Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1844181

Anatolian Curr Med J. 2026;8(2):179-186

Maternal separation disrupts emotional, cognitive, and metabolic development in rats: protective and regulatory effects of environmental enrichment

Serap Ata*[1], Hamit Özyürek[2]

[1]Department of Pediatrics, Ümraniye Training and Research Hospital, İstanbul, Turkiye

[2]Department of Pediatric Neurology, Ankara Bilkent City Hospital, Ankara, Turkiye

Cite this article as: Ata S, Özyürek H. Maternal separation disrupts emotional, cognitive, and metabolic development in rats: protective and regulatory effects of environmental enrichment. *Anatolian Curr Med J.* 2026;8(2):179-186.

Received: 17.12.2025 • **Accepted**: 17.01.2026 • **Published**: 10.03.2026

ABSTRACT

Aims: Early-life maternal separation (MS) induces long-term metabolic and neurobehavioral disturbances, whereas environmental enrichment (EE) is considered a protective intervention; however, potential sex-dependent responses remain unclear. This study evaluated whether adolescent EE mitigates MS-related alterations in growth and emotional-cognitive outcomes and whether these effects differ between males and females.

Methods: Sprague-Dawley litters were allocated to control, MS, or MS+EE groups. MS consisted of daily 180-minute separations from postnatal day (PND) 2-14, and EE was initiated after PND14. Body weight was monitored at several developmental stages. Neurobehavioral assessments included the open field test (OFT), elevated plus maze (EPM), forced swim test (FST), and passive avoidance test (PAT). Data were analyzed using ANOVA with Tukey-Kramer post hoc comparisons.

Results: MS resulted in significantly reduced weight gain on PND7 ($p<0.001$). By PND28, both MS and MS+EE groups displayed greater weight gain than controls ($p<0.05$ and $p<0.0001$, respectively), with EE animals showing the highest weight by week 10 ($p<0.0001$), particularly males. MS decreased locomotor activity in the OFT ($p<0.001$), increased anxiety-like behavior in the EPM ($p<0.05$), elevated FST immobility ($p<0.0001$), and impaired PAT memory ($p<0.05$). EE reversed these deficits, enhancing locomotion ($p<0.0001$), reducing immobility ($p<0.05$), and improving memory retention ($p<0.001$). Females exhibited more pronounced anxiolytic and cognitive benefits.

Conclusion: MS produces persistent metabolic and neurobehavioral impairments, whereas EE confers significant restorative effects with notable sex-specific variation.

Keywords: Maternal separation, environmental enrichment, anxiety-like behavior, cognitive function, sex differences, body weight regulation,rats

INTRODUCTION

Experimental and clinical studies have demonstrated that early-life stressors induce endocrine, autonomic, and behavioral stress responses by creating long-lasting vulnerability in corticotropin-releasing factor and other neurotransmitter systems. As a consequence of this heightened susceptibility, repeated exposure to stress plays a critical role in the development of depression, anxiety disorders, and other psychophysiological conditions.[1-3] Clinical and epidemiological evidence further indicates that adverse experiences during early development increase vulnerability to psychopathology in adulthood.[4,5] Accordingly, experimental models of early-life stress-particularly the MS paradigm-are widely employed to investigate the neurobiological mechanisms underlying stress-related disorders.

During the first postnatal weeks, rat pups depend entirely on maternal care for thermoregulation, nutrition, and appropriate maturation of the hypothalamic-pituitary-adrenal (HPA) axis.[6] Prolonged MS disrupts mother–infant interactions and has been shown to induce HPA axis hyperresponsiveness, reduced neurogenesis, decreased glucocorticoid receptor expression, and alterations in synaptic plasticity.[7,8] At the behavioral level, MS has been consistently associated with increased anxiety-and depressive-like behaviors, as well as impairments in learning and memory.[7,8]

The early postnatal period represents a critical window of heightened neuroplasticity, characterized by intense hippocampal neurogenesis, elevated synaptic plasticity, and rapid maturation of neuronal networks.[9] During this sensitive developmental phase, environmental experiences

*Corresponding Author: Serap Ata, serapataaydemir@hotmail.com

This work is licensed under a Creative Commons Attribution 4.0 International License.

can profoundly shape neural and behavioral trajectories. In this context, EE has emerged as a potent modulator of brain function, known to enhance synaptic plasticity, increase dendritic complexity and spine density, promote learning and memory, and attenuate stress reactivity.[10,11] EE has also been shown to upregulate neuroplasticity-related molecules, including synaptophysin and brain-derived neurotrophic factor (BDNF), particularly within the hippocampus.[12]

Accumulating evidence suggests that EE may partially counteract the deleterious behavioral and neurobiological consequences of early MS.[12,13] However, the extent to which EE administered during adolescence can mitigate or reverse the long-term effects of early postnatal MS on emotional behaviors (anxiety and depression) and cognitive functions (learning and memory) remains insufficiently understood. Moreover, despite well-documented sex-dependent differences in HPA axis regulation and hippocampal plasticity,[14] relatively few studies have systematically examined whether MS and EE exert differential effects in males and females.

This study aimed to investigate the adverse effects of maternal deprivation during the early neonatal period on somatic development, depression- and anxiety-like behaviors, and learning processes in young adulthood and adulthood. In addition, the study evaluated whether a short-term, 15-day EE applied during the neonatal period could mitigate or reverse these negative effects, and whether such effects differ according to sex.

METHODS

Ethics

In this study, 15 pregnant Sprague–Dawley rats obtained from the Ondokuz Mayıs University Laboratory Animal Breeding and Research Center were used with the approval of the Ondokuz Mayıs University Local Animal Experiments Ethics Committee (Date: 01.03.2010 Decision No: 2010/10). All procedures were conducted in accordance with the ARRIVE guidelines and the national/international guidelines for the care and use of laboratory animals.

Animals

To prevent environmental changes and stress, all animals were housed throughout the experimental period at the Ondokuz Mayıs University Laboratory Animals Breeding and Research Center under controlled conditions with a 12-hour light/dark cycle, ambient temperature of 22±1°C, and 55-60% humidity. Animals were provided standard rat chow and tap water ad libitum. For behavioral testing, two male and two female animals were selected from each litter to control for potential litter effects and to ensure a balanced sex distribution across experimental groups.

Rodent postnatal development is categorized into defined stages: PND 1-14 corresponds to infancy, PND15-21 to early childhood, PND22-34 to the juvenile period, PND35-59 to adolescence, and PND60 onwards to adulthood. In the present study, MS was conducted during the infantile period (PND2-14), while EE was initiated after PND14 and maintained throughout adolescence.[15]

Maternal Separation Method and Study Groups

Fifteen pregnant Sprague-Dawley rats were included in the study. All dams were allowed to deliver naturally, and 24 hours postpartum, the mother-offspring units were randomly assigned to three groups (**Figure 1, Table 1**). Each dam and her litter were housed in a separate cage. The day of birth was considered as PND 1.

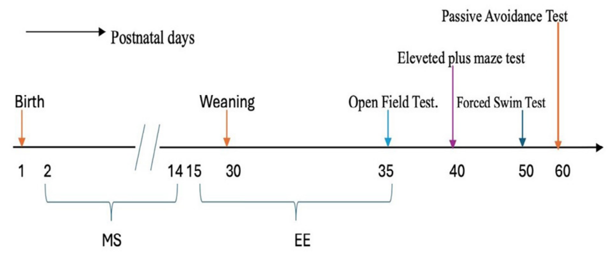


Figure 1. Experimental design

Table 1. Distribution of female and male offspring in study groups

Group 1	1A	1B	1C	1D	1E	Total: 53
(Control grup)	5 female	7 female	7 female	5 female	4 female	28 female
	4 male	5 male	7 male	4 male	5 male	25 male
Group 2	**2A**	**2B**	**2C**	**2D**	**2E**	**Total :48**
(MS)	4 female	7 female	6 female	7 female	4 female	25 female
	4 male	4 male	7 male	3 male	2 male	23 male
Group 3	**3A**	**3B**	**3C**	**3D**	**3E**	**Total: 55**
(MS +EE)	6 female	7 female	6 female	4 female	6 female	29 female
	6 male	7 male	6 male	3 male	4 male	26 male

Total: 156, Female: 74, Male: 82 MS: Maternal separation, EE: Environmental enrichment

Group I (Control; n=5): Mothers and pups were left undisturbed except for routine cage cleaning.

Group II (Maternal Separation, MS; n=5): Rats were subjected to a MS protocol consisting of daily 180-min separation (09:00-12:00) between postnatal days (PND) 2 and 14. During separation, the dam was removed from the home cage and placed individually in a separate cage within the same room. At the end of the separation period, the dam was returned to the litter. After PND 14, pups exposed to MS were housed in standard cages (42×34×15 cm).

Group III (MS+Enriched Environment, MSE; n=5): The same MS protocol was applied, and after PND 14, pups were transferred to a larger enriched environment cage until behavioral and motor evaluations were completed. The enriched environment cage (50×35×52 cm) contained plastic tunnels, ropes, swings, balls, ladders, shelters, toys, and a running wheel arranged to provide sensory and motor stimulation (**Figure 1**).

All pups were weaned on PND 30, after which dams were removed from the cages. All animals had free access to food and water. Handling was performed briefly during cage cleaning, and all cages were cleaned weekly throughout the experiment.[16]

Animals in each experimental group underwent behavioral assessments according to the timeline presented in **Figure 2**, including body weight measurements, the open field test, the elevated plus maze, and the Morris water maze.

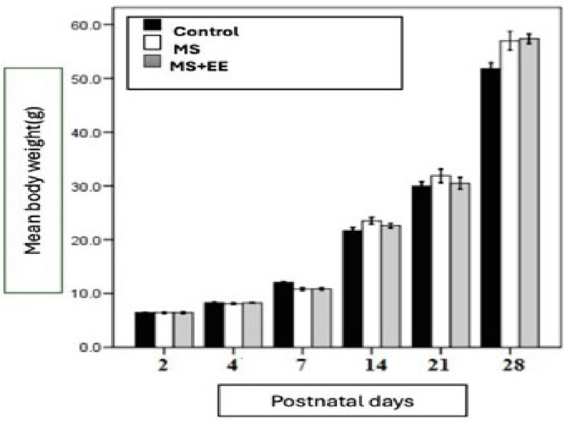


Figure 2. Mean body weight±S.E.M. among the groups

Body Weight

Body weight was recorded on postnatal days 2, 4, 7, 14, 21, and 28 for all offspring from each dam. Additionally, to evaluate the long-term effects of MS and environmental enrichment, body weight measurements were repeated at postnatal week 10.

Motor and Behavioral Assessments

For behavioral and motor evaluations, two male and two female offspring were randomly selected from each mother to eliminate hormonal variability. The following behavioral tests were performed:

Open-field test for locomotor activity: The open-field test was used to evaluate locomotor activity (number of squares crossed and rearings) and anxiety-related behaviors. Testing was conducted at PND 35 using a 100×100 cm arena divided into 16 equal sections. Each rat was placed in the center, and behavior was recorded for 5 minutes. Locomotor parameters and time spent in the center versus periphery were assessed, with increased peripheral activity indicating anxiety-like behavior and greater center time reflecting an anxiolytic effect.

Elevated plus-maze test for anxiety-like behavior: The elevated plus-maze test was used to assess anxiety-like behavior based on avoidance of open arms. The apparatus consisted of two open arms and two enclosed arms (50×10 cm each), with enclosed arms surrounded by 40 cm high walls. Testing was conducted on PND 40. Each rat was placed at the center of the maze facing an open arm, and behavior was recorded for 5 minutes. Time spent in open and enclosed arms was analyzed, with increased enclosed-arm time indicating higher anxiety levels.

Forced swim test for depressive-like behavior: The FST was used to evaluate depressive-like behavior, with increased immobility time indicating behavioral despair. Rats were tested at PND 50 in transparent cylinders (28 cm diameter, 45 cm height) filled with water at 25 °C, with a depth preventing tail contact with the bottom. Animals underwent a 15-minute habituation swim 24 hours before testing. During the test session, rats were placed in the water for 5 minutes, and immobility time was recorded.

Passive avoidance test for learning and memory: The PAT was used to assess spatial learning and memory. The apparatus consisted of a brightly lit compartment and a larger dark compartment equipped to deliver a mild foot shock. Latency to enter the dark compartment from the light compartment was recorded. The test was repeated after 24 hours, and increased latency or avoidance of the dark compartment during the second trial was interpreted as successful learning. Latency times from both sessions were analyzed.

Statistical Analysis

Data were analyzed using SPSS version 15.0. Normality of distribution was assessed using the Shapiro-Wilk test. Variables with normal distribution were expressed as mean±standard error of the mean (SEM). For multiple comparisons, one-way ANOVA followed by the Tukey-Kramer post hoc test was applied. A p-value <0.05 was considered statistically significant. In this study, sex was not included as an interaction term in the statistical models; instead, sex-specific effects were evaluated through predefined subgroup analyses.

RESULTS

This study examined the effects of EE following MS in rats. Short- and long-term changes in body weight, including sex-specific differences, were analyzed. Anxiety-like behavior and locomotor activity were assessed using the EPM and open field tests, learning and memory using the passive avoidance test, and depression-like behavior using the forced swim test, with all outcomes evaluated in a sex-dependent manner.

Body Weight

Body weights did not differ among groups on PND 2. At PND7, both maternal MS groups showed significantly reduced weight gain compared with controls (p<0.0001); however, no group differences were observed during PND14-21. By PND28, pups exposed to EE following maternal separation (MS+EE) exhibited the greatest weight gain, significantly exceeding controls (p<0.0001), while the MS group also weighed more than controls (p<0.05) (**Table 2, Figure 2**).

Table 2. Body weight by postnatal days

Group	PND2 (g)	PND7 (g)	PND14 (g)	PND21 (g)	PND28 (g)	PNW10 (g)
Control	6.3±0.8	8.1±0.7	11±0.13	21±0.33	30±0.49	51±0.78
MS	6.4±0.8	8.1±0.1	10±0.42	22±0.4	29±0.4	55±1.2
MS+EE	6.4±0.5	8.2±0.7	10±0.11	22±0.2	30±0.3	57±0.72
p value	0.91	0.88	<0.001	0.34	<0.005	<0.001

Data are presented as mean±SEM. p values indicate overall group comparisons at each postnatal time point. Values of p <0.05 were considered statistically significant. PND: Postnatal day, MS: Maternal separation, EE: Environmental enrichment

Sex-specific analyses revealed no differences at PND2. At PND7, control males and females had significantly higher body weights than both MS groups (p<0.001). By PND28, females in the MS+EE group displayed significantly higher body weights than control and MS females, with no significant sex differences observed within groups (**Table 2, 3**).

At postnatal week 10, long-term assessment demonstrated significantly higher body weights in both MS and MS+EE groups compared with controls, with the MS+EE group showing the greatest increase (p<0.0001), followed by the MS group (p<0.05 vs. control) (**Table 4, Figure 3**).

When sex was considered, males in the MS+EE group exhibited the highest body weights, exceeding females in the same group and both sexes in other groups, whereas MS females did not differ significantly from control females (**Table 3**).

Behavioral Experiments

Open Field Activity

In the open field test, square crossings, rearing, and time spent in the center were assessed (**Figure 4, Table 5**). Square crossing frequency differed significantly among groups (p<0.0001), with reduced locomotor activity in the MS group and markedly increased activity in the MS + enriched environment (MS+EE) group compared with both controls and MS animals. Sex-specific analysis indicated the greatest reduction in MS males and the highest activity in MS+EE males, while no significant within-group sex differences were observed.

Rearing activity showed a nonsignificant decrease in the MS group and a significant increase in the MS+EE group compared with MS animals (p<0.05), with no sex-related differences.

Time spent in the center was significantly reduced in the MS group, indicating increased anxiety-like behavior, whereas control and MS+EE groups exhibited comparable center times, suggesting normalization of anxiety-related behavior with environmental enrichment.

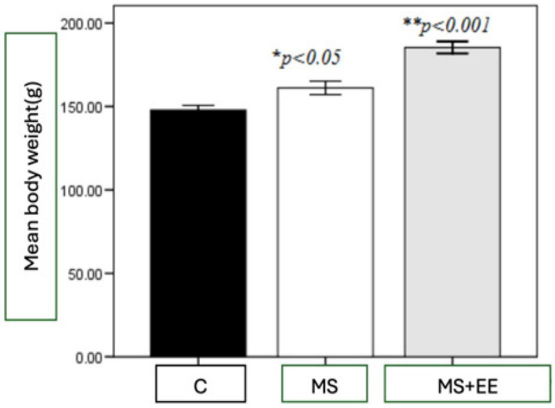
Elevated Plus Maze Test

Rats in the MS group spent significantly more time in the closed arms than both control and enriched environment groups (p<0.05), indicating increased anxiety-like behavior. No differences were observed between the control and enriched groups. Sex-specific analyses revealed no within-group differences; however, both male and female rats in

Table 4. Body weight at week 10

Groups	(n)	Mean body weight (g)±S.E.M	p value
C male	25	160±4.4.	-
C female	28	138±2.9	-
MS male	23	180±3.4	<0.005
MS female	25	143±4.8	0.18
MS+EE male	26	210±4.2	<0.001
MS+EE female	29	169±2.5	<0.001

Data are presented as mean ± SEM. p values indicate comparisons with the corresponding control group at postnatal week 10. A p value <0.05 was considered statistically significant. MS: Maternal separation, EE: Environmental enrichment



Figüre 3. Mean body weight±S.E.M values of the groups at the 10th week

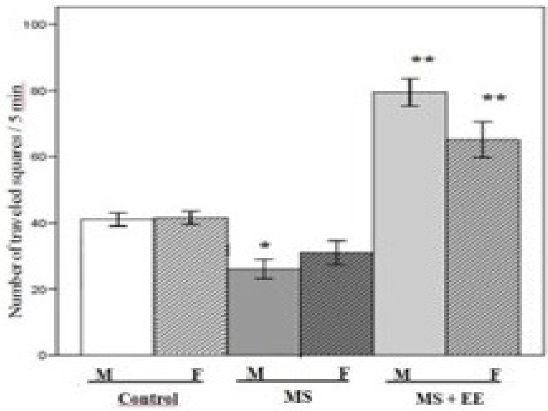


Figure 4. Effects of Maternal Separation and Environmental Enrichment on Locomotor Activity.In the open-field test, maternal separation significantly decreased the number of square crossings relative to controls (p<0.001), indicating reduced locomotor and exploratory activity. Conversely, environmental enrichment markedly increased square crossings compared with controls (*p<0.0001)

Table 3. Body weight measurements of male and female offspring across experimental groups

Groups	2.day	4.day	7.day	14.day	21.day	28.day
C male (n:25)	6.4±0.12	8.2±0.12	12.±0.19	21.6±0.6	29.3±0.86	52±1.3
C female (n:28)	6.2±0.12	8.07±0.13	11.±0.18	21.4±0.38	29.3±0.60	51.1±1.1
MS male (n:23)	6.4±0.14	8.11±0.11	10.±0.26*	23.5±0.48	31.2±1.24	56.9±1.7
MS female (n:25)	6.4±0.18	8.35±0.14	11±0.40	21.9±0.45	30.6±0.78	54.7±1.8
MS+EE male (n:26)	6.3±0.15	8.24±0.12	10.±0.21*	22.6±0.68	30.7±1.09	*57.3±1
MS+EE female (n:29)	6.5±0.07	8.39±0.16	10±0.18*	22.6±0.13	30.8±0.97	**57.2±12
p value	0.94	0.81	<0.001	0.18	0.07	<0.001

Data are presented as mean±SEM. p values indicate overall group comparisons at each postnatal time point. Values of p <0.05 were considered statistically significant. MS: Maternal separation, EE: Environmental enrichment

Table 5. Open field activity

	Group I (C)	Group II (MS)	Group III (MS+EE)	p value
Number of traveled squares	43±1.4*	26.5±0.7	78±3.9	<0.001
Number of vertical activity	18±1.6	13.6±2.1	23±1.8	<0.001
Time spent at the center (second)	47.5±3.3****	24.6±1.7	45.2±1.9	<0.001

Data are presented as mean±SEM. p values indicate overall group comparisons. A p value <0.05 was considered statistically significant. MS: Maternal separation, EE: Environmental enrichment

the MS group showed significantly greater closed-arm time compared with their respective control and enriched counterparts. EE normalized closed-arm time, with values comparable to controls (**Figure 5**).

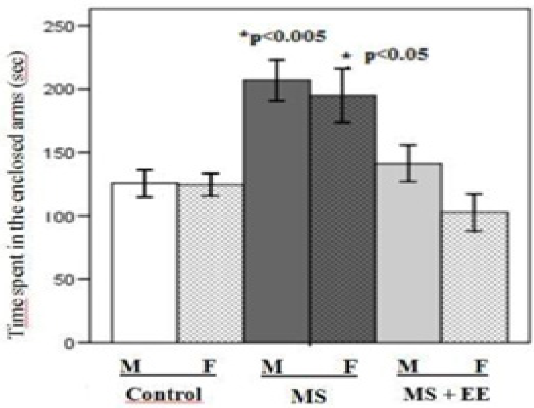


Figure 5. Effects of Maternal Separation and Environmental Enrichment on Anxiety-Like Behavior in the Elevated Plus Maze Test. The time spent in the closed arms was evaluated in male (E) and female (D) offspring across control, maternal separation (MS), and MS plus enriched environment groups. Both male and female MS offspring exhibited significantly increased closed-arm time relative to controls (*p<0.005, *p<0.05), reflecting heightened anxiety-like behavior. Environmental enrichment partially reduced this anxiety response

Forced Swim Test

Maternally separated rats exhibited significantly increased immobility time compared with controls (p<0.0001), indicating enhanced depressive-like behavior. In contrast, EE significantly reduced immobility relative to controls (p<0.05). Sex-specific analyses showed no within-group differences; however, both male and female MS rats displayed greater immobility than all other groups (**Figure 6**).

Passive Avoidance Test

During training, latency to enter the dark compartment did not differ significantly among groups. In the test session, maternally separated rats showed significantly shorter crossover latency than both control and enriched environment groups, indicating impaired learning and memory (p<0.05 vs. control; p<0.001 vs. EE), while no difference was observed between control and EE animals (**Figure 7**).

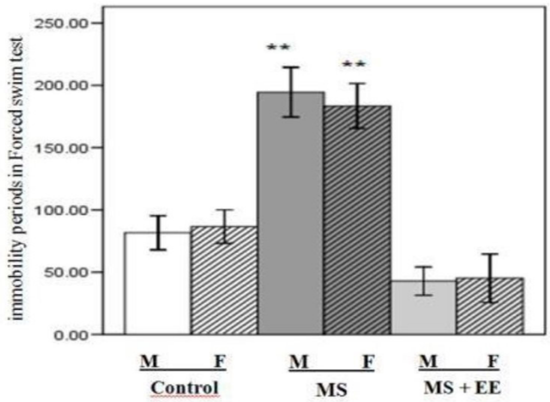


Figure 6. Immobility Time in the Forced Swim Test. Maternal separation significantly increased immobility time in both male and female offspring, indicating enhanced depressive-like behavior (**p<0.0001)

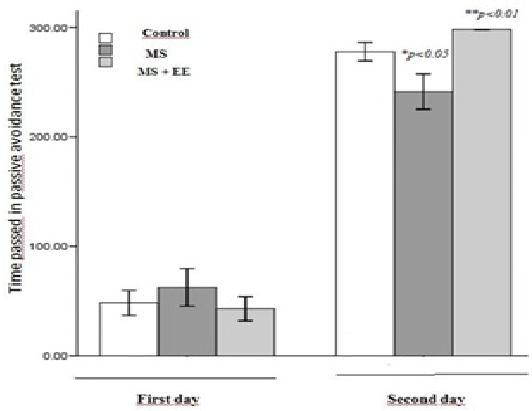


Figure 7. Passive Avoidance Test Performance Across Experimental Groups. The maternal separation group exhibited a significantly shorter latency to enter the dark compartment compared with the control group (p<0.05) and the enriched-environment group (*p<0.01), indicating impaired learning and memory retention

DISCUSSION

Our study demonstrated that neonatal MS induces both depression-like and anxiety-like behaviors in young adulthood. Pups separated from their mothers exhibited reduced locomotor activity in the open field test, increased immobility in the forced swim test, and significantly greater time spent in the closed arms of the elevated plus maze. Moreover, the time spent in the center of the open field-an indicator of reduced anxiety-was markedly decreased in the MS group. These findings are consistent with previous studies employing similar separation paradigms.[17-19]

Previous studies have shown that repeated MS during the neonatal period can transiently alter body weight gain in offspring.[20,21] In our separation model, body weight was monitored on postnatal days 2,4,7,14,21, and 28, as well as at 10 weeks of age to assess long-term effects. This approach allowed us to evaluate both the early- and late-stage effects of MS and EE on body weight. In a study by Lee et al.[22] investigating the effects of maternal deprivation on the serotonergic system, Sprague–Dawley rats were subjected to a 3-hour daily MS protocol from postnatal days 1 to 14. That study reported a marked increase in body weight gain beginning on PND 36, accompanied by increased daily food intake, with the elevated body weight persisting until PND 61.

At postnatal week 10, maternally separated rats showed significantly greater body weight gain than controls, consistent with evidence linking early-life stress to altered serotonergic regulation and increased food intake. The greatest weight gain was observed in the MS plus enriched environment group, suggesting additive effects of MS and environmental enrichment, in line with previous reports that enrichment can enhance weight gain following chronic stress exposure.[21-25] The increased body weight observed in the enriched environment group may reflect an adaptive response related to enhanced physical activity and developmental compensation; however, in the absence of detailed metabolic assessments, it should also be interpreted cautiously with regard to potential maladaptive metabolic outcomes.

Our study demonstrated that neonatal MS results in depression-and anxiety-like behaviors during young adulthood. Compared with controls, maternally separated rats exhibited reduced locomotor activity in the open field test, increased immobility in the forced swim test, and spent more time in the closed arms and less time in the open arms of the elevated plus maze. In addition, time spent in the center of the open field-an established parameter of anxiety-was markedly reduced in the maternal deprivation group. These findings are consistent with previous studies employing similar MS paradigms.[23-25] In line with our results, Kalinichev et al.[26] reported that a 3-hour daily MS protocol applied for two weeks induced depressive-like behaviors in the EPM and resulted in a fivefold increase in corticosterone levels compared with controls in male rats. Fuente et al.[9] investigated the neurological, immunological, and endocrine effects of maternal deprivation by applying a single 24-hour separation on PND 9 in 13-day-old rats, corresponding to the early periadolescent period. Compared with controls, maternally separated animals exhibited significantly elevated plasma corticosterone levels, increased immobility time in the forced swim test, reduced locomotor activity, and decreased hippocampal cell numbers-particularly in males-along with impaired immune responses. Based on these findings, the authors concluded that maternal deprivation exerts early periadolescent effects and that this model may be relevant for studying the neurodevelopmental mechanisms underlying schizophrenia.[9] In contrast, Farkas et al.,[27] using a neonatal MS model involving daily 3-hour separations from postnatal days 1 to 14, assessed outcomes during postnatal weeks 3 to 5 and reported no evidence of early neurobehavioral disturbances. These observations underscore the critical importance of timing when evaluating the consequences of MS. Indeed, several studies have demonstrated that the early and late effects of maternal deprivation differ substantially: while early postnatal assessments often reveal no significant changes in body weight or neuromotor development, later-life evaluations consistently demonstrate increased anxiety- and depressive-like behaviors as well as alterations in body weight.

In the open field test, MS reduced horizontal locomotor activity in offspring rats, whereas an enriched environment increased both horizontal and vertical activity. These findings indicate that MS induces stress-related behavioral alterations, which may be partially ameliorated by environmental enrichment.[28]

Another key objective of our study was to identify sex-specific differences in the long-term effects of MS and environmental enrichment. Sex differences are increasingly recognized across multiple domains of neuroscience and provide critical insight into the development, prevalence, and heterogeneity of stress-related disorders. Previous studies have demonstrated that MS induces sex-dependent neuronal and astroglial alterations in the hippocampus and cerebellar cortex, with more pronounced effects in males. Viveros et al.,[14] using a single 24-hour MS on PND 9, reported greater locomotor suppression and increased depressive-like behavior in male rats compared with females, while FST outcomes were similar across sexes. The same study showed significant reductions in plasma leptin levels in both sexes, accompanied by anorexigenic effects, and a higher number of cerebellar GFAP-positive cells in males. The effects of MS on sex, body weight, and leptin appear to depend strongly on the timing and protocol of separation. Gruss et al.[4] applied single 24-hour separations on postnatal days 4,9, and 18 and found no comparable effects at days 9 or 18, suggesting a critical early postnatal window coinciding with peak leptin levels during the first 10 days of life.

Consistent with these findings, we observed several sex-dependent outcomes. At postnatal week 10, males in the MS group exhibited significantly greater body weight gain than controls. In the elevated plus maze, maternally separated males spent significantly more time in the closed arms than all other sex-matched groups. In contrast, females housed in enriched environments spent the least amount of time in the closed arms among all groups, indicating a pronounced anxiolytic effect of EE in females.

Previous studies report heterogeneous effects of EE following MS. Cevik et al.[28] showed that MS increased memory performance and anxiety without altering gene expression, and that EE alone was insufficient to modify behavioral or molecular outcomes, although combined MS and enrichment improved memory performance. In contrast, Dandi et al.[29] demonstrated that EE exerted protective effects, reducing anxiety and reversing spatial memory deficits, accompanied by increased hippocampal BDNF and synaptophysin expression and normalization of corticosterone responses. These findings suggest that the impact of EE on MS-induced outcomes may depend on developmental timing, experimental design, and neurobiological targets.

Limitations

This study has several limitations. First, maternal caregiving behaviors, such as licking, grooming, and nursing posture, were not quantitatively assessed. Given the well-established influence of these behaviors on offspring neurodevelopment, they may have contributed to the observed behavioral and neurobiological outcomes. Second, biochemical and molecular analyses were not performed, which limits mechanistic interpretation of the findings. Reliance primarily on behavioral measures may therefore restrict the ability to directly link the observed outcomes to underlying biological pathways. Despite these limitations, the study provides valuable insights into the age-and sex-specific effects of early-life stress and environmental enrichment.

CONCLUSION

Neonatal MS induces persistent alterations in emotional behavior, cognitive function, and metabolic growth. EE alleviates many of these adverse effects, resulting in improved anxiety- and depression-like behaviors as well as enhanced learning and memory. Sex-specific analyses reveal greater susceptibility to early-life stress in males, while females derive greater benefit from enrichment-based interventions. Additionally, MS is associated with increased weight gain, dysregulated appetite control, and heightened metabolic vulnerability in later life, supporting its relevance as a translational model for studying stress-related mechanisms underlying pediatric obesity.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study has been approved by the Ondokuz Mayıs University Local Animal Experiments Ethics Committee (Date: 01.03.2010, Decision No: 2010/10).

Informed Consent

Since this study involves animal research, written consent is not required.

Peer Review Process

This manuscript was subject to external peer review.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

Financial Disclosure

The authors received no financial support for the conduct or publication of this research.

Author Contributions

Conceptualization: SA, HÖ; Methodology: SA, HÖ; Software: SA, HÖ; Validation: SA, HÖ; Formal Analysis: SA, HÖ; Investigation: SA, HÖ; Resources: SA, HÖ; Data Curation: SA, HÖ; Writing-Original Draft Preparation: SA, HÖ; Writing-Review & Editing: SA, HÖ; Visualization: SA, HÖ; Supervision: SA, HÖ; Project Administration: SA, HÖ.

Acknowledgements

The authors would like to express their gratitude to the staff of the Experimental Animals Research and Application Center of Ondokuz Mayıs University for their technical assistance and valuable contributions throughout the study.

REFERENCES

1. MacMillan HL, Fleming JE, Streiner DL, et al. Childhood abuse and lifetime psychopathology in a community sample. *Am J Psychiatry.* 2001;158(11):1878-1883. doi:10.1176/appi.ajp.158.11.1878
2. Agid O, Kohn Y, Lerer B. Environmental stress and psychiatric illness. *Biomed Pharmacother.* 2000;54(3):135-141. doi:10.1016/S0753-3322(00)89046-0
3. Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM. Long-term behavioral and neuroendocrine adaptations to adverse early experience. *Prog Brain Res.* 2000;122:81-103. doi:10.1016/s0079-6123(08)62132-9
4. Gruss M, Braun K, Frey JU, Korz V. Maternal separation during a specific postnatal time window prevents reinforcement of hippocampal long-term potentiation in adolescent rats. *Neuroscience.* 2008;152(1):1-7. doi:10.1016/j.neuroscience.2007.12.033
5. Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry.* 2001;49(12):1023-1039. doi:10.1016/s0006-3223(01)01157-x
6. Levine S. Primary social relationships influence the development of the hypothalamic-pituitary-adrenal axis in the rat. *Physiol Behav.* 2001;73(3):255-260. doi:10.1016/s0031-9384(01)00496-6
7. Korosi A, Naninck EF, Oomen CA, et al. Early-life stress mediated modulation of adult neurogenesis and behavior. *Behav Brain Res.* 2012;227(2):400-409. doi:10.1016/j.bbr.2011.07.037
8. Aisa B, Tordera R, Lasheras B, Del Río J, Ramírez MJ. Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. *Psychoneuroendocrinology.* 2007;32(3):256-266. doi:10.1016/j.psyneuen.2006.12.013
9. De la Fuente M, Llorente R, Baeza I, et al. Early maternal deprivation in rats: a proposed animal model for the study of developmental neuroimmunoendocrine interactions. *Ann N Y Acad Sci.* 2009;1153:176-183. doi:10.1111/j.1749-6632.2008.03979.x
10. Cortese GP, Olin A, O'Riordan K, Hullinger R, Burger C. Environmental enrichment improves hippocampal function in aged rats. *Neurobiol Aging.* 2018;63:1-11. doi:10.1016/j.neurobiolaging.2017.11.004
11. Brenes Sáenz JC, Villagra OR, Fornaguera Trías J. Factor analysis of forced swimming test, sucrose preference test and open field test on enriched, social and isolated reared rats. *Behav Brain Res.* 2006;169(1):57-65. doi:10.1016/j.bbr.2005.12.001
12. Vivinetto AL, Suárez MM, Rivarola MA. Neurobiological effects of neonatal maternal separation and post-weaning environmental enrichment. *Behav Brain Res.* 2013;240:110-118. doi:10.1016/j.bbr.2012.11.014
13. Francis DD, Diorio J, Plotsky PM, Meaney MJ. Environmental enrichment reverses the effects of maternal separation on stress reactivity. *J Neurosci.* 2002;22(18):7840-7843. doi:10.1523/JNEUROSCI.22-18-07840.2002
14. Viveros MP, Llorente R, López-Gallardo M, et al. Sex-dependent alterations in response to maternal deprivation in rats. *Psychoneuroendocrinology.* 2009;34(1):S217-S226. doi:10.1016/j.psyneuen.2009.05.015
15. Sengupta P. The Laboratory rat: relating its age with human's. *Int J Prev Med.* 2013;4(6):624-30.
16. Dandi E, Kalamari A, Touloumi O, et al. Beneficial effects of environmental enrichment on behavior, stress reactivity and synaptophysin/BDNF expression in hippocampus following early life stress. *Int J Dev Neurosci.* 2018;67:19-32. doi:10.1016/j.ijdevneu.2018.03.003
17. Newport DJ, Stowe ZN, Nemeroff CB. Parental depression: animal models of an adverse life event. *Am J Psychiatry.* 2002;159(8):1265-1283. doi:10.1176/appi.ajp.159.8.1265
18. Daniels W.M., Pietersen C.Y., Carstens M.E., Stein D.J. Maternal separation in rats leads to anxiety-like behavior and altered neurotransmitter levels. *Metab Brain Dis.* 2004;19:3–14. doi:10.1023/b:mebr.0000027412.19664.b3
19. El Khoury A., Gruber S.H., Mørk A., Mathé A.A. Adult behavioral consequences of early maternal separation are alleviated by escitalopram. *Prog Neuropsychopharmacol Biol Psychiatry.* 2006;30:535–540. doi:10.1016/j.pnpbp.2005.11.011
20. Iwasaki S, Inoue K, Kiriike N, Hikiji K. Effect of maternal separation on feeding behavior of rats in later life. *Physiol Behav.* 2000;70(5):551-556. doi:10.1016/s0031-9384(00)00305-x
21. Zhang Y, Wang R, Wang L, Chen R. Disorder of intestinal amino acid pathway in depression-like offspring rats induced by maternal separation. *J Zhejiang Univ Med Sci.* 2021;50:298-304. doi:10.3724/zdxbyxb-2021-0102
22. Lee JH, Kim HJ, Kim JG, et al. Depressive behaviors and decreased expression of serotonin transporter in rats exposed to neonatal maternal separation. *Neurosci Res.* 2007;58:32–39. doi:10.1016/j.neures.2007.01.008
23. Curzon G. Serotonin and appetite. *Ann N Y Acad Sci.* 1990;600:521-530. doi:10.1111/j.1749-6632.1990.tb16907.x

24. Roy V, Belzung C, Delarue C, Chapillon P. Environmental enrichment in BALB/c mice: effects in classical tests of anxiety and exposure to a predatory odor. *Physiol Behav.* 2001;74(3):313-320. doi:10.1016/s0031-9384(01)00561-3
25. Chu J.Y. *The effects of environmental enrichment on stress-induced eating disturbances in rats.* Master's thesis. University of Canterbury; 2008.
26. Kalinichev M, Easterling KW, Holtzman SG. Long-lasting changes in morphine-induced locomotor sensitization and tolerance in Long-Evans mother rats as a result of periodic postpartum separation from the litter: a novel model of increased vulnerability to drug abuse?. *Neuropsychopharmacology.* 2003;28(2):317-328. doi:10.1038/sj.npp.1300068
27. Farkas J, Reglodi D, Gaszner B, et al. Effects of maternal separation on the neurobehavioral development of newborn Wistar rats. *Brain Res Bull.* 2009;79(3-4):208-214. doi:10.1016/j.brainresbull.2008.12.011
28. Cevik OS, Cevik K, Temel GO, Sahin L. Maternal separation increased memory function and anxiety without effects of environmental enrichment in male rats. *Behav Brain Res.* 2023;441:114280. doi:10.1016/jbbr.2022.114280
29. Dandi E, Kalamari A, Touloumi O, et al. Beneficial effects of environmental enrichment on behavior, stress reactivity and synaptophysin/BDNF expression in hippocampus following early life stress. *Int J Dev Neurosci.* 2018;67:19-32. doi:10.1016/j.ijdevneu.2018.03.003