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ORIGINAL ARTICLE

Effects of maternal deprivation on melatonin production and cognition in adolescent male and female rats

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Abstract

OBJECTIVES: It is known that maternal deprivation (MD) may alter cognitive functions such as learning and memory in adult life by effecting normal growth and development. However, the mechanisms of these cognitive alterations are unknown. The aim of this study is to investigate the effects of maternal deprivation on cognition and melatonin production in adolescent male and female rats.
METHODS: The litters were separated daily from their mothers for 6 hours on postnatal days 2 to 20. The spatial memory performance was evaluated using a Morris water maze between the postnatal 26th and 32nd days. Plasma melatonin levels were determined on postnatal days 42.
RESULTS: MD-rats had longer escape latencies at the second, third and fifth days of training days and spend significantly less time in probe trial, compared to control animals.
MAIN FINDINGS: The repeated maternal deprivation caused low blood melatonin levels and there was a significant negative correlation between blood melatonin levels and spatial memory performance in both of male and female adolescent rats.
CONCLUSION: These results suggest an association between melatonin production and neurodevelopment. Further studies are needed to determine the interaction between maternal deprivation and pineal gland maturation/function.

Abbreviations

ACTH – adrenocorticotropin hormone
HPA: – hypothalamic–pituitary–adrenal
MD: – maternal deprivation
ROS: – reactive oxygen species

Introduction

The developing brain is much more sensitive to environmental exposures. Early-life stress may cause dysfunctional changes in the central nervous system, and be associated with increased risks of depressive psychopathology and neurodegenerative diseases in adult life.

The repeated maternal deprivation might influence neuronal development during infancy and increase the susceptibility to neuropsychiatric disorders such as anxiety, personality disorders, schizophrenia and depression [4, 12, 29].

Maternal deprivation can induce several developmental abnormalities including the neurochemical and endocrinologic changes, alterations of hippocampal neuroplasticity, and infralimbic cortex synaptic connections in adulthood [9, 13, 24, 26]. Several studies have mainly focused on measuring levels of stress-related hormones, receptors and neurotransmitters such as corticotropin-releasing factor, glucocorticoid receptors, and norepinephrine to identify the pathogenetic mechanisms of neurobehavioral changes observed following maternal deprivation [10, 15, 17, 20, 30, 33, 37].

Melatonin, a potent free radical scavenger and antioxidant, enhances stress management by counteracting the immunosuppressive influence of acute stress [18]. Melatonin has been suggested to reduce the affinity of glucocorticoid receptor in rat brain and affected on regulation of glucocorticoid receptor gene expression [19, 27]. Melatonin secretion is altered in subjects suffering from affective disorders, eating disorders, and schizophrenia [25]. Low melatonin levels in infants and children were shown to be linked to epilepsy, an apparent life-threatening event, and sudden infant death syndrome [3, 32, 38]. However, the effect of maternal separation on the melatonin production is not known in developing rats.

Maternal deprivation activates the hypothalamic–pituitary–adrenal (HPA) axis, demonstrating that the separation experience is stressful for the neonate [17]. Exposure to high levels of glucocorticoids during this critical period in development has been shown to lead to numerous detrimental effects on the developing central nervous system [31]. Stress-induced elevations in corticosterone may play a critical role in activation of dopamine transmission and may interact with mesocorticolimbic brain regions [21]. Maternal deprivation increases dopamine turnover in mesolimbic region of brain in laboratory animals [34]. Inhibition of dopamine release by melatonin has been demonstrated in specific areas of the central nervous system including hypothalamus, hippocampus, medulla-pons, and retina. The interaction of melatonin with the dopaminergic system and its antioxidant nature has been suggested to be beneficial in the treatment of dopamine-related disorders [40,41].

Thus, the aim of the present study was to evaluate the effects of maternal deprivation on melatonin production and spatial memory in adolescent rats.

Methods

Subjects and experimental design

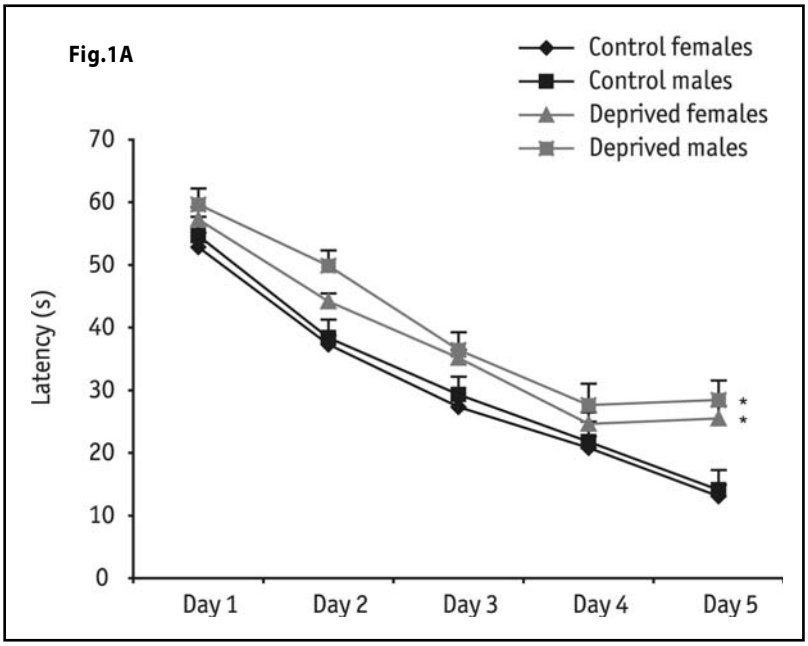
Wistar rats with dated pregnancies (n=4) were maintained at the same center and housed in individual cages. Thirty-two litters delivered spontaneously. The date of birth was designated day 0. The animals were maintained under standard colony conditions with a 12 h light/dark cycle (lights on 07:00 h) at constant room temperature (23 ± 2 °C), and humidity (60%) and ad lib food and water throughout the experiments. Experiments were carried out between 9:00–12:00 in a sound-attenuated and air-regulated experimental room. 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. Rats were divided into four groups: (1) maternal deprived male group (MD-male) (n=8), (2) Maternal deprived female group (MD-female) (n=8), (3) male control group (C-male) (n=8), (4) female control group (C-female) (n=8).

Maternal deprivation procedure

The litters were separated daily from their mothers for 6 hours between 09:00 and 15:00 h on postnatal days 2 to 20. During separation, the pups of each litter were kept together in small compartment filled with clean bedding. This compartment was in a separate room and warmed by a heating pad (33 ± 0.2 °C), while the other half of the pups remained with the dam. At the conclusion of the separation period, pups were returned to their maternity cage, followed by reunion with the dam. Bedding in the pup and transfer cages was never changed. After weaning (postnatal day 22), the siblings were separated by sex and housed together in standard plastic cages until postpartum day 42.

Morris water maze testing

The spatial memory performance was evaluated using a Morris water maze. All rats were subjected to water maze tests between the postnatal 26th and 32nd days. The Morris water maze was 200 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 platform. The pool was filled with opaque water to prevent visibility of the platform in the pool. The animals were tested for 6 days in a water maze task. On each day, rats were placed in the water (22 ± 1 °C) and allowed to swim (maximum swim time 60 s) until they located a hidden, but fixed escape platform, using extra maze cues. The escape platform was placed in the middle of one of the random quadrants of the pool. If the animal failed to locate the platform in 60 s, the experimenter placed the rat on the platform and left it there for 20 s. Each rat was tested for four consecutive trials per day, with an inter-trial interval of 60 s. Each rat was exposed to the task for 5 consecutive days (a total of 20 trials), and on day 6 a probe trial was run in which the platform was removed from the pool, and rats were placed into the pool and swam for 60 s. The data were analyzed for latency to find the platform and time spent in the correct quadrant. Morris water maze training was recorded using a video camera mounted to the ceiling.



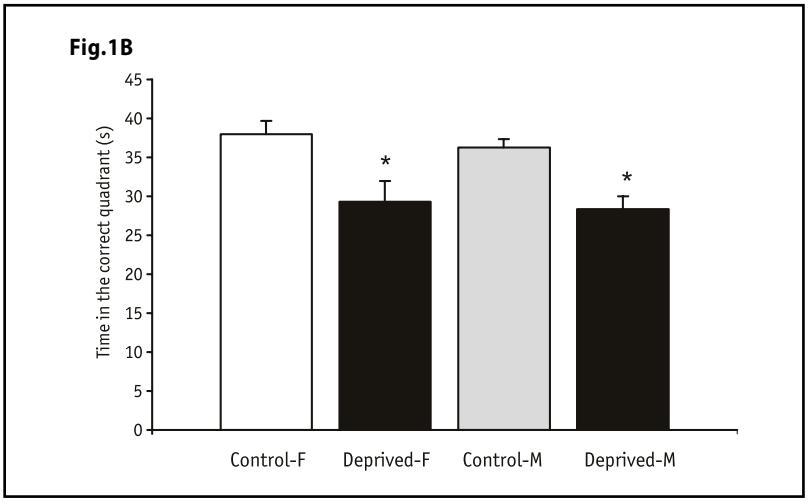


Figure 1. Effects of maternal deprivation on spatial learning in the Morris water maze (**Fig.1A**) and time spent in the correct quadrant in the probe trial (**Fig.1B**). Data are presented as the mean ± SEM. *p<0.05 compared with the control group.

Determination of Plasma Melatonin Levels

Plasma melatonin levels were determined on postnatal days 42 in S.U. Meram Medical School Hospital, Central Biochemistry Laboratory using melatonin kit "IBL-Hamburg (Germany)" according to ELISA method as pg/ml.

Statistical analysis

The data from water maze testing were analyzed by using one-way ANOVA with repeated measures on the day factor. Comparisons between groups (control and deprived rats) were performed using the Kruskal–Wallis H-test analysis of variance by ranks. In the case of a significant H value (significance level was set at $p \leq 0.05$), the Mann–Whitney U-test was used for matched pairs post hoc comparisons. Correlation between spatial memory, and blood melatonin levels was calculated using Spearman correlation analyze.

Results

Morris water maze

The present study showed that MD induced significant cognitive deficits in rats. The mean latency to find the platform declined progressively during the period of postnatal day 26–32 in all animals. However, MD-rats had longer escape latencies at the second ($p<0.05$), third ($p<0.001$) and fifth ($p<0.001$) days of training days than control animals (*Figure 1A*). In probe trials (quadrant time), time spent in target quadrant was used to evaluate long-term memory. MD-animals spend significantly less time than control groups ($p<0.001$, *Figure 1B*). These results indicated that MD significantly impaired water maze performance in male and female rats.

Plasma melatonin levels

The mean melatonin levels are demonstrated in *Figure 2*. Melatonin levels were significantly decreased by MD in both female and male rats compared with the control group ($p<0.001$). The female rats had higher

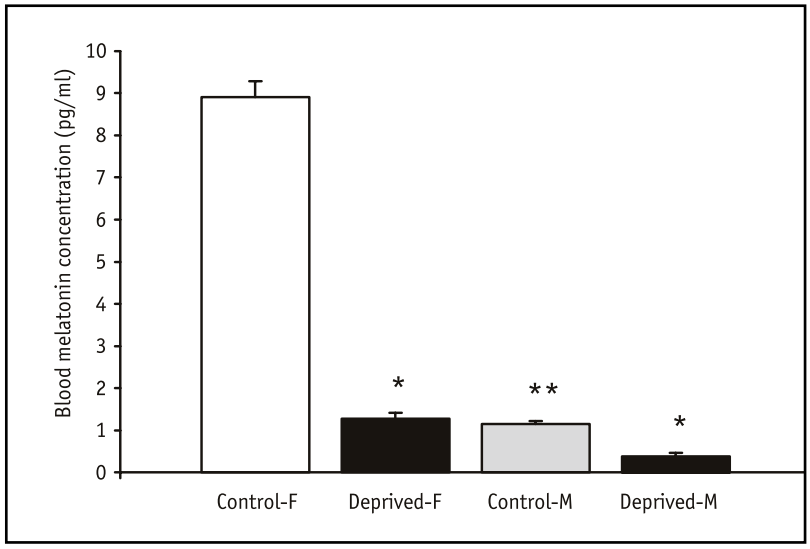


Figure 2. Effects of maternal deprivation on blood melatonin levels. Data are presented as the mean ± SEM. *p<0.05 compared with the control group, **p<0.05 compared with the control-F group.

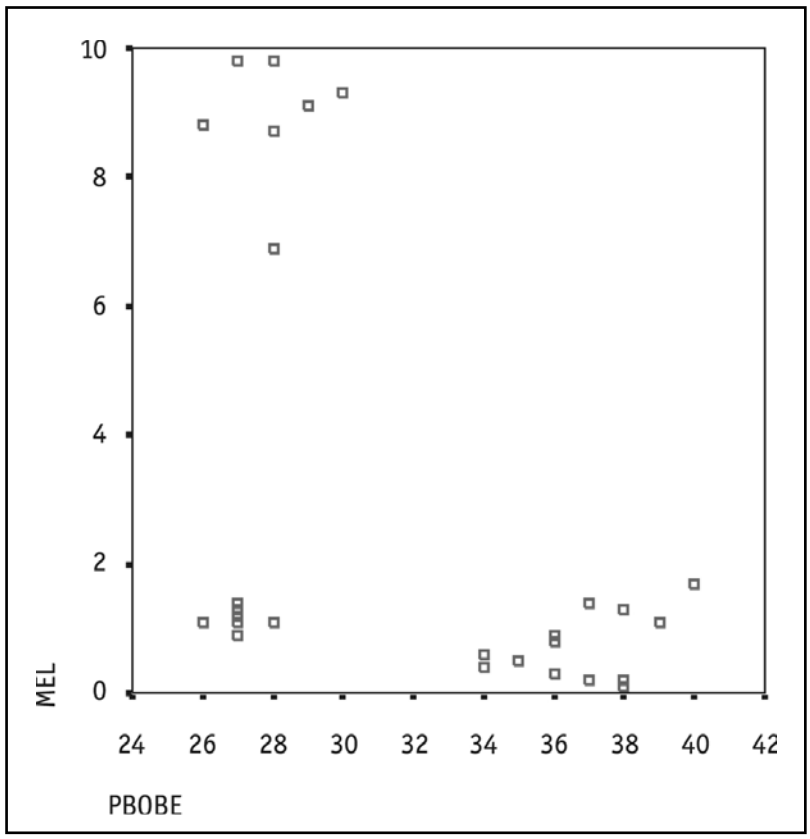


Figure 3. The scattergrams of probe trials. P-significance for blood melatonin levels, p<0.05.

melatonin levels than those of males. We assessed the correlation between mean latency of probe trial and blood melatonin levels and found a significant negative correlation (p=0.029, r=–0.420). The scattergram of the correlated values is presented in *Figure 3*.

Discussion

The results of the present study indicate the long-term maternal deprivation cause low melatonin production and spatial memory deficits in adolescent period. It is well known that the repeated maternal deprivation cause the long-term consequences including stress hyper-responsiveness, anxiety-like behavior and cognitive deficits, which can persist throughout adulthood [4,12,29]. Additionally, even a single episode of maternal deprivation for 24 h during infancy is sufficient to induce cognitive deficits in the rats as adults [23]. Maternal deprivation had been shown to alter normal brain development by increasing the neuronal death and decreasing in cell proliferation in the dentate gyrus of the hippocampus in the infant rats [14, 16, 39]. Hippocampal neurons normally play a crucial role in the processing of spatial memory and learning [22].

While the long-term neurobehavioral sequel of maternal deprivation has been well documented, there is still relatively little known as to how maternal deprivation affects brain development of the infant rat. Prolonged maternal deprivation can disrupt the developmental pattern of the HPA axis: it enhances the sensitivity of

adrenals to adrenocorticotropin hormone (ACTH) and/or plasma ACTH levels, which then increases plasma corticosterone levels [10,15,17,20,30,33,37]. Corticosterone increases the toxicity of oxygen radical generators, and may increase the basal level of reactive oxygen species (ROS) in the hippocampus and cortex. Stress-induced elevations in corticosterone may play a critical role in activation of dopamine transmission and may interact with mesocorticolimbic brain regions [21]. Maternal deprivation increases dopamine turnover in mesolimbic region of brain in laboratory animals [34]. Increased turnover of dopamine evokes an oxidative stress derived from increased production of hydrogen peroxide. Generation of ROS can be a major component of decreased cell function and eventual death [1,7]. In a previous study, we showed that maternal deprivation reduced enzyme activities and increased lipid peroxidation in brain of the infant rats [36].

In the present study, maternally deprivated rats exhibit low melatonin levels in adolescent period, which was parallel with spatial learning deficits. To our knowledge, this is the first report indicating that maternal deprivation cause low melatonin production in the adolescent period. Melatonin is secreted by pineal gland which is a neuroendocrine transducer in mammals. Although its role in child development is unclear, the classical functions of melatonin are associated with the control of the circadian rhythms and the regulation of HPA [2,28]. Absolute melatonin production has been shown to remain constant during childhood and adolescence [6]. However, it was demonstrated a relationship between low melatonin production in the infancy and impaired psychomotor development [35]. Several studies reported the beneficial effect of exogeneous melatonin in brain function and neurologic development. Recently, exogenous melatonin has been reported to decrease the expression of glucocorticoid receptor and to increase cell proliferation in the dentate gyrus of maternally deprivated rats [14].

The female rats had higher melatonin levels than those of males in the present study. Such difference may be related with the interaction between melatonin and corticosterone. Corticosterone may influence melatonin levels via affecting on serotonergic mechanism [5]. Females tend to have higher basal levels of corticosterone than males and this may partially account for the sex difference [8]. An interaction between melatonin and sex was mentioned by Hill et al. [11], and they claimed that greater increase in activity in females than males may be related with the melatonin.

In summary, the present study suggested that maternal deprivation cause low melatonin levels and spatial memory deficits in adolescent period, revealing an association between melatonin production and neurodevelopment. Further studies are needed to determine the interaction between maternal deprivation and pineal gland maturation/function.

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