Caffeine/sleep-deprivation interaction in mice produces complex memory effects

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KEY WORDS

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ABSTRACT

Background: Sleep deprivation negatively impacts memory, causing deficits in memory processes. Of interest is any agent that can offset such deficits. Mice were given varying doses of caffeine for 14 days and then deprived of sleep for 6 hours by the 'gentle handling' method. Memory was assessed using the Novel Object Recognition Test and Y maze alternation.
Purpose: The study was designed to ascertain the impact of varying doses of caffeine combined with total sleep-deprivation on spatial and non spatial memory in mice.
Methods: Adult Swiss Webster mice of both sexes were assigned to six groups viz., vehicle (distilled water), or one of five selected doses of caffeine (10, 20, 40, 80 and 120 mg/kg) for 14 days via the oral route. Open field novel object recognition test and Y maze spatial working memory tests were carried out on day 14. Results were analysed using multi-factorial ANOVA followed by Tukey HSD test and expressed as mean ± S.E.M, with p values less than 0.05 were considered statistically significant.
Results: Novel object recognition tests (NOR) revealed that pre-training and pre-test sleep deprivation and caffeine combination impaired non spatial and spatial memory in male and female mice.
Conclusion: The study shows the complex interactions with memory that may arise when total sleep deprivation is superimposed on caffeine administration.

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Introduction

The mammalian brain normally goes through cycles of neural and metabolic activity associated with distinct biological states of wake and sleep.[1] Sleep is a natural physiological process whose hallmark is the cyclic occurrence of two main stages: non-rapid-eye movement (non-REM) sleep followed by a rapid-eye movement (REM) sleep with the duration of the REM sleep being shorter.[2] Of crucial importance is sleep in the adequate functioning of memory and neuronal plasticity,[3–5] coupled with reinforcement of connections between neuronal networks for memory consolidation in the hippocampus with optimal sleep.[6,7] Increased hippocampal activity is seen during sleep following a learning task;[8] this increment enhances hippocampal dependent memory.[9] In humans however, pressure to excel often result in self-imposed sleep restriction. Considering how many hours of overnight sleep is actually 'enough'; seven to eight hours of sleep has been deemed vital for optimal cognitive performance.

Sleep deprivation (SD) has been defined as either a complete or partial lack of sleep.[10] Deficits in cognitive function as a result of sleep loss have been reported globally.[11] Ample evidence exists in support of a correlation between sleep deprivation and memory impairment in humans and animals.[5,12–17] Some studies have reported that SD prior to learning reduced learning ability and impaired memory;[18,19] others argue that SD following learning impairs memory formation.[20–22] While sleep loss causes a significant decrease in hippocampal activity[23] the hippocampus becomes more active when subjects are allowed to sleep following a learning task.[8]

Stimulants, mostly caffeinated beverages are increasingly being relied upon to maintain alertness, increase awareness and repress the need for sleep in situations of sleep loss. Caffeine is a common central nervous system stimulant[24] which has also been shown to be important in the modulation of learning and memory functions.[25] Caffeine has been shown to enhance cognitive function in both clinical and animal studies,[26] it also alleviates memory impairment in animal models of brain disorders including Alzheimer disease[25,27,28] and Parkinson disease.[29] Although several studies have shown that caffeine and sleep deprivation have both positive and negative effects respectively on learning and memory, the impact of caffeine and sleep deprivation, in combination, on cognitive function is still a matter of debate. Little or no work has yet been done on the effect of caffeine and sleep deprivation on spatial and non spatial learning and memory and hence this study was designed. Our intention was to study the effect of caffeine and sleep deprivation combination on spatial and non spatial learning and memory.

Methods

Subjects

Swiss Webster mice (Empire Breeders, Osogbo, Osun State, Nigeria) weighing 18–20 *g* at the commencement of this study were used. Mice were housed in plastic cages measuring 16 × 12 × 10 inches (6 mice in each cage). Housing is a temperature-controlled (22.5°C ± 2.5°C) quarters with 12 hours of light. Mice had free access to food and water except during the

behavioural tests. The experimental protocol was approved by the University Animal Ethics Committee. All guidelines applicable applying to animal safety and care were observed.

Apparatus/Behavioural testing

The behavioural models included the open field for the novel object recognition (NOR) test and Y maze for spatial learning and memory tests. Mice in respective groups received vehicle (distilled water) or one of five doses of caffeine (10, 20, 40, 80 and 120 mg/kg) orally for a period of 14 days. Neurobehavioural study was carried out on day 14 for Y maze spontaneous alternation (last dose of caffeine or vehicle was administered 30 minutes before the test). Mice were sleep deprived for 6 hours before test; and for the NOR test, acclimatization commenced on day 10; the acquisition phase was on day 13 (mice received caffeine/ vehicle 30 minutes before training) and test was on day 14 (caffeine or vehicle was administered 30 minutes before test), mice were sleep-deprived for 6 hours pre-training and pre-test. Mice in the sleep deprivation group were subjected to 'gentle handling' which consists of keeping the animal awake by tapping on the cage and, if necessary, by gently touching them with a soft brush if behavioural signs of sleep are observed. The animals were gently handled for 6 hours and immediately submitted to behavioural tasks. At the beginning of the behavioural test, each animal was placed in the apparatus and videotaped for subsequent analysis.

Novel object recognition test (Open Field Box) or NOR test

The experimental apparatus consisted of an open-field box ($36 \times 30 \times 26$ cm). The apparatus was located in a quiet room. The NOR test was performed according to the method reported.[30,31] All groups of mice were subjected to three phases*:* acclimation, acquisition and test, and scores were recorded. For acclimation, the mouse was placed into the NOR chamber (an open field box) and allowed to explore freely for 10 minutes daily for three days. No objects were placed in the box during the acclimation trial. Following acclimation, the acquisition trial was conducted by placing the mouse in the field; two novel objects were symmetrically fixed to the floor of the box 5 cm from the walls. The objects were constructed from a ping-pong ball, a cylindrical bottle cover and a piece of Lego brick, which were different in shape and colour but similar in size. Mice were allowed to explore the two objects for 10 minutes (day 4), and exploratory activity (i.e., the time spent exploring each object) was recorded. After 24 hours (day 5), mice were re-exposed to one of the objects of the acquisition phase, together with a novel object (not used in acquisition phase). Once again, animals were allowed to explore freely for 5 minutes and the time spent exploring each object was recorded. A mouse was considered to be involved in exploratory behaviour when its head was oriented directly towards the object and within approximately 1–2 cm from it. For test data, the percentage of exploration time spent at the novel object was determined.

The choice for novel or familiar object was counterbalanced, and the position of each object was also alternated between trials to avoid any misinterpretation of data. After each exposure, the objects and test chamber were cleaned with 5% ethanol to eliminate odour cues. Subsequently, the following indices were estimated. Discrimination ratio (DR) was the exploration difference between the novel(N) and familiar(F) object divided by the total ET (DR = N-F/N + F).[32] Recognition index (RI) consisted of dividing novel object exploration by the total ET (N/N + F).[33,34]

Y maze analysis

Spontaneous alternation is a measure of spatial working memory. The Y-maze can be used as a measure of short term memory. In this study spontaneous alternation was assessed using a Y- maze composed of three equally spaced arms (120°, 41 cm long and 15 cm high). The floor of each arm is made of wood and is 5 cm wide. Each mouse was placed in one of the arm compartments and was allowed to move freely until its tail completely entered another arm. The arms were labeled A, B and C.

An alternation is defined as entry into all three arms consecutively, for instance if the animal makes the following arm entries; ACB,CA,B,C,A,CAB,C,A, in this example, the animal made 13 arm entries, 8 of which are correct alternations. The number of maximum spontaneous alternations consists of total number of arms entered minus two, and the percentage alternation is calculated as {(actual alternations /maximum alternations) x 100}. For each animal, the Y-maze testing was carried out for 5 minutes. The apparatus was cleaned with 5% alcohol and allowed to dry between sessions.

Statistical Analysis

Data was analysed using Chris Rorden's ezANOVA statistical package, (version 0.98). Hypothesis testing was performed using analysis of variance (ANOVA). Multi-factorial ANOVA models were used to test effects of dose, gender and sleep deprivation on novel object recognition test parameters and Y maze spontaneous alternation. Tukey HSD test was used for within and between group comparisons. Results are expressed as mean ± S.E.M, p values less than 0.05 were considered statistically significant.

Results

Novel object recognition test

Total exploration time (Acquisition phase)

First, we ascertained the effect of six hours of total sleep deprivation and caffeine (10, 20, 40, 80 and 120 mg/kg) administration on the total exploration time during the acquisition phase in male and female mice following the novel object recognition test. There was a significant effect of caffeine dose ($p = 0.000$) and sleep deprivation ($p = 0.000$). A strong association between caffeine dose and gender ($p = 0.000$), caffeine dose and sleep deprivation and between all three main factors (caffeine dose, gender and sleep deprivation) was also observed, even though the lack of a effect of gender ($p = 0.532$) alone was also evident.

Figure 1 summarises the comparative response of sleep deprived and non sleep deprived male and female mice administered with increasing doses of caffeine to corresponding vehicle. Significant reduction in total exploration time was seen at all doses of caffeine in sleep deprived females and at 10 ($p = 0.000$), 20 ($p = 0.002$) and 40 ($p = 0.000$) mg/kg in non sleep deprived females when compared to vehicle control. In non sleep deprived males there was a significant ($p = 0.000$) increase in total exploration time at caffeine doses at caffeine doses of 20, 80 and 120 mg/kg while in sleep deprived males the total exploration time increased at 40 mg/kg dose of caffeine as compared to vehicle.

Comparing response of SD mice to NSD mice with vehicle, there was a significant increase in total exploration time in both SD

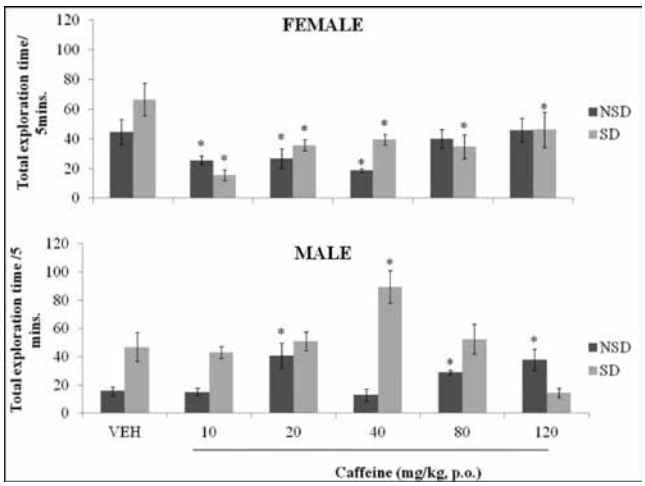

Fig. 1: Effect of Caffeine on total exploration time in non sleep deprived (NSD) and sleep deprived (SD) mice in the novel object recognition test. Each bar represents Mean ± S.E.M, *p<0.05 compared to vehicle n = 6.

male (p = 0.000) and female (p = 0.003) mice. Comparisons of the effect of sleep deprivation in male and female mice at varying doses of caffeine revealed increase in total exploration time at 20 (p = 0.000) and 40 (p = 0.000) mg/kg in SD female mice and at 10 (p = 0.000), 20 (p = 0.046), 40 (p = 0.000) and 80 (p = 0.000) mg/kg in SD male mice as compared to NSD mice, at 10 (p = 0.000) mg/kg in SD females and at 120 (p = 0.000) mg/kg in male mice total exploration time reduced significantly when compared to NSD mice as shown in Table 1.

Effects of gender following vehicle or caffeine administration in sleep deprived and non sleep deprived mice showed that in females compared to males, total exploration time of sleep deprived mice increased significantly in VEH (p = 0.002) and at 10 (p = 0.000), 40 (p = 0.011) and 80 (0.002) mg/kg of caffeine, while at 20 mg/kg total exploration time decreased significantly. In non sleep deprived mice, total exploration time decreased significantly at 10 (p = 0.001), 20 (p = 0.000), 40 (p = 0.000) and 80 (p = 0.010) mg/kg, and increased significantly following VEH (p = 0.010) and at 120 (p = 0.000) mg/kg of caffeine as shown in Table 2.

Novel object exploration time

We also examined the effect of six hours of total sleep deprivation and caffeine (10, 20, 40, 80 and 120 mg/kg) administration on the novel object exploration time in male and female mice. Caffeine dose (p = 0.000) and sleep deprivation (p = 0.000) exerted significant effects while sleep deprivation as an independent factor (P = 0.187) did not have a significant effect. Strong associations were seen between caffeine dose and gender (p = 0.000), caffeine dose and sleep deprivation and between all three main factors (caffeine dose, gender and sleep deprivation).

Figure 2 compares the response of sleep deprived and non sleep deprived male and female mice respectively to corresponding vehicle (distilled water). Compared to mice administered with vehicle, sleep deprived female mice showed a significant increase in novel object exploration time at 20 mg/kg (p = 0.000) and a significant reduction at 40 (p = 0.01) and 120 (p = 0.000) mg/kg of caffeine, in non sleep deprived females novel object exploration time increased significantly at 10 (p = 0.000) and 40 (p = 0.000) mg/kg. Sleep deprived male mice showed a significant increase in novel object exploration time at 20 (p = 0.000) and 40 (p = 0.000) mg/kg and a significant reduction in novel object exploration time was seen at 10 (p = 0.000), 20 (p = 0.001) and 80 (p = 0.000) mg/kg in non sleep deprived male mice administered caffeine compared to vehicle.

Comparing sleep deprived to non sleep deprived mice following administration of vehicle, novel object exploration time increased significantly in female (p = 0.000) and decreased

Table 1: Effects of sleep deprivation on total exploration time in mice. Mean ± S.E.M, VEH: Vehicle. *$^{\alpha}$p<0.05. Comparison of means between sleep deprived (SD) and non sleep deprived (NSD) mice of same gender, n = 6

Dose groups	Male NSD, Mean (S.E.M)	Male SD Mean (S.E.M)	F	Tukey HSD Test	p value
VEH	15.67 ± 2.94	46.83 ± 10.17	61.2	7.21	0.000*
10	15 ± 2.53	43 ± 4.38	"	13.56	0.000*
20	40.67 ± 8.89	51 ± 6.66	"	2.28	0.046*
40	12.83 ± 4.4	89.5 ± 11.48	"	15.27	0.000*
80	28.83 ± 1.47	52.5 ± 10.63	"	5.40	0.000*
120	37.67 ± 7.5	14.33 ± 3.14	"	7.03	0.001$^{\alpha}$
Dose groups	**Female NSD Mean (S.E.M)**	**Female SD Mean (S.E.M)**	**F**	**Tukey HSD Test**	**p value**
VEH	44.5 ± 8.31	66.33 ± 10.93	61.2	3.39	0.003*
10	25.5 ± 2.81	15.5 ± 3.62	"	5.35	0.000$^{\alpha}$
20	26.83 ± 6.74	35.67 ± 3.83	"	2.79	0.02*
40	18.67 ± 1.21	39.5 ± 3.67	"	13.19	0.000*
80	39.83 ± 6.4	34.5 ± 8.02	"	1.27	0.232
120	46 ± 8.05	46.17 ± 11.87	"	0.03	0.978

Table 2: Effects of gender on total exploration time in male and female mice: Mean ± S.E.M, VEH: Vehicle. *$^{\alpha}$p<0.05 Comparison of means between male and female mice, n = 6.

Dose groups	Male NSD, Mean (S.E.M)	Female NSD Mean (S.E.M)	F	Tukey HSD test	p value
VEH	15.67 ± 2.94	44.5 ± 8.31	42.8	8.01	0.000*
10	15 ± 2.53	25.5 ± 2.81	"	6.80	0.000*
20	40.67 ± 8.89	26.83 ± 6.74	"	3.04	0.013$^{\alpha}$
40	12.83 ± 4.4	18.67 ± 1.21	"	3.13	0.011*
80	28.83 ± 1.47	39.83 ± 6.4	"	4.10	0.002*
120	37.67 ± 7.5	46 ± 8.05	"	1.86	0.093
Dose groups	**Male SD Mean (S.E.M)**	**Female SD Mean (S.E.M)**	**F**	**Tukey HSD test**	**p value**
VEH	46.83 ± 10.17	66.33 ± 10.93	42.8	3.20	0.01*
10	43 ± 4.38	15.5 ± 3.62	"	11.85	0.000$^{\alpha}$
20	51 ± 6.66	35.67 ± 3.83	"	4.89	0.001$^{\alpha}$
40	89.5 ± 11.48	39.5 ± 3.67	"	10.61	0.000$^{\alpha}$
80	52.5 ± 10.63	34.5 ± 8.02	"	3.31	0.008*
120	14.33 ± 3.14	46.17 ± 11.87	"	6.35	0.000*

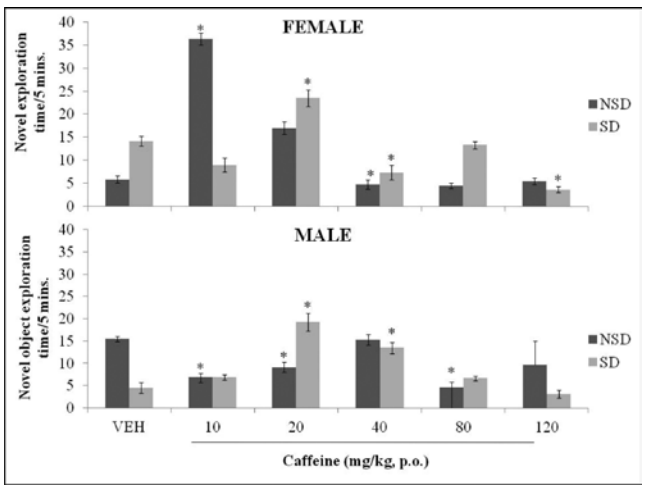

Fig. 2: Effect of Caffeine on novel object exploration time in non sleep deprived (NSD) and sleep deprived (SD) mice in the novel object recognition test. Each bar represents Mean ± S.E.M, *p<0.05 compared to vehicle, n = 6.

significantly in male (p = 0.000) sleep deprived mice compared to non sleep deprived mice. Between group comparisons of the effect of sleep deprivation in male and female mice at varying doses of caffeine revealed a significant increase in novel object exploration time at 20 (p = 0.000) and 80 (p = 0.000) mg/kg in female mice and at 20 (p = 0.001) mg/kg in male mice as shown in Table 3.

Effects of gender following vehicle or caffeine administration in sleep deprived and non sleep deprived mice was also analysed. Result showed that in females compared to males novel object exploration time was significantly increased following VEH (p = 0.000) and at 80 (p = 0.000) mg/kg of caffeine in sleep deprived mice and in non sleep deprived mice at 10 (p = 0.000). Novel object exploration time decreased significantly in females compared to males at 40 (p = 0.012) mg/kg in SD mice and in non sleep deprived mice following VEH (p = 0.000) and at 20 (0.037) mg/kg of caffeine as shown in Table 4.

Recognition index

The effect of six hours of total sleep deprivation and caffeine (10, 20, 40, 80 and 120 mg/kg) administration on the recognition index in male and female mice following the novel object recognition test was also evaluated. Multifactorial ANOVA of all three main factors revealed significant {caffeine dose (p = 0.000), sleep deprivation (p = 0.000) and gender (p = 0.043) effect and very strong interactions between caffeine dose and gender (p = 0.000), caffeine dose and sleep deprivation and within all three main factors (caffeine dose, gender and sleep deprivation).

Comparing within group effects in sleep deprived and non sleep deprived male and female mice respectively against vehicle (distilled water) showed statistically significant increase in the recognition index (RI) in NSD female mice at 10 (p = 0.000) 20 (p = 0.001), 40 (p = 0.002) and 80 (p = 0.000) mg/kg and a significant reduction in recognition index in NSD males at 10 (p = 0.003), 40 (p = 0.002), 80 (p = 0.000) and 120 (p = 0.000) mg/kg, in SD females recognition index increased significantly at 20 (p = 0.013)mg/kg and decreased significantly at 10 (p = 0.043), 80 (p = 0.000) and 120 (p = 0.001) mg/kg, SD males showed a significant reduction in recognition index at 120 (p = 0.000) mg/kg of caffeine compared to corresponding vehicle as shown in Figure 3.

Comparing recognition indices between SD and NSD male and female mice respectively following administration of vehicle revealed a significant reduction in recognition index in SD males (p = 0.001) and an increase in SD females (p = 0.008) when compared to NSD mice administered vehicle. Between group comparisons of the effect of sleep deprivation in male and female mice at varying doses of caffeine revealed a

Table 3: Effects of sleep deprivation on novel object exploration time in mice. Mean ± S.E.M, VEH: Vehicle. *$^{\alpha}$p<0.05 Comparison of means between sleep deprived (SD) and non sleep deprived (NSD) mice of same gender, n = 6.

Dose groups	Male NSD Mean (S.E.M)	Male SD Mean (S.E.M)	F	Tukey HSD test	p value
VEH	15.5 ± 0.56	4.5 ± 1.15	27.0	8.61	0.000$^{\alpha}$
10	6.83 ± 1.01	6.83 ± 0.6	"	0.00	1.000
20	9.17 ± 1.19	19.33 ± 1.99	"	4.37	0.001*
40	15.33 ± 1.15	13.5 ± 1.26	"	1.08	0.3065
80	4.67 ± 1.12	6.67 ± 0.49	"	1.64	0.1322
120	9.67 ± 5.41	3.17 ± 0.83	"	1.19	0.2623
Dose groups	**Female NSD Mean (S.E.M)**	**Female SD Mean (S.E.M)**	**F**	**Tukey HSD test**	**p value**
VEH	5.83 ± 0.83	14.17 ± 1.05	27.0	6.23	0.000*
10	36.33 ± 1.28	9 ± 1.51	"	13.82	0.000$^{\alpha}$
20	4.83 ± 1.35	23.5 ± 1.77	"	8.39	0.000*
40	17 ± 1	7.33 ± 1.58	"	5.16	0.000$^{\alpha}$
80	4.5 ± 0.56	13.33 ± 0.8	"	9.01	0.000*
120	5.5 ± 0.72	3.67 ± 0.61	"	1.94	0.081

Table 4: Effects of gender on novel object exploration time in mice: Mean ± S.E.M, VEH: Vehicle. *$^{\alpha}$p<0.05 Comparison of means between male and female mice, n = 6.

Dose groups	Male NSD Mean (S.E.M)	Female NSD Mean (S.E.M)	F	Tukey HSD test	p value
VEH	15.5 ± 0.56	5.83 ± 0.83	20.2	9.61	0.000$^{\alpha}$
10	6.83 ± 1.01	36.33 ± 1.28	"	18.05	0.000*
20	9.17 ± 1.19	4.83 ± 1.35	"	2.40	0.037$^{\alpha}$
40	15.33 ± 1.15	17 ± 1	"	1.10	0.2986
80	4.67 ± 1.12	4.5 ± 0.56	"	0.13	0.8965
120	9.67 ± 5.41	5.5 ± 0.72	"	0.76	0.4626
Dose groups	**Male SD Mean (S.E.M)**	**Female SD Mean (S.E.M)**	**F**	**Tukey HSD test**	**p value**
VEH	4.5 ± 1.15	14.17 ± 1.05	20.2	6.23	0.000*
10	6.83 ± 0.6	9 ± 1.51	"	1.34	0.260
20	19.33 ± 1.99	23.5 ± 1.77	"	1.56	0.148
40	13.5 ± 1.26	7.33 ± 1.58	"	3.05	0.012$^{\alpha}$
80	6.67 ± 0.49	13.33 ± 0.8	"	7.07	0.000*
120	3.17 ± 0.83	3.67 ± 0.61	"	2.12	0.06

significant reduction in recognition index at 10 (p = 0.000), 20 (p = 0.036) and 80 (p = 0.010) mg/kg in SD female mice and at 20 (p = 0.012) mg/kg in SD male mice compared to NSD mice as shown in Table 5.

Effects of gender following vehicle or caffeine administration in sleep deprived and non sleep deprived mice showed statistically significantly increase in recognition index at 10 (p = 0.001) and 40 (p = 0.034) mg/kg of caffeine in non sleep deprived female mice compared to males. SD mice showed significant increase at 20 (p = 0.015), 40 (0.013) and 120 (p = 0.047) mg/kg and significant reduction at 80 (p = 0.001) mg/kg in females compared to male mice as shown in Table 6.

Discrimination ratio

Effects of six hours of total sleep deprivation and caffeine (10, 20, 40, 80 and 120 mg/kg) administration on discrimination ratio in male and female mice using the novel object recognition test was also studied. Multifactorial ANOVA revealed significant main effect of caffeine dose (p = 0.000), sleep deprivation (p = 0.000), gender (p = 0.001) and very strong group interactions between caffeine dose and gender (p = 0.000),

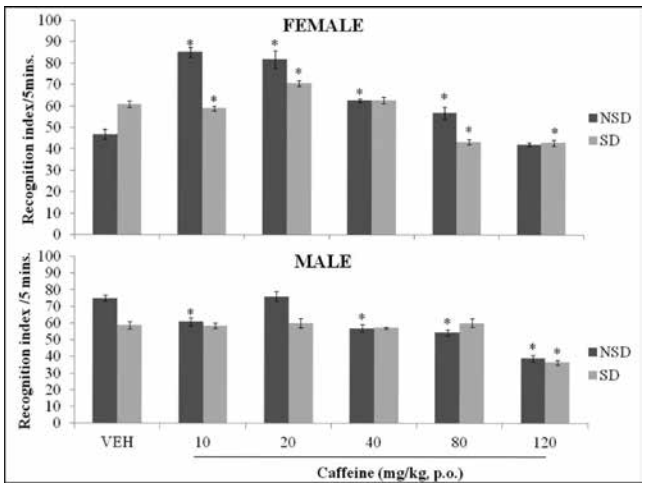

Fig. 3: Effect of Caffeine on recognition index in non sleep deprived (NSD) and sleep deprived (SD) mice in the novel object recognition test. Each bar represents Mean ± S.E.M, *p<0.05 compared to vehicle, n = 6.

caffeine dose and sleep deprivation (p = 0.000) and within all three main factors (caffeine dose, gender and sleep deprivation) (p = 0.000).

Comparing the response seen within sleep deprived and non sleep deprived groups in both male and female mice respectively (distilled water) revealed statistically significant increase in the discrimination ratio in NSD female mice at 10 (p = 0.000), 20 (p = 0.000) and 40 (0.042) mg/kg, and in SD females at 20 (p = 0.006) mg/kg, discrimination ratio reduced significantly in NSD females at 120 (p = 0.000) mg/kg and in SD females at 80 (p = 0.047) and 120 (p = 0.001) mg/kg. In NSD males discrimination ratio decreased significantly at 10 (p = 0.000), 40 (p = 0.000) and 120 (p = 0.000) mg/kg and increased significantly at 20 (p = 0.000), while in SD males discrimination ratio decreased significantly at 120 mg/kg (p = 0.000) compared to corresponding vehicle as shown in Figure 4.

Comparing discrimination ratios between sleep deprived and non sleep deprived male and female mice respectively following administration of vehicle, revealed a significant reduction

Table 5: Effects of sleep deprivation on novel object recognition index in male mice: Mean ± S.E.M, VEH: Vehicle. *$^{\alpha}$p<0.05 Comparison of means between sleep deprived (SD) and non sleep deprived (NSD) mice, n = 6.

Dose groups	Male NSD, Mean (S.E.M)	Male SD Mean (S.E.M)	F	Tukey HSD Test	p value
VEH	74.9 ± 1.78	58.83 ± 2.22	9.67	6.72	0.001$^{\alpha}$
10	61 ± 2.42	58.45 ± 1.6	"	0.74	0.494
20	76.0 ± 3.02	60.09 ± 2.83	"	3.88	0.012$^{\alpha}$
40	57.16 ± 2.14	57.21 ± 0.56	"	0.02	0.983
80	54.5 ± 1.77	60.24 ± 2.6	"	2.54	0.052
120	39.18 ± 1.99	36.66 ± 1.33	"	0.94	0.389
Dose groups	**Female NSD Mean (S.E.M)**	**Female SD Mean (S.E.M)**	**F**	**Tukey HSD Test**	**p value**
VEH	46.9 ± 2.29	60.93 ± 1.59	9.67	4.32	0.008*
10	85.22 ± 2.4	59 ± 0.98	"	3.46	0.000$^{\alpha}$
20	81.8 ± 4.19	70.72 ± 1.44	"	2.84	0.036$^{\alpha}$
40	62.71 ± 0.79	62.93 ± 1.53	"	0.13	0.904
80	56.82 ± 2.98	43.42 ± 1.23	"	4.00	0.010$^{\alpha}$
120	42.24 ± 0.71	42.94 ± 1.41	"	0.54	0.609

Table 6: Effects of gender on novel object recognition index in mice: Mean ± S.E.M, VEH: Vehicle. *$^{\alpha}$p<0.05 Comparison of means between male and female mice, n = 6.

Dose groups	Male NSD, Mean (S.E.M)	Female NSD Mean (S.E.M)	F	Tukey HSD test	p value
VEH	74.9 ± 1.78	46.9 ± 2.29	20.5	12.13	0.000$^{\alpha}$
10	61 ± 2.42	85.22 ± 2.4	"	7.07	0.001*
20	76.0 ± 3.02	81.8 ± 4.19	"	0.87	0.4243
40	57.16 ± 2.14	62.71 ± 0.79	"	2.91	0.034*
80	54.5 ± 1.77	56.82 ± 2.98	"	0.82	0.447
120	39.18 ± 1.99	42.24 ± 0.71	"	1.99	0.103
Dose groups	**Male SD Mean (S.E.M)**	**Female SD Mean (S.E.M)**	**F**	**Tukey HSD test**	**p value**
VEH	58.83 ± 2.22	60.93 ± 1.59	20.5	1.16	0.299
10	58.45 ± 1.6	59 ± 0.98	"	0.46	0.664
20	60.09 ± 2.83	70.72 ± 1.44	"	3.65	0.015*
40	57.21 ± 0.56	62.93 ± 1.53	"	3.78	0.013*
80	60.24 ± 2.6	43.42 ± 1.23	"	5.30	0.003$^{\alpha}$
120	36.66 ± 1.33	42.94 ± 1.41	"	2.62	0.047*

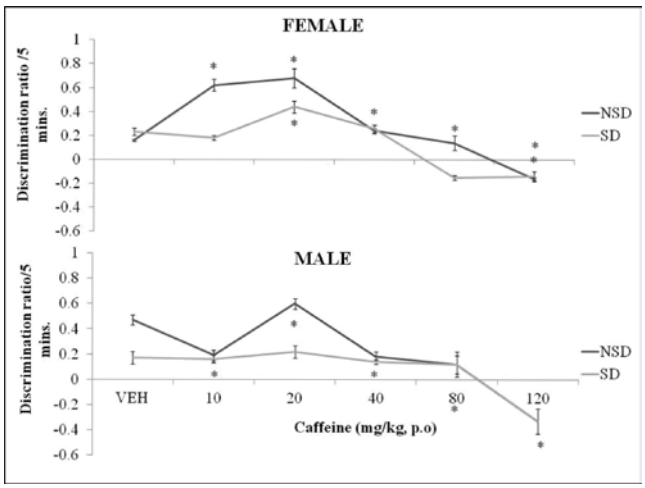

Fig. 4: Effect of Caffeine on discrimination ratio in non sleep deprived (NSD) and sleep deprived (SD) mice in the novel object recognition test. Each bar represents Mean ± S.E.M, *p<0.05 compared to vehicle, n = 6.

in males (p = 0.01) and a slight increase in females (p = 0.082) but non-significant. Between group comparisons of the effect of sleep deprivation in male and female mice at varying doses of caffeine revealed a significant reduction in discrimination ratio at 10 (p = 0.000), 20 (p = 0.029),80 (p = 0.000) and 120 (p = 0.001) mg/kg in female mice and at 20 (p = 0.000) mg/kg in male mice, at all other doses in both male and females, discrimination ratio was reduced although not statistically significant as shown in Table 7.

Effects of gender following vehicle or caffeine administration in sleep deprived and non sleep deprived mice showed that in females compared to males discrimination ratio decreased significantly following VEH (p = 0.000) and at 120 (p = 0.000) mg/kg and increased significantly at 10 (p = 0.000) mg/kg of caffeine in non sleep deprived mice. In sleep deprived mice however discrimination ratios increased significantly at 20 (p = 0.001) mg/kg and decreased significantly at 40 (p = 0.001), 80 (p = 0.013) and 120 (p = 0.000) mg/kg in females compared to males as shown in Table 8.

Effect of caffeine on Y maze spontaneous alternation

The effect of six hours of total sleep deprivation and caffeine (10, 20, 40, 80 and 120 mg/kg) administration on spontaneous alternation following 5 minutes of exploration in the Y maze was assessed in male and female mice. Results from Multifactorial ANOVA showed significant effects in two of the three main factors in this study, {caffeine dose (p = 0.000) and sleep deprivation (p = 0.000)} while gender (p = 0.296) did not exert a significant effect. Strong associations also exist between caffeine dose and gender (p = 0.000), caffeine dose and sleep deprivation, gender and sleep deprivation (p = 0.000) and also within all three main factors; caffeine dose, gender and sleep deprivation (p = 0.000).

Within group interaction in sleep deprived and non sleep deprived animals male and female mice respectively compared to vehicle (distilled water) showed significant decrease in spontaneous alternation at 20 (p = 0.012) and 80 (p = 0.001) mg/kg in NSD females. In SD females however, spontaneous alternation increased significantly at 20 (p = 0.003) and 40 (p = 0.002) mg/kg and decreased significantly at 80 (p = 0.011) mg/kg. Non sleep deprived male mice showed a significant increase in spontaneous alternation at 10 (p = 0.002), 20 (p = 0.017), 40 (p = 0.000) and 80 (p = 0.010) mg/kg while SD males showed significant increments at 20 (p = 0.005) and 80 (p = 0.012) mg/kg of caffeine compared to vehicle as shown in Figure 5.

Comparisons between sleep deprived and non sleep deprived male and female mice respectively following administration of vehicle revealed no significant difference in either male or female mice. Between group comparisons of the effect of sleep deprivation in male and female mice at varying doses of

Table 7: Effects of sleep deprivation on discrimination ratio in mice: Mean ± S.E.M, VEH: Vehicle. *$^{\alpha}$p<0.05 Comparison of means between sleep deprived (SD) and non sleep deprived (NSD) male and female mice, n = 6.

Dose groups	Male NSD Mean (S.E.M)	Male SD Mean (S.E.M)	F	Tukey HSD test	p value
VEH	0.47 ± 0.04	0.17 ± 0.05	9.98	4.86	0.001$^{\alpha}$
10	0.19 ± 0.04	0.16 ± 0.03	"	0.83	0.426
20	0.6 ± 0.04	0.22 ± 0.05	"	5.91	0.000$^{\alpha}$
40	0.18 ± 0.04	0.14 ± 0.02	"	1.09	0.299
80	0.12 ± 0.1	0.12 ± 0.07	"	1.64	0.1322
120	−0.33 ± 0.1	−0.33 ± 0.1	"	0.00	0.9623
Dose groups	**Female NSD Mean (S.E.M)**	**Female SD Mean (S.E.M)**	**F**	**Tukey HSD test**	**p value**
VEH	0.16 ± 0.01	0.23 ± 0.03	9.98	1.93	0.082
10	0.62 ± 0.05	0.18 ± 0.02	"	8.78	0.000$^{\alpha}$
20	0.68 ± 0.08	0.44 ± 0.05	"	2.55	0.029$^{\alpha}$
40	0.24 ± 0.02	0.26 ± 0.03	"	0.59	0.567
80	0.14 ± 0.06	−0.15 ± 0.02	"	9.01	0.000$^{\alpha}$
120	−0.16 ± 0.01	−0.14 ± 0.04	"	6.94	0.001$^{\alpha}$

Table 8: Effects of gender on discrimination ratio in mice: Mean ± S.E.M, VEH: Vehicle. *$^{\alpha}$p<0.05 Comparison of means between male and female mice, n = 6.

Dose groups	Male NSD Mean (S.E.M)	Female NSD Mean (S.E.M)	F	Tukey HSD test	p value
VEH	0.47 ± 0.04	0.16 ± 0.01	12.0	8.11	0.000$^{\alpha}$
10	0.19 ± 0.04	0.62 ± 0.05	"	7.23	0.000*
20	0.6 ± 0.04	0.68 ± 0.08	"	0.92	0.381
40	0.18 ± 0.04	0.24 ± 0.02	"	1.41	0.189
80	0.12 ± 0.1	0.14 ± 0.06	"	0.33	0.747
120	−0.33 ± 0.1	−0.16 ± 0.01	"	7.11	0.000$^{\alpha}$
Dose groups	**Male SD Mean (S.E.M)**	**Female SD Mean (S.E.M)**	**F**	**Tukey HSD test**	**p value**
VEH	0.17 ± 0.05	0.23 ± 0.03	12.0	0.90	0.392
10	0.16 ± 0.03	0.18 ± 0.02	"	0.78	0.454
20	0.22 ± 0.05	0.44 ± 0.05	"	3.02	0.001*
40	0.14 ± 0.02	0.26 ± 0.03	"	3.20	0.001$^{\alpha}$
80	0.12 ± 0.07	−0.15 ± 0.02	"	7.07	0.013$^{\alpha}$
120	−0.33 ± 0.1	−0.14 ± 0.04	"	2.12	0.000$^{\alpha}$

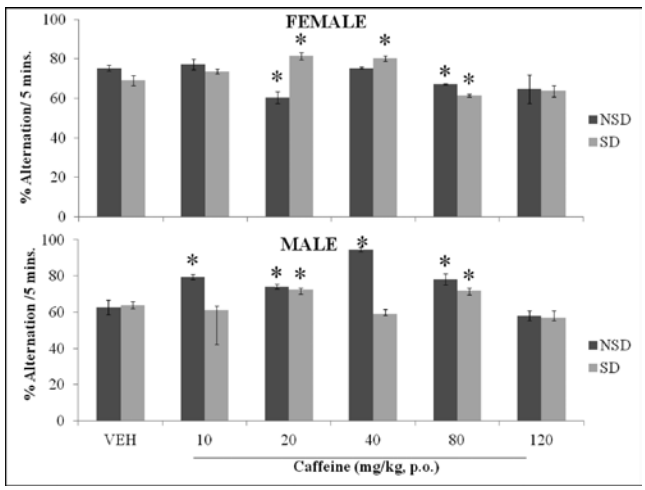

Fig. 5: Effect of Caffeine on spontaneous alternation in non sleep deprived (NSD) and sleep deprived (SD) mice in the Y maze. Each bar represents Mean ± S.E.M, *p<0.05 compared to vehicle, n = 6.

caffeine revealed a significant increase in spontaneous alternation at 20 (p = 0.000) and 40 (p = 0.008) mg/kg and a significant reduction at 80 (p = 0.000) mg/kg in SD compared to NSD female mice. SD male mice showed significant reduction in spontaneous alternation at 10 (p = 0.000) and 40 (p = 0.000) mg/kg of caffeine compared to NSD males as shown in Table 9.

Effects of gender following vehicle or caffeine administration in sleep deprived and non sleep deprived mice showed significant increase in spontaneous alternation following VEH (p = 0.012) and significant reduction in alternation at 20 (p = 0.002), 40 (p = 0.000) and 80 (p = 0.006) mg/kg in NSD females compared to males. In SD mice spontaneous alternation increased significantly at 10 (p = 0.001), 20 (p = 0.002) and 40 (p = 0.000) mg/kg and reduced significantly at 80 (p = 0.000) mg/kg in females compared to males as shown in Table 10.

Discussion

This study was designed to examine how certain aspects of memory may be affected by sub chronic oral caffeine of increasing doses and total sleep deprivation by the gentle handling method; study was done using adult male and female mice. Examinations of effects of caffeine dose, gender and total sleep deprivation in a three factor model using spatial and non spatial memory tasks were conducted. The findings of this study show all three factors independently have strong impacts on memory and also reveal very strong interactions among these factors.

The object recognition task exploits the natural exploratory activity of rodents toward spatial novelty to assess the detection of spatial relocation of a known object and is critically dependent on the hippocampus.[35] Caffeine administration before 30 minutes of training impaired memory acquisition in NSD female mice and improved it in NSD male mice. However caffeine administered 30 minutes pre-test improved memory retention in NSD females at lower doses and had no effect at higher doses, while in NSD males, it showed a biphasic response. In NSD female mice, ability to recognize novel object improves with caffeine administration while it is impaired in males. Pre-test caffeine administration improves object discrimination in females at lower doses and worsens it at higher doses. In males object discrimination gave a biphasic response. Results of Y maze spontaneous alternation revealed that pre test caffeine administration impaired memory in female mice while in male mice, memory was improved. This finding is consistent with our previous study where we gave acute intraperitoneal caffeine to mice. This leads to the understanding that caffeine response is not affected by duration or route of administration, corroborating other studies that have reported the ability of caffeine to enhance memory irrespective of the route of administration

Table 9: Effects of sleep deprivation on Y maze spontaneous alternation in mice: Mean ± S.E.M, VEH: Vehicle. *$^{\alpha}$p<0.05 Comparison of means between sleep deprived (SD) and non sleep deprived (NSD) male and female mice, n = 6.

Dose groups	Male NSD Mean (S.E.M)	Male SD Mean (S.E.M)	F	Tukey HSD test	p value
VEH	62.65 ± 3.78	63.87 ± 2.19	12.6	0.28	0.786
10	79.52 ± 1.46	61.15 ± 2.37	"	6.59	0.000$^{\alpha}$
20	74.18 ± 1.35	72.45 ± 0.99	"	1.04	0.324
40	97.68 ± 1.24	59.23 ± 2.22	"	15.11	0.000$^{\alpha}$
80	78.23 ± 3.19	72 ± 1.49	"	1.77	0.1072
120	58.12 ± 2.77	56.82 4.17	"	0.26	0.800
Dose groups	**Female NSD Mean (S.E.M)**	**Female SD Mean (S.E.M)**	**F**	**Tukey HSD test**	**p value**
VEH	75.32 ± .61	69.05 ± 2.38	12.6	2.18	0.054
10	77.25 ± 2.61	73.68 ± 1.29	"	1.23	0.248
20	60.43 ± 2.92	81.43 ± 1.98	"	5.96	0.000*
40	75.28 ± 0.46	80.12 ± 1.38	"	3.32	0.008*
80	67.08 ± 0.28	61.42 ± 0.64	"	8.14	0.000$^{\alpha}$
120	64.73 ± 7.29	63.68 ± 2.96	"	0.13	0.897

Table 10: Effects of gender on Y maze spontaneous alternation in mice: Mean ± S.E.M, VEH: Vehicle. *$^{\alpha}$p<0.05 Comparison of means between male and female mice, n = 6.

Dose groups	Male NSD Mean (S.E.M)	Female NSD Mean (S.E.M)	F	Tukey HSD test	p value
VEH	62.65 ± 3.78	75.32 ± .61	32.9	3.09	0.012*
10	79.52 ± 1.46	77.25 ± 2.61	"	0.76	0.466
20	74.18 ± 1.35	60.43 ± 2.92	"	4.28	0.002$^{\alpha}$
40	97.68 ± 1.24	75.28 ± 0.46	"	7.08	0.000$^{\alpha}$
80	78.23 ± 3.19	67.08 ± 0.28	"	3.48	0.006$^{\alpha}$
120	58.12 ± 2.77	64.73 ± 7.29	"	0.85	0.416
Dose groups	**Male SD Mean (S.E.M)**	**Female SD Mean (S.E.M)**	**F**	**Tukey HSD test**	**p value**
VEH	63.87 ± 2.19	69.05 ± 2.38	32.9	1.60	0.140
10	61.15 ± 2.37	73.68 ± 1.29	"	4.65	0.001*
20	72.45 ± 0.99	81.43 ± 1.98	"	4.07	0.002*
40	59.23 ± 2.22	80.12 ± 1.38	"	7.98	0.000*
80	72 ± 1.49	61.42 ± 0.64	"	6.52	0.000$^{\alpha}$
120	56.82 4.17	63.68 ± 2.96	"	1.34	0.209

or subject.[26,28] Mechanisms underlying memory changes with caffeine remain a matter of continuous debate and study. Enhancement of cholinergic transmission is believed to be one of the prime candidates. We also observed in previous studies that the ability of caffeine to enhance memory is more obvious against a background of central cholinergic deficit as it occurs with aging or scopolamine administration.

Sleep plays a critical role in learning and memory formation. Studies have demonstrated that sleep deprivation in animals lead to memory deficits.[6,17] Pre-training and pre-test sleep deprivation improved total exploration time, novel object exploration time, novel object recognition index and discrimination ratio in female vehicle treated mice. In males it worsens novel exploration time, discrimination ratio and novel object recognition index but improves total exploration time. This suggests that in females pre training SD enhanced memory acquisition and allowing post training sleep may have facilitated memory consolidation and hence retention, but pre test SD did not alter memory retrieval in them. In males pre training SD impaired memory acquisition, pre test SD impaired memory retention and retrieval. Administration of caffeine in sleep deprived male and female mice showed mixed effects, asserting both gender sensitive and dose dependent response. The impact of SD on

memory processes i.e., acquisition, consolidation and retrieval is a complex process, believed to depend on the neurobehavioural model used.[36] Several studies have reported that pre-training SD alters acquisition and memory retention in humans and rodents.[23,37] Impairment in memory tasks have been reported in rodents using varying behavioural models[38–41] which support our finding in male mice, where SD impairs memory. Combination of caffeine and sleep deprivation results in a biphasic response in males and females and for the most part the effect seen was deterioration from SD control.

Pre-training SD in Y maze tests showed impairment of spatial learning in female SD controls and no significant effect in males. Administration of caffeine led to spatial memory improvement in females and in males biphasic response was recorded. Studies related to the effect of SD on spatial learning reported mixed results, with some studies reporting that pre-training SD slows memory acquisition[41] and others finding no effect,[42] support the biphasic response of our study.

We conclude that effects of SD and caffeine on memory depend on various competing factors in the experimental animals. As we have said earlier, caffeine is a known enhancer of central cholinergic transmission and this is believed to be responsible for improvement in our study. On the other hand, sleep deprivation is known to impair memory by its negative effects on glutamatergic neurotransmission. We hypothesize that enhanced cholinergic transmission is pitted against impaired glutamatergic transmission for memory enhancement, which is further modulated by the influence of sex hormones. Overall, we observed that caffeine administration cannot fully compensate for memory deficits occurred by total sleep deprivation.

Conclusion

In conclusion, the study shows the complex nature of memory formation, retention and retrieval in opposite sex of animals in response to total sleep deprivation superimposed with caffeine administration.

Authorship Contributions

Olakunle J Onaolapo, **Adejoke Y Onaolapo:** Conceived and designed the work that led to the submission, **Olakunle J Onaolapo**, **Adejoke Y Onaolapo:** Were also responsible for the collection, collation and analysis of data, interpretation of results and drafting of manuscript. Also responsible for the care of animals, **Moses A Akanmu**, **Gbola Olayiwola:** interpretation of data, supervision of the entire study and drafting of manuscript.

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References

1. Matthew P. Walker. Sleep-Dependent Memory Processing. Harv Rev Psychiatry. 2008; 16: 287–8.
2. Smith C. Sleep states and memory processes. Behav Brain Res. 1995; 69: 137–45.
3. Blissitt PA. Sleep, memory, and learning. J Neurosci Nurs. 2001; 33: 208–15.
4. Peigneux P, Laureys S. Delbeuck X, et al., Sleeping brain, learning brain. The role of sleep for memory systems. Neuroreport. 2001; 12: A111–24.
5. McDermott CM, LaHoste GJ, Chen C, et al. Sleep deprivation causes behavioural, synaptic, and membrane excitability alterations in hippocampal neurons. J Neurosci. 2003; 23: 9687–95.
6. Kim EY, Mahmoud GS, Grover LM. REM sleep deprivation inhibits LTP *in vivo* in area CA1 of rat hippocampus. Neurosci Lett, 2005; 388: 163–67.
7. McDermott CM, Hardy MN, Bazan NG, et al. Sleep deprivation-induced alterations in excitatory synaptic transmission in the CA1 region of the rat hippocampus. J Physiol, 2006; 570: 553–65.
8. Gais S, Albouy G, Boly M, et al., Sleep transforms the cerebral trace of declarative memories. Proc Natl Acad Sci U S A, 2007; 104: 18778–83.
9. Cai DJ, Shuman T, Gorman MR, et al. Sleep selectively enhances hippocampus-dependent memory in mice. Behav Neurosci, 2009; 123: 713–19.
10. Orzel-Gryglewska J., Consequences of Sleep Deprivation. Int J Occup Med Environ Health. 2010; 23: 95–114.
11. Ibrahim A. A The protective effects of chronic caffeine treatment on the Cognitive function and synaptic plasticity in acute sleep deprivation lhaider, Abdulaziz M. Aleisa. The protective effects of chronic caffeine treatment on the Cognitive function and synaptic plasticity in acute sleep deprivation. Sleep. 2010; 33(4): 457–44.
12. Youngblood BD, Zhou J Smagin GN et al. Sleep deprivation by the "flower pot" technique and spatial reference memory. Physiol Behav. 1997; 61: 249–56.
13. Smith CT, Conway JM Rose GM. Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. Neurobiol Learn Mem. 1998; 69: 211–17.
14. Guan Z, Peng X, Fang J. Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. Brain Res. 2004; 1018: 38–47.
15. Guzman-Marin R, Ying Z, Methippara M, et al. Suppression of hippocampal plasticity-related gene expression by sleep deprivation in rats. J Physiol. 2006; 575: 807–19.
16. Ishikawa A, Kanayama Y, Matsumura H, et al. Selective rapid eye movement sleep deprivation impairs the maintenance of long-term potentiation in the rat hippocampus. Eur J Neurosci. 2006; 24: 243–48.
17. Ferrara M, Laria G, Tempesta D, et al. Sleep to find your way: the role of sleep in the consolidation of memory for navigation in humans. Hippocampus. 2008; 18: 844–51.
18. Yang RH, Hu SJ, Wang Y, et al. Paradoxical sleep deprivation impairs spatial learning and affects membrane excitability and mitochondrial protein in the hippocampus. Brain Res. 2008; 1230: 224–32.
19. Hagewoud R, Havekes R, Novati A, et al. Sleep deprivation impairs spatial working memory and reduces hippocampal AMPA receptor phosphorylation. Sleep Res. 2009; 19(2): 280–88.
20. Harrison Y, Horne JA, Sleep loss and temporal memory. Q J Exp Psychol A. 2000; 53: 271–79.
21. Li S, Tian Y, Ding Y, et al. The effects of rapid eye movement sleep deprivation and recovery on spatial reference memory of young rats. Learn Behav. 2009; 37: 246–53.
22. Wang J. H., van den Buuse M., Tian SW, et al. Effect of paradoxical sleep deprivation and stress on passive avoidance behavior. Physiol. Behav. 2003; 79, 591–96.
23. Yoo SS, Hu PT, Gujar N, et al. A deficit in the ability to form new Human memories without sleep. Nat Neurosci. 2007; 10: 385–92.
24. Ferre S, An update on the mechanisms of the psychostimulant effects of caffeine. J Neurochem. 2008; 105: 1067–179.
25. Dall'Igna OP, Fett P, Gomes MW, et al. Caffeine and adenosine A(2a) receptor antagonists prevent beta-amyloid induced cognitive deficits in mice. Exp Neurol. 2007; 203: 241–45.
26. Angelucci ME, Cesario C, Hiroi RH, et al. Effects of caffeine on learning and memory in rats tested in the Morris water maze. Braz J Med Biol Res. 2002; 35: 1201–08.
27. Arendash GW, Schleif W, Rezai-Zadeh K, et al. Caffeine protects Alzheimer's mice against cognitive impairment and reduces brain beta-amyloid production. Neuroscience. 2006; 142: 941–952.

28. Arendash GW, Mori T, Cao C, et al. Caffeine reverses cognitive impairment and decreases brain amyloid-beta levels in aged Alzheimer's disease mice. J Alzheimers Dis,2009; 17: 661–80.
29. Gevaerd MS, Takahashi RN, Silveira R, et al. Caffeine reverses the memory disruption induced by intra-nigral MPTP-injection in rats. Brain Res Bull, 2001; 55: 101–06.
30. Ennaceur A. One-trial object recognition in rats and mice: methodological and theoretical issues. Behav Brain Res 2010; 215: 244–54.
31. Kamei H, Nagai T, Nakano H, et al. Repeated methamphetamine treatment impairs recognition memory through a failure of novelty-induced ERK 1/2 activation in the prefrontal cortex of mice. Biol Psychiatry 2006; 59: 75–84.
32. Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats 1: Behavioral data. Behav Brain Res. 1988; 31: 47–59.
33. Costa MS, Botton PH, Mioranzza S, 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.
34. Leite MR, Wilhelm EA, Jesse CR, et al. Protective effect of caffeine and a selective A2A receptor antagonist on impairment of memory and oxidative stress of aged rats. Exp Gerontol 2011; 46: 309–15.
35. Stupien G, Florian C, Roullet P. Involvement of the hippocampal CA3-region in acquisition and in memory consolidation of spatial but not in object information in mice. Neurobiol Learn Mem. 2003; 80: 32–41.
36. Graves LA, Heller EA, Pack AI, et al. Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. Learn Mem, 2003; 10: 168–76.
37. Alvarenga TA, Patti CL, Andersen ML, et al. Paradoxical sleep deprivation impairs acquisition, consolidation, and retrieval of a discriminative avoidance task in rats. Neurobiol Learn Mem 2008; 90: 624–632.
38. Danguir J, Nicolaidis S. Impairments of learned aversion acquisition following paradoxical sleep deprivation in the rat. Physiol Behav, 1976; 17: 489–92
39. Palchykova S, Winsky-Sommerer R, Meerlo P, et al. Sleep deprivation impairs object recognition in mice. Neurobiol Learn Mem. 2006; 85: 263–71.
40. Bueno OF, Lobo LL, Oliveira MG, et al. (1994) Dissociated paradoxical sleep deprivation effects on inhibitory avoidance and conditioned fear. Physiol Behav. 1994; 56: 775–79.
41. Ruskin DN, Liu C, Bazan NG, et al. Sleep deprivation impairs hippocampus-dependant contextual learning but not amygdala-mediated cued learning in rats. Eur J Neurosci. 2004; 19: 3121–24.
42. Ward CP, McCarley RW, Strecker RE. Experimental sleep fragmentation impairs spatial reference but not working memory in Fischer/Brown Norway rats. J Sleep Res. 2009; 18: 238–44.