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Sleep Deprivation Affects Brain Acetylcholinesterase Activity and Cognitive Ability in Young and Aged Rats

Neha Raina[1], Dushyant Singh Chauhan[1], Sandeep Tripathi[2*]

[1]Department of Advanced Science & Technology, Nims University Rajasthan, Jaipur, India
[2]Professor, Department of Advanced Science & Technology, Nims University Rajasthan, Jaipur, India

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***Corresponding author:** Sandeep Tripathi

Abstract **Original Research Article**

Sleep plays an important role in maintaining neuronal circuitry, signalling and helps maintain overall health and wellbeing. Sleep deprivation (SD) disturbs the circadian physiology and exerts a negative impact on brain and behavioural functions. SD impairs the cellular clearance of misfolded neurotoxin various proteins which are involved in major neurodegenerative diseases like Alzheimer's disease and Parkinson's disease. SD affects the immunological and redox system resulting in neuroinflammation and oxidative stress. The aim of the present study is to investigate sleep deprivation (SD) induced oxidative stress in different parts of the brain and in vivo behavioural changes associated with aging. Lipid peroxidation and reduced antioxidant status were significantly ($p<0.05$) increased along with the altered learning and memory ability. The most affected part of the brain was hippocampus followed by cerebral cortex. Acetyl cholinesterase was found to be altered in SD treated rats. These are remarkably correlated with the cognitive impairment. Aging is the affects more deleterious effect. It is also evident that SD induces premature aging in the study.

Keywords: Sleep Deprivation; Aging; oxidative stress.

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INTRODUCTION

The Sleep deprivation (SD) is a known physiological adverse condition and it is considered as a major health problem in population (Wu *et al*., 2011; Gaine *et al*., 2018). In today's modern era of society, the population is greatly affected by not getting enough or sufficient rest each night (Bishir *et al*., 2020). The combined effects of consistent deprivation of sleep over time can be a leading factor of neuronal degeneration (Hudson *et al*., 2020). Continuous lack of sleep can also be leads to death. The eye function may begin to generate even less coherent images possibly resulting in temporary insanity" during sleep deprivation (Frau *et al*., 2020). Sleep deprivation also caused weight fluctuations, memory loss, sleep paralysis, weakened immune system, and high blood pressure (Havekes *et al*., 2015). Further, Selvi *et al*., (2007) documented that the link between sleep deprivation and psychosis. Sleep deprivation caused deterioration in alertness, reasoning and response (Ben *et al*., 2020) and high order of cognitive ability (Atrooz and Salim 2019).

Various study has been documented that SD potentiate oxidative stress and changes in lipid peroxidation and antioxidant defence system (Villafuerte *et al*., 2015). Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen derived oxidants commonly known as reactive oxygen species (ROS). ROS are reported to damage almost all macromolecules of the cell, including polyunsaturated fatty acids of membranes, thus causing impairment of cellular functions (Cobley *et al*., 2018). Several hypotheses about the functions of sleep rest on the assumption that wakefulness represents an oxidative challenge for the brain. It has been claimed, for instance, that sleep may allow the removal of free radicals accumulated in the brain during wakefulness (Tripathi *et al*., 2008). Moreover, it has been proposed that, during sleep, uridine and glutathione may facilitate the oxidative detoxification of the brain by potentiating GABAergic transmission and inhibiting glutamatergic transmission, respectively (Villafuerte *et al*., 1995).

It is hypothesized that, sleep architecture changes with age (Kryger *et al*., 2004), and lack of adequate sleep is common in older adults, but sleep loss may also induce oxidative stress in the brain (Hipólide *et al*., 2002). To the best of our knowledge, no study has directly tested the effects of SD on oxidative stress in the different part of the brain and no comparison was made between young and old animals. Therefore, in the

present study attempt has been made to compare the effect of sleep on young and adult.

2. MATERIAL AND METHODS

2.1. Animals

Healthy young (n=12, age- 6 months, weight-140 ± 5) and old (n=12, age- 24 months, weight- 480±5) male albino rats, Charles Foster strain, obtained from the Institutional animal house were used in the study. The animals were separately housed in polypropylene cages in a room, which was maintained at a temperature of 22±2 ^{0}C, relative humidity of 50±10 % and 12h light dark cycles and allowed access to water ad libitum. The Institutional Animal Ethics Committee approved the study prior to the initiation of the experiment and also approved all experimental protocols. Animals were divided in to two subgroups namely control and SD treated group.

2.2. Mode of treatment

Chronic sleep deprivation was induced using columns-in-water (modified multiple platform) model as described (Alhaider *et al*., 2011; Alzoubi *et al*., 2011). Briefly, experimental animals were placed on platforms; 20 cm high and 5 cm diameter, 7 cm apart edge-to-edge) surrounded by water (24 ± 1 ◦C) in an aquarium where water and food were accessible to animals. The water level in the aquarium was about 4 cm below the edge of the platform. Same were applied in the control without water. The sleep SD treatments were administered between 8:00 and 9:00 am with 8 h/day for 45 days.

2.3. Water maze test

The ability to learn and memorize was tested by Morris water maze after experimental period for 45 days. The water maze consisted of a large, circular, galvanized steel pool (1.8 in diameter, 0.6 m in height). A white platform (10 cm in diameter) was placed inside and the tank was filled with water (22°C) until the top of the platform was submerged 1 cm below the water's surface. A sufficient amount of white paint was added to make the water opaque and render the platform invisible. An automated tracking system (Genheart Co.USA) was used to analyze the swim path of each subject and calculated escape latencies (the time between being placed in the water and finding the hidden platform) and total path lengths.

2.4. Tissue Homogenate Preparation

After 45 days of experimental period, rats were sacrificed by anesthetic overdose and the brain was removed immediately and dissected into different parts (hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) for biochemical estimations. Ten percent (w/v) homogenate of different brain regions was prepared with the aid of York's homogenizer fitted with Teflon plunger in KCl (0.15M) or 0.1 M phosphate buffer (pH 7.1), as per requirement.

2.5. Protein estimations: (Lowry et al., 1951)

To 0.1 ml of tissue homogenate was added 0.9 ml normal saline and 1.5 ml of 10% TCA and kept at 40C for 4 hours. Protein precipitate was recovered by centrifugation. Protein was dissolved in 1.0 ml 0.1 N NaOH. 0.5ml alkaline copper sulfate (mixture of 1ml of 1.0%w/v copper sulphate + 1ml of 2% w/v sodium potassium tartarate + 48 ml of 2.0%w/v sodium carbonate in 0.1N NaOH) was added to the diluted sample, and incubated at 37oC, after 30 min 0.5 ml folin-calteau reagent. Optical density of the blue colour was read at 625 nm exactly after 30 min.

2.6. Acetylcholinesterase: Ellman et al., (1961)

The activity of acetylcholinesterase enzyme was determined by the modified method of Ellman *et al*., (1961). The substrate used in the assay system is acetylthiocholine iodide. The mercaptan formed because of hydrolysis of ester then reacts with an oxidizing agent, DTNB that splits into two products, one of which is 5 thio –nitro benzoate, which absorbs at 412 nm. The enzyme activity is measured by the increase in absorbance at 412 nm. The results were expressed as nmoles of acetylcholine hydrolyzed/mg protein/min.

3. RESULTS

3.1. Body Weight

In the present study, male Charles Foster rats of two age groups were taken for the study. The young rats (6 months, 120 ± 5.5-gram weight) and old rats (24 months, 520 ± 5.5-gram weight) were sleep deprived treated the terminal body weight and brain weight were found to be significantly reduced of SD treated old rats when compared with the old control rats, while, insignificant difference was observed in SD treated young and their age matched control rats (Table-1). The piloerection and hair loss were observed in SD treated young and old rats as physical sign of toxicity.

3.2. Behavioral study

The behavioral studies were also performed by Morris water maze test in term of path length, escape of latency and swimming speed (Table 2). The result show that the path length and escape latency to find the platform increased significantly ($p<0.05$) in SD treated rats when compared with the controls. Moreover, the path length and escape latency were found to be reduced significantly ($p<0.05$) in both SD treated young and old rats, when, compared with the age matched controls. However, the swimming speed had no difference between control and experimental groups.

3.3. Concentration of Total protein

The concentration of protein in the various regions (namely, hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain of young and old rats after the sleep deprivation is depicted in the table-3. The concentration of protein was found to be significantly ($p>0.05$) changed between groups. The maximum reduction of proteins was observed in brain

stem (-28.5 %) of SD treated young rats and in cerebrum (- 49%) of SD treated old rats when compared with their respective controls. While, there was least reduction in protein content of hippocampus (-19 %) of SD treated young rats and in cerebellum (-14.5%) of SD treated old rats, when compared with their respective controls. It may be seen in the table -3 that the concentration of protein was decreased in aged rats in all the brain regions, however, their levels varied significantly. The age dependent reduction of proteins was found to be maximum in cerebellum (-24%) and minimum in cerebrum (-14%) of old controls, when compared with the young controls. In SD treated old rats, there was a significant reduction in protein content of cerebrum (-31%) and least in hypothalamus (-21 %), when compared with the SD treated young rats, respectively.

3.4. Acetylcholinesterase activity

The activity of acetylcholinesterase (AChE) in various regions (namely, hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain of SD treated young and old rats are presented in the Table-4. The AChE was found to be increased following 90 days of SD administration in rats. The maximum increment was observed in brain stem (122%) of SD treated young rats and hippocampus (165%) of SD treated old rats when compared with their respective controls. While the least increment of AChE was observed in cerebrum (52%) of SD treated young rats and brain stem (76%) of SD treated old rats as compared with their respective controls. An age dependent insignificant changes in the activity of AChE were observed between young and old rats. An age dependent effect of Al on AChE activity was found in different group of rats. The activity of AChE differed significantly in SD treated old rats as compared with the SD treated young rats. Maximum increment was found in hippocampus (67.5%) and least in brain stem (29%) of SD treated old rats, when, compared with the SD treated young rats.

Table 1: Terminal body weight and brain weight

Groups	Terminal body Wt (g)	Brain weight (g/kg)
Young Control	255.8 ± 8.3	4.64 ± 0.07
Young treated	237.5 ± 9.2[a]	3.89 ± 0.15[a]
Old Control	569.2 ± 11	3.92 ± 0.17
Old treated	505.8 ± 8.7[c]	3.12 ± 0.21[c]

The value of the terminal body weight and brain weight are presented as mean ± SEM of six animals per group. Superscripts relate significant ($p< 0.05$) comparison with young control (a), young Al treated (b), aged control (c) and aged treated rats

Table 2: Escape latency (sec), path length (cm), and swimming speed (cm)

Groups	Swimming speed (cm)	Path length (cm)	Escape latency (sec)
Young Control	18.2 ±1.3	324.2 ± 44.7	14.5 ± 1.4
Young Treated	17.8 ±1.6	586.8 ± 46.5[a]	29.2 ± 2.6[a]
Old Control	16.8 ±1.1	424.7 ± 67.1	20.5 ± 1.5
Old Treated	16.2 ±1.6	797.8 ± 52.2[abc]	49.7 ± 5.5[abc]

Path length is the distance that the rats find the platform. Escape latency meant the time it takes the rats to find the platform. The values are presented as mean ± SEM of twelve animals per group. Superscripts relate significant ($p< 0.05$) comparison with young control (a), young Al treated (b), aged control (c) and aged treated rats

Table 3: concentration of total protein in different parts of the control and experimental rats

	Young Control (YC)	Young Treated (YT)	Old Control (OC)	Old Treated (OT)
Hippocampus	87.9 ± 5.27	70.7 ± 4.63	75.2 ± 5.43	60.7 ± 6.16
Hypothalamus	89.1 ± 5.17	70.9 ± 4.35	75.4 ± 6.35	56.1 ± 4.57
Cerebrum	72.5 ± 3.78	54.3 ± 4.10	63.1 ± 5.05	37.3 ± 3.66
Cerebellum	81.7 ± 6.27	61.7 ± 4.53	62.3 ± 5.33	53.3 ± 4.59
Brain Stem	57.8 ± 4.88	41.3 ± 4.66	51.7 ± 5.04	31.9 ± 2.62

The values of the total protein are presented as mean ± SEM of twelve animals per group. Superscripts relate significant ($p< 0.05$) comparison with young control (a), young Al treated (b), aged control (c) and aged treated rats

Table 4: Activity of acetylcholinesterase in different parts of the control and experimental rats

	Young Control (YC)	Young Treated (YT)	Old Control (OC)	Old Treated (OT)
Hippocampus	14.4 ± 1.7	10.6 ± 1.0[a]	11.2 ± 1.3	7.92 ± 0.6[ac]
Hypothalamus	14.5 ± 1.5	11.4 ± 1.5 [a]	11.9 ± 1.6	8.89 ± 0.7 [ac]
Cerebrum	12.6 ± 1.8	9.56 ± 1.1[a]	9.34 ± 0.8	6.34± 0.6 [ac]
Cerebellum	14.7 ± 1.6	11.2 ± 1.3 [a]	11.5 ± 1.2	8.92 ± 0.9 [ac]
Brain Stem	13.7± 1.4	11.3 ± 1.4 [a]	8.35 ± 0.8	6.80 ± 0.8 [ac]

The values of the acetylcholinesterase are presented as mean ± SEM of twelve animals per group. Superscripts relate significant ($p< 0.05$) comparison with young control (a), young Al treated (b), aged control (c) and aged treated rats

4. DISCUSSION

Sleep plays an important role in maintaining neuronal circuitry, signalling and helps maintain overall health and wellbeing. Sleep deprivation (SD) disturbs the circadian physiology and exerts a negative impact on brain and behavioural functions. SD impairs the cellular clearance of misfolded neurotoxin various proteins which are involved in major neurodegenerative diseases like Alzheimer's disease and Parkinson's disease. SD affects the immunological and redox system resulting in neuroinflammation and oxidative stress. Hence, it is important to understand the molecular and biochemical alterations that are the causative factors leading to these pathophysiological effects on the neurochemical and neurobehavioral function.

In the present study, we found that the SD treatment to young and old rats had a detrimental effect on the body and brain weights of old rats when compared with the controls (Table 1). Loss in body weight, following exposure to heavy metals, has also been attributed to the increased mobilization of fat deposits, owing to enhanced synthesis of glucose from noncarbohydrate sources (Ganrot, 1986). The loss in brain weights after subacute treatment could be attributed to the retarded development of the animal. Moreover, these changes seem to be linked with SD and as they were more apparent in the old rats, it is likely that they may be due to the perturbation in cellular and molecular metabolism of aged rats.

The alterations in the neuronal activity are accompanied by changes in concentration of protein. The specific neuronal functions, such as conduction of action potentials and synaptic transmission, are known to be mediated by proteins. Mcllwain and Bachelard, (1971) have reported that many environmental and nutritional factors may perturb the brain protein levels. In the present study, we found reduced concentration of total protein following 45 days of SD administration to young and old rats when compared with their age matched control rats. The decrease of protein content suggests high rate of utilization of protein in ageing as well as SD. The decrement of protein may also be due to increased proteolytic activity necessiated by (Pickering AM 2012) greater energy demands under toxic stress. It has been reported (Sabeo *et al*., 2009) that the increased neuronal activity inhibits the synthesis of protein. Age-related decline in the rate of protein synthesis is widely described in the central nervous system (Dwyer et al., 1980), whereas other studies show no changes. These divergent results of different studies are difficult to interpret. However, reduction of protein content can be correlated well with DNA loss. According to Le *et al*., (2001) there is also evidence of protein damage and increased rate of intracellular proteolysis in aged animals. The accumulation of damaged proteins in the ageing brain may be compatible with a concurrent increase in levels of reactive oxygen species, since the former event represents a cumulative process while the latter assay reflects only current activity. Increased proteolytic activity in the SD toxicity and associated ageing may reflect an adaptation to deal with an elevated amount of non-functional denatured proteins (Pickering and Davies, 2012). Such damaged proteins appear abnormally early in diseases of premature ageing such as progeria and Werner's syndrome (Agrelo *et al*., 2006). The lipofuscin content of neurons increases with senescence and this pigment consists of a proteinaceous complex that is resistant to break-down (Zhou *et al*., 2017). The accumulation of lipofuscin with SD induced neuronal aging appears to be a consistent parameter (Williams *et al*., 2017) that holds true over a wide range of species (Tuo *et al*., 2007). Such proteins may be cross-linked products produced by oxidative events, which are no longer substrates for proteolytic break-down and thus accumulate within the cell (Sitte *et al*., 2000). In other words, altered protein structure and function may be a contributing factor to senescence. Our results support this evidence because lipofuscin pigment was found to be increased with ageing as well as following SD toxicity.

The most conspicuous changes that occur during sleep loss are the neuromodulatory transitions and effective control of these transitions is critical for fitness and survival. Acetylcholine (ACh) is a fast-acting, steeplechase cholinergic neurotransmitter present at the neuromuscular junction and in the autonomic ganglia. These cholinergic neurons apart from its role in wakefulness have been included in control of much wake-promoting behaviours such as attention, sensory procession and learning. It was found that behaviourally pertinent signals from the sensory inputs induce a transient increase in the ACh levels and the subsequent activation of cholinergic transmission improves the performance of sustained attention task. The data obtained showed that the administration of SD caused

highly activation of AChE activity in different regions of the rat brain. Highest elevation of AChE activity was observed in the hippocampus of both SD treated young and old rats. Increased AChE activity following SD exposure may be due to the allosteric interaction between the cation and the peripheric anionic site of the enzyme (Le *et al*., 1992).

In the present study, behavioural tests were carried out by morris water maze for the assessment of cognitive impairment following SD treatment in young and old rats. In the SD group, the distance and the time to find the platform submerged in the water increased, which indicates that the ability to learn and memorize were degraded. We found that interesting inverse relationship between age dependent SD accumulation and learning & memory performance in rats. This relationship also influences by altered AChE and increased oxidative stress, as we found in SD treated young and old rats when compared with the controls. The concept was advanced by Dauter *et al*., (2001) that showed a similar inverse correlation between mice maze performance and oxidative modification in brain.

5. CONCLUSION

On the basis of result it may conclude that a close relationship exists between SD induced oxidative stress and cognitive impairment. Sleep deprivation (SD) disturbs the circadian physiology and exerts a negative impact on brain and behavioural functions. Hippocampus is main part of the brain that affects maximum in SD. SD may impairs the cellular clearance of misfolded neurotoxin various proteins which are involved in major neurodegenerative diseases. Aging is the affects more deleterious effect. It is also evident that SD induces premature aging in the study. The results of the study recommend prevention of SD and supplementation of antioxidants for health brain.

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