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Acute footshock-stress increases spatial learning–memory and correlates to increased hippocampal BDNF and VEGF and cell numbers in adolescent male and female rats

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ABSTRACT

It is well known that the acute-stress enhances cognitive functions in adults, but is not known in adolescents. The purpose of this study is to investigate the effects of low and high intensities of acute-stress on hippocampus and spatial memory in the adolescent male and female rats. Thirty-eight days aged rats were subjected to 0.2 and 1.6 mA intensity of footshock-stress for 20 min. Spatial memory performance was assessed in the Morris water maze. Learning had been positively affected in stress groups. Neuron density in the CA1 hippocampal region and the gyrus dentatus as well as VEGF and BDNF levels of hippocampus increased in all stress groups. In females, learning process and BDNF levels increased in low-intensity-stress group than high-intensity-stress group. There was no difference in hippocampal apoptosis among groups.

We conclude that adolescent hippocampus is affected positively from acute-stress; however, while there is no difference in male response with respect to intensity of stress, females are affected more positively from low-intensity of stress.

Stress is an environmental factor that affects our behavior and can cause many disease, and plays an important role in human and animal biology. Stress causes a new regulation for response to stress in the human body. Inadequate regulation to stress may occur depression, panic disorders, drug addiction and cognitive disorders [36].

In mammals, brain development begins in intrauterine period and continues until the end of the adolescence. Many disorders among humans often begin during adolescence. The adolescent period in rats starts at postnatal 38–42 days and continues postnatal 60 days [36]. During the adolescent period, the volume of the rodent hippocampus, the number of cells and the dendrite density undergo development. Learning and memory also develop throughout adolescence [21].

During adolescent period, the daily life-stresses are perceived as larger that in other ages. In human studies, adolescents had a higher stress levels in stressor tasks and increased stressor-induced cortisol levels and increased cardiac reactivity to stressors compared adults [15,37]. Also in animal studies, adolescent animals show more anxious than adults in anxiety tests [16,43]. In our previously study, we have shown that both low and high intensity footshock-stress increased glutathione peroxidase (GPx) enzyme activity, an anti-oxidant enzyme, in the prefrontal cortex of adolescent male rats, while only high intensity stress increased activity of GPx in the same region of adult males [14,40].

It is known that stress is an important regulator for brain functions and cognition. In adults, prolonged and/or severe stress negatively affects learning and memory; acute-stress can affect learning differently, depending on stress duration, severity, type and time. Many studies in adults show that acute-stress affects negatively the hippocampus-related spatial learning [45]; in some studies, positively affects the cognitive functions of the adaptive response to stress [31]. However, effects of acute-stress on cognitive functions in adolescents are unknown.

Acute-stress stimulates the sympathetic nervous system, causing the release of noradrenaline [39] and results in activation of the dopamine (DA) system which are neuromodulators

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[2,30]. Also, acute-stress results in activation of the hypothalamic–pituitary–adrenal (HPA) axis, which regulates the release of glucocorticoids [39]. Acute-stress is essential for adaptation and maintenance of homeostasis. In previous studies, men and female were given different endocrinological response and memory effects to acute-stress [42,44].

The adult hippocampal neurogenesis is regulated by angiotrophic factors such as VEGF and by neurotrophic factors such as BDNF [24,46]. It is known that stress affects both VEGF and BDNF levels in adult hippocampus. Chronic-stress, reduces both the level of these factors [34,35], but how they are affected by acute-stress is not known. Whether effects of acute-stress on hippocampal VEGF and BDNF levels is unknown in adolescents.

The aim of this study is to investigate the effects of acute-footshock-stress on hippocampal cells and VEGF and BDNF levels and spatial learning and memory in adolescent male and female rats.

There are the same stress hormone levels (corticosterone and ACTH) with adults after postnatal 46 days [21]. So that, 38 days old Wistar-Albino female and male rats ($n = 7$) were used. All experiments were performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the Dokuz Eylul University, School of Medicine. All rats were maintained on a constant 12-h-light/dark cycle at constant room temperature (21 °C), and humidity (60%). The animals were divided into six groups: control-males, 0.2 mA footshock-males (low-intensity), 1.6 mA footshock-males (high-intensity), control-females, 0.2 mA footshock-females, 1.6 mA footshock-females. Rats were exposed to electric foot-shocks of 160 ms duration with a 160 ms interval for 20 min.

One hour after at the end of stress period, all rats were subjected to Morris-water-maze tests [22]. The Morris-water-maze was 140 cm in diameter and 75 cm in height. The water level in the tank was 50 cm, which was 1.0 cm above the height of the escape invisible platform. Each rat was exposed to the task for 4 consecutive days (total 20 trials), and on day 5 a probe trial was run. The learning tests recorded and evaluated with HVS-image video-tracking-system.

After the water maze test at postnatal days 42, all animals were decapitated under ether anesthesia. Brain tissues were removed and half of hemisphere fixed in 10% formalin in phosphate-buffer for histological-examination. Other hemisphere hippocampus was used biochemical-analysis.

The brain was sectioned coronally into sequential 6 μm sections using a microtome (Leica RM2255, Köln, Germany). Each sample was subjected to the estimation of neuron number by taking three coronal sections through the hippocampus that corresponded to plates 21,23,25 in the rat atlas of Paxinos and Watson [25]. All sections were stained by cresyl violet. The images were analyzed by using a computer assisted image analyzer system consisting of a microscope (OlympusBH-2 Tokyo, Japan) equipped with a high-resolution video camera (JVC TK-890E, Japan). The numbers of hippocampal neurons were counted by help of a 6000 μm^2 counting frame viewed through a 20× Nikon-lens at the monitor. The counting frame was placed randomly five times on the image analyzer system monitor and neuron numbers were counted (UTHSCA-Image-Tool for windows version-3.0 software) and the average was taken. The numbers of hippocampal neurons were counted in CA1, CA2, CA3 and gyrus dentatus (GD) regions and neuron density was calculated.

To detect DNA-fragmentation in cell nuclei, terminal deoxynucleotidyl-transferase-mediated dUTP-nick end-labeling (TUNEL) reaction was applied to the paraffin sections by using a kit (G7130-Promega, USA).

The serum corticosterone levels were measured with the radioimmunoassay method using a double antibody kit (ImmuChem, MP-Biomedicals, Orangeburg, NY).

Hippocampus tissue homogenate was analyzed by enzyme immunoassay for BDNF (Catalog Number EK0308, Boster Immunoleader, Wuhan, China) with assay sensitivity <2 pg/ml and range 31.2–2000 pg/ml and VEGF (Catalog Number EK0308, Boster Immunoleader, Wuhan, China) with assay sensitivity <1 pg/ml and range 15.6–1000 pg/ml.

VEGF expression was detected by avidin-biotin-complex method using Santa-Cruz biotechnology (SC-7629) (R&D-Systems) according to the manufacturers. Immunoreactivity was graded as follows: more than 10% of the cells staining were graded as positive. No detectable staining or <10% of cells staining was graded as negative. The qualitative intensity of staining for VEGF was assessed using a scale between 0 and +++. With 0 representing no detectable stain and +++ representing strongest stain.

Difference between the learning days in MWM was analyzed by using GLM-repeated measure post hoc Bonferroni. Differences between the groups were analyzed using two-way-ANOVA post hoc Bonferroni. Results are presented as mean ± S.E.M (significance level was $p \leq 0.05$).

The present study showed that acute-stress significantly improved spatial learning–memory. The mean latency to find the platform declined progressively in all animals ($F_{1,34} = 2.88$, $p < 0.05$). Stressed rats had shorter escape latencies at the second (in stressed males), third (in all stressed groups) and fourth (in all stressed groups) days of training days than controls (Fig. 1A). An effect of sex ($F_{3,33} = 5.48$, $p = 0.004$) and interaction between sex × stress in learning days ($F_{3,33} = 4.73$, $p = 0.008$), but no interaction only stress on subject's learning was observed. In females, low-intensity-stress more decreased escape latency than high-intensity-stress (second-day, $F_{2,17} = 7.95$, $p = 0.004$; third-day, $F_{2,17} = 28.04$, $p = 0.000$, fourth-day, $F_{2,17} = 6.52$, $p = 0.009$). In males, the escape latency was reduced in all stressed-males compared to control-males, but there was not any difference between high and low-intensity-stress groups (second-day, $F_{2,20} = 9.16$, $p = 0.002$; third-day, $F_{2,20} = 6.68$, $p = 0.007$, fourth-day, $F_{2,20} = 9.46$, $p = 0.002$).

In probe trials (quadrant time), time spent in target quadrant was used to evaluate long-term memory. All stressed animals spent more time in the target-quadrant and spent less time in the opposite-quadrant (Fig. 1B), compared to controls (males, target-quadrant, $F_{2,20} = 19.70$, $p = 0.000$, opposite-quadrant, $F_{2,20} = 111.43$, $p = 0.000$; females, target-quadrant, $F_{2,17} = 9.16$, $p = 0.003$, opposite-quadrant, $F_{2,17} = 8.24$, $p = 0.004$). The cell numbers increased in CA1 hippocampal region and the GD (males, CA1, $F_{2,18} = 10.87$, $p = 0.001$, GD, $F_{2,18} = 3.80$, $p < 0.05$; females, CA1, $F_{2,16} = 12.84$, $p = 0.001$, GD, $F_{2,16} = 22.56$, $p = 0.000$) (Fig. 2). Hippocampal VEGF and BDNF levels increased in all stress groups (males, VEGF, $F_{2,14} = 19.70$, $p = 0.000$, BDNF, $F_{2,14} = 6.85$, $p < 0.05$; females, VEGF, $F_{2,14} = 4.22$, $p < 0.05$, BDNF, $F_{2,14} = 6.43$, $p < 0.05$) (Fig. 3). However, BDNF levels of the females showed significant differences between low- and high-intensity of stress. VEGF immune staining and marking were increased in all stress groups. There was a positive correlation between time spent in the target quadrant and cell numbers of CA1 and gyrus dentatus, VEGF and BDNF levels (CA1 cell-numbers, $r = 0.548$, $p = 0.001$; GD cell-numbers, $r = 0.341$, $p = 0.042$; VEGF, $r = 0.423$, $p = 0.020$; BDNF, $r = 0.438$, $p = 0.016$), and a negative correlation between the time spent in the opposite quadrant and cell numbers of CA1 and gyrus dentatus, VEGF and BDNF levels (CA1 cell numbers, $r = -0.643$, $p = 0.000$; GD cell numbers, $r = -0.455$, $p = 0.005$; VEGF, $r = -0.376$, $p = 0.040$; BDNF, $r = -0.388$, $p = 0.034$). Few cells were seen to be TUNEL-positive in both control and stress groups in females and males. There was no statistical difference of hippocampal apoptosis and basal corticosterone levels among groups (5 days after stress).