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Age and Sex-Specific Effects of Maternal Deprivation on Memory and Oxidative Stress in the Hippocampus of Rats

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Abstract

Early-life stress (ELS) is a risk factor for a variety of neuropsychiatric disturbances, including anxiety and depression. ELS in the form of maternal deprivation (MD) can be linked also to memory impairment during adulthood. However, the short-term behavioral consequences of MD have not been evaluated in detail. In addition, the mechanism by which MD induces these impairments is far from fully understood. Therefore, in the present work, we exposed 9-day old. Wistar rats to 24 h MD with the objective to investigate the effects of ELS on memory performances and oxidative stress (OS) in the hippocampus (HPC) of rats across different ages (adolescence, emerging adulthood, and middle adulthood) and the possible existence of sexual dimorphisms. The rat pups were exposed to MD in the early postnatal period. Then working and recognition memory were tested at different time points during adolescence, emerging adulthood and middle adulthood, using the Y-maze and Object Recognition (OR) tests, respectively. Additionally, we examined the effect of MD on the level of lipid peroxidation and nitric oxide in the hippocampus. Interestingly, compared to controls, rats in the MD groups scored significantly an altered working memory performances in Y-maze (but not in the ORT) in both genders, which are linked to an increased OS in the HPC of rats. In addition, gender differences in response were observed for most measures. Our results suggest that MD induces short-term and long-term changes in memory performance, and in OS. Such effects differ according to age and sex.

Keywords: Maternal deprivation, Memory response, Oxidative stress, Dimorphism, Wistar Rat

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1. Introduction

Factors such as parental loss, childhood abuse, viral infection, malnutrition or low socio-economic status cause early life stress (ELS), this ELS is linked to a significant risk of depression and mental health problems during childhood or adolescence [1,2] a high risk of several pathologies, such as cardiovascular diseases [3], oncological and metabolic diseases [4]. Recent studies showed that ELS constitutes a risk factor for the development of neuropsychiatric diseases in the later phases of life, including anxiety and depression disorders [5], memory troubles, cognitive impairment, and personality disorders [6,7].

several animal models are used to study early stress and to explore the links between early adversities and psychopathologies that occur in adolescence or adulthood [8]. Maternal deprivation (MD) in early life in rodents, like a period of 24-hour on 9th day age (PND 9), was mostly used as an animal model to follow various behavioral changes in these animals [9]. According to this model, early loss of maternal support (emotional care and feeding) during the low stress reactivity period (SHRP) (from PND 4 to 14) can influence infants' quality of life [10]. Deprivation of puppies from their mother for a single prolonged period leads to an increase in serum corticosterone, which would have an action on the hypothalamus-pituitary-adrenal (HPA) axis and an impact on the neurodegenerative effects of glucocorticoids [11]. Studies have shown that the P9 MD model has a long-term impact on cognitive and emotional functions. [5]. Additionally, the effect of DM on individuals' neuropsychiatry has been linked to anatomical and neurochemical biomarkers in specific brain regions, especially the prefrontal cortex (PFC) [12].

Recent studies have attempted to determine the physiological mechanisms explaining the relationship between neurobehavioral disorders and ELS [13]. The effects of DM on neuropsychiatry could be explained by neuroinflammation, disruptions of the HPA axis (excess release of neurotransmitters and stress hormones) or epigenetic modifications [14,15]. other mechanisms have been proposed to explain the effects of MD, such as oxidative stress (OS) which would affect brain structures [16]. OS which reflects an imbalance between oxidant and antioxidant has been linked to psychiatric disorders such as depression and anxiety in humans and animals [17,18]. as has been

shown in our previous studies, MD is responsible for neurobehavioral alterations translated by oxidative damage in certain regions of the brain, such as the PFC [5], brain region implicated in several cognitive functions like memory, mood, and learning processes [19].

According to the literature, most studies have focused on the impacts of MD on the behavior of adult animals. Thus, the effect of deprivation of adolescents and young adult animals from their mother is rarely studied (short- and long-term effects). In our previous studies in rat models, we showed that P9 MD is responsible for anxiety and depressive behaviors and memory disorders in adults [5]. To our knowledge, the majority of studies evaluating the effects of MD have not explored the effect of sex and use exclusively male rodents and often give varied results [20]. Based on these studies, further research on the influence of age and gender on the impact of MD on affective and cognitive disorders is needed. Thus, the present study was designed to evaluate the effect of MD on the neurochemistry of brain HPC area and memory in rats at different age periods (adolescents, young adults and adults) and different sexes.

2. Materials and Methods

2.1. Animals and procedure

To carry out the experiments, pregnant female Wistar rats provided by the Laboratory of Biology and Health, Department of Biology, Faculty of Sciences, Ibn Tofail University, Morocco, were individually housed in standard plastic cages (210 × 290 × 430 mm) at a fixed ambient temperature (24 ± 1 °C), humidity (50-60%) and ventilation. Rats were maintained in a 12 h light/dark cycle (lights on at 7:00 am to 19:00 pm) with free access to food and water. A total of 80 pups are given by pregnant females. The day of delivery was marked as P0. Then, the half of the litters were subjected to the MD protocol on PND9 according to the protocol described by Llorente et al. [20]. Briefly, 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. For this experiment, for each gender 24 animals were divided into two groups, out of which four control and four MD rats were submitted to the behavioral tests and sacrificed on P60, which corresponds to adolescence. Four control and four MD rats were submitted to the behavioral tests and sacrificed at the period of young adulthood (P90). The other two groups (four control and four MD rats) were sacrificed at the 10 months-old age (middle adulthood). Every effort was made to reduce animal suffering and the number of rats used in the experiment. All experimental procedures were performed according to the National Institutes of Health guide for the care and use of Laboratory animals and were authorized by the Doctoral Study Center at the university.

2.2. Memory tests

Behavioral tests were performed to assess the working memory (Y-maze) and learning and memory performances (Object Recognition Test (ORT), respectively.

2.2.1. Y Maze

Spatial working memory was evaluated by spontaneous alternation behavior in the Y-maze test [21]. This three-arm apparatus (A, B and C; 61*35*12 cm^3) was made of brown wood, at a 120° angle from each other. The animals were placed individually in the center of the apparatus and allowed to freely move over an 8-minute session. The sequence of alternation (i.e. ACBCABCAABCABAC, etc.) was recorded and analyzed to obtain the % correct alternation which is calculated according to the following formula: % of Spontaneous alternation = [(Number of alternations) / (Total arms entries-2)] × 100.

2.2.2. Object recognition Test (ORT)

To assess learning and memory performances in rats, the ORT is used in this experiment [22]. The protocol is well detailed in our previous work [23]. Generally, deficits in short-term recognition memory are expressed by a reduction in the recognition index (% RI), which is calculated as follows: [The total time spent exploring the novel object / (The total time spent exploring the novel object + The total time spent exploring the familiar object)] x 100.

2.3. Biochemical analysis

The day after finishing the behavioral tests, the rats were anesthetized and euthanized by a rapid decapitation, the prefrontal cortex was dissected by free hand technique, and the tissue were 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) [24]. Levels of NO were estimated using Griess reagent [25].

2.4. 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 and the main actions of two factors "age" (three levels – adolescent, emerging adulthood and middle adulthood) and "sex" (two levels – female and male) were examined. Data were expressed in the form of mean ± SEM. A $p < 0.05$ was considered a significant difference.

3. Results and Discussion

3.1. Effects of Maternal deprivation on memory

3.1.1. Y-maze test

Working memory was assessed in rats using the Y-maze test as shown in Figure 1. In males, the percentage of spontaneous alternation on the Y-maze was significantly decreased in adolescent and young adult MD groups by 39% ($p < 0.05$) and 31% ($p > 0.05$), respectively, when compared with control rats. Whereas in female rats, only the adult MD rats had a lower % of alternation when compared with their controls (–42%; $p < 0.01$). While a non-significant decrease was observed in the two other ages ($p > 0.05$). In addition, an age effect was noted. The comparison between different age groups of males revealed that the adolescent and young adult MD groups had a decreased percentage of spontaneous alternation in comparison with adult MD group ($p < 0.01$ and $p < 0.05$, respectively). In contrast, in the females, the adolescent and young adult MD groups had an increased %

of alternation in comparison with adult MD group ($p < 0.001$).

Importantly, at the different ages, the statistical analysis revealed a main impact of sex, with adolescent and young adult males maternally deprived showing an impaired working memory, as indicated by a decreased % of alternation when compared to females' group counterparts ($p < 0.05$ and $p < 0.01$, respectively). However, Tukey test showed that adult female rats made a lower % of alternation in comparison with the male's group counterparts ($p < 0.01$).

3.1.2. OR test

Recognition performance was evaluated by ORT test and was showed in Figure 2. The statistical analysis showed that there were no obvious changes in the Recognition Index in all MD groups of both genders ($p > 0.05$). Additionally, in all MD groups no significant effect of age and sex on recognition index was revealed ($p > 0.05$) (Figure 2).

3.2. Effects of Maternal Deprivation on Oxidative Stress parameters in the hippocampus

3.2.1. Effects on NO levels

The statistical analysis revealed that NO levels were significantly elevated only in the adult rats of both genders maternally deprived, when compared with the control rats (+143% in males; $p < 0.001$ and +31% in females; $p > 0.05$). While, in the other two age groups, differences between males and females and their controls was not statistically significant ($p > 0.05$).

Additionally, in male and female MD groups, a significant effect of age and sex on NO levels was showed (Figure 3). The adult rats had significantly higher NO concentrations in comparison to both adolescent and young adult rats ($p < 0.001$ in males; $p < 0.01$ and $p < 0.05$ in females, respectively). Also, the statistical analysis revealed also that the adult rats of both genders presented a higher NO levels when compared to the adult females (***+143***% vs. ***+31***%; $p < 0.01$). In the other two age groups, differences between males and females was not statistically significant ($p > 0.05$).

3.2.2. Effects on TBARS levels

As shown in Figure 4, the TBARS levels were significantly increased only in young adult male rats maternally deprived when compared to control groups (+31% in males; $p < 0.01$ and +0% in females; $p > 0.05$). In addition, a non-significant increase in TBARS concentrations was noted in adult male and female animals ($p > 0.05$). While, the comparison between TBARS levels of the adolescent groups of both genders and the control groups revealed no significant difference ($p > 0.05$). On the other hand, in both genders, the comparison between different age groups revealed that the adult MD groups had a higher TBARS levels compared to both adolescent and young adult MD groups ($p < 0.01$ in males and $p < 0.01$ in females) (Fig. 4). Concerning the sex impact, the adult male rats maternally deprived had significantly higher TBARS compared to the young adult female rats (+31% vs. +0%; $p < 0.01$). Also, in adolescent and adult MD groups no significant effect sex was evident ($p > 0.05$).

In this study, we utilized a MD model to explore the influence of ELS on memory state and oxidative stress in the hippocampus of male and female rats. The main results of this research are as follows: (1) MD-exposed rats show impaired memory, particularly in the Y-maze test; (2) MD-exposed rats revealed an increased levels of NO and LPO in the hippocampus; MD affects memory and oxidative stress in an age- and sex-dependent manner. Regarding the effect on memory, the results of the present experiment indicated that MD led to a significant decline in memory performance in experimental rats. These conclusions were drawn from the reduced % of alternations in the Y maze. However, our work detects no significant impact of MD in rodents when tested in the ORT. These findings are consistent with recent studies reported that MD affect memory performance, especially the working memory in adolescent and adult rats [26,27]. In addition, it has been demonstrated that ELS impaired recognition memory in adolescence and adulthood in rodents [28,29], while others notice no difference [30,31].

Sex is another crucial aspect of the MD model. Male and female rats differ in neuroendocrine and behavioral responses, and susceptibility to stress is sex-dependent [32]. In this regard, in our study, we observed that memory impairment was significantly more marked in adolescent and young adult male rats compared to female rats. These results are in accordance with the other works. For example, following ELS, the male rodents are impaired, but stressed females showed enhanced performance in comparison with their control counterparts on the Y-maze [33]. It seems that adolescent and young adult female rats are more resistant than males to the adverse effects of stress, as they show better performance on the Y-maze, whereas males show cognitive deficits [34,35]. Also, our study in association with some studies detect no sex difference in rodents when performing recognition memory in the OR [36,37]. These discrepancies may be linked to the type of spatial task, the strategy used, exposure to stress and activation of the stress axis, the cues that can be used to solve the task, and hormonal levels. Interestingly, the apparent female resistance to MD effects on working memory may be the result of both organizational and activational influences of estrogen [33]. Also, the increase in basal corticosterone levels in male rodents compared to females following stress may be the cause of the pronounced effects observed in males [38]. As known, corticosteroids are essential for memory [39]. However, excessive corticosterone responses to stress have been associated with memory impairment in different tests [40]. Glucocorticoids can affect memory by activating glucocorticoid receptors in the hippocampus [41]. Then, the impaired glucocorticoid receptor function leads to altered cognitive and spatial abilities [42]. However, it is noteworthy that several other factors including differences in neuronal circuitries, ultrastructural and molecular mechanisms, independent of hormonal influence, may constitute the biological substrates of such dichotomies in male–female responses.

On the other hand, our current work also reported an age-specific effects of MD. ELS did not affect adult male performance on the Y-maze, and these results differ from those of male adolescents and young adults, who experience reduced performance following MD. In contrast, female rats are no longer enhanced, the normal female advantage seen on memory dissipates with age.

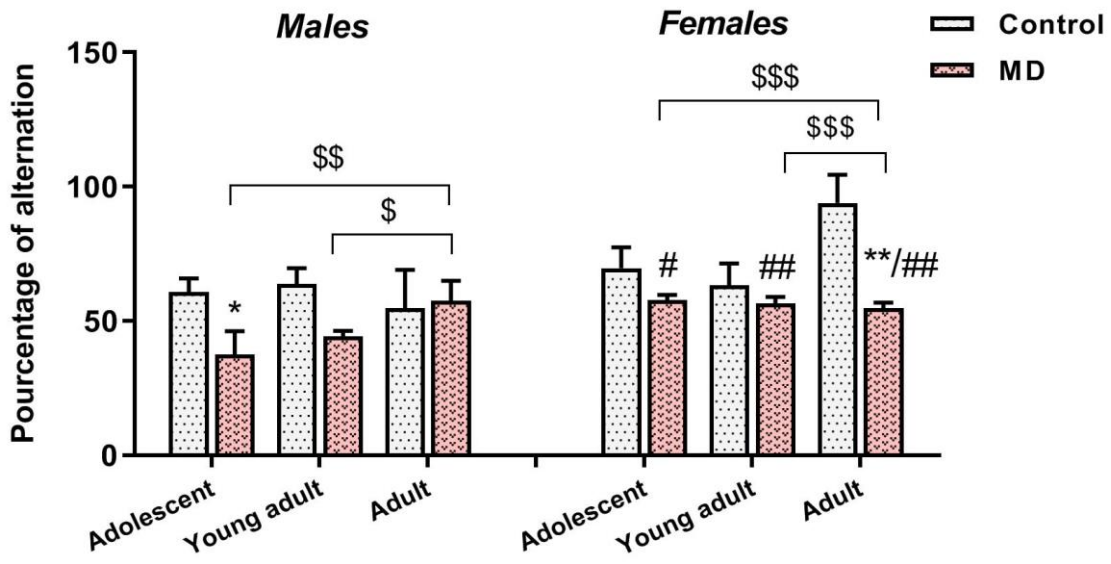

Figure 1: Effect of Maternal deprivation on % of alternation in adolescent, young adult and adult females and male rats in Y-maze. The symbols *, # and $ denote significant differences compared to control, or to gender counterparts, or between different age groups, respectively. The significance level is 0.05 with (*, #, $) $p < 0.05$, (**, ##, $$) $p < 0.01$, and (***, ###, $$$) $p < 0.001$.

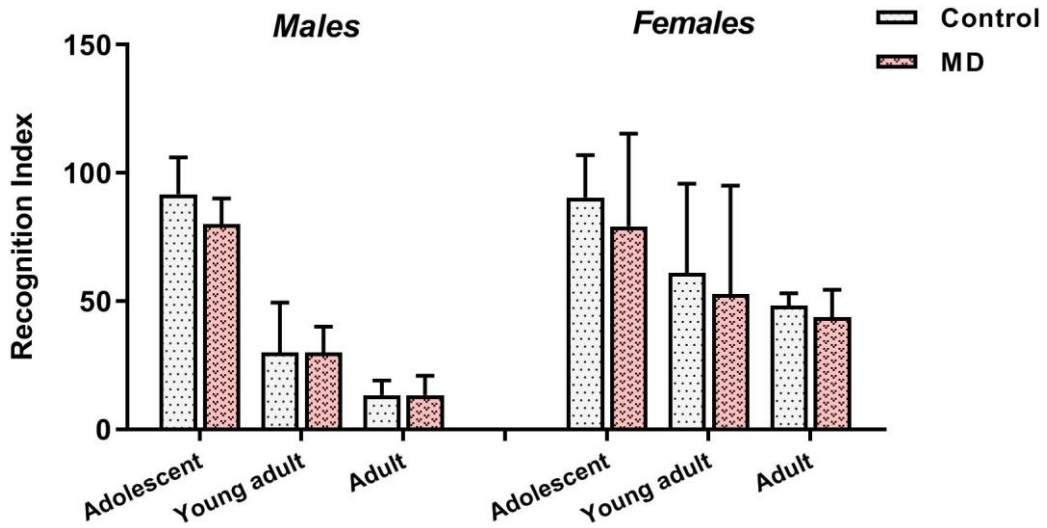

Figure 2: Effect of Maternal deprivation on recognition memory in adolescent, young adult and adult females and male rats measured in the object recognition test. The symbols *, # and $ denote significant differences compared to control, or to gender counterparts, or between different age groups, respectively. The significance level is 0.05 with (*, #, $) $p < 0.05$, (**, ##, $$) $p < 0.01$, and (***, ###, $$$) $p < 0.001$.

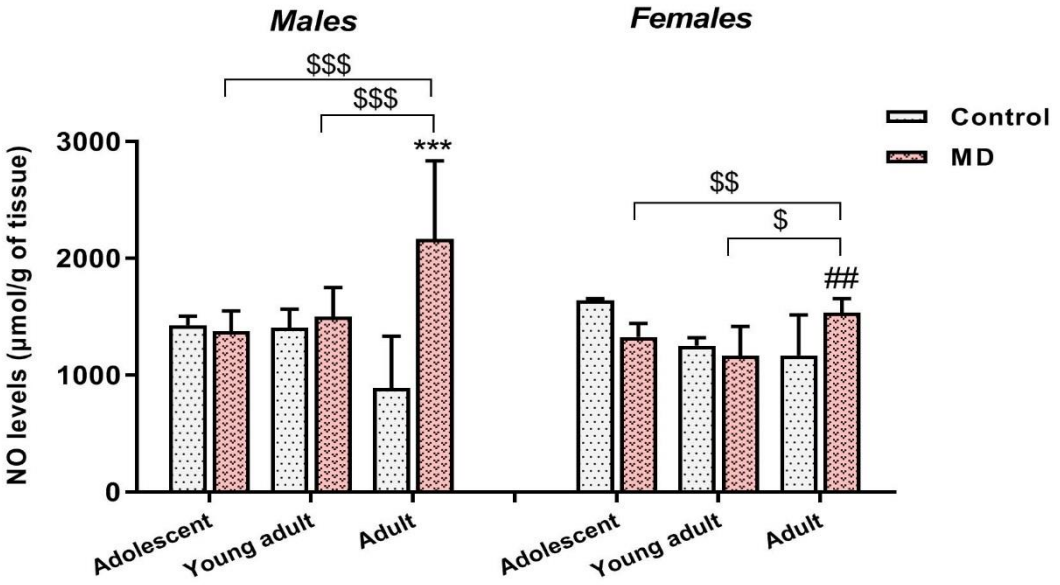

Figure 3: Effect of maternal deprivation on nitric oxide (NO) in the hippocampus of adolescent, young adult and adult females and male rats. The symbols *, # and $ denote significant differences compared to control, or to gender counterparts, or between different age groups, respectively. The significance level is 0.05 with (*, #, $) $p < 0.05$, (**, ##, $$) $p < 0.01$, and (***, ###, $$$) $p < 0.001$.

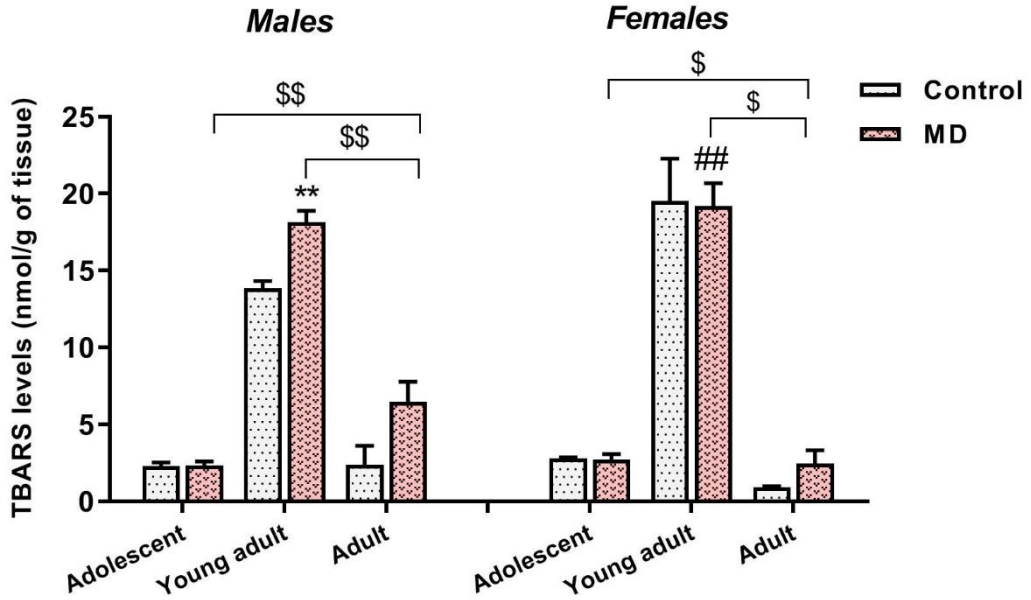

Figure 4: Effect of maternal deprivation on thiobarbituric acid reactive substances (TBARS) content in the hippocampus of adolescent, young adult and adult females and male rats. The symbols *, # and $ denote significant differences compared to control, or to gender counterparts, or between different age groups, respectively. The significance level is 0.05 with (*, #, $) $p < 0.05$, (**, ##, $$) $p < 0.01$, and (***, ###, $$$) $p < 0.001$.

The underlying mechanisms for the MD effects on cognitive performance in adolescent, young adult and adult male and female rats are unclear but may include changed sensitivities of the HPA axis and changes in gonadal hormone levels with age [35]. As mentioned above, estrogens are known to influence female resistance to deleterious stress effects on memory performance [35] and, thus, a reasonable hypothesis is that the increased levels of estrogens and testosterone detected in the adult males are conferring stress resistance on memory performance [43].

It appears that adult male rats are responding to restraint stress exposure in a similar fashion to adolescent and young adult females who are thought to be protected from the deleterious effects of stress possibly by their ovarian hormones. Furthermore, the fact that estrogen levels decline with age makes adult females more vulnerable to the damaging effects of MD. The mechanisms of the cognitive alterations induced by ELS are unknown. In the present work, we examined also the impacts of MD on OS in the hippocampus of rats, region of the brain implicated in memory processes and consolidation [44]. Damage to this structure has been associated with cognitive and motor dysfunction [45]. Furthermore, a correlation between oxidative state and memory impairment has also been documented [46,47]. LPO, along with other biomarkers of OS such NO, have recently been linked to memory impairment[17,48]. In line with this, we demonstrated that MD leads to an increase in LPO and NO concentrations in the hippocampus only in young adult and adult males.

These findings were parallel to several experiments, in which, adult rats maternally deprived showed increases in LPO and NO concentrations in HPC [5,16,49]. However, the experiment of Drastichova et al., showed that maternal separation for 3 hours a day during the first three weeks of life did not provoke OS in the hippocampus of both juvenile and adult rats [50]. The generation and severity of OS in the brains of pups separated from their mothers clearly depend on the experimental conditions such as the duration of separation [51,52]. It is conceivable that short-term maternal separation may be responsible for the generation of OS, while prolonged maternal separation may result in the restoration of the initial state of the antioxidant defense system [50].

The increased NO and LPO levels revealed in this study during adulthood might be explained by increased corticosterone levels following ELS [53]. Excessive corticosterone can lead to increased toxicity of free radicals, such as NO, in the hippocampus [54]. Subsequently, at high levels, NO initiate a cascade of chemical events that will exacerbate LPO, resulting in an impairment in brain function due to increased damage to lipids, proteins and a deficiency in the modulation of neurotransmitters, such as acetylcholine and glutamate [55], contributing to the altered memory observed in such cases. Additionally, the present study demonstrates that MD induces OS in a sex dimorphic manner. In contrast to males, the young adult and adult female rats are apparently protected from the MD-induced OS. Females did not show any difference for NO and LPO levels in the hippocampus. The less pronounced impacts of MD in females suggest the role of protective mechanisms in this sex, at least regarding the parameters evaluated in this experiment. Previous study in adult rats already showed sexual dimorphisms regarding the effects of stress on hippocampus, with males being more vulnerable [5]. These differences may depend on protecting effects of the female gonadal hormone estrogens, possibly through a radical scavenging and antioxidant actions of this hormone [56], making the females less vulnerable to the effects of MD.

5. Conclusions

Cumulatively, our data indicate that exposure to MD protocol during the first two weeks of development provoked memory impairment in adolescent, young adult and adult Wistar rats. Also, the findings of the present work revealed that neurotoxic effects of MD may be, at least partially, mediated via the induction of OS in the HPC. Moreover, the altered memory and OS responses to MD differ according to age and sex.

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Compliance with ethical standards

All experimental procedures were performed according to the NIH Guidelines for the Care and Use of Laboratory Animals and under observation and authorization of the doctoral studies center at Ibn Tofail University Kenitra, Morocco.

Conflict of interests

All the Authors declare that have no conflict of interest.

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