Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Chronic restraint stress impairs spatial memory while decreasing hippocampal BDNF levels in rats

Sinan Saral[a,*], Aysegul Sumer[b], Ali Koray Kaya[a], Aykut Ozturk[c], Atilla Topcu[c]

[a] *Recep Tayyip Erdogan University, Faculty of Medicine, Department of Physiology, Rize, Türkiye*
[b] *Recep Tayyip Erdogan University, Faculty of Health Sciences, Department of Nursing, Rize, Türkiye*
[c] *Recep Tayyip Erdogan University, Faculty of Medicine, Department of Pharmacology, Rize, Türkiye*

ARTICLE INFO

Keywords:
Acetylcholine
Brain-derived neurotrophic factor
Cognitive impairment
Memory
Restraint stress

Received: Jul 07, 2021
Accepted: Nov 09, 2021
Available Online: June 24, 2022

DOI:
10.5455/annalsmedres.2021.07.487

Abstract

Aim: This study investigated the potential role of chronic restraint stress (CRS) on spatial memory, recognition memory, brain-derived neurotrophic factor (BDNF) and acetylcholine (ACh) levels in young adult rats.

Material and Methods: In the study, 16 female rats of 12 weeks old were used. Rats were divided into two groups as control and CRS (n=8). CRS was applied 5 hours a day for 21 days. Following the end of CRS, recognition memory of rats was evaluated with new object recognition test (NORT) and spatial memory was evaluated with Morris water maze (MWM) test. At the end of the study, rats were euthanized and hippocampal tissue homogenates were obtained. Hippocampal BDNF and ACh levels were determined by ELISA method.

Results: Exposure to CRS did not significantly change the exploratory behavior and discrimination index of rats ($p > 0.05$). In the test phase in which spatial memory was evaluated, CRS decreased the time spent in the target quadrant ($p > 0.01$). There was no significant difference between days in the training phase. CRS significantly decreased BDNF level in hippocampus ($p > 0.05$). Hippocampal ACh levels were not statistically significant ($p > 0.05$).

Conclusions: CRS weakened cognitive functions in rats. This effect was mainly accompanied by a decrease in hippocampal BDNF levels. Our findings point to the potential role of BDNF in understanding the molecular mechanism of CRS-induced cognitive impairment.

Introduction

Stress is a necessary and important mechanism for living things at the physiological level. However, the constant stress experienced by modern humans has devastating effects on the organism. One of the processes that are significantly affected by the destructive effects of stress is learning and memory performance. Chronic stress has been reported to cause atrophy and dysfunction in the frontal cortex, hippocampus and some other areas of the brain. [1]. High cognitive functions such as learning and memory can be negatively affected by chronic stress. Importantly, the hippocampus, which is among the limbic brain regions, has been reported to be susceptible to the detrimental effects of stress [2]. In a previous study, it was reported that prolonged exposure to stress reduces long-term potentiation (LTP) in the hippocampus, characterized by impairments in neurogenesis, spatial learning, and working memory [3].

Chronic restraint stress (CRS) is one of the most common experimental models of psychoemotional stress. In CRS, animals are placed in an unavoidable stress environment, resulting in structural and functional changes associated with learning and memory impairment [4]. In a previous report, CRS was reported to reduce cell proliferation and expression of brain-derived neurotrophic factor (BDNF) in rat hippocampal tissue [5]. BDNF is an important neurotrophin that plays a pivotal role in synaptic plasticity. CRS has been reported to reduce BDNF levels of the hippocampus in different physiological conditions [6]. In addition, chronic stress has been reported to cause shrinkage of the dendrites of hippocampal CA3 and dentate gyrus neurons, and loss of spines in CA1 neurons [7]. On the other hand, there is evidence showing that CRS may cause neuronal dysfunction and cell damage in the hippocampus via the cholinergic system [8].

Cholinergic synapses have a widespread anatomical distribution in the central nervous system. Localization in the striatum and neocortex, especially in the limbic sys-

*Corresponding author:
Email address: sinan.saral@erdogan.edu.tr (Sinan Saral)

tem including the hypothalamus, suggests that cholinergic transmission has critical importance on learning, memory, attention and cognitive functions [9]. Acetylcholine (ACh) is an important neurotransmitter functional in the basal ganglia cortex and basal forebrain. An increase in ACh release in the hippocampus has been reported after acute stress [10]. Moreover, a previous study has shown that increased acetylcholinesterase (AChE) levels cause memory impairment [11]. Taken together, these findings support the hypothesis that CRS may cause cognitive impairment by affecting hippocampal neurochemistry. However, the molecular and behavioral mechanism of the effect of CRS on memory performance is not clear.

The aim of this study was to examine the effects of CRS exposure on young adult rats on recognition memory, spatial memory, hippocampal ACh and BDNF levels.

Materials and Methods

Animals

Female Wistar albino rats (12 weeks old) were purchased from Karadeniz Technical University Surgical research center (Trabzon, Turkey). 16 rats were divided into 2 groups (n=8). Groups were formed as 1) control, 2) chronic restraint stress (CRS). Rats were housed in laboratory conditions standardized for temperature (23±1 °C, 50±5% humidity) and light (12 h light/dark cycles). Food and water restrictions were not applied except for the CRS protocol. Ethics committee approval of the study was obtained from the Animal Experiments Local Ethics Committee of Recep Tayyip Erdogan University.

Restraint stress protocol

After one week of adaptation to housing conditions, the CRS paradigm was applied to rats randomly divided into two groups of similar weights. The restraint stress apparatus is made of plexiglass with the dimensions of 23 x 6.5 x 6.5 cm. Stress protocol modified from a previous study (12). Accordingly, CRS was applied for 5 hours every day for 21 days. During the stress period, food restriction was applied to all groups. Rats were returned to their normal cages after restraint stress.

New object recognition test (NORT)

NORT was modified from previous studies (13). A black platform made of plexiglass with the dimensions of 90 x 90 x 45 cm was used in the experiment. The experiment was performed on three consecutive days. Accordingly, in the 1st day session (habit), rats were placed on the platform for 5 minutes and allowed to acclimate. On day 2, rats were allowed to explore the same two objects placed on the platform for 5 minutes. On day 3 (test session), one of the familiar objects was replaced with a new object and the rats were allowed to explore the objects for 3 minutes. Discovery of an object was defined as approaching the object in the range of 0-1 cm. Before each test, the objects and platform were cleaned of previous animal remains (urine, etc.) with 70% ethanol. The tests were carried out daily between 14:00 and 17:00. Cognitive behaviors were recorded with the ANY maze program (ANY maze 5.2, Dublin / Ireland).

Morris water maze test (MWM)

The test protocol used in the study was modified from our previous study (13). In this experiment, a water pool with a diameter of 150 cm was divided into 4 equal quadrants. The rats' escape platform (15 cm diameter) was placed at the midpoint of the target quadrant. The water in the tank was adjusted to the ideal temperature (25±2 °C). On the first day of the experiment, rats were floated for 60 seconds to reach the platform placed 1 cm above the water. The rats that found the platform were allowed to stay there for 30 seconds. The water was made opaque on the first day of the learning trials. Next, the platform was lowered 2 cm underwater. Then, the rats were left in the water 3 times a day for 4 days and waited to find the platform. The time taken to locate the platform was determined as the escape latency. On the test day (day 5), the platform was removed from the water and the time spent by the rats in the target quadrant, average velocity and the number of quadrant entries were recorded with the software program.

Measurement of BDNF and ACh levels in the hippocampus

At the end of the experiment, rats were euthanized under high-dose anesthesia (ketamine and xylazine), and the hippocampus was dissected on a cold platform and stored at -80 C. At the analysis stage, the hippocampus tissues were thawed under appropriate conditions and homogenates were prepared. Briefly, cold PBS (phosphate buffered saline, pH:7.4) was added to the hippocampus at 2 times the tissue weight. Afterwards, the samples were homogenized with a 30 Hertz/5 minutes (min) homogenizer. Then, the supernatants were collected by centrifugation at 3600 rpm for 15 minutes at +4 °C. BDNF and ACh levels in hippocampal homogenates were measured using rat-specific ELISA kits according to the manufacturer's instructions (Elabscience, Texas, USA).

Statistical analysis

SPSS 24.0 (IBM Corp., Armonk, NJ, USA) and GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA, USA) software programs were used for statistical analysis and generation of graphics. Data are given as mean standard error (± SEM). The normality of the data distribution was evaluated with the Shapiro Wilk test. Then, Mann Whitney-U test was used for pairwise comparisons. $P < 0.05$ was considered significant.

Results

Effects of CRS on recognition memory

The exploratory behavior and discrimination ratio of rats are shown in Figure 1. There was no difference between the groups in the training session with two identical objects. Similarly, in the test session in which a familiar object was replaced with a new object, the total time rats spent recognizing both objects were not significant between groups (Fig 1A, $p > 0.05$). On the other hand, although there was a moderate increase in the discrimination ratio in stress group rats, this value was not significant (Fig 1B, 0.21±0.03, 0.26±0.01, $p > 0.05$). These results indicate that CRS had no significant effect on exploratory behavior and discrimination ratio in rats.

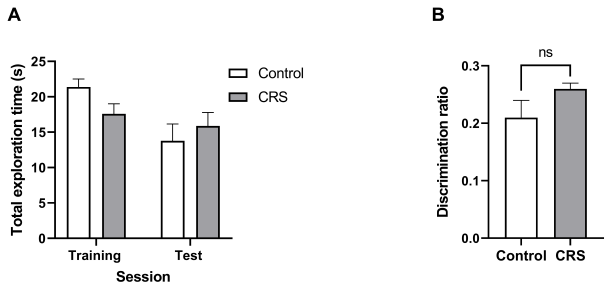

Figure 1. Effect of CRS on exploratory behavior and discrimination ratio in rats. Data are presented as SEM (n=8). Abbreviations: CRS; chronic restraint stress.

The effect of CRS on spatial learning and memory functions

Spatial memory tests of rats were evaluated by MVM. As shown in Figure 2A, we found that in the memory test session on day 5, the time spent by the stress group in the target quadrant was significantly reduced compared to the control ($p<0.01$). This result indicates that CRS weakens memory performance. As another finding, no significant difference was found between the mean velocities of the groups (Fig 2B, $P>0.05$). On the other hand, we observed that the number of entry into the target quadrant of the stress group rats was lower than the control (Fig 2C, $p<0.01$). In addition, although there was a moderate increase in the escape latency of the stress group on the 2nd day of the training trials on consecutive days, these values were not statistically significant (Fig 2D, $p>0.05$).

The effect of CRS on BDNF levels in the hippocampus

In this study, we evaluated BDNF levels in homogenates prepared from hippocampus tissues by ELISA method. As seen in Figure 3, BDNF levels in rats exposed to 5 hours of restriction per day for 21 days were found to be significantly lower in the stress group compared to the control (4.323 ± 0.49, 5.472 ± 1.14, $P<0.05$, respectively).

The effect of CRS on ACh levels in the hippocampus

ACh content was analyzed in rat hippocampal tissue homogenates to further evaluate the potential effects of CRS. Accordingly, a moderate decrease in ACh level was observed in stress group rats compared to the control group (207.6 ± 35.8, 171.8 ± 38.9, respectively). However, this difference was not statistically significant (Fig 4, $p>0.05$).

Discussion

In the present study, we examined the potential effects of CRS on cognitive behaviors, neuronal plasticity and cholinergic system in young adult rats. We used NOR and MWM tests to investigate whether CRS can affect cognitive functions such as learning and memory. Object exploration time in the NOR test did not differ significantly in both sessions. Moreover, there was no difference between the groups in terms of discrimination ratio. Our results showed that CRS did not affect the discrimination ratio. However, a recent study has shown that restraint

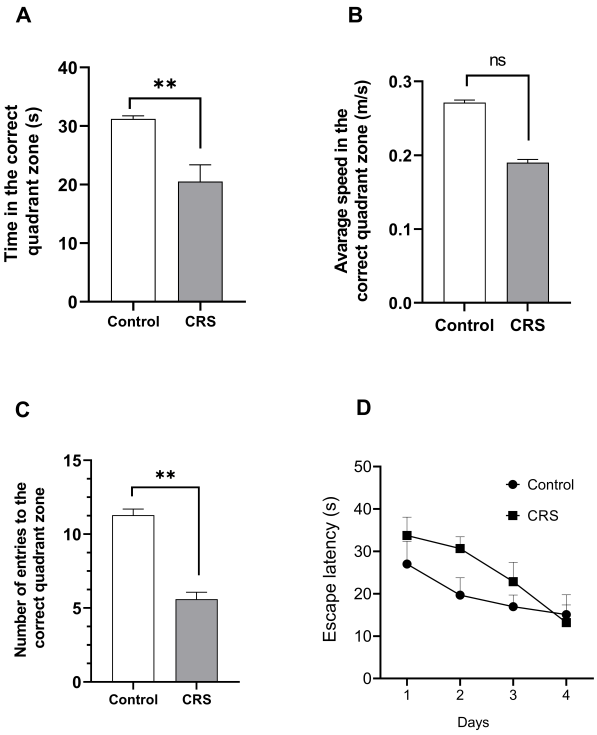

Figure 2. Effect of CRS on learning and memory functions in rats. Data are presented as SEM (n=8). **p<0.01. Abbreviations: CRS; Chronic restraint stress.

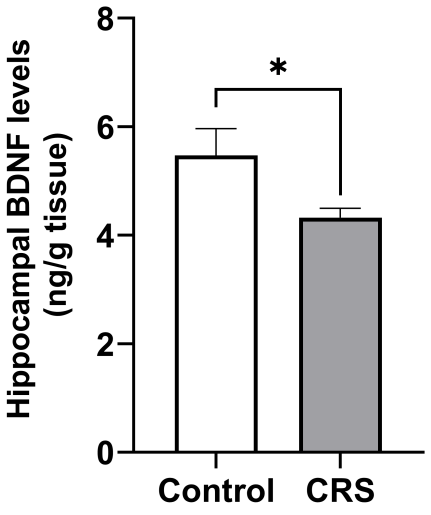

Figure 3. The effect of CRS on hippocampal BDNF levels in rats. Data are presented as SEM (n=8). *p<0.05. Abbreviations: CRS; Chronic restraint stress, BDNF; Brain-derived neurotrophic factor.

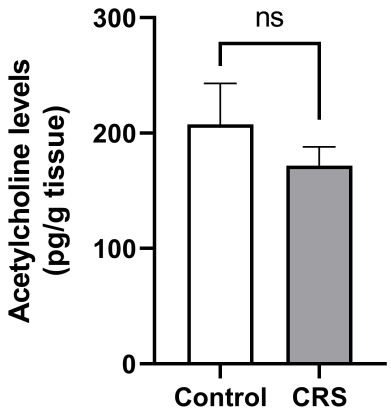

Figure 4. The effect of CRS on hippocampal acetylcholine levels in rats. Data are presented as SEM (n=8). Abbreviations: CRS; Chronic restraint stress.

stress for seven days reduces the discrimination index [14]. The variable results in the NOR test suggested that it may be related to the duration of exposure to stress and age. On the other hand, in the MWM test, in which spatial memory was evaluated, it was determined that the time spent in the target quadrant of the stress group rats was less than that of the control. This result points to the role of CRS in the deterioration of spatial memory. Similarly, CRS reduced the number of rats entering the target quadrant. In a previous study, it was reported that CRS caused a longer escape latency in acquisition trials and significantly reduced the time spent in the target quadrant in the test phase [15]. In another study, it was reported that spatial memory was impaired in MWM tests of male rats exposed to restraint stress for three hours a day for 14 days [16]. Taken together, the present results revealed that exposure to prolonged restraint stress could potentially impair spatial memory in rats.

It has been stated that chronic stress generally impairs various hippocampal-dependent memory tasks. Experimental studies have revealed that stress alters the synaptic plasticity and firing patterns of hippocampal neurons [17]. The hippocampus is an important limbic structure where brain functions such as learning and memory are regulated. BDNF is a neurotrophin widely expressed in the hippocampus and cortex [18]. Neuronal and glial cell development, synaptic plasticity, memory functions and neuroprotective effects of BDNF have been reported [19]. In a previous study, CRS was shown to cause cognitive impairment by reducing hippocampal BDNF expression [20]. In another study, it was reported that acute stress as well as chronic stress caused a significant reduction in BDNF mRNA in the hippocampus [21]. Consistent with the MWM test results, we found that CRS exposure for 21 days significantly reduced the level of BDNF in the hippocampus of rats. In a previous study, it was reported that intrahippocampal BDNF infusion in rats prior to chronic restraint stress protocol may protect against deficits in learning and memory in MWM and long-term potentiation [22]. Taken together, these results point the role of BDNF in explaining the molecular mechanisms underlying the cognitive impairment caused by CRS.

Chronic stress has been associated with Alzheimer's disease (AD), a neurodegenerative disease due to its effects on neuronal atrophy and synaptic degeneration [23]. It is known that cholinergic insufficiency plays an important role in the etiology of AD. Therefore, examining the potential relationship between chronic stress and cognitive impairment remains important. We observed that restraint stress for 21 days moderately reduced the hippocampal ACh level, but this result was not statistically significant. In a previous study, it was shown that two hour restraint stress markedly increased ACh levels in the hippocampus, but ACh returned to baseline after one hour [24]. In addition, Masuda et al. reported that restraint stress for one hour a day for 4 days caused a significant increase in ACh release in the hippocampus of female rats [25]. Variable results in hippocampal ACh levels have been associated with difference in duration of stress exposure. On the other hand, in a recent study, it was shown that the level of AchE increased in the brain tissue of rats administered with CRS for 4 hours a day for 28 days [26]. AchE level could not be measured in this study. Therefore, we suggest investigating the effect of CRS on AchE enzyme activity in future studies.

Limitations

We state that our study has some limitations. In fact, we have potential questions for understanding the molecular mechanism of cognitive impairment caused by CRS. However, in the presented study, we focused on the effect of CRS on cognitive behavior and hippocampal BDNF and ACh levels. Therefore, we suggest that future studies investigate the effects of CRS on markers of hippocampal neuroplasticity.

Conclusion

In this study, we examined the potential effects of CRS on memory functions. The results of the study showed that CRS reduced the level of BDNF in the hippocampus and impaired spatial memory. We suggest that BDNF may be a potential target for the prevention of CRS-induced learning and memory impairments.

Ethical approval

Ethics committee approval of the our study was obtained from the Animal Experiments Local Ethics Committee of Recep Tayyip Erdogan University.

References

1. Wang Y, Kan H, Yin Y, et al. Protective effects of ginsenoside Rg1 on chronic restraint stress induced learning and memory impairments in male mice. Pharmacol Biochem Behav 2014;120:73–81.
2. Nagata K, Nakashima-Kamimura N, Mikami T, et al. Consumption of molecular hydrogen prevents the stress-induced impairments in hippocampus-dependent learning tasks during chronic physical restraint in mice. Neuropsychopharmacology 2009;34:501-8.

3. Bhagya VR, Srikumar BN, Veena J, Shankaranarayana Rao BS. Short-term exposure to enriched environment rescues chronic stress-induced impaired hippocampal synaptic plasticity, anxiety, and memory deficits. J Neurosci Res 2017;95:1602–10.
4. Miao Z, Wang Y, Sun Z. The relationships between stress, mental disorders, and epigenetic regulation of bdnf. Int J Mol Sci 2020;21:1375.
5. Xu H, Chen Z, He J, et al. Synergetic effects of quetiapine and venlafaxine in preventing the chronic restraint stress-induced decrease in cell proliferation and BDNF expression in rat hippocampus. Hippocampus 2006;16:551–9.
6. Yeh CM, Huang CC, Hsu K Sen. Prenatal stress alters hippocampal synaptic plasticity in young rat offspring through preventing the proteolytic conversion of pro-brain-derived neurotrophic factor (BDNF) to mature BDNF. J Physiol 2012;590:991–1010.
7. Eisch AJ, Petrik D. Depression and hippocampal neurogenesis: A road to remission? Science 2012;338:72–5.
8. Zhao D, Xu X, Pan L, et al. Pharmacologic activation of cholinergic alpha7 nicotinic receptors mitigates depressive-like behavior in a mouse model of chronic stress. J Neuroinflammation 2017;14:1–15.
9. Hampel H, Mesulam MM, Cuello AC, et al. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain 2018;141:1917–33.
10. Paul S, Jeon WK, Bizon JL, Han JS. Interaction of basal forebrain cholinergic neurons with the glucocorticoid system in stress regulation and cognitive impairment. Front Aging Neurosci 2015;7:1–11.
11. Croxson PL, Kyriazis DA, Baxter MG. Cholinergic modulation of a specific memory function of prefrontal cortex. Nat Neurosci 2011;14:1510–2.
12. Sántha P, Pákáski M, Fazekas ÖC, et al. Restraint stress in rats alters gene transcription and protein translation in the hippocampus. Neurochem Res 2012;37:958–64.
13. Saral S, Topçu A, Alkanat M, et al. Apelin-13 activates the hippocampal BDNF/TrkB signaling pathway and suppresses neuroinflammation in male rats with cisplatin-induced cognitive dysfunction. Behav Brain Res 2021;408:113290.
14. Sun DS, Zhong G, Cao HX, et al. Repeated restraint stress led to cognitive dysfunction by nmda receptor-mediated hippocampal CA3 dendritic spine impairments in juvenile sprague-dawley rats. Front Mol Neurosci 2020;13:1–12.
15. Zhang Q, Wang X, Bai X, et al. Resveratrol reversed chronic restraint stress-induced impaired cognitive function in rats. Mol Med Rep 2017;16:2095–100.
16. Guedri K, Frih H, Chettoum A, Rouabhi R. Chronic restraint stress induced neurobehavioral alterations and histological changes in rat. Toxicol Environ Health Sci 2017;9:123–9.
17. Kim EJ, Pellman B, Kim JJ. Stress effects on the hippocampus: A critical review. Learn Mem 2015;22:411–6.
18. Yamada K, Nabeshima T. Current Perspective Brain-Derived Neurotrophic Factor / TrkB Signaling in Memory Processes. J Pharmocol Sci 2003;91:267–70.
19. Kowiański P, Lietzau G, Czuba E, et al. BDNF: A key factor with multipotent impact on brain signaling and synaptic plasticity. Cell Mol Neurobiol 2018;38:579–93.
20. 20. Kwon DH, Kim BS, Chang H, et al. Exercise ameliorates cognition impairment due to restraint stress-induced oxidative insult and reduced BDNF level. Biochem Biophys Res Commun 2013;434:245–51.
21. Murakami S, Imbe H, Morikawa Y, et al. Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. Neurosci Res 2005;53:129–39.
22. Radecki DT, Brown LM, Martinez J, Teyler TJ. BDNF protects against stress-induced impairments in spatial learning and memory and LTP. Hippocampus 2005;15:246–53.