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Maternal exercise decreases maternal deprivation induced anxiety of pups and correlates to increased prefrontal cortex BDNF and VEGF

Nazan Uysal[a,*], Ali Riza Sisman[b], Ayfer Dayi[a], Ilkay Aksu[a], Ferihan Cetin[a], Celal Gencoglu[a], Aysegul Tas[a], Erkan Buyuk[c]

[a] Dokuz Eylul University, School of Medicine, Department of Physiology, Turkey
[b] Dokuz Eylul University, School of Medicine, Department of Biochemistry, Turkey
[c] Gynecology & Women's Health Albert Einstein College of Medicine/Montefiore Medical Center, Division of Reproductive Endocrinology & Infertility Department of Obstetrics, New York, USA

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ABSTRACT

Maternal deprivation (MD) may cause neuropsychiatric disorders such as anxiety disorder by negatively affecting the cognitive functions and behavior in pups. The aim of this study is to investigate whether maternal exercise during pregnancy has beneficial effects on anxiety that increases with MD, and on the levels of VEGF and BDNF which have anxiolytic effects on the prefrontal cortex, the anxiety-related region of the brain. The anxiety level in the deprivation group was greater than the control group and found more in male than female pups. The prefrontal cortex VEGF and BDNF levels were decreased in the deprivation group compared to control group while serum corticosterone levels were increased in the deprivation group. Anxiety and serum corticosterone levels were decreased in maternally exercised female and male pups, while the prefrontal cortex VEGF and BDNF levels were increased, compared to sedentary mother's pups. These results indicate that maternal exercise may attenuate the negative effect of stresses such as maternal deprivation that can be encountered early in life.
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In mammals, normal development and growth is significant for mother–pup relation; maternal deprivation (MD) is a major stress which may cause physiological and behavioral changes during brain development. Internal and external factors can affect the brain development [24]. Stressful situations in early development period, such as MD, may cause neuropsychiatric diseases which affect the later periods of life, such as anxiety disorder [17].

MD is a stress model that is widely used in rodents in their early life periods [29–32]. In later periods of life, permanent changes may occur in the endocrine systems of the maternally deprived animals [31,32,34]. MD may cause anxiety-like behavior by causing structural changes in the prefrontal cortex (PFC) [21]. Also, the hypothalamic levels of the neurotrophic factors such as brain derived neurotrophic factor (BDNF), with known anxiolytic effects, change during maternal deprivation, and this change alters the cell proliferation and maturation differentially in females and males [34].

Approximately on postnatal day 4, the infant rat enters the so-called stress-hyporesponsive period (SHRP) [24]. This period lasts until postnatal day 14 and is characterized by reduction of adrenal sensitivity. It appears that low levels of corticosterone during SHRP are essential for a normal development, because exposure to high levels of corticosterone has widespread deleterious effects on the developing brain [24]. In our previous studies, we showed that the infant brain protected from MD in SHRP. Thus, in this study MD was applied after SHRP (postnatal days 18) [29,30].

It has been showed that males and females were affected differentially from MD. Prolonged MD increased the glucocorticoid receptor expression in male rat brains [26]. When maternally deprived males become adults, they feel much anxiety and fear than females [26,34]. We have previously reported that the SOD enzyme activity increases in the striatum and like GPx enzyme activity and TBARS levels in the PFC in maternally deprived males. No change is observed in maternally deprived females [31].

It is known that regular exercise ameliorates the neural structures and functions. We have been demonstrated that exercise enhanced the cognitive functions [33]. It was also showed that exercise increased the neurotrophic factors such as BDNF and angiotrophic factors such as Vascular Endothelial Growth Factor (VEGF) in hippocampus and blood [25].

Recent research is directed towards emending the negative interaction in pups due to MD by the positive effects of exercise. In studies on this topic, it was found that both the forced exercise and the voluntary exercise ameliorated behavior and learning negatively affected by MD [10,13]. However, the effects of the maternal

* Corresponding author. Tel.: +90 232 4124460; fax: +90 232 4124459.
E-mail address: nazan.uysal@deu.edu.tr (N. Uysal).

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exercise during pregnancy on pup's anxiety caused by MD and interrelated on the PFC are not known.

The aim of this study is to investigate the effects of maternal regular aerobic exercise on the anxiety levels of the pups in preadolescent period, and on the prefrontal cortex BDNF and VEGF levels.

Eight-week-old male and female Wistar Albino rats were purchased from Experimental Animal Laboratory. The pregnancies of the groups were controlled by estrous monitoring. The female rats ($n = 8$) were equally divided into sedentary and exercised groups. The day of birth was designated as postnatal day 0. Pups were divided into two groups: maternally exercised, sedentary. Each group consists of two sub-groups: maternally deprived, control. Each group consists of female and male rats ($n = 8$). The animals were maintained under standard colony conditions with a 12 h light/dark cycle (lights on 07:00 h), at constant room temperature ($22 \pm 1\,^{\circ}C$), and humidity (60%). Food and water were available ad lib. 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.

Exercised mothers were trained to the treadmill before pregnancy at 5 m/min for 10 min/day, 5 days. After breeding with estrous monitoring, the mothers started to exercise at 8 m/min for 30 min/day, 5 days/week. In the last week of pregnancy, the pace of the exercise was reduced to 6 m/min and the exercise had continued until birth [10].

MD took place on PND 18 as previously described [29,30]. In brief, mothers from the deprived groups were removed in the morning (beginning at 09:00 h) and pups remained in their home cages for 24 h. On PND 19 the mothers were returned to pups. During separation, the pups of each litter were kept together and warmed by a heating pad ($33 \pm 0.2\,^{\circ}C$) and they were provided access to food and water.

The remaining rats were weaned on day 22 and when the pups were 24 days old, their anxiety levels were evaluated using the open field and elevated plus maze tests. The recording and analysis of the anxiety levels were performed with the HVS image video tracking system.

Locomotor activity was measured with an open-field test; 1 m × 1 m and 50 cm in height, video camera installed 2.5 m above apparatus. Each rat was placed in the center of the open-field and then locomotor activity (ambulation) was measured for 5 min in a sound-proof observation room illuminated with controlled light (100 lx).

The animals were tested for anxiety behavior in the elevated plus maze. This consisted of a central platform (5 cm × 5 cm) with two open arms (50 cm long, 10 cm wide and 0.5 cm high borders) and two closed arms (same dimensions as the open arms only with 40 cm high walls) that were elevated 50 cm above the ground. Rats were placed on the platform facing the open arm and were observed for 5 min. The total number of entries into the open and closed arms as well as the time spent in the open and closed arms was measured.

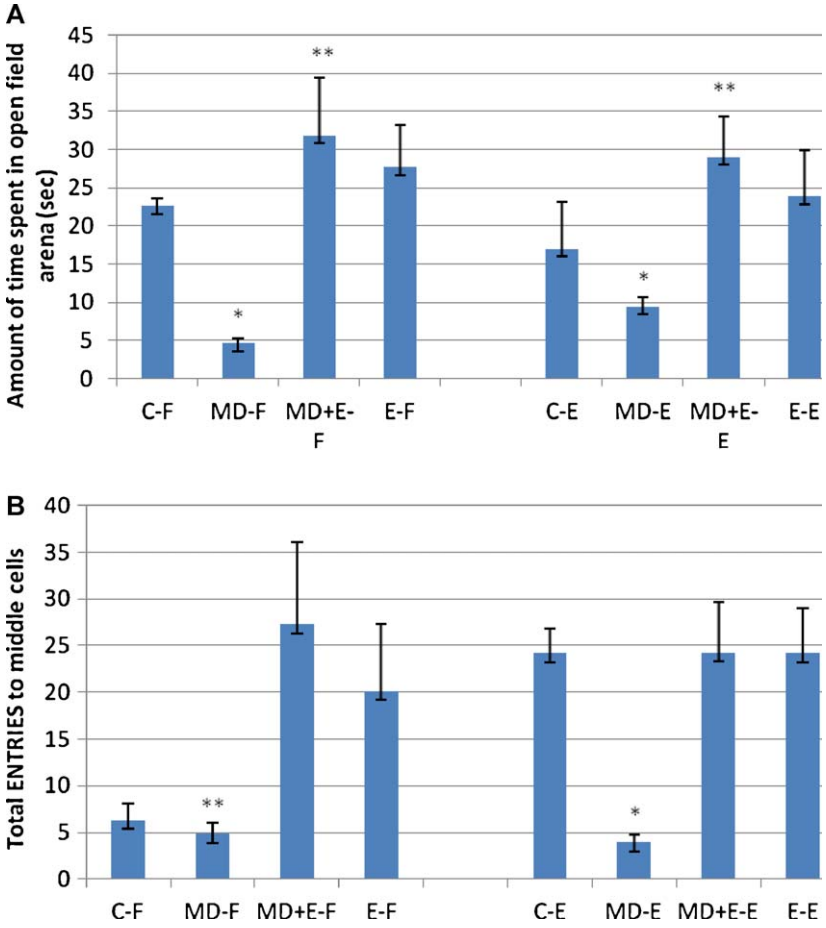


Fig. 1. Open field test results; (A) Percentage of the time they are moving the open field arena, (B) Number of entries in the open field arena in the middle cells. $^{*}p < 0.05$ compared with the same sex of control group; $^{**}p < 0.05$ compared with the MD+E-F group (C-F: Control females, MD-F: deprived females, MD+E-F: maternally exercised deprived females, E-F: Maternally exercised females, C-E: Control males, MD-F: deprived males, MD+E-F: maternally exercised deprived males, E-F: Maternally exercised males).

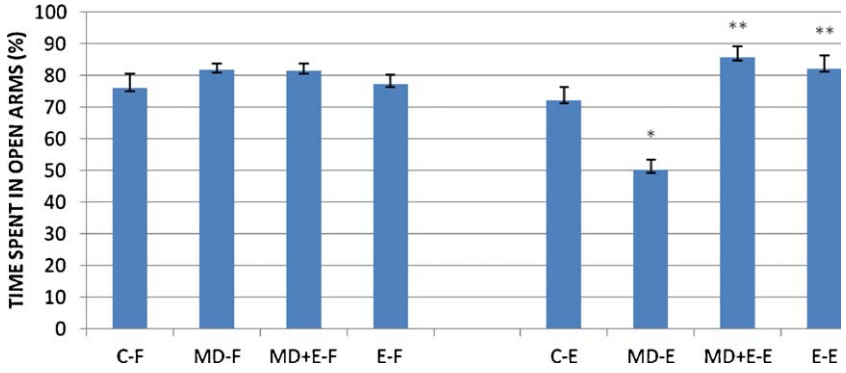


Fig. 2. Elevated plus maze test results; $^{*}p < 0.05$ compared with the same sex of control group; $^{**}p < 0.05$ compared with the MD-E group (C-F: Control females, MD-F: deprived females, MD+E-F: maternally exercised deprived females, E-F: maternally exercised females, C-E: Control males, MD-F: deprived males, MD+E-F: maternally exercised deprived males, E-F: maternally exercised males).

At the end of the behavior tests, after a light ether anesthesia, the blood of the subjects were drawn, and the PFC regions were separated after removing the brain tissue.

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

PFC 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.

Differences in the behavioral and biochemical parameters were performed using the three way ANOVA post hoc Sheefe comparisons. Results are presented as mean ± S.E.M (significance level was set at $p \leq 0.05$).

The moving time in the deprived pups and their total entries into cell were lower (moving time; females, $p < 0.05$; males, $p < 0.05$) (total entries into cell; females, $p < 0.05$; males, $p < 0.004$), compared to non-deprivation group (Figs. 1A and 2B). With maternal exercise, both male and female pups were more active in the open field test (females $p < 0.001$; males $p < 0.05$). Deprived pups spent more time in the open arms of elevated plus maze test ($p < 0.05$) (Fig. 2) and their anxiety levels were lower with the maternal exercise ($p < 0.001$). We found that the prefrontal cortex VEGF and BDNF levels decreased with the effect of MD, and these levels returned to normal with the exercise the mother had done during pregnancy (Fig. 3A and B). The basal corticosterone levels of the deprived pups, both female and male, were higher compared to non-deprived pups and maternal exercise returned to normal, blood corticosterone levels (females, $p < 0.05$; males, $p < 0.007$) (Fig. 4).

In this study, we found increased anxiety levels in the female and male maternally deprived rats. We also observed that with maternal exercise, the anxiety levels were decreased, maternally exercised rats took more risks, they had more exploring behaviors (spent more time in the open arms of elevated plus maze) and increased the levels of VEGF and BDNF in PFC, which is decrease with MD.

It is well-known that the maternal deprivation causes behavioral and neuroendocrine findings seen in anxiety disorders [13,26]. We found that the anxiety levels increased in maternally deprived rats. We also observed that the locomotor activities of deprived males increased in open field test, and they spent less time in the open arms of elevated plus maze. The locomotor activity increased in open field test in deprived females, however there were not any statistically significant difference between the groups in elevated plus maze test. This finding indicates that the anxiety levels of deprived males are higher than females. Open field and elevated plus maze tests are devices widely used in testing the anxiety levels in rodents. However, elevated plus maze causes more anxiety in the subjects than open field [12]. It is known that males are more affected from maternal deprivation compared to females. Previous studies showed that the cognitive functions of maternally deprived males were more affected adversely, and their anxiety levels were higher, compared to females [12]. In this study, we found that maternal exercise during pregnancy decreased the maternal deprivation induced anxiety. To our knowledge, this is the first study regarding the effect of maternal regular aerobic exercise on the maternal deprivation induced anxiety. It was previously showed that the exercise decreased anxiety levels in humans and animals [3,4,15,16].

MD is causes long-term hyperactivity of the hypothalamus-pituitary-adrenal axis in pups [21,34]. Maternally deprived rat's blood glucocorticoid levels are high in rest and in mild stress [34]. In this study, the basal corticosteroid levels were higher in maternally deprived female and males. The close contact between mother–infant and mother's licking the pups changes the glucocorticoid receptor expression in brain regions situated in the glucocorticoid feedback areas such as hippocampus. In previous studies it was found that glucocorticoid receptor expression decreased in deprived rats, which is indicated decreased negative feedback [27]. In this study, maternal exercise decreased the basal blood corticosterone levels in deprived rats. Stress, during the development of dopaminergic system, can cause permanent behavioral changes by altering dopamine transmission in the mesocorticolimbic regions of the brain [14,19]. MD increases the dopamine turnover in mesocorticolimbic brain regions [2,15]. Stress-induced activation of the mesocortical dopaminergic path has a significant role in the pathophysiology of mood disorders such as anxiety disorder [9]. Exercise stimulates the calcium metabolic hormone and supports the blood calcium levels, increases the brain calcium, and supports the endogenous dopamine synthesis and secretion in the dopamine metabolism related regions such as PFC [35]. Thus, the balanced dopamine level may be considered to be useful in treating diseases such as anxiety disorder, in which the dopamine transmission is increased.

Neurotrophic factors are important agents in the growth, development and plasticity of the brain. The change of neurotrophic factor levels during growth can affect the normal growth and maturation of brain, thus can cause long term changes in the brain functions and activity. It has been previously reported that BDNF, which is an important neurotrophic factor in neuronal survival, differentiation and synaptic plasticity, is also significant in the pathophysiology of mood disorders [5]. In previous studies, it was observed that BDNF-deficient rats were more aggressive and their anxiety levels were higher. In humans, who had a BDNF gene variant which causes decreased secretion of BDNF, neuropsychiatric

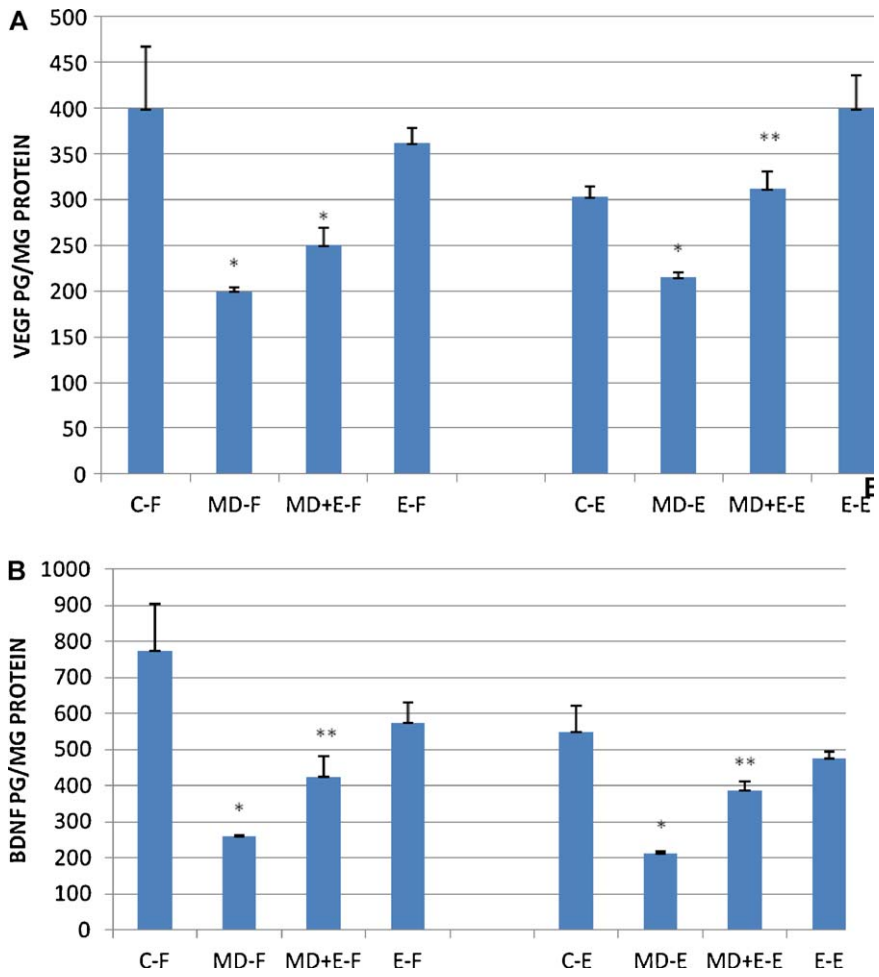


Fig. 3. Prefrontal cortex VEGF (vascular endothelial growth factor) and BDNF (brain derive growth factor) results; (A) VEGF levels, (B) BDNF levels. $^{*}p < 0.05$ compared with the same sex of control group; $^{**}p < 0.05$ compared with the same sex of deprived group (C-F: Control females, MD-F: deprived females, MD+E-F: maternally exercised deprived females, E-F: Maternally exercised females, C-E: Control males, MD-F: deprived males, MD+E-F: maternally exercised deprived males, E-F: Maternally exercised males).

disorders such as anxiety disorder were more common [8]. Irregularities has been detected in VEGF signaling in anxious and depressed individuals, thus angiotrophic and neurotrophic factors had a role in the pathophysiology of psychiatric diseases [20]. VEGF, which is an angiogenic, neurogenic, and neurotrophic cytokine, induces the proliferation, migration and vasopermeability of the vascular endothelial cells. Regulation of neurotrophic factors, changes in levels of hormones and neurotransmitters, can easily be affected by stress. It was showed that MD decreased BDNF levels in PFC and hippocampus [6]. In this study, we observed that BDNF and VEGF levels decreased in PFC of maternally deprived rats. We could not find any data about effects of MD on the brain VEGF levels. In addition, we also showed that the maternal regular aerobic exercise increased the pup's prefrontal cortex VEGF and BDNF levels.

It is well known that the PFC controls the stress response and emotional responses. In previous studies, it was showed that people with a damaged PFC had problems in emotional regulation in both their daily lives and in laboratory tests. In disorder of emotional regulation, which is so-called frontal disinhibition syndrome, it is seen that the individuals experience emotional bursts disproportionate to the provocation and take excessive risks [1]. In neuroimaging studies, it was demonstrated that the PFC inhibits the autonomous and endocrine response to stress, and reduce anxiety [22]. In PFC damage, the inhibition of psychological and physiological responses to stress disappears [22]. In addition to these, PFC regulates the secretion of dopamine from the mesocortical projection system, which is an important neuromodulator in fear and anxiety [7]. Cortical and limbic dopamine dysfunction causes to the emergence of anxiety; decreased dopamine input from cortical structures and amygdala to the nucleus accumbens causes to an exacerbation of symptoms in people with anxiety disorders [28]. In this study, we also investigated the VEGF and BDNF levels in the PFC, which is an important brain region in anxiety disorders. BDNF, with known anxiolytic effect, has also trophic and modulatory effects on the growth and plasticity of dopaminergic, serotonergic and other neurons [5]. The dopaminergic neurons in the ventral midbrain express the BDNF-mRNA. In various studies on cerebral cortex cultures, it was found that dopamine was induced by BDNF [11]. The stimulation of dopamine causes to increase the BDNF-mRNA and protein. The BDNF infusion to substantia nigra increases the dopamine turnover in striatum [18], however, the loss of BDNF expression causes dopaminergic neuronal death [23]. It was showed that dopamine secretion from the PFC was related to the regulation of anxiety and fear [22]. The VEGF, with reduced levels in the brain in anxiety, has protective effect on the dopaminergic neurons [36]. In this study, the decreased levels of VEGF and BDNF, due to MD, might have affected the dopaminergic neurons, which have regulatory effects on anxiety and fear.

With respect to these findings, the anxiety levels of these rats were high. Maternal exercise during pregnancy increased the BDNF and VEGF levels of pups PFC and may be affected the dopaminergic neurons, thus decreased the anxiety levels.

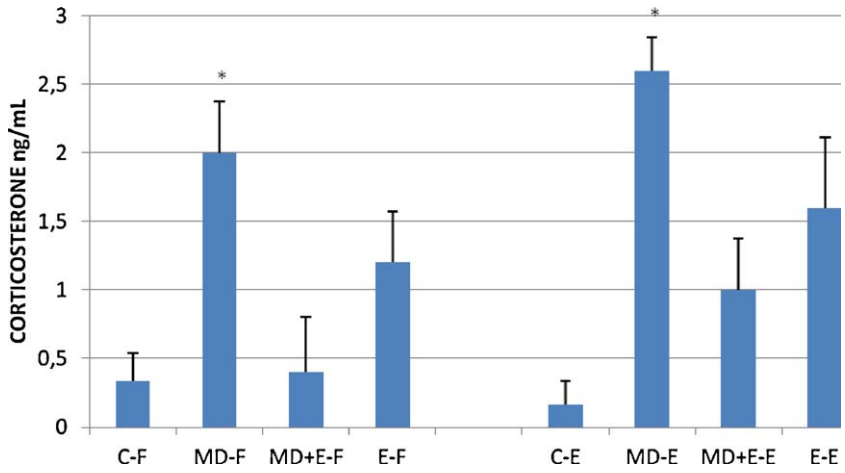


Fig. 4. Basal blood corticosteroid levels; $^{*}p < 0.05$ compared with the other groups (C-F: Control females, MD-F: deprived females, MD+E-F: maternally exercised deprived females, E-F: maternally exercised females, C-E: Control males, MD-F: deprived males, MD+E-F: maternally exercised deprived males, E-F: maternally exercised males).

In conclusion, MD decreases the prefrontal VEGF and BDNF levels and increases the anxiety levels of pups; light maternal regular aerobic exercise increases the BDNF and VEGF levels of these pups PFC, and decreases the anxiety levels. Further studies are needed to elucidate the role of BDNF and VEGF on the MD induced changes in dopamine metabolism.

References

[1] S. Bishop, J. Duncan, M. Brett, A.D. Lawrence, Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli, Nat. Neurosci. 7 (2004) 184–188.
[2] K. Braun, E. Lange, M. Metzger, G. Poeggel, Maternal separation followed by early social deprivation affects the development of monoaminergic fiber systems in the medial prefrontal cortex of *Octodon degus*, Neuroscience 95 (2000) 309–318.
[3] B. Cakır, O. Kasımay, M. Kolgazi, Y. Ersoy, F. Ercan, B.C. Yeğen, Stress-induced multiple organ damage in rats is ameliorated by the antioxidant and anxiolytic effects of regular exercise, Cell Biochem. Funct. 28 (2010) 469–479.
[4] V. Carola, F. D'Olimpio, E. Brunamonti, F. Mangia, P. Renzi, Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice, Behav. Brain Res. 134 (2002) 49–57.
[5] Z.Y. Chen, D. Jing, K.G. Bath, A. Ieraci, T. Khan, C.J. Siao, D.G. Herrera, M. Toth, C. Yang, B.S. McEwen, B.L. Hempstead, F.S. Lee, Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior, Science 314 (2006) 140–143.
[6] D. Floden, M.P. Alexander, C.S. Kubu, D. Katz, D.T. Stuss, Impulsivity and risk-taking behavior in focal frontal lobe lesions, Neuropsychologia 46 (2008) 213–223.
[7] L.E. Goldstein, A.M. Rasmusson, B.S. Bunney, R.H. Roth, Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat, J. Neurosci. 16 (1996) 4787–4798.
[8] P. Gormanns, N.S. Mueller, C. Ditzen, S. Wolf, F. Holsboer, C.W. Turck, Phenome–transcriptome correlation unravels anxiety and depression related pathways, J. Psychiatr. Res. 45 (2011) 973–979.
[9] J.W. Jahng, V. Ryu, S.B. Yoo, S.J. Noh, J.Y. Kim, J.H. Lee, Mesolimbic dopaminergic activity responding to acute stress is blunted in adolescent rats that experienced neonatal maternal separation, Neuroscience 171 (2010) 144–152.
[10] H. Kim, S.H. Lee, S.S. Kim, J.H. Yoo, C.J. Kim, The influence of maternal treadmill running during pregnancy on short-term memory and hippocampal cell survival in rat pups, Int. J. Dev. Neurosci. 25 (2007) 243–249.
[11] E. Küppers, C. Beyer, Dopamine regulates brain-derived neurotrophic factor (BDNF) expression in cultured embryonic mouse striatal cells, Neuroreport 12 (2001) 1175–1179.
[12] C.O. Ladd, K.V. Thrivikraman, R.L. Huot, P.M. Plotsky, Differential neuroendocrine responses to chronic variable stress in adult Long Evans rats exposed to handling-maternal separation as neonates, Psychoneuroendocrinology 30 (2005) 520–533.
[13] H.H. Lee, H. Kim, J.W. Lee, Y.S. Kim, H.Y. Yang, H.K. Chang, T.H. Lee, M.C. Shin, M.H. Lee, M.S. Shin, S. Park, S. Baek, C.J. Kim, Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups, Brain Dev. 28 (2006) 147–154.
[14] D. Liu, J. Diorio, B. Tannenbaum, C. Caldji, D. Francis, A. Freedman, S. Sharma, D. Pearson, P.M. Plotsky, M.J. Meaney, Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress, Science 277 (1997) 1659–1662.
[15] R. Llorente, E. O'Shea, M.D. Gutierrez-Lopez, A. Llorente-Berzal, M.I. Colado, M.P. Viveros, Sex-dependent maternal deprivation effects on brain monoamine content in adolescent rats, Neurosci. Lett. 479 (2010) 112–117.
[16] B.C. Long, R.V. Satvel, Effects of exercise training on anxiety: a meta analysis, J. Appl. Sports Psychol. 7 (1995) 167–189.
[17] L. Marais, S.J. van Rensburg, J.M. van Zyl, D.J. Stein, W.M. Daniels, Maternal separation of rat pups increases the risk of developing depressive-like behavior after subsequent chronic stress by altering corticosterone and neurotrophin levels in the hippocampus, Neurosci. Res. 61 (2008) 106–112.
[18] M.T. Martin-Iverson, K.G. Todd, C.A. Altar, Brain-derived neurotrophic factor and neurotrophin-3 activate striatal dopamine and serotonin metabolism and related behaviors: interactions with amphetamine, J. Neurosci. 14 (1994) 1262–1270.
[19] J.L. McIntosh, E.K. Hong, R.M. Sapolsky, Glucocorticoids may alter antioxidant enzyme capacity in the brain: baseline studies, Brain Res. 791 (1998) 209–214.
[20] E. Ognibene, W. Adriani, A. Caprioli, O. Ghirardi, S.F. Ali, L. Aloe, G. Laviola, The effect of early maternal separation on brain derived neurotrophic factor and monoamine levels in adult heterozygous reeler mice, Prog. Neuropsychopharmacol. Biol. Psychiatry 32 (2008) 1269–1276.
[21] R. Pascual, S.P. Zamora-León, Effects of neonatal maternal deprivation and postweaning environmental complexity on dendritic morphology of prefrontal pyramidal neurons in the rat, Acta Neurobiol. Exp. (Wars.) 67 (2007) 471–479.
[22] M.A. Pezze, J. Feldon, Mesolimbic dopaminergic pathways in fear conditioning, Prog. Neurobiol. 74 (2004) 301–320.
[23] M.J. Porritt, P.E. Batchelor, D.W. Howells, Inhibiting BDNF expression by antisense oligonucleotide infusion causes loss of nigral dopaminergic neurons, Exp. Neurol. 192 (2005) 226–234.
[24] D. Rice, S. Barone Jr., Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models, Environ. Health Perspect. 108 (2000) 511–533.
[25] S. Rojas Vega, A. Knicker, W. Hollmann, W. Bloch, H.K. Strüder, Effect of resistance exercise on serum levels of growth factors in humans, Horm. Metab. Res. 42 (2010) 982–986.
[26] D.R. Russell, A. Mueller, H.M. Sisti, S. Ogawa, B.S. McEwen, W.G. Brake, Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation, Horm. Behav. 43 (2003) 561–567.
[27] S. Salim, N. Sarraj, M. Taneja, K. Saha, M.V. Tejada-Simon, G. Chugh, Moderate treadmill exercise prevents oxidative stress-induced anxiety-like behavior in rats, Behav. Brain Res. 208 (2010) 545–552.
[28] K.B. Seroogy, K.H. Lundgren, T.M. Tran, K.M. Guthrie, P.J. Isackson, C.M. Gall, Dopaminergic neurons in rat ventral midbrain express brain-derived neurotrophic factor and neurotrophin-3 mRNAs, J. Comp. Neurol. 342 (1994) 321–334.
[29] K. Tugyan, N. Uysal, D. Ozdemir, U. Sonmez, C. Pekcetin, G. Erbil, A. Sonmez, Protective effect of melatonin against maternal deprivation-induced acute hippocampal damage in infant rats, Neurosci. Lett. 398 (2006) 145–150.
[30] N. Uysal, S. Gonenc, O. Acikgoz, C. Pekçetin, B.M. Kayatekin, A. Sonmez, I. Semin, Age-dependent effects of maternal deprivation on oxidative stress in infant rat brain, Neurosci. Lett. 384 (2005) 98–101.
[31] N. Uysal, A.R. Sisman, S. Gonenc, O. Acikgoz, B.M. Kayatekin, G. Yalaz, Effects of repeated maternal separation on oxidative stress in adolescent male and female rat brains, J. Neurol. Sci. (Turk.) 25 (2008) 155–162.
[32] N. Uysal, D. Ozdemir, A. Dayi, G. Yalaz, A.K. Baltaci, C.S. Bediz, Effects of maternal deprivation on melatonin production and cognition in adolescent male and female rats, Neuro Endocrinol. Lett. 26 (2005) 555–560.
[33] N. Uysal, K. Tugyan, B.M. Kayatekin, O. Acikgoz, H.A. Bagriyanik, S. Gonenc, D. Ozdemir, I. Aksu, A. Topcu, I. Semin, The effects of regular aerobic exercise

in adolescent period on hippocampal neuron density, apoptosis and spatial memory, Neurosci. Lett. 383 (2005) 241–245.
[34] M.P. Viveros, R. Llorente, F. Díaz, S.Y. Romero-Zerbo, F.J. Bermudez-Silva, F. Rodríguez de Fonseca, J. Argente, J.A. Chowen, Maternal deprivation has sexually dimorphic long-term effects on hypothalamic cell-turnover, body weight and circulating hormone levels, Horm. Behav. 58 (2010) 808–819.
[35] G.J. Wang, N.D. Volkow, J.S. Fowler, D. Franceschi, J. Logan, N.R. Pappas, C.T. Wong, N. Netusil, PET studies of the effects of aerobic exercise on human striatal dopamine release, J. Nucl. Med. 41 (2000) 1352–1356.
[36] T. Yasuhara, T. Shingo, K. Muraoka, M. Kameda, T. Agari, Y. Wen Ji, H. Hayase, H. Hamada, C.V. Borlongan, I. Date, Neurorescue effects of VEGF on a rat model of Parkinson's disease, Brain Res. 1053 (2005) 10–18.