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# Neuroanatomical, Neurochemical and DNA Fragmentation Effects of Maternal Sleep Deprivation on the Hippocampal CA1 Region of F1 Female Wistar Rat Offspring

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**ABSTRACT**
Maternal sleep deprivation (MSD) during pregnancy may adversely affect offspring neurodevelopment, particularly hippocampal structure and function. This study investigated the effects of gestational sleep deprivation on the hippocampal CA1 region of female F1 Wistar rat offspring, focusing on pyramidal cell number, glutamate concentration, and DNA fragmentation. Adult male and female Wistar rats were acclimatized for two weeks before mating. Female estrous cycles were monitored, and successful mating was confirmed by spermatozoa and vaginal plug detection, designated as gestational day (GD) 0. Pregnant dams were randomly assigned to control or MSD groups. Dams in the MSD group underwent 20 hours of daily sleep deprivation from GD5 to GD14 using the modified multiple-platform method, targeting a critical period of fetal brain development. Female F1 offspring were euthanized on postnatal day 43 for tissue collection and analysis. Glutamate concentration in the CA1 region was quantified using high-performance liquid chromatography (HPLC), while thionine staining combined with unbiased stereological analysis was used to estimate pyramidal cell number. DNA fragmentation was assessed using Feulgen staining and optical density measurements. Compared with controls, MSD offspring exhibited significant reductions in CA1 glutamate concentration and pyramidal cell number, accompanied by significantly increased DNA fragmentation optical density. These findings demonstrate that gestational sleep deprivation induces persistent neurochemical, structural, and epigenetic alterations in the hippocampal CA1 region of female offspring, indicating that prenatal sleep loss may disrupt hippocampal development and impair long-term hippocampal function.

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## INTRODUCTION

Sleep is a fundamental biological process essential for normal brain development, memory consolidation, synaptic plasticity, and emotional regulation (Walker, 2009; Hyndych *et al.,* 2025). During pregnancy, adequate sleep is important not only for maternal physiological homeostasis but also for fetal growth and neurodevelopment (Satyapal *et al.,* 2025). However, sleep disruption and insufficient sleep are common during pregnancy, arising from hormonal fluctuations, physical discomfort, and metabolic changes (Mindell & Jacobson, 2000; Delgado & Louis, 2022). Maternal sleep deprivation (MSD) may therefore represent an important prenatal stressor with potential consequences for offspring brain development.

Experimental evidence indicates that gestational sleep deprivation can alter endocrine function, increase oxidative stress and inflammation, and disrupt neurodevelopmental processes in offspring (Calegare *et al.,* 2010; Hodor *et al.,* 2014; Liu *et al.,* 2025). These effects are of particular concern in the hippocampus, a brain region critical for learning, memory, and spatial navigation (Clark, 2000). The CA1 subfield is especially vulnerable to metabolic and stress-related insults because its pyramidal neurons are highly sensitive to alterations in glucocorticoid signaling, synaptic activity, and neuronal homeostasis (Kim & Diamond, 2002). Sleep deprivation in adult animals has been associated with impaired hippocampal neurogenesis and synaptic plasticity, altered dendritic architecture, and neuronal loss (Havekes & Abel, 2017; Bishir *et al.,* 2020). These findings raise the possibility that exposure to sleep deprivation during a critical period of gestation may produce persistent alterations in hippocampal development.

Despite increasing evidence linking maternal sleep disturbance with adverse offspring neurobehavioral outcomes, an important knowledge gap remains. Specifically, the effects of gestational MSD on the structural, neurochemical, and epigenetic characteristics of the hippocampal CA1 region in female F1 offspring have not been adequately characterized. Previous studies have largely emphasized behavioral outcomes or generalized hippocampal changes, providing limited information on CA1 pyramidal cell populations, glutamatergic neurochemistry, and DNA fragmentation. This gap is particularly relevant because female offspring may exhibit distinct neurodevelopmental and stress-response profiles following prenatal insults (McCarthy *et al.,* 2017; Soti *et al.,* 2022).

Accordingly, this study investigated whether gestational MSD produces persistent alterations in the hippocampal CA1 region of female F1 Wistar rat offspring. We specifically assessed CA1 pyramidal cell number, glutamate concentration, and DNA fragmentation. We hypothesized that gestational MSD would (1) reduce CA1 pyramidal cell number, (2) decrease CA1 glutamate concentration, and (3) increase DNA fragmentation in female F1 offspring compared with controls. Collectively, these measures were used to determine whether prenatal sleep deprivation produces coordinated structural, neurochemical, and epigenetic alterations in the developing hippocampus.

## MATERIALS AND METHODS

### Experimental Animals

#### *Animal Welfare and Ethics*

Forty (40) Wistar rats consisting of (15) females and (15) males weighed 90 and 100 grams, were purchased from the Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU), Zaria. The rats were transported in a separate cage according to their sex to the Department of Human anatomy animal facility. The rats were housed separately according to their sex in plastic cages (24cm by 28cm by 40cm) containing wooden bedding and a plastic nest to mimic natural habitat with a lead aluminum wired cover and maintained in standard condition (temperature, humidity, 12 hours' light and dark circle). All rats were given chicken feed and water allowed at libitum. The rats were acclimatized for four weeks in the plastic cages prior to the commencement of the experiments. Animals were treated in accordance with the guidelines of the Ahmadu Bello University, Zaria committee on Animal Use and Care. With all efforts, animal suffering was minimized to reduce the number of animals used for this experiment.

### Determination of the Estrous Cycle Phases

After four weeks of acclimatization and maturity, the estrous cycle of female Wistar rats was determined using the vaginal cytology method, which is widely regarded as a simple, non-invasive, economical, rapid, and reliable technique for assessing reproductive status in rodents (Marcondes *et al.,*2002; Goldman *et al.,*2007; de Boer & Koolhaas, 2024). Initially, the vaginal opening of each rat was visually inspected to assess reproductive maturity. Thereafter, each animal was gently restrained by holding the loose skin around the neck and upper back while elevating the tail to allow clear visualization of the vaginal orifice.

Vaginal smears were obtained by lavage using a sterile plastic pipette containing approximately 100 µL of normal saline. The saline was gently introduced into the vaginal canal and aspirated repeatedly (3–4 times) to collect vaginal epithelial cells. Subsequently, two to three drops of the vaginal suspension were placed onto a clean glass slide and examined under a light microscope at 10× objective magnification without staining (Cora *et al.,* 2015). The phase of the estrous cycle was identified based on the relative proportions of the different cell types present in the vaginal smear, following established cytological criteria (Marcondes *et al.,* 2002; Ibiyeye *et al.,* 2026).

***Proestrus*:** This phase was characterized predominantly by numerous rounds, nucleated epithelial cells that were relatively uniform in size and morphology. The presence of these cells reflects increasing estrogen secretion and follicular maturation.

***Estrus*:** The estrus phase was identified by the predominance of anucleated cornified epithelial cells. These cells appeared irregular in shape with granular cytoplasm and were present in large numbers. This stage corresponds to ovulation and maximal sexual receptivity.

***Metestrus*:** Metestrus was characterized by a mixed population of cells, including leukocytes, nucleated epithelial cells, and cornified epithelial cells. The proportions of these cell types were generally comparable, indicating the transitional phase following ovulation.

***Diestrus*:** Diestrus was distinguished by the predominance of polymorphonuclear leukocytes, accompanied by relatively few nucleated epithelial and cornified epithelial cells. This phase represents a period of reproductive inactivity before the commencement of a new cycle.

Fifteen out of twenty-five female rats were confirmed to be in their estrus phase and were selected for mating because this stage is associated with peak sexual receptivity and the highest likelihood of successful fertilization (Goldman *et al.,* 2007; Kuru & Kuru, 2023). Estrous females were subsequently paired with proven fertile male rats for breeding.

**Confirmation of Mating and Pregnancy**

Following mating, female rats were examined the next morning between 07:00 and 08:00 hours for evidence of successful copulation. The vaginal opening was visually inspected for the presence of a whitish or translucent copulatory (vaginal) plug, which is considered an initial indicator of successful mating (Hamid & Zakaria, 2013). To further confirm copulation, gentle examination of the vaginal opening was performed, followed by the collection and microscopic examination of vaginal smears for the presence of spermatozoa. The detection of sperm cells in the vaginal smear was taken as confirmation of successful mating and fertilization (Yugesh *et al.,* 2023). The day on which spermatozoa were identified in the vaginal smear was designated as Gestational Day 0 (GD0), providing a standardized reference point for accurate pregnancy dating and subsequent experimental procedures (Yugesh *et al.,* 2023). Following confirmation of pregnancy, the pregnant rats were subjected to the experimental protocol. Beginning on Gestational Day 5 (GD5), animals assigned to the experimental group underwent a chronic sleep deprivation regimen for a period of two weeks, while control animals were maintained under standard housing conditions.

**Experimental Design**

After the confirmation of mating and fertilization, Rats were divided into two groups composing of 5 pregnant rats per group:

***Group 1*** – contains 5 animals which served as the control group. They are non-sleep deprived pregnant rats.

***Group 2*** – contains 5 pregnant rats which were subsequently sleep deprived. At the gestational day 5/6, the group 2 pregnant rats were subjected to 20 hours sleep deprivation for 14 days.

**Sleep Deprivation Protocol**

Maternal sleep deprivation was induced using a Customized Multiple Platform Model (CMPM), adapted from the method described by Sontakke *et al*. (2026). The CMPM apparatus consisted of a plastic tank measuring 30 cm × 42 cm × 60 cm, containing ten fixed metal platforms evenly distributed within the tank. Water was added to the tank to a level approximately 1–2 cm below the surface of the platforms. The water was allowed to stand for approximately 3 hours before the commencement of each experimental session to equilibrate to room temperature (Sontakke *et al*. 2026).

To ensure adequate ventilation and prevent escape, the tank was covered with an iron gauze wire mesh. Food was provided in suspended aluminum feeders, while water was supplied through hanging plastic bottles, allowing unrestricted access to food and water throughout the sleep deprivation period.

Pregnant rats assigned to the experimental group were subjected to sleep deprivation for 20 hours per day over a period of 14 consecutive days. The sleep deprivation sessions were conducted daily from 3:00 p.m. to 11:00 a.m. of the following day. During this period, when the animals entered rapid eye movement (REM) sleep and experienced muscle atonia, contact with the surrounding water caused arousal, thereby preventing sustained sleep and maintaining the sleep deprivation condition (Gulyani *et al.,*2000; Sontakke *et al*. 2026).

At 11:00 a.m. each day, the rats were removed from the CMPM apparatus and returned to their home cages, where they had free access to food and water and were allowed a 4-hour recovery sleep period. During this interval, the CMPM tanks were thoroughly cleaned and refilled with fresh water maintained at room temperature. The animals were subsequently reintroduced into the CMPM apparatus at 3:00 p.m. to commence the next sleep deprivation cycle.

Following completion of the 14-day sleep deprivation protocol, the pregnant rats were allowed to carry their pregnancies to term and deliver naturally. The resulting F1 offspring were reared under standard laboratory conditions and subsequently utilized for the experimental analyses conducted in the present study.

Figure 1: Modified Multiple Platform for Sleep Deprivation

**Euthanasia**

After the pregnant Wistar rats had littered, control and sleep deprived dams gave care to their offsprings under a standard laboratory condition. Ten female offsprings (F-1 generation) comprises of 5 control and MSD were euthanized by cervical dislocation on the post-natal day 43 (PND43). The brains were divided into two left and right hemispheres; half of the hemispheres were randomly selected and immersed in 10% formal saline for morphological analyses. And the other half of the hemispheres were frozen and stored at -80 $^{O}$C which were used for essential amino acid analysis using High-Performance Liquid Chromatography (HPLC).

**High-Performance Liquid Chromatography (HPLC) Analysis**

High-performance liquid chromatography (HPLC), formerly known as high-pressure liquid chromatography, is a widely utilized analytical technique for the separation, identification, and quantification of individual components within complex mixtures (Snyder *et al.,* 2012; Carla *et al.,* 2024). HPLC has broad applications in pharmaceutical, biomedical, environmental, agricultural, forensic, and food sciences due to its high sensitivity, accuracy, and reproducibility (Câmara *et al* 2022; Kumar *et al.,* 2025).

The technique operates on the principle of differential interactions between analytes and two phases: a mobile phase and a stationary phase. In HPLC, high-pressure pumps deliver a solvent or mixture of solvents, known as the mobile phase, through the chromatographic system. The sample mixture, dissolved in a suitable solvent, is introduced into the flowing mobile phase and transported through a chromatographic column packed with fine adsorbent particles that constitute the stationary phase (Meyer, 2010).

As the sample components pass through the column, they interact differently with the stationary phase based on their physicochemical properties, resulting in varying migration rates. Components with weaker interactions elute more rapidly, whereas those with stronger interactions are retained longer within the column. This differential retention facilitates the separation of the individual constituents of the sample mixture (Kaur *et al.,* 2026).

Following separation, the eluted compounds are detected using an appropriate detector, commonly an ultraviolet-visible (UV-Vis) detector. The detector generates a chromatogram, which is a graphical representation of detector response versus retention time. Each peak on the chromatogram corresponds to a specific analyte, with the retention time serving as a qualitative indicator for compound identification, while the peak area is proportional to the concentration or quantity of the analyte present in the sample (Dong, 2019).

In the present study, HPLC was employed to separate, identify, and quantify the target analytes in the collected biological samples. The technique was selected because of its high analytical precision, sensitivity, and suitability for detecting trace levels of biomolecules in complex biological matrices (Snyder *et al.,* 2012).

**Hippocampal Amino Acid Analysis by High-Performance Liquid Chromatography (HPLC)**

The selected hemispheres for amino acid analysis. Were dissected on an ice-chilled surface and the hippocampus CA1 was identified and dissected according to established neuroanatomical landmarks (Paxinos, 2014). Briefly, hippocampal tissues were homogenized in perchloric acid, neutralized with potassium carbonate, and centrifuged to remove cellular debris. The resulting pellets were air-dried and subjected to acid hydrolysis using 6 N hydrochloric acid at 120°C for 24 hours to liberate protein-bound amino acids. Following hydrolysis, samples were centrifuged, evaporated to dryness, reconstituted in 1 N hydrochloric acid, filtered, and transferred into HPLC vials for analysis.

Amino acids were derivatized using o-phthalaldehyde (OPA) and fluorenyl-methyloxy-carbonyl chloride (FMOC)

reagents prior to chromatographic separation. Separation and quantification were performed using an Agilent 1260 Series HPLC system equipped with fluorescence detection. Mobile phases consisting of sodium acetate buffer and organic solvent mixtures were prepared according to standard protocols, and amino acid standards were used for calibration and identification of chromatographic peaks (Henderson *et al.,* 2021). The concentrations of amino acids were determined from the chromatographic peak areas using calibration curves generated from known standards. Final amino acid concentrations were expressed as nmol/mg tissue after correction for dilution factors and tissue weight.

**Stereological Estimation of Hippocampus CA1 Neuron**

The total number of neurons in the hippocampal CA1 region was estimated using an unbiased design-based stereological approach with the physical dissector method on Thionin-stained sections, as described by Sterio (1984) and Gundersen *et al*. (1988). Briefly, the CA1 subfield of the hippocampus, designated as the region of interest (ROI), was examined under a light microscope, and images were captured at the appropriate magnification. A stereological counting frame was systematically and randomly superimposed on each captured image, and CA1 pyramidal neurons were counted according to the inclusion and exclusion criteria of the physical dissector method. This stereological technique provides an unbiased and reliable estimate of neuronal number that is independent of tissue volume, orientation, or cell size (Sterio, 1984; Gundersen *et al.,* 1988).

**Feulgen Staining and Image Analysis for DNA Fragmentation**

Serial hippocampal sections (8 µm thickness) from postnatal day 43 (PND43) rats were processed for Feulgen staining following standard histochemical procedures for DNA visualization (Soyab, 2020). A total of 20 sections were deparaffinized in xylene and rehydrated through descending grades of ethanol before rinsing in distilled water. The sections were then immersed in cold 1 N hydrochloric acid and incubated at 60°C for controlled acid hydrolysis. Following hydrolysis, the sections were again rinsed in cold 1 N HCl and immediately transferred into Schiff's reagent for specific DNA staining. After staining, the sections were washed in running tap water to develop color, followed by treatment with potassium metabisulfite solution to remove excess reagent. The slides were counterstained with Light Green, rinsed in distilled water, and subsequently dehydrated through ascending grades of ethanol. The sections were cleared in xylene and mounted using DPX mounting medium. Slides were air-dried at room temperature and examined under a light microscope for histological evaluation of DNA integrity.

For quantitative assessment of DNA fragmentation, digital images of stained sections were captured and analyzed using ImageJ software. Each section was individually uploaded into the software, followed by color deconvolution to isolate the Feulgen signal. Mean optical density values were then calculated to estimate the degree of DNA fragmentation, in accordance with established image analysis protocols for histochemical quantification (Schneider *et al.,* 2012).

**Data Analysis**

Data obtained were analyzed using GraphPad Prism version 8.3 and expressed as mean ± standard error of the mean (SEM). Differences between the MSD and control F-1 female Wistar rats were analyzed using an independent-samples Student's *t*-test. A *P*-value < 0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

**Effect of MSD on glutamine concentration**

HPLC hippocampi CA1 tissues glutamine revealed, MSD F-1 (SD) had significant increase in glutamine concentration compared with control F-1 (CT) Wistar rats [$t$ = 5.752, p=0.0012] (Figure 2).

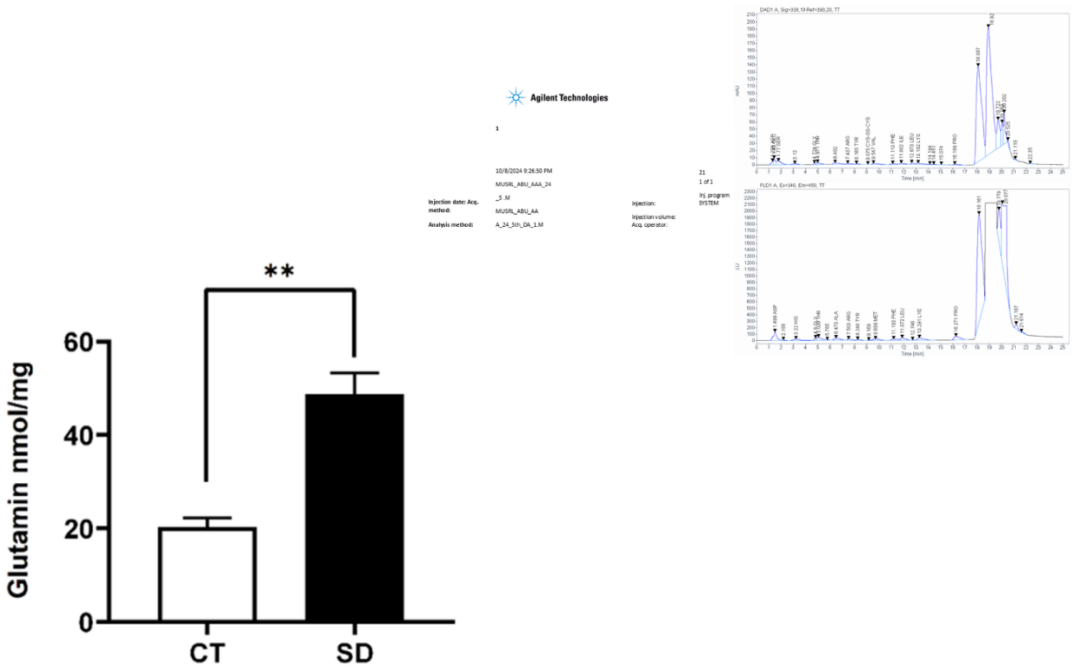


Figure 2: Effect MSD on SD hippocampus CA-1 glutamine concentration in CT = Experimental Control and SD = Sleep deprived. n=5, student *t-test*, **P<0.0012

**Effect of MSD on CA1 pyramidal cell number**

Every 1/20 thionine stained CA1 sections and unbiased designed-based stereology method, showed significant decreased in number of pyramidal cells in the pyramidal cell layer of SD female Wistar rats compared with CT female Wistar rats $t$ = 3.048, p=0.0381] (Figure 3). Indicating a remarkable decrease in number of CA1 pyramidal cells in the pyramidal cell layer of MSD.

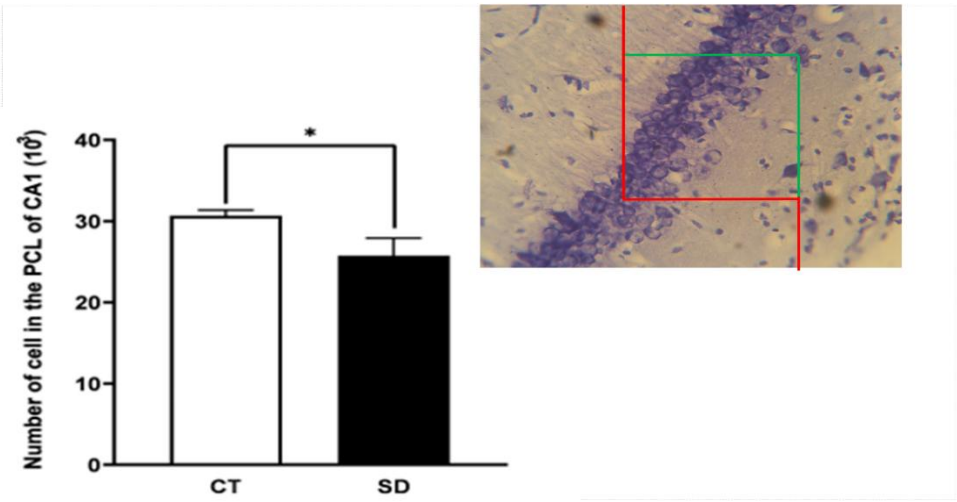


Figure 3: Effect MSD on SD hippocampus CA-1 glutamine concentration in CT = Experimental Control and SD = Sleep deprived. n=5, student *t-test*, **P<0.0381

**Effect of MSD on CA1 DNA fragmentation**

Every 1/15 Feulgen stained CA1 sections showed a significantly high mean value of DNA fragmented optical density in SD compared to CT F-1 female Wistar rats student $t$ = 4.634, p=0.0082] (Figure 4).

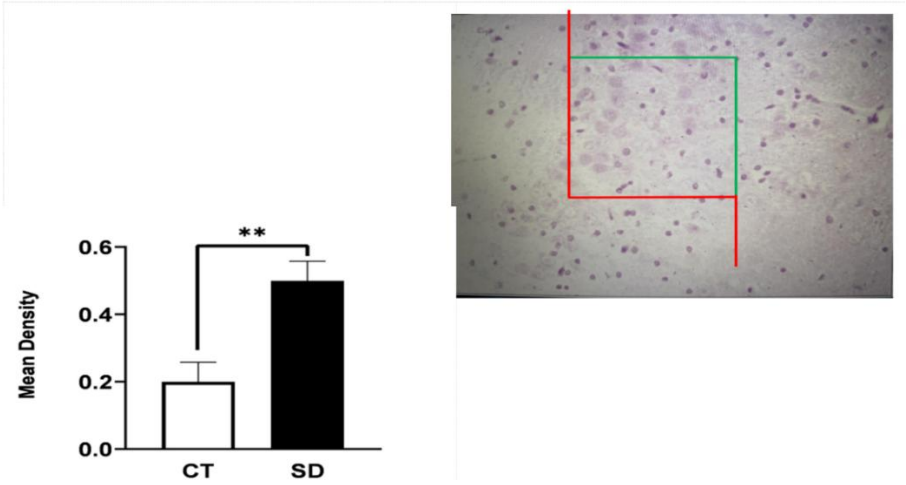


Figure 4: Effect MSD F-1 hippocampus CA-1 DNA fragmentation using Feulgen reaction (mean optical density) in CT = Experimental Control and SD = Sleep deprived. n=5, student *t-test*, **P<0.005

**Discussion**

The present study demonstrated a significant increase in glutamine concentration in the hippocampal CA1 region of F1 female Wistar rat offspring exposed to maternal sleep deprivation (MSD) compared with control F1 (CT) offspring. This finding suggests that maternal sleep deprivation during pregnancy may induce persistent alterations in amino acid metabolism and glutamatergic neurotransmission in the developing offspring hippocampus. Such alterations are of particular importance because the hippocampus is highly dependent on tightly regulated excitatory neurotransmission and is vulnerable to developmental and environmental stressors (McEwen, 2000; Magariños & McEwen, 2000).

Glutamine is a major component of the glutamate–glutamine cycle, which is essential for the maintenance of normal excitatory neurotransmission in the central nervous system. Glutamate released from presynaptic neurons is primarily taken up by astrocytes and converted to glutamine by the enzyme glutamine synthetase. Glutamine is subsequently transported back to neurons, where it can be converted to glutamate by phosphate-activated glutaminase. This metabolic pathway ensures the continuous supply of glutamate for synaptic transmission while helping to regulate extracellular glutamate concentrations (Bak et al., 2006; Hertz & Rothman, 2016). Therefore, the significant elevation of glutamine observed in the MSD F1 offspring may indicate an alteration in the normal regulation or turnover of the glutamate–glutamine cycle.

One possible explanation for the increased glutamine concentration is an adaptive response to altered glutamatergic activity following maternal sleep deprivation. Sleep deprivation and chronic disruption of sleep have been associated with changes in neuronal activity, metabolic homeostasis and synaptic function. Because sleep plays an important role in maintaining neuronal energy balance and synaptic homeostasis, disruption of maternal sleep during pregnancy may alter the maternal physiological environment and consequently influence fetal brain development (Xie et al., 2013; Tononi & Cirelli, 2014). The increased glutamine detected in the offspring hippocampus may therefore represent a compensatory metabolic response aimed at maintaining glutamate availability and neurotransmitter homeostasis.

Maternal sleep deprivation may affect this system indirectly through maternal stress and neuroendocrine mechanisms. Sleep disruption can influence hypothalamic–pituitary–adrenal (HPA) axis activity and glucocorticoid regulation, while excessive or prolonged exposure to glucocorticoids during critical periods of development has been associated with changes in hippocampal structure and function (McEwen, 2007; Seckl & Holmes, 2007). Since the hippocampus is an important target of glucocorticoid action, developmental alterations in maternal stress physiology may contribute to subsequent changes in hippocampal neurotransmitter metabolism in the offspring. Thus, the increased glutamine concentration in the MSD F1 group may represent one neurochemical consequence of developmental exposure to maternal sleep disturbance.

The finding also has potential implications for hippocampal neuronal function. Glutamate is the principal excitatory neurotransmitter in the mammalian brain and is essential for synaptic plasticity, learning and memory. In the CA1 region, glutamatergic neurotransmission is fundamental to long-term potentiation and hippocampal information processing (Malenka & Bear, 2004). Consequently, persistent changes in glutamine concentration may indicate an alteration in the metabolic support of glutamatergic

neurotransmission. However, the present increase in glutamine should not, by itself, be interpreted as evidence of neuronal injury or excitotoxicity. Rather, it indicates that maternal sleep deprivation may have modified the biochemical environment supporting excitatory neurotransmission in the offspring hippocampus.

The observed increase may also represent an attempt by the developing hippocampus to maintain neurotransmitter balance following maternal sleep deprivation. The glutamate–glutamine cycle links neuronal signaling with astrocytic metabolism and energy utilization. Changes in glutamine availability can therefore influence the capacity of neurons to synthesize glutamate and sustain synaptic transmission. Hertz and Rothman (2016) emphasized that glutamate neurotransmission is closely coupled to cellular metabolism, making alterations in glutamine concentration potentially relevant to both neurotransmitter recycling and energy metabolism. In this context, the elevated glutamine concentration in MSD F1 offspring may reflect an adaptive response to increased metabolic or neurotransmitter demands imposed by altered developmental conditions.

Furthermore, the present observation provides evidence that maternal sleep deprivation may have effects that persist into the offspring period rather than being restricted to the maternal or gestational period. Developmental exposure to adverse maternal conditions can influence the maturation of neural circuits and alter subsequent neurochemical regulation. Previous experimental studies have demonstrated that maternal stress and adverse prenatal environments can affect offspring hippocampal development, synaptic plasticity and cognitive-related functions (Maccari et al., 2003; Van den Bergh et al., 2005). The current finding of increased glutamine in F1 female offspring is therefore consistent with the broader concept that maternal environmental conditions can program aspects of offspring brain function.

The sex-specific nature of the present study is also noteworthy. Female offspring may exhibit distinct neuroendocrine and metabolic responses to developmental stress compared with males. Sex differences in the effects of prenatal stress on hippocampal structure and function have been reported, with hormonal and developmental factors potentially contributing to differential vulnerability (McEwen & Milner, 2017). Therefore, the increased glutamine concentration observed specifically in F1 female offspring may reflect sex-dependent neurochemical adaptation to maternal sleep deprivation. Nevertheless, direct comparison with male offspring would be required before a definitive sex-specific effect could be established.

Overall, the significant increase in hippocampal glutamine concentration in F1 female Wistar rat offspring following maternal sleep deprivation suggests that MSD can alter neurochemical homeostasis within the hippocampal CA1 region. The elevation of glutamine may reflect increased activity of the glutamate–glutamine cycle, altered astrocytic metabolism, compensatory regulation of glutamatergic neurotransmission, or developmental programming of hippocampal metabolic pathways. Since glutamine was assessed without simultaneous measurement of glutamate, glutamine synthetase, glutaminase or glutamate transporters, the precise mechanism responsible for the observed increase cannot be determined from the present finding alone. Nevertheless, the result provides important evidence that maternal sleep deprivation is associated with persistent neurochemical alterations in the offspring hippocampus and may contribute to changes in hippocampal synaptic function and plasticity.

Taken together, the increased glutamine concentration observed in the MSD F1 group may represent an adaptive neurochemical response to maternal sleep deprivation and its developmental consequences. When considered alongside structural, stereological and other biochemical alterations in the hippocampal CA1 region, this finding supports the hypothesis that maternal sleep deprivation can adversely influence the neurodevelopmental and functional organization of the offspring hippocampus. Further investigations involving glutamate concentration, glutamine synthetase activity, astrocytic markers, oxidative stress indices and glutamate receptor expression would provide additional evidence for the mechanisms underlying the altered glutamine profile.

The present study demonstrated that maternal sleep deprivation (MSD) significantly reduced the number of pyramidal neurons in the CA1 pyramidal cell layer of F1 female Wistar rats, as determined using every 20th Thionin-stained hippocampal section and an unbiased design-based stereological approach. Quantitative stereological analysis revealed a significant decrease in the number of CA1 pyramidal neurons in the SD group compared with CT (Figure 3). These findings suggest that maternal sleep deprivation adversely affects hippocampal neuronal survival, resulting in a marked loss of pyramidal cells within the CA1 subfield.

The hippocampal CA1 region is particularly susceptible to metabolic stress, oxidative damage, and excitotoxicity because of its high synaptic activity and energy demand (Small *et al.,* 2011). Maternal sleep deprivation has been shown to disrupt the intrauterine environment by elevating glucocorticoid levels, increasing oxidative stress, and promoting neuroinflammation, all of which can impair neuronal proliferation and survival during critical periods of fetal brain development (Mueller & Bale, 2008; Sontakke *et al.,* 2026). Consequently, these pathological processes may contribute to the reduced neuronal population observed in the CA1 region of the offspring.

The present findings are consistent with previous studies reporting that prenatal stress and sleep deprivation

negatively affect hippocampal morphology by reducing neuronal density, suppressing neurogenesis, and increasing neuronal apoptosis, particularly within the CA1 and dentate gyrus regions (Mirescu *et al.,*2004; Havekes & Abel, 2017). The reduction in CA1 pyramidal neuron number observed in the present study may underlie impairments in hippocampal-dependent learning, memory formation, and spatial navigation, as the CA1 region plays a critical role in information processing and memory consolidation (Small *et al.,* 2011; Andersen *et al.,* 2014). Therefore, the stereological evidence of neuronal loss supports the hypothesis that maternal sleep deprivation induces long-lasting structural alterations in the hippocampus of F1 female offspring, with potential consequences for cognitive function.

To further support the hypothesis that maternal sleep deprivation induces neuronal loss in F1 female Wistar rats, the present study demonstrated that maternal sleep deprivation (MSD) significantly increased DNA fragmentation in F1 female Wistar rats, as evidenced by the higher mean optical density of Feulgen-positive fragmented DNA compared with the normal control group (Figure 3). The Feulgen reaction is a highly specific histochemical technique for DNA detection, and increased staining intensity is indicative of enhanced DNA fragmentation, a hallmark of apoptotic cell death (Mello & de Campos, 2017; Biggiogera *et al.,* 2024). The observed increase in DNA fragmentation suggests that maternal sleep deprivation may induce apoptotic processes in the offspring through mechanisms involving oxidative stress, mitochondrial dysfunction, and activation of intrinsic apoptotic signaling pathways. Sleep deprivation has been reported to increase the production of reactive oxygen species (ROS), impair antioxidant defense systems, and promote neuronal DNA damage, ultimately leading to apoptosis (Everson *et al.,* 2014; Houldsworth, 2024). Furthermore, oxidative DNA damage may activate p53-dependent pathways, resulting in increased expression of pro-apoptotic proteins such as Bax and caspases, thereby accelerating programmed cell death (Elmore, 2007). These findings are consistent with previous studies demonstrating that prenatal or maternal stress, including sleep deprivation, adversely affects fetal brain development by increasing neuronal apoptosis and genomic instability in offspring (Mirescu *et al.,* 2004; Mueller & Bale, 2008; Satyapal *et al.,* 2025). The elevated DNA fragmentation observed in the MSD F1 female rats therefore indicates that maternal sleep deprivation may compromise neuronal integrity and survival, which could contribute to long-term structural and functional deficits in the developing brain.

## CONCLUSION

Maternal sleep deprivation (MSD) significantly increased hippocampal glutamine concentration, decreased CA1 pyramidal cell number, and increased DNA fragmentation in F1 female Wistar rat offspring compared with the control F1 group. These findings suggest that maternal sleep deprivation adversely affects hippocampal neurochemical homeostasis and neuronal integrity. Collectively, the alterations indicate that MSD may impair the structural and functional integrity of the hippocampal CA1 region in female offspring.

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