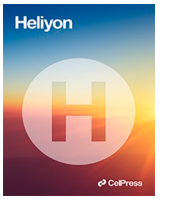
Heliyon 10 (2024) e34853

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Vitamin D injection into the dorsal-CA1 hippocampus improves short-term sleep deprivation induced cognitive impairment in male rats

Amir Rezagholizadeh [a], Amin Firoozi [b], Zohreh Tavassoli [a], Amir Shojaei [a], Narges Hosseinmardi [c], Javad Mirnajafi-Zadeh [a], Kristi Anne Kohlmeier [d], Yaghoub Fathollahi [a,*]

[a] Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, PO Box 14115-111, Tehran, Iran
[b] Department of Anatomy, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Department of Physiology, Medical School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

ARTICLE INFO

Keywords:
Hippocampus
Learning and memory
Sleep deprivation
Astrocytes
Glial metabolic inhibitor
Glial activation suppressor

ABSTRACT

This study was conducted with aim of investigating the consequences of sleep deprivation (SD) on cognitive functions. For this purpose, adult male rats were subjected to SD protocol for 5 h. The SD and the control rats were trained in the Morris water maze (MWM) to assess spatial behavioral deficits due to the SD protocol. To determine the role of astrocytes in spatial navigation deficits associated with SD, an inhibitor of astrocyte activation, fluorocitrate (FC), or a suppressor of astrocyte activation, vitamin D, was injected into the dorsal-CA1 hippocampus before subjecting rats to the SD protocol and the effects of these compounds on spatial navigation deficits associated with SD in the MWM were assessed. As expected, 5 h of SD impaired the Morris water navigation task in rats. FC injection into the dorsal-CA1 hippocampus before the SD protocol did not prevent the SD-induced cognitive deficits. Interestingly, injection of vitamin D into the dorsal-CA1 hippocampus prior to the SD protocol alleviated the SD-induced severe spatial navigation deficit in the MWM. Sequential injection of FC and vitamin D prior to the SD protocol did not reduce the SD-induced spatial memory impairment, suggesting a role for astrocytes. In sum, vitamin D can improve cognitive dysfunction associated with sleep deprivation, possibly dependent on astrocyte function. The results show that maintaining adequate levels of vitamin D offers a promising avenue to improve cognitive function in sleep-deprived conditions.

1. Introduction

Sleep deprivation (SD) due to insufficient sleep is a widespread issue in today's modern society and affects millions of people worldwide every day [1]. One important consequence of SD is its adverse effect on the brain, specifically when it comes to memory and learning processes that rely on the hippocampus. The hippocampus is an important part of the brain that is responsible for memory formation and consolidation. When sleep is disrupted or insufficient, the ability to learn and remember information that relies on the

* Corresponding author.
E-mail address: fatolahi@modares.ac.ir (Y. Fathollahi).

https://doi.org/10.1016/j.heliyon.2024.e34853
Received 20 March 2024; Received in revised form 29 June 2024; Accepted 17 July 2024
Available online 18 July 2024

hippocampus is negatively affected [2]. During prolonged periods of wakefulness, extracellular adenosine levels are increased in the forebrain, basal cortex, and hippocampus [3,4], raising the possibility that adenosine accumulation during SD may contribute to impaired learning and memory.

Adenosine is produced from the rapid conversion of ATP released from neurons and astrocytes in the tripartite synaptic space by enzymes called ectonucleotidases [5]. Adenosine can act on the adenosine A1 receptor (A1R) located in the presynaptic neuron, and the source of adenosine from ATP conversion has been shown to play an important role in transmission at excitatory synapses [6]. Inhibition of soluble N-ethylmaleimide-sensitive factor attached protein (SNARE)- related protein specifically in astrocytes, which is a protein involved in gliotransmitter release, leads to decreased extracellular adenosine associated with impaired synaptic transmission and plasticity in hippocampal brain slices [7]. It is expected that the effect of the increased level of adenosine from astrocytes on synaptic plasticity during prolonged wakefulness has a negative role in learning and memory processes. This is supported by findings that when gliotransmission was inhibited, mice were resistant to the effects of SD on recognition memory as assessed in the novel object recognition task, leading to the epilogue that ATP/adenosine derived from astrocytes during wakefulness causes cognitive impairment after brief periods of SD [8].

Because SD due to insufficient sleep is likely to remain a societal problem, it is important to explore strategies through which negative cognitive effects on learning and memory can be ameliorated. Targeting astrocyte activity could be one approach, and therefore, we wanted to study the effects of inhibiting astrocyte function and suppressing astrocyte activation on SD-affected learning and memory performance. In this study, the effects of fluorocitrate (FC), a reversible inhibitor of astrocyte metabolism, and vitamin D on the cognitive behavior of sleep-deprived adult male rats were investigated. FC is preferentially taken up by astrocytes rather than neurons, possibly due to its specific uptake mechanism in astrocytic cells. Studies have shown that within a certain concentration range and duration of exposure, FC only damages astrocytes [9–12]. In astrocytes, FC disrupts the Krebs cycle by blocking aconitase activity, thereby disrupting ATP synthesis and astrocytic energy metabolism, leading to a significant impairment in important astrocytic functions [13,14].

While vitamin D is primarily known as a hormone associated with calcium balance, bone formation, and maintenance, numerous studies have shown that it plays a direct and indirect role in sleep regulation. Astrocytes, specifically those in the hippocampus, express

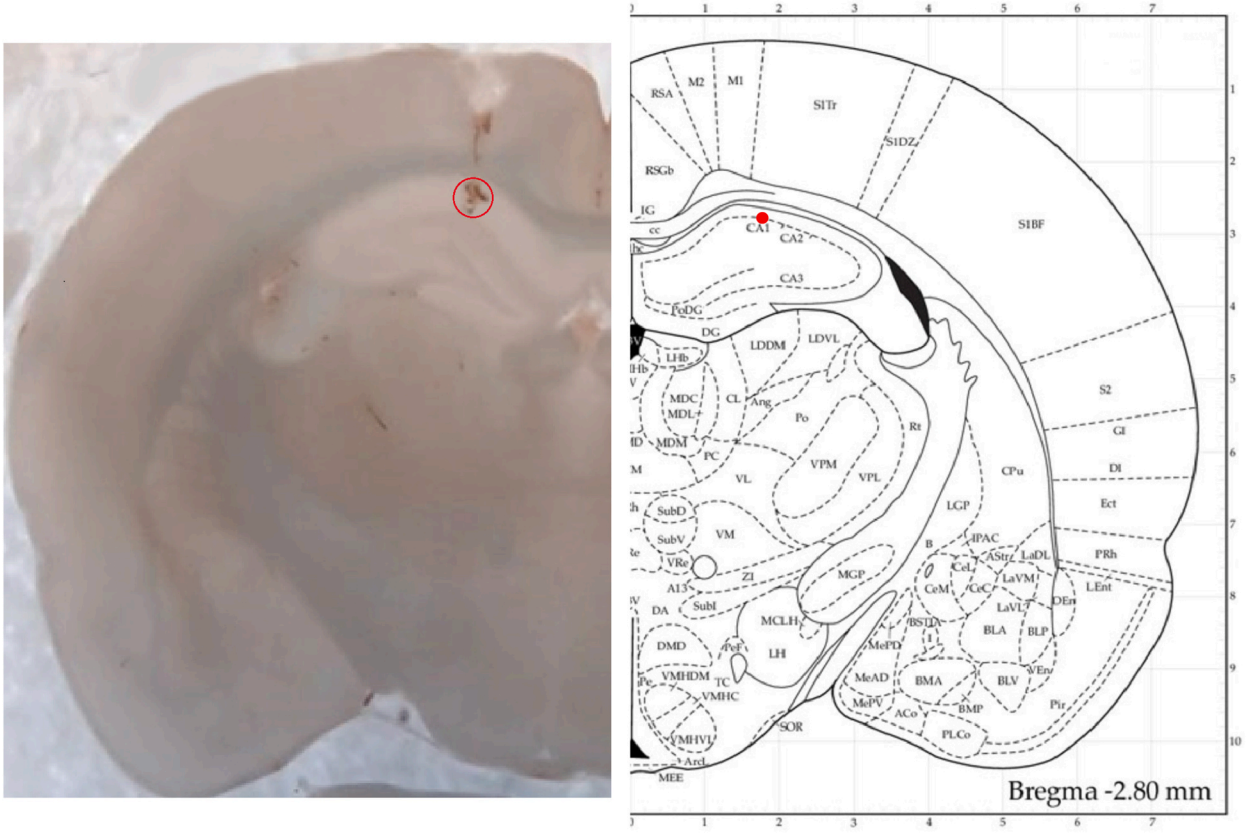


Fig. 1. Schematic representations of coronal sections of the rat brain and histological verification of accurate positioning of the cannula tips in the CA1 region of the dorsal hippocampus. For histological confirmation of sites of injection, we created electrolytic lesions in the hippocampal CA1 region by applying direct current (0.2 mA, 20 s), which can be visualized as a brown scar shown in the red circle in the left panel. Shown to the right is a cartoon showing with a red dot the cannula position (Image modified from Rat Brain Atlas at gaidi.ca). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the nuclear and transmembrane vitamin D receptors (VDR) as well as the enzyme CYP27B1, which is responsible for conversion of vitamin D3 into its active form, suggesting that astrocytes have the machinery to respond to vitamin D signals [15,16]. Some studies have shown that vitamin D can potentially suppress or reduce the activation of astrocytes [17,18]. However, the role of vitamin D in astrocyte function is not fully understood. Furthermore, the mechanisms behind the action of the active form of vitamin D3, known as 1,25(OH)2D3, in regulating sleep and cognitive function are not fully understood and could be due to effects on astrocytes or neurons.

Our studies provide additional information on whether targeting astrocytes could be a therapeutic approach to reduce SD-mediated cognitive impairment. Our results suggest that inhibition of glial function does not rescue SD-associated cognitive impairments, however, vitamin D appears to reduce the short-term negative cognitive effects of SD via astrocytes, possibly involving a reduction in the release of factors that are responsible for the mechanisms of learning and memory.

2. Materials and methods

2.1. Animals

Male Wistar rats (n = 36) weighing between 250 and 280 g and an age range of 10–14 weeks were obtained from our breeding colony for this study. The rats were housed in an air-conditioned room maintained at 22 ± 2 °C with a 12-h light/dark cycle, and they had ad libitum access to water and food. All experiments were conducted during the light cycle, specifically between 6:00 a.m. to 2:00 p.m. This study adhered to the ethical guidelines outlined by the Faculty of Medical Sciences Ethics Committee, Tarbiat Modares University (Approval No. IR.MODARES.AEC.1401.021.2303043), based on the NIH Guide for the Care and Use of Laboratory Animals

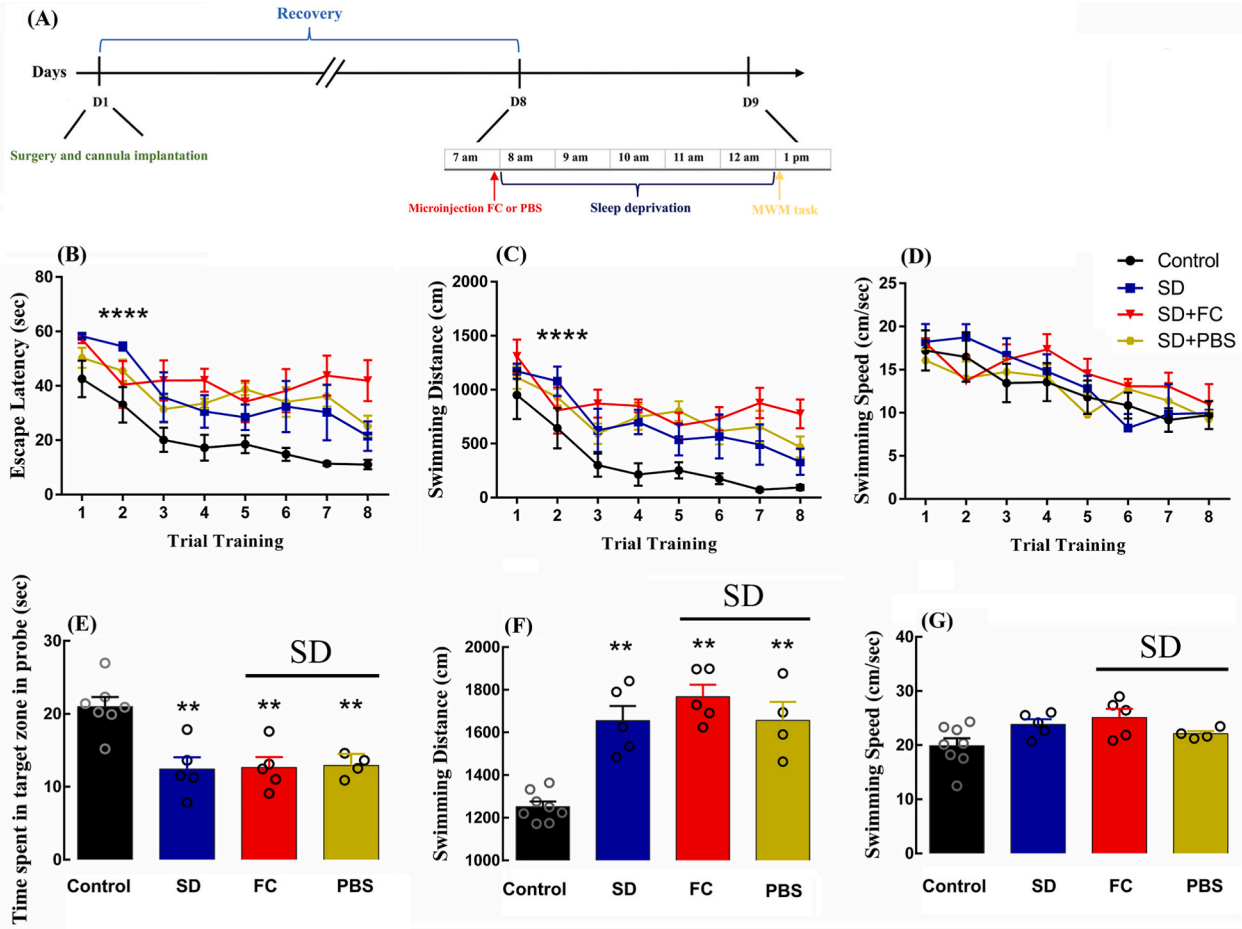


Fig. 2. FC infusion into the dorsal-CA1 hippocampus did not improve SD-induced spatial learning and memory impairment. The experimental timeline (A). Latency to find a hidden platform (B), and swimming distance (C) during the training trials were longer in sleep-deprived animals. Swimming speed did not change among groups (D). FC infusion did not have any effect on this deficit. The probe trial was conducted 24 h after the last training trial, SD animals spent less time in the target zone (E) and traveled a longer distance (F) in the probe test. The swimming speed (G) did not show any significant changes during the probe test. The number of animals in each group is as follows: control (n = 8), SD and SD + FC groups (n = 5 each), and SD + PBS group (n = 4). Data presented as mean ± S.E.M. ****$p < 0.0001$, **$p < 0.01$ Control vs. SD. (SD, Sleep deprivation; FC, Fluorocitrate; PBS, Phosphate Buffered Saline).

(Publication no. 85:23, updated 1985). We made all efforts to minimize animal suffering and the number of animals used.

2.2. Stereotaxic cannulation and intra-hippocampal infusion

The animals were first anesthetized using an intraperitoneal injection of a combination of ketamine (100 mg/kg) and xylazine (2.5 mg/kg). After anesthesia and shaving of the surgical area, the animal was placed in a stereotactic frame (Steolting, USA). The scalp was cleaned using a solution of ethanol (70 %) and povidone-iodine (10 %). The coordinates for the injection site in the CA1 region of the dorsal part of the hippocampus were determined based on the Paxinos and Watson rat brain atlas. The coordinates were as follows: Anterior-Posterior (AP): −2.8 mm from bregma, Midline (ML): ±1.8 mm from the midline, Dorsoventral (DV): −2.8 mm from the surface of the skull (Fig. 1). A hole was drilled at the specified coordinates, and a stainless-steel guide cannula was bilaterally implanted into the CA1 region of the dorsal hippocampus. These cannulas were used to guide the infusion of the experimental drug. The guide cannulas were fixed to the skull using dental cement and secured with sterile stainless-steel screws to keep them in place. To prevent occlusion, a sterile stylet with a 30-gauge needle was inserted into each guide cannula, which extended at least 1 mm beyond the intracerebral tip. During the procedure, every effort was made to minimize pain and the risk of postoperative infection. An injection of 0.15 ml of procaine was administered around the margins of the scalp incision. The surgical site was secured with dental cement, and the rats were allowed to recover from anesthesia.

To assess the effect of calcitriol, the active form of vitamin D3 (Vit D), and fluorocitrate (FC) on learning and memory impairment induced by SD, animals were divided into seven groups including control (n = 8), SD (n = 5), SD plus Vit D (n = 5), SD plus DMSO (n =

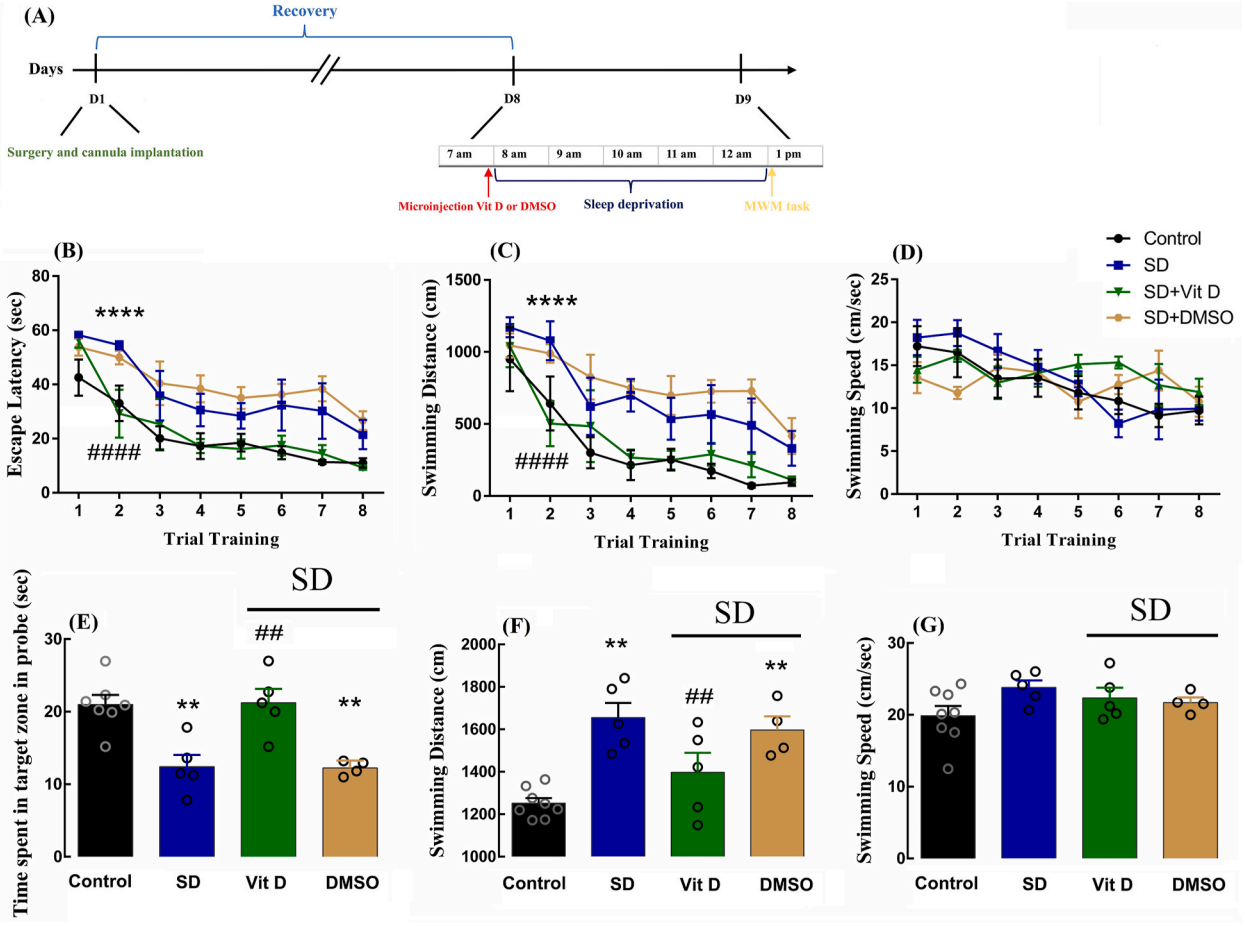


Fig. 3. The infusion of Vitamin D into the dorsal-CA1 hippocampus improved sleep deprivation induced spatial memory impairment. The experimental timeline (A). SD led to impaired spatial learning and memory. In animals that were administered Vitamin D before experiencing sleep deprivation, there was a significant decrease in the latency to find a hidden platform (B) and swimming distance (C) compared to the sleep-deprived animals during the training trials. Swimming speed (D) did not change across the groups during the training trials. On the probe day, animals that received Vitamin D before undergoing sleep deprivation spent more time in the target zone (E) and traveled a shorter distance (F) compared to the sleep-deprived animals. The swimming speed (G) did not exhibit any significant change during the probe test. The sample sizes are n = 8 for the Control group, n = 5 for both the SD and SD + Vit D groups, and n = 4 for the SD + DMSO group. Data presented as mean ± S.E.M. ****P < 0.0001, Control vs. SD; ####P < 0.00001, SD vs. SD + Vit D. SD, Sleep deprivation; Vit D, Vitamin D; DMSO, Dimethyl sulfoxide.

4), SD plus FC (n = 5), SD plus PBS (n = 4), and SD plus Vit D and FC (n = 5). PBS and DMS are solvents for fluorocitrate and vitamin D, respectively.

Eight days after surgery, rats were gently held in place while the stylet was taken out and replaced with sterile needles (30 gauge) that extended 1 mm below the tip of the guide cannula. One dose of calcitriol (1μg/1 μl, Mefar Ilac Sanayii A.S., Istanbul, Turkey) [19] or DMSO (1 μl), fluorocitrate (FC, 1mM/1 μl, Sigma-Aldrich, St. Louis, MO, USA) [20–22] or PBS (1 μl) or a combination of calcitriol and FC was infused into the CA1 region of the dorsal hippocampus bilaterally. The drug is administered into the intra-CA1 region using a syringe pump (Steolting, USA). It is delivered gradually over 2 min through a 30-gauge cannula. The needles remained in place for 2 min after the infusion. Five minutes after the microinjection, the rats were deprived of sleep for 5 h, following which a behavioral test was conducted. In both in vitro and in vivo paradigms, low doses of FC which target inhibition of astrocytes reach peak inhibition approximately 4 h after administration and gradually subside within 24–48 h, suggesting relevant pharmacological actions during the period of SD [23].

2.3. Sleep deprivation protocol

Animals were SD by gentle handling consisting of engaging in stimulating activities including gently tapping the cage, gently shaking the cage, and, when required, disturbing the sleeping nests. This method of SD has been validated and considered to induce only a mild, but not significant stress in the animals [2,24]. In this study, the rats were subjected to SD for 5 h at the beginning of the light phase (8:00 a.m.) [25], during which they normally would sleep approximately 76 %, spending the remaining 3 h of the light phase in active wakefulness, or quiet wakefulness [26]. The duration of sleep deprivation and behavior tests are the same in all experimental groups. As shown in the experimental timeline (A) in Figs. 2–4, all groups subjected to a 5-h period of sleep deprivation

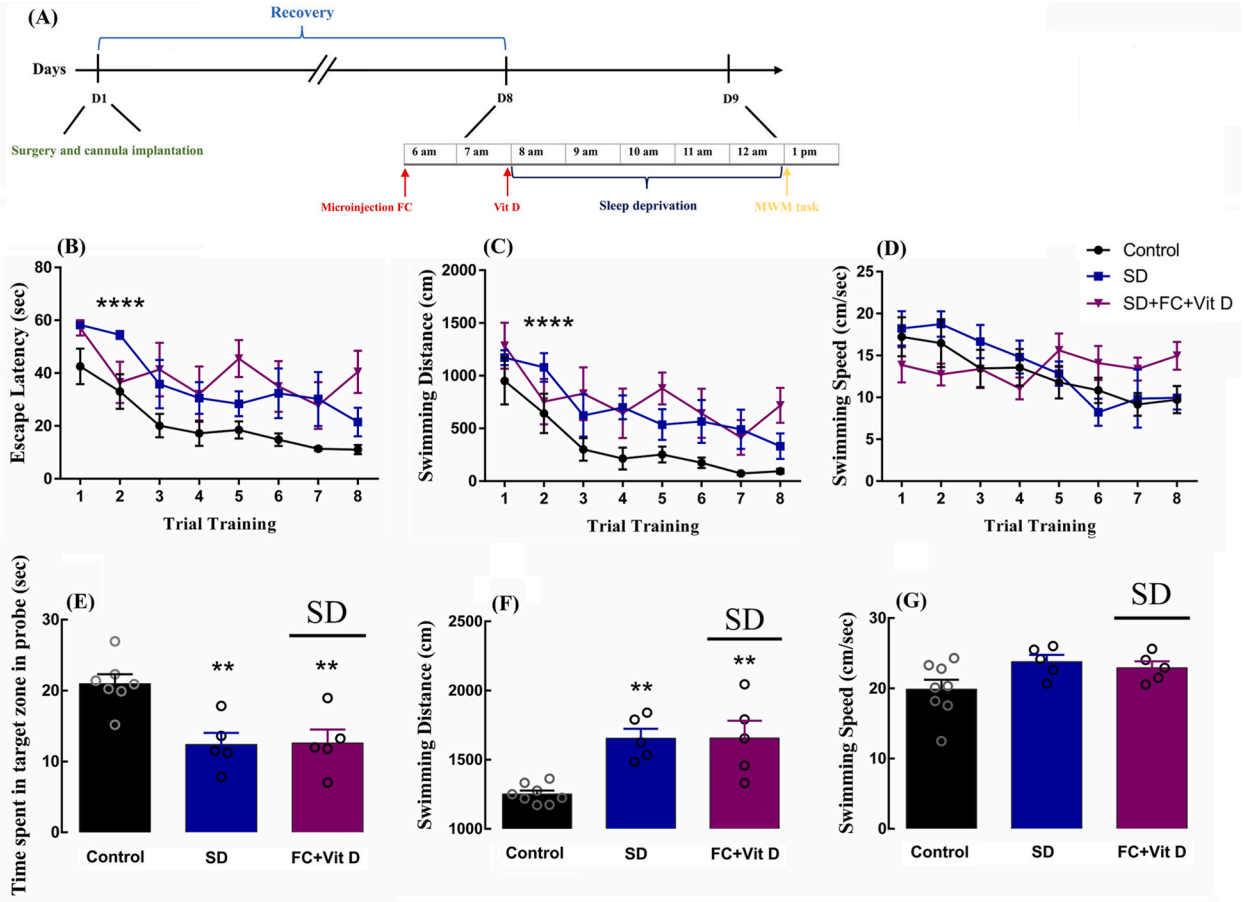


Fig. 4. The sequential injection of fluorocitrate and Vitamin D into the dorsal-CA1 hippocampus did not alleviate the SD-induced spatial impairments. The experimental timeline (A). SD resulted in impaired spatial learning and memory. The groups that received FC and Vit D injections before SD did not exhibit significant differences in escape latency (B), traveled distance (C), and swimming speed (D) compared to the SD groups. Also, FC and Vit D injection in the dorsal hippocampus did not significantly affect the time spent in the target zone (E), swimming distance (F), and swimming speed (G) during the probe test. The sample size is 8 for the Control group and 5 for both the SD and SD + FC + Vit D groups. Data presented as mean ± S.E.M. ****$p < 0.0001$, **$p < 0.01$, Control vs. SD. SD, Sleep deprivation; FC, Fluorocitrate; Vit D: Vitamin D.

from 8:00 a.m. to 1:00 p.m.

2.4. MWM task

The Morris water maze (MWM) consisted of a black lined cylindrical tank: 150 cm in diameter, 60 cm high, and 30 cm depth, filled with water. A round platform with a diameter of 10 cm was placed about 2 cm below the surface of the water in the center of one of the 4 quadrants of the tank, which is the only place where the animal can sit and escape from swimming. The water temperature of the tank was set between 23 and 26 °C. At higher or lower temperatures, exposure of the animal to water may cause excessive stress. Visual cues were present in the room where the tank was located to assist the animal in finding the platform easily once its position is detected. The movements of the rats were recorded by the camera (Panasonic Inc., Japan) and analyzed using Ethovision software (XT11, Netherlands).

To allow habituation, 24 h before training began, rats were allowed to swim in the tank for 1 min. The training schedule for this experiment consisted of eight trials with a time interval of 20-s between trials. The platform was fixed in one of the quadrants. In each trial, the animal was released into the water from one of the quadrants which was considered as four starting points (north, south, east, and west) with its face toward the wall of the tank. If the animal could not find the platform after 60 s, it was guided to swim to the platform by the investigator and allowed to sit on the platform for 20 s. After each training, the animal was dried with a towel and returned to the cage. The probe test was done after 24 h of the last training. In the probe test, the platform was removed from the tank and animals were allowed to swim for 60 s. Escape latency (i.e., time to reach the platform), distance moved, velocity, and time spent in the target quadrant were calculated for subsequent analyses. 15 min after the probe test, the visible platform test was performed. The visible platform test was conducted to evaluate the visual and motivational ability of the animals by covering the platform with aluminum foil and placing it above the water level in the opposite quadrant from the quadrant where it had been hidden [27].

2.5. Statistical analysis

The data is presented as means ± SEM. GraphPad Prism (GraphPad®9.0 software) was used for statistical analysis. Depending on the levels of independent variables, we utilized either one-way or two-way ANOVA. A Bonferroni or Tukey's test was used as the post hoc test. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. FC injection into the dorsal-CA1 hippocampus cannot recover sleep deprivation induced spatial memory impairment

The experimental timeline for this set of groups is shown in Fig. 2A. As expected, the escape latency to find the hidden platform was significantly greater in sleep-deprived animals ($n = 5$) when compared to that in the control group ($n = 8$) [$F(3, 142) = 26.51$, $p < 0.0001$, two-way ANOVA followed by Tukey's test, Fig. 2B], and the SD animals swam longer distances during the training trials compared to the control animals [$F(3, 142) = 26.32$, $p < 0.0001$, Fig. 2C]. The animals treated with FC before SD ($n = 5$) did not exhibit a lower escape latency when compared to the sleep-deprived animals ($p > 0.05$, two-way ANOVA followed by Tukey's test, Fig. 2B). In the SD groups, no significant effect of FC was observed on swimming distance ($p > 0.05$, Fig. 2C). The animals that were administered PBS ($n = 4$) before SD did not demonstrate a difference in escape latency or traveled distance as compared to the SD animals ($p > 0.05$, Fig. 2B and C). There were no significant changes in swimming speed during training among the groups [$F(3, 142) = 1.423$, $P = 0.2386$, two-way ANOVA followed by Tukey's test, Fig. 2D].

To evaluate memory, a probe trial was conducted 24 h after the final training trial on day 1. The animals that experienced sleep deprivation spent less time in the target zone (control: 21.20 s, $n = 8$, SD: 12.40 s $n = 5$, $p < 0.001$, one way ANOVA, Bonferroni test, Fig. 2E) and swam a longer distance in the probe test ($p < 0.001$, Fig. 2F). FC infusion into the CA1-dorsal hippocampus did not affect these SD-associated memory impairments as there was no notable distinction in the time spent in the target zone and swimming distance during the probe test between animals that received FC treatment before SD and the SD group ($p > 0.05$, Fig. 2E and F). The infusion of PBS did not impact the time spent in the target zone or the swimming distance during the probe test in the SD group ($p > 0.05$, one-way ANOVA, Bonferroni test, Fig. 2E and F). The swimming speed did not demonstrate any difference during the probe test among the groups ($p > 0.05$, Fig. 2G). In the visible test conducted after the probe test, all animals successfully located the platform ($p > 0.05$, one-way ANOVA, Bonferroni test).

3.2. Vitamin D injection into the dorsal-CA1 hippocampus improves sleep deprivation induced spatial memory impairment

The experimental timeline for these groups is shown in Fig. 3A. While inhibition of hippocampal glial cells with FC did not demonstrate an effect on SD-associated cognitive deficits in the MWM, administration of vitamin D could reduce SD's negative impact on spatial learning and memory. Interestingly, there were no significant differences in escape latency and traveled distance between the SD animals treated with vitamin D ($n = 5$) and the control group ($p > 0.05$, two-way ANOVA followed by Tukey's test, Fig. 3B and C). Animals that received vitamin D before experiencing SD showed a significant decrease in escape latency [$F(3, 142) = 24.96$, $P < 0.0001$, two-way ANOVA followed by Tukey's test, Fig. 3B], as well as in the traveled distance, compared to the SD animals [$F(3, 142) = 26.51$, $P < 0.0001$, two-way ANOVA followed by Tukey's test, Fig. 3C]. The animals that were given DMSO before SD did not demonstrate a significant decrease in escape latency and traveled distance compared to the SD group ($p > 0.05$, two-way ANOVA

followed by Tukey's test, Fig. 3B and C), suggesting differences were not due to the vehicle. No significant changes were observed in swimming speed during training among the groups [$F(3, 142) = 0.7734$, $P = 0.5107$, two-way ANOVA followed by Tukey's test, Fig. 3D].

The probe trial was conducted 24 h after the last training trial. Animals treated with vitamin D before SD exhibited a significant increase in time in the target zone (control: 21.20 s, $n = 8$; SD: 12.40 s, $n = 5$; SD + vitamin D: 20.99 s, $n = 5$, $p < 0.001$, one-way ANOVA, Bonferroni test, Fig. 3E), as well as a decrease in the traveled distance, compared to the SD animals ($p < 0.001$, one-way ANOVA, Bonferroni test, Fig. 3F). The administration of DMSO did not have an impact on the time spent in the target zone or the swimming distance in comparison to the SD group during the probe test ($p > 0.05$, one-way ANOVA, Bonferroni test, Fig. 3E and F). During the probe test, there were no significant differences in swimming speed among the groups ($p > 0.05$, one-way ANOVA, Bonferroni test, Fig. 3G). In the subsequent visible test conducted after the probe test, all animals successfully located the platform ($p > 0.05$, one-way ANOVA, Bonferroni test).

3.3. Sequential injection FC and vitamin D into the dorsal-CA1 hippocampus has no effect on sleep deprivation induced spatial memory impairment

The experimental timeline for this group is shown in Fig. 4A. The animals that received FC and vitamin D ($n = 5$) before SD showed a significant increase in escape latency [$F(2, 117) = 22.88$, $P < 0.0001$, two-way ANOVA followed by Tukey's test, Fig. 4B], and swimming distance compared to the control group [$F(2, 117) = 19.13$, $P < 0.0001$, two-way ANOVA followed by Tukey's test, Fig. 4B]. There were no significant differences observed in escape latency and traveled distance between the SD animals treated with FC plus vitamin D and the SD group ($p > 0.05$, two-way ANOVA followed by Tukey's test, Fig. 4B and C). Swimming speed did not show significant changes during training among the groups [$F(2, 117) = 0.5270$, $P = 0.5917$, two-way ANOVA followed by Tukey's test, Fig. 4D].

The probe test was performed 24 h after the final training trial. The infusion of FC and vitamin D before SD did not influence the time spent in the target zone and swimming distance in comparison to the SD group (Control: 21.20 s, $n = 8$; SD: 12.40 s, $n = 5$; SD + FC + vitamin D: 12.81 s, $n = 5$, $p < 0.001$, one-way ANOVA, Bonferroni test, Fig. 4E and 4F). There were no significant differences in swimming speed during the probe test among the groups ($p > 0.05$, one-way ANOVA, Bonferroni test, Fig. 4G). In the visible test performed after the probe test, all animals were able to find the platform successfully ($p > 0.05$, one-way ANOVA, Bonferroni test).

4. Discussion

The main findings of the current research can be summarized as follows: a) Five-hour SD impairs spatial learning and memory in the Morris water maze (MWM), b) Inhibition of the dorsal-CA1 hippocampal glial cells before SD did not prevent the SD induced cognitive impairment, c) Vitamin D injection into the dorsal-CA1 hippocampus prior to SD ameliorates the adverse effect of sleep deprivation on spatial learning and memory in the MWM, and d) The co-application of FC and vitamin D did not reduce spatial memory impairment caused by SD, indicating the role of the VDR on astrocytes in the positive effects of vitamin D on cognitive impairment associated with SD.

Lack of sleep can negatively affect neuronal physiological processes that are critical to cognitive function, and astrocyte activity has been shown to play a role. Several studies have shown that SD impairs various types of learning and memory that rely on the hippocampus [2]. SD was found to negatively affect the acquisition and retrieval of spatial learning and memory in the MWM [28], as well as influence learning in the passive avoidance test [29] and in contextual fear conditioning [30]. Brief SD (5 h) impairs long-term potentiation (LTP) in the hippocampal CA1 region, cAMP/PKA/ERK-dependent LTP, and hippocampus-dependent memory [25]. In SD, activation of the inhibitory G protein-coupled adenosine A1 receptor on the presynaptic neuron by astrocyte-derived adenosine can inhibit adenylyl cyclase, leading to a reduction in cAMP levels and phosphorylation of extracellular signal-regulated kinase (ERK, which is another intracellular molecule that is crucial for the LTP maintenance [25]. Following changes in intracellular signaling, astrocytic adenosine in the hippocampus has been shown to reduce neuronal excitability by modulating postsynaptic ion currents or by reducing presynaptic neurotransmitter release [6,31]. Furthermore, astrocyte-derived adenosine was implicated in the cognitive impairments induced by 6 h SD [8]. Thus, changes in neuronal excitability and synaptic processes underlying plasticity that are associated with SD could be due to an effect of astrocyte-derived adenosine at A1 receptors.

Here, we showed that FC injection into the dorsal-CA1 hippocampus before SD not only had no effect on the SD induced learning and memory impairment but also partially exacerbated the observed impairment. Our data are consistent with previous studies showing that FC-mediated inhibition of glial cell activity in the dorsal hippocampus leads to glutamate toxicity and impaired synaptic transmission [22]. In addition, our previous research showed that the intracerebroventricular microinjection of FC in intact animals resulted in impaired reversal memory acquisition and reference memory retrieval in the MWM [32]. FC mediated inhibition of astrocytes likely impaired fundamental processes underlying learning and memory [33] and thus could explain why we did not observe any SD-induced cognitive impairments, and instead, we observed exacerbation of cognitive impairment.

Interestingly, vitamin D pretreatment significantly improved cognitive deficits in SD animals. The vitamin D injection into the dorsal-CA1 hippocampus before SD reduced the escape latency and traveled distance to find the hidden platform during the acquisition phase. In addition, it reversed the effects of SD on the retrieval phase of the MWM task. Our findings are consistent with previous studies showing that chronic REM sleep deprivation caused spatial memory impairment that was prevented by vitamin D treatment [34]. In cell culture, vitamin D administration suppresses astrocyte activation, which includes suppressing the expression of pro-inflammatory factors, cytokines, and interleukin, which can promote inflammatory processes detrimental to neuronal function

[17]. Also, vitamin D modulates morphological changes in astrocytes during activation [18].Therefore, it is possible that the observed positive effects on cognitive behaviors affected by SD could be due to the suppressed activity of astrocytes by vitamin D, which prevents the production of neurotoxic factors. Studies have shown the positive effect of vitamin D on astrocytes in various pathological conditions, and vitamin D3 deficiency is associated with the development of neurological diseases [35,36]. Vitamin D injection in the 5xFAD Alzheimer's mouse model reduced the release of pro-inflammatory cytokines, chemokines, reactive oxygen, and nitrogen species from reactive astrocytes, and consistent with their role in aggravating disease progression, reducing neurotoxic factors improved functional outcomes [37]. The effects of vitamin D on astrocytes have been shown to increase cell viability and reduce the incidence of necrotic and apoptotic events in Parkinson's disease [38]. Vitamin D play a role in sleep regulation, and its effects could be due to the activation of VDRs located on astrocytes, which leads to changes in astrocyte function. Through co-administration studies with FC and vitamin D, we were able to rule out the effects of vitamin D on neurons or other glial cells in short sleep deprivation. Our findings show that during glial cell, specifically astrocyte, dysfunction, vitamin D cannot prevent short sleep deprivation-induced cognitive impairment and suggest that inhibition of astrocytes by FC abrogates the benefical effects of vitamin D. The benefical effects of vitamin D on cognitive impairment associated with SD could involve the regulation of adenosine levels, as suppression of astrocytes would be expected to lower adenosine at the tripartite synapse. As SD affects millions worldwide, especially in night shift jobs, vitamin D therapy can help manage cognitive impairments and improve mental alertness and acuity.

5. Conclusion

We have shown that vitamin D can improve cognitive impairment caused by short-term sleep deprivation. Considering the low cost and availability of this intervention, this research can have significant implications for people who suffer from sleep deprivation due to irregular working hours or social participation. Maintaining adequate levels of vitamin D offers a promising avenue to improve cognitive performance under such demanding physiologically conditions.

Data availability statement

The data will be made available upon request.

CRediT authorship contribution statement

Amir Rezagholizadeh: Writing – original draft, Methodology, Investigation. **Amin Firoozi:** Methodology, Formal analysis. **Zohreh Tavassoli:** Methodology, Investigation. **Amir Shojaei:** Software, Data curation. **Narges Hosseinmardi:** Methodology, Investigation. **Javad Mirnajafi-Zadeh:** Formal analysis, Data curation. **Kristi Anne Kohlmeier:** Writing – review & editing. **Yaghoub Fathollahi:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research work is financially supported by the Faculty of Medical Sciences, Tarbiat modares University, Tehran, Iran. The graphical elements in this graphical abstract were created using BioRender.com.

References

[1] V.K. Chattu, M.D. Manzar, S. Kumary, D. Burman, D.W. Spence, S.R. Pandi-Perumal, The global problem of insufficient sleep and its serious public health implications, Healthcare 7 (1) (2019) 1–16.
[2] T.M. Prince, T. Abel, The impact of sleep loss on hippocampal function, Learn. Mem. 20 (10) (2013) 558–569.
[3] T. Porkka-Heiskanen, R.E. Strecker, M. Thakkar, A.A. Bjørkum, R.W. Greene, R.W. McCarley, Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness [cited 2023 Nov 4], Science [Internet] 276 (5316) (1997 May 23), 1265–7. Available from: https://pubmed.ncbi.nlm.nih.gov/9157887/.
[4] T. Porkka-Heiskanen, R.E. Strecker, R.W. McCarley, Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep: an in vivo microdialysis study, Neuroscience 99 (3) (2000) 507–517.
[5] D. Boison, J.F. Chen, B.B. Fredholm, Adenosine signaling and function in glial cells, Cell Death Differ. 17 (7) (2010) 1071–1082.
[6] R. Havekes, C.G. Vecsey, T. Abel, The impact of sleep deprivation on neuronal and glial signaling pathways important for memory and synaptic plasticity, Cell Signal [Internet] 24 (6) (2012) 1251–1260, https://doi.org/10.1016/j.cellsig.2012.02.010. Available from:.
[7] O. Pascual, K.B. Casper, C. Kubera, J. Zhang, R. Revilla-Sanchez, J.Y. Sul, et al., Neurobiology: astrocytic purinergic signaling coordinates synaptic networks, Science (80-) 310 (5745) (2005) 113–116.
[8] M.M. Halassa, C. Florian, T. Fellin, J.R. Munoz, S.Y. Lee, T. Abel, et al., Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss [Internet], Neuron 61 (2) (2009) 213–219, https://doi.org/10.1016/j.neuron.2008.11.024. Available from:.
[9] J.S. Erlichman, J.C. Leiter, Glia modulation of the extracellular milieu as a factor in central CO 2 chemosensitivity and respiratory control, J. Appl. Physiol. 108 (6) (2010) 1803–1811.
[10] T. Paquette, M. Piché, H. Leblond, Contribution of astrocytes to neurovascular coupling in the spinal cord of the rat [cited 2023 Dec 31], J Physiol Sci [Internet] 71 (1) (2021 Dec 1) 1–9. Available from: https://jps.biomedcentral.com/articles/10.1186/s12576-021-00800-6.

[11] C.R. Sobrinho, C.M. Gonçalves, A.C. Takakura, D.K. Mulkey, T.S. Moreira, S. Paulo, Control of Homeostasis Fluorocitrate-Mediated Depolarization of Astrocytes in the Retrotrapezoid Nucleus Stimulates Breathing, 2017, pp. 1690–1697.
[12] L.A. Volobouéva, S.W. Suh, R.A. Swanson, R.G. Giffard, Inhibition of mitochondrial function in astrocytes: implications for neuroprotection, J. Neurochem. 102 (4) (2007) 1383–1394.
[13] F. Fonnum, A. Johnsen, B. Hassel, Use of fluorocitrate and fluoroacetate in the study of brain metabolism, Glia 21 (1) (1997) 106–113.
[14] R.A. Swanson, S.H. Graham, Fluorocitrate and fluoroacetate effects on astrocyte metabolism in vitro, Brain Res. 664 (1–2) (1994) 94–100.
[15] M.C. Langub, J.P. Herman, H.H. Malluche, N.J. Koszewski, Evidence of functional vitamin D receptors in rat hippocampus, Neuroscience 104 (1) (2001) 49–56.
[16] A.L. Lardner, Vitamin D and hippocampal development-the story so far, Front. Mol. Neurosci. 8 (2015) 1–7. OCT.
[17] K.P. Jiao, S.M. Li, W.Y. Lv, M.L. Jv, H.Y. He, Vitamin D3 repressed astrocyte activation following lipopolysaccharide stimulation in vitro and in neonatal rats, Neuroreport 28 (9) (2017) 492–497.
[18] B.J. Curletto, The effects of 1,25-dihydroxyvitamin D3 on rat primary astrocytes [cited 2023 Nov 4]; Available from: https://open.bu.edu/handle/2144/12080, 2013.
[19] X. Guo, J. Yuan, J. Wang, C. Cui, P. Jiang, Calcitriol alleviates global cerebral ischemia-induced cognitive impairment by reducing apoptosis regulated by VDR/ERK signaling pathway in rat hippocampus [Internet], Brain Res. 1724 (April) (2019) 146430, https://doi.org/10.1016/j.brainres.2019.146430.
[20] V. Affairs, S. Francisco, Fluorocitrate and fluoroacetate effects on astrocyte metabolism in vitro, in: V. Affairs, S. Francisco (Eds.), Fluorocitrate and Fluoroacetate Effects on Astrocyte Metabolism in Vitro, 1994, p. 664.
[21] J. Berg-Johnsen, R.E. Paulsen, F. Fonnum, I.A. Langmoen, Changes in evoked potentials and amino acid content during fluorocitrate action studied in rat hippocampal cortex, Exp. Brain Res. 96 (2) (1993) 241–246.
[22] C. Largo, P. Cuevas, G.G. Somjen, R. Martín Del Río, O. Herreras, The effect of depressing glial function in rat brain in situ on ion homeostasis, synaptic transmission, and neuron survival [cited 2023 Sep 9], J Neurosci [Internet] 16 (3) (1996 Feb 1) 1219–1229. Available from: https://pubmed.ncbi.nlm.nih.gov/8558250/.
[23] B. Hassel, R.E. Paulsen, A. Johnsen, F. Fonnum, Selective inhibition of glial cell metabolism in vivo by fluorocitrate, Brain Res. 576 (1) (1992) 120–124.
[24] R. Hagewoud, R. Havekes, P.A. Tiba, A. Novati, K. Hogenelst, P. Weinreder, et al., Coping with sleep deprivation: shifts in regional brain activity and learning strategy, Sleep 33 (11) (2010) 1465–1473.
[25] C.G. Vecsey, G.S. Baillie, D. Jaganath, R. Havekes, A. Daniels, M. Wimmer, et al., Sleep deprivation impairs cAMP signalling in the hippocampus [Internet], Nature 461 (7267) (2009) 1122–1125, https://doi.org/10.1038/nature08488.
[26] J.J. Clancy, D.F. Caldwell, M.J. Villeneuve, S. Sangiah, Daytime sleep-wake cycle in the rat, Physiol. Behav. 21 (3) (1978 Sep 1) 457–459.
[27] S. Mahboubi, M. Nasehi, A. Imani, M.S. Sadat-Shirazi, M.R. Zarrindast, N. Vousooghi, et al., Benefit effect of REM-sleep deprivation on memory impairment induced by intensive exercise in male wistar rats: with respect to hippocampal BDNF and TrkB, Nat. Sci. Sleep 11 (2019) 179–188.
[28] Z. Guan, X. Peng, J. Fang, Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus, Brain Res. 1018 (1) (2004) 38–47.
[29] R.H. Silva, A.B. Chehin, S.R. Kameda, A.L. Takatsu-Coleman, V.C. Abílio, S. Tufik, et al., Effects of pre- or post-training paradoxical sleep deprivation on two animal models of learning and memory in mice, Neurobiol. Learn. Mem. 82 (2) (2004 Sep 1) 90–98.
[30] L.A. Graves, E.A. Heller, A.I. Pack, T. Abel, Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning [cited 2023 Nov 4], Learn Mem [Internet] 10 (3) (2003 May) 168–176. Available from: https://pubmed.ncbi.nlm.nih.gov/12773581/.
[31] C. Florian, C.G. Vecsey, M.M. Halassa, P.G. Haydon, T. Abel, Astrocyte-derived adenosine and A1 receptor activity contribute to sleep loss-induced deficits in hippocampal synaptic plasticity and memory in mice, J. Neurosci. 31 (19) (2011) 6956–6962.
[32] A. Rezagholizadeh, S.A. Karimi, N. Hosseinmardi, M. Janahmadi, M. Sayyah, The effects of glial cells inhibition on spatial reference, reversal and working memory deficits in a rat model of traumatic brain injury (TBI) [cited 2023 Sep 9], Int J Neurosci [Internet] 132 (3) (2022) 226–236. Available from: https://pubmed.ncbi.nlm.nih.gov/32799586/.
[33] P.G. Haydon, Glia: listening and talking to the synapse, Nat. Rev. Neurosci. 2 (3) (2001) 185–193, https://doi.org/10.1038/35058528. PMID: 11256079.
[34] S.E. Bdear, N.A. Raafat, R.M. Al-Sayed, Shaimaa Abozaid, Role of vitamin D in memory impairment induced by rapid eye movement sleep deprivation in albino rats, Zagazig Univ. Med. J. 28 (5) (2022) 957–965.
[35] R. Nair, A. Maseeh, Vitamin D: the sunshine vitamin, J. Pharmacol. Pharmacother. 3 (2) (2012) 118–126.
[36] Q. Gao, T. Kou, B. Zhuang, Y. Ren, X. Dong, Q. Wang, The association between vitamin D deficiency and sleep disorders: a systematic review and meta-analysis, Nutrients 10 (10) (2018).
[37] J. Kang, M. Park, E. Lee, J. Jung, T. Kim, The role of vitamin D in Alzheimer's disease: a transcriptional regulator of amyloidopathy and gliopathy, Biomedicines 10 (8) (2022).
[38] E.A. de Siqueira, E.P. Magalhães, R.R.P.P.B. de Menezes, T.L. Sampaio, D.B. Lima, C. da Silva Martins, et al., Vitamin D3 actions on astrocyte cells: a target for therapeutic strategy in Parkinson's disease? Neurosci. Lett. (2023) 793. September 2022.