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ORIGINAL RESEARCH

Memory Impairment Induced by Chronic Psychosocial Stress Is Prevented by L-Carnitine

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Introduction: Psychosocial stress (STS) negatively influences memory. This might be associated to oxidative stress-induced progressive destruction of numerous brain structures and functions. L-carnitine (L-CAR) is a widely used antioxidant compound that is endogenously made in mammalian species. The current study investigated the effect of L-CAR on STS-induced memory impairment in the rat hippocampus.

Methods: The STS was induced using intruder model, where two rats were randomly switched from each one cage to another, once/day for 6 weeks. Concurrently, L-CAR (300mg/kg/day) was intraperitoneally administered for 6 weeks. After that, radial arm water maze (RAWM) was used to assess spatial learning memory in rats. Hippocampal biomarkers of oxidative stress, including thiobarbituric acid reactive substance (TBARs), oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD), and Brain-derived neurotrophic factor (BDNF) were examined.

Results: The results showed impairment of short-term memory ($P < 0.05$) during STS, whereas L-CAR treatment protected against this effect. Furthermore, while no change was observed in GSH, GSSG, GPx, catalase, and SOD, L-carnitine normalized STS-induced reduction in the hippocampal BDNF levels and increase in TBARS levels.

Discussion: Chronic psychosocial stress-induced memory impairment was prevented via L-CAR administration, which could have been achieved via normalizing changes in lipid peroxidation (TBARs) and BDNF levels in the hippocampus.

Keywords: L-carnitine, psychosocial stress, maze, hippocampus, memory, BDNF, oxidative stress

Introduction

Psychosocial stress (STS) is common in modern societies.[1] Long-lasting effects of stress induces changes in the hippocampal brain structure and function, which is an essential component of memory systems.[2–4] Chronic stress was also shown to negatively modulate learning and memory processes and influences important signaling pathways of learning and memory functions in the hippocampus.[4–11]

Oxidative stress induces lipid peroxidation reaction as a consequence of excess free radicals produced in the body leading to marked damage to cells and organs.[12] As shown by several previous studies, brain is mostly sensitive to free radical insults.[13–16] In fact, a number of stressors were revealed to impact lipid peroxidation activity in the brain including immobilization stress,[17] high-fat diet,[18] and sustained prolonged stress exposure.[19]

Brain-derived neurotrophic factor (BDNF), on the other hand, is a member of the neurotrophin family which is widely expressed in the central nervous system, especially

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in the hippocampus and cerebral cortex.[20] BDNF is one of the vital contributors in the progress, survival, preservation of neurons and memory formation.[20] Hippocampal BDNF levels are reduced by various stressors including chronic psychosocial stress.[21–23] This reduction can lead to loss of pyramidal neurons in the hippocampus, synaptic deficiencies, and sometimes cell death that might be related to apoptotic signaling. In that respect, deficiency of serum BDNF associated with psychiatric disorders patients was accompanied by hippocampal atrophy.[24,25]

L-Carnitine (3-hydroxy-4-N-trimethylammoniobutanoate) is derived from L-lysine that is mainly acquired from diet.[26] It is mainly synthesized in the mammalians' liver, kidney, and muscles.[27] It works as a long-chain fatty acid mediator that facilitates β-oxidation cycle.[28] Additionally, the antioxidant action of L-CAR is through superoxide radicals and hydrogen peroxide scavenging, which protects cells from lipid peroxidation.[29] L-CAR is efficient in protection against oxidative stress alterations that are related to many health condition including Alzheimer's disease,[7,30] chronic sleep deprivation,[31] cerebrovascular disease,[32] and aging.[33] In the present study, we examined the potential neuroprotective influence of L-CAR on memory tasks and antioxidative mechanisms in rats, which are exposed to chronic psychosocial stress.

Materials and Methods

Animals and Treatments

Adult male Wistar rats (180–220 g) were obtained from the animal care facility at Jordan University of Science and Technology (JUST). Animals were housed under hygienic conditions in a climate-controlled room (24 ± 2°C), in plastic cages (5/cage), with free access to water and rat chow. They were put on 12-hr light/dark cycle. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of Jordan University of Science and Technology (Approval number 16/3/3/170). The animal welfare guide used was the ARENA/OLAW IACUC Guidebook, 2nd Edition 2002 of the Office of Laboratory Animals Welfare at National Health Institute, USA. The experimental manipulation started after one week of acclimatization. Rats were distributed into four groups (12–15 rats in each group): control, psychosocial stress (STS), L-CAR treatment alone (L-CAR) and psychosocial stress with L-CAR treatment (STS+L-CAR). The L-CAR and STS+L-CAR groups were administrated L-CAR (300mg/kg/day, intraperitoneally, Sigma Chemical CO., Saint Louis, MO) which was given one injection every day for 6 weeks as previously described.[31] The STS and control groups were administrated normal saline (0.9% w/v NaCl, Sigma Chemical CO., Saint Louis, MO) intraperitoneally daily for 6 weeks. Concomitantly, the STS +L-CAR and STS groups were exposed to chronic psychosocial stress. Psychosocial stress and L-CAR administration started on the 8th day of the experiment and continued for 6 weeks, during behavioral test day until animals' killing day.[2] For every animal, we carried out the RAWM procedure on the next day following the 6 weeks of STS and/or L-CAR treatments.

Induction of Chronic Psychosocial Stress

The intruder stress model was previously detailed.[4,6,8,34] Briefly, animals were kept with the same cage mates in home cages for a minimum of week allowing establishment of social hierarchy. Afterwards, two rats from each cage were transferred once a day from one cage to another for 6 weeks. We have previously shown from our same laboratory that animals, which were subjected to this stress chronically, have developed hypertension,[35] and had elevated plasma levels of corticosterone.[36]

The Radial Arm Water Maze (RAWM)

The RAWM procedure was previously described in detail.[37–43] Briefly, the RAWM is a circular black tank, which was filled with water with six radiating swim paths that extend out from an open central area. An escape platform was located at the end of one arm (the goal arm). During the learning or acquisition phase, training of each animal consisted of 12 successive trials. Five minutes resting time was given to every animal after the first six acquisition trials. After 30 mins of the end of the 12 trials, the short-term memory test was carried out, followed by the long-term memory tests done after 5 hrs and 24 hrs of the end of the 12-trial learning phase.

Hippocampus Dissection and Biochemical Assays

Decapitation applied to the animals and the brain was straightaway dissected out. Afterwards, we placed the brain over a normal saline-impeded filter paper over a cold glass dish that was already filled with crushed ice. The hippocampus was quickly isolated and put in an Eppendorf tube that was labeled formerly. Then, the Eppendorf tubes were transferred to a container filled with liquid nitrogen, at −70°C freezer until time of tissue processing. At analysis time, we placed 200μL of homogenization

buffer over the hippocampus tissues, which were next homogenized using plastic pestles. The homogenization buffer was prepared by reconstituting two tablets of protease inhibitor (Sigma Chemical CO., Saint Louis, MO), and one tablet of phosphate-buffered saline (Sigma Chemical CO., Saint Louis, MO) in 200 mL of distilled water. To remove insoluble materials, the homogenized hippocampus tissues were centrifuged (10 min at 15,000 ×g, at 4°C). The obtained supernatant was stored for additional analysis. We estimated the total protein concentration in the obtained supernatant via a commercially available kit (Bio-Rad, Hercules, CA, USA).

To measure total glutathione, 5% 5-sulfosalicylic acid (SSA; Sigma Chemical CO., Saint Louis, MO) was used to deproteinize hippocampal tissue homogenates. Then, the homogenate was centrifuged at 10,000 xg for 10 mins at 4°C in order to remove the precipitated protein. Next, samples were examined glutathione photometrically according to kit's instructions (Glutathione assay kit, Sigma-Aldrich, MI, USA). The GSSG was calculated by adding 10 µL of 1M 2-vinylpyridine (Sigma-Aldrich, MI, USA) per 1 mL of supernatant from the sample. Afterward, the kit's procedures, as described above, were carried out to measure total glutathione. The GSH levels were calculated by subtracting GSSG value from total glutathione. The activity of Glutathione peroxidase (GPx) was measured by means of cellular activity assay kit (CGP1, Sigma-Aldrich, MI, USA). Catalase and superoxide dismutase (SOD) activities were evaluated using commercially available kits in accordance with the instructions of the kit's manufacturer (SOD: Sigma-Aldrich Corp; Catalase: Cayman Chem, Ann Arbor, MI, USA). To measure the levels of Thiobarbituric acid reactive substance (TBARs) in the homogenized hippocampus tissues, TBARS assay kit (Cayman Chem. Com. Ann Arbor, MI, USA) was used. BDNF was evaluated through using R&D assay kit (DuoSet ELISA development system. MN, USA). Plates were read at the kit's specific wavelengths by Epoch BioTek microplate reader (Highland Park, Winooski, USA).

Statistical Analysis

Statistics were completed by means of GraphPad Prism software version 6.0 (GraphPad Software, La Jolla, CA). Two-way analysis of variance (ANOVA) has been used to compare the number of errors in the RAWM procedure followed by Bonferroni post-test. The two independent variables were time (repeated measures factors) and treatment (between-subjects factor). For biochemical assays results, one-way ANOVA was used followed by Bonferroni post-test. $P < 0.05$ was considered Significant. All values were presented as mean ± SEM.

Results

The Effect of L-Carnitine and/or Psychosocial Stress on Learning and Memory

In the acquisition phase, all experimental groups did high number of errors. As learning trials continued, the number of errors was gradually reduced, with no significant difference among all experimental groups (Figure 1).

In the 30-min short-term memory test, significantly higher number of errors were committed by the STS group ($P<0.05$, Figure 2A) compared to the number of errors were made in all other groups (control, L-CAR, and STS+L-CAR). No significant difference was observed among experimental groups in the 5 and 24 hrs long-term memory tests (Figure 2B and C).

The Effects of Psychosocial Stress and/or L-CAR on Oxidative Stress Biomarkers in the Hippocampus

Neither GSH nor GSSG levels were altered in any of the experimental groups (Figure 3A and B). For anti-oxidative defense enzymes, chronic STS has not changed the levels of GPx, SOD, and catalase (Figure 4) compared to the control group. Moreover, STS+L-CAR groups showed similar activities of GPx, catalase or SOD compared to the L-CAR, and control groups. Remarkably, the psychosocial stress significantly increased the levels of TBARs compared with other groups ($P < 0.05$). L-CAR administration prevented this increase in TBARS levels as shown in Figure 5A (L-CAR/STS and L-CAR group).

Effect of STS and/or L-CAR on BDNF Levels in the Hippocampus

In the STS group, the levels of BDNF were significantly reduced compared to control, L-CAR and STS+L-CAR groups. Moreover, STS+L-CAR groups showed similar BDNF levels to those in the L-CAR, and control groups (Figure 5B).

Discussion

The present study aimed at investigating the possible preventive effects of chronic L-CAR treatment on memory impairment induced by STS. Using intruder model of STS

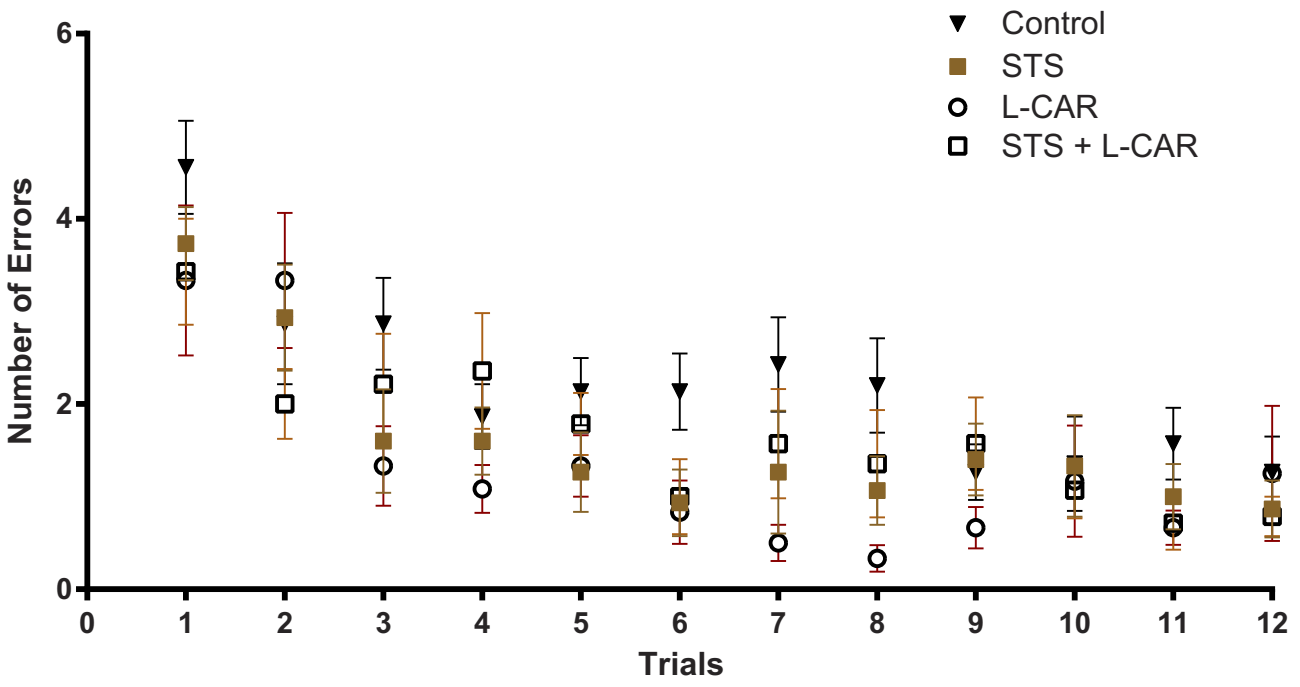

Figure 1 Comparison of rats' performance during the learning phase. The number of errors made by animals dropped with continued learning trials with no significant difference among all groups. Each point is the mean ± SEM of 12–15 rats.

and RAWM to test memory, the current data showed that STS induces impairment of short-term memory. These results confirm the findings of previous studies that STS leads to brain neuronal damage and short memory impairment.[2,4–6] In fact, animals from all groups had progressively learned with continued training. Administration of L-CAR prevented impairment of short-term memory during STS. Such preventive effect for L-CAR against impairment of memory that is revealed in the present study is in accordance with results from previous research that tested the beneficial effects of L-CAR on impairing memory in other health conditions such as Alzheimer's disease animal models,[7,30] chronic sleep deprivation,[31] cerebrovascular disease,[32] and aging.[33]

The current study showed that treatment with the neuronal antioxidant, L-CAR, protected from chronic STS-induced impairment of short-term memory through preventing change in the lipid peroxidation biomarker (TBARS) and BDNF. The L-CAR is a natural component of all mammalian cells, and its L-isoform is biologically active.[26] The present results displayed no alteration in the levels of catalase, GPX, SOD, GSH, GSSG, and GSH/GSSG ratio. Previously, it was shown that chronic social isolation in rats had not altered activities of SOD or catalase, whereas it reduced activity of GPx in the hippocampus.[44] Notably, evaluations of these biomarkers in the current study were not conducted in isolated mitochondria, which could be a reason for discrepancy of results of various studies. Yet, alternative mechanisms to antioxidant enzymatic pathways could be the ability of L-CAR to prevent for changes in lipid peroxidation and BDNF.

The STS group exhibited high level of TBARs that are byproducts of lipid peroxidation supporting data from other studies that showed elevated brain levels of TBARs in animals exposed to stress.[45] L-CAR decreased these elevations, which goes in line with previous results that showed L-CAR reduces TBARs levels in other animal model with elevated oxidative stress status such as in the brain of rats exposed to restraint stress,[46] and to the chemical toxin, arsenic,[47] and in the muscle tissues of rat exposed to intermittent hypoxia.[48] Moreover, L-carnitine supplementation was shown to reduce elevated TBARs in the serum of humans with exercise-induced muscle damage,[49] and in isolated blood platelets.[50]

The levels of BDNF, which is a neurotrophin is crucially involved in memory processes,[20] were shown to be reduced in the hippocampus during chronic STS exposure and by exposure to single prolonged stress.[22,23] Moreover, the BDNF signaling pathway in hippocampus was shown to mediate memory deficits of rats subjected to chronic unpredictable mild stress.[51] Thus, BDNF impacts learning and memory in a robust way. However, in the current study, the STS-impaired only short-term memory. The current study

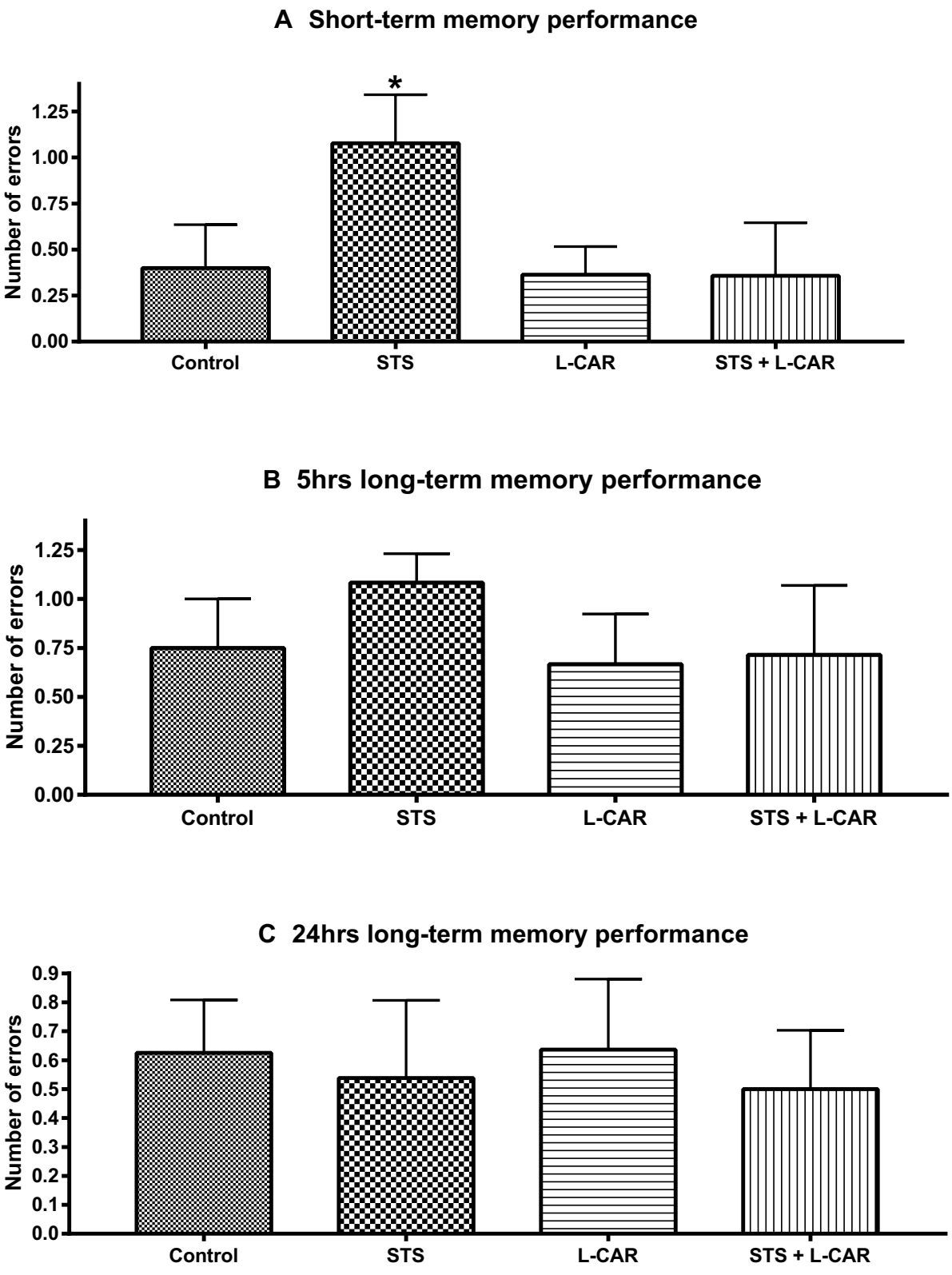

Figure 2 L-CAR prevents short-term memory impairment induced by the STS model. (**A**) Short-term memory test (30 mins) post learning phase. The STS group committed significantly higher number of errors in short-term memory test compared to other groups. On the other hand, the number of errors in the STS + L-CAR group was similar to that in the control and L-CAR groups. Long-term memory tests at (**B**) 5 hrs and (**C**) 24 hrs. All experimental groups showed no significant difference from control. Each point is the mean ± SEM of 12–15 rats. *P < 0.05 indicates significant difference from control.

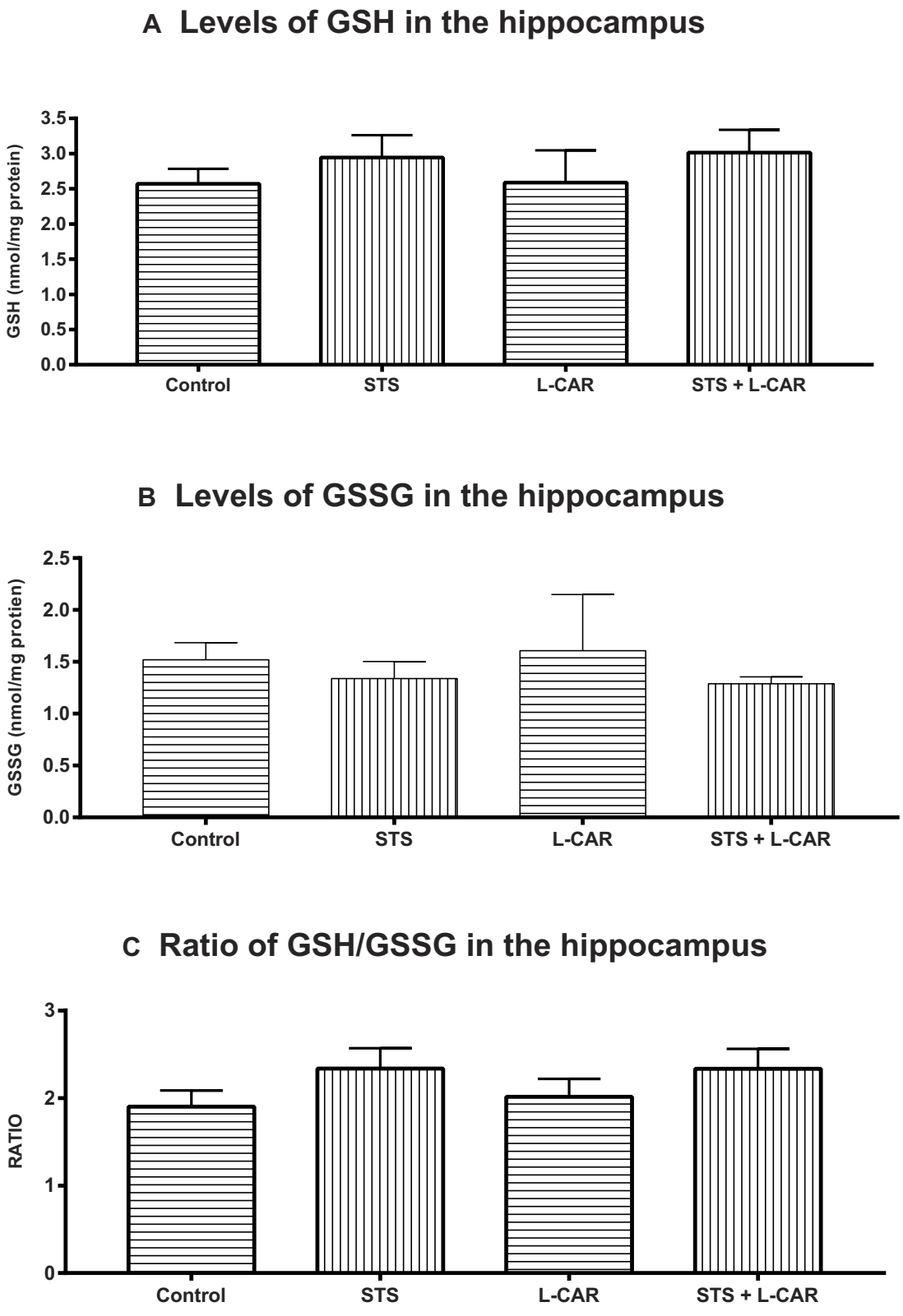

Figure 3 Hippocampal GSH and GSSG levels: No change was observed in the levels of (**A**) GSH,and (**B**) GSSG, and (**C**) ratio of GSH/GSSG among all experimental groups. Mean values ± SEM of 15 rats per group are presented.

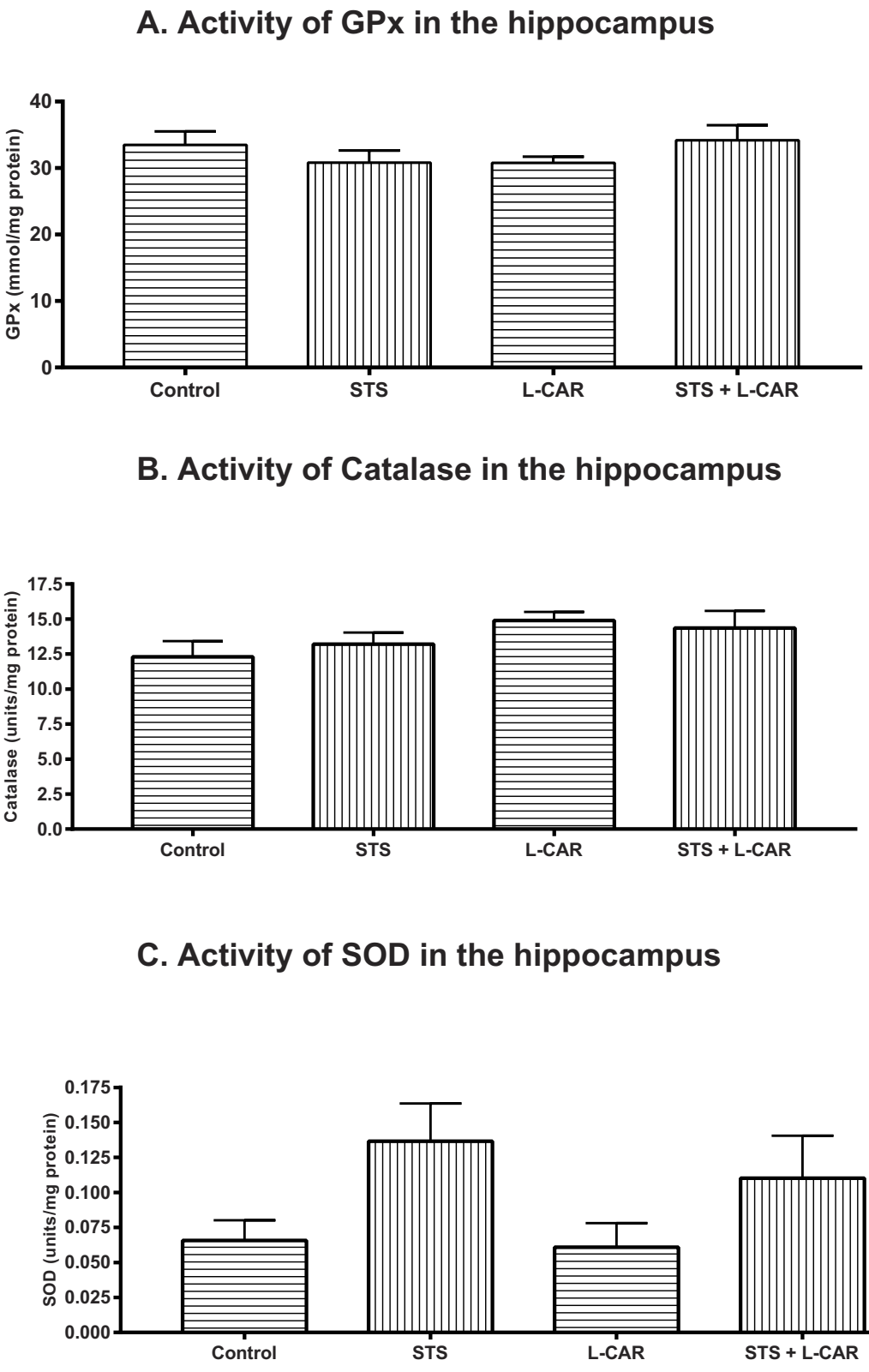

Figure 4 Effect of L-CAR and/or STS on the activity of anti-oxidative stress capacity enzymes in the hippocampus tissue. (**A**) GPx activity, (**B**) catalase activity, and (**C**) SOD activity were similar among all experimental groups in the hippocampal tissue. Mean values ± SEM of 15 rats per group are presented.

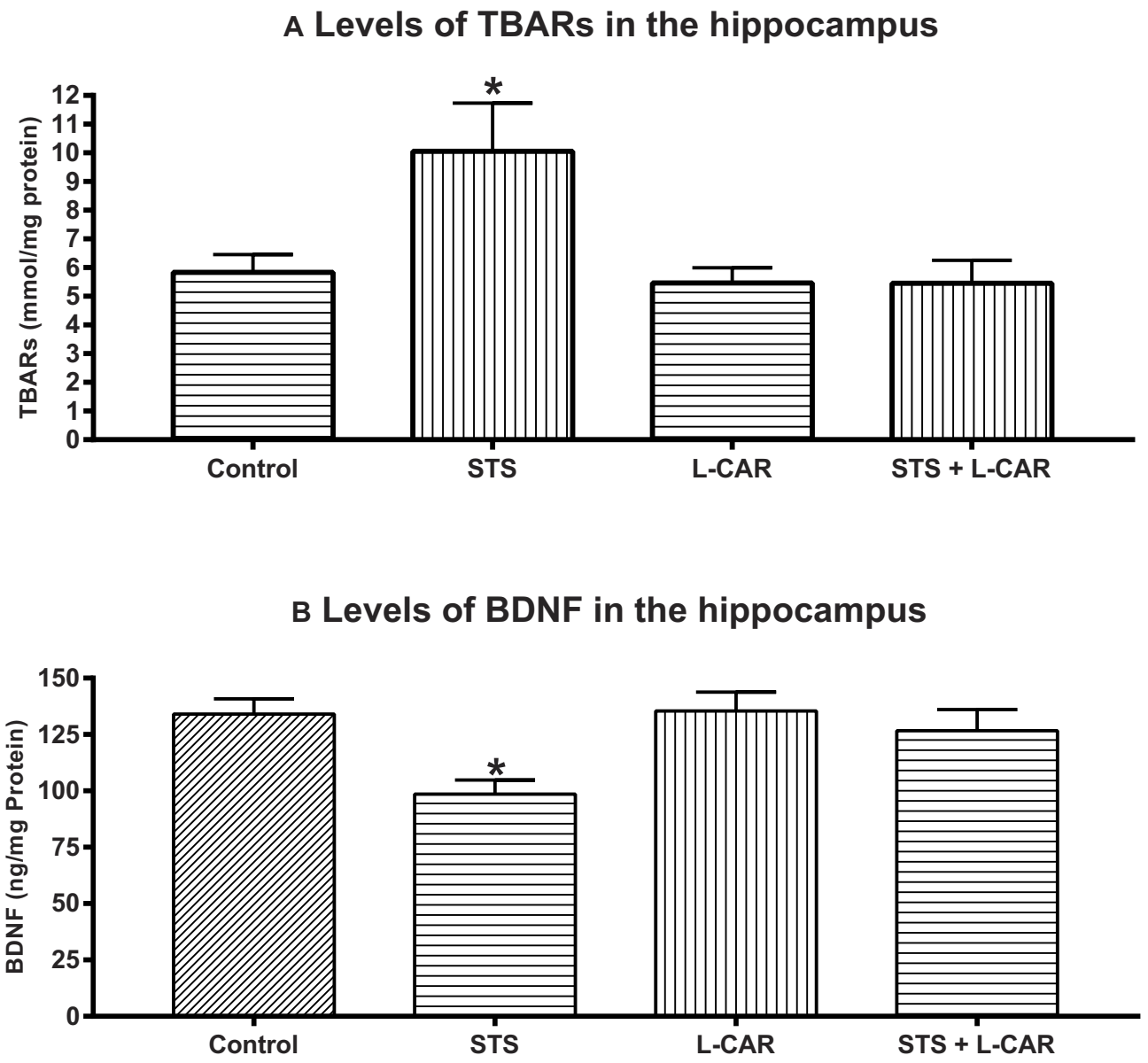

Figure 5 (**A**) Effect of L-CAR and/or STS on the hippocampal levels of TBARS in STS rats. The STS group revealed significant increase in the hippocampal TBARs levels compared to other groups. On the other hand, the levels of TBARs in the STS + L-CAR group were similar to those in the control and L-CAR groups. (**B**) Levels of brain-derived neurotrophic factor (BDNF) in the hippocampus. BDNF levels were significantly decreased in the hippocampus of the STS group as compared to the control group. Moreover, the levels of BDNF in the STS + L-CAR group were similar to that in the control and L-CAR groups. Mean ± SEM, n = 15 for each group, *p < 0.05 indicates significant difference compared with all other groups.

reported reduced levels of hippocampal BDNF during STS. This effect was prevented by administration of L-CAR. L-CAR was formerly shown to protect memory during chronic sleep deprivation through normalizing neuroprotective antioxidant mechanisms although BDNF was not included in that model.[31] Additionally, BDNF levels decreased in PTSD patients who suffered memory and cognition impairment.[52]

The psychosocial stress model used in the current study has been previously validated in our laboratory setting, where animals subjected to chronic psychosocial stress, had elevated blood pressure and plasma levels of corticosterone.[35,36] Further model validation via measuring hippocampal and plasma cytokine that are known to be higher in the STS model used would be informative for the validity of L-carnitine as potential therapeutics for chronic STS. Moreover, immunohistochemical analysis of markers such as microglial activation marker, oxidative marker (8-oxo-dG), astrocyte activation marker, and blood–brain barrier integrity marker would aid in understanding the mechanism of L-CAR

action. Doing such analysis of further biomarkers is a strongly recommended future study.

Conclusion

The current results showed that L-CAR protects memory impairment induced by chronic psychosocial stress probably via preventing increase in lipid peroxidation (TBARs) and decrease in BDNF levels associated with psychosocial stress condition.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Damron KR. Review of the relationships among psychosocial stress, secondhand smoke, and perinatal smoking. *J Obstet Gynecol Neonatal Nurs*. 2017;46(3):325–333. doi:10.1016/j.jogn.2017.01.012
2. Alzoubi K, Abdul-Razzak K, Khabour O, Al-Tuweiq G, Alzubi M, Alkadhi K. Adverse effect of combination of chronic psychosocial stress and high fat diet on hippocampus-dependent memory in rats. *Behav Brain Res*. 2009;204(1):117–123. doi:10.1016/j.bbr.2009.05.025
3. Olver JS, Pinney M, Maruff P, Norman TR. Impairments of spatial working memory and attention following acute psychosocial stress. *Stress Health*. 2015;31(2):115–123. doi:10.1002/smi.v31.2
4. Alzoubi KH, Abdul-Razzak KK, Khabour OF, Al-Tuweiq GM, Alzubi MA, Alkadhi KA. Caffeine prevents cognitive impairment induced by chronic psychosocial stress and/or high fat-high carbohydrate diet. *Behav Brain Res*. 2013;237:7–14. doi:10.1016/j.bbr.2012.09.018
5. Aleisa AM, Alzoubi KH, Gerges NZ, Alkadhi KA. Nicotine blocks stress-induced impairment of spatial memory and long-term potentiation of the hippocampal CA1 region. *Int J Neuropsychopharmacol*. 2006;9(4):417–426. doi:10.1017/S1461145705005912
6. Gerges NZ, Alzoubi KH, Park CR, Diamond DM, Alkadhi KA. Adverse effect of the combination of hypothyroidism and chronic psychosocial stress on hippocampus-dependent memory in rats. *Behav Brain Res*. 2004;155(1):77–84. doi:10.1016/j.bbr.2004.04.003
7. Shenk JC, Liu J, Fischbach K, et al. The effect of acetyl-L-carnitine and R-alpha-lipoic acid treatment in ApoE4 mouse as a model of human Alzheimer's disease. *J Neurol Sci*. 2009;283(1–2):199–206. doi:10.1016/j.jns.2009.03.002
8. Aleisa AM, Alzoubi KH, Gerges NZ, Alkadhi KA. Chronic psychosocial stress-induced impairment of hippocampal LTP: possible role of BDNF. *Neurobiol Dis*. 2006;22(3):453–462. doi:10.1016/j.nbd.2005.12.005
9. Alzoubi KH, Aleisa AM, Alkadhi KA. Molecular studies on the protective effect of nicotine in adult-onset hypothyroidism-induced impairment of long-term potentiation. *Hippocampus*. 2006;16(10):861–874. doi:10.1002/(ISSN)1098-1063
10. Alzoubi KH, Bedawi AS, Aleisa AM, Alkadhi KA. Hypothyroidism impairs long-term potentiation in sympathetic ganglia: electrophysiologic and molecular studies. *J Neurosci Res*. 2004;78(3):393–402. doi:10.1002/(ISSN)1097-4547
11. Alzoubi KH, Gerges NZ, Aleisa AM, Alkadhi KA. Levothyroxin restores hypothyroidism-induced impairment of hippocampus-dependent learning and memory: behavioral, electrophysiological, and molecular studies. *Hippocampus*. 2009;19(1):66–78. doi:10.1002/hipo.v19:1
12. Slimen IB, Najar T, Ghram A, Dabbebi H, Ben Mrad M, Abdrabbah M. Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. *Int J Hyperthermia*. 2014;30(7):513–523. doi:10.3109/02656736.2014.971446
13. Alqudah MAY, Alzoubi KH, Ma'abrih GM, Khabour OF. Vitamin C prevents memory impairment induced by waterpipe smoke: role of oxidative stress. *Inhal Toxicol*. 2018;30(4–5):141–148. doi:10.1080/08958378.2018.1474977
14. Alzoubi KH, Hasan ZA, Khabour OF, et al. The effect of high-fat diet on seizure threshold in rats: role of oxidative stress. *Physiol Behav*. 2018;196:1–7. doi:10.1016/j.physbeh.2018.08.011
15. Alzoubi KH, Mayyas FA, Mahafzah R, Khabour OF. Melatonin prevents memory impairment induced by high-fat diet: role of oxidative stress. *Behav Brain Res*. 2018;336:93–98. doi:10.1016/j.bbr.2017.08.047
16. Alzoubi KH, Rawashdeh NQ, Khabour OF, et al. Evaluation of the effect of moringa peregrina extract on learning and memory: role of oxidative stress. *J Mol Neurosci*. 2017;63(3–4):355–363. doi:10.1007/s12031-017-0986-x
17. Samarghandian S, Azimi-Nezhad M, Farkhondeh T, Samini F. Anti-oxidative effects of curcumin on immobilization-induced oxidative stress in rat brain, liver and kidney. *Biomed Pharmacother*. 2017;87:223–229. doi:10.1016/j.biopha.2016.12.105
18. Alzoubi KH, Khabour OF, Salah HA, Hasan Z. Vitamin E prevents high-fat high-carbohydrates diet-induced memory impairment: the role of oxidative stress. *Physiol Behav*. 2013;119:72–78. doi:10.1016/j.physbeh.2013.06.011
19. Alzoubi KH, Al-Ibbini AM, Nuseir KQ. Prevention of memory impairment induced by post-traumatic stress disorder by cerebrolysin. *Psychiatry Res*. 2018;270:430–437. doi:10.1016/j.psychres.2018.10.008
20. Lu B, Nagappan G, Lu Y. BDNF and synaptic plasticity, cognitive function, and dysfunction. *Handb Exp Pharmacol*. 2014;220:223–250.
21. Murakami S, Imbe H, Morikawa Y, Kubo C, Senba E. Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. *Neurosci Res*. 2005;53(2):129–139. doi:10.1016/j.neures.2005.06.008
22. Li XH, Liu NB, Zhang MH, et al. Effects of chronic multiple stress on learning and memory and the expression of Fyn, BDNF, TrkB in the hippocampus of rats. *Chin Med J (Engl)*. 2007;120(8):669–674. doi:10.1097/00029330-200704020-00011
23. Alzoubi KH, Khabour OF, Ahmed M. Pentoxifylline prevents post-traumatic stress disorder induced memory impairment. *Brain Res Bull*. 2018;139:263–268. doi:10.1016/j.brainresbull.2018.03.009
24. Shimizu E, Hashimoto K, Okamura N, et al. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry*. 2003;54(1):70–75. doi:10.1016/S0006-3223(03)00181-1
25. Licznerski P, Jonas EA. BDNF signaling: harnessing stress to battle mood disorder. *Proc Natl Acad Sci USA*. 2018;115(15):3742–3744. doi:10.1073/pnas.1803645115
26. Ferreira GC, McKenna MC. L-carnitine and acetyl-L-carnitine roles and neuroprotection in developing brain. *Neurochem Res*. 2017;42(6):1661–1675. doi:10.1007/s11064-017-2288-7
27. Lai HS, Chen Y, Chen WJ. Carnitine contents in remnant liver, kidney, and skeletal muscle after partial hepatectomy in rats: randomized trial. *World J Surg*. 1998;22(1):42–46. discussion 46–47. doi:10.1007/s002689900347.
28. Hollis VW Jr., Blecher M. Carnitine stimulated transport of the intermediates of long chain fatty acid beta oxidation in liver and heart mitochondria. *Proc Soc Exp Biol Med*. 1967;125(4):1201–1206. doi:10.3181/00379727-125-32313
29. Alves E, Binienda Z, Carvalho F, et al. Acetyl-L-carnitine provides effective in vivo neuroprotection over 3,4-methylenedioximethamphetamine-induced mitochondrial neurotoxicity in the adolescent rat brain. *Neuroscience*. 2009;158(2):514–523. doi:10.1016/j.neuroscience.2008.10.041

30. Ando S, Kobayashi S, Waki H, et al. Animal model of dementia induced by entorhinal synaptic damage and partial restoration of cognitive deficits by BDNF and carnitine. *J Neurosci Res*. 2002;70(3):519–527. doi:10.1002/(ISSN)1097-4547
31. Alzoubi KH, Rababa'h AM, Owaisi A, Khabour OF. L-carnitine prevents memory impairment induced by chronic REM-sleep deprivation. *Brain Res Bull*. 2017;131:176–182. doi:10.1016/j.brainresbull.2017.04.004
32. Suslina ZA, Fedorova TN, Maksimova M, Kim EK. Antioxidant activity of mildronate and L-carnitine in the treatment of patients with cerebrovascular diseases. *Eksp Klin Farmakol*. 2003;66(3):32–35.
33. Rani PJ, Panneerselvam C. Effect of L-carnitine on brain lipid peroxidation and antioxidant enzymes in old rats. *J Gerontol a Biol Sci Med Sci*. 2002;57(4):B134–137. doi:10.1093/gerona/57.4.B134
34. Alzoubi KH, Aleisa AM, Alkadhi KA. Effect of chronic stress or nicotine on hypothyroidism-induced enhancement of LTD: electrophysiological and molecular studies. *Neurobiol Dis*. 2008;32(1):81–87. doi:10.1016/j.nbd.2008.06.008
35. Alkadhi KA, Alzoubi KH, Aleisa AM, Tanner FL, Nimer AS. Psychosocial stress-induced hypertension results from in vivo expression of long-term potentiation in rat sympathetic ganglia. *Neurobiol Dis*. 2005;20(3):849–857. doi:10.1016/j.nbd.2005.05.020
36. Gerges NZ, Stringer JL, Alkadhi KA. Combination of hypothyroidism and stress abolishes early LTP in the CA1 but not dentate gyrus of hippocampus of adult rats. *Brain Res*. 2001;922(2):250–260. doi:10.1016/S0006-8993(01)03181-X
37. Alzoubi KH, Mhaidat NM, Obaid EA, Khabour OF. Caffeine prevents memory impairment induced by hyperhomocysteinemia. *J Mol Neurosci*. 2018;66(2):222–228. doi:10.1007/s12031-018-1158-3
38. Mhaidat NM, Alzoubi KH, Khabour OF, Tashtoush NH, Banihani SA, Abdul-razzak KK. Exploring the effect of vitamin C on sleep deprivation induced memory impairment. *Brain Res Bull*. 2015;113:41–47. doi:10.1016/j.brainresbull.2015.02.002
39. Nuseir KQ, Alzoubi KH, Alhusban A, Bawaane A, Al-Azzani M, Khabour OF. Sucrose and naltrexone prevent increased pain sensitivity and impaired long-term memory induced by repetitive neonatal noxious stimulation: role of BDNF and beta-endorphin. *Physiol Behav*. 2017;179:213–219. doi:10.1016/j.physbeh.2017.06.015
40. Hei Y, Chen R, Yi X, Wei L, Long Q, Liu W. The expression of hippocampal NRG1/ErbB4 correlates with neuronal apoptosis, but not with glial activation during chronic cerebral hypoperfusion. *Front Aging Neurosci*. 2018;10:149. doi:10.3389/fnagi.2018.00149
41. Hutchinson KM, McLaughlin KJ, Wright RL, et al. Environmental enrichment protects against the effects of chronic stress on cognitive and morphological measures of hippocampal integrity. *Neurobiol Learn Mem*. 2012;97(2):250–260. doi:10.1016/j.nlm.2012.01.003
42. Vanelzakker MB, Zoladz PR, Thompson VM, et al. Influence of pre-training predator stress on the expression of c-fos mRNA in the hippocampus, amygdala, and striatum following long-term spatial memory retrieval. *Front Behav Neurosci*. 2011;5:30. doi:10.3389/fnbeh.2011.00030
43. Wolf G, Lotan A, Lifschytz T, et al. Differentially severe cognitive effects of compromised cerebral blood flow in aged mice: association with myelin degradation and microglia activation. *Front Aging Neurosci*. 2017;9:191. doi:10.3389/fnagi.2017.00191
44. Djordjevic J, Djordjevic A, Adzic M, Radojcic MB. Chronic social isolation compromises the activity of both glutathione peroxidase and catalase in hippocampus of male wistar rats. *Cell Mol Neurobiol*. 2010;30(5):693–700. doi:10.1007/s10571-009-9493-0
45. Celikbilek A, Gocmen AY, Tanik N, Yaras N, Yargicoglu P, Gumuslu S. Serum lipid peroxidation markers are correlated with those in brain samples in different stress models. *Acta Neuropsychiatr*. 2014;26(1):51–57. doi:10.1017/neu.2013.32
46. Derin N, Aydin S, Yargicoglu P, Agar A. Changes in visual evoked potentials, lipid peroxidation and antioxidant enzymes in rats exposed to restraint stress: effect of L-carnitine. *Int J Neurosci*. 2006;116(3):205–221. doi:10.1080/00207450690969805
47. Sepand MR, Razavi-Azarkhiavi K, Omidi A, et al. Effect of acetyl-L-carnitine on antioxidant status, lipid peroxidation, and oxidative damage of arsenic in rat. *Biol Trace Elem Res*. 2016;171(1):107–115. doi:10.1007/s12011-015-0436-y
48. Dutta A, Ray K, Singh VK, Vats P, Singh SN, Singh SB. L-carnitine supplementation attenuates intermittent hypoxia-induced oxidative stress and delays muscle fatigue in rats. *Exp Physiol*. 2008;93(10):1139–1146. doi:10.1113/expphysiol.2008.042465
49. Parandak K, Arazi H, Khoshkhahesh F, Nakhostin-Roohi B. The effect of two-week L-carnitine supplementation on exercise - induced oxidative stress and muscle damage. *Asian J Sports Med*. 2014;5(2):123–128.
50. Saluk-Juszczak J, Olas B, Wachowicz B, Glowacki R, Bald E. L-carnitine modulates blood platelet oxidative stress. *Cell Biol Toxicol*. 2010;26(4):355–365. doi:10.1007/s10565-009-9148-4
51. Luo Y, Kuang S, Li H, Ran D, Yang J. cAMP/PKA-CREB-BDNF signaling pathway in hippocampus mediates cyclooxygenase 2-induced learning/memory deficits of rats subjected to chronic unpredictable mild stress. *Oncotarget*. 2017;8(22):35558–35572. doi:10.18632/oncotarget.16009
52. Autry AE, Monteggia LM. Brain-derived neurotrophic factor and neuropsychiatric disorders. *Pharmacol Rev*. 2012;64(2):238–258. doi:10.1124/pr.111.005108