Research Article

Age and Sex Dependent Effects of Maternal Deprivation on Anxiety-Like and Depressive-Like Behaviors and Oxidative Stress in the Prefrontal Cortex of Rats

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Abstract | Early life stress, represented by maternal deprivation (MD), is associated with numerous behavioral disorders in later life. In young adulthood, a single 24-hour period of MD has been proven to produce behavioral changes such as affective and cognitive disorders in rats. However, the short-term and long-term behavioral consequences of MD have not been examined in detail. Also, the mechanism by which MD causes these disorders is far from fully understood. Therefore, this study was designed to examine the impact of MD on anxiety- and depression-like behaviors and oxidative stress (OS) in the prefrontal cortex (PFC) of rats across different ages (adolescence, emerging adulthood, and middle adulthood) and the possible existence of sexual dimorphisms. Rat pups were maternally deprived on postnatal day 9 for 24 hours. The rats of both genders were then tested in the open field and the elevated plus-maze tests for anxiety, and in the forced swimming test for depression at different time points. Additionally, we removed the PFC for biochemical analysis, specifically measuring nitric oxide and lipid peroxidation. The results indicate that, in both genders, MD generates an anxiogenic and depressive effect compared to the control group, which are linked to an increased OS in the PFC of rats, with greater impact during adolescence and adulthood. Interestingly, most measures showed sex differences in responses, particularly in the MD group. Our findings suggest that MD causes short-term and long-term changes in anxiety-like and depression-like behaviors and OS. Such effects depend on gender and age.

Keywords | Maternal deprivation, Anxiety, Depression, Oxidative stress, Rat

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INTRODUCTION

Early life stress (ELS) such as parental loss, low socioeconomic status, childhood maltreatment, and malnutrition or viral infection, has a lasting impact on lifelong health outcomes (Taylor *et al*., 2011). Researchers have linked ELS with an increased risk of various pathologies, including cardiovascular disorders (Galobardes *et al*., 2006), oncological diseases, metabolic disturbances (Danese *et al*., 2009) and mental illnesses. In particular, recent works revealed that stress during early developmental periods represents a significant contributing

factor to the onset and endurance of neuropsychiatric disorders in the later phases of life, including depression and anxiety disorders (Benmhammed *et al*., 2019), cognitive impairment, personality disorders, autism spectrum disorder (Jawahar *et al*., 2015), and hyper/ deficit activity changes (Babenko *et al*., 2015). Most of these disorders begin during adolescence (Spear, 2000).

The use of animal models to study ELS is a largely utilized research approach to investigate the connection between early adversities and the development of psychopathologies during adolescence or adulthood (Mhillaj *et al*., 2015). Maternal deprivation (MD) in rodents, notably a singlular 24-hour period on postnatal day (PND) 9, has been suggested as a potential animal model employed to provoke various behavioral changes (Hennessy *et al*., 2017; Nassiri *et al*., 2023). This model is based on the evidence that early loss of maternal care (emotional attachment and necessary feeding) during the stress hypo-responsive period (SHRP) (from PND 4 to PND 14) can affect the vulnerability of infants throughout their lives (Rice and Barone, 2000). When pups are separated from the dam during this specific time frame for an extended duration, this results in an elevation of circulating corticosterone, which is believed to impact the hypothalamus-pituitary-adrenal (HPA) axis and enhance the neurodegenerative actions of glucocorticoids (Llorente *et al*., 2007). Additionally, the P9 MD model has shown a strong long–term impact on emotional and cognitive performances (Benmhammed *et al*., 2019; Nassiri *et al*., 2023). Also, the link between MD and neuropsychiatric disorders has been related to abnormal neurochemical and neuroanatomical biomarkers in specific brain regions, particularly the prefrontal cortex (PFC) (Malter Cohen *et al*., 2013).

A growing number of works published over the past two decades have attempted to identify the biological mechanisms implicated in the development of neurobehavioral disorders as a result of ELS (Yang *et al*., 2021). The impacts of MD might involve a variety of mechanisms, for example, changes in the HPA axis (excessive releases of different stress hormones and neurotransmitters), neuroinflammation, and epigenetic alterations (Johnson and Kaffman, 2018; Abbott *et al*., 2018). Additionally, oxidative stress (OS) has also been proposed as one of the mechanisms behind the harmful effect of MD (Abelaira *et al*., 2021). As known, OS as an oxidant/antioxidant imbalance is linked to psychiatric disorders in humans and animals like depression and anxiety (Zghari *et al*., 2023a, b). In this context, our previous studies have shown that MD causes neurobehavioral alterations accompanied by oxidative damage to different regions of the brain, especially at PFC (Benmhammed *et al*., 2019), brain structures that have long been involved in mood, learning, and memory processes (Belzung *et al*., 2014).

On the other hand, the majority of studies only focus on the impacts of MD on the behavior of young adult animals. The effect of maternally deprived adolescents and adult animals (the short-term and long-term consequences) has been researched relatively small in comparison with young adult studies. In our previous work, we have revealed that P9 MD causes depression-like, anxiety-like behaviors and, cognitive alterations in adult rats (Benmhammed *et al*., 2019). In addition, as far as we are aware, experiments assessing the effects of MD have been little explored in the context of sex. Most of these studies predominantly involve male rodents, and when examining the influence of gender on the effects of MD, they frequently yield varied outcomes (De Melo *et al*., 2018). These observations highlight the need for additional research to explore the interplay of age, gender, and MD in shaping affective and cognitive disorders. Therefore, this research was conceived to examine the influence of MD on depression-related and anxiety-like behaviors, memory impairment, and neurochemical changes in the PFC area of the brain rats across different ages (adolescent, young adult, and adult rats) and the possible existence of sexual dimorphisms.

MATERIALS AND METHODS

Animals and procedure

To carry out the experiments, pregnant female Wistar rats provided by the Laboratory of Biology and Health, Faculty of Sciences, Ibn Tofail University, Morocco, were individually housed in standard plastic cages (210 × 290 × 430 mm) under controlled conditions of ambient temperature (24 ± 1 °C), humidity (50-60%), and ventilation. They were subjected to a 12-hour light/dark cycle (lights on from 7:00 am to 19:00) and provided unrestricted access to both food and water. A total of 80 pups are given by pregnant females. Half of the litters underwent the MD protocol on PND9, following the procedure outlined by Llorente *et al*. (2007). In short, the pups were removed from the cage at 10:00 am and kept in a separate cage until the next day, when at 10:00 am the dams were returned to their corresponding home cage. In PND21, the litters were classified according to gender; at this point, the animals were weighed. In this experiment, 24 animals were allocated to two groups for each gender. Within each gender group, four control rats and four MD animals were submitted to the behavioral tests and sacrificed on P60, which corresponds to adolescence. Four control and four MD rats were exposed to the behavioral tests and sacrificed at the period of young adulthood (P90). The remaining two groups, consisting of four control rats and four maternal deprivation (MD) rats, were euthanized at the age of 10 months (middle adulthood) (Nassiri *et al*.,

2023a). Every possible measure was taken to minimize animal distress and limit the quantity of rats utilized in the study. All experimental protocols adhered to the guidelines outlined in the National Institutes of Health for the care and use of laboratory animals and received approval from the Doctoral Study Center at the University.

Behavioral tests

Behavioral tests were performed to assess levels of anxiety and level of depression (forced swimming test), respectively.

Anxiety-Like measurement

The open field test (OFT)

An OF test was performed to examine anxiety-like symptoms and locomotors activity in rodents (Carola *et al*., 2002). Every rat was carefully introduced into the middle of a white box measuring 100 × 100 × 40 cm, and their behaviors were observed and recorded over 10 minutes: (a) the time spent in the center of the area (TCA; (b) the number of returns to the center (NRC); (c) and total squares traveled (NTS).

The elevated plus maze (EPM)

The EPM is an approved test for assessing anxiety in rats (Naranjo-Rodriguez *et al*., 2000). The apparatus consisted of two arms: an open one measuring 10 × 50 cm and a closed one measuring 10 × 50 × 40 cm, connected by a platform (10 × 10 cm). During the test, each rat was positioned in the central area, oriented towards one of the open arms, and their behaviors were measured for 5 minutes: (a) time spent on the open arms (TOA); (b) open-arm entries (EOA); and (c) total arm entries (TAE).

Depression-Like measurement in the forced swim test (FST)

The FST was conducted following the procedure outlined by Porsolt *et al*. (1978). Briefly, the test was made from a cylindrical transparent tank of 30 cm in diameter and was filled with tap water (23 ± 2°C) at a height of 35 cm. Each rat was subjected to the FST for a 5-minute session to calculate the immobility time (TIM). The immobility duration (TIM) was recorded for 5 min.

Biochemical analysis

The day after finishing the behavioral tests, the animals were anesthetized and euthanized by rapid decapitation, the prefrontal cortex was dissected by free hand technique, and the tissue was homogenized in 10 volumes (50 mM W/V) of phosphate buffer, pH 7.4. The homogenates were centrifuged at 1500 rpm for 10 min at 4 °C and the supernatant was used for the analysis of thiobarbituric acid reactive substances (TBARS) and nitric oxide (NO) levels. Calorimetrically, TBARS levels in the prefrontal cortex were quantified according to Draper and Hadley (1990). Levels of NO were estimated using Griess reagent (Chao *et al*., 1992).

Statistical analysis

In this study, statistical analysis was carried out using SPSS (version 22). Two-way ANOVA was used to investigate the actions of sex and aging on behavioral indices (adolescent, emerging adulthood, and middle adulthood). Data were presented as the mean ± SEM. A $p < 0.05$ was interpreted as a significant difference.

RESULTS AND DISCUSSION

Effects of maternal deprivation on the anxiety-like behavior evaluated in the OFT

The OFT was conducted to assess anxiety-related behaviors (Figure 1A). Regarding the TCA, our findings indicate that MD led to anxiety-like behavior only in adolescent rats of both genders. These rats exhibited reduced TCA compared to their control counterparts (–42% in males; $p < 0.01$ and -41% in females; $p < 0.05$). In addition, the comparison between different groups of males revealed that the adolescent and adult MD groups spent significantly less time in the center area in comparison to the young adult MD group ($p < 0.01$). Conversely, in females, no significant age-related effect was observed ($p > 0.05$).

Moreover, the NRC was significantly decreased only in adolescent male and female rats maternally deprived as compared to the control rats (–42% in males; $p < 0.001$ and -47% in females; $p < 0.001$). Interestingly, in MD groups, no significant effect of age and sex on NRC was evident ($p > 0.05$) (Figure 1B).

Besides, at 10 months of age, both MD males and female rats had significantly higher NTS than their controls ($p < 0.05$ and $p < 0.001$, respectively) (Figure 1C). Also, in both genders, NTS increased significantly between adolescence, young adulthood and middle adulthood age, with females having significantly higher NTS than males at adult age ($p < 0.01$).

Effects of maternal deprivation on the anxiety-like behavior evaluated in the EPM

In the EPM, the results demonstrated that the MD provoked a notable anxiogenic impact (Figure 2A), marked by a significant reduction in TOA only in adolescent females, when compared to the control rats (–46%; $p < 0.01$), while a non-significant decrease in TOA was observed in the other ages of both genders ($p > 0.05$). Additionally, an age effect was observed only in females, it was observed that the adolescent rats spent less time on the open arm as compared to the young adult animals ($p < 0.05$). Besides, the TOA was unaffected by the sex factor in all MD groups ($p > 0.05$).

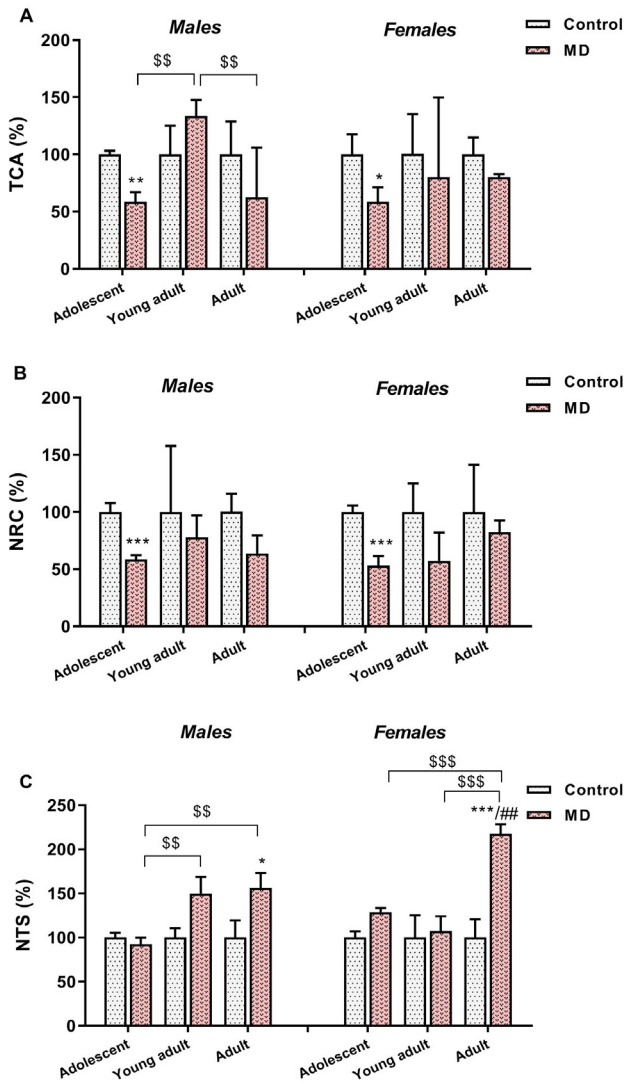

Figure 1: Behavioral performances of adolescent, young adult and adult females and male rats in the open field test. A – Total amount time spent in the center (TCA); B – Number of return into center area (NRC), C – Number of total squares crossed (NTS). The symbols *, # and $ denote significant differences compared to control, or to gender counterparts, or between different age groups, respectively. The significance level is 0.05.

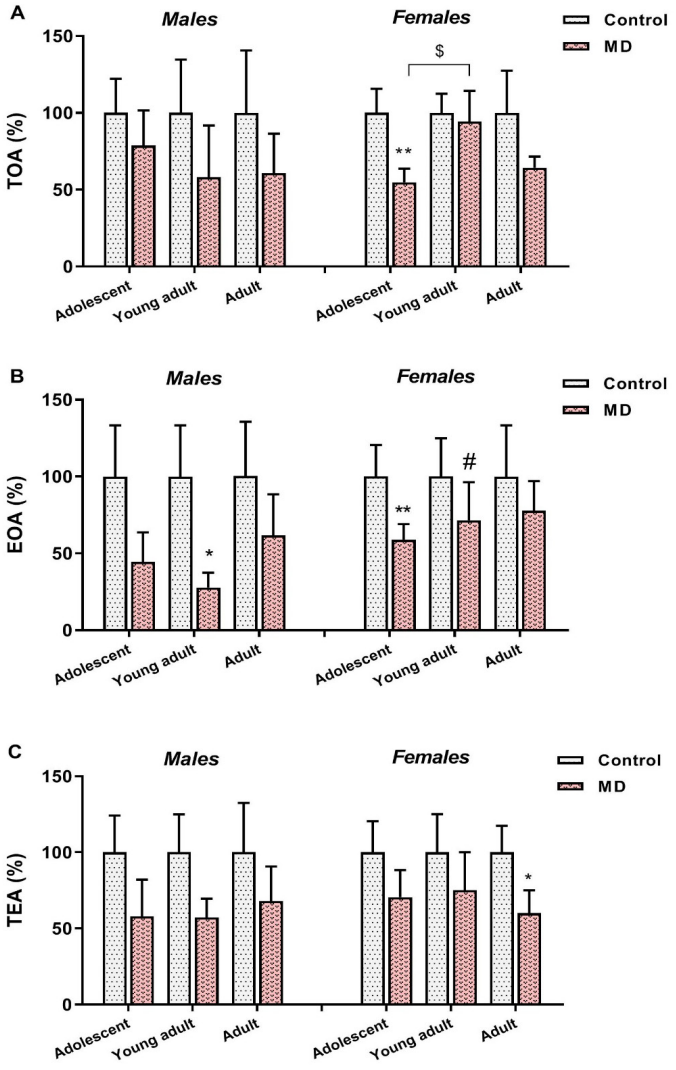

Figure 2: Behavioral performances of adolescent, young adult and adult females and male rats in to the elevated plus maze. A – Time spent on the Open Arms (TOA); B – Entries into Open Arms (EOA); C – Total Entries into the Arms (TEA). The symbols *, # and $ denote significant differences compared to control, or to gender counterparts, or between different age groups, respectively. The significance level is 0.05.

Similar to TOA, only adolescent females showed a significant decrease in EOA than the control group (–42%; $p < 0.01$), whereas in male rats, only the young adult males visited less the open arms in comparison with their controls (–73%; $p < 0.05$). Also, the statistical analysis revealed that the young adult male rats made less EOA when compared to the young adult females (–73% vs.–29%; $p < 0.05$). In the remaining two age groups, there were no statistically significant differences between males and females ($p > 0.05$). Additionally, the comparison of male and female groups across various age ranges did not indicate any notable distinctions ($p > 0.05$) (Figure 2B).

Concerning the locomotors activity represented by TEA, there were no apparent alterations in the overall number of arm entries across all MD groups for both males and females ($p > 0.05$), except for adult females, which showed a significant decrease in TEA in comparison with control animals ($p < 0.05$) (Figure 2C).

Effects of maternal deprivation on depressive-like behavior evaluated in the FST

The FST was applied to evaluate the depression-like effect in the MD rats (Figure 3). The statistical analysis showed that in males, the average duration of TIM in the adolescent MD group significantly surpassed the corresponding values in the male control group (+94%; $p < 0.05$), whereas the comparison between TIM of the young

adult and adult MD groups and their controls revealed no significant difference ($p > 0.05$). In females, as shown in Figure 3, the immobility time in young adult and adult MD groups was significantly increased compared to the female control group (+154%; $p < 0.01$ and +187%; $p < 0.05$, respectively).

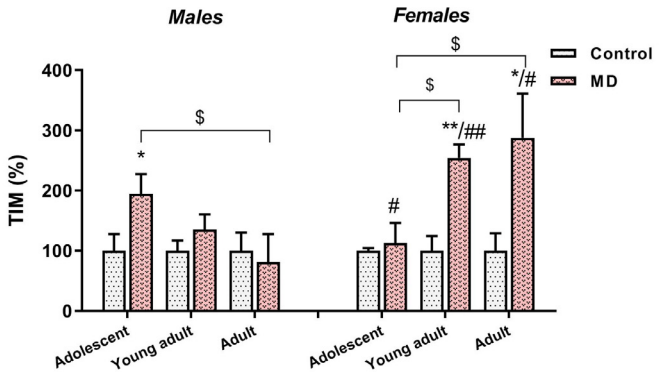

Figure 3: Effect of maternal deprivation on depression-related behavior of adolescent, young adult and adult females and male rats in the forced swimming test. TIM: immobility time. The symbols *, # and $ denote significant differences compared to control, or to gender counterparts, or between different age groups, respectively. The significance level is 0.05.

An age effect was noted also, the adolescent male rats maternally deprived had significantly higher TIM compared to both young adult ($p > 0.05$) and adult ($p < 0.05$) rats. In contrast, the young adult and adult female rats had significantly higher immobility time compared to adolescent female rats ($p < 0.05$).

Interestingly, at the different ages, the statistical analysis revealed a main impact of sex, with adolescent males maternally deprived showing a greater depressive-like behavior, as indicated by an increased TIM when compared to female group counterparts. However, Tukey test demonstrated that young adult and adult female rats made a higher TIM in comparison with the males' group counterparts ($p < 0.01$ and $p < 0.05$, respectively).

Effects of maternal deprivation on oxidative stress parameters in prefrontal cortex

Effects on NO levels

Our results also showed that the NO levels were significantly increased only in adolescent male and female rats maternally deprived as compared to control groups (+107% in males; $p < 0.01$ and +23% in females; $p > 0.05$). Whereas the comparison between NO levels of the young adult and adult MD groups and the control groups revealed no significant difference ($p > 0.05$). In addition, the comparison between different age groups of males revealed that the adolescent MD groups had higher NO levels compared to both the young adult and adult MD groups ($p < 0.01$) (Figure 4). On the contrary, there was no noteworthy impact of age observed in females ($p > 0.05$).

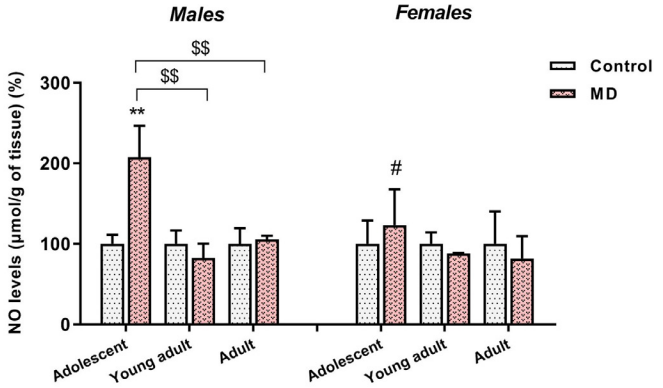

Figure 4: Effect of maternal deprivation on nitric oxide (NO) in the prefrontal cortex of adolescent, young adult and adult females and male rats. The symbols *, # and $ denote significant differences compared to control, or to gender counterparts, or between different age groups, respectively. The significance level is 0.05.

Concerning the sex effect, the adolescent male rats maternally deprived had significantly higher NO compared to the adolescent female rats (+107% vs. +23%; $p < 0.05$). Also, in young adult and adult MD groups, no significant effect of sex was evident ($p > 0.05$).

Effects on TBARS levels

Figure 5 reveals a noteworthy increase in TBARS levels among young adult rats subjected to MD, demonstrating a significant elevation compared to control rats (+29% in males; $p < 0.05$ and +58% in females; $p < 0.05$). However, in the two other age groups, the differences between males and females and their respective controls did not reach statistical significance ($p > 0.05$). Furthermore, within the MD groups, no significant impact of age and sex on TBARS levels was observed ($p > 0.05$) (Figure 5).

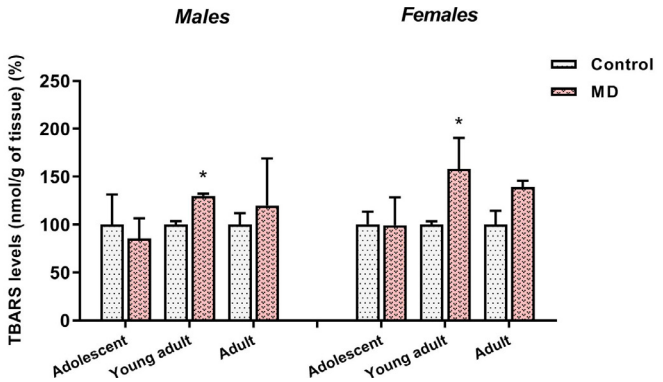

Figure 5: Effect of maternal deprivation on thiobarbituric acid reactive substances (TBARS) content in the prefrontal cortex of adolescent, young adult and adult females and male rats. The symbols *, # and $ denote significant differences compared to control, or to gender counterparts, or between different age groups, respectively. The significance level is 0.05.

In this research, we investigated how MD affects anxiety-related and depression-like behaviors, as well as OS alterations in the PFC of rats at various life stages (adolescent, young adult, and adult), while also exploring the potential presence of sexual dimorphism.

As measured in the OFT and EPM, two tests suitable for assessing anxiety in rodents (Naranjo-Rodriguez *et al*., 2000; Carola *et al*., 2002), MD leads to anxiety-like behavior in either sex in adolescent and young adult rats. These effects did not persist into middle adulthood for both genders. This data goes in line with the study of Banqueri *et al*. (2019) which reported that ELS increased anxious behavior in both adolescence and young adulthood (Banqueri *et al*., 2019). Also, our previous work showed that MD at PND9 provoked an anxiety-like behavior in young adult rats of both genders (Benmhammed *et al*., 2019). However, these findings are in contrast with numerous other works employing diverse ELS protocols in rats and mice demonstrating the impact of maternal separation on affective behaviors in young adulthood (Loi *et al*., 2017; Tan *et al*., 2017). The causes of these variations need to be explained, although, maternal separation protocol, animal strains, and experimental environment may be explanatory factors.

Interestingly, the findings from our work indicate that anxiety-like behavior responses to MD differ according to sex and age. Maternally deprived males showed greater anxiety-like behavior than females during young adulthood. However, during adolescence and middle adulthood, no discernible gender distinctions were identified among the groups. Our results are in concordance with our recent findings and others works that support a sexually dimorphic response to ELS in rodents, with young adult males displaying more anxious behavior compared to females (Benmhammed *et al*., 2019; Prusator and Greenwood-Van Meerveld, 2015; Guadagno *et al*., 2018; Cabbia *et al*., 2018). The differential impact of MD on genders can be partially ascribed to the female cycle profile, with rats in the pro-estrous and estrous phases exhibiting reduced anxiety compared to males (Molina-Hernández *et al*., 2006; Ramos-Ortolaza *et al*., 2017). This difference can also be explained by the selective dendritic hypertrophy and a higher number of spines in the basolateral amygdala of male rodents noted following MD (Guadagno *et al*., 2018). Additionally, our results revealed also that male and female adolescent MD rats appeared to be more affected by anxiety-like behavior in comparison with young adult and adult rats. The anxiogenic effect of MD did not persist into young adulthood (in females) or middle adulthood (in both genders). As known, adolescence is a period of heightened behavioral and psychiatric susceptibility, and of rapid structural and functional neurological developments (Sturman and Moghaddam, 2011). The elevated anxiety-like behavioral sensitivity to MD in adolescence may be caused, at least partially, by the accelerated development of limbic structures, particularly the central nucleus of the amygdala, compared to the PFC (Arain *et al*., 2013). This distinct developmental pace of limbic structures may render the PFC even more vulnerable to the detrimental effects of stress during the adolescent period (Arnsten, 2009). Furthermore, it is suggested that the ELS may prepare rats to cope with adversity in young adulthood and middle adulthood in a more adaptive manner (Loi *et al*., 2014). Another explication is that the severity of the MD protocol used in our study was not intense enough to influence anxiety-related behaviors in young adult and adult rats. Further investigation is needed to clarify the contribution and significance of these factors in controlling the effect of MD through adolescence, young adulthood, and middle adulthood.

In the case of assessing depressive-like behavior, the FST has been used as a valid test in rodents (Porsolt *et al*., 1978). Our results showed that MD led to an emergent depressive-like behavior that was present during early adolescence, young adulthood, and middle adulthood. Our results align with prior studies that have shown an elevation in depression-like behaviors in both adolescent and adult animals following ELS (Réus *et al*., 2015; Lukkes *et al*., 2017; Benmhammed *et al*., 2019). Additionally, in the study of Marco, the MD at P9 or P12 facilitated depressive-like symptoms during both adolescence and adulthood (Marco *et al*., 2009). However, with the other protocol of maternal separation, the recent study by Banqueri reported that long-term maternal separation (P1-P21) did not induce depression-like behavior in adolescence and adulthood (Banqueri *et al*., 2019). This variation in results may be linked to the timing and duration of maternal separation, important factors in manifesting the ELS action.

Importantly, our current work also reported age- and gender-specific effects of MD. This ELS increased depression levels in adolescent males, but not in adolescent females. In contrast, sexually differentiated MD impacts on depression-related behavior appear reversed with age, exposure to ELS exerts depressogenic effects on young adult and adult females, but not effects on males. In adolescent female rats, estrogen may influence the action of MD. Gender differences have already been identified (Ibrahim *et al*., 2016), showing that male rats display longer TIM in the FST than females. In this context, it has been documented that estrogen reduces susceptibility to stress-provoked depression in female rats. Along the same lines, ovariectomized rodent models have been protected from depressive behavior by female hormones (Ibrahim *et al*., 2016). The estrogen has the ability to upregulate dopamine receptors (Lee and Mouradian, 1999). The downregulation

of the dopamine receptors is regarded as a pivotal factor in the onset of depression (Zhu *et al*., 2011). These findings may clarify why adolescent female rats are less sensitive to MD than adolescent males when evaluating depression-associated outcomes. In addition, the marked depressogenic effect in young adult and adult female rats may be attributed to the decreasing levels of estrogen in females and the corresponding increase in males as they age. Further studies into the mechanism driving these age and gender-selective, MD-caused depressive-like behaviors are necessary.

On the other hand, one mechanism hypothesized as a factor causing anxiety- and depression-like behaviors following stress is an elevated OS, especially in the PFC, a structure of the brain implicated in mood regulation (Belzung *et al*., 2014). In this regard, a correlation between mood changes and OS was reported (Nassiri *et al*., 2023b; Zghari *et al*., 2023a, b). LPO levels are raised in psychiatric disturbances, such as depression (Ozcan *et al*., 2004). Furthermore, recent works have highlighted the involvement of NO in the pathogenesis of depression and anxiety (Dhir and Kulkarni, 2011). In our study, we found that affective impairment following the MD paradigm is linked to oxidative damage, reflected in increased levels of MDA and NO in the PFC in rats during adolescence and young adulthood. These results are in line with recently published data in which adolescent and young adult rats exposed to MD exhibited higher concentrations of LPO and NO in both the HPC and PFC (Réus *et al*., 2018; Benmhammed *et al*., 2019; Abelaira *et al*., 2021). However, the study of Drastichova revealed that maternal separation for 3 hours a day during the first three weeks of life did not provoke OS either in PFC or HPC of both juvenile and adult rats (Drastichova *et al*., 2021). It is demonstrated that the generation and severity of OS in the brain of maternally separated rats depend on the experimental conditions such as the duration of separation (Diehl *et al*., 2012; Marković *et al*., 2017). Short-term maternal separation may be related to the onset of OS, while prolonged maternal separation may restore the initialized state of the antioxidant defense system (Drastichova *et al*., 2021).

The elevated levels of NO and LPO noted in our work may be attributed to the effects of corticosterone. Higher plasma corticosterone levels were detected in maternally separated rats (Ladd *et al*., 2000). This hormone leads to increased basal levels of free radicals in the PFC and HPC (McIntosh *et al*., 1998). Briefly, stress-provoked corticosterone elevations may play a key role in activating dopamine transmission and interact with mesocorticolimbic brain regions (McIntosh *et al*., 1998). The increased DA metabolism by stress is limited to PFC if the ELS severity is sufficiently low, and exposure duration is short. As a result, increased DA turnover causes OS due to increased production of free radicals. In excess, free radicals such as NO cause serious deterioration of biological macromolecules such as lipids (Shields *et al*., 2021), and a lack of regulation of neurotransmitters like serotonin (Siwek *et al*., 2013), contributing to the affective disorders detected in such cases. Finally, we found that MD induces OS in a sex-dimorphic manner. Interestingly, in contrast to males, adolescent females are clearly protected from maternal MD-induced OS. Female rats showed no difference in NO and LPO levels in PFC. A possible explanation is the protective effects of circulating female hormones. Since estrogens appear to have potent antioxidant and radical-scavenging properties (Ventura-Clapier *et al*., 2017), it may be assumed that female rats are less vulnerable to the pro-oxidant activity of MD. Estrogen has been shown to inhibit LPO in both in vivo and in vitro experiments (Miura *et al*., 1996).

CONCLUSIONS AND RECOMMENDATIONS

In summary, our study demonstrated a significant induction of anxiety and depression-like behaviors in adolescent, young adult, and adult rats subjected to ELS through 24-hour MD. Furthermore, our findings suggest that the neurotoxic effects of MD may be mediated by the induction of OS in the PFC. Additionally, the altered behavioral and OS responses to MD vary based on age and sex.

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NOVELTY STATEMENT

To our knowledge, this study is the pioneering exploration into the modulation of anxiety-like and depression-like disorders as well as OS through the interaction of age, sex, and MD.

AUTHORS' CONTRIBUTION

All authors contributed equally to the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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