, which is installed at a height of 50 cm. All the groups were trained for three days before the surgery. On postoperative days 1, 3, 7, 14, and 21, locomotor activity evaluation was performed based on how long the rat remained on the wire. The test took 60 s (23).

Evaluation of spatial memory with Morris water maze

Morris water maze (MWM) was employed to assess spatial memory. Each rat was tested four times a day for 5 days. On each day of the experiment, the starting point was from one of four directions: east, west, north, or south, while the position of the platform and its coordinates were constant. On the days of the experiment, each rat was given 60 s to search for the platform; after the platform was found, the experiment would end, but if the rat could not find the platform, it would be led to the platform by the experimenter and remain on the platform for 30 s to remember its spatial position. On the day of the probe, the platform was removed and the distance traveled by the rat was recorded from all directions for 60 s. The animal's movements and trajectories were recorded by the camera. The latency to find the platform and the distance traveled to the platform were considered a measurement of spatial memory (24).

Cortisol assessment

At the end of the behavioral tests, the animals underwent deep anesthesia with ketamine and xylazine (100 and 10 mg/kg, respectively). After the skin of the neck was opened, blood samples were collected from the jugular vein to check plasma cortisol levels. The blood was then centrifuged at 1300 *g* for 5 min and the plasma was kept at -20 °C (25). Blood cortisol concentration (µg/dL) was measured in a clinical laboratory using a medical diagnostic kit (Cortisol ELISA Kit 96t Cat. No. 2924-96; Ideal Company, Iran).

Biochemical tests

After blood serum collection, the brain was removed and the hippocampus was isolated for oxidative stress measurement tests.

Malondialdehyde assessment

To measure the amount of malondialdehyde (MDA), the brain tissue was homogenized with 1.5% potassium chloride (KCl) solution, then, 1 mL of the homogenized brain tissue solution containing 2 mL of trichloroacetic acid / thiobarbituric acid / hydrochloric acid was prepared and boiled at 100 °C for 45 min until a purple mixture was formed. After being cooled down, it was centrifuged for 10 min and the absorbance was measured at 535 nm. The MDA concentration was calculated as follows:

$$\text{Concentration (nmol/g tissue)} = \text{Absorbance} / 1.56 \times 10^5 \quad (1)$$

Total thiol measurement

5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) reacts with thiol groups to form a yellow complex. The procedure is summarized as follows: 1 mL of tris-EDTA buffer (pH 8.6) was added to 50 µL of homogenized brain tissue, and the sample absorbance was read at 412 nm (A1). Then, 20 µL of DTNB reagent was added to the mixture and the sample was re-read after 15 min (A2). The adsorption rate of the DTNB reagent was referred to as blank (B). The concentration of thiol was calculated as follows:

$$\text{Total thiol concentration (nanomol/g tissue)} = (A2\text{-}A1\text{-}B) \times 1.07 / (0.05 \times 13.6) \quad (2)$$

Statistical analysis

Following the assessment of normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests, one-way ANOVA followed by the LSD post hoc test and two-way ANOVA followed by Tukey's multiple comparisons test were conducted using GraphPad Prism version 9.0. For the MDA and total thiol tests, where the data did not follow a normal distribution, the Kruskal-Wallis test followed by Dunn's multiple comparisons was employed. The results are expressed as mean ± SEM, and the *P*-values < 0.05 were considered statistically significant.

RESULTS

Effect of global ischemia acute and chronic stress on neurological deficit score and locomotor activity

To evaluate motor activity in the animals on postoperative days 1, 3, 7, 14, and 21, the neurological deficit score was measured and the animal's performance in the wire hanging test was examined. The results showed that all the animals were neurologically healthy and received a score of 18 and completely performed the wire hanging test (data not shown).

Evaluation of body weight changes following global ischemia and exposure to acute and chronic stress

To assess the effects of acute and chronic stress on body weight, animals in the sham and global ischemia groups received acute stress for one day and chronic stress for 10 days. Body weight in these animals was measured on the day before the surgery and on days 1, 3, 7, and 14 after the surgery. Based on Fig. 1, stress and surgery did not significantly change the weight of the animals. Data analysis with two-way ANOVA revealed the significant main effect of time (df = 5, F (5, 195) = 7.61, $P < 0.001$) and intervention (df = 4, F (4, 195) = 32.97, $P < 0.001$) on body weight, but no significant effect of time × intervention interaction (df = 20, F (20, 195) = 0.43, $P = 0.98$) was found.

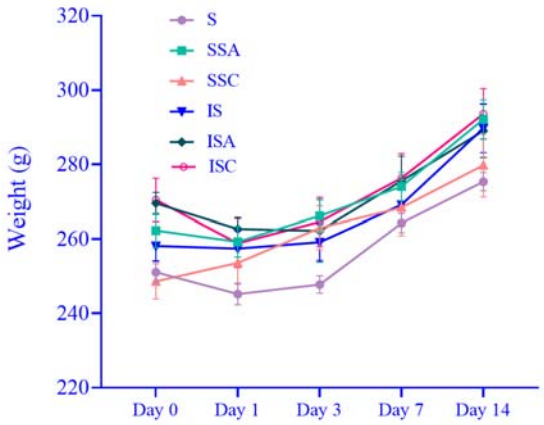

Fig. 1. Body weight changes of rats before surgery (day 0) and on days 1, 3, 7, and 14 after surgery. Data are shown as mean ± SEM, n = 6-9 in each group). There were no significant differences between the groups. S, Sham; SSA, sham-acute stress; SSC, sham-chronic stress; IS, ischemia; ISA, ischemia-acute stress; ISC, ischemia-chronic stress.

Effect of global ischemia and acute and chronic stress on latency time and distance in the MWM test

In the MWM test, the animal has to create a "spatial orientation map" in the brain by using the visual stimuli placed around the device and find a hidden platform to escape by swimming in the pool. Therefore, to assess the animals' spatial memory, the amount of time taken to reach the platform to escape from water (time latency) and the distance traveled to the platform are evaluated. The animals in the sham group managed to find the location of the platform after one day of training.

The time spent to reach the platform was significantly different between the IS and ISA groups, the ISA and SSC groups, and the ISC and SSC groups on the 1st day of training. On the 2nd day, the sham group showed a significant reduction in the time to find the platform in contrast to the performance exhibited by the IS, ISA, and ISC groups. A significant difference was also observed between SSC and ISC. On the 3rd day, the IS and ISC groups markedly spent more time in the MWM test compared to the sham group. Moreover, the SSA group had a significant decrease in the time spent compared to the ISC group. On the 4th and 5th days, the time spent to reach the platform was not significantly different amongst the groups (Fig. 2A).

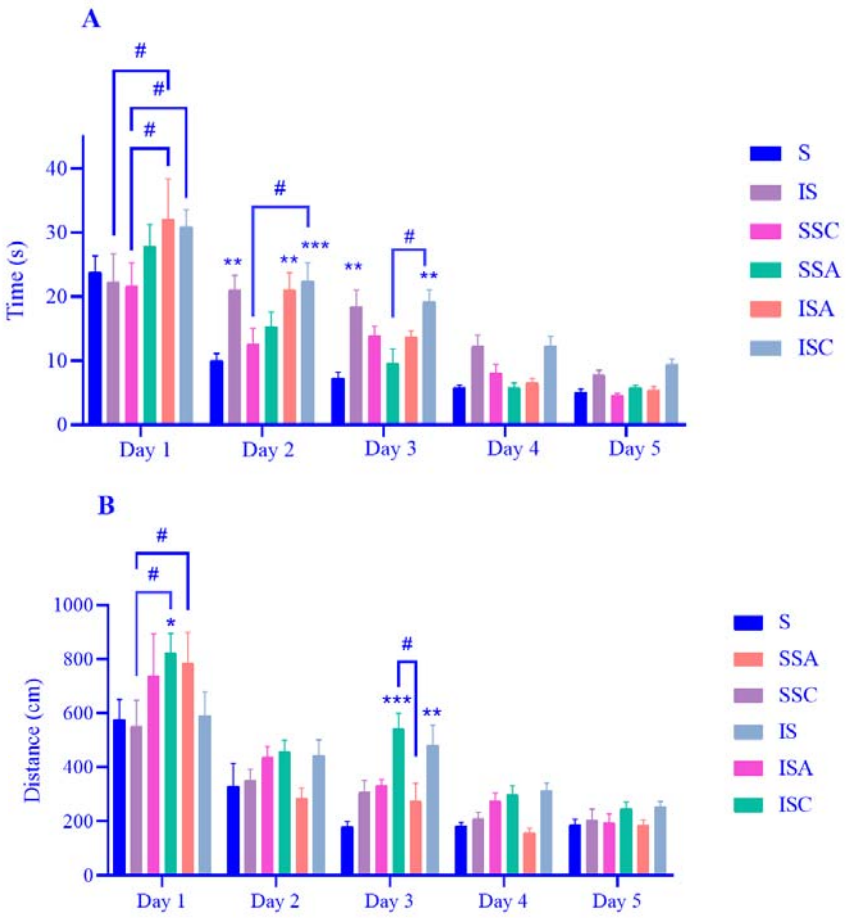

Fig. 2. Effect of global ischemia and acute and chronic stress on (A) time latency and (B) traveled distance to reach the hidden platform in the MWM test. Data are shown as mean ± SEM, n = 6-9 in each group). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significant differences compared to the sham group, $^{\#}P < 0.05$ demonstrates significant differences between the defined groups. S, Sham; SSA, sham-acute stress; SSC, sham-chronic stress; IS, ischemia; ISA, ischemia-acute stress; ISC, ischemia-chronic stress.

Figure 2B displays the distance traveled by the groups. On the 1st day, the distance traveled by the ISC group significantly increased compared to the sham group. Furthermore, the SSC group traveled a considerably shorter distance than the ISC and SSA groups. On the 3rd day of training, there was a significant increase in distance traveled by the ISC and IS groups in comparison with the sham group. On this day, there was a significant reduction in distance traveled by the SSA group versus the ISC group. However, on the 2nd, 4th, and 5th days of training, there was no significant difference between the study groups.

Data analysis with two-way ANOVA revealed the significant main effect of time (df = 5, F (5,195) = 59.28, $P < 0.001$) and intervention (df = 4, F (4,195) = 6.8, $P < 0.001$) on the distance traveled, but no significant interaction effects for time × intervention (df = 20, F (20,195) = 1.45, $P = 0.102$). There was a significant main effect for time (df = 5, F (5,195) = 10.39, $P < 0.001$), intervention (df = 4, F (4,195) = 77.64, $P < 0.001$), and time × intervention interaction (df = 20, F (20,195) = 1.64, $P < 0.05$) on latency time.

Time and the distance traveled significantly decreased in the IS and ISC groups compared to the sham group in the probe test (Fig. 3A and B). The parameter time also decreased significantly in the IS and ISC groups compared to the SSA group (Fig. 3A). There were significant main effects of the intervention on time spent (df = 5, F (5,36) = 5.51, $P < 0.001$) and distance traveled (df = 5, F (5,36) = 4.16, $P < 0.01$).

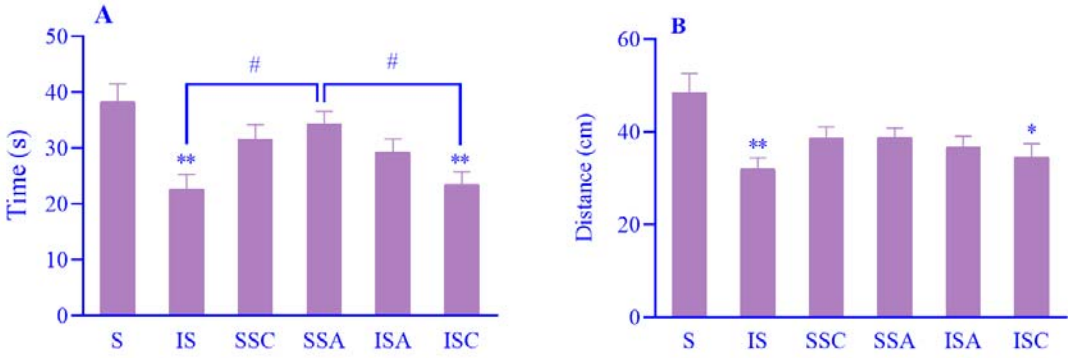

Fig. 3. Effect of global ischemia and acute and chronic stress on the (A) time and (B) traveled distance in the target quadrant in the probe day in the Morris water maze test. Data are shown as mean ± SEM, n = 6-8 in each group. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences compared to the sham group, $^{\#}P < 0.05$ demonstrates significant differences between the defined groups. S, Sham; SSA, sham-acute stress; SSC, sham-chronic stress; IS, ischemia; ISA, ischemia-acute stress; ISC, ischemia-chronic stress.

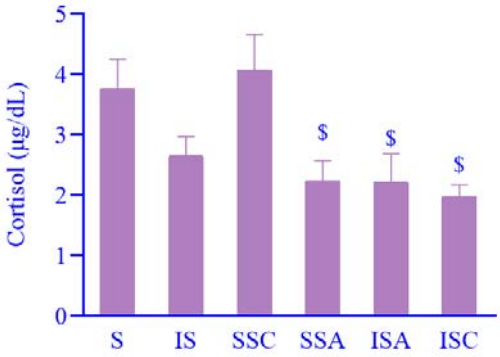

Fig. 4. Plasma concentrations of cortisol. Data are shown as mean ± SEM, n = 6-8 in each group. $^{\$}P < 0.05$ indicates significant differences compared to the SSC group. S, Sham; SSA, sham-acute stress; SSC, sham-chronic stress; IS, ischemia; ISA, ischemia-acute stress; ISC, ischemia-chronic stress.

Effects of global ischemia and acute and chronic stress on serum cortisol

To evaluate the effect of stress, cortisol as a key stress hormone was measured at the end of the experiment. The intervention had a significant effect on cortisol levels (df = 5, F (5,30) = 4.32, $P < 0.01$). Figure 4 shows that cortisol levels were significantly lower in the SSA, ISA, and ISC groups than in the SSC group.

Evaluation of hippocampal and cortical MDA and total thiol following global ischemia and exposure to acute and chronic stress

The hippocampal MDA concentration significantly increased in the IS, ISA, and ISC groups compared with the sham group. This parameter decreased in the SSA and SSC groups compared with the IS group. There was also a significant difference between the ISC and ISA groups with the SSA group. The hippocampal MDA concentration of the ISC group was significantly higher than that of the SSC group (Fig. 5A). The intervention had a significant effect on hippocampal MDA concentration (df = 5, F (5,18.76) = 17.04, $P < 0.001$). The cortical MDA concentration significantly decreased in the SSA group compared with the IS group, but there were no significant differences among the other groups (Fig. 5B). Besides, there were no significant differences between the groups in terms of hippocampal and cortical total thiol (Figs. 6A and B).

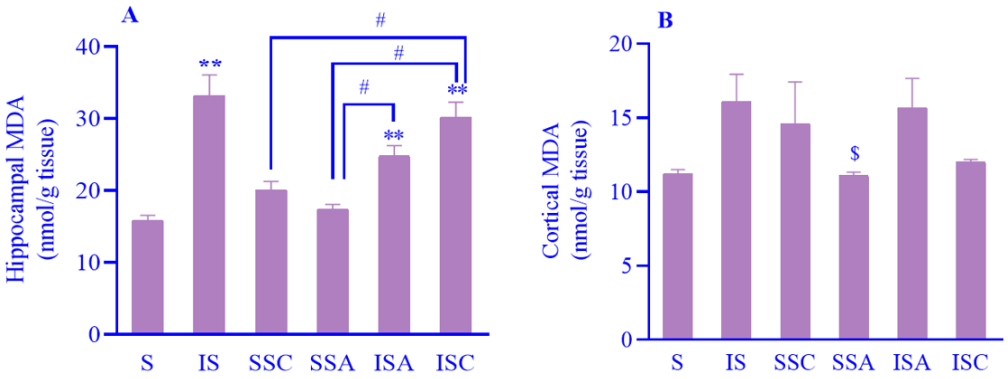

Fig. 5. Comparison of (A) the hippocampal MDA concentrations and (B) cortical MDA concentrations between groups. Data are shown as mean ± SEM, n = 6-8 in each group. $^{**}P < 0.01$ indicates significant differences compared to the sham group, $^{\#}P < 0.05$ demonstrates significant differences between the defined groups; $^{\$}P < 0.05$ indicates significant differences compared to the IS group. S, Sham; SSA, sham-acute stress; SSC, sham-chronic stress; IS, ischemia; ISA, ischemia-acute stress; ISC, ischemia-chronic stress; MDA, malondialdehyde.

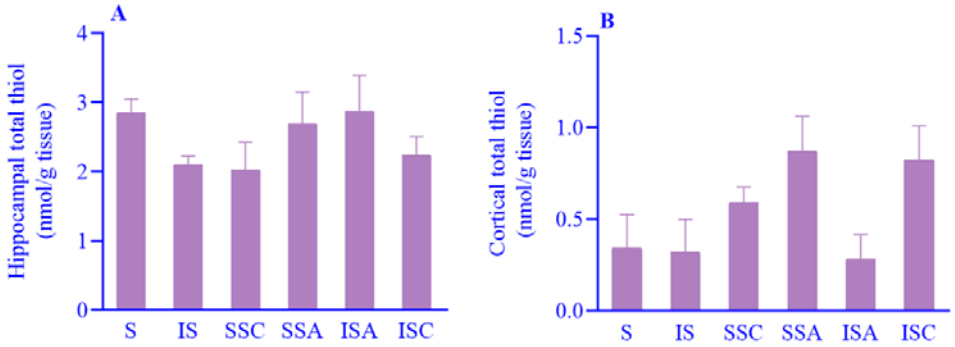

Fig. 6. Comparison of (A) the hippocampal thiol contents and (B) cortical thiol contents between the groups. Data are shown as mean ± SEM, n = 6-8 in each group. There were no significant differences between the groups in total thiol of the hippocampus and cortex. S, Sham; SSA, sham-acute stress; SSC, sham-chronic stress; IS, ischemia; ISA, ischemia-acute stress; ISC, ischemia-chronic stress.

DISCUSSION

Cerebral blood flow is reduced in global ischemia, leading to nerve death in the CA1 area of the hippocampus (21). Our results showed that the animals developed memory impairment following global ischemia induction surgery, but their motor function remained intact. The neurological deficit score, which includes sensory and motor parameters, also received the highest score in all the animals, similar to the sham group. According to our results, the induction of global ischemia is a specific model for memory impairment. In addition, acute and chronic stress did not affect the animals' motor function and neurological deficit score, and no body weight changes were observed between the groups.

The present study and similar studies showed that exposure to stress, especially chronic stress, can increase cortisol levels. A rise in stress hormones, including cortisol, can affect cognitive function by impacting corticosteroid receptors in the brain and reducing the volume of the hippocampus, amygdala, and frontal cortex due to the neurotoxic effect of the long-term release of these hormones (26,27). According to our results, stress in both chronic and acute states increased the oxidant and decreased the antioxidant factors in the hippocampus, ultimately leading to cognitive disorders. Our findings are consistent with other results in this field (9,28-31).

On the other hand, studies have shown that global ischemia for 5 min can initiate nerve

death processes in the CA1 region of the hippocampus which, if continued for up to 20 min, causes irreversible damage (32). The hippocampus suffers the most damage following the reduction of blood supply to the whole brain, probably because of its low level of antioxidant capacity and proteasome activity (9,33,34). In addition, the animals that underwent global ischemia induction could not recognize the new object in the novel object recognition test. These findings suggest that global ischemic injury is not limited to the hippocampus but also affects other areas such as the entorhinal cortex, thalamus, and cingulate cortex (35). The findings of the present study revealed that global ischemia increased the time to find the platform in the MVM test, while the combination of global ischemia with the induction of stress, especially chronic stress, significantly raised this parameter. This memory impairment is probably due to the significant elevation of oxygen free radicals (oxidants) during cerebral ischemia, which plays an important role in post-stroke brain damage. In addition to cell damage, oxidants disrupt mitochondrial function and play a role in cell death signaling pathways. Following increased oxidative stress and inflammation, the apoptotic pathway also begins in vulnerable neurons in the hippocampus 2-3 days after global ischemia (9,36,37). As our results showed, the oxidative stress factors in the hippocampus of groups that underwent global ischemia induction and were also under stress significantly increased, which confirms the findings of similar abovementioned studies.

Stress, especially if it is chronic, can lead to biochemical and structural alterations such as the loss of dendritic spines in the hippocampus (38). Moreover, the effects of acute and chronic stress on the amygdala have been reported to differ from those of the hippocampus. Acute stress increases the density of dendritic spines in the basolateral nucleus of the amygdala, and chronic stress decreases the amygdala dendritic spines (38,39).

Acute and chronic stress reduces dendritic spines in parts of the prefrontal cortex but increases the dendritic spines in neurons of the orbitofrontal cortex, possibly associated with higher alertness (40). Recently, research has shown that oxidative stress plays a role in depression and anxiety. The literature reports a link between oxidative stress and psychological disorders (41). Our results demonstrated that chronic stress as a risk factor could have a significant effect on increasing the latency time in the MWM, which is probably due to increased oxidative factors and is confirmed by other studies. However, acute stress strengthened spatial memory.

Lucca *et al*. reported for the first time that mild and chronic stress increases oxidative damage and changes in superoxide dismutase activity and ultimately exacerbates stress-related disorders such as depression (15). Stress leads to anxiety-like behaviors and raises oxidative stress in the brain, as well as memory impairment in rats (42). Both chronic and acute stress models have been reported to significantly impair memory, which is associated with elevated corticosteroid levels (43). In addition, cortisol and adrenal corticosteroids are essential for the body's health and their changes following stress or various injuries can lead to inflammation and cognitive impairment. Increased plasma levels of cortisol affect the pituitary gland and, therefore, the brain controls its secretion using negative feedback (43). Increasing stress hormones such as cortisol at the beginning and end of stress is critical for protecting the body and preparing it for emergencies. However, the continuous increase of cortisol following chronic stress inhibits the negative feedback function of the hypothalamic-pituitary-adrenal gland and thus raises inflammation and oxidative stress in the body and brain (25).

Therefore, based on these results, stress alone can cause memory impairment; nevertheless, if it is followed by ischemia, due to the disruption of the biochemical balance of the brain caused by the increase of oxidant factors, the destructive effect of cerebral ischemia is multiplied playing an important role as a main risk factor. On the other hand, Kirby *et al*. stated that acute stress can increase neurogenesis in the hippocampus of newborn mice (44). It seems that acute stress leads to biochemical changes, but its main effect is in increasing alertness.

CONCLUSION

The results revealed that chronic stress as a risk factor can impair spatial memory and increase oxidants in hippocampal damage due to the induction of global ischemia, while acute stress does not cause these effects.

Acknowledgments

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Conflicts of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

F. Vafaee conceptualized the study; F. Vafaee and R. Assaran-Darban designed the study; N. Forghani conducted the experiments; F. Vafaee, S. Hosseinian, and A. Abroumand Gholami carried out the statistical analysis; N. Forghani, F. Vafaee, and Z. Akhoond-Ali wrote the manuscript; S. Hosseinian, Z. Akhoond-Ali, R. Assaran-Darban and A. Abroumand Gholami manuscript reviewed and edited the manuscript; F. Vafaee supervised the research. The finalized version of the article was thoroughly read and approved by all authors.

REFERENCE

1. Salim S. Oxidative stress and psychological disorders. Curr Neuropharmacol. 2014;12(2):140-147. DOI: 10.2174/1570159X11666131120230309.
2. De Kloet ER, Oitzl MS, Joëls M. Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci. 1999;22(10):422-426. DOI: 10.1016/S0166-2236(99)01438-1.
3. Vogel S, Schwabe L. Learning and memory under stress: implications for the classroom. NPJ Sci Learn. 2016;1:16011,1-10. DOI: 10.1038/NPJSCILEARN.2016.11.
4. Yegorov YE, Poznyak AV., Nikiforov NG, Sobenin IA, Orekhov AN. The link between chronic stress and accelerated aging. Biomedicines. 2020;8(7):198, 1-14. DOI: 10.3390/BIOMEDICINES8070198.
5. Harrison FE, Hosseini AH, McDonald MP. Endogenous anxiety and stress responses in water maze and Barnes maze spatial memory tasks. Behav Brain Res. 2009;198(1):247-251. DOI: 10.1016/J.BBR.2008.10.015.
6. Mariotti A. The effects of chronic stress on health: new insights into the molecular mechanisms of brain-body communication. Futur Sci OA. 2015;1(3):1-6. DOI: 10.4155/fso.15.21.
7. McEwen BS. Stress and hippocampal plasticity. Annu Rev Neurosci. 1999;22:105-122. DOI: 10.1146/annurev.neuro.22.1.105.
8. Schiavone S, Jaquet V, Trabace L, Krause KH. Severe life stress and oxidative stress in the brain: from animal models to human pathology. Antioxidants Redox Signal. 2013;18:1475-1490. DOI: 10.1089/ars.2012.4720.
9. Alipourfard F, Shajjee H, Nazari-Serenjeh F, Hojati V, Alirezaie M. Betaine attenuates oxidative stress and cognitive dysfunction in an amyloid β-induced rat model of Alzheimer's disease. Res Pharm Sci. 2023;18:270-278. DOI: 10.4103/1735-5362.371583.
10. Juszczyk G, Mikulska J, Kasperek K, Pietrzak D, Mrozek W, Herbet M. Chronic stress and oxidative stress as common factors of the pathogenesis of depression and Alzheimer's disease: the role of antioxidants in prevention and treatment. Antioxidants. 2021;10(9):1439,1-31. DOI: 10.3390/ANTIOX10091439.
11. Choi IY, Hwang L, Jin JJ, Ko IG, Kim SE, Shin MS, *et al*. Dexmedetomidine alleviates cerebral ischemia-induced short-term memory impairment by inhibiting the expression of apoptosis-related molecules in the hippocampus of gerbils. Exp Ther Med. 2017;13:107-116. DOI: 10.3892/etm.2016.3956.
12. Mohammadi-Farani A, Haghighi A, Ghazvineh M. Effects of long-term administration of testosterone and estradiol on spatial memory in rats. Res Pharm Sci. 2015;10:407-418. PMID: 26752989.
13. Quartu M, Serra MP, Boi M, Pillolla G, Melis T, Poddighe L, *et al*. Effect of acute administration of *Pistacia lentiscus L*. essential oil on rat cerebral cortex following transient bilateral common carotid artery occlusion. Lipids Health Dis. 2012;11:8,1-10. DOI: 10.1186/1476-511X-11-8.
14. Zamir-Nasta T, Abbasi A, Kakebaraie S, Ahmadi A, Pazhouhi M, Jalili C. Aflatoxin G1 exposure altered the expression of BDNF and GFAP, histopathological of brain tissue, and oxidative stress factors in male rats. Res Pharm Sci. 2022;17(6):677-685. DOI: 10.4103/1735-5362.359434.
15. Lucca G, Comim CM, Valvassori SS, Réus GZ, Vuolo F, Petronilho F, *et al*. Effects of chronic mild stress on the oxidative parameters in the rat brain. Neurochem Int. 2009;54(5-6):358-362. DOI: 10.1016/j.neuint.2009.01.001.
16. Shaki F, Shayeste Y, Karami M, Akbari E, Rezaei M, Ataee R. The effect of epicatechin on oxidative stress and mitochondrial damage induced by homocysteine using isolated rat hippocampus mitochondria. Res Pharm Sci. 2017;12(2):119-127. DOI: 10.4103/1735-5362.202450.

17. Mansoorali KP, Prakash T, Kotresha D, Prabhu K, Rama Rao N. Cerebroprotective effect of *Eclipta alba* against global model of cerebral ischemia-induced oxidative stress in rats. Phytomedicine. 2012;19(12):1108-1116. DOI: 10.1016/j.phymed.2012.07.004.
18. Saito A, Maier CM, Narasimhan P, Nishi T, Yun SS, Yu F, *et al*. Oxidative stress and neuronal death/survival signaling in cerebral ischemia. Mol Neurobiol. 2005;31(1-3):105-116. DOI: 10.1385/MN:31:1-3:105.
19. Vázquez-León P, Martínez-Mota L, Quevedo-Corona L, Miranda-Páez A. Isolation stress and chronic mild stress-induced immobility in the defensive burying behavior and a transient increased ethanol intake in Wistar rats. Alcohol. 2017;63:43-51. DOI: 10.1016/J.ALCOHOL.2017.03.005.
20. Xing B, Liu P, Jiang WH, Liu F, Zhang H, Cao GF, *et al*. Effects of immobilization stress on emotional behaviors in dopamine D3 receptor knockout mice. Behav Brain Res. 2013;243:261-266. DOI: 10.1016/J.BBR.2013.01.019.
21. Kamali Dolatabadi L, Emamghoreishi M, Namavar MR, Sarkala HB. Curcumin Effects on memory impairment and restoration of irregular neuronal distribution in the hippocampal CA1 region after global cerebral ischemia in male rats. Basic Clin Neurosci. 2019;10(5):527-540. DOI: 10.32598/BCN.9.10.365.
22. Vafaee Bagheri F, Emamghoreishi M, Shahpari M. Effect of recombinant insulin-like growth factor-2 injected into the hippocampus on memory impairment following hippocampal intracerebral hemorrhage in rats. Galen Med J. 2018;7:1353,1-10. DOI: 10.22086/gmj.v0i0.1353.
23. Vafaee F, Hosseini M, Sadeghinia HR, Hadjzadeh MAR, Soukhtanloo M, Rahimi M. The effects of soy extract on spatial learning and memory damage induced by global ischemia in ovariectomised rats. Malays J Med Sci. 2014;21(3):19-30. PMID: 25246832.
24. Vélez-Marín M, Salazar AH, Uribe-Velásquez LF. Plasma cortisol activity in rats under conditions of chronic stress supplemented with resveratrol. Colomb Méd C. 2012;43(3):221-225. DOI: 10.25100/cm.v43i3.842.
25. Vyas S, Rodrigues AJ, Silva JM, Tronche F, Almeida OFX, Sousa N, *et al*. Chronic stress and glucocorticoids: From neuronal plasticity to neurodegeneration. Neural Plast. 2016;2016:1-15. DOI: 10.1155/2016/6391686.
26. Lupien SJ, Juster RP, Raymond C, Marin MF. The effects of chronic stress on the human brain: From neurotoxicity, to vulnerability, to opportunity. Front Neuroendocrinol. 2018;49:91-105. DOI: 10.1016/j.yfrne.2018.02.001.
27. Kim EJ, Pellman B, Kim JJ. Stress effects on the hippocampus: a critical review. Learn Mem. 2015;22(9):411-416. DOI: 10.1101/LM.037291.114.
28. Gueroui M, Kechrid Z. Evaluation of some biochemical parameters and brain oxidative stress in experimental rats exposed chronically to silver nitrate and the protective role of vitamin E and selenium. Toxicol Res. 2016;32(4):301-309. DOI: 10.5487/TR.2016.32.4.301.
29. Nabaee E, Kesmati M, Shahriari A, Khajehpour L, Torabi M. Cognitive and hippocampus biochemical changes following sleep deprivation in the adult male rat. Biomed Pharmacother. 2018;104:69-76. DOI: 10.1016/J.BIOPHA.2018.04.197.
30. Azadbakht AA, Radahmadi M, Javanmard SH, Reisi P. The effects of doxepin on stress-induced learning, memory impairments, and TNF-α level in the rat hippocampus. Res Pharm Sci. 2015;10(5):460-465. PMID: 26752995.
31. Baron JC, Yamauchi H, Fujioka M, Endres M. Selective neuronal loss in ischemic stroke and cerebrovascular disease. J Cereb Blood Flow Metab. 2014;34(1):2-18. DOI: 10.1038/jcbfm.2013.188.
32. Ravindran S, Kurian GA. Eventual analysis of global cerebral ischemia-reperfusion injury in rat brain: a paradigm of a shift in stress and its influence on cognitive functions. Cell Stress Chaperones. 2019;24(3):581-594. DOI: 10.1007/s12192-019-00990-4.
33. Nili-Ahmadabadi A, Ali-Heidar F, Ranjbar A, Mousavi L, Ahmadimoghaddam D, Larki-Harchegani A, *et al*. Protective effect of amlodipine on diazinon-induced changes on oxidative/antioxidant balance in rat hippocampus. Res Pharm Sci. 2018;13(4):368-376. DOI: 10.4103/1735-5362.235164.
34. Bachevalier J, Meunier M. Cerebral ischemia: Are the memory deficits associated with hippocampal cell loss? Hippocampus. 1996;6(5):553-560. DOI: 10.1002/(SICI)1098-1063(1996)6:5<553:AID-HIPO8>3.0.CO;2-J.
35. Chen H, Yoshioka H, Kim GS, Jung JE, Okami N, Sakata H, *et al*. Oxidative stress in ischemic brain damage: mechanisms of cell death and potential molecular targets for neuroprotection. Antioxidants Redox Signal. 2011;14(8):1505-1517. DOI: 10.1089/ars.2010.3576.
36. Friberg H, Wieloch T, Castilho RF. Mitochondrial oxidative stress after global brain ischemia in rats. Neurosci Lett. 2002;334(2):111-114. DOI: 10.1016/S0304-3940(02)01116-3.
37. McEwen BS. Stress-induced remodeling of hippocampal CA3 pyramidal neurons. Brain Res. 2016;1645:50-54. DOI: 10.1016/j.brainres.2015.12.043.
38. Ajaji V, Rupshi M, Rao BSS, Chattarji S, *et al*. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J Neurosci. 2002;22(15):6810-6818. DOI: 10.1523/JNEUROSCI.22-15-06810.2002.
39. Bennur S, Shankaranarayana Rao BS, Pawlak R, Strickland S, McEwen BS, Chattarji S. Stress-induced spine loss in the medial amygdala is mediated by tissue plasminogen activator. Neuroscience. 2007;144(1):8-16. DOI: 10.1016/j.neuroscience.2006.08.075.

40. Radley JJ, Sisti HM, Hao J, Rocher AB, McCall T, Hof PR, *et al*. Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. Neuroscience. 2004;125(1):1-6.
DOI: 10.1016/j.neuroscience.2004.01.006.
41. Bouayed J, Rammal H, Soulimani R. Oxidative stress and anxiety Relationship and cellular pathways. Oxid Med Cell Longev. 2009;2(2):63-67.
DOI: 10.4161/oxim.2.2.7944.
42. Patki G, Solanki N, Atrooz F, Allam F, Salim S. Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. Brain Res. 2013;1539:73-86.
DOI: 10.1016/j.brainres.2013.09.033.
43. Arlt W, Stewart PM. Adrenal corticosteroid biosynthesis, metabolism, and action. Endocrinol Metab Clin North Am. 2005;34(2):293-313.
DOI: 10.1016/J.ECL.2005.01.002.
44. Kirby ED, Muroy SE, Sun WG, Covarrubias D, Leong MJ, Barchas LA, *et al*. Acute stress enhances adult rat hippocampal neurogenesis and activation of newborn neurons via secreted astrocytic FGF2. Elife. 2013;2:1-23.
DOI: 10.7554/eLife.00362.