Access this Article online
Quick Response Code:

Website: jecajournal.com
Doi: doi.org/10.4314/jeca.v21i2.15

Original Article

Chronic sleep deprivation induces spatial memory impairment, chromatolysis, and histoarchitectural changes in the CA3 region of the hippocampus

[1,2]Ochai, J.; [1]Umana, U.E.; [1]Musa, S.A.; [3]Oladele, S.B.

Abstract

BACKGROUND AND AIM: Sleep is a vital bodily function involving the activity of brain networks. Disruption in sleep patterns can lead to problems with memory and even changes in brain structure. Hence, this study aimed to assess the effects of chronic sleep deprivation on spatial memory and histopathological changes in the CA3 region of the hippocampus of adult male Wistar rats.

METHODOLOGY: 12 male rats were divided into two groups of six rats each. Group (I) received 2ml/kg of distilled water, while Group II were sleep-deprived for 18 hours daily for 21 consecutive days using the modified multiple platform method. The Morris water maze test was conducted to assess spatial learning and memory. At the end of the experiment, the rats were euthanized using 75mg/kg of ketamine hydrochloride intraperitoneally, and the brain tissues were harvested. Hippocampal tissue homogenate of some rats were used to assess glutamate levels, whereas, the whole brains of the remaining rats were fixed, and processed using Haematoxylin & Eosin, and Cresyl Fast Violet stains to demonstrate the general histoarchitecture and Nissl substance expression in the hippocampal CA3 regions respectively. Cavalieri's principle was employed for estimation of the number of pyramidal cells in the CA3 region of the hippocampus of Wistar rats.

RESULTS: There was a significant increase (p<0.001) in the time spent locating the escape platform in the sleep-deprived group compared to the control group in the MWM test. No significant difference (p>0.05) was observed in hippocampal glutamate activity levels when the sleep deprived group was compared with the control group. Histological findings revealed degenerative changes such as cytoplasmic vacuolation, karyorrhexis and pyknosis in the pyramidal cells of the CA3 region of the hippocampus of Wistar rats. Additionally, the number of pyramidal cells in the CA3 region was significantly decreased (p>0.05) in the sleep-deprived group compared to the control. Furthermore, the Nissl substance in the hippocampal sections of the sleep-deprived group exhibited a significant (p>0.05) decrease in staining intensity compared to the control group, suggesting disruptions in neuronal function and protein synthesis.

CONCLUSION: The findings of this study revealed spatial memory impairment, histological alterations, reduced pyramidal cell count, and protein depletion in the CA3 region of the sleep-deprived rats.

Keywords:
Memory deficit; sleep deprivation; histological changes; cornu ammonis; pyramidal cells; Morris water maze.

Submitted: 25th September, 2024
Revised: 8th November, 2024
Accepted: 10th November, 2024
Published: 31st December, 2024

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, Nigeria; [2] Department of Anatomy, Faculty of Basic Medical Sciences, Federal University of Health Sciences, Otukpo; [3]Department of Veterinary Pathology, Faculty of Basic Veterinary Medicine, Ahmadu Bello University, Zaria.

Address for Correspondence:
Ochai, J.
Department of Anatomy, Faculty of Basic Medical Sciences, Federal University of Health Sciences, Otukpo.
joyochai@gmail.com

INTRODUCTION

Sleep deprivation is a widespread problem in modern society, with significant impacts on cognitive performance that are only beginning to be understood scientifically (Killgore, 2010; Khan and Aljadali, 2023; Ochai *et al*., 2024). The prevalence of excessive daytime sleepiness due to sleep deprivation ranges from 9% to 24%, and it is a leading cause of visits to sleep clinics (Ng *et al*., 2005; Kolla *et al*., 2020).

While it is well-established that insufficient sleep leads to slower response times and increased variability in performance, particularly for measures of alertness, attention, and vigilance, the effects on higher-level cognitive abilities, such as perception, memory, and executive function remain unclear (Killgore, 2010; Kusztor *et al*.,2019). Sleep deprivation is also associated with the development of multiple health risks, which can reduce quality of life and increase mortality (Grandner *et al*., 2011).

Various factors contribute to sleep deprivation, including lifestyle factors like shift work, stress, and the use of electronic devices before bed, which can disrupt melatonin secretion (Gamble *et al*., 2014; Abbot *et al*., 2020). The aging process also disrupts sleep physiology and reduces total sleep time (Ohayon *et al*., 2004). Individuals with sleep disorders, such as insomnia, restless leg syndrome, periodic limb movements, and sleep-

How to cite this article: Ochai, J.; Umana, U.E.; Musa, S.A.; Oladele, S.B. Chronic Sleep Deprivation Induces Spatial Memory Impairment, Chromatolysis, and Histoarchitectural Changes in the CA3 region of the Hippocampus. J Exp Clin Anat 2024; 21(2): 252-261.
https://dx.doi.org/10.4314/jeca.v21i2.15

breathing disorders, are also prone to sleep deprivation (Sateia, 2014; Roth, 2007; Bassetti *et al*., 2015).

The consequences of sleep deprivation are significant, with an increased risk of major road traffic accidents and occupational-related accidents and errors (Horne & Reyner, 1995; Dinges, 1995). Despite the large proportion of our lives spent sleeping, there is still little scientific consensus on the exact function of sleep (Siegel, 2005). Some individuals view sleep as merely a minor inconvenience in the daily routine, preferring to devote time to more productive, lucrative, or entertaining pursuits (Tempesta *et al*., 2018; Ferrara & De Gennaro, 2001). This attitude often leads people to trade sleep for additional time to devote to work, school, or social demands (Patel & Hu, 2008). Additionally, insufficient sleep is widespread in certain professions, such as medical residents, military personnel, and shift workers (Williamson & Feyer, 2000).

The consequences of sleep deprivation extend far beyond a mere inconvenience. It can have significant impacts on behavior, mood, cognitive performance, and motor function (Durmer & Dinges, 2005). Studies have shown that sleep deprivation negatively affect long-term memory, working memory, attention, executive function, and decision-making processes (Eugene & Masiak, 2015; Durmer & Dinges, 2005).

Sleep deprivation is known to have a detrimental effect on physical and psychological functions (Krause *et al*., 2017). Rapid eye movement (REM) sleep deprivation, in particular, has been shown to influence insight, attentiveness, and memory functions regulated by brain areas such as the neocortex, hippocampal formation, and amygdala (Goldstein & Walker, 2014). The hippocampus and dentate gyrus, which play key roles in mediating short-term and long-term memory as well as spatial recognition, are also affected by sleep deprivation (Deng *et al*., 2010).

Given the widespread prevalence of sleep deprivation and its far-reaching consequences, scientific study of its effects can provide valuable insights, not only about the nature and function of sleep but also practical importance of enhancing the health and well-being of workers who must perform optimally despite the periods of little to no sleep (William D.S. Killgore, 2010). Therefore, this study aims to investigate the effects of chronic sleep deprivation on spatial memory, glutamate levels in the brain, protein synthesis, and the histology of the hippocampus in a rat model.

MATERIALS AND METHODS

Ethical approval

The study protocol, including the ethical treatment and care of the experimental animals, was reviewed and approved by the Ethics Committee on Animal Use and Care at Ahmadu Bello University, Zaria with approval number ABU/CAUC/2024/034.

Experimental design

For this study, a total of 12 apparently healthy male rats weighing between 150-200 g were obtained from the Animal House Centre, Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria. They were housed in clean cages and provided with a standard pellet diet (rat chow-vital feed) as well as unrestricted access to clean drinking water. The rats were then divided into two groups, with each group comprising six animals. Group I served as the control group and received 2 ml/kg of body weight of distilled water throughout the experiment. Group II rats were subjected to 18 hours of sleep deprivation daily from 2:00 pm to 8:00 am, for a consecutive period of 21 days.

Rapid Eye Movement-Sleep Deprivation Procedure

The sleep deprivation protocol utilized in this study employed a modified multiple-platform method using a rapid eye movement (REM) technique. The setup consisted of a Plexiglas water tank measuring 127.0 cm in length, 44.0 cm in width, and 45.0 cm in height, containing 15 cylindrical platforms spaced horizontally and vertically at distances of 13 cm and 10 cm, respectively. The tank was filled with 1 cm of water, and six Wistar rats from the same cage were placed in each tank for 18 hours daily, with access to food and water. When the rats entered REM sleep and experienced a loss of muscle tone, they would touch the water and wake up. This model did not restrict the rats' movement or isolate them socially (Javad-Moosavi *et al*., 2017).

Morris Water Maze Test (MWM test)

The spatial learning and memory of the rats was assessed using the Morris Water Maze (MWM) test, as described by Barnhart *et al*. (2015) and Mahdipour *et al*. (2022). The MWM setup consisted of a black circular pool (136 cm in diameter, 60 cm high, and 30 cm deep) filled with water (23–25 ℃) and located in the center of a small room. A circular platform (10 cm in diameter and 28 cm high) was submerged about 2cm beneath the water surface in the center of the northeast quadrant. The rats performed a trial on each of the five consecutive days, where they were placed in the pool and allowed to swim until they found and remained on the platform for 20 seconds. Rats that failed to find the platform within 60 seconds were guided to it. On the sixth day, the platform was removed, and the rats were allowed to swim for 60 seconds, with the time spent and distance traveled in the target quadrant (Q1) being compared among the groups. (Ekpo *et al*., 2023; Onimisi *et al*., 2024). The test was carried out by 2 pm daily throughout the experiment.

Animal euthanization

After the experiment, the animals were humanely euthanized through an intraperitoneal injection of 75 mg/kg ketamine hydrochloride. After the animals were anesthetized, their skulls were carefully opened along the midline to access the tissues. The hippocampus, a specific region of interest, was meticulously dissected and separated from any surrounding tissues. A portion of the hippocampal tissues was homogenized and used for the

assessment of glutamate levels, while the remaining tissues were immersed in a 10% formal saline solution for 24 hours to ensure proper fixation. Following the fixation process, the prepared tissue samples were transferred to the histology laboratory at the Department of Human Anatomy, Ahmadu Bello University in Zaria, where they underwent staining with H&E (hematoxylin and eosin) to facilitate the visualization and study of the general histology of the hippocampus.

Evaluation of glutamate activity levels

The hippocampal tissues were homogenized in 0.1M phosphate buffered 7.4 pH solution and immediately centrifuge at 3000rpm. The supernatant obtained was used to evaluate the concentration of glutamate in the hippocampus using a rat standard-ELISA kit purchased from Fine Test Company, Wuhan Fine Biotech Co. LTD, Wuhan, China) following the manufacturer's instructions.

Light Microscopy and Cresyl fast violet stain.

The brain tissues underwent a series of processing steps, including fixation, dehydration, clearing, infiltration, and finally embedding in paraffin wax. Sectional slices (5–6 um) were cut from the embedded tissue blocks, mounted on glass slides, and stained using hematoxylin and eosin to visualize the overall histological cytoarchitecture of the hippocampal areas, as well as Cresyl violet staining to detect the presence of Nissl material within the hippocampus (Carson, 1990). The processed slides from each group were examined under a light microscope, and photomicrographs were captured using an AmScope MD 900 digital microscope camera at magnifications of 250 for the H&E and Cresyl-stained samples, respectively.

Quantification of Nissl substance in the hippocampus

To assess the quantity of Nissl substance in CA3 pyramidal neurons, Cresyl fast violet (CFV) stain was utilized, a highly effective stain that specifically targets neuronal cell bodies (Yurt *et al*., 2018; Ekpo *et al*., 2024). The staining intensity of the CFV-stained micrographs was measured as a means to quantify the reactivity of Nissl substances, following the instructions provided by the manufacturer (Eluwa *et al*., 2013). The Image J region of interest (ROI) manager tool for analysis of specific areas of micrographs was employed to limit bias values resulting from non-identical image quality (image acquisition setting and exposure time) (Amber *et al*., 2020). The modal gray values for three ROI was obtained, means computed and analyzed.

Estimation of pyramidal cell number in the CA3 region of the hippocampus

An unbiased estimation technique, known as the physical fractionator method was employed, to determine the pyramidal cell number in the CA3 hippocampal region of Wistar rats (Bolon & Butt, 2011; Gundersen *et al*., 1988). The hippocampus of the Wistar rat per group was isolated, processed and sectioned at 8 µm after pilot study on how many slices could be derived. Tissue sections were selected using systematic uniform random sampling method. The sections derived were stained using H and E stain. To count the number of pyramidal cells, a transparent counting frame with acceptance and rejection region was used on the two consecutive sections. One of the section planes was taken as the "look up" section and the other as the "reference" section. Pyramidal cells were selected in the reference section and counting was done in the look up section, pyramidal cells that appear in the reference section but not in the lookup section were also counted. The total number of pyramidal cells was then computed using the formula as reported by Gundersen *et al*., 1988.

Data analysis

The data was analyzed using IBM SPSS Statistics software (version 14.0 for the window evaluation) where an independent sample t-test was conducted on the results, which were presented as mean ± standard error. A p-value <0.05 was considered significant.

RESULTS

Spatial learning and memory of Wistar rats following sleep deprivation

In the present study, there was a significant increase ($p<0.001$) in the time spent in locating the escape platform in the sleep-deprived group when compared to the control group **(Figure 1).**

Hippocampal glutamate activity levels of Wistar rats following sleep deprivation

No significant difference ($p>0.05$) was observed in hippocampal glutamate activity levels when the sleep deprived group was compared with the control group. **(Figure 2).**

Histological assessment of the CA3 region of the hippocampus following sleep deprivation

Histological analysis of the hippocampal CA3 region in Wistar rats, using hematoxylin and eosin staining, revealed normal histoarchitecture with large and sparsely distributed pyramidal neurons in the control group. In contrast, the sleep-deprived group exhibited a disoriented histoarchitecture in the CA3 region, accompanied by clear degenerative changes, such as cytoplasmic vacuolation, karyorrhexis and pyknosis. **(Figure 3).**

Estimation of pyramidal cell number in the CA3 region of the hippocampus

The result of the pyramidal cell count showed that the number of pyramidal cells in the CA3 of the hippocampus of the Wistar rats significantly decreased ($p< 0.0001$) in the sleep-deprived group when compared to the control group **(Figure 4).**

Nissl substance expression in the CA3 region of the hippocampus following sleep deprivation

The Nissl substance of hippocampal sections of the control group revealed the normal appearance of distinct, intensely stained CA3 region when compared to the sleep-deprived group which showed chromatolysis. The result of Cresyl Fast Violet staining intensity revealed a decrease in the staining intensity in the sleep

deprived group when compared to the control group suggesting protein depletion **(Figure 5 and 6).**

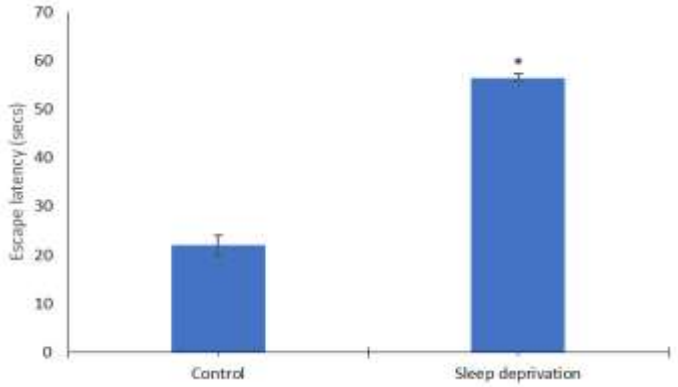

Figure 1: Morris water maze latency for Wistar rats following chronic sleep deprivation. n=5; mean ± SEM, Student *t*-test, *Tukey post hoc* test, * statistically different ($p<0.05$). Control (2ml/kg of distilled water), SD= sleep deprivation(18hours), MWM= Morris Water Maze.

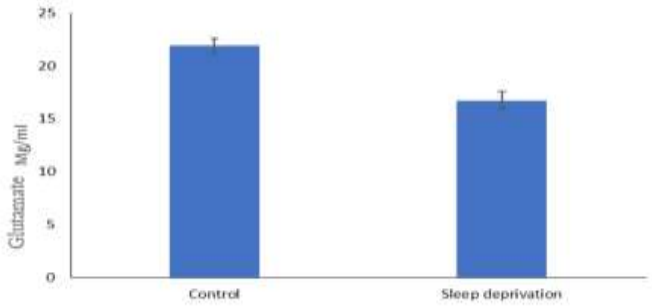

Figure 2: Hippocampal glutamate (GLU) activity levels of Wistar rats following sleep deprivation. n=5; mean ± SEM, Student *t*-test, *Tukey post hoc* test, not statistically different ($p>0.05$). Control (2ml/kg of distilled water), SD= sleep deprivation (18hours).

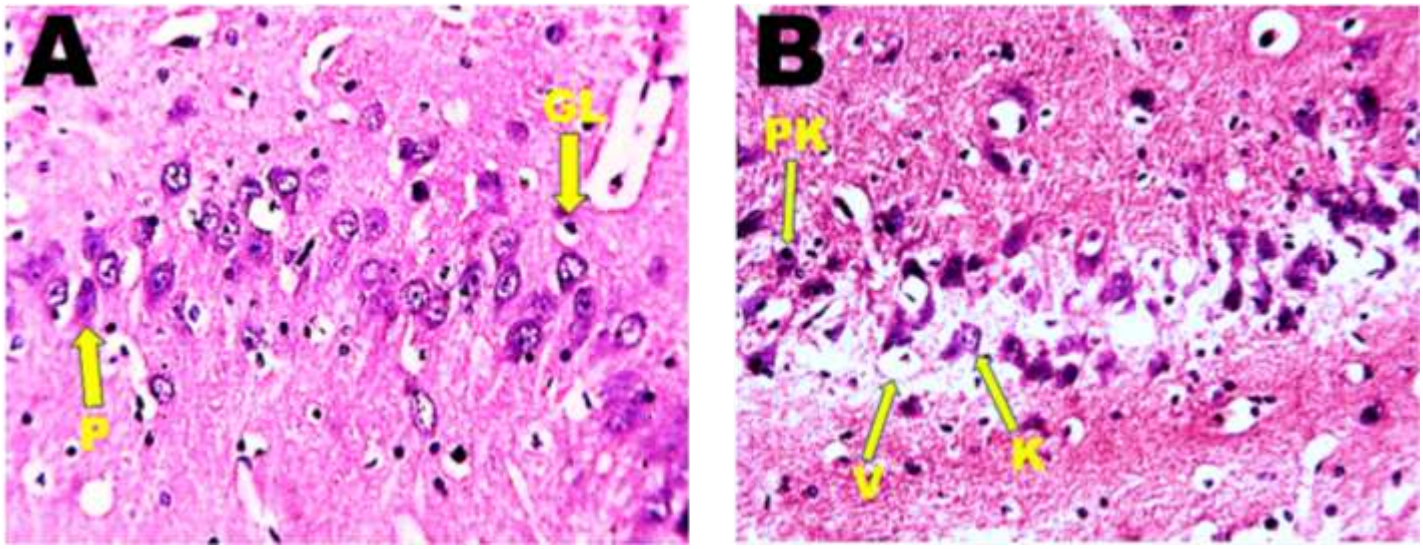

Figure 3: Section of the hippocampus (CA3) of Wistar rat. H and E (Mag x250). (A) Control group (2 ml/kg distilled water) with normal histoarchitecture of the CA3. Large and sparse pyramidal cells. (B) Chronic sleep deprived (18 hours) group with marked disorientation of the histoarchitecture and neuronal degeneration. Sleep deprivation (SD); Cytoplasmic Vacuolation (V); Karyorrhexis (K); Pyknosis (PK)

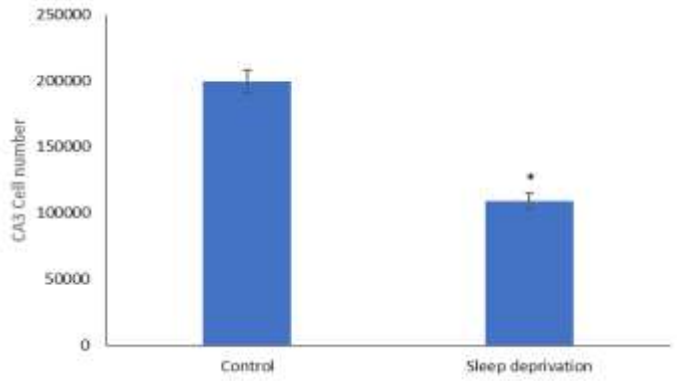

Figure 4: The CA3 pyramidal cell count of the hippocampus of sleep-deprived Wistar rats. n=5; mean ± SEM, Student *t*-test, *Tukey post hoc* test, * statistically different (p<0.05). Control (2ml/kg of distilled water), SD= sleep deprivation (18hours).

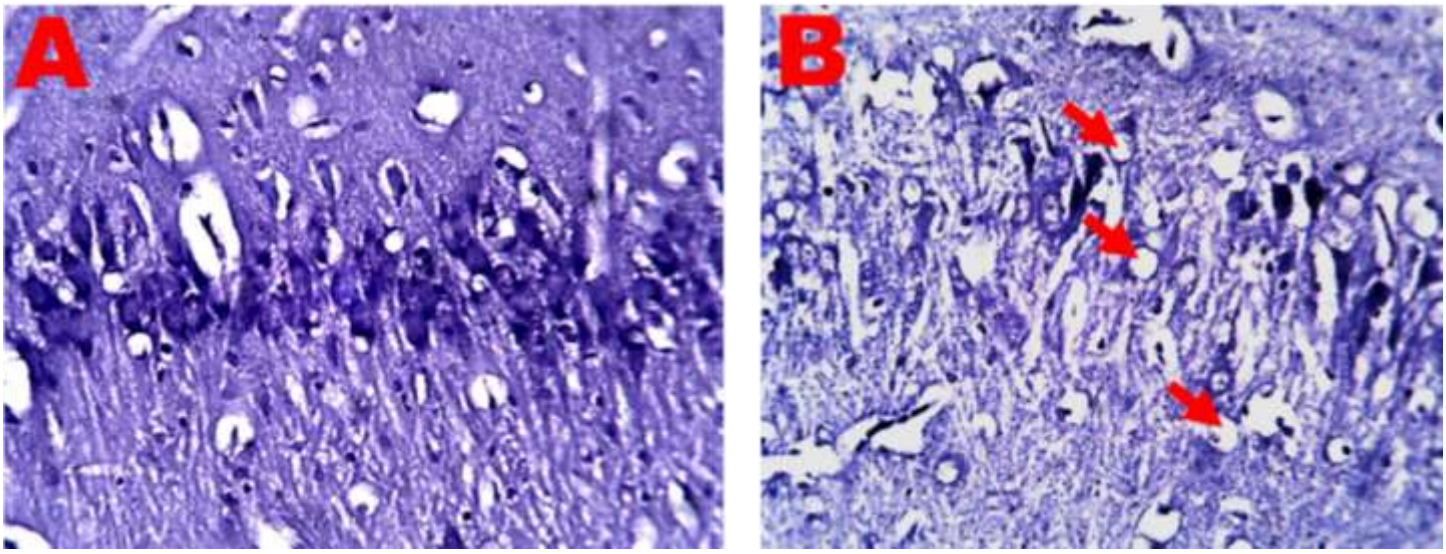

Figure 5: Section of the hippocampus (CA3) of Wistar rat. CFV (Mag x250). (A) The control group (2ml/kg distilled water) with normal appearance of Nissl granules. (B) Chronic sleep deprived (18 hours) group showing depletion of Nissl granules (arrows). Sleep deprivation (SD); Cresyl Fast Violet (CFV) stain; Chromatolysis (arrow).

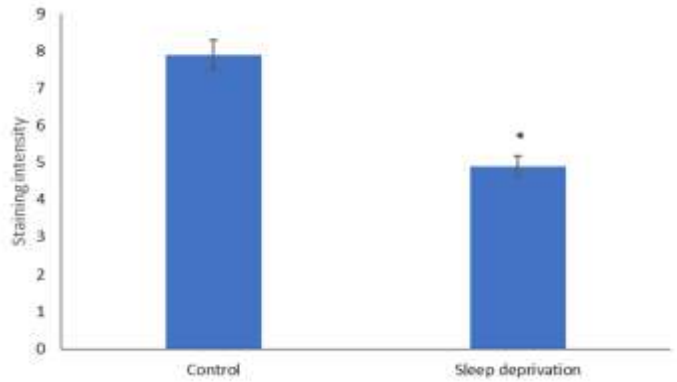

Figure 6: The CA3 Cresyl Fast Violet Staining Intensity of Sleep Deprived Rats. n=5; mean ± SEM, Student *t*-test, *Tukey post hoc* test, * statistically different (p<0.05). Control (2ml/kg of distilled water), SD= sleep deprivation (18hours).

DISCUSSION

Deficits or impairments in memory are typically associated with hippocampal pathologies (Belayev *et al.*, 2018). One of the most popular tasks in behavioural neuroscience for investigating the brain mechanics and psychological processes of spatial learning and memory is the Morris water maze (Vorhees *et al.*, 2021). The time it takes to find the escape platform in the Morris Water Maze test is a good indicator of learning and memory skills when compared to pre-experiment session performance. The results of this study showed an increase in the time taken for Wistar rats in the sleep-deprived group to locate the escape platform when compared to the control group, which suggests learning and memory impairment. This could be attributed to oxidative stress, which may have lowered acetylcholine levels at the synaptic cleft and hampered hippocampal synaptic connections. According to reports, acetylcholine decrease impairs memory and learning in

experimental animals (Bauer *et al*., 2017). Rahman *et al*. (2010) found that acetylcholine depletion was the cause of the deficit in spatial learning and memory observed in the experimental mice. The results of this investigation are consistent with those of Yabesh *et al*. (2014), who noted memory impairment in Wistar rats after sleep deprivation where the radial arm maze error scores of sleep-deprived Wistar rats were significantly higher than those of the control group.

Comparing 48-hour sleep-deprived rats to a control group, Li *et al*. (2009) found that the former group had less retention of learned spatial reference memory in the Morris water maze.

The primary excitatory neurotransmitter in the central nervous system is glutamate. An estimated 40% of synapses release it, and it is in charge of a variety of neurological processes, such as memory, cognition, behavior, movement, sensation, and the development of neural networks (Riedel *et al*., 2003). For long-term potentiation (LTP), which underlies synaptic plasticity in the hippocampal regions, glutamate is an essential neurotransmitter. Changes in the brain's glutamate neurotransmitter levels have a profound effect on cognitive function and can lead to a number of neurological and psychiatric conditions. Excitotoxicity, or overstimulation of glutamate receptors, especially the NMDA (N-methyl-D-aspartate) receptors, can result from high glutamate levels. This overstimulation can cause an excessive calcium influx into neurons, which can cause oxidative stress, mitochondrial dysfunction, and ultimately, neuronal damage or death (Yang *et al*., 2011; Gasiorowska *et al*., 2021; Zhang *et al*., 2023). However, low glutamate levels in the hippocampus can damage these hippocampal-dependent cognitive processes, making it difficult to perform tasks including contextual fear conditioning, object-place recognition, and spatial navigation. Deficits in learning and memory formation can result from hippocampus neurons' inability to undertake these activity-dependent modifications when glutamate levels are low (Menard and Quirion, 2012).

The current study's findings did not show any statistical difference in glutamate activity levels between the sleep-deprived group and the control. This result conflicts with that of Panaccione *et al*. (2017), who observed that, in comparison to control rats, sleep-deprived rats exhibited a significant decrease in the expression of type-2 metabotropic glutamate (mGlu2) receptors in the hippocampus.

The major pyramidal cell fields in the hippocampus, the hippocampal CA1 and CA3 areas, are crucial for the establishment of memory (Lee *et al*., 2004; Leutgeb *et al*., 2005). Cognitive impairment may result from neurotoxic injuries or from pharmacologically inactivating these regions (Lee & Kesner 2004; Daumas *et al*. 2005). Quality sleep promotes neural plasticity, which in turn helps the brain and cognitive processes. Consequently, insufficient sleep causes unfavorable brain changes that affect cellular and molecular levels (Fjell and Walhovd, 2010; Tuyet *et al*., 2017). Hematoxylin and eosin microscopy used in this investigation showed that the histoarchitecture of the hippocampal CA3 region appeared normal with large and sparsely distributed pyramidal cells with intact cytoplasm. Conversely, the sleep-deprived group showed significant disorientation of the histoarchitecture of the CA3 region along with vacuolation, pyknosis, and karryohexsis, which are signs of substantial neuronal degenerative alterations. The altered histoarchitecture of the CA3 area in the sleep deprived group may be the consequence of elevated oxidative stress and neuroinflammation. The hippocampus produces more reactive oxygen species (ROS) when it is sleep-deprived over an extended period. The buildup of reactive oxygen species (ROS) beyond the endogenous antioxidant capacity can initiate and maintain an inflammatory response, harming cellular constituents such proteins, lipids, and DNA, and ultimately leading to apoptosis (Verma *et al*., 2021). Sleep deprivation can also affect neurogenesis and synaptic plasticity through oxidative stress, which can change the structure and function of the hippocampal regions (Momoh *et al*., 2024). The results of this investigation are in line with those of Suresh *et al*. (2022), who found that sleep deprivation impairs memory and other cognitive functions by causing degenerative changes in the hippocampal region.

In biomedical research, unbiased stereology is acknowledged as the optimal approach for quantitative histology. It is employed to precisely measure the area and volume of biological structures or regions, as well as the number of cells and fiber lengths. Lack of sleep has been demonstrated to significantly affect the hippocampal cell count estimation (Suntsova, 2003; Havekes *et al*., 2016; Raven *et al*., 2019). Results of this study showed that the sleep-deprived group had a significantly lower number of pyramidal cells in the CA3 region when compared to the control group. A possible reason for this finding could be sustained oxidative stress, which can activate microglia and cause them to secrete proinflammatory cytokines, both of which significantly increase the risk of neuronal injury (Chen *et al*., 2023).

This result is consistent with a study by Guzman-Marin *et al*. (2006) that found sleep deprivation inhibits the growth of cells in the dorsal DG of the hippocampal region. Additionally, it has been shown that sleep deprivation reduces neurogenesis and hippocampal cell proliferation in the dorsal and ventral hippocampus (Murata *et al*., 2018). Furthermore, it has been documented that prolonged sleep deprivation causes morphological alterations in the hippocampus and other brain regions of rodents, with the hippocampus shrinking most prominently. But in lab mice, brief period of sleep deprivation does not seem to have an impact on the size of the hippocampus (Guzman-Marin *et al*., 2006; Tung *et al*., 2005; Mueller *et al*., 2008).

Rough endoplasmic reticulum aggregates called Nissl granules are found in the cell bodies of neurons and are an essential part of the physiological machinery that controls protein production in neurons. They are essential for the synthesis of proteins that are

necessary for the structure and function of neurons. According to Bhati *et al*. (2023), these granules are enriched in ribosomes, which create a characteristic cytoplasmic inclusion in the neuronal soma. A decrease in metabolic activity, such as that seen in stroke, trauma, or neurodegenerative illnesses like Alzheimer's, can result in neuronal injury, damage, or degeneration, as indicated by the depletion of Nissl material (Bhati *et al*., 2023). Lack of sleep has a profound effect on a cell's ability to synthesize proteins, changing its functionality. This is brought on by elevated oxidative stress and inflammatory reactions in the brain, which can damage the endoplasmic reticulum's structure and function, hence lowering protein production in important brain areas (Vaccaro *et al*., 2020; Coulson *et al*., 2022).

Reduced Cresyl fast staining intensity in the sleep-deprived alone group indicated a decrease in the expression of Nissl material, according to the histochemical evaluation of the CA1 and CA3 areas using Cresyl fast violet stain. Axon damage or neuronal exhaustion brought on by intense or protracted stimulation, including long-term sleep deprivation, reduces the amount of Nissl bodies in the body. This modification, known as chromatolysis, lowers the RNA level and happens concurrently with nuclear migration to the periphery of the perikaryon (Ajibade *et al*., 2009). As demonstrated by the results of the Morris water maze test and the degree of glutamate activity, this study concluded that long-term sleep deprivation led to an impairment in spatial memory. In the hippocampal CA3 region, there were also noticeable changes in histoarchitectural features, which were followed by a notable decrease in the number of pyramidal cells. Additionally, the CA3 region's histochemical test revealed protein loss. In light of the negative impacts that sleep has on the nervous system, this study encourages people to get adequate sleep in order to maintain good health and prevent neurological conditions.

Acknowledgments

The staff of Histology and Neuroscience Laboratory, Department of Human Anatomy, Faculty of Basic Medicine are greatly appreciated by the authors for their invaluable contributions to the success of this work.

Authors contributions

Conceptualization: OJ, UEU, SAM, SBO. Data acquisition: OJ, UEU, SAM, SBO. Data analysis or interpretation: OJ, UEU, SAM, SBO. Drafting of the manuscript: JO. Critical revision of the manuscript: UEU, SAM, SBO. Approval of the final version of the manuscript: all authors.

REFERENCES

Abbott, S. M., Malkani, R. G., & Zee, P. C. (2020). Circadian disruption and human health: A bidirectional relationship. European Journal of Neuroscience, 51(1), 567-583.

Ajibade, A. J., Adeeyo, O. A., Ofusori, D. A., Adenowo, T. K., Ishola, O. O., Ashamu, E. A., & Nwangwu, S. C. (2009). Microstructural observations on Nissl substances in the cerebellar cortex of adult Wistar rats following quinine administration. Tropical Journal of Pharmaceutical Research, 8 (2).

Amber, W.S., Musa, S.A., Sambo, S.J., and Agbon, A.N (2020). Neuroprotective effect of *Citrus sinesis L*. on mercury exposed Wistar rats. Ann Trop Path; 11(2): 157-165

Barnhart, C. D., Yang, D., & Lein, P. J. (2015). Using the Morris water maze to assess spatial learning and memory in weanling mice. PloS one, 10(4), e0124521.

Bassetti, C. L., Ferini-Strambi, L., Brown, S., Adamantidis, A., Benedetti, F., Bruni, O., Cajochen, C., Dolenc-Groselj, L., Ferri, R., Gais, S., Huber, R., Khatami, R., Lammers, G. J., Luppi, P.H., Manconi, M., Nissen, C., Nobili, L., Peigneux, P., Pollmächer, T., Randerath, W., Riemann, D., Santamaria, J., Schindler, K., Tafti, M., Van Someren, E & Wetter, T. C. (2015). Neurology and psychiatry: waking up to opportunities of sleep: State of the art and clinical/research priorities for the next decade. European Journal of Neurology, 22(10), 1337-1354.

Bauer, J. A., Henn, B. C., Austin, C., Zoni, S., Fedrighi, C., Cagna, G., Placidi, D., White, R.F., Yang, Q., Coull, B.R., Smith, D., Lucchini, R.G., Wright, R.O & Arora, M. (2017). Manganese in teeth and neurobehavior: Sex-specific windows of susceptibility. Environment International, 108, 299-308.

Belayev, L., Hong, S. H., Menghani, H., Marcell, S. J., Obenaus, A., Freitas, R. S., Khoutorova, L., Balaszczuk, V., Jun, B., Oriá, R.B & Bazan, N. G. (2018). Docosanoids promote neurogenesis and angiogenesis, blood-brain barrier integrity, penumbra protection, and neurobehavioral recovery after experimental ischemic stroke. Molecular Neurobiology, 55, 7090-7106.

Bhati, M., Thakre, S., & Anjankar, A. (2023). Nissl granules, axonal regeneration, and regenerative therapeutics: a comprehensive review. Cureus, 15 (10).

Bolon, B., & Butt, M. (Eds.). (2011). Fundamental neuropathology for pathologists and toxicologists: principles and techniques. John Wiley & Sons.

Carson, F. L. (1990). Histotechnology. A self-instructional text.

Chen, K., Wang, H., Ilyas, I., Mahmood, A., & Hou, L. (2023). Microglia and astrocytes dysfunction and key neuroinflammation-based biomarkers in Parkinson's disease. Brain Sciences, 13 (4), 634.

Cirelli, C., & Tononi, G. (2022, May). The why and how of sleep-dependent synaptic down-selection. In Seminars in cell & developmental biology (Vol. 125, pp. 91-100). Academic Press.

Coulson, R. L., Mourrain, P., & Wang, G. X. (2022). Sleep deficiency as a driver of cellular stress and damage in neurological disorders. Sleep Medicine Reviews, 63, 101616.

Daumas, S., Halley, H., Francés, B., & Lassalle, J. M. (2005). Encoding, consolidation, and retrieval of contextual memory: differential involvement of dorsal CA3 and CA1 hippocampal subregions. Learning & Memory, 12 (4), 375-382.

Deng, W., Aimone, J. B., & Gage, F. H. (2010). New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory?. Nature reviews neuroscience, 11(5), 339-350.

Durmer, J. S., & Dinges, D. F. (2005, March). Neurocognitive consequences of sleep deprivation. In Seminars in neurology (Vol. 25, No. 01, pp. 117-129). Copyright© 2005 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA.

Ekpo, U. U., Umana, U. E., & Sadeeq, A. A. (2023). Zingiber officinale Ameliorates Tramadol-induced Histopathological Distortions in CA1 and CA3 of the Hippocampus of Adult Wistar Rats. The Journal of Neurobehavioral Sciences, 10(2), 29-40.

Ekpo, U. U., Umana, U. E., Sadeeq, A. A., Shuaib, Y. M., Oderinde, G. P., Dauda, K., & Teryila, G. A (2023). Zingiber officinale Exhibits Neuroprotective Properties against Tramadol-Induced Spatial Memory Impairment and Histopathological Changes. Nigerian Journal of Neuroscience. 14(3):86-95.

Ekpo, U. U., Ikpa, J. O., Onimisi, B. O., Ochai, J., & Gbenga, O. P (2024). Tramadol Causes Weight, Nissl Substance, and Astrocytic Changes in the Hippocampal Formation's Structures.

Eluwa, M. A., Ekanem, T. B., Udoh, P. B., Ekong, M. B., Asuquo, O. R., Akpantah, A. O., & Nwakanma, A. O. (2013). Teratogenic effect of crude ethanolic root bark and leaf extracts of Rauwolfia vomitoria (Apocynaceae) on nissl substances of albino wistar rat fetuses. Neuroscience Journal, 2013(1), 906731.

Eugene, A. R., & Masiak, J. (2015). The neuroprotective aspects of sleep. MEDtube science, 3(1), 35.

Ferrara, M., & De Gennaro, L. (2001). How much sleep do we need?. Sleep medicine reviews, 5(2), 155-179.

Fjell, A. M., & Walhovd, K. B. (2010). Structural brain changes in ageing: courses, causes and cognitive consequences. Reviews in the Neurosciences, 21 (3), 187-222.

Gamble, K. L., Resuehr, D., & Johnson, C. H. (2013). Shift work and circadian dysregulation of reproduction. Frontiers in endocrinology, 4, 92.

Gasiorowska, A., Wydrych, M., Drapich, P., Zadrozny, M., Steczkowska, M., Niewiadomski, W., & Niewiadomska, G. (2021). The biology and pathobiology of glutamatergic, cholinergic, and dopaminergic signaling in the aging brain. Frontiers in Aging Neuroscience, 13, 654931.

Goldstein, A. N., & Walker, M. P. (2014). The role of sleep in emotional brain function. Annual review of clinical psychology, 10(1), 679-708.

Grandner, M. A., Hale, L., Moore, M., & Patel, N. P. (2010). Mortality associated with short sleep duration: the evidence, the possible mechanisms, and the future. Sleep medicine reviews, 14(3), 191-203.

Gundersen, H. J. G., Bendtsen, T. F., Korbo, L., Marcussen, N., Møller, A., Nielsen, K., Nyengaard, J.R., Pakkenberg, B., Sorensen, F.B., Vesterby, A & West, M. J. (1988). Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. Apmis, 96(1-6), 379-394.

Guzman-Marin, R., Ying, Z., Suntsova, N., Methippara, M., Bashir, T., Szymusiak, R., Gomez-Pinilla, F & McGinty, D. (2006). Suppression of hippocampal plasticity-related gene expression by sleep deprivation in rats. The Journal of Physiology, 575 (3), 807-819.

Havekes, R., Park, A. J., Tudor, J. C., Luczak, V. G., Hansen, R. T., Ferri, S. L., & Abel, T. (2016). Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area CA1. Elife, 5, e13424.

Javad-Moosavi, B. Z., Vaezi, G., Nasehi, M., Haeri-Rouhani, S. A., & Zarrindast, M. R. (2017). Critical role of CA1 muscarinic receptors on memory acquisition deficit induced by total (TSD) and REM sleep deprivation (RSD). Progress in Neuro-Psychopharmacology and Biological Psychiatry, 79, 128-135.

Killgore, W. D. (2010). Effects of sleep deprivation on cognition. Progress in brain research, 185, 105-129.

Killgore, W. D. (2010). Effects of sleep deprivation on cognition. Progress in brain research, 185, 105-129.

Killgore, W. D. (2010). Effects of sleep deprivation on cognition. Progress in brain research, 185, 105-129.

Kolla, B. P., Mansukhani, S., & Mansukhani, M. P. (2016). Consumer sleep tracking devices: a review of mechanisms, validity and utility. Expert review of medical devices, 13(5), 497-506.

Krause, A. J., Simon, E. B., Mander, B. A., Greer, S. M., Saletin, J. M., Goldstein-Piekarski, A. N., & Walker, M. P. (2017). The sleep-deprived human brain. Nature Reviews Neuroscience, 18(7), 404-418.

Krusztur, A., Raud, L., Juel, B.E., Nilsen, A, S., Storm, J.F and Huster, R.J. (2019). Sleep deprivation differentially affects suncomponents of cognitive control. Sleep. 42(4); zsz016

Khan, M.A and Aljahdali, H. (2023). The consequences of sleep deprivation on cognitive performance. Neuroscience Journal. 28(2); 91-99

Lee, I., & Kesner, R. P. (2004). Differential contributions of dorsal hippocampal subregions to memory acquisition and retrieval in contextual fear-conditioning. Hippocampus, 14 (3), 301-310.

Lee, I., Rao, G., & Knierim, J. J. (2004). A double dissociation between hippocampal subfields: differential time course of CA3 and CA1 place cells for processing changed environments. Neuron, 42 (5), 803-815.

Leutgeb, J. K., Leutgeb, S., Treves, A., Meyer, R., Barnes, C. A., McNaughton, B. L., Moser, M., & Moser, E. I. (2005). Progressive transformation of hippocampal neuronal representations in "morphed" environments. Neuron, 48 (2), 345-358.

Li, S., Tian, Y., Ding, Y., Jin, X., Yan, C., & Shen, X. (2009). The effects of rapid eye movement sleep deprivation and recovery on spatial reference memory of young rats. Learning & Behavior, 37, 246-253

Mahdipour, R., Ebrahimi, V., Hosseini, M., Soukhtanloo, M., Rastegar-Moghaddam, S. H., Malvandi, A. M., & Mohammadipour, A. (2022). Maternal exposure to silicon dioxide nanoparticles reduces hippocampal neurogenesis and synaptogenesis and induces neurodegeneration in rat offspring hippocampus. Toxicology and Industrial Health, 38(1), 41-52.

Ménard, C., & Quirion, R. (2012). Group 1 metabotropic glutamate receptor function and its regulation of learning and memory in the aging brain. Frontiers in Pharmacology, 3, 182.

Momoh, I. J., Alhassan, A., Dawud, F. A., Abubakar, S. A., Abba, S., Ogohi, D. A., Haruna, S.O & Jonah, A. C. Bayero Journal of Medical Laboratory Science Journal/Bayero Journal of Medical Laboratory Science 9 (1), 24.

Mueller, A. D., Pollock, M. S., Lieblich, S. E., Epp, J. R., Galea, L. A., & Mistlberger, R. E. (2008). Sleep deprivation can inhibit adult hippocampal neurogenesis independent of adrenal stress hormones. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 294 (5), R1693-R1703.

Murata, Y., Oka, A., Iseki, A., Mori, M., Ohe, K., Mine, K., & Enjoji, M. (2018). Prolonged sleep deprivation decreases cell proliferation and immature newborn neurons in both dorsal and ventral hippocampus of male rats. Neuroscience Research, 131, 45-51.

Ng, E. P., Ng, D. K., & Chan, C. H. (2009). Sleep duration, wake/sleep symptoms, and academic performance in Hong Kong secondary school children. Sleep and Breathing, 13, 357-367.

Ochai, J., Umana, U. E., Musa, S. A., & Oladele, S. B. (2024, July). Omega 3 fatty acid alleviates sleep deprivation-induced oxidative stress, inflammation, and cognitive impairment in the hippocampus of Male Wistar rats. In *Alzheimer's Association International Conference*. ALZ.

Onimisi, O. B., Musa, S. A., Umana, U. E., Sambo, S. J., & Makena, W. (2024). Tetrapleura tetraptera fruit phenolics fraction protects against the impact of ischemic stroke-induced hippocampal distortions and memory deficits in Wistar rats. *Anatomy & Cell Biology*.

Ohayon, M. M., Carskadon, M. A., Guilleminault, C., & Vitiello, M. V. (2004). Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. Sleep, 27(7), 1255-1273.

Panaccione, I., Iacovelli, L., di Nuzzo, L., Nardecchia, F., Mauro, G., Janiri, D., Blasi, A.D., Sani, G., Nicoletti, F & Orlando, R. (2017). Paradoxical sleep deprivation in rats causes a selective reduction in the expression of type-2 metabotropic glutamate receptors in the hippocampus. Pharmacological Research, 117, 46-53.

Patel, S. R., & Hu, F. B. (2008). Short sleep duration and weight gain: a systematic review. Obesity, 16(3), 643-653.

Rahman, H., Muralidharan, P., Sivaraman, D., & Saha, D. (2010). Continuous sleep deprivation for 5 days produces loss of memory in mice and may be a cause of Alzheimer's disease. Annals of Biological Research, 1 (4), 185-93.

Raven, F., Meerlo, P., Van der Zee, E. A., Abel, T., & Havekes, R. (2019). A brief period of sleep deprivation causes spine loss in the dentate gyrus of mice. Neurobiology of Learning and Memory, 160, 83-90.

Riedel, G., Platt, B., & Micheau, J. (2003). Glutamate receptor function in learning and memory. Behavioural Brain Research, 140 (1-2), 1-47.

Roth, T. (2007). Insomnia: definition, prevalence, etiology, and consequences. Journal of clinical sleep medicine, 3(5 suppl), S7-S10.

Sateia, M. J. (2014). International classification of sleep disorders. Chest, 146(5), 1387-1394.

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. Journal of neurology, neurosurgery, and psychiatry, 20(1), 11.

Siegel, J. M. (2005). Clues to the functions of mammalian sleep. Nature, 437(7063), 1264-1271.

Suntsova, N. V., & Dergacheva, O. Y. (2003). Dynamics of neuron activity in the lateral preoptic area of the hypothalamus during the sleep–waking cycle. Neuroscience and Behavioral Physiology, 33, 651-658.

Suresh, S., Singh, A., & Vellapandian, C. (2022). Bisphenol A exposure links to exacerbation of memory and cognitive

impairment: A systematic review of the literature. Neuroscience & Biobehavioral Reviews, 104939.

Tempesta, D., Socci, V., De Gennaro, L., & Ferrara, M. (2018). Sleep and emotional processing. Sleep medicine reviews, 40, 183-195.

Tung, A., Takase, L., Fornal, C., & Jacobs, B. (2005). Effects of sleep deprivation and recovery sleep upon cell proliferation in adult rat dentate gyrus. Neuroscience, 134 (3), 721-723.

Tuyet, L. T., Nhung, B. T., Dao, D. T. A., Hanh, N. T. H., Tuyen, L. D., Binh, T. Q., & Thuc, V. T. M. (2017). The brain-derived neurotrophic factor val66met polymorphism, delivery method, birth weight, and night sleep duration as determinants of obesity in Vietnamese children of primary school age. Childhood Obesity, 13 (5), 392-399.

Vaccaro, A., Dor, Y. K., Nambara, K., Pollina, E. A., Lin, C., Greenberg, M. E., & Rogulja, D. (2020). Sleep loss can cause death through accumulation of reactive oxygen species in the gut. Cell, 181 (6), 1307-1328.

Van Dongen, H. P., Maislin, G., Mullington, J. M., & Dinges, D. F. (2003). The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep, 26(2), 117-126.

Verma, N., Maiti, R., Mishra, B. R., Jha, M., Jena, M., & Mishra, A. (2021). Effect of add-on melatonin on seizure outcome, neuronal damage, oxidative stress, and quality of life in generalized epilepsy with generalized onset motor seizures in adults: A randomized controlled trial. Journal of Neuroscience Research, 99 (6), 1618-1631.

Vorhees, C. V., & Williams, M. T. (2024). Tests for learning and memory in rodent regulatory studies. Current Research in Toxicology, 6, 100151.

Williamson, A. M., & Feyer, A. M. (2000). Moderate sleep deprivation produces impairments in cognitive and motor performance equivalent to legally prescribed levels of alcohol intoxication. Occupational and environmental medicine, 57(10), 649-655.

Yabesh, J. M., Prabhu, S., & Vijayakumar, S. (2014). An ethnobotanical study of medicinal plants used by traditional healers in silent valley of Kerala, India. Journal of Ethnopharmacology, 154 (3), 774-789.

Yang, J. L., Sykora, P., Wilson III, D. M., Mattson, M. P., & Bohr, V. A. (2011). The excitatory neurotransmitter glutamate stimulates DNA repair to increase neuronal resiliency. Mechanisms of Ageing and Development, 132 (8-9), 405-411.

Yurt, K. K., Kivrak, E. G., Altun, G., Mohamed, H., Ali, F., Gasmalla, H. E., & Kaplan, S. (2018). A brief update on physical and optical disector applications and sectioning-staining methods in neuroscience. Journal of chemical neuroanatomy, 93, 16-29.

Zhang, M., Shan, K., Song, S., Bai, J., Jiang, P., Zhao, D., Ke, G., Zhou, G & Li, C. (2023). Serine, glutamate, and proline in a high-fat diet exacerbated metabolite reduction-induced memory and cognitive decline. Food Frontiers, 4 (2), 883-901.