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Early Maternal Separation as a Precursor of Neurodevelopmental and Neurobehavioural Disorders

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Abstract

The mother-infant bond plays a pivotal role in early neurodevelopment, and disruptions to this relationship can lead to significant behavioral and neurochemical alterations. This study explores the impact of maternal separation on cognitive and exploratory behaviors, redox status, and neuroanatomy in the prefrontal cortex of juvenile rats. Female rats were bred and assigned to either a control or maternal separation (MS) group, with maternal separation pups experiencing a 24-hour separation from their mothers on postnatal day nine. Behavioral assessments using the open field test revealed marked reductions in total line crossings and rearing frequency in the MS group, indicating deficits in cognitive and exploratory behavior. Biochemical assays showed significant increase in oxidative stress markers, including malondialdehyde, and reduction in antioxidant enzyme activity, namely superoxide dismutase and catalase, in the prefrontal cortex of MS rats. Histological observation further revealed signs of cellular degeneration, including poorly stained nuclei and increased chromatolytic cells, in MS rats compared to control. Additionally, increased astrocyte reactivity, as evidenced by hypertrophied astrocytes and higher astrocyte counts, was observed in the prefrontal cortex of MS rats. These findings suggest maternal separation induces oxidative stress and cellular degeneration in the prefrontal cortex, potentially underlying behavioral deficits observed in MS rats. This model provides insights into the neurobiological mechanisms by which early-life adversity impacts neurodevelopment, with potential implications for understanding the development of neuropsychiatric disorders in humans exposed to early-life stress.

Keywords: behavior, maternal separation, neurodevelopment, oxidative stress

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INTRODUCTION

Human interaction in pregnancy and immediately after birth is crucial to the development of mental health in the growing child. The process of breastfeeding and cuddling a newborn has relevance beyond nutrition, and forms a foundation for emotional security and wellbeing. Early attachment between a mother and her baby is beneficial for initiation of neonatal sleep, modulation of hormones, regulation of behavioural responses, and psychosocial maturation [1]. From animal studies to human research, the implications of early maternal separation on the behavioural patterns of the offspring have been described. Lorenz explained imprinting to highlight how animals such as geese follow the mother or object they see immediately after hatching [2]. The importance of his theory was foundational to the understanding of attachment and the lifelong effects of early childhood events. John Bowlby took this further by his human studies that showed the relationship between maternal bonding and mental health, as well as the deleterious effects of maternal separation, and consequent deprivation[3]. Deprivation in the critical phase of early childhood leads to progressive stages of protest, distress and eventual detachment [3]. This sets the tone for psychosocial problems in later life.

Maternal separation has been linked with adolescent depression [4] and is also associated with an increased risk for psychosis in childhood by up to 53% [5]. The critical period appears to be the first two years, as maternal separation of as little as a week within this time frame is associated with child negativity and aggression [6]. Children who grow outside of a home setting could have poor psychosocial outcomes later in life [7].

Animal research has shown that exposure to stress during neurodevelopment affects brain structure and function. Various rodent models, including maternal separation and maternal separation with early weaning, are employed to study early-life stress, particularly to reduce maternal compensation effects and enhance the impact of maternal deprivation on offspring [8]. Such models help elucidate the neurobiological mechanisms behind these neuropsychiatric disorders.

Maternal separation refers to the detachment of an infant or child from their primary caregiver (typically the mother) during crucial developmental phases, causing emotional turmoil whose intensity varies based on the nature of the separation whether traumatic, mild or intentional. Regardless of the cause, maternal separation can have enduring consequences for both mother and child. Factors like the age of the child, the duration and intensity of separation, and the quality of care received during and after separation influence the extent of impact [9].

Various mechanisms underlie the structural and functional brain changes attributable to parental separation. For instance, maternal separation stimulates a neuroinflammatory response with oxidative stress and mitochondrial dysfunction at the cellular level. Furthermore, maternal separation represents a stressful life event that causes dysregulation of the hypothalamic-pituitary axis, ultimately affecting brain plasticity [10].

The mother-infant relationship during the ontogenetic period is crucial for the normal formation of neural circuits and emotional development. Changes in early social experiences can alter the hypothalamic-pituitary-adrenal (HPA) axis and affect stress responses [11]. Early maternal separation overstimulates the stress response system,

raising cortisol levels, altering HPA axis function, and disrupting the balance of neurotransmitters such as serotonin, dopamine, and GABA that regulate mood, motivation, and emotional well-being [12]. This disruption hampers brain development and maturation, with significant consequences for neuroendocrine systems governing stress response, leading to behavioral and cognitive abnormalities. Maternal separation (MS) heightens the risk of emotional changes, mood disorders, and neuropsychiatric conditions in adolescence and adulthood. It has been observed to increase aggressive play in juveniles and reduce social interaction in adults [13]. Early-life stress during critical brain development phases can have long-lasting physical and mental health impacts. Oxytocin, a crucial regulator of social behavior and stress response, exhibits high plasticity during early postnatal and adolescent periods [14]. Stress during these times can cause long-term alterations in oxytocin receptor expression and signaling [15]. Early life adversity is strongly linked to future psychopathologies with deficits in emotional regulation, reward processing, and cognitive function, increasing the risk of anxiety, depression, and stress-related neuropsychiatric disorders. Hence, oxytocin is recognized as a promising target for treating these conditions [15].

The prefrontal cortex, essential for decision-making, personality expression, emotional stability, and cognitive functions, continues maturing after birth, making it vulnerable to stressors like maternal separation during postnatal neurodevelopment. Exposure to such stress has been associated with neurodevelopmental issues, impairing emotional regulation and cognitive function in adulthood [16].

Animal studies have demonstrated the neuroprotective nature of kolaviron and vitamin B complex against neurological sequelae in the hippocampus and prefrontal cortex arising from maternal separation in early postnatal period [17, 18]. These studies suggest a possible supplementary role of micronutrients in the treatment of schizophrenia and other psychotic illnesses that have been found to have oxidative stress as pivotal to the pathophysiology. The present study investigated the impact of maternal separation on the oxidative balance and histomorphology of the prefrontal cortex in rats.

MATERIALS AND METHODS

Animal procurement and care

Mature 20 nulliparous female and 10 male rats with a weight range of 180-200 g were used for the study. The rats were housed in a wire-gauzed cage in the Animal holding facilities of the Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria. The rats were allowed to acclimatize for two weeks; kept under standard conditions of a 12-hour light/dark cycle and fed with pelletized grower feed and water *ad libitum*. The oestrus cycle of the female rats was done using vaginal smear and thereafter exposed to male rats for mating [19].

Animal grouping

The rats were grouped into a control and a maternal separation group. After the pregnant rats were delivered, the mother rats in the maternal separation group were separated from the pups on postnatal day 9 for 24 hours, after which both the mother and pups were re-united in the same cage and re-established breastfeeding [18].

Open Field Test

The open field test was conducted following the method outlined by Gould et al. [20]. The apparatus used was a wooden box measuring 100 cm by 100 cm with 38 cm high walls, open at the top, and positioned in a quiet room with standard lighting and temperature. The floor of the box was marked with straight lines to create 10 cm squares. A video recording system, set at an angle, captured the movement of rats within the arena. Before postnatal day 40, each group of rats was individually transported to the arena in separate cages to prevent any agitation and minimize stress. Each rat was then placed in the arena alone, and its exploratory activity was recorded for 10 minutes. This process was repeated for all groups. Between tests, the apparatus was cleaned with methylated spirit to eliminate any scent left by previous rats. A neutral observer remained out of view during the test, later reviewing the footage to count the number of squares each rat explored.

Tissue collection and processing

On postnatal day 40, some rats from the control and maternal separation group were anaesthetized intramuscularly for histology using 20 mg/kg of ketamine and perfused transcardially with normal saline, followed by 4% paraformaldehyde (PFA). The brains were excised and post-fixed for 24 hr in 4% PFA. The prefrontal cortex was excised, processed and stained in hematoxylin and eosin (H&E) stain and cresyl fast violet (CFV) stain. Meanwhile, rats processed for enzymatic studies were sacrificed by cervical dislocation and the prefrontal cortex was placed in 30% sucrose solution, homogenized and centrifuged at 3000 rpm for 10 min and the supernatant was extracted for further enzymatic analysis. Enzymatic studies were carried out using the enzyme-linked immunosorbent assay to quantify the levels of superoxide dismutase, catalase and malondialdehyde. Using PFA-fixed tissues, the expression of astrocytes was carried out with antibody against anti-glial fibrillary acidic protein through immunohistochemistry.

Data analysis

The results obtained were subjected to statistical analysis using the GraphPad Prism software, Version 6 (GraphPad Software Inc., San Diego, CA, USA). Malondialdehyde, catalase and superoxide dismutase results were plotted in the T-test with Tukey's multiple comparisons test. Data obtained were presented as mean ± standard error of mean, with the determination of the level of significance. The outcomes were represented in bar charts with error bars to show the mean and standard error of mean, respectively.

RESULTS

Maternal separation resulted in cognitive and exploratory deficits

The cognitive and exploratory behaviors of the animals in both groups were assessed with an open field test and the total lines crossed (Fig. 1a) and frequency of rearing (Fig. 1b) were recorded. The results revealed a significant decrease in the total line crossed ($p<0.001$) and rearing frequency ($p<0.005$) in the maternal-separated group in comparison to that of the control.

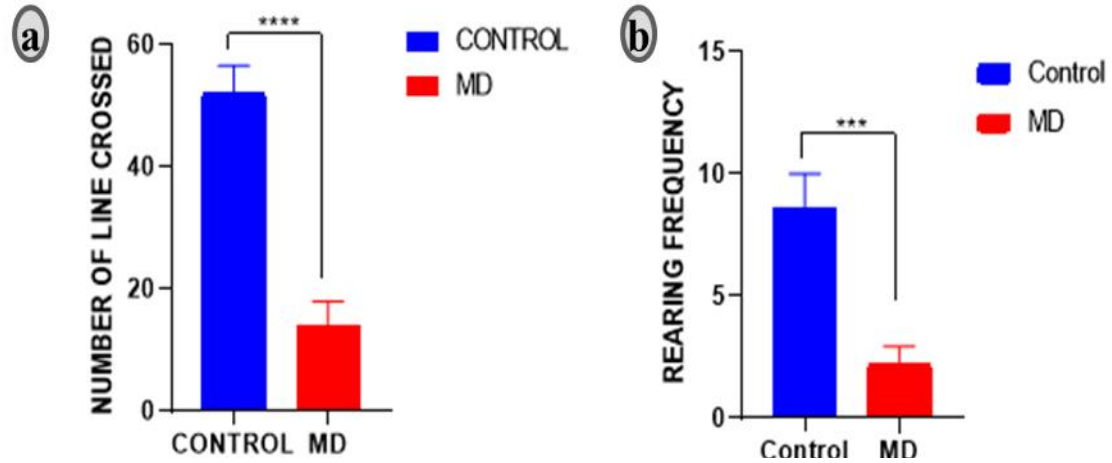

Figure 1: Open field test outcomes among experimental groups. (a): Number of lines crossed; (b): Rearing frequency. The results showed a significant reduction in both the rearing frequency and total line crossed in the maternal-separated group when compared to the control group. ****= ($p<0.001$; n=5); ***= ($p<0.005$; n=5).

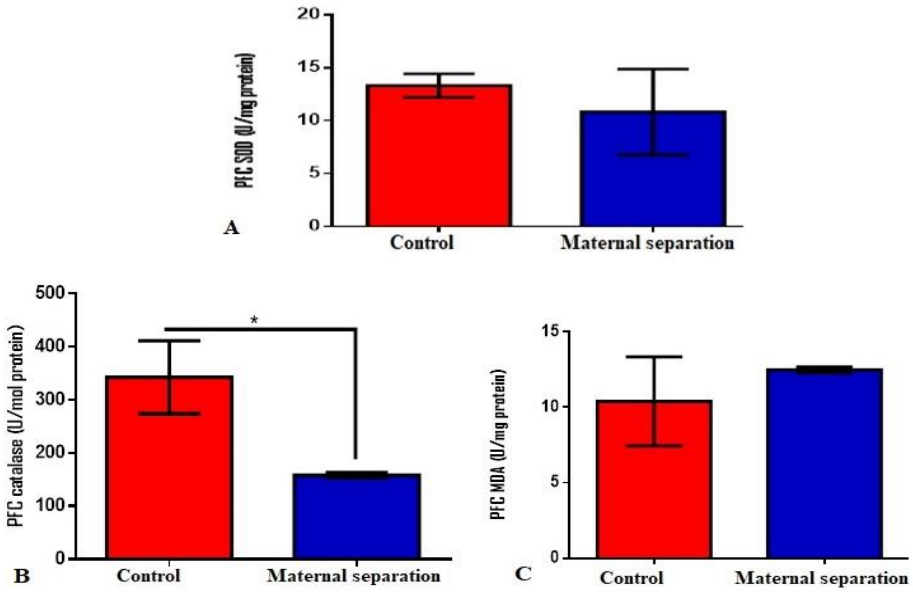

Figure 2: Reduced activities of enzymatic oxidative markers: superoxide dismutase (SOD; A) and catalase (B) and elevation of malondialdehyde (MDA) in the prefrontal cortex (PFC) maternal separation rats compared with the control. * $p<0.05$.

Maternal separation elevates redox reactions and reduced antioxidant activity

The results in Fig. 2a-c showed the level of superoxide dismutase, catalase and malondialdehyde in the prefrontal cortex lysate of rats. The result showed a higher stress level in the maternal separated group when compared to control as indicated by a significant decrease in antioxidant activity (catalase; $p<0.05$) and increased lipid peroxidation which is shown by elevated cortical malondialdehyde level.

Histological and histochemical changes in the prefrontal cortex due to maternal separation

Morphological and Nissl substance integrity was assessed with H&E and CFV respectively. Maternal separation of early litters resulted in cortical assaults which is indicated in this study by poorly stained nuclei denoting cellular degeneration (Fig. 3a) and the presence of increased chromatolytic cells (Fig. 3b) when compared with the control.

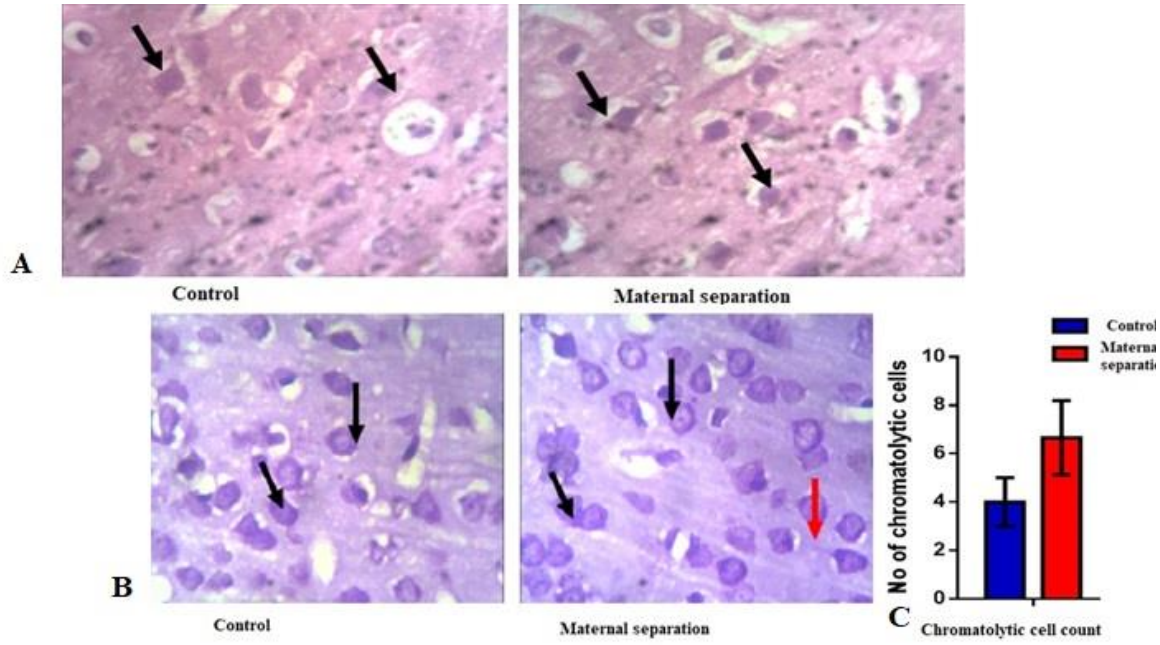

Figure 3: Photomicrographs of the prefrontal cortex showing (A) hematoxylin & eosin (H&E) and (B) cresyl fast violet (CFV) stained sections and (C) chromatolytic cell counts. The control revealed deeply stained nuclei of neuronal and non-neuronal cells (H&E-arrows) within their respective neuropils and abundant Nissl substances within the neurons (CFV-arrows). Cortical sections of maternal separation group showed numerous cellular degeneration indicated by poorly stained nuclei (H&E-arrows), and presence of chromatolytic cells (red arrow) which was more in maternal separation group (C).

Astrocytic reactivity and morphological changes due to maternal separation

Prefrontal cortical sections of maternal separation group revealed activation of more astrocytes with the presence of hypertrophied astrocytes and increased number of astrocytic cells, compared with the control (Fig. 4).

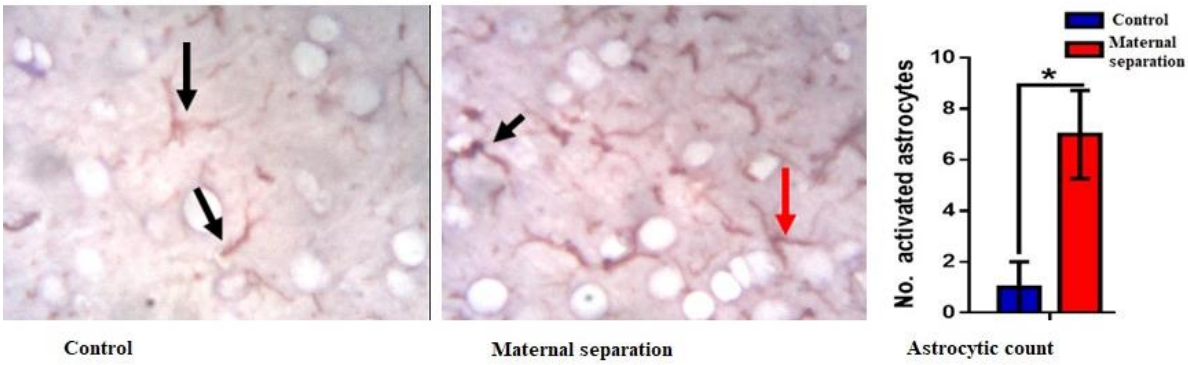

Figure 4: Expression of astrocytes in the prefrontal cortex of the control and maternal separation groups showing immunopositivity for anti-GFAP antibody. Maternally separated rats presented with increased number of activated astrocytes (red arrow) and astrocytic count.

DISCUSSION

The maternal separation paradigm is a model frequently employed in rodents to investigate how early-life adversities can change an individual's sensitivity to challenges later in life and how they can influence their resilience or susceptibility to psychopathologies like addiction, depression, or anxiety [21, 22]. Different models of maternal separation have been employed by different researchers, and these involve subjecting pups to recurrent maternal absence during the early postnatal days, or for a varied length of time [23, 17], after which both the mother and pups are re-united and breastfeeding is re-established. The current examined the implication of maternal separation on the behavior, oxidative status and histomorphology of the prefrontal cortex of rats.

Maternal separation, a well-characterized early life stressor, has a profound effect on numerous behavioral aspects of cognition and exploration in model organisms of different species. This stressor interrupts brain development and neuroendocrine functions and often leads to anxiety, hypo-locomotor activity, and exploratory deficits [24]. The open field results in this study show a significant reduction in both the rearing frequency and total line crossed in the maternal-separated rats when compared to the control. The results of this study agree with the findings of previous studies that reported a significant decline in both the cognitive and exploratory behavior in animals following maternal separation [13, 25]. Oxytocin, a key hormone involved in regulating social behavior and stress responses, shows significant adaptability during the early postnatal and adolescent stages. Maternal separation, however, may lead to lasting changes in oxytocin receptor expression and signaling [15],

resulting in impairments in emotional regulation, reward processing, and cognitive function, which elevate the risk of anxiety, depression, and other stress-related neuropsychiatric disorders. This might be the reason for the decreased cognitive and exploratory activities reported in this study.

Perturbation of the cellular redox balance in the prefrontal cortex occurred following maternal separation. This was clearly revealed in the level of activities of oxidative enzyme markers: superoxide dismutase and catalase which was low, and the increased level of malondialdehyde in the prefrontal cortex lysates of rats separated from their mother on postnatal day 9 compared with the control that were not separated. The findings indicate heightened stress in the maternal separation rats, as evidenced by a significant reduction in antioxidant activity and an increase in lipid peroxidation. Lipid peroxidation, in conjunction with various oxidative and nitrosative stress biomarkers, has been linked to childhood physical neglect [26]. The brain is more vulnerable to oxidative injury, especially the infant brain due to its lower antioxidant capability when compared to other parts of the body. A key role of the antioxidant defense system is to protect against the damaging effects of reactive oxygen species (ROS). Increased ROS production and lipid peroxidation can disrupt mitochondrial function, reducing ATP production in the electron transport chain and potentially damaging neurons and impairing their development. In a previous study, we observed significantly elevated stress levels and reduced antioxidant activity following maternal separation [27], findings that are consistent with our current study. The elevated stress levels observed in this study may underlie the negative morphological changes observed in the prefrontal cortex of maternally separated rats, as redox imbalances are known to significantly compromise cell membrane and organelle integrity, potentially leading to cell death. The degeneration of Nissl substance in the prefrontal cortex of these animals may inhibit the protein synthesis mechanisms within neurons, ultimately disrupting cellular processes and neurological functions. Nissl bodies, composed of rough endoplasmic reticulum in neurons, are essential for protein synthesis. Furthermore, the endoplasmic reticulum is involved in ROS production, with redox interactions between the mitochondria, endoplasmic reticulum, and peroxisomes contributing to oxidative stress in cells. Our report is consistent with another study that shows prolonged exposure to chronic stress renders neurons vulnerable and makes them prone to degeneration through pathways mediated by neuro-inflammation and oxidative damage as well [28]. Translating these findings into human implications, childhood trauma and abuse have been linked to similar morphological changes in the PFC in the form of decreased cortical volume and disrupted neuronal architecture [4]. Such disruption can affect the development of cognition and lead children towards mental health issues, such as anxiety, depression, and executive dysfunction.

Astrocytes are essential for maintaining synaptic balance and responding to CNS stressors. Their activation, while initially protective, can become maladaptive with prolonged stress, leading to impaired synaptic plasticity and cognitive deficits. These changes in astrocytic behavior align with findings that early adversity disrupts HPA axis regulation and increases pro-inflammatory cytokine activity. Our study demonstrated notable astrocytic reactivity and morphological changes in the prefrontal cortex following maternal separation. This

indicates an adaptive glial response to early-life stress that could result in chronic neuroinflammation and potential long-term neuronal dysfunction [4]. A study has shown that glial scars formed by reactive astrocytes may hinder axon regeneration, exacerbating the neurological dysfunctions often seen in psychiatric disorders modelled by maternal separation. This astrocytic reactivity sheds light on how childhood trauma can lead to structural and functional changes in the brain. The disrupted glial function is linked to mood and cognitive disorders, highlighting the prefrontal cortex's susceptibility to early-life stress and underscoring the need for targeted interventions to counter these effects.

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