ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 1, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

EFFECT OF SLEEP DEPRIVATION ON REMINISCENCE AND APPREHENSION BEHAVIOR IN WISTAR ALBINO RATS

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Received: 01 August 2019, Revised and Accepted: 29 November 2019

ABSTRACT

Objective: The aim of the present study is to investigate the effect of paradoxical sleep deprivation (SD) on learning and memory impairment and anxiety-like behavior in female Wistar albino rats.

Methods: Eight-arm radial maze, open-field test, and light and dark test were used to assess the animals learning and memory and anxiety-like behavior.

Results: SD associated with weaker learning and memory and increased anxiety- and depressive-like behavior in animals.

Conclusion: Animals were exposed to SD showed learning and memory impairment and also exhibited increased anxiety- and depressive-like behavior when compared to control animals.

Keywords: Behavior, Sleep deprivation, Cognition.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i1.35187

INTRODUCTION

Memory is typically defined as the ability of retaining and manipulates information previously acquired by means of neuronal plasticity [1]. This memory process is dramatically dynamic and can be modified by several factors which include drugs [2-4] as well as experimental conditions such as sleep deprivation (SD) [5-7] and stress [8,9], which interfere with acquisition, retention, and/or retrieval processes. SD has been shown to affect physiological and psychological functioning.

It is well established that sleep plays a critical role in learning and memory formation. Indeed, Myria studies have demonstrated that paradoxical SD (PSD) in animals leads to memory deficits in several behavioral models [10,11]. It has been proposed that the deleterious effects caused by SD are due to its effects on the long-term potential town in the hippocampus, which is important for encoding memories [12,13]. Alternatively, some studies have also suggested that the memory impairments observed in SD animals are induced by other behavioral alterations, such as decreased reaction time [14], motor function alterations [15], stress response [16], and modification in exploratory and fear responses [17] as memory can be state dependent when a response that has been acquired in a certain condition may not be retrieved when in another condition [18], these behavioral alterations induced by SD could contribute to its amnestic effects through a state-dependency phenomenon.

Although the consequences of total SD in animal models have been studied for many years, the effects of PSD on learning/memory as well as anxiety and locomotor activity in animal models have been overlooked. The aim of the present study was to investigate the participation of state-dependent learning in memory impairment induced by PSD by eight-arm radial maze, open-field, and light and dark task, it is an animal model which evaluates learning/memory and anxiety- and depression-like behavior concomitantly.

Sleep, especially rapid eye movement (REM) sleep, has an essential role in learning and memory process in the hippocampus [19].

Aim

The aim of the present study was to investigate the participation of state-dependent learning and memory impairment induced by PSD in female Wistar albino rats.

METHODS

Animals

Wistar albino female rats weighing 180–200 g were used for the study. Animals were housed in a group of three rats per cage and animals were maintained in controlled room temperature 23°C±2°C with 12:12-h light: dark cycle and allowed to free access to food and water. All animal procedures were approved by the Institutional Animal Ethical Committee and CPCSEA (IAEC No: 01/44/2015). All efforts were made to minimize both the number of animals used and unwanted suffering to the animals during experimental procedures.

Experimental design

Animals were divided into two groups; each group consists of six animals: Group I – control and Group II – SD (Table 1). To avoid circadian rhythm-induced variation, all the experiments have been carried out between 9 and 10 AM.

Sleep deprivation procedure

We have employed the columns-in-water model of SD as previously described [31]. Basically, 4–6 rats were placed in a sleep-deprived cage (28 cm × 16 cm × 22 cm) filled with water at room temperature. It contains six platforms (diameter: 2.5 cm, with platforms 3 cm above the water level), spaced 7 cm apart (edge to edge) (Fig. 1) and arranged in two rows such that rats could move freely from one platform to another over the water.

The water level is kept 2 cm below the top of the platforms the rats get minimal water exposure after they learn to balance themselves properly on the platforms. In addition, animals were group housed which allow them to form stable social groups and reduce the chances of psychosocial or isolation stress within the tank environment. Rats have free access to

food and water during the entire length of the experiment with food pellets and water bottle placed on top of the tank. When rats begin the REM sleep phase, they exhibit inherent muscle paralysis, which causes them to fall into the water and are awakened. On falling into the water, the rats quickly recovered back onto the platforms to dry themselves without any outside interference. Moreover, video recordings show that rats rarely fall off the narrow platform after approximately the 1st h of the experiment, probably because they wake up as soon as their snouts touch the water.

Behavioral tests

Open-field test

Open-field test is used to measure the exploratory and anxiety-related behavior in the novel place. The test was performed accordingly previously described method [23]. The apparatus is illuminated with white light in the center. The test provides a unique opportunity to assess three independent behavioral dimensions relating to locomotor activity, exploration, and emotional activity, by placing the animal in the corner of brightly lighted a large rectangular box, Fig. 2. The floor was cleaned after every entry of animal with 70% alcohol. Emotional status of the animal and locomotor activity of the rat was assessed by the parameters such as peripheral ambulation, central ambulation, rearing, grooming, immobilization, and defecation.

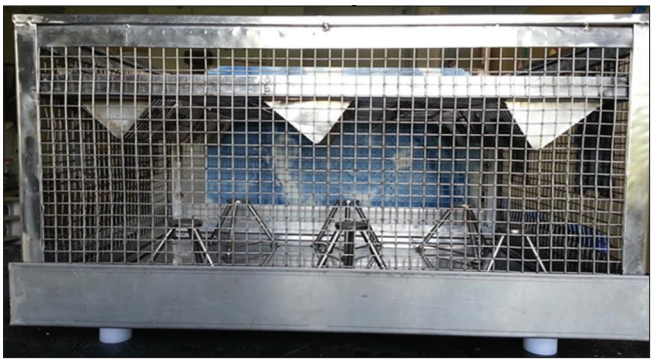

Fig. 1: PSD set up

Fig. 2: Open field test

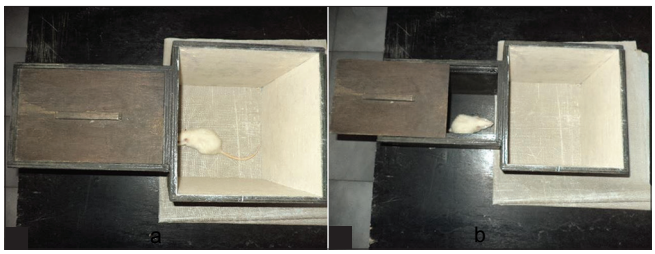

Fig. 3: Light and dark test/place preference task

Light and dark box test/place preference task

The light/dark box also was used to assess the anxiety level of rodents (Fig. 3). At the beginning of testing, each animal was placed in the center of the light compartment. Behavior subsequently was videotaped for 5 min. Behaviors were scored by an observer who was blind to the treatment conditions. The measures scored were (1) initial latency to enter the dark compartment, (2) time spent in brighter area, and (3) time spent in the dark compartment [24].

Radial arm maze (RAM)

Spatial learning and memory was tested using an eight-arm radial maze apparatus as described before [25]. Stress group and control group animals were tested in the eight-arm radial maze for testing memory. The eight-arm radial maze made of steel material, had an octagonal central platform, 33.5 cm wide, around which were arranged 60 cm long by 12 cm wide arms (Fig. 4). The whole apparatus was elevated 40 cm from the floor in a soundproof chamber.

During behavioral training, the food is kept as its reward; animals were fasted 2 h before the behavioral training, so that they could become habituated to the apparatus. Initially, animals were allowed to freely explore the maze for 3 consecutive days with all arms baited with sucrose pellets for 10 min. By the 4th day of training on the spatial task, only four arms (fixed for that animal) were always baited and food rewards placed at the end of the arms.

After the adaptation, maze test was performed between 09:30 am and 11:00 am every day. Each individual rat had its own set of four rewarded arms. The room contained several visual reference cues on the wall. Each trial began with the placement of the animal on the central platform facing arm number one and ended when the rat had visited the four baited arms or after a period of 5 min. The following parameters were measured.

Based on olton's definition

1. Number of reference memory errors (i.e., each entry into a non-baited arm)
2. Number of working memory errors (i.e., reentries into already visited baited arms were noted)
3. Time taken to visit all the baited arms.

Statistical analysis

Data were presented in the form of the bar diagram with mean±standard error of mean. Data are analyzed by paired samples *t*-test, chosen for group comparison which was applied mean performance in the eight-arm radial maze, open-field behavior, and place preference test. $p<0.05$ was considered as statistically significant.

RESULTS

Effect of paradoxical sleep deprivation on eight-arm radial maze working memory error

The number of working memory error is summarized in Fig. 5a. The working memory error was more in PSD group, whereas there was no working memory error observed in the control group Fig. 5a.

Values are expressed as mean±standard deviation (SD), n=6 *compared with saline control; the symbol represents statistical significance: *$p<0.05$.

Table 1: Experimental design

Serial number	Group	Animals
Group I	Control	6
Group II	Sleep deprivation (48 h)	6
Total		12

Reference memory error

The number of reference memory errors is summarized in Fig. 5b. The reference memory error was more in PSD group compared to the control Fig. 5b.

Values are expressed as mean±SD, n=6.*compared with saline control; the symbol represents statistical significance:*p<0.05.

Time taken to visit the entire baited arm

PSD group animals taken significantly (p<0.05) more time to visit all baited arms as compared to control. The times taken to visit all baited arms are summarized in Fig. 5c.

Values are expressed as mean ± SD, N=6. *compared with saline control; the symbol represents statistical significance: *p<0.05.

Effect of paradoxical sleep deprivation on place preference task:

Time spent in light area

The PSD group showed less time spent in brighter area when compared to control group. Time spent in brighter area of all groups is shown in Fig. 6a.

Values are expressed as mean±SD, n=6. *compared with saline control; the symbol represents statistical significance: *p<0.05.

Time spent in darker area

The PSD group animals showed increased in time spent in darker area when compared with the control group. Time spent in darker area of all groups is shown in Fig. 6b.

Values are expressed as mean±SD, n=6. *compared with saline control; the symbol represents statistical significance: *p<0.05.

Effect of PSD in locomotor activity in open field

All the animals appeared healthy and no mortality was observed. The results of open field are shown in Fig. 2 with mean ± SD. The PSD animals showed a marked decrease in ambulation peripheral square, ambulation central square, grooming, increase in the rearing, fecalbolus, and immobilization when compared to the control group Values are expressed as mean±SD, n=6. *compared with saline control; the symbol represents statistical significance: *p<0.05.

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Values are expressed as mean±SD, n=6. *compared with saline control; the symbol represents statistical significance: *p<0.05.

Values are expressed as mean±SD, n=6. Ambulation (peripheral and central squares) was expressed in number of square entry.

Fig. 4: Eight-arm radial maze

Immobilization was expressed in seconds. Rearing and grooming were expressed in number of attempts. The symbol represents statistical significance: *p<0.05. *Compared with control.

Effect of paradoxical sleep deprivation on weight

In this present study, the PSD group showed a substantial weight loss Fig. 8 when compared to the control group, suggesting that their energy expenditure was far higher than that of control animals. Social instability can amplify the stress effect on food intake and body weight loss.

Values are expressed as mean±SD, n=6. *compared with control; the symbol represents statistical significance: *p<0.05.

DISCUSSION

Sleep has important homeostatic functions, and SD is a stressor that has consequences for the brain, as well as many-body systems. Whether SD is due to anxiety, depression, or a hectic lifestyle, there are consequences of chronic SD (CSD) that impairs brain functions and contribute to allostatic load throughout the body [26]. CSD in young healthy volunteers has been reported to increase appetite and energy expenditure, increase levels of pro-inflammatory cytokines, decrease parasympathetic and increase sympathetic tone, increase blood pressure, increase evening cortisol levels, as well as elevate insulin and blood glucose.

Repeated stress in animal models causes structural remodeling of brain regions in hippocampus, amygdala, and prefrontal cortex, which are responsible for memory and emotions, which results in increased memory impairment and anxiety and aggression behaviors. Structural and functional magnetic resonance imaging studies in depression and Cushing's disease, as well as anxiety disorders provide evidence that the human brain may be similarly affected. Moreover, brain regions such as the hippocampus are sensitive to glucose and insulin, and both type 1 and type 2 diabetes mellitus are associated with cognitive impairment and increased risk for Alzheimer's disease [27].

Animal models of CSD indicate that memory is impaired along with depletion of glycogen stores and increases in oxidative stress and free radical production, which includes markers such as 8-isoprostane and malondialdehyde levels in the serum as well as in the cortex, amygdala, and the hippocampus.

PSD disrupts a number of physiological systems, which results in hyperphagia, hypothermia, and impaired immune function. PSD is a common feature of several sleep disorders in humans, such as insomnia, obstructive sleep apnea syndrome, and periodic leg movements. Therefore, the need to develop animal models to induce PSD to evaluate its health consequences is justified. Human subjects deprived of sleep showed increased irritability, aggressiveness, and restlessness with reduced alertness, discrimination, and learning recall.

In rats, SD results in a variety of behavioral changes. SD rats show increased aggression and locomotor and exploratory activity but decreased emotionality, less fear, and greater sensitivity to environmental stimuli than controls based on this information, this study conducted to evaluate the relationship between PSD on reminiscence and apprehension behaviors.

Several authors in their reviews suggested that sleep acts to conserve energy, involved in thermoregulatory processes, detoxification of the brain, and restorative function, and involved in neural plasticity [28]. Vertes and Eastman (2000) suggested that it is a mechanism for activating the sleeping brain in slow-wave sleep in a waking state. In support of this view, persons awoken from slow-wave sleep were impaired on cognitive tasks relative to those woken from REM [28]. Smith (1996) suggested that REM sleep is associated with learning. REM sleep is not a singular requirement for effective learning and memory consolidation [32]. However, alterations in REM patterns

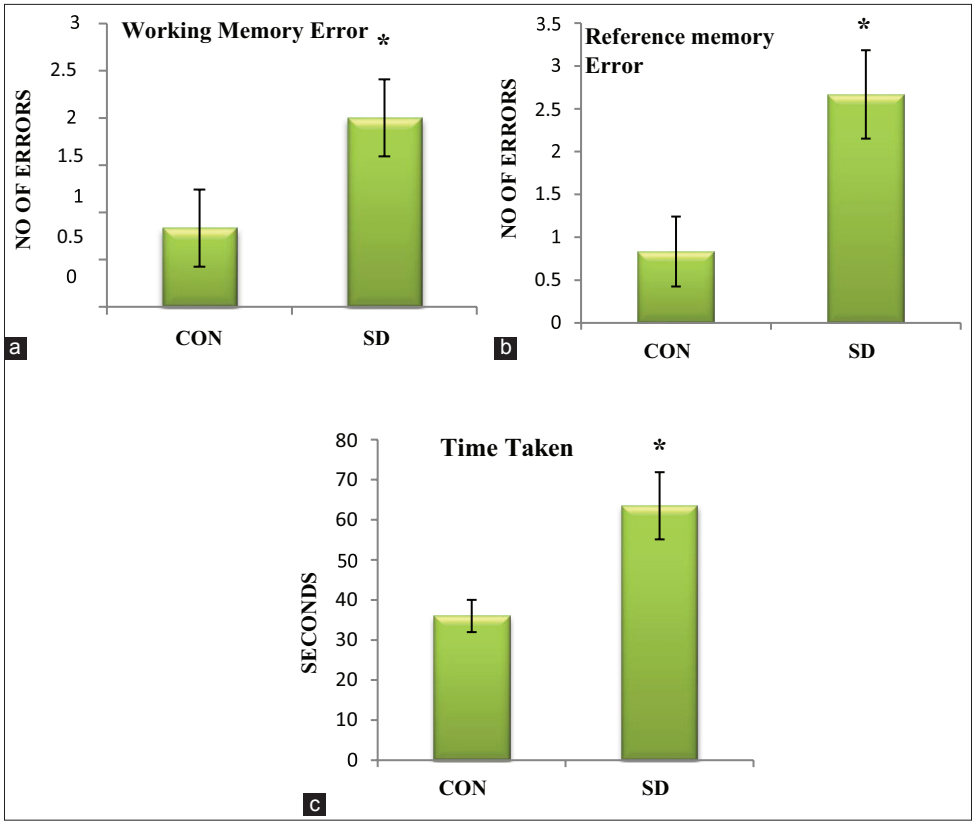

Fig 5: Eight arm radial maze

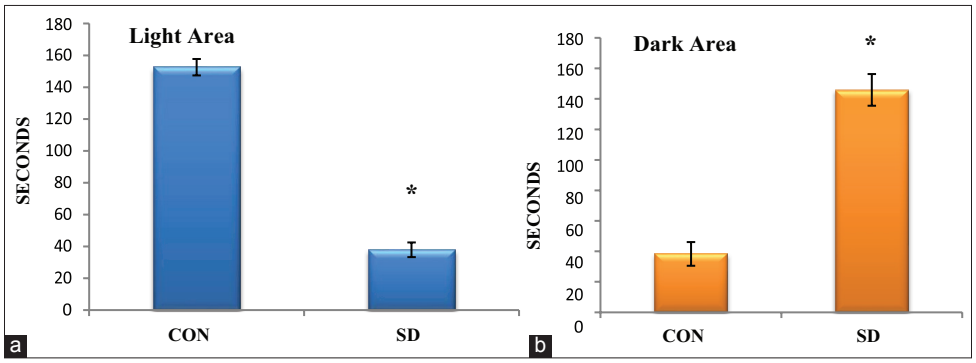

Fig. 6: Place preference task

have an impact on consolidation processes which are time- dependent process that converts recent experience of lasting memory.

Rodent experiments have shown that REM and learning may be related. Thus, REM increased after mice learned a shock avoidance task. REM SD blocked cortical mass increases resulting from enriched environments and impaired learning of spatial memories from the Morris water maze [20] and the RAM task results implicate REM sleep in learning and memory.

Selective deprivation of paradoxical sleep (PS) after learning results in memory deficits in a variety of tasks. The present experiment was designed to examine the effects of PSD on spatial working and reference memory.

Studies in rats have shown that the PS is necessary for consolidating particular memories and is confined to distinct post-training periods which are termed as PS Windows. It was recently demonstrated for spatial memory in the Morris water maze [30]. In the present study, we sought to extend these findings by comparing the effect of PSD on spatial memory in a different task the RAM. Using this apparatus allowed us to examine simultaneously the consequences of PSD on spatial working and spatial reference memory. The results of the RAM for working memory error, reference memory error, and time taken to complete the task show significantly increased in the SD group when compared to the control group.

Sleep loss was associated with memory impairment in both human and animal studies. The previous studies have shown that acute SD for 24–72 h impairs hippocampus-dependent short- and long-term memory [31]. Even a recent study showed that 3 h/day for 14 days of SD also resulted in memory impairment. The present results also reveal that SD of 48 h in female rats also impairs spatial memory.

In animals, uncontrollable stress has been shown to induce changes in a wide range of behavior parameters, including decreases in general locomotor activity and explorative behavior, impairment of feeding and drinking, and sexual behaviors. Studying such behavioral changes and the underlying physiological mechanisms may be useful to obtain more insight into human psychiatric disorders.

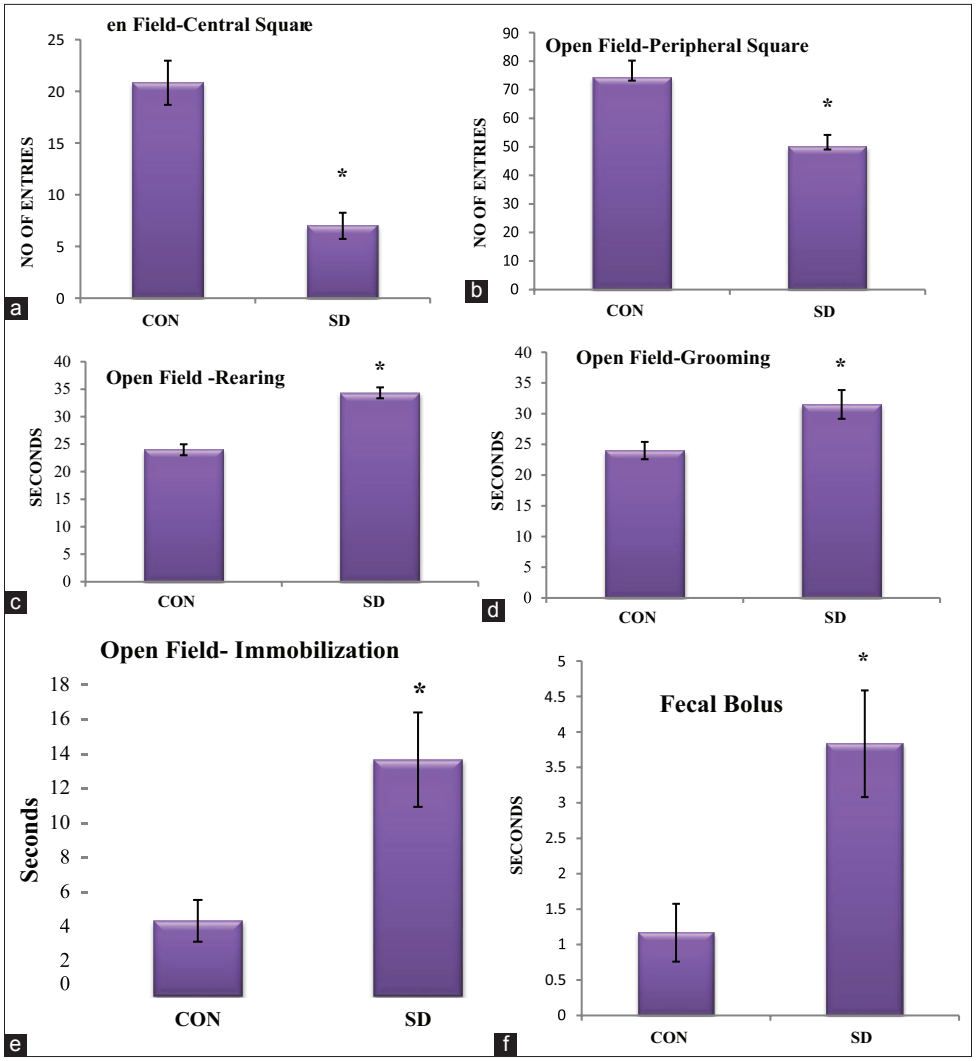

Fig. 7: Open field test

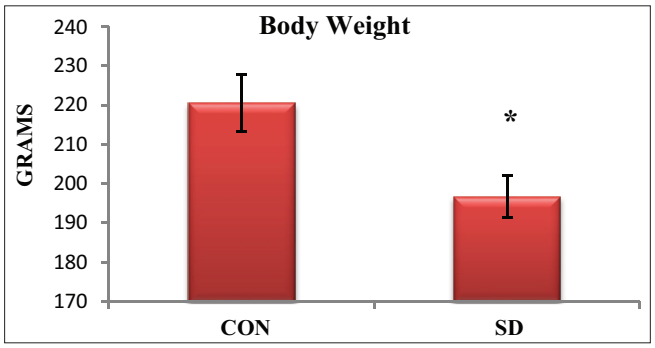

Fig. 8: Body weight

Locomotion in an open field is considered to reflect general or exploratory activity, whereas decrease in the overall mobility and ambulation is considered to represent increased anxiety in the animals. In the present experiment, the consequences of PSD on open-field behavior in female rats were studied, the results show that the PSD group exhibited a marked increase in immobilization and fecal bolus, rearing and grooming with the decrease in ambulation of central and peripheral square entries when compared to the control group.

Fecal pellets are commonly used as a sign of fear since emotional subjects are likely to defecate in stressful situations, resulting from the emotional-induced parasympathetic activity and high ambulation together with low defecation is commonly interpreted as low emotionality and exploratory behavior which is seen in the PSD group.

The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors. That is novel environment and light. A natural conflict situation occurs when an animal is exposed to an unfamiliar environment or novel objects [32]. Our results show that 48 h SD increases anxiety-like behavior of rats assessed via light–dark behavior test. In light and dark behavior, the SD group spent more time in dark area and less time in brighter area, whereas the control group spent more time in brighter area rather than the dark area. Latency for entering the dark area was more in the control group. Display of increased anxiety-like behavior is indicated by reduced time spent in the lit area of the light and dark box this indicates that SD animals exhibit anxiety-like behavior.

CONCLUSION

Over the past few decades, a remarkable explosion of research has allowed us to construct a much more complete picture of the genetic, cellular, neurophysiological, and behavioral changes that are affected by SD. It seems to disrupt vital biological processes necessary for cognitive function and physical health, yet the ways in which the body is compromised are not fully understood. There is a pressure in modern society to carry out an increasing variety and number of activities

during wakefulness. This trend toward SD and irregular sleep-wake patterns with resultant impaired concentration and memory reduces the quality of life and the ability to enjoy and complete activities. This study also reveals that PSD alters the memory function and locomotor activity in female rats. The balance may need to swing back toward awareness that adequate and regular sleep is required to promote a state of well-being during wakefulness. Once aware of this relationship, it becomes the responsibility of each individual to select his/her own combination of sleep and wakefulness by prioritizing the opportunities that present themselves every day.

AUTHORS' CONTRIBUTIONS

First author – Keerthi priya C.S – experiments and manuscript preparation and formulation of experimental design were done. Second author – Dr. Malathi.S – manuscript edition and formulation of experimental design were done. Corresponding author and second author – Ravindran Rajan – formulation of experimental design and approved.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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