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RESEARCH ARTICLE

Investigation of Learning and Anxiety States in Postpartum Female Rats

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ABSTRACT

The present study seeks to examine the impact of the postpartum period on anxiety levels and learning abilities in female rats, utilizing established behavioral paradigms. Adult female Wistar rats were divided into two groups: a nulliparous control group and a postpartum group. Anxiety-related behavior was assessed using the Elevated Plus Maze (EPM), while spatial learning and memory performance were evaluated through the Morris Water Maze (MWM) test. In the EPM, postpartum rats demonstrated a significant reduction in the time spent in the open arms compared to controls ($p < 0.05$), indicating elevated anxiety levels following parturition. Conversely, no significant differences were observed in the time spent in closed or central zones. In the MWM, although postpartum rats initially located the hidden platform more quickly, their performance plateaued over time, whereas control animals exhibited a robust learning curve across trials. These findings suggest that physiological and neurobiological changes during the postpartum period may increase anxiety and moderately impair learning progression. The study contributes to the growing body of evidence on the neurobehavioral impact of the postpartum period and underscores the utility of animal models in understanding anxiety and cognitive alterations associated with maternal adaptation.

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1. INTRODUCTION

Pregnancy is a complex physiological period in which the zygote, the result of fertilization of a sperm cell from a man and an oocyte from a woman, undergoes developmental, hormonal and structural changes in the uterus over a period of approximately 37-40 weeks. Although the pregnancy process is largely initiated by sexual intercourse, today, thanks to advances in assisted reproductive technologies, alternative methods of conception have become widespread. At the end of this process, which lasts for an average of nine months, a healthy delivery takes place in the absence of complications [1]. Although the pregnancy process is largely initiated by sexual intercourse, today, thanks to advances in assisted reproductive technologies, alternative methods of conception have become widespread [2].

In recent years, there has been increasing evidence that pregnancy and labor have significant effects not only on physiological but also on neuropsychiatric and cognitive levels. For example, Oatridge et al. [3] observed shrinkage in maternal brain volume during pregnancy and reported that this shrinkage reversed within a few months after delivery. In parallel with these findings, Kim et al. [4] found an increase in gray matter volume in the prefrontal cortex and midbrain regions in the early postpartum period and suggested that the mother's positive thoughts about the baby may facilitate this increase. However, a study published in 2017 claimed that these morphologic changes may be persistent for at least two years after birth and reached contradictory results with the previous literature [5]. Finally, a 2019 study showed that the decrease in gray matter volume during pregnancy averaged 0.09 mm^3 per month and was associated with morphological parameters such as cortical thickness, surface area and sulcus depth [6].

Psychiatric conditions observed during pregnancy and postpartum also have important clinical consequences. Postpartum anxiety and depression are among the most common postpartum mood disorders and the prevalence of postpartum depression is 10-15%, especially in industrialized societies [7]. Anxiety symptoms are often caused by concerns about motherhood and may have both positive and negative effects on the development of the baby. In the emergence of postpartum depression, the woman's level of social support, especially the emotional support she receives from her husband and the quality of marital relations are among the important determinants [7]. Atkinson and colleagues showed that increasing the time spent by the mother with her baby led to a decrease in depression levels [8]. On the other hand, "postpartum blues", which is observed in the postpartum period and usually starts on the 3rd day and resolves spontaneously within 2 weeks, occurs in 50-80% of women and is considered a risk factor for the development of depression [7]. Postpartum psychosis, which is a rare but serious condition, is observed at a rate of 0.01-0.02% and is characterized by hallucinations, delusions and irregular behaviors in which the perception of reality is impaired.

In addition to these, cognitive deficits commonly experienced by women during pregnancy and the postpartum period have received increasing attention in the scientific literature. This phenomenon, defined as "maternal amnesia" or popularly known as "momnesia", is characterized by transient impairments in attention, memory and executive functions [9]. In studies, a large proportion of women (e.g., 81%) reported postpartum memory problems. In their study on prospective memory, it is reported that sleep disorders, depressive symptoms and increased cognitive load may lead to impairment in such memory functions. It is also suggested that the decrease in estrogen levels in the postpartum period reinforces this process [10]. However, the neurobiological basis of these cognitive changes has not yet been fully elucidated.

The aim of this study was to investigate the effects of pregnancy and postpartum period on women's anxiety levels and learning abilities through an experimental model. Compared to clinical observation and questionnaire-based studies, cognitive and behavioral studies with experimental animal models are limited in the literature. In this study, for the first time, the effects of the maternal period will be evaluated in the context of cognitive performance and emotional behaviors using an experimental animal model. Our hypothesis is that hormonal and neuroanatomical changes in the postpartum period are associated with increased anxiety levels and impaired learning processes in maternal individuals. The findings are expected to contribute to a better understanding of possible cognitive and psychiatric risks related to the postpartum period in humans.

2. MATERIAL METHOD

2.1 Animal Studies

This study was conducted at Ankara Medipol University Animal Experiments Laboratory and was carried out in accordance with the approval numbered ... obtained from the Local Ethics Committee for Animal Experiments of the relevant institution. The animals used in the study were adult female Wistar albino rats (Rattus norvegicus) obtained from Ankara Medipol University Animal Experiments Laboratory. Two experimental groups were formed in total: Control Group (Nulliparous): The 7 female rats included in this group did not experience pregnancy or parturition and were subjected to experimental procedures when they reached 3 months of age. Postpartum Group: The 6 female rats included in this group were mated and allowed to become pregnant. Since the experimental process could not be initiated after the first birth, they waited for one month after the second birth and were included in the experimental studies in the first month of postpartum. Both groups were subjected to Elevated Plus Maze (EPM) and Morris Water Maze (MWM) tests, respectively. Behavioral tests were performed at different age stages according to the experimental group of rats: Control Group: At 3 months of age, the rats were subjected to the tests. Postpartum Group: Testing was initiated on the 30th day after the second birth. All animals were housed in standard laboratory cages with a 12 h light/dark cycle, constant temperature (22 ± 2 °C) and humidity, and ad libitum access

to water and standard feed. All experimental applications and data recordings were performed at the same time interval, and environmental variables were minimized to ensure the reliability of inter-animal comparisons. All procedures involving animals were conducted in accordance with the ethical standards of the Animal Experiments Local Ethics Committee of Ankara Medipol University. The experimental protocol was reviewed and approved under the decision number HDY 2/2, dated 30/11/2023.

2.2 Behavioral Test

2.2.1 Elevated Plus Maze (EPM) Method

The Elevated Plus Maze (EPM) is a behavioral test commonly used to assess anxiety-like behavior in rodents. The maze consists of two open and two closed arms, located approximately 50 cm above the ground and extending perpendicular to each other from the center. The animal is placed in the center of the maze and allowed to move freely between the open and closed arms, usually for a period of 5 minutes. Exploration of the open arms (duration and number of entries) correlates with a lower level of anxiety. The data obtained in this test are often used to assess the anxiolytic or anxiogenic effects of pharmacological agent. This test is based on the animal's natural tendency to avoid high and open spaces. Increased time spent in open arms may indicate a decreased level of anxiety. During the test, behaviors are recorded manually or through video monitoring systems [11].

2.2.2 Morris Water Maze (MWM) Method

The Morris Water Maze (MWM) is a standardized test used to assess spatial learning and memory processes in rodents. Animals are released into a large circular pool filled with water and are expected to find an escape platform hidden under the water. The water is made opaque to obscure the visibility of the platform so that the animal must orient to the location using visual cues from the external environment. The test usually includes a learning phase (multiple trials with the hidden platform in the same location) followed by a "probe" test (the platform is removed and the time it takes the animal to locate the previous platform is assessed). Parameters such as time to find the platform, swimming route and time spent in the target area are evaluated as indicators of cognitive performance [12].

2.3 Statistic

To statistical evaluations and graphical representations of the results were conducted using GraphPad Prism 10.0 software (GraphPad, USA). The data analysis involved the application of an unpaired t-test and Two-Way ANOVA to assess the significance of differences among various groups. These groups included the control group and experiment group.

3. RESULTS

3.1 Elevated Plus Maze (EPM)

Figure 1 shows a comparison of the time spent in open arms between control and postpartum female rats in the Elevated Plus Maze (EPM) test, which is used to assess open space preference. This test is widely used to measure anxiety-like behavior based on rodents' natural tendency to avoid open and elevated areas. The length of time spent in open arms is often interpreted as a marker indicating that the animal is experiencing lower levels of anxiety. Accordingly, exploring open arms more indicates lower levels of anxiety, whereas avoiding open arms indicates higher levels of anxiety. According to the findings obtained in this study, parturient female rats spent significantly less time in open arms compared to female rats in the control group ($p < 0.05$). This difference may indicate increased anxiety levels after parturition. In other words, parturient female rats tended to avoid areas perceived as open and threatening, suggesting that the postpartum period may increase anxiety levels.

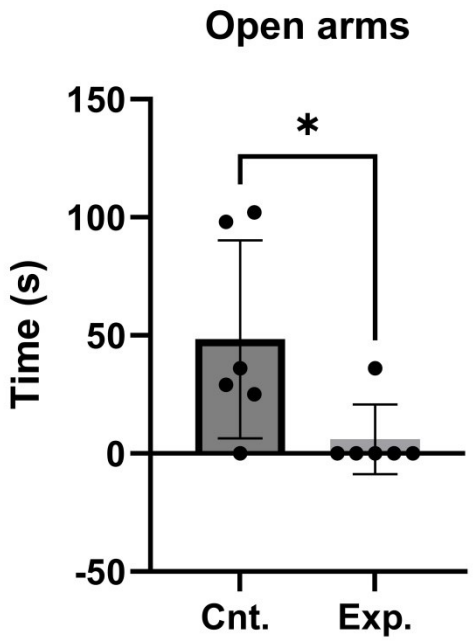

Figure 1. Comparison of the Time Spent in the Open Arms of Control (Cnt.) and Post-Pregnancy Mother Rats (Exp.). The difference between the groups was analyzed by t-test and *p<0.05 level of significance.

Enclosed arms are the preferred areas when anxiety is high. In this graph, it is seen that the time spent in the closed arms of female rats that gave birth after pregnancy did not show a significant difference compared to the control group (ns; p>0.05) (Figure 2). This finding suggests that the indoor preferences of postpartum rats do not indicate a statistically significant increase in anxiety, but individual variations are present.

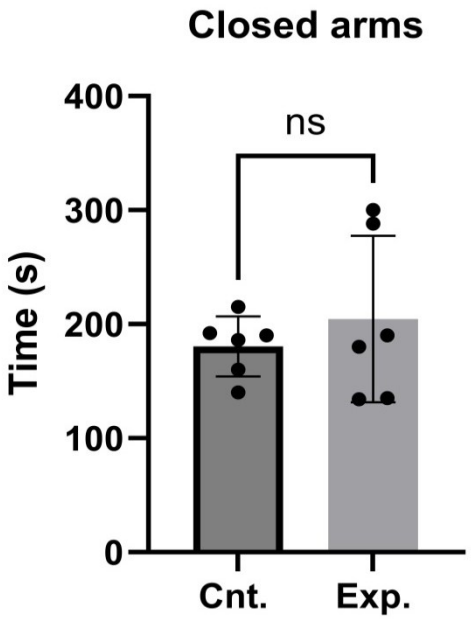

Figure 2. Comparison of Time Spent in Closed Arms between Control (Cnt.) and Post-Pregnancy Mother Rats (Exp.). The difference between the groups was analyzed by t-test and *p<0.05 level of significance, ns: not significance.

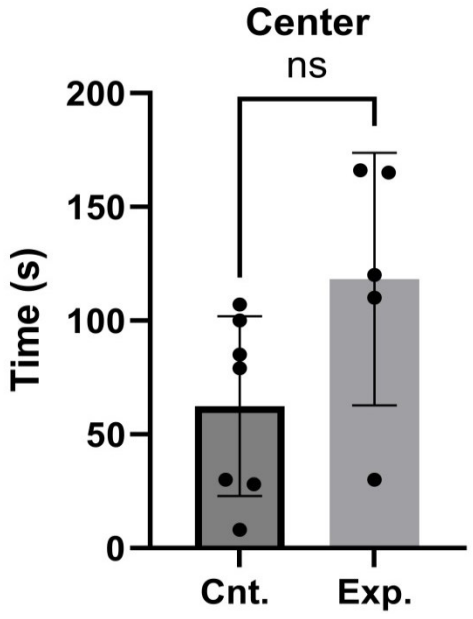

Figure 3. Comparison of the Time Spent in the Center by Control (Cnt.) and Post-Pregnancy Mother Rats (Exp.). The difference between the groups was analyzed by t-test and *p<0.05 level of significance, ns: not significance.

As shown in the Figure 3, the time spent in the center region by control female rats and parturient female rats in the plus maze test. There is no statistically significant difference between the two groups (ns: non-significant). The fact that there was no significant change in the time spent in the central region may indicate that postpartum anxiety is not at a level that can affect the central region preference. However, the presence of significant differences in open and closed arms suggests that the effect of anxiety on the central region may be evaluated differently from other areas.

3.2 Morris Water Maze Test

Figure 4 compares the time to find the platform (in seconds) of control (triangle) and parturient mother rats (circle) during the Morris Water Maze Test on a daily basis. Two-Way ANOVA results revealed that there was a significant interaction between time and group factors, and a statistically significant difference was observed between day 2 and day 5 data, especially in the control group (*p<0.05). It is noteworthy that from the first day, the parturient female rats in the experimental group found the platform in a shorter time compared to the control group, but in the following days, the control group showed a significant learning curve in performance, whereas this progress was relatively weaker in the experimental group. Especially on day 5, there was a significant decrease in the time it took the control group to find the platform, whereas no significant improvement was observed in the experimental group. This suggests that the physiological and neurobiological changes that occur in the postpartum period may negatively affect the learning process.

In conclusion, these findings point to the effect of the postpartum period on cognitive functions and suggest that learning and memory processes may progress more slowly, especially in the postpartum period. It is thought that hormonal, synaptic or neuroinflammatory changes in the postpartum period may play a role in this cognitive slowdown.

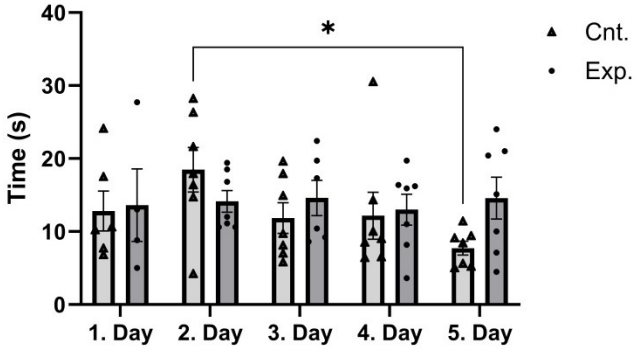

Figure 4. Evaluation of the Effects of Pregnancy on the Time to Find the Platform (sec) of Control (Cnt.) and Post-Pregnancy Mother Rats (Exp.) in the Morris Water Maze Test by Two-Way ANOVA Test.

4. DISCUSSION

The results of this study indicate that postpartum female rats exhibit increased anxiety-like behaviors, as demonstrated by a significant reduction in time spent in the open arms of the Elevated Plus Maze (EPM). These findings align with previous research suggesting that hormonal fluctuations particularly decreases in estrogen and progesterone following parturition contribute to heightened anxiety responses [13].

The EPM is known for its sensitivity to subtle neuroendocrine changes, making it an ideal instrument for

detecting postpartum anxiety states. Consistent with our findings, other rodent studies have also shown elevated anxiety during early postpartum and hormonal manipulation states, including altered GABA receptor sensitivity and stress reactivity [14]. Similarly, in models involving environmental stress or pharmacological manipulation, anxiety and learning deficits often diverge, suggesting distinct underlying circuits [15].

In the Morris Water Maze, postpartum rats demonstrated efficient initial acquisition but exhibited attenuated improvement across trials. This may reflect disrupted consolidation processes or diminished cognitive flexibility. Indeed, hippocampal plasticity during the postpartum period is known to fluctuate, including reductions in dendritic spine density and neurogenesis [16].

Dissociation between anxiety levels and spatial learning is also supported by findings in rat models of neurodevelopmental disruption and hormonal imbalance, where hippocampal-dependent memory remains relatively stable despite changes in anxiety [17,18]. Collectively, these findings reinforce the notion that anxiety and learning are modulated by different, though interacting, neural systems particularly the amygdala and hippocampus [19]. Moreover, the postpartum behavioral profile may represent an adaptive recalibration of neural priorities, enhancing vigilance for offspring protection while de-emphasizing novelty-seeking or spatial updating.

While the current study provides valuable insights into the behavioral alterations during the postpartum period in female rats, certain limitations must be acknowledged. First, the relatively small sample size may limit the generalizability of the findings and reduce statistical power. Second, the study focused exclusively on behavioral endpoints without parallel neurobiological or hormonal analyses, which restricts the ability to directly correlate observed behaviors with underlying physiological mechanisms. Third, the use of only two time points (nulliparous vs. early postpartum) does not capture the potential longitudinal dynamics of anxiety and learning processes throughout the postpartum trajectory. Future studies incorporating larger cohorts, molecular markers, and extended observation periods would provide a more comprehensive understanding of postpartum neuroadaptations.

This study contributes to the expanding literature on postpartum neurobiology by providing behavioral evidence of increased anxiety and altered learning dynamics and highlights the utility of rodent models in elucidating the neuroendocrine and cognitive mechanisms underlying maternal adaptation.

5. CONCLUSION

In conclusion, this study presents compelling evidence that the postpartum period in female rats is associated with heightened anxiety-like behaviors and subtle disruptions in the progression of spatial learning. The employment of validated behavioral paradigms, namely the Elevated Plus Maze and Morris Water Maze, facilitated precise evaluation of neurobehavioral changes indicative of postpartum neuroendocrine adaptations. Our findings support the hypothesis that the postpartum state induces functional alterations in neural circuits related to anxiety and cognition, particularly within the amygdala and hippocampus. Notably, these behavioral patterns may represent adaptive mechanisms rather than pathological impairments. Future studies incorporating molecular and neuroimaging techniques are warranted to elucidate the underlying mechanisms and to explore potential interventions for mood and cognitive disturbances associated with the postpartum period.

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