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Total Sleep Deprivation Modulates Working Memory and Depression in Aged Rats

Cristina Dumitru
Department of Educational Sciences, Faculty of Educational Sciences, Social Sciences and Psychology, The National University of Science and Technology POLITEHNICA Bucharest, Pitești. University Centre, Targul din Vale, 1, Pitesti, Romania.
cristina.dumitru81@upb.ro
https://orcid.org/0000-0002-2130-6661

Florin Zamfirache
Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Splaiul Independentei 91-95, Bucharest, Romania.
zamfirache.florin@s.bio.unibuc.ro

Adina Trimbitas
Department of Educational Sciences, Faculty of Educational Sciences, Social Sciences and Psychology, The National University of Science and Technology POLITEHNICA Bucharest, Pitești University Centre, Targul din Vale, 1, Pitesti, Romania.
adina.duta_429@student.upit.ro

Beatrice Mihaela Radu
Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Splaiul Independentei 91-95, Bucharest, Romania.
beatrice.radu@bio.unibuc.ro

Abstract: *Sleep deprivation has shown promise in rapidly alleviating depression symptoms without adverse effects, its impact on memory remains unclear, especially in depressed individuals. The study investigates the effects of total sleep deprivation on depression symptomatology and memory in aged rats with induced depression. The experiments were carried out on 8 adult male Sprague Dawley rats. A depression model was induced using the chronic retention stress paradigm. Total Sleep Deprivation was achieved using the "gentle handling" procedure. Behavioral tests, including the open field test, Y-maze, and novel object recognition test, were conducted to assess locomotor activity, spatial working memory, object recognition memory and depressive symptomatology. Sleep deprivation resulted in an initial increase in locomotor activity and exploration behaviors across different experimental setups. This heightened activity tended to normalize over time, suggesting a transient response to the lack of sleep. However, the total time spent in these zones was not significantly different from controls, suggesting that the fundamental engagement with the environment might remain intact despite altered entry behaviors. This suggests that the cognitive components of these behaviors may be resilient to short-term sleep loss. Overall, these findings suggest that the role of sleep is facilitating rather than essential in memory.*

Keywords: *depression; induced-depression rat model; memory; sleep deprivation; working memory.*

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1. Introduction

Mental disorders impose a significant economic and social burden on society due to their widespread prevalence, early onset, and tendency towards chronicity. Among these, depressive spectrum disorders represent a prominent subgroup with a prevalence of 16 % (Kessler et al., 2003). The global point prevalence of elevated self-reported depressive symptoms between 2001 and 2020 was 34%, highlighting the widespread nature of depression and its impact on public health (Shorey et al., 2022). The efficacy of typical antidepressants currently ranges between 65-80%, alongside the significant concern regarding adverse medication effects (Avrutsky & Neduva, 1988). More recent studies (Rush et al., 2004) show that less than 50% of depressed patients respond to the first antidepressant treatment and only 30% achieve full remission. As an alternative to pharmacological intervention, one may consider the empirically identified method from the 1960s for treating depression through total or partial sleep deprivation (SD) (Caliyurt & Guducu, 2005; Giedke & Schwäzler, 2002). Unlike the effect of medications, SD rapidly improves the condition of patients with depression, sometimes proving effective even in medication-resistant cases, and additionally, it does not provoke adverse effects (Vein & Airapetov, 1986). Furthermore, SD improves depression symptomatology by inducing the activation of serotonergic neurons due to prolonged wakefulness, leading to serotonergic adaptive processes (Adrien, 2002). However, despite being verified in numerous clinical conditions, SD is still inadequately studied experimentally. Decades of research have revealed inconsistent effects of SD on depression, with a focus on the duration of SD (Vaseghi et al., 2023). However, the role of SD in depression appears to be more complex than just its duration. Several other important factors are implicated in the effects of SD on cognitive functions and mood, including neurotrophic factors like brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), serotonin, cortisol, and tumor necrosis factor-alpha (TNF-α) (L. Neto et al., 2011). Vaseghi et al. (2023) emphasise the necessity of studying the impact and intricate role of SD in modulating depression.

The complex and mysterious effects of SD on animals have raised several questions, emphasizing the necessity of comprehending how SD, often recommended for individuals with depression, will impact memory. A large amount of evidence suggests that sleep plays a key role in memory consolidation (Alzoubi et al., 2016; Diekelmann & Born, 2010; Lu et al., 2023; Peigneux et al., 2001; Rajizadeh et al., 2018; Stickgold et al., 2001). The effects of SD on memory have been extensively explored, especially using rodents (Colavito et al., 2013; Gais & Born, 2004). SD produces a memory deficit (Gais & Born, 2004; Peigneux et al., 2001). Studies investigating shorter-term SD (<24 hours) have yielded conflicting results, with some showing no effect (Harris et al., 1982; Lyamin et al., 2023) while others indicating improved memory retrieval (Azogu et al., 2015; Takatsu-Coleman et al., 2013) in passive avoidance tasks. Other results show that SD improves memory in old mice, not in young ones (Yuan et al., 2021). One research gap that emerges from the existing literature is the lack of understanding regarding the specific mechanisms of how SD affects memory in rats with induced depression. While previous studies have extensively explored the effects of SD on memory, particularly in rodents, there remains a need to delve deeper into how SD affects memory processes in the context of depression.

This study aims to address this gap by exploring the impact of SD on memory in rats with induced depression. To better understand how lack of sleep affects memory in rats, and to further help understand the impact on individuals with depression, this study aims to answer the following questions:

Research Questions:

- *RQ1*: What are the effects of SD on depression symptomatology in aged rats with induced depression? What effects does SD have on depression symptoms in aged rats with experimentally induced depression?

- *RQ2:* Does SD impair working memory and novel object recognition in aged rats with induced depression, in line with previous findings that link SD to cognitive deficits?

Therefore, we are interested in investigating the effects of SD on depression symptomatology, as well as the potential negative impacts of SD on memory in rats with induced depression.

The following *hypotheses* were formulated:

- *H1:* Changes in working memory and novel object recognition would be observed in aged rats with induced depression from the experimental group (EG) after short periods of total SD.
- *H2:* Short periods of total SD in aged rats with induced depression would affect the manifestation of depression symptoms.

2.1. Ethics statement

Experimental procedures were performed following the University of Bucharest (Romania) Health Guide for the Care and Use of Laboratory Animals, based on “NIH Guide for the Care and Use of Laboratory Animals”. They were approved by the University Bucharest Ethical Committee (Approval No: 14/28.04.2021).

2.2. Experimental animals

The experiments were carried out on 8 adult male Sprague Dawley rats (~500 g, 1.5 years of age) obtained from the "Cantacuzino" National Medical-Military Development Research Institute, Bucharest, Romania. For this study, the number of subjects was minimised to eight to reduce potential pain, distress, and discomfort experienced by the animals during the experimental procedures.

The rationale for the sample selection in our study was driven by the need to address a notable gap in the literature concerning the impact of SD and chronic stress on aged rats, particularly those with depressive-like behavior. To the best of our knowledge, studies focusing on these factors in aged rats are limited, which prompted us to select this specific population to contribute new insights into the interaction between aging, SD, and cognitive decline. Aging is associated with significant changes in sleep architecture and an increased risk of cognitive impairments, making it a relevant focus for understanding the effects of SD. Additionally, depression is more prevalent in older individuals, further supporting the relevance of our chosen model. Female rats were excluded due to the potential confounding effects of hormonal fluctuations related to the estrous cycle, which could have impacted our findings, especially considering our already limited sample size (n=8, with four per group). The number of subjects was minimised in accordance with ethical guidelines to reduce potential pain and distress, further limiting the inclusion of additional variables. Therefore, the selection of aged male rats was necessary to maintain consistency and address key research gaps in this field.

Limiting the number of rats not only aligns with ethical considerations but also allows for better management and monitoring of each animal's well-being. Upon their arrival, a depression model was induced using the chronic retention stress paradigm (Mao et al., 2022; Pucilowski et al., 1993). The chronic stress model was carried out within eight weeks, and the preference for sucrose solution test was used to assess depressive-like behavior (Pucilowski et al., 1993). This model, extensively documented in previous literature, is recognised for its effectiveness in inducing depression (Willner, 2005). It has been validated rigorously (Henningsen et al., 2009; Willner et al., 2019), and is known to elicit phenotypic changes over time, encompassing both behavioral and physiological alterations. Notably, this stress model has been associated with hippocampal CA3 pyramidal cell atrophy (Havekes et al., 2016; Tang et al., 2019) and increased corticosteroid levels (Ayensu et al., 1995; Longordo et al., 2009; Mizoguchi et al., 2001). To induce the depression

model, rats were subjected to restraint for two hours daily over two weeks (Wang et al., 2017). This can be compared with the chronic stress experienced by humans in demanding work environments or challenging life circumstances.

2.3. Total sleep deprivation protocol

Total Sleep Deprivation (TSD) was achieved using the well-established "gentle handling" procedure (Franken et al., 1991; Longordo et al., 2011), involving "a direct interaction with the experimenter, who actively keeps the animal awake, and is by far the most popular method of total SD in the rodent" (Colavito et al., 2013) being a popular procedure for total SD in rodents. The procedure is designed to reduce the negative effects of stress and forced movement caused by other methods (Colavito et al., 2013). Highly regarded for its ability to minimise excessive stress, forced locomotor activity, or discomfort, this method requires the continuous presence of the experimenter throughout the entire SD period. The technique involves gently stimulating the animals to prevent them from falling asleep. Such interaction may include activities like lightly stroking with a brush, tapping the cage, softly touching the animal, or introducing novel objects for exploration. Gentle handling technique proves highly effective in significantly inhibiting almost all sleep patterns in both rats and mice (Colavito et al., 2013), however, we have to consider the possibility of microsleep occurrences (Colavito et al., 2013).

Before behavioral tests, rats were acclimated to the experimenter for 5 minutes per day over 5 days. TSD commenced around 9:00 a.m. and lasted for 6 hours, repeated over 3 consecutive days. For our experiments, rats were easily kept awake by gentle or mild stroking with a soft brush, when the SD was 6 hours, and direct handling for the 24-hour SD procedure. Based on researchers' recommendations (Colavito et al., 2013), the number of subjects exposed to SD was five, to adjust more effectively the level of stimulation to match each animal's current alertness level, thereby minimizing variability and stress. This approach ensures that the animals receive optimal care and attention throughout the experimental period while also adhering to ethical guidelines for animal research.

2.4. Behavioral tests

The experiments were carried out on 8 rats with induced depression. Two experimental groups were designated randomly (n = 4 per group): control (CG) and sleep deprivation (SD) group. All subjects (n=8) were individually housed in a temperature- and humidity-controlled room (23±1 °C), with ad libitum access to food and water. The animals were kept on a 12-hour light/dark schedule, with lights on at 8 a.m. Each testing and SD session was performed in the same time frame to avoid effects associated with the circadian rhythm. Manipulations with animals were carried out in accordance with ethical standards in Bucharest (14/28.04.2021).

The first experiment consisted of two conditions: (1) a total sleep deprivation (SD) schedule involving a single 12-hour session, resulting in a total of 24 hours without sleep (12 hours of wakefulness followed by 12 hours of total SD), and (2) a total SD schedule consisting of three sessions of 6 hours each, implemented over three consecutive days. Behavioral tests were performed between 9:00 a.m. and 12:00 a.m. (i.e., in the first half of the rest phase when sleep pressure is typically high). The overall experimental design of this study is presented in Fig. 1.

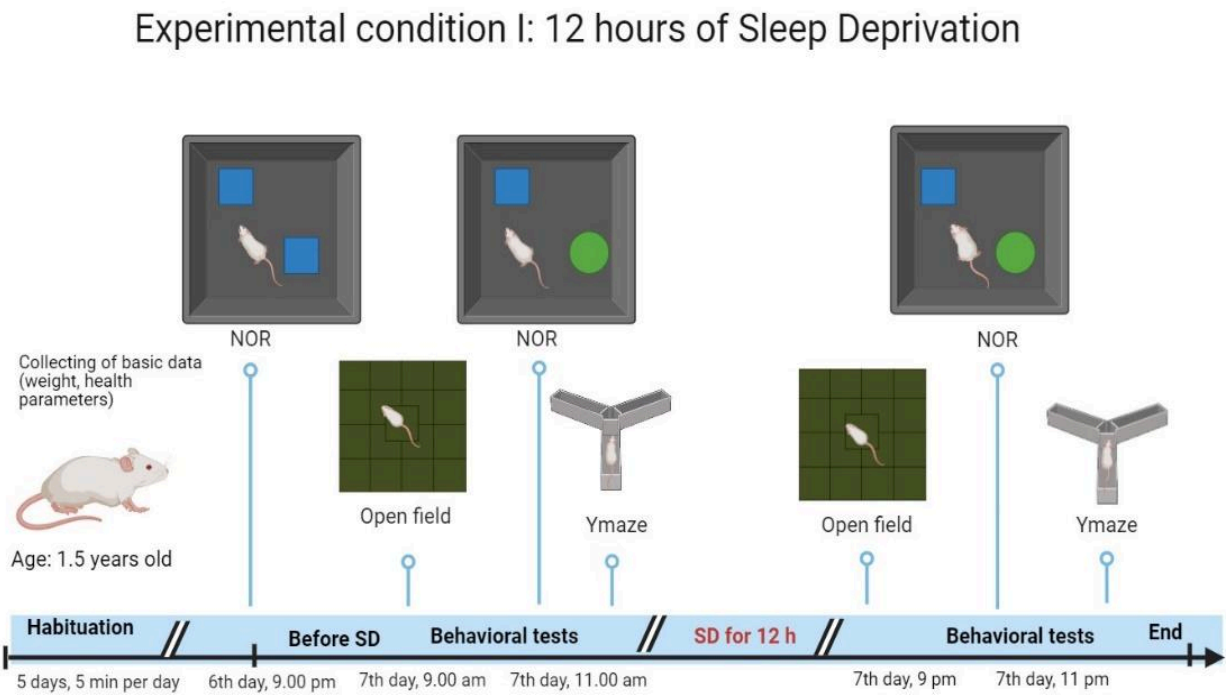


Figure 1. Summary of the experimental methodology used in the current study

2.4.1. Open field test

The open field test was used to assess depression symptomatology and evaluate the locomotor activities of our sample. The open field arena consisted of a high-walled chamber (60 × 60 × 50 cm^3). The same procedure was consistently applied across all tests before and after the SD condition, all rats were placed in the center of the chamber and allowed to move freely for 5 min. After each session, the arena was cleaned with 5% alcohol. Behavior was recorded using a camera fixed on a tripod; indexes, such as total distance, latency, and number of entries in central and peripheral zones were recorded, through an automated behavioral analysis tracking system and software ANY-maze (Dublin, Ireland).

2.4.2. Y maze

To assess spatial working memory, we used a Y-maze, three arms arranged in a Y-shape apparatus, where each arm represents a different choice or direction. Each rat was placed at the center of the Y-maze and allowed to freely explore and navigate the arms. The spontaneous alternation behavior, where the animal alternates between visiting different arms of the maze (Kraeuter et al., 2019). We used it to observe the rat's ability to remember and navigate spatial information.

2.4.3. NOR

For object recognition memory we used the novel object recognition (NOR) test, where we tested the rats' spontaneous preference for novelty (Colavito et al., 2013). The rats were exposed to a familiar and novel (new) object. The test relies on the animals' natural inclination to prefer exploring novel objects over familiar ones. The amount of time the animal spends exploring the new object compared to the familiar one(s) reflects its recognition of the objects it has previously encountered, showing their ability to recognise and remember the novel object thus allowing us to assess their object recognition memory. The NOR test provides valuable insights into cognitive processes related to memory and recognition in animal models (Broadbent et al., 2010).

2.5. Statistical analysis

Statistical analysis was performed using an independent non-parametric Mann-Whitney U test, $p < 0.05$ was considered statistically significant. All the data were shown as the mean ± SEM. We have selected Mann-Whitney U to assess the relationship between the variables in our study, with non-normally distributed data and a limited sample size.

3. Results

To investigate whether SD affects depression symptomatology and memory in male rats, we designed two experimental conditions with 12h TSD, and consequently 6h TSD for three consecutive days. We used two behavioral tests: the NOR, for object recognition memory, and the Y maze, for working memory. Our results showed that SD condition had no effects on working memory and object recognition in rats with induced depression and on depression symptomatology tested with Open field behavioral test. However, SD increased irritability, responsiveness, and aggressive behavior of rats in response to environmental stimuli.

3.1. Experiment 1: effects of 12 hours SD on depressive symptomatology

Before and after the experimental condition, the exploration behavior was tested using the Open Field test. Analysis of the exploration time of the central zone revealed no significant effect of 12 hours of SD.

No significant difference was observed in average speed, the frequency of entries, and the duration spent in the peripheral zone, as well as the frequency of entries and time spent in the central zone, measured for both groups (see Supplementary material).

However, the Mann-Whitney U test showed a statistically significant difference between the groups ($p=0.043$) regarding the average distance traveled after SD, with a higher distance traveled by the SD G (Fig.2). Cohen's d effect size metric of 1.67 indicates that the group means differ by 1.67 standard deviations. Also, for the time spent in the central zone at T1, T2, and T3 we ran Friedman Test statistics (similar to ANOVA for repeated measures, but for nonparametric data, due to our small sample size), but the results indicated a non-significant value ($p=0.24$). Therefore, SD did not affect depression symptomatology in old rats with induced depression.

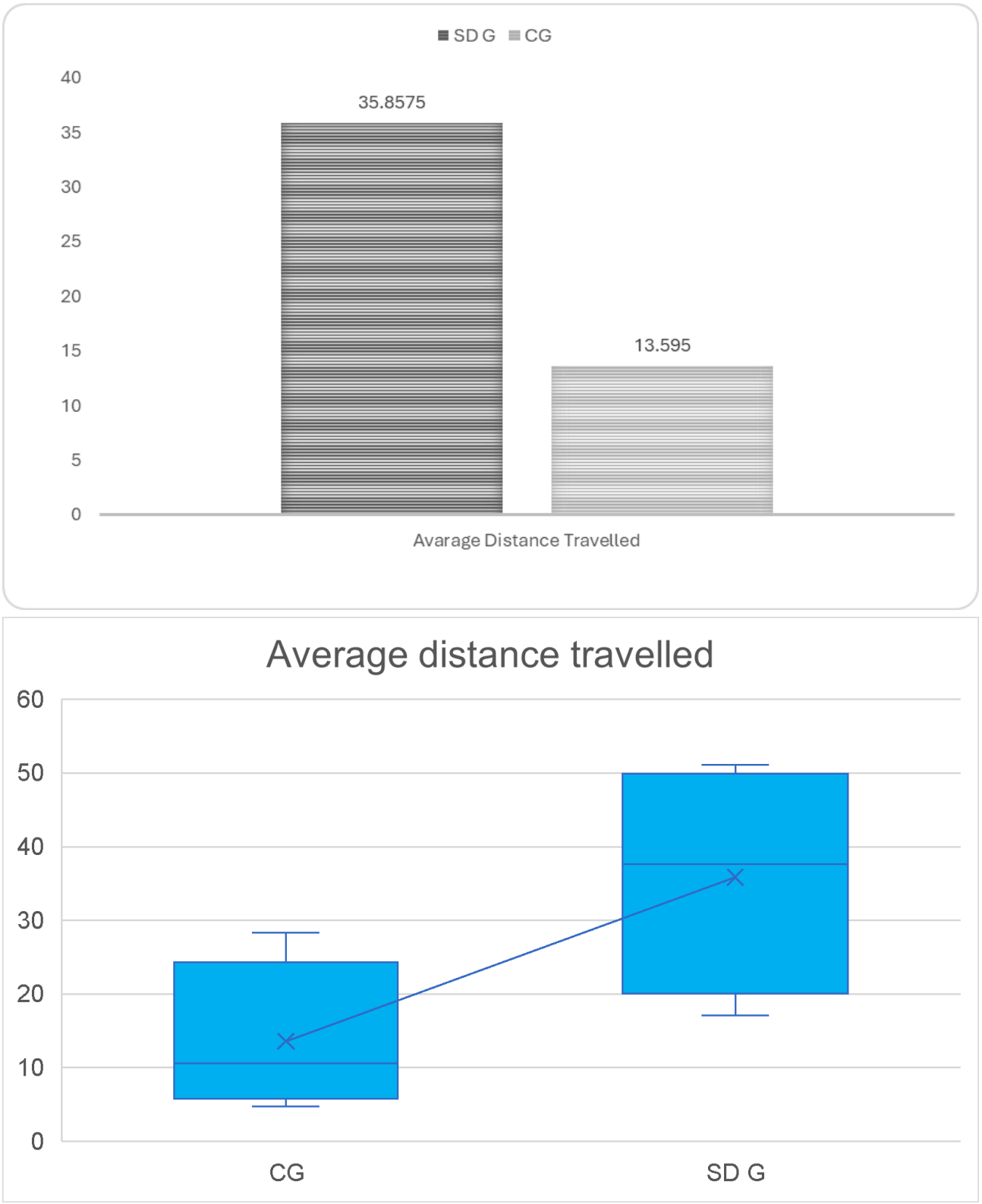


Figure 2. Experiment 1: Average distance traveled after SD (T3) for SD G and CG

3.2. Experiment 2: effects of 12 hours SD on spatial working memory and on object recognition memory

During the testing phase after the SD, the locomotor behavior of sleep-deprived animals was reduced. For the statistical analysis of the data, the Mann-Whitney U test showed that the overall time spent investigating both objects found no significant group effect (p=0.56), indicating that the control group and 12 h TSD groups showed equivalent levels of exploring both objects.

Table 1 presents the results of 12 h of SD on exploring all three arms of the Y maze experiment. During the testing phase after the SD, the locomotor behavior of sleep-deprived animals was reduced. For the statistical analysis of the data, the Mann-Whitney U test showed that the time variation coefficient found no significant group effect (p= 0.14) indicating that the control group and 12 h TSD groups showed similar levels of arms exploration.

Table 1. Behavioral Characteristics of Animals in Y maze

		CG	SD G
Total distance traveled (cm)	T1	11.94 (4.43)	26.46 (8.51)
	T2	12.65 (7.72)	23.98 (6.73)
Average speed (cm/s)	T1	0.04 (0.02)	0.07 (0.04)
	T2	0.04 (0.02)	0.06 (0.03)
A, B, C entries variation coefficient	T1	0.47 (0.29)	0.44 (0.06)
	T2	0.59 (0.20)	0.36 (0.20)
Time spent A, B, C arms (variation coefficient)	T1	0.58 (0.08)	0.49 (0.23)
	T2	0.83 (0.45)	0.54 (0.22)

*Note: The data presented show the results of CG (n=4) and SD G (n=5) for various characteristics related to the behavior of subjects in Y maze.

3.3. Experiment 3: effects of short SD on depressive symptomatology

We considered using short periods of TSD (6 hours/per day/during three consecutive days) because most human pathological conditions do not involve continuous SD throughout the day. Instead, individuals often experience SD intermittently during the night and are unable to fully compensate for it on subsequent days due to social obligation (Machado et al., 2006). However, the results were similar to the 12h condition, with no statistically significant differences between the two groups (see table 2)

Table 2. Effects of short SD on depressive symptomatology

		CG	SD G
Total distance traveled (cm)	T1	14.34 (7.72)	42.96 (12.30)
	T2	30.98 (7.79)	38.96 (15.54)
	T3	11.88 (2.57)	27.11 (16.52)
	T4	8.69 (6.59)	14.90 (9.41)
Average speed (cm/s)	T1	0.05 (0.25)	0.14 (0.04)
	T2	0.10 (0.26)	0.13 (0.05)
	T3	0.04 (0.01)	0.09 (0.05)
	T4	0.03 (0.02)	0.05 (0.03)
Number of entries Peripheral Zone (#)	T1	2.00 (1.54)	10.00 (5.77)
	T2	2.50 (1.73)	9.25 (3.09)
	T3	1.25 (0.5)	7.50 (9.94)
	T4	1.50 (0.57)	3.25 (3.30)
Time spent Peripheral Zone (s)	T1	288.88 (9.52)	291.58 (2.96)
	T2	285.39 (16.62)	294.13 (3.94)
	T3	284.15 (21.99)	294.40 (1.28)
	T4	292.30 (6.34)	293.78 (6.97)
Number of entries Central Zone (#)	T1	5.63 (5.70)	1.75 (1.5)
	T2	3.75 (3.95)	1.75 (1.70)

	T3	3.63 (7.61)	0.50 (0.57)
	T4	2.00 (2.61)	1.00 (0.81)
Time spent Central Zone (s)	T1	7.44 (10.50)	3.08 (3.35)
	T2	10.93 (15.73)	1.63 (1.19)
	T3	11.04 (23.05)	1.68 (2.08)
	T4	4.74 (6.80)	4.40 (7.94)

**Note: The data presented show the results of CG (n=4) and SD G (n=5) for various characteristics related to the behavior of subjects in an Open Field task.*

There was no significant difference between the two groups in terms of distance traveled, average speed, time spent in the peripheral zone, or the number of entries and time spent in the central zone (Table 2). According to the Mann-Whitney U test, the only significant difference between groups (p=0.03) was shown for the number of entries in the peripheral zone after the first short period of TSD (Figure 3).

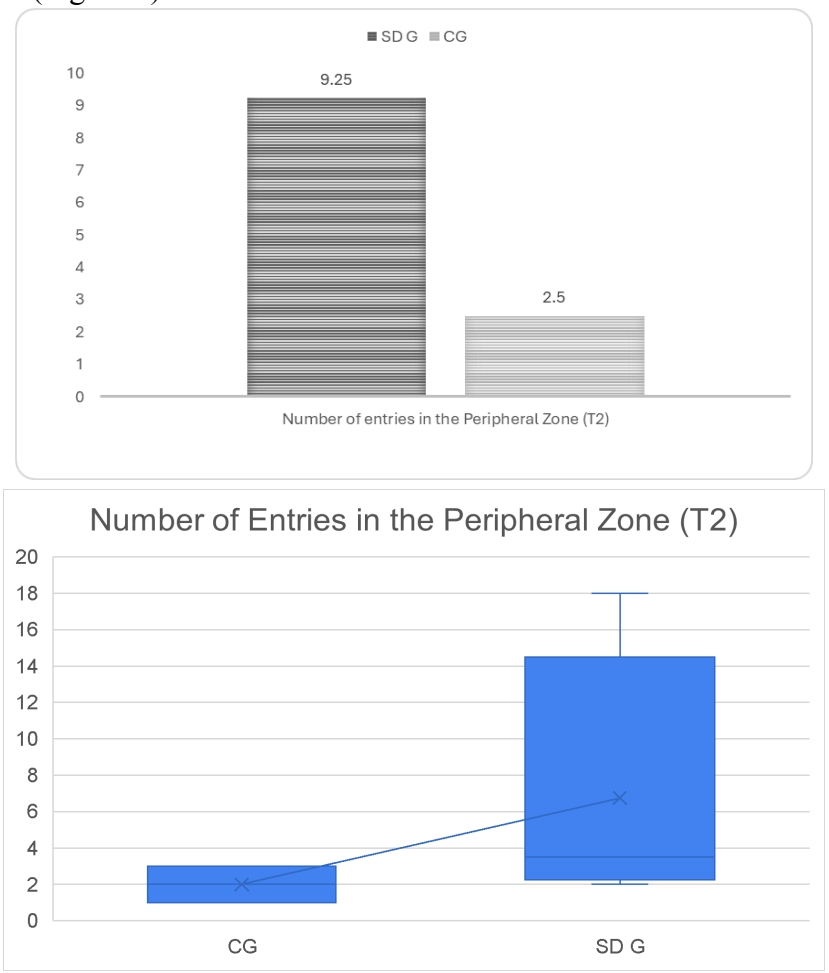


Figure 3. Experiment 2: Average number of entries Peripheral Zone for CG and SD G in T2

In T2, the subjects in the EG group showed a significant increase in both average speed (p=0.02) and motor activity (p=0.02). For the statistical analysis of the data, the Mann-Whitney U test showed no significant differences between groups for all four times considering the time spent in the central zone (p=0.24, p=0.08, p=0.56, and p=0.66). Therefore, SD did not affect depression symptomatology in old rats with induced depression. According to the Mann-Whitney U test, the only significant difference between groups (p=0.03) was shown for the number of entries in the

peripheral zone after the first short period of TSD. Cohen's d effect size metric of 1.69 indicates that the group means differ by 1.69 standard deviations.

Short-term effects of SD appear to increase exploratory behavior or reduce anxiety, as indicated by increased distance traveled, average speed, and entries into the central zone immediately following SD (T1). However, these effects seem to diminish over time (T2 and T3). In the long term, the behavior of sleep-deprived animals appears to normalise somewhat towards that of the control group, with reduced exploratory activity and central zone entries by T3. The exploration behavior in the central zone, which might be indicative of anxiety-like behavior or willingness to explore open, potentially dangerous areas, initially increases in sleep-deprived animals but reduces over time, suggesting a temporary change in risk assessment or anxiety due to SD. While there are differences observed, the analysis mentioned in the prompt indicates that these were not statistically significant, suggesting that the variations might not be reliably attributable to SD alone, and could be within the range of normal variability. SD leads to an initial increase in locomotor activity and exploration, particularly in familiar areas, which could be due to a transient hyperactivity or stress response following the deprivation period. Over time, sleep-deprived animals show a tendency to spend more time in familiar zones, possibly indicating a shift towards safety-seeking behavior or a decrease in the motivation to explore new stimuli. The engagement with new stimuli is minimal for both groups, which might suggest that the task design or the animals' condition (including the control group) limits novel object exploration. By T3, sleep-deprived animals show a notable decrease in overall activity, including time spent in the open arena, which could be attributed to accumulating fatigue or reduced exploration drive as the effects of SD persist or worsen.

3.4. Experiment 4: effects of short SD on spatial working memory

Table 3 presents the results of a short SD on exploring all three arms of the Y maze experiment. During the testing phase after the SD, the locomotor behavior of sleep-deprived animals was reduced. For the statistical analysis of the data, the Mann-Whitney U test showed that the time variation coefficient found no significant group effect indicating that the control group and 12 h TSD groups showed similar levels of arms exploration.

Table 3. Effects of short SD on spatial working memory (Y maze)

		CG	**SD G**
Total distance traveled (cm)	T1	11.94 (4.43)	25.06 (9.13)
	T2	15.79 (4.11)	23.40 (6.73)
	T3	12.65 (7.34)	15.11 (5.67)
	T4	15.05 (5.59)	14.88 (3.47)
Average speed (cm/s)	T1	0.06 (0.03)	0.04 (0.01)
	T2	0.07 (0.02)	0.05 (0.01)
	T3	0.05 (0.02)	0.04 (0.02)
	T4	0.05 (0.01)	0.05 (0.01)
A, B, C entries variation coefficient	T1	0.48 (0.33)	0.44 (0.07)
	T2	0.59 (0.22)	0.36 (0.23)
Time spent on A, B, C arms (variation coefficient)	T1	0.59 (0.22)	0.36 (0.23)
	T2	0.58 (0.10)	0.49 (0.26)

**Note: The data presented show the results of CG (n=4) and SD G (n=5) for various characteristics related to the behavior of subjects in Y maze.*

Short-term SD may initially alter locomotors and exploration behaviors in a spatial memory task, these effects are not sustained, and there is no significant long-term impact on the uniformity of exploration across different spatial areas within the Y maze.

Despite these observations, the Mann-Whitney U test results indicate no significant difference in the total amount of time spent examining both objects between the groups, suggesting that while SD may alter locomotor and exploration behaviors, it does not significantly impact the overall engagement with or memory for novel objects in this context. These findings should be interpreted with caution, as the limited sample size may affect the generalizability and statistical strength of the results.

4. Discussion

The present study aimed to explore the effect of SD on memory in rats with induced depression and on depression symptomatology. Aligned with our hypotheses, we found that short periods of SD (12 h or 6 h for 3 consecutive days) showed no effect on depression symptomatology, working memory, and novel object recognition in aged rats with induced depression. To our knowledge, few studies have previously examined how SD impacts memory in older rodents with induced depression. In our two experiments, we tested object recognition memory and working memory. Studies have shown that rodents' memory performance declines with SD (Alzoubi et al., 2016; Chen et al., 2023), but determining the specific effects remains challenging. It's unclear if sleep loss affects learning or memory consolidation. Some studies conduct training before SD, followed by testing to understand memory processes. Others explore learning capacity by training after SD.

SD leads to noticeable changes in behavior, such as increased initial activity and altered exploration patterns. However, these changes do not appear to significantly impact the overall capacity for spatial exploration or engagement with the environment, as evidenced by the lack of statistically significant differences in key measures such as the time variation coefficient in the Y maze and the exploration of objects in the novel object recognition task. These findings suggest that while SD alters certain aspects of behavior, animals can maintain core functional behaviors in exploratory and memory tasks.

Rodent memory assessment paradigms

Before discussing our results, it is important to understand how to assess memory in rodents. The operational definition of memory in rodents involves categorizing memory into three stages: acquisition, consolidation, and retrieval (Bimonte-Nelson, 2015). For memory assessment in rodents, researchers use mazes, such as the Moris Water Maze (Morris, 1984), the radial arm maze, the T or Y maze, and Novel Object Recognition (Konakanchi et al., 2022; Morellini, 2013). Reviews and meta-analysis show a prevalence of the Morris Water Maze in assessing memory in sleep-deprived rodents (Diao et al., 2023). The discussion of our findings will consider also the studies that used similar protocols for the SD paradigm, the behavioral tests used for memory assessment, and the phases of memory testing, acquisition, consolidation, or retrieval.

Sleep Deprivation Effects on Object Recognition

Experimental studies showed impairments in cognitive function, measured as reduced latency in performing several tasks, in several paradigms such as Morris Water Maze (Pei et al., 2021; Rajizadeh et al., 2018; Sur & Lee, 2022), Radial Arm Water Maze (Alzoubi et al., 2018, 2020; Massadeh et al., 2022), T maze (Xie et al., 2015), 8-Arm RAM task (Sur & Lee, 2022), Hebb Williams Maze (Konakanchi et al., 2022) and passive and active avoidance or Multiple Trial Inhibitory Avoidance Task (Ota et al., 2013; Rajizadeh et al., 2018). Therefore, a great body of research confirms the important correlation between sleep and cognitive functions, from the SD paradigm approach. Several animal studies also showed that sleep disorders impair hippocampus-dependent memory formation (Chen et al., 2023; Mahboubi et al., 2019), memory consolidation (Shi et al., 2011), emotional memory (Kumar & Jha, 2012), fear memory (Kumar & Jha, 2012) and working memory (Xie et al., 2015), as well as increase anxiety levels (Konakanchi et

al., 2022; Vollert et al., 2011). Currently, researchers have mainly studied the effects of short-term and long-term SD separately. This makes it hard to compare how behavior and brain chemistry change based on how long SD lasts. It's tricky because different studies use different types of rodents and ways to keep them from sleeping. However, the lack of consistency in the methodology used to induce SD, which might produce changes in sleep architecture, including the use of an appropriate control condition, also produces a challenge in summarizing findings across studies. In addition, other factors could interfere with the outcomes received. The absence of movement resulting from the single platform method employed in behavioral testing could serve as a potential confounding factor. Rajizadeh et al. (2018) noted that other variables should be considered when assessing memory outcomes, such as the use of active or passive coping strategies in knockout and mutant rodents. Moreover, disruption of circadian rhythms complicates the interpretation of any resulting cognitive deficits (Colavito et al., 2013).

Sleep Deprivation Effects on Spatial Working Memory

We found no effect of SD on the spatial working memory in any of the present experiments, based on Y maze testing. The number of animals in each group was not optimal, with only 4 animals. However, the effect size statistics did not suggest that a lack of power resulted in an inability to see significant effects of SD. Our sample consisted of old rats with induced depression, therefore, the results should be discussed considering the sample characteristics. SD in aged animals causes memory impairments after 1 day of SD, but not after 30 days (Vecsey et al., 2015). Other studies show that 4 h SD immediately after the sample phase (early SD group) disrupted object-place recognition but not novel-object recognition memory (Ishikawa et al., 2014). Other studies align with our results and recent evidence suggests that REM sleep is not essential for spatial learning and memory. The studies on the effects of SD on memory acquisition show controversial results. Hunter (2019) found no evidence of memory impairment because of REM SD while testing memory with Morris Water Maze, which implies a learning phase. The lack of memory impairment was noted, regardless of when REM SD occurs or how training is distributed (Hunter, 2019).

It is important to note that a great number of experiments used the Moris Water Maze to assess spatial memory (Diao et al., 2023), while the use of the Y maze and NOR is not that present among reported results. Their systematic review of the effects of REM SD on memory consolidation offers a detailed understanding of how different memory types (e.g., procedural vs. declarative) are affected. Gao et al. (2020) used Ymaze to assess the impact of SD on rats' memory, reporting a statistically significant difference in the level of maze arms alternation ($p<0.001$ compared to the control group), however, locomotor activity showed no significant differences (Gao et al., 2020). Hunter (2019) considers that animals have differential responses. Experiments on fur seals showed that a 108-h TSD did not affect cognitive abilities (Lyamin et al., 2023).

Sleep Deprivation Effects on Depression Symptomatology

In our study, we intended to confront the effects of SD on depression symptomatology, which according to the existing literature yields positive outcomes (Caliyurt & Guducu, 2005; Giedke & Schwäzler, 2002; Vein & Airapetov, 1986), as well as the potential negative effects on memory in rats with induced depression, expressed as well in a great body of research mentioned above. However, our results showed no statistically significant difference between EG and CG on depression symptomatology and memory abilities in old rats with induced depression. Decades of research have shown the inconsistent effects of SD on depression, focusing on SD duration (Vaseghi et al., 2023). However, the inconsistent role of SD seems to be more complicated, and SD duration cannot be the only factor.

Our findings that SD did not significantly affect working memory, object recognition, or depression symptomatology in rats with induced depression may indeed suggest that SD has no measurable impact on these parameters under the conditions tested. However, given the small

sample size (n=8), it is also possible that the study was underpowered to detect subtle but potentially important effects. This limitation could have increased the risk of Type II errors, where true effects were not detected due to insufficient statistical power.

5. Conclusions and future directions

Sleep, an evolutionarily conserved behavior throughout the animal kingdom, is fundamental for healthy physiological functions (Vaseghi et al., 2023). Overall, these findings suggest that the role of sleep is facilitating rather than essential (Leenaars et al., 2013). SD resulted in an initial increase in locomotor activity and exploration behaviors across different experimental setups (Open Field, Novel Object Recognition, Y Maze). This heightened activity tended to normalise over time, suggesting a transient response to the lack of sleep. In some tasks, sleep-deprived animals showed increased entries into certain zones (e.g., peripheral zones in the Open Field task), which could indicate altered anxiety-like behaviors or risk assessment. However, the total time spent in these zones was not significantly different from controls, suggesting that the fundamental engagement with the environment might remain intact despite altered entry behaviors. Despite changes in activity levels and exploration patterns, SD did not significantly impair the animals' performance in spatial memory tasks (e.g., uniform exploration of arms in the Y Maze). This suggests that the cognitive components of these behaviors may be resilient to short-term sleep loss. The Open Field task designed to assess depressive-like symptomatology showed altered behaviors in sleep-deprived animals that could be interpreted as indicative of increased stress or depressive-like states. However, these findings were not definitive and warrant further investigation.

Future research could extend the observation period to explore the long-term effects of SD and the potential for behavioral recovery with rest. Integrating physiological, molecular, and neurobiological measures could provide deeper insights into the mechanisms underlying SD's effects on behavior and cognitive function. Expanding the research to include various animal models, species, and strains could enhance the generalizability of the findings and provide a more comprehensive understanding of SD's impact.

Other future research should incorporate neurobiological markers such as Brain-Derived Neurotrophic Factor (BDNF), serotonin, corticosterone, and inflammation markers to provide a more comprehensive understanding of the molecular and neurochemical processes underlying SD’s effects on cognitive functions, mood regulation, and stress responses.

6. Limitations

Learning and memory are complex processes and difficult to grasp and study. Several limitations have however to be kept in mind when assessing learning and memory functions in animals. First, several behavioral paradigms are available, with their specific advantages and disadvantages. In our case, the SD protocol implied the individual and the physical presence of the experimenter that could influence the behavioral performance. Moreover, the reliance on behavioral measures without corresponding physiological or neurobiological data limits the understanding of the underlying mechanisms driving the observed changes. Second, the number of participants in the experiment was limited, thereby diminishing the generalizability of the results obtained. An important limitation of this study is the small sample size, which may have affected our ability to detect significant effects. Future research with larger sample sizes is necessary to confirm these preliminary findings and to gain a clearer understanding of the potential interactions between SD, memory, and depressive-like behavior The limited number of subjects reduces the robustness of our statistical conclusions. Future studies with larger cohorts will be essential to validate these findings and ensure their reproducibility and broader applicability. Another limitation was the focus on short-term SD effects. The long-term consequences and the potential for recovery with subsequent sleep opportunities remain unclear.

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