Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Caffeine as a potential arousal enhancer: altered NMDA subunit gene expression without improving cognitive performance in REM sleep deprived rats

Leyla Sahin[1*], Ozge Selin Cevik[1], Dilan Deniz Koyuncu[1], Sayad Kocahan[2]

[1] Department of Physiology, Faculty of Medicine, Mersin University, Mersin, Turkey
[2] Department of Physiology, Faculty of Medicine, Adıyaman University, Adıyaman, Turkey

Correspondence to: leyladrm@gmail.com

Received May 8, 2018; **Accepted** February 19, 2019; **Published** February 28, 2019

Doi: http://dx.doi.org/10.14715/cmb/2019.65.2.10

Abstract: Caffeine is one of the most extensively consumed stimulants in the world and has been suggested to induce wakefulness by antagonizing the function of the adenosine A2A receptor. Therefore, we investigated the effects of chronic caffeine consumption on learning and memory in the REM sleep-deprived rats. Male Wistar rats (n = 50), were randomly assigned into 5 groups: Control (C), Caffeine (Cf), Pedestal Control (PC), Sleep Deprivation (SD), Sleep Deprivation and Caffeine (SD + Cf). Sleep deprivation procedure was applied as the flower-pot technique. SD and SD + Cf groups were deprived for 18 hours in a day for 21 days. Caffeine was administered daily in drinking water (0.3 g/L) for 5 weeks. For evaluated learning and memory function, Morris Water Maze Test (MWM) was used. Fluidigm Access Array was used for Grin2a, Grin2b, BDNF, cdk5/cdk5r1, CaMKIIa genes expression in the hippocampus. Distance moved and escape latency were decreased through trial days ($p<0.05$). However, there is no significant difference between groups for time spent in targeted quadrant during probe test for memory performance. Grin2a up-regulation was found in Cf and SD+Cf ($p<0.05$), and cdk5r1 increased in Cf and PC control ($p<0.05$). Also, BDNF up-regulation was found in PC group. Grin2b, Cdk5, CaMKIIa expression levels were not changed significantly. We showed chronic caffeine altered some of the hippocampal genes without changing learning and memory in REM sleep deprived rats. Chronic consumption of caffeine caused up-regulation in Grin2a that subunit of NMDA receptor. We supposed that chronic caffeine consumption maintained arousal without affecting learning and memory performance.

Key words: Caffeine; CaMKIIa; MWMT; NMDA receptors; REM-sleep deprivation.

Introduction

It is clearly explained from molecular to phenomenological levels that sleep greatly contributes to the processes of memory and learning (1). Sleep can be primarily divided into 2 main stages as the non-rapid eye movement (non-REM) followed by a shorter period of the rapid eye movement (REM) sleep that especially important in terms of the consolidation and retention of memories (2-5). Not surprisingly, many studies have indicated a strong correlation between the sleep deprivation and memory impairment both in humans and animals (6-8).

Caffeine-contained soft drinks and medications are widely preferred by many individuals to reduce the deleterious effects of sleeplessness. Additionally, caffeine is an important pharmacological substance in the involvement of adenosine receptors, which have significant roles in the cognitive functions and arousal (9,10). The molecular mechanism of caffeine can be explained as follows: Particularly, adenosine A1 receptor is coupled with inhibitory G- protein (Gi) that decreases the cyclic adenosine monophosphate (cAMP) levels. Adenosine is also known to prevent the release of glutamate from the presynaptic nerve and stabilizes Mg^{2+} ion on N-methyl D-aspartate (NMDA) receptors, in turn; inhibits NMDA receptor excitation (11,12). Adenosine also increases postsynaptic K^+ conductance, and decreases presynaptic Ca2+ conductance. Especially during sleep deprivation, extracellular adenosine level increases and causes activation of Gi protein-coupled adenosine A1 receptor and elucidates a decrease in transmitter release from the excitatory presynaptic terminals. Alhaider et al., (13) have shown that caffeine as an adenosine antagonist has attenuated the spatial short-term memory and E-LTP impairment induced after 24 h sleep deprivation. In addition, Sahu et al., (14) have found that caffeine has promoted the adult neuronal cell proliferation after 48 h total sleep deprivation in the rat dentate gyrus.

Memory is one of the most important cognitive functions and occurs in the hippocampus, especially in the CA1 region. Cyclin-dependent kinase (Cdk) 5 is highly expressed in the hippocampus and is required for binding of a regulatory subunit protein, p35 with its activator Cdk5r1 (15). Cdk5 also controls the neurotransmitter release from presynaptic sites, endocytosis of the NMDA receptors at the postsynaptic sites, in turn; regulates the synaptic plasticity and memory processes (16). Brain-derived neurotropic factor (BDNF) plays a major role in synaptic plasticity of the hippocampus and hippocampal-dependent learning and memory. According to the studies, it was known that there is strong interaction between BDNF, Cdk5 and NMDA receptor activations (17). Alhaider et al., (18) suggested that chronic caffeine treatment may protects the sleep-deprived brain; possibly via preserving the levels of phos-

phorylated cyclic AMP-responsive transcription factor (P-CREB) and BDNF of the 24 h sleep-deprived rats. Costa et al., (19) have found that caffeine has improved the performance of adult mice in object recognition task and has increased the level of BDNF in the hippocampus.

To the best of our knowledge, there is no study about the simultaneous effects of chronic caffeine and long-term REM sleep deprivation on the hippocampus-dependent learning and memory in literature. Hence, the aim of this study was to evaluate the possible effects of chronic caffeine treatment on long-term REM sleep deprived rats via learning and memory test, Morris Water Maze Task (MWMT) and investigate the expression levels of hippocampal learning-related genes like Grin 2a, Grin 2b, Cdk5/Cdk5r1, BDNF, CaMKIIa.

Materials and Methods

Experimental protocol

All experimental procedures were carried out according to the guidelines of National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. The ethical approval of the study was taken from the local ethical committee of Mersin University (Approval No: 52602694.050/58). The study has included 50 post-natal, 28 days Wistar Albino rats which were randomly assigned into 5 groups (n = 10 per group) as control (C), caffeine (Cf), pedestal control (PC), sleep deprivation (SD) and sleep deprivation/caffeine (SD +Cf). The rats were fed with tap water and purina rodent chow ad lib. All applications and tests were carried out in the Physiology laboratory of Mersin University Medical Faculty. During all procedures, both the numbers of animals used and their suffering were attentively watched out.

Caffeine treatment

Caffeine (Sigma Aldrich, Saint Louis, MO, USA) was administered via drinking water (0.3 g / L) chronically for 4 weeks and prepared daily (13). The daily consumption of caffeine by each rat was ensured to be nearly 16–20 mg. The amount of water consumed by rats in the caffeinated groups for 4 weeks was found similarly with the control group.

REM sleep deprivation procedure

REM sleep deprivation was provided via placing the experimental animals into a Plexiglas tank with multiple small platforms filled with water (24°C±1°C). In the literature, this technique is known as the flower-pot method (20). The small platforms with 6 cm diameter prevents animals to fall asleep; otherwise, they fall into the water and wake up. When rats on 6-cm platforms lapse into REM sleep, they lose muscle tone, make facial contact with or fall into the surrounding water, rapidly awaken, and the cycle is repeated. Therefore, the flower-pot method is selective for eliminating REM sleep. PC animals were also placed in a similar water tank with 10 cm diameter platforms that allowed rats to sleep. All SD, SD +Cf and PC animals have remained on the platforms from 14:00 p.m. to 08:00 a.m. and stayed in the vivarium from 08:00 a.m. to 14:00 p.m. for 21-days. Control rats have normally remained in their home cages all day.

Behavioral experiments: Morris water maze task procedure

A circular stainless-steel tank that has a diameter of 1.5 m and 0.6 m in depth was used in the MWMT. The tank was filled with water to a depth of 0.5 m. The water and room temperature were adjusted to the temperature of 22±1°C. A curtain ornamented with several marked visual cues surrounded the tank. Inside the tank, there was a circular platform that has a diameter of 15 cm. In order to record the swimming track of the test animals, a camera was used that placed above the tank. The behavioral data were recorded and analyzed by a visual analysis system (EthoVision, Noldus Information Technology, Wageningen, NL). The parameters have recorded during the experiments including latency, the path length of rats to reach the platform in meter (m), swimming velocity in cm/s, time spent in the targeted quadrant. All experiments were conducted between 9:00 a.m. to 13:00 p.m. for 6 days.

Long-term spatial memory training

The classic MWMT training method has initiated (20, 21). The day before the training period, the animal was gently released into the water without a platform, facing the wall. The animal was allowed to swim freely for 120 s to get used to the tank environment. The whole water surface was equally divided into 4 quadrants (southeast, southwest, northeast, and northwest) without physical boundaries. The first day of the training platform was fixed at all of the quadrants and center, 1.5 cm underneath the water surface. Each animal was trained 5 times (120 s for each, interval=5 min). Animals were semi-randomly released into the water from the east, south, west and north directions, respectively. If animals did not found the platform in 120 s, experimenter directed the rat gently towards to it and let the animal rest for 10 s. Each animal was trained 5 times (60 s for each, interval=5 min) per day for 4 successive days. During training, the platform was placed southwestern quadrant, the animal was allowed to rest for 5 s. If animal would find the platform, we assumed that animal has remembered the platform location.

Cognitive assessment

A final memory test was also conducted while the platform removed from the target quadrant. The animal was allowed to explore the tank for 60 s; then the time spent in target quadrant which previously contained the platform (22). Then, animals were sacrificed after MWMT was completed (Fig. 1). All rats were decapitated under ketamine-xylazine at nearly 09:00 am ± 30

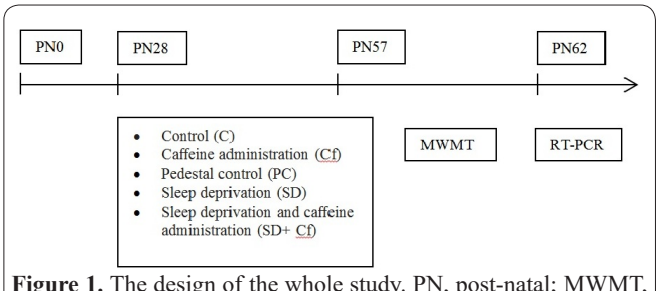

Figure 1. The design of the whole study. PN, post-natal; MWMT, Morris Water Maze Test.

min.

Chemicals

All chemicals utilized in the experiments were purchased from Sigma–Aldrich, USA unless otherwise stated specifically.

Extraction of total RNA and reverse transcription

Both sides of the hippocampus of rats were immediately isolated inside phosphate buffer solution (PBS) for analysis after decapitation procedure. First, total RNA isolation was prepared manually by using TRIzol (Invitrogen). The manually sectioned hippocampal specimens were put into Eppendorf tubes including RiboZol as 1 ml RiboZol for each 5-10 mg tissue sample. After incubation (10 min), 200-µl chloroform was added and mixed. Then the solution was centrifuged for 15 min at 12000 RCF. After centrifugation, 500 ml of isopropanol was added and all samples have waited for 10 min at room temperature. An additional centrifugation was done for 10 min, again. The supernatant was removed, and the pellet was collected. Ethanol was added to the pellet as 1 ml of ethanol for every 1 ml of RiboZol. Then, the mixture was centrifuged at 7500 RCF for 5 min.

Finally, ethanol was removed and collected RNA was dissolved in an RNase-free solution for cDNA synthesis. 4 ul total RNA extraction was used as a template into synthesis of cDNA using Fluidigm Reverse Transcription Kit in accordance with the manufacturer's instructions to obtain cDNA. Individual reactions were carried out with using thermal condition (Bioer, Gene Pro Thermal Cycler): 25 ˚C for 5 minutes, 42 ˚C for 30 minutes, 85 ˚C for 5 minutes, 4°C for ∞.

Quantitative Real-time polymerase chain reaction

Quantitative real-time (RT)-PCR was run on a Fluidigm Biomark Real-Time PCR using Taqman GE Master Mix. The reaction was carried out using 40 amplification cycles of 50 °C for 2 minutes, 70 °C for 30 minutes, 25 °C for 10 minutes, 96 °C for 15 seconds and 60 °C for 1 minutes. Assay ID numbers of primers have shown in Table 1. The relative expression of genes was calculated by the comparative $2^{-\Delta\Delta Ct}$ method using Rpl5 RNA levels as internal control.

Statistical analysis

All data are expressed as mean values ± SEM. The normal distribution of data in each group was controlled by the Shapiro Wilk test. For normally distributed data of behavioral test and genes expression level, statistical analysis was performed by using one-way ANOVA, repeated measures analysis of variance (ANOVA) and Tukey post hoc comparisons. The significance level of $p < 0.05$ was considered for statistically significance.

Table 1. Primers for quantitative RT-PCR.

Gene Symbol	TaqMan Assay ID
Grin2a	Rn00561341_m1
Grin2b	Rn00680474_m1
BDNF	Rn02531967_s1
Cdk5	Rn04219635_m1
Cdk5r1	Rn02132948_s1
CaMKIIa	Rn01258147_m1
Rpl5	Rn01531116_g1

Results

Spatial learning and memory after chronic sleep deprivation and caffeine treatment

The distance move to reach the platform was significantly differed between trial days (F=4,702, p=0.005). The distance moved significantly decreased in 3rd and 4th trial days compared to the 1st day of training period of MWMT (F =5.31, p=0.026; F=11.95, p=0.001). According to the between-group comparisons, the distance moved significantly differed between all groups (p<0.001 for each trial days). Both the SD and SD+Cf groups have moved significantly higher distances compared the other groups (p<0.05, and p<0.001, respectively) (Fig. 2A).

There was no significant difference in terms of the swimming velocity between trial days (p>0.05). However, it has changed significantly between all groups in 4 trial days (p<0,001) (Fig. 2B).

The escape latency for rats to reach the platform significantly differed between trial days (F=5,077 p=0.003). In addition, escape latency significantly differed between all groups (F=10.17, p<0.001) (Fig. 2C). There was no significant difference between all groups in time spent in the targeted quadrant during trial days and probe test (p>0.05).

Gene expression level by the qRT-PCR method

The mRNA expression levels of Grin2a were significantly higher in SD and SD+Cf groups than other groups (p<0.05), while Grin2b expression levels did not significantly differ between all groups (Fig. 3A). The fold change expression level of BDNF was significantly higher in the PC group than other groups (p<0.05),

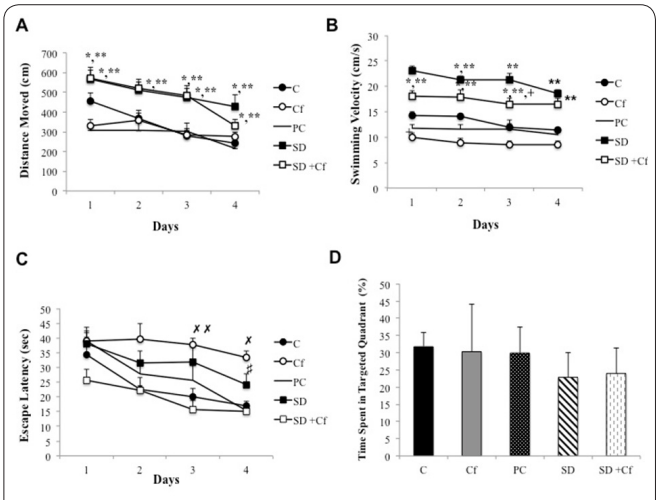

Figure 2. Morris water maze performances. MWMT performances of C, Cf, PC, SD and SD +Cf groups, the distance moved to reach the platform (cm) (A), swimming velocity (cm/s) (B), escape latency (s), and time spent in targeted quadrant (%). All values represent the mean ± SD from 10 male rats in each group. '*' p<0.05: Significant difference from C, Cf, PC rats, '**' p<0.001: Significant difference from C, Cf, PC rats, '+' p<0.001: Significant difference from SD rats, X' p<0.001: Significant difference from C, PC, SD+Cf rats, X X' p<0.001: Significant difference from C, SD+Cf rats and '♯' p<0.05: Significant difference from C, PC rats.

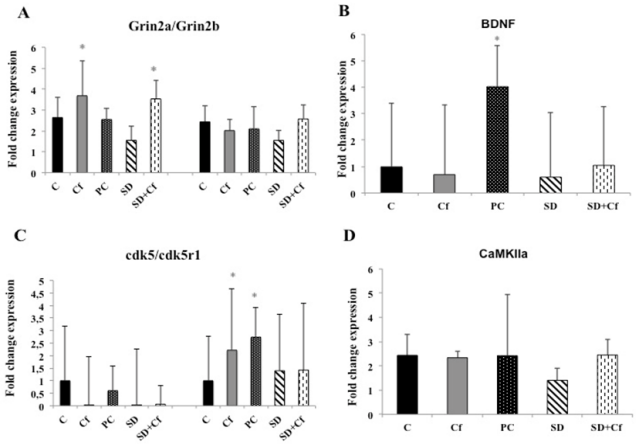

Figure 3. Expression levels of Grin2a, Grin2b, BDNF, cdk5/cdk5r1, CaMKIIa. Graphs showing the levels of fold change expression of Grin2a and Grin2b (A), BDNF (B), cdk5/cdk5r1 (C), CaMKIIa (D) genes in hippocampal regions of 5 groups. All values represent the mean ± SD from 5 male rats in each group * $p < 0.05$: Significant difference from C rats.

(Fig. 3B). There was no significant difference between all groups in terms of Cdk5 expression levels, but the expression level of Cdk5r1 was significantly higher in Cf and PC groups (p>0,05), (Fig. 3C). In addition, the fold change expression levels of CaMKIIa did not significantly differ between all groups (p>0.05), (Fig. 3D).

Discussion

Many studies have reported that acute sleep deprivation causes certain impairments in spatial memory (23). In a study with acute sleep deprivation indicated that, the maze performance of sleep-deprived rats has decreased compared to the control group after an acute (48 h) sleep deprivation (24). In our study, we have observed a lower MSWT performance during trial days in the sleep-deprivated groups (SD and SD+Cf) as they have significantly longer path length distances to reach the platform and shorter time spent in the targeted quadrant compared to the other groups although it was not significant. In addition, we have also observed the path length to reach the platform and escape latency decreased gradually during trial days showing that rats learned the location of the platform before test day but they have failed in the memory test. In another striking study including acute sleep-deprived individuals has indicated a decline in memory performances, but surprisingly while the duration of deprivation was prolonged, people have increased in mental effort and perceptual motor skills (25). Possibly, extended the duration of stress for chronic period have caused habituation to the perceived stress. Because in our another study, stress protocol was applied chronically as social isolation and rats cognitive performances did not change significantly, also their corticosterone blood concentration remained constant between group (26).

Caffeine is a non-specific adenosine receptor antagonist and uses as a psychostimulant in animal models of learning and memory. In our study, we have administered caffeine chronically for 4 weeks. According to the data of training period of MWMT, caffeine administration did not change learning (trial days) process of rats since both Cf and SD+Cf groups have exhibited close performance compared with other groups. Similarly, we have not observed any significant difference in performance of our caffeinated groups during probe test according to the variable of time spent in the targeted quadrant. From our results, we can indicate that chronic caffeine consumption has not affect hippocampal-dependent spatial memory. Angelucci et al. have indicated the post- water maze training administration of caffeine has improved the memory retention at the doses of 0.3-10 mg/kg (27). However, the caffeine administration in the pre-training period has not change the performance of the animals in the maze. In addition, Soussi et al., (28) have reported that caffeine has attenuated the detrimental effects of the sleep deprivation for 36 h on the cognitive performance. Similarly, Wadhwa et al., (29) have indicated that caffeine has lessened the effects of acute sleep deprivation via modulating the memory of object recognition and the synaptic proteins of rat hippocampus after 48 h sleep deprivation. Based on this information, caffeine administration in acute REM sleep deprivation improved the learning and memory processes, but not when it was chronically applied. In Stickgol study (30), they have found that when subjects deprived of sleep for 30 hours and tested after 2 full nights of recovery sleep; they showed no significant improvement in visual discrimination task, despite normal levels of alertness. During the deprivation days in our chronic period, rats have allowed to sleep from 08:00 a.m. to 14:00; this recovery sleep may be replaced the effects of REM sleep deprivation on cognitive tasks.

The main targets of caffeine are A1 and A2A receptors and have a psychostimulant effect, involves in memory normalization (31) and the motor activating processes (32). In our results, up-regulated Grin2a (NR2A) gene expression level was found in chronic caffeine application with sleep deprivation group (SD+Cf). In addition, we have observed a significantly higher Grin2a expression level in the Cf group, in which rats also consumed caffeine chronically. Our results also have shown that all the expression levels of Grin2b (NR2B), BDNF and CaMKII have not changed significantly. The enrichment effect of expanded environment may induce an increment in BDNF level of PC compared to control group. On the other hand, up-regulated Cdk5r1 gene expression level was found in Cf and PC groups compared to other groups. Up-regulation of Cdk5r1 depends on down-regulation of Cdk5 expression. BDNF mediates NMDAR potentiation through activation of TrkB and phosphorylation of the NR2B subunit (33). In addition, the Cdk5/p35 pathway has been known closely related to NR2B gene in spatial learning and memory (17, 34). In our results; BDNF, Cdk5 and Grin2b expression levels have remained constant after caffeine and sleep deprivation protocol. Hence, it can be said that caffeine has no potential effect on neurons through the modulation of the cdk5/p35, BDNF and related NR2B genes in the chronic period. Together with gene analysis and MWMT, we could say that chronic sleep deprivation has not affect learning and memory. Nevertheless, surprisingly, we can clearly point out that consuming caffeine chronically caused alterations in Grin2a expression level.

Adenosine receptors are not just involved in lear-

ning and memory, they also responsible for arousal and motor activity (31). Especially the locus coeruleus (LC) is a main noradrenergic brain nucleus that adjusts states of arousal that has NMDA receptors like hippocampus (35). The neurotransmitter glutamate appears to be involved in most LC functions (36). The study have indicated that glutamatergic activation of the LC induces the release of norepinephrine in the hippocampus and initiates the delayed synaptic potentiation of perforant path input to the dentate gyrus in awake rats (37). Moreover, we have known that Grin2a subunit involves in motor activity, especially in Parkinson Disease. In a study of Simon et al., they have demonstrated the strong relationship between caffeine, Grin2a, and Parkinson Disease (PD) progress. In addition, they have indicated that the association might be influenced by the genotype of Grin2a, which encodes a NMDA glutamate-receptor subunit (38). A role of caffeine and other A2a receptor antagonists in protection against cell death was also noticed both in animal models and in vitro studies of PD (39-41). Our results strongly supported the overexpression of Grin2a subunit genes in caffeinated groups. Also in our MWMT results, SD+Cf group's velocity and total distance moved higher compare to the other groups. We can say that caffeine may improve locomotor activity in sleep-deprived rats. Moreover, caffeine was applied through systemic circulation in our study; in contrast to the direct infusion to the hippocampal region. It can be hypothesized that caffeine may triggers LC activity, causes up-regulation of Grin2a subunit genes, and delays the synaptic potentiation of the hippocampus. Surprisingly, in our results Grin2b expression level did not change significantly. These alterations also demonstrated that chronic caffeine consumption did not improve learning and memory performances, but possibly related to arousal.

In literature, our study is the first to indicate the effects of chronic caffeine consumption on prolonged sleep deprivation by hippocampal related gene expression profile. We indicate the effects of caffeine on the chronic period without altering the cognitive processes and the strong relationship between Grin2a and caffeine. However, we have not still obvious knowledge about the molecular function of Grin2a gene. Studies about the neuroprotective effects of caffeine should be evaluated to elucidate the underlying mechanism on NMDA gene expression.

Acknowledgments

This research was supported entirely by the Department of Scientific Research Projects (No. BAP-2015-AP4-1313).

References

1. Stickgold R. Sleep-dependent memory consolidation. Nature 2005; 437: 1272-8.

2. Dos Santos AC, Castro MA, Jose EA, Delattre AM, Dombrowski PA, Da Cunha C, et al. REM sleep deprivation generates cognitive and neurochemical disruptions in the intranigral rotenone model of Parkinson's disease. J Neurosci Res 2013; 911: 508-16.

3. Proenca MB, Dombrowski PA, Da Cunha C, Fischer L, Ferraz AC, Lima MM. Dopaminergic D2 receptor is a key player in the substantia nigra pars compacta neuronal activation mediated by REM sleep deprivation. Neuropharmacology 2014; 761:18-26.

4. Smith C. Sleep states and memory processes. Behav Brain Res 1995; 69: 137-45.

5. Stone MJ. Memory performance after arousal from different sleep stages. Br J Psychol 1977; 681: 77-81.

6. Ferre S. An update on the mechanisms of the psychostimulant effects of caffeine. J. Neurochem 2008; 105: 1067-79.

7. Kim EY, Mahmoud GS, Grover LM. REM sleep deprivation inhibits LTP in vivo in area CA1 of rat hippocampus. Neurosci Lett 2005; 388: 163-7.

8. Polzella DJ. Effects of sleep deprivation on short-term recognition memory. Journal of experimental psychology. Exp Psychol Hum Learn 1975: 104; 194-200.

9. Dall'Igna OP, Souza DO, Lara DR. Caffeine as a neuroprotective adenosine receptor antagonist. Ann Pharmacother 2004: 387; 17-8.

10. Kopf SR, Melani A, Pedata F, Pepeu G. Adenosine and memory storage: effect of A(1) and A(2) receptor antagonists. Psychopharmacology 1999: 146; 214-9.

11. De Mendonca A, Sebastiao AM, Ribeiro JA. Inhibition of NMDA receptor-mediated currents in isolated rat hippocampal neurons by adenosine A1 receptor activation. Neuroreport 1995: 6; 1097-100.

12. Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 2001: 243; 1-55.

13. 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 2010: 33; 437-44.

14. Sahu S, Kauser H, Ray K, Kishore K, Kumar S, Panjwani U. Caffeine and modafinil promote adult neuronal cell proliferation during 48 h of total sleep deprivation in rat dentate gyrus. Exp Neurol 2013: 248; 470-81.

15. Tsai LH, Delalle I, Caviness VS, Jr Chae T, Harlow E. p35 is a neural-specific regulatory subunit of cyclin-dependent kinase 5. Nature 1994: 317; 419-23

16. Kawauchi T. Cdk5 regulates multiple cellular events in neural development, function, and disease. Dev Growth Differ 2014: 56; 335-48.

17. Plattner F, Hernández A, Kistler TM, Pozo K, Zhong P, Yuen EY, Guo, A. Memory enhancement by targeting Cdk5 regulation of NR2B. Neuron 2014: 8; 1070-83.

18. Alhaider IA, Aleisa AM, Tran TT, Alkadhi KA. Sleep deprivation prevents stimulation-induced increases of levels of P-CREB and BDNF: protection by caffeine. Mol Cell Neurosci 2011: 46; 742-51.

19. Costa MS, Botton PH, Mioranzza S, Ardais AP, Moreira JD, Souza DO, et al. Caffeine improves adult mice performance in the object recognition task and increases BDNF and TrkB independent on phospho-CREB immunocontent in the hippocampus. Neurochem Int 2008: 53; 89-94.

20. Süer C, Dolu N, Artis AS, Sahin L, Yilmaz A, Cetin A. The effects of long-term sleep deprivation on the long-term potentiation in the dentate gyrus and brain oxidation status in rats. Neurosci Res 2011: 70; 71-77.

21. Akillioglu K, Babar Melik E, Melik E, Kocahan S. The investigation of neonatal MK-801 administration and physical environmental enrichment on emotional and cognitive functions in adult Balb/c mice. Pharmacol Biochem Behav 2012: 102; 407-14.

22. Ancoli-Israel S. Sleep and aging: prevalence of disturbed sleep and treatment considerations in older adults. J Clin Psychiatry 2005: 66; 24-30.

23. Xie M, Yan J, He C, Yang L, Tan G, Li C, et al. Short-term sleep deprivation impairs spatial working memory and modulates expression levels of ionotropic glutamate receptor subunits in hippocampus. Behav Brain Res 2015: 286; 64-70.

24. Alhaider IA, Aleisa AM, Tran TT, Alkadhi KA. Caffeine pre-

vents sleep loss-induced deficits in long-term potentiation and related signaling molecules in the dentate gyrus. Eur J Neurosci 2010: 311; 368-76.
25. Samkoff JS, Jacques CH. A review of studies concerning effects of sleep deprivation and fatigue on residents' performance. Acad Med 1991: 66; 687-93.
26. Cevik OS, Sahin L, Tamer L. Long term treadmill exercise performed to chronic social isolated rats regulate anxiety behavior without improving learning. Life Sci J 2018: 200; 126-133
27. Angelucci ME, Cesario C, Hiroi RH, Rosalen PL, Da Cunha C. Effects of caffeine on learning and memory in rats tested in the Morris water maze. Braz J Med Biol Res 2002: 351; 201-8.
28. Souissi M, Chtourou H, Abedelmalek S, Ghozlane IB, Sahnoun Z. The effects of caffeine ingestion on the reaction time and short-term maximal performance after 36 h of sleep deprivation. Physiol Behav 2014: 13; 11-6.
29. Wadhwa M, Sahu S, Kumari P, Kauser H, Ray K, Panjwani U. Caffeine and modafinil given during 48 h sleep deprivation modulate object recognition memory and synaptic proteins in the hippocampus of the rat. Behav Brain Res 2015: 294; 95-101.
30. Stickgold R, James L, Hobson JA. Visual discrimination learning requires sleep after training. Nature Neurosci 2003: 121; 237.
31. Cunha RA, Agostinho PM. Chronic caffeine consumption prevents memory disturbance in different animal models of memory decline. J Alzheimers Dis 2010: 20; 95-116.
32. Huang ZL, Qu WM, Eguchi N, Chen JF, Schwarzschild MA, Fredholm BB, et al. Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. Nature Neurosci 2005: 88; 58-9.
33. Hildebrand ME, Xu J, Dedek A, Li Y, Sengar AS, Beggs S, Salter MW. Potentiation of synaptic GluN2B NMDAR currents by Fyn kinase is gated through BDNF-mediated disinhibition in spinal pain processing. Cell Rep 2016: 17; 2753-2765.
34. Mishiba T, Tanaka M, Mita N, He X, Sasamoto K, Itohara S, Ohshima T. Cdk5/p35 functions as a crucial regulator of spatial learning and memory. Mol Brain 2014: 7; 82.
35. Noriega NC, Garyfallou VT, Kohama SG, Urbanski HF. Glutamate receptor subunit expression in the rhesus macaque locus coeruleus. Brain Res 2007: 11; 53-65.
36. Brann DW, Mahesh VB, Glutamate: a major neuroendocrine excitatory signal mediating steroid effects on gonadotropin secretion. J Steroid Biochem Mol Biol 1995: 53; 325–329.
37. Walling SG, Harley CW. Locus ceruleus activation initiates delayed synaptic potentiation of perforant path input to the dentate gyrus in awake rats: a novel β-adrenergic-and protein synthesis-dependent mammalian plasticity mechanism. J Neurosci 2004: 24; 598-604.
38. Simon DK, Wu C, Tilley BC, Lohmann K, Klein C, Payami H, Hauser RA. Caffeine, creatine, GRIN2A and Parkinson's disease progression. J Neurol Sci 2017: 375; 355-359.
39. Schwarzschild MA, Xu K, Oztas E, Petzer JP, Castagnoli K, Castagnoli N, Chen JF. Neuroprotection by caffeine and more specific A2A receptor antagonists in animal models of Parkinson's disease. Neurology 2003: 61; 55–61.
40. Kachroo A, Irizarry MC, Schwarzschild MA. Caffeine protects against combined paraquat and maneb-induced dopaminergic neuron degeneration. Exp Neurol 2010: 223; 657–661.
41. Kachroo MA, Schwarzschild MA. Adenosine A2A receptor gene disruption protects in an alpha-synuclein model of Parkinson's disease. Ann Neurol 2012: 71; 278–282.