Original Article

Caffeine improved spatial learning and memory deficit in sleep deprived female rat

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Abstract

Previous studies have shown that caffeine has beneficial effects on cognitive impairment in sleep deprived male rats. Therefore in the present study, we examined the effects of chronic caffeine administration on learning and memory impairments induced by sleep deprivation (SD) in the intact and ovarectomized (OVX) female rats. Two sets of animals including intact and OVX were randomly recruited into the following subgroups: control, SD, wide platform (Sham platform), caffeine, and caffeine plus SD. Multiple platform method was used for SD induction. Spatial learning and memory were determined using Morris water maze (MWM) task. Throughout behavioral investigation, significant learning impairment was observed in sleep-deprived OVX rats compared to the intact and the other OVX groups (P<0.05). Short term memory impairment was observed in both sleep-deprived OVX and intact groups (P<0.05). 4weeks caffeine administration improved these impairments.

Based on these findings we propose that sleep deprivation impaired cognitive function whereas caffeine treatment reversed these impairments.

Introduction

Sleep is a natural physiological process that plays a pivotal role in normal biological functions (Smith, 1995). Growing reports show that sleep may be a fundamental contributor to memory consolidation (Blissitt, 2001; Diekelmann and Born, 2010). It is also know that there is a strong correlation between sleep deprivation and memory impairment (Ferrara et al., 2008). For instance, sleep deprivation causes the impairment of long term potentiation in the dentate gyrus (DG) area (McDermott et al., 2003; Marks and Wayner, 2005), and hippocampus becomes more active when the individuals are allowed to sleep after learning task. In fact, increased hippocampal activity during sleep contributes to the acquisition and consolidation of memory (Gais et al., 2007).

It seems that some features such as sleep patterns, sleep quality, learning and memory capacity and even sleep disorders are gender dependent (Reyner et al., 1995; Tsai and Li, 2004). Moreover, it has been shown that daily functioning in men who sleep less than seven hours at night is better than women, suggesting that women are more vulnerable to sleep deprivation (Barnett et al., 1987). In addition, sleep difficulty increases markedly at menopause because of unclear hormonal mechanisms (Eichling and Sahni, 2005). Evidence indicates that steroid hormones have some effects on sleep and central nervous system processes

related to sleep (Manber and Armitage, 1999). Previous studies suggested a neural basis for sex difference in hippocampus dependent learning task (Maren et al., 1994). Estrogen level can alter memory during the lifespan of the female. Indeed, when estrogen increases, dendrite spine density increase on pyramidal cells in the prefrontal cortex and hippocampus and improve memory functions (Luine and Frankfurt, 2013). Estrogen in its acute form facilitates the effect of glutamatergic transmission, and long-term potentiation (LTP) which provides a potential explanation for the considerable influence of steroid on behavior pattern. Recent works have identified the mechanism which underlies this synaptic action (Kramar et al., 2013).

Caffeine is used commonly as a central nervous system stimulant (Ferré, 2008) which is found in several beverages and food. Caffeine has beneficial effects on cognitive impairment and neurodegenerative disease including Alzheimer's and Parkinson's disease because of its neuroprotective effects (Dall'Igna et al., 2004; Dall'Igna et al., 2007).

Previous studies have shown that low doses of caffeine administration have positive effects on learning and memory (Angelucci et al., 2002); additionally chronic caffeine consumption prevents learning and memory impairment in Alzheimer disease (Dall'Igna et al., 2007). The beneficial effects of caffeine administration on memory may be related to its nonselective antagonistic effects on adenosine receptors (Takahashi et al., 2007; Pires et al., 2009).

Indeed, another study has shown that caffeine prevents spatial short-term memory and E-LTP impairments induced by 24-h sleep deprivation in CA1 area of the hippocampus of male rats (Alhaider et al., 2010b).

However, no information is available about the effect of caffeine administration on the damaging effects of SD on the cognitive function in female rats. The aim of the present study was to examine the effects of chronic caffeine treatment on SD-induced impairment of learning and memory in the intact and ovarectomized female rats.

Materials and methods

All experimental protocols and treatments were approved by the Ethical Committee of the Kerman Neuroscience Research Center (EC/KNRC/92-30) that was completely in agreement with the "NIH Guide for the Care and Use of Laboratory Animals". Adult intact and OVX female Wistar rats, weighing 200– 250 g, were randomly assigned into five subgroups: Control, SD, wide platform (sham platform), Caffeine and Caffeine∕SD. A sham surgery was also performed on a separate group of rats. All OVX and sham surgery (submitted to surgery without removing the ovaries) underwent ovariectomy surgical procedure (n = 8 for each group). Rats were caged individually and maintained under constant temperature (23 ± 1 °C) and 12-h light–dark cycle (light on at 07:00). They had free access to standard food and water.

All female were bilaterally ovariectomized under general anesthesia (60 mg/kg ketamin and 10mg/kg xylazin). All of the ovarectomized rats were used after 1 month of surgery to prevent or minimize any hormonal influence originating from their reproductive cycle (Saadati et al., 2015)

Caffeine (Sigma Aldrich) was orally consumed for 4 weeks in drinking water (0.3 g ∕ L) (Alhaider et al., 2010b). The range of the daily consumption of caffeine by each rat was between 16–20 mg. The amount of water consumed by rats in the caffeinated groups during the 4 weeks was not significantly different than that of the control group. During sleep deprivation of the caffeine/sleep deprivation group, we added the same concentration of caffeine in the aquarium water in case the rats drink from aquarium water rather than water bottles.

The rats in SD and caffeine∕SD groups were sleep-deprived for 72 h using the columns-in-water method (modified multiple platform model). Four rats from the same cage were placed in an aquarium containing 10 circular platform platforms (10 cm height, 7 cm diameter) whose tops were 2 cm above the water. Platforms arranged in two rows and spaced 10 cm apart (edge to edge) so that the rats could move freely around by leaping from one platform to another. Because the animals can move freely within such multiplatform chamber, it is reported that they experience less immobilization stress compared to the widely used single platform model. Rats woke as they fell into the water each time they reached the REM sleep phase because of loss of muscle tone. During the

sleep deprivation period, rats had free access water bottles and food pellets baskets hanging from the top of the chamber. We also tested the effects of possible stress causing elements of the chamber environment by using a wide platform (15 cm in diameter), which allowed the rats to sleep without falling into the water (Hajali et al., 2012).

The Morris water maze is one of the most greatly. Widely used models in behavioral neuroscience for evaluating the potential effects on spatial learning and memory in experimental manipulation.

This device is a black circular pool (160 cm in diameter and 80 cm in height) that was filled to a depth of 40 cm with water and kept at room temperature. The pool was separated into four equal quadrants. A black square platform (10 cm in diameter) was centered in northeast quadrant of the pool and submersed 1.5 cm into the water so that it was imperceptible at water level. The experiments were done in the dimly lit light room with spatial cues which were attached to the walls around the maze at different points. Noldus Ethovision system, version 7.1, is a smart video tracing system that recorded the performance of rats. Also, it can be traced on the monitor of the computer.

In this test there are three blocks with 30 minutes. Each block included four sequential trials each taking 60s (Inter-trial interval=30s). Rats were release randomly in the water maze while facing the wall of each quadrant. After each rat found the platform, it was allowed to stay there for 20s and then it was relocated to its cage to rest for 10s before starting the next trial. In case a rat did not discover the platform in 60s it was led to that platform by the experimenter. The data on time and distance to find the hidden platform were collected and analyzed later.

A single probe trial was given 2 h after the last training trial to test the short term spatial memory in the water maze. In this trial the platform was removed and rat was allowed to swim for 60 s. The time and distance spent in the target quadrant (quadrant 4) were analyzed as a measure of spatial memory retention (Saadati et al., 2015).

All comparisons among the groups were also analyzed with two-way ANOVA followed by Tukey's post hoc multiple comparison test. The averages for different groups also were compared using one-way ANOVA,

followed by Tukey test. Data was explained as Means ± SEM of eight rats per group. The statistical significant level was considered as $P < 0.05$.

Results

To investigate whether sleep deprivation and/or caffeine administration affects learning and memory in female rats, the Morris water maze was used in the present study. The result of mean distance and escape latency to find the hidden platform in the Morris water maze test in the intact female rats were shown in figs. 1 and 2A. Our results showed that there was no significant difference (P>0.05) in spatial learning among control, wide platform, SD, caffeine and caffeine/SD in the intact female rats. Therefore SD and caffeine couldn't impair the spatial learning in intact female rats.

As shown in Figs. 1 and 2B, the mean distance and escape latency were increased in the SD-OVX group compared to other groups. These result showed that SD impaired the spatial learning in SD-OVX female rats.

In the MWM test the mean of the traveled distance (986.03 ± 47.08 vs. 598.32 ± 12.82; P<0.001) and escape latency (46.43 ±1.37 vs. 36.87 ± 2.37; P <0.05) were also both increased in the SD-OVX group in comparison with the SD- intact female rats. Meanwhile, the ability of the sleep deprived OVX rats to find the hidden platform was obviously enhanced through caffeine administration (Figs. 1 and 2B). This was also indicated by the significant reduction in their swimming distance (577.75 ± 46.15 vs. 986.03 ± 47.08, P = 0.001; Fig 1B) and a reduction in the escape latencies (25.74 ± 2.04 vs. 46.43 ± 1.37; P = 0.001; Fig 2B) in caffeine /SD group compare to SD group in OVX female rats in the MWM test.

Among sham (wide platform), control, caffeine and caffeine /SD groups of intact and OVX female rats, the distance and escape latency to find the hidden platform in the water maze was did not show any major significant (Figs.1 and 2). Therefore, the caffeine and caffeine/sleep deprived rats of intact and OVX female groups did not perform significantly better than those of the control groups in their learning phase.

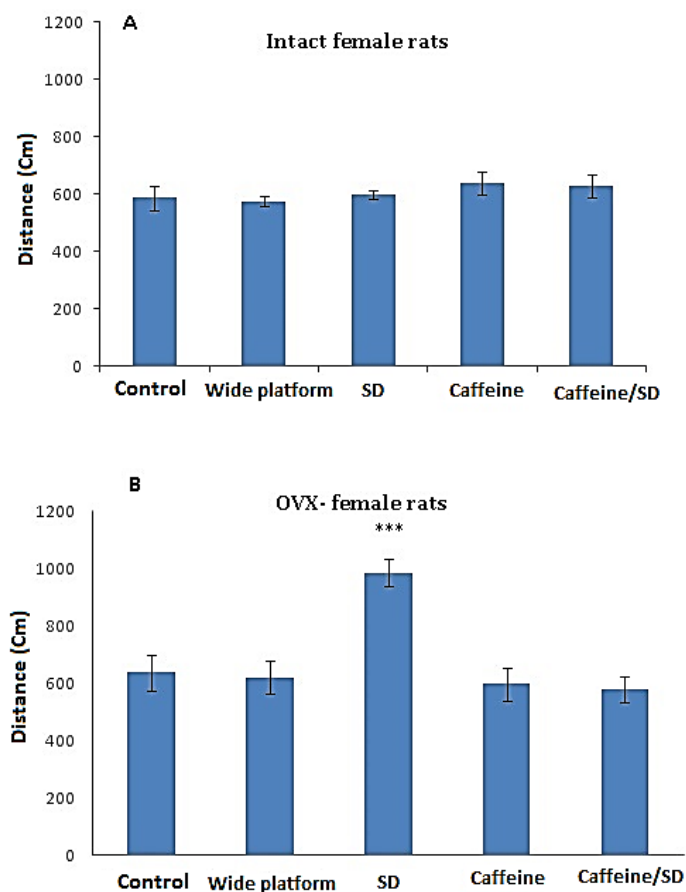Two hours after the acquisition phase, a probe test was performed to determine short term spatial memory

Fig. 1. Effect of sleep deprivation (SD) and/or chronic caffeine treatment on the spatial learning in the Morris water maze test in the intact (A) and ovariectomized (OVX)(B) female rats. Traveled distance to find the hidden platform in the OVX (B) female rats increased compared with other groups .Therefore 4 weeks caffeine treatment attenuated this impairment. Each point is the Mean± SEM. ***P<0.001 indicating the significant differences from all the other groups.

retention. The results of the probe test indicated that the sleep deprived intact and OVX female rats spent less time and shorter distance in the target quadrant compared to the control, sham (wide platform), caffeine and caffeine/SD groups (intact females: P<0.05 for time and distance in the target quadrant; OVX females: P < 0.01 for time and P<0.05 for distance in the target quadrant; Fig. 3 A and B respectively), which showed short term memory deficit. However, this impairment was treated by a 4-week caffeine administration in the caffeine/ sleep deprived (intact and OVX female) groups since they spent more time and distance in the target quadrant than the sleep deprived rats (Fig. 3). There was no significant difference between the performance of the caffeine and caffeine / sleep deprived rats, and that of the control and sham (wide platform) group in the short term memory test in both intact and OVX female rats.

Additionally, the sham operation and control groups of intact and OVX female rats had similar performance in MWM test (results not shown).

The result of the swimming speed and visual test showed no difference in escape latency in search for the visible platform among all female rats (Table 1).

Discussion

In the present study, the effect of chronic caffeine treatment on SD-induced impairment of spatial learning and memory were investigated in intact and ovarectomized female rats. Our findings indicated that 72h SD could impair the learning ability only in the OVX and memory in both intact and OVX rats, but this memory impairment severely worsened in SD-OVX group. Meanwhile caffeine administration reversed these impairments.Much evidence verify the harmful
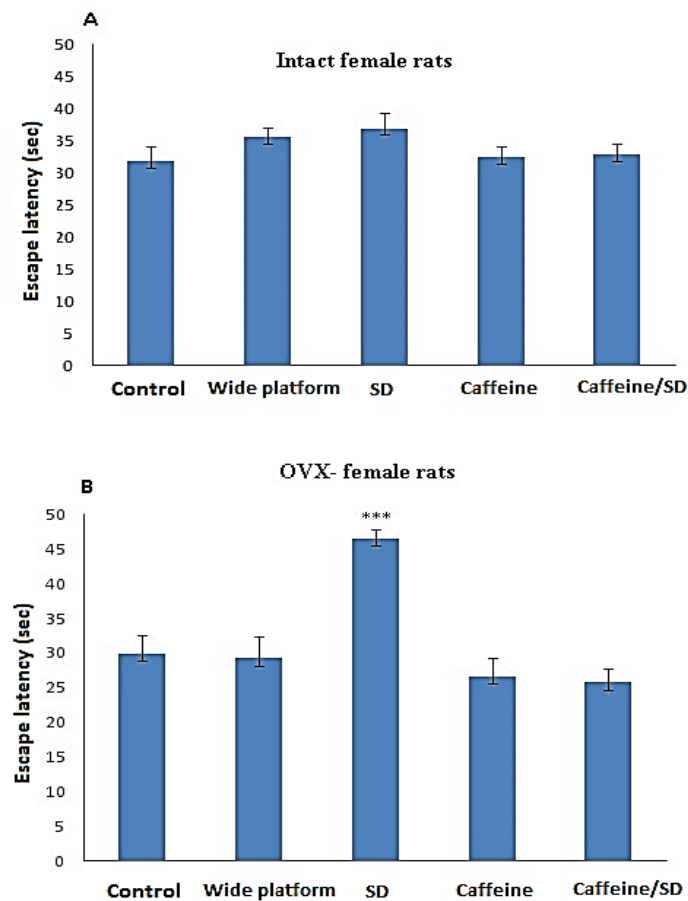
Fig. 2. Effect of sleep deprivation (SD) and/or chronic caffeine treatment on the spatial learning in the Morris water maze test in the intact (A) and ovariectomized (OVX) (B) female rats. Escape latency to reach the hidden platform in OVX female rats enhanced compared with other groups. Meanwhile caffeine administration improved this impairment. Each point is the mean±SEM. ***P<0.001 vs. all groups.

impact of SD on hippocampus-dependent learning and memory (Alvarenga et al., 2008). Additionally, numerous studies have shown that sleep deprivation unfavorably, effects functions of the central nervous system, it especially impairs the ability to form new information and disrupts memory consolidation (Dubiela et al., 2010). Our previous studies indicated that sleep deprivation impaired cognitive function (Hajali et al., 2012; Saadati et al., 2015) and long term potentiation in the CA1 area of the hippocampus of female rats (Saadati et al., 2014b).

In addition, our results show that the sleep deprived OVX female rats have the most impaired learning ability in the MWM task. Therefore, sleep deprived OVX female rats are more susceptible to the effects of sleep deprivation on cognitive functions as compared with intact female animals (Hajali et al., 2012; Hajali et al., 2015; Saadati et al., 2015), furthermore cognitive

impairment is induced by sleep deprivation probably by decreasing BDNF protein and mRNA expression in the ovariectomized female rats (Saadati et al., 2014a).

In addition, other experiments has provided evidence that estrogens have been suggested as possible therapeutic agents for improving cognition in postmenopausal women, Indeed certain types of hormone therapy improve cognition and reduce the incidence of Alzheimer's disease in postmenopausal women (Barha and Galea, 2013). Moreover it was previously shown that estrogen therapy given to postmenopausal women might protect against specific cognitive declines (Sherwin and Henry, 2008). Therefore, it is reasonable to postulate that reproductive hormones have protective effects against cognitive impairments.

In the current investigation, we used the multiple platform method to induce sleep deprivation. This
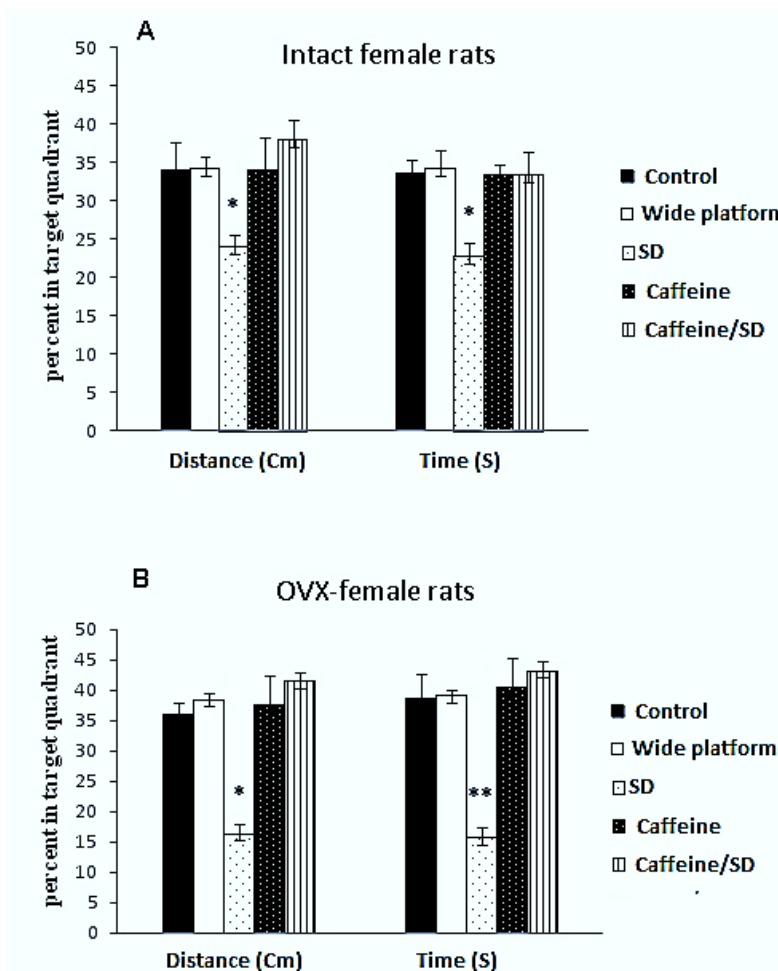
Fig. 3. Effect of sleep deprivation (SD) and/or chronic caffeine treatment on the spatial short term memory in the Morris water maze test in intact (A) and ovariectomized (OVX)(B) female rats. The time and distance in the target quadrant decreased significantly in the SD intact and OVX female groups in compared to the other groups. However, impairment in short- term memory caused by SD in intact and OVX female rats was reversed by 4 weeks caffeine administration.
Each point is the mean± SEM. **P< 0.01 and *P<0.0 5 indicating the significant differences from all the other groups.

method disrupt 95% rapid eye movement (REM) sleep because of loss of muscle tone and can also interferewith NREM sleep (Machado et al., 2004). The best way to control for the aquarium environment in SD studies was the use of wide platform columns. The advantages of these platforms allow rats to be undisturbed. Evidence indicated that cognitive ability of rats on a wider platforms held in aquarium for 24 hours was not significantly different from the home cage control rats (Zagaar et al., 2012). Because animals in the wide platform groups did not show any significant alterations in cognitive performance compared with control groups, as well as the result of the visible platform experiment and swimming speed we have evidence to suggest that 72h of SD has no significant

effect on mood, motivation or sensorimotor coordination of rats. Additionally, there was no significant difference in plasma corticosterone levels among all groups of the female rats in the recent study (Hajali et al., 2012; Saadati et al., 2015). Therefore, we can conclude that such observed impairments do not appear to be related to a non-specific effect of multiple platform technique.

Our data indicated that 4 weeks caffeine administration prevented cognitive impairments induced by sleep deprivation in female rats. Previous studies reported that caffeine prevents the SD-induced reduction in the levels of P-CAMKII and subsequent impairment of LTP in the DG region in male rats (Alhaider et al., 2010a). The protective mechanism of chronic caffeine treatment

Table 1: Swimming speed and latency to escape onto the visible platform in the intact and ovariectomized female rats.

Group	Swimming speed (cm/s)	Escape latency (s)
Intact		
Control	23.25 ± 0. 4	25.33 ± 1.97
Wide platform	21.08 ± 0.48	25.28 ± 3.53
SD	22.57 ± 0.58	27.14 ± 3.76
Caffeine	23.67 ± 0.50	22.71 ± 2.65
Caffeine/SD	23.16 ± 0.48	26.71 ± 1.30
OVX		
Control	21.48 ± 1. 83	23.13 ± 2.37
Wide platform	24.35 ± 1.48	24.37 ± 1.43
SD	23.72 ± 1.15	21.54 ± 3.43
Caffeine	21.64 ± 1.12	22.86 ± 2.48
Caffeine/SD	24.48 ± 1.74	24.31 ± 2.87

Comparisons of swimming speed and latency to escape onto the visible platform in Morris water maze among groups were performed using one way analysis of variance (ANOVA) (the differences were not significant). Data are means ± S.E.M. (8 rats/ group).

against SD-induced E-LTP impairment may involve preventing the decrease in the level of P-CAMKII in sleep-deprivation rats (Pettit et al., 1994). Although caffeine can act by way of several mechanisms of action inclusive of antagonism of adenosine receptors (Kerr et al., 1991). It has been proposed that the concentration of caffeine typically consumed by human may act mostly by inhibiting adenosine receptors (Fredholm, 1995). Nevertheless, some reports show that caffeine has negative effects on cognitive performance (Angelucci et al., 1999). Such discrepancy may be attributed to differences in type of memory test experimental protocols.

Under our experimental situation, 4 weeks caffeine treatment alone has no effect on spatial learning and memory in control groups of intact and OVX animals. However, in the presence of sleep deprivation, caffeine antagonizes the deleterious effect of sleep deprivation on learning and short-term memory. This is consistent with a recent report (Alhaider et al., 2010a). These findings support the concept that maybe caffeine exerts positive effects, when there is cognitive impairment. Therefore, caffeine seems to be a protector rather than a enhancer of cognitive performance.

Conclusion

Collectively, our result showed that sleep deprivation for 72h impaired cognitive performance in the intact and OVX female rats. Also we found that caffeine administration for 4 weeks attenuated this impairment.

Acknowledgments

Conflict of interest

Authors have no declarations of interest to report.

References

Alhaider IA, Aleisa AM, Tran TT, Alkadhi KA. Caffeine prevents sleep loss-induced deficits in long-term potentiation and related signaling molecules in the dentate gyrus. European Journal of Neuroscience 2010a; 31: 1368-1376.

Alhaider IA, Aleisa AM, Tran TT, Alzoubi KH, Alkadhi KA. Chronic caffeine treatment prevents sleep deprivation-

induced impairment of cognitive function and synaptic plasticity. Sleep 2010b; 33: 437.

Alvarenga TA, Patti CL, Andersen ML, Silva RH, Calzavara MB, Lopez GB, et al. Paradoxical sleep deprivation impairs acquisition, consolidation, and retrieval of a discriminative avoidance task in rats. Neurobiology of learning and memory 2008; 90: 624-632.

Angelucci M, Cesario C, Hiroi R, Rosalen P, Cunha CD. Effects of caffeine on learning and memory in rats tested in the morris water maze. Brazilian Journal of medical and biological Research 2002; 35: 1201-1208.

Angelucci ME, Vital MA, Cesário C, Zadusky CR, Rosalen PL, Da Cunha C. The effect of caffeine in animal models of learning and memory. European journal of pharmacology 1999; 373: 135-140.

Barha CK, Galea LA. The hormone therapy, premarin, impairs hippocampus-dependent spatial learning and memory and reduces activation of new granule neurons in response to memory in female rats. Neurobiology of aging 2013; 34: 986-1004.

Barnett RC, Biener LE, Baruch GK. Gender and stress: Free Press, 1987.

Blissitt PA. Sleep, memory, and learning. Journal of Neuroscience Nursing 2001; 33: 208-215.

Dall'Igna OP, Fett P, Gomes MW, Souza DO, Cunha RA, Lara DR. Caffeine and adenosine A(2a) receptor antagonists prevent β-amyloid (25–35)-induced cognitive deficits in mice. Experimental neurology 2007; 203: 241-245.

Dall'Igna OP, Souza DO, Lara DR. Caffeine as a neuroprotective adenosine receptor antagonist. Annals of Pharmacotherapy 2004; 38: 717-718.

Diekelmann S, Born J. The memory function of sleep. Nature Reviews Neuroscience 2010; 11: 114-126.

Dubiela FP, Oliveira MGMd, Moreira KDM, Nobrega JN, Tufik S, Hipólide DC. Inverse benzodiazepine agonist β-ccm does not reverse learning deficit induced by sleep deprivation. Neuroscience letters 2010; 469: 169-173.

Eichling P, Sahni J. Menopause related sleep disorders. J Clin Sleep Med 2005; 1: 291-300.

Ferrara M, Iaria G, Tempesta D, Curcio G, Moroni F, Marzano C, et al. Sleep to find your way: The role of sleep in the consolidation of memory for navigation in humans. Hippocampus 2008; 18: 844-851.

Ferré S. An update on the mechanisms of the psychostimulant effects of caffeine. Journal of neurochemistry 2008; 105: 1067-1079.

Fredholm BB. Adenosine, adenosine receptors and the actions of caffeine. Pharmacology & toxicology 1995; 76: 93-101.

Gais S, Albouy G, Boly M, Dang-Vu TT, Darsaud A, Desseilles M, et al. Sleep transforms the cerebral trace of declarative memories. Proceedings of the National Academy of Sciences 2007; 104: 18778-18783.

Hajali V, Sheibani V, Esmaeili-Mahani S, Shabani M. Female rats are more susceptible to the deleterious effects of paradoxical sleep deprivation on cognitive performance. Behavioural brain research 2012; 228: 311-318.

Hajali V, Sheibani V, Mahani SE, Hajializadeh Z, Shabani M, Aliabadi HP, Saadati H, and Esmaeilpour K. Ovariectomy does not exacerbate the negative effects of sleep deprivation on synaptic plasticity in rats. Physiology & behavior 2015;44: 73-81.

Kerr J, Sherwood N, Hindmarch I. Separate and combined effects of the social drugs on psychomotor performance. Psychopharmacology 1991; 104: 113-119.

Kramar E, Babayan A, Gall C, Lynch G. Estrogen promotes learning-related plasticity by modifying the synaptic cytoskeleton. Neuroscience 2013; 239: 3-16.

Luine V, Frankfurt M. Interactions between estradiol, bdnf and dendritic spines in promoting memory. Neuroscience 2013; 239: 34-45.

Machado RB, Hipólide DC, Benedito-Silva AA, Tufik S. Sleep deprivation induced by the modified multiple platform technique: Quantification of sleep loss and recovery. Brain research 2004; 1004: 45-51.

Manber R, Armitage R. Sex, steroids, and sleep: A review. Sleep 1999; 22: 540-55.

Maren S, De Oca B, Fanselow MS. Sex differences in hippocampal long-term potentiation (ltp) and pavlovian fear conditioning in rats: Positive correlation between LTP and contextual learning. Brain research 1994; 661: 25-34.

Marks CA, Wayner MJ. Effects of sleep disruption on rat dentate granule cell ltp in vivo. Brain research bulletin 2005; 66: 114-119.

McDermott CM, LaHoste GJ, Chen C, Musto A, Bazan NG, Magee JC. Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. The Journal of neuroscience 2003; 23: 9687-9695.

Pettit D, Perlman S, Malinow R. Potentiated transmission and prevention of further ltp by increased camkii activity in postsynaptic hippocampal slice neurons. Science 1994; 266: 1881-1885.

Pires VA, Pamplona FA, Pandolfo P, Fernandes D, Prediger RD, Takahashi RN. Adenosine receptor antagonists improve short-term object-recognition ability of spontaneously hypertensive rats: A rodent model of attention-deficit hyperactivity disorder. Behavioural pharmacology 2009; 20: 134-145.

Reyner L, Horne J, Reyner A. Gender-and age-related differences in sleep determined by home-recorded sleep logs and actimetry from 400 adults. Sleep 1995; 18: 127-134.

Saadati H, Esmaeili-Mahani S, Esmaeilpour K, Nazeri M, Mazhari S, Sheibani V. Exercise improves learning and memory impairments in sleep deprived female rats. Physiology & Behavior 2015; 138: 285-291.

Saadati H, Sheibani V, Esmaeili-Mahani S, Darvishzadeh-Mahani F, Mazhari S. Prior regular exercise reverses the decreased effects of sleep deprivation on brain-derived neurotrophic factor levels in the hippocampus of ovariectomized female rats. Regulatory Peptides 2014a; 194–195: 11-15.

Saadati H, Sheibani V, Esmaeili-Mahani S, Hajali V, Mazhari S. Prior regular exercise prevents synaptic plasticity

impairment in sleep deprived female rats. Brain Research Bulletin 2014b; 108: 100-105.

Sherwin BB, Henry JF. Brain aging modulates the neuroprotective effects of estrogen on selective aspects of cognition in women: A critical review. Frontiers in neuroendocrinology 2008; 29: 88-113.

Smith C. Sleep states and memory processes. Behavioural brain research 1995; 69: 137-145.

Takahashi RN, Pamplona FA, Prediger R. Adenosine receptor antagonists for cognitive dysfunction: A review of animal studies. Frontiers in bioscience: a journal and virtual library 2007; 13: 2614-2632.

Tsai L-L, Li S-P. Sleep patterns in college students: Gender and grade differences. Journal of Psychosomatic Research 2004; 56: 231-237.

Zagaar M, Alhaider I, Dao A, Levine A, Alkarawi A, Alzubaidy M, et al. The beneficial effects of regular exercise on cognition in rem sleep deprivation: Behavioral, electrophysiological and molecular evidence. Neurobiology of Disease 2012; 45: 1153-1162.