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Acute Administration of Caffeine: The Effect on motor coordination, higher brain cognitive functions and the social behavior of BLC57 Mice

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Abstract:

Heavy caffeine consumption is associated with adverse health effects. The effects of moderate and high doses of caffeine mixed with drinking water on the motor coordination, learning and memory and the social behavior in mice were studied in mice.
Animals were divided into 3 groups: control group, moderate dose group (Ac MD) and high dose group (Ac HD). The animals were tested after 7 days of caffeine administration.
Rota rod test for motor coordination showed that the mice of the moderate dose group could stay more time on the rotating rod before they fall than the control group and the high dose group. Water maze test for learning and memory showed better performance of mice receiving moderate dose of caffeine compared to the other groups. Animals that were administered moderate as well as high doses of caffeine showed no sociability and no preference for social novelty in the three-chamber test used to test the social behavior. In elevated plus maze, control animals showed no anxiety- like behavior while mice administered with caffeine were both showing anxiety-like behaviors.
We concluded that acute administration of moderate dose of caffeine to mice could enhance their spatial memory and motor coordination. High doses however caused defects in memory and learning. The social behavior as the level of anxiety and sociability was affected negatively by moderate as well as high dose caffeine administration.

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1. Introduction:

Caffeine, found in tea, coffee, energy drinks and others, is the most wildly consumed psycho-stimulant substance known to man. Recently, it was estimated that 90% of U.S. population and 80% of the world's population consume caffeine on a daily basis [1]. It has been reported that caffeine can enhance memory in both animal models and humans [2][3]. In fact, while healthy people can tolerate moderate intakes of caffeine, heavy caffeine consumption has been associated with severe adverse health effects [4]
Memory is significantly affected by sleep, in which the brain's neural connections are strengthened. The strengthening of the neural connections enhances the brain's ability to retain memory. During sleep, different parts of the brain process the memories and convert them into long-term memory. Insomnia or sleep deprivation results in the lower retention rate of memories due to the neural connections not being as strong as they need to be [5]. Sleep deprivation, in general, is associated with deterioration of memory [6]
Acute administration of caffeine improves the performance of adult rodents in various learning and memory tasks [7], whereas chronic caffeine administration prevents mnemonic deficits in experimental models of Alzheimer´s disease as well as age-related cognitive decline [8]. However, caffeine can cause delayed sleep onset in both humans and rodents [9]. It was shown that it stimulates locomotor activity. Caffeine is recognized as a non-selective adenosine receptor antagonist, especially for A1R and A2AR and it exerts a stimulating effect on locomotor activity at low to moderate doses, however, at higher doses it has less stimulating and even depressive effects at higher doses [10]

Anxiety disorder, which is a mental disorder characterized by out of proportion feelings of fear and anxiety in frequency and/or duration to the actual situation [11][12][13]. Interestingly, the effects of caffeine on anxiety differ based on the dose ingested. It has been observed that high doses can cause anxiety, whereas low doses can have anxiolytic effects [14][15][16][17].

This study aimed to measure the effect of caffeine on learning, memory, cognitive functions, anxiety and the social behavior on mice.

2. Materials and methods

2.1. Animals:

Male BLC57 mice were bred in the animal department of the Arabian Gulf University. They were housed in cages on sawdust. The animals were divided into the following: control group (n=8, were given normal water), acute moderate dose (n=8, were given moderate dose caffeine (0.1g/L) for 1 week, Ac MD), acute high dose (n=8, were given high dose caffeine for 1 week, Ac HD 1g/L). All tests were performed when the animals were six weeks of age. All experiment procedures followed the animal care ethics of Arabian Gulf University, Manama, Bahrain.

2.2. Tests:

2.2.1. Rotarod (RR)

The accelerating rotarod assesses motor coordination and balance. Mice were placed on a cylinder that rotates at a pre-assigned speed of 45 revolutions per minute (rpm). Each mouse was habituated for a total of a 6-minute period divided into three trials of 2 minutes. During

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each trial, the mouse was replaced on the rod when it falls. Habituation was not recorded. Following the habituation, the test was performed with three trials per test. Latency to fall from the rotating rod was recorded. The six groups of mice were tested within the same experiment to allow comparison of baseline motor performance. Habituation and testing were done on the same day for each group.

2.2.2. Elevated plus maze test (EPM)

The elevated plus maze tests anxiety-like behavior [17]; it consists of two open arms (25 cm x 5 cm) and two enclosed arms of the same size at opposite sides of each other. The enclosed arms are surrounded by 15 cm high walls. The edges, 3 mm high, surround the open arms, minimizing the likelihood of animals falling from the apparatus. Both arms are 55 cm above the floor. Between the arms is a central square area (5 cm x 5 cm) where the mouse is placed. The entire apparatus was cleaned using 70% ethanol between each subject. Each mouse was individually placed in the central square of the maze and was allowed to freely explore the apparatus. The mice's behavior was recorded for the test period of 10 minutes and then analyzed. The number of entries per arm and the time spent in the open arms were recorded. An entry is recorded when all four paws enter the arm. The numbers of entries and time spent in the open arm reflect the general behavior of the mice. The less common entrance of the mice into the open arms of the maze, as well as the decreased amount of time spent in them, was considered as anxiety-like behavior.

2.2.3. Morris Water maze test (MWM)

Water maze measures spatial learning and memory [18][19]. The apparatus consisted of a circular swimming pool (140 cm diameter and 50 cm height, filled to a depth of 30 cm); the water was maintained at room temperature (26ºC- 28ºC). The maze was housed in a darkened room with visual cues and illuminated by sparse red light. It was divided into four equal quadrants by two diagonal lines set by the program.
Each mouse was given five acquisition trials per day for the first day (training day) to learn the position of a hidden 'escape' platform, which is submerged 2 cm below the water surface, at a fixed location inside the pool. On each trial, the mice were released from one of four predetermined positions on the perimeter of the pool. Animals were given a maximum of 120 seconds (s) to find the platform and were allowed to remain on the platform for 20 s. Mice that failed to locate the platform were put onto it by the experimenter and allowed to stay there for 20 s.
The position and movement of the animals, in the pool, was captured and analyzed every 0.2 s, using a video-camera computer system, and ANY-maze video- tracking system [Stoelting Co., Wood Dale, IL, USA]. Outcome measures were latency time and distance swum to reach the platform. Performance in each trial was averaged to yield one data point per mouse per test. The speed of swimming (a measure of motor function [18]) was measured as a control between the groups. Following the test, a probe test was performed in which the platform was removed, and each animal was allowed to swim for 120 s.

2.2.4. Three-chambers social apparatus [Crawley's sociability and preference for social novelty test] (3C)

The Three-chambers social apparatus, assess sociability and preference for social novelty [20][21][22]. The rectangular three-chambered box consists of chambers with the dimensions of 20 cm x 40 cm x 22 cm. The walls of the box are made of clear Plexiglas. The dividing

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walls (also made of Plexiglas) had small rectangular openings (5 cm × 3 cm) allowing access to each chamber. Each chamber contained a cage which was 11 cm high, with a bottom diameter of 9 cm. The test consists of habituation for 5 minutes and two 10 minutes sessions. The subject mouse was first placed in the middle chamber to habituate for 5 min. Session 1 is started by placing an unfamiliar mouse (stranger 1) that had no prior contact with the subject mouse inside the wire cage in one of the side chambers. The subject mouse was then allowed to explore the entire apparatus freely. The time spent in each chamber, as well as the number of chamber entries, were recorded.
Session 2 was started by placing a second unfamiliar mouse in the wire cage inside the chamber which was empty during session 1. The test mouse is then left to freely choose between the chamber containing the already investigated mouse (stranger 1), and the one containing the novel unfamiliar mouse (stranger 2). The said strangers were of the same species and gender. The same parameters recorded for session 1 were recorded for session 2. The apparatus was cleaned with 70% ethanol between subjects. Session 1 tests for sociability, which is evident by the subject spending more time in the chamber containing a mouse than in the empty chamber. Preference for social novelty, measured in session 2, is indicated by spending more time in the chamber containing the novel mouse than the one containing the already investigated mouse.

3. Statistical analysis

Data are presented as average ± SEM unless indicated otherwise. Comparisons between and within groups are made using ANOVA, and post-hoc paired or unpaired two-tail t-tests. All statistical tests performed with Microsoft ExcelTM 2010 incorporating the Analysis Tool Pak add-in. Data were expressed as mean ± SEM. Statistical significance is set at a P value of less than 0.05.

4. Results:

4.1. Rota Rod test: Ac MD displayed better motor coordination than the other groups.

In comparison to the Cont (29.83 ± 2.5 s) and Ac HD (26.2 ± 2.5 s) groups, Ac MD spent significantly more time on the rotating rod before falling (41.1 ± 4.3 s, ANOVA test, p‹

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0.05). (Fig 1)

4.2. Improved performance displayed by Ac MD group in Morris water maze test.

Cognitive function was assessed by using Morris Water Maze test (Fig 2). Latency (Fig 2A) to reach the platform of Ac MD group was significantly better (30.45 ± 7.3 s) compared to the control group latency (54.4 ± 6.8 s, ANOVA test, $p< 0.05$). However, the Ac HD group took significantly more time to reach the platform compared to the control group (73.4 ± 6.4 s, ANOVA test, $p< 0.05$). There were significant differences in the distances (Fig 2B) traveled by each group to reach the platform in which the Ac MD group traveled less distance (5.8 ± 0.96 s) compared to the other groups (ANOVA test, $p<0.05$). Another outcome that was noted was in the swimming velocity (Fig 2C) where the velocity of the Ac MD group was the highest (0.30 ± 0.02). The probe test (Fig 2D) revealed that the Ac MD group exhibited learning behavior as they spent more time (36 ± 2.1 s) in the disc zone than the other groups (ANOVA test, $p<0.05$). On the other hand, the Ac HD group spent the least amount of time in the disc zone (30.2 ± 1.8 s).

4.3. Increased Anxiety in Caffeine Treated Mice

Anxiety was tested using the Elevated Plus Maze test. Control animals showed less anxiety- like behavior by spending more time in the open arms than in the closed arms during the test. On the contrary, the caffeine-treated mice spent significantly less time in the open arm which indicates an increase in anxiety (ANOVA test, $p<0.05$, Fig 3). However, there was no significant difference between the Ac MD and Ac HD groups.

4.4. Lack of sociability and preference for social novelty in caffeine treated mice

In Three Chambers Test, the control group animals showed normal sociability (Session 1) by preferring to be in the chamber containing another mouse rather than staying in the empty chamber (315.4 ± 17.9 s versus 217 ± 22.4 s, respectively). They also showed a normal preference for social novelty (Session 2) by spending more time in the chamber that contained a novel mouse than the chamber containing the old mouse (347 ± 38.1 s versus 184.8 ±29.5 s, respectively). On the other hand, caffeine-treated animals demonstrated a lack in both sociability (Session 1) and preference for social novelty (Session 2) with no significant difference between the two treated groups. (Fig 4)

5. Discussion and Conclusion:

Caffeine administration affects the functions of the cardiovascular, respiratory, renal, and nervous systems. The proposed mechanisms of action differ for different physiological effects. Caffeine action could be mediated via several mechanisms: the antagonism of

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adenosine receptors, the inhibition of phosphodiesterase, the release of calcium from intracellular stores, and antagonism of benzodiazepine receptors

The ability of caffeine to inhibit adenosine receptors appears to be highly important in its effects on behavior and cognitive function. This ability results from the competitive binding of caffeine and paraxanthine to adenosine receptors and is of importance in contributing to CNS effects, especially those involving the neuromodulatory effects of adenosine. Due to the blocking of adenosine inhibitory effects through its receptors, caffeine indirectly affects the release of norepinephrine, dopamine, acetylcholine, serotonin, glutamate, gamma-aminobutyric acid (GABA), and perhaps neuropeptides [23].

There are two main classes of adenosine receptors: A_1 and A_2; caffeine and paraxanthine are nonselective antagonists at both the specificity of inhibition depends on the concentration in the blood [24].

The research in hand investigates the effects of different doses of caffeine on four behavioral parameters in mice. There was a lack of research studying this number of variables under the effect of different doses of caffeine for short and long durations in one study. Thus, the idea for this research came to life. The different parameters measured; motor and cognitive function, anxiety, and social behavior showed a variation in results. The performance of Ac MD group gave the most robust results concerning cognitive and motor function whereas the Ac HD gave the weakest. Furthermore, caffeine increased the anxious behavior and decreased the sociability and the preference for social novelty.

According to the results collected and analyzed, mice treated with moderate doses of caffeine showed an enhanced motor and cognitive function, as they notably had the most anxious behavior and decreased sociability and preference for social novelty. On the other hand, mice treated with high doses of caffeine showed deterioration in motor and cognitive functions with an increased anxious behavior and similarly decreased sociability and social novelty as the group mentioned above.

Based on other research, the effects of caffeine on motor function are highly dose dependent. In fact, caffeine has biphasic effects, in which low doses increase motor function while high doses decrease it [19][20][21][22][25]. The earliest proposed mechanism of action for caffeine involved the mobilization of intracellular calcium by increasing calcium release from the sarcoplasmic reticulum. Specific actions of caffeine in skeletal muscle appear to involve ionic calcium (Ca++). Caffeine in high concentrations (1–10 mM) was found to interfere with the uptake and storage of calcium in the sarcoplasmic reticulum of striated muscle and to increase the translocation of Ca++ through the plasma membrane [26]. Caffeine may also increase myofilament sensitivity to Ca++ through its binding to ryanodine receptors in calcium channels of muscle and brain [27].

Low doses of caffeine selectively decrease the activity of striatopallidal Neurons in the striatum and their counterparts in the nucleus accumbens. It is known that the basal expression of mRNA for NGFI-A [28] and NGFI-B [29] is relatively high in the striatum. Two studies examined the expression of NGFI-A and NGFI-B mRNA [30]. They showed that lower doses of caffeine (7.5-25 mg/kg) decrease the expression of mRNA for NGFI-A and NGFI-B in the striatum. This suggests that low doses of caffeine, similar to that of typical human caffeine consumption, are stimulant. This is caused by the blockade of adenosine receptors which are abundant in the striatum. This is further supported by the finding that caffeine-induced changes are located in the striatopallidal neuron, which expresses adenosine A2 receptors in high abundance.

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The research at hand showed similar results; mice treated with moderate doses of caffeine showed enhancement in motor functions, while those treated acutely with high doses showed the opposite.

When examining the effects on the cognitive function, it was apparent that low doses of caffeine enhance the cognitive function while high doses deteriorate it. In fact, studies have concluded that activation of adenosine A_1 receptors strongly inhibits the release of acetylcholine from pyramidal hippocampal neurons [31][32][33]. Acetylcholine has been known to be important for memory storage [34].

To our knowledge, the literature contains few pieces of research on the effect of caffeine on memory retrieval, a topic that deserves further questioning. The results obtained in those studies agree with our results that caffeine improves retention of cognitive function on short-term ingestion when given with lower doses [35].

Our result support previous studies, in that lower doses of caffeine enhances the cognitive function. In fact, AC MD group had significant improvement in memory retrieval, while the AC HD group was negatively affected compared to the controlled group [36].

Caffeine administration can exert anxiolytic or anxiogenic effects in rodents depending on the anxiety test employed, the rat strain, and the sex of the rat [37]. Recent reports have associated caffeine with anxiety-related behaviors, and the suggested mechanisms include; blockade of benzodiazepine binding sites on GABAA receptors, stimulation of central noradrenergic activity, or antagonism of adenosine receptors. [17][18][19]. It has also been shown that the mesocortical cholinergic neurons are tonically inhibited by adenosine and that caffeine consequently increases their firing rate [38]. It was proposed that this effect is of importance in the electroencephalogram (EEG) arousal following caffeine ingestion. Because dopamine and noradrenaline neurons also are involved in arousal, there is ample neuropharmacological basis for assuming that central stimulatory effect of caffeine could be related to inhibition of adenosine A1 receptors. Also, there is an increase in 5-hydroxytryptamine receptors, muscarinic receptors, and delta–opioid receptors following higher doses of caffeine [39]. It is also known that caffeine increases the turnover of several monoamine neurotransmitters, including 5hydroxytryptamine (5-HT) dopamine, and noradrenaline [40][41]. As well as increasing the rate of firing of noradrenergic neurons in the locus ceruleus [42]. The increase in noradrenaline turnover is probably the explanation for the fact that methylxanthines also reduce the number of β-adrenoceptors in rat brain [39]. Caffeine modifies or antagonizes the effects of benzodiazepines on behavior in both animals and humans [43][44]. The mechanism for this antagonism was proposed to be the blocking of benzodiazepine receptors by caffeine. Caffeine does have weak antagonistic properties at these receptors. However, this mechanism requires very high concentrations of caffeine [45][46]. More recent evidences [26][47] suggests that the interaction between caffeine and benzodiazepines is mediated through caffeine's effects on adenosine receptors. There is some evidence suggesting that caffeine may also be a histamine receptor antagonist [48].

Our research showed anxiogenic effects of caffeine on the treated mice; This finding is opposing the idea that high doses of caffeine exert an anxiogenic effect while low doses exert an anxiolytic effect) [49]. In fact, there was no clear relationship between the dose and the effect, Ac MD group was more anxious than Ac HD group.

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There is a lack of researches concerning caffeine and its effects on sociability and preference for social novelty. Several studies showed that caffeine decreased social interaction in mice and rats [50][51]. The suggested mechanism can be related to its anxiogenic actions [50][51][52]. Another study demonstrated that AC HD of caffeine decreases the level of social interaction [53].

In the three chambers test, the control group that was treated with saline solution spent more time exploring the conspecific than the unknown object. This pattern of behavior was preserved after administration of the moderate dose of caffeine, but not after the high dose. Which further indicates a lack of preference for the conspecific after receiving the high doses of caffeine. The same study showed that the high doses of caffeine significantly decrease the time spent sniffing the novel conspecific. As a result, AC HD of caffeine reduces the sociability and preference for social novelty [47].

Our study demonstrates that the acutely treated groups of mice were less social than the Cont group. Such results indicate that the decrease in sociability is not significantly dose-dependent. Same results were shown regarding preference for social novelty in previous pieces of research. However, Ac HD group preferred to spend more time with the novel mice than the Ac MD group.

Overall this study documents the diverse effects of caffeine on different parameters. Ac MD group showed better results in motor and cognitive functions, whereas Ac HD group was less anxious than Ac MD group. The most striking result was that sociability was not dose-dependent but had an equal effect on mice when administered. Since no concrete evidence proves the link between the dose of caffeine and the level of decrease in sociability, further investigation is advised.

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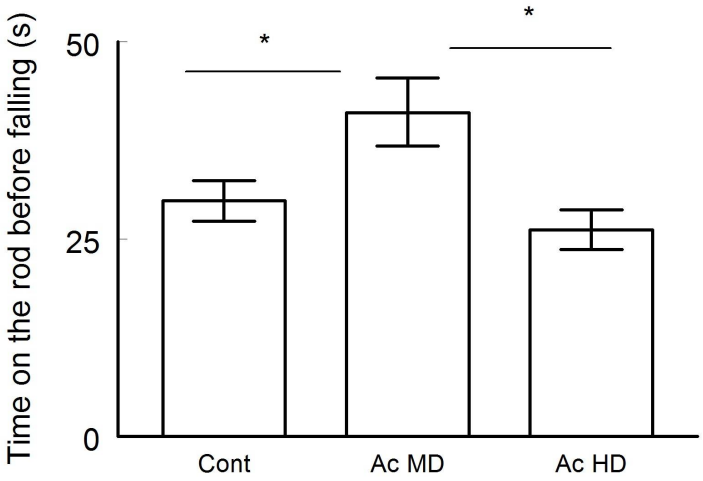

Figure 1. Latency to fall (mean ± SEM seconds) in the rotarod test for the control, moderate dose, and high dose groups. The time spent by Ac MD was significantly more than the other groups (ANOVA test, $p<0.05$).

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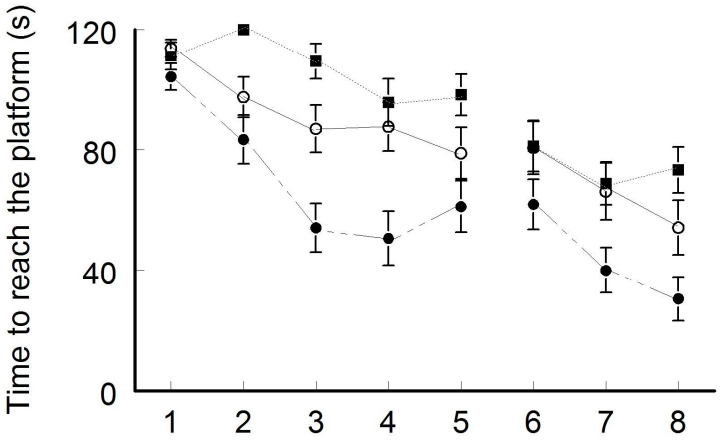
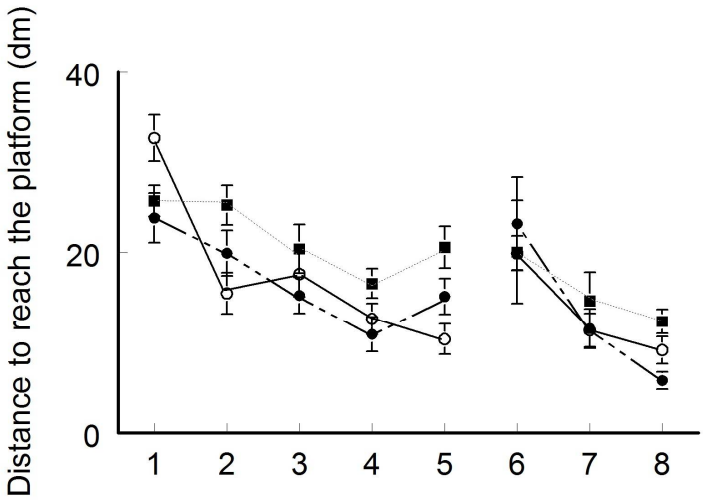


A



B

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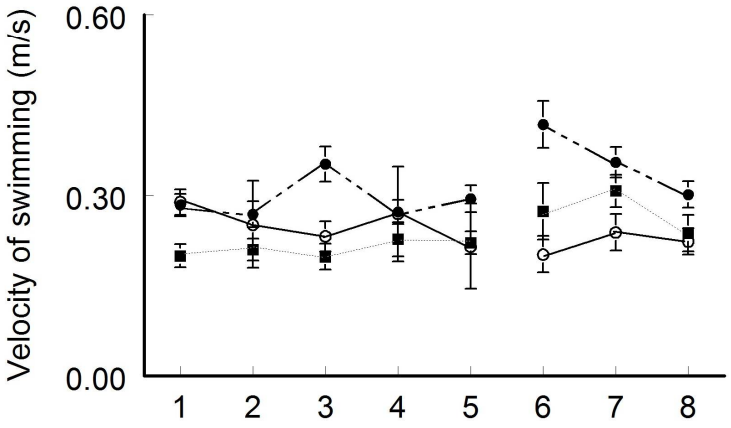
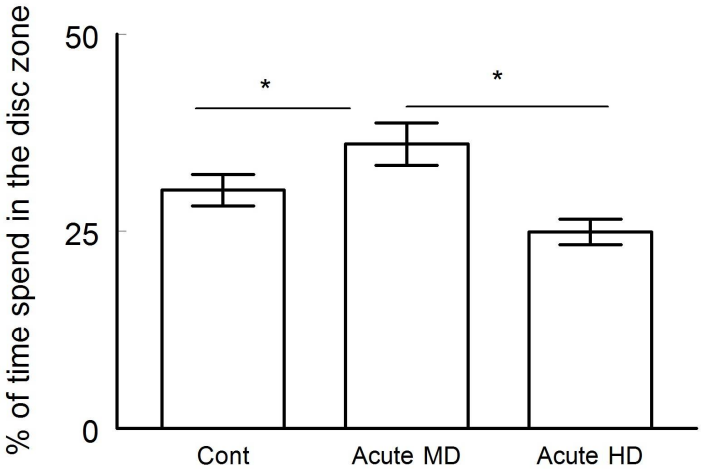


C



D

Figure 2. Morris Water Maze test used to assess cognitive function of the control, moderate dose, and high dose groups. (A) Time (latency) to reach the platform, (B) Distance to reach platform, (C) Velocity of swimming (m/s) and (D) % of time spent in the disc zone (Probe Test). In all the parameters tested, the Ac MD group showed better results than the other groups (ANOVA Test, $p<0.05$).

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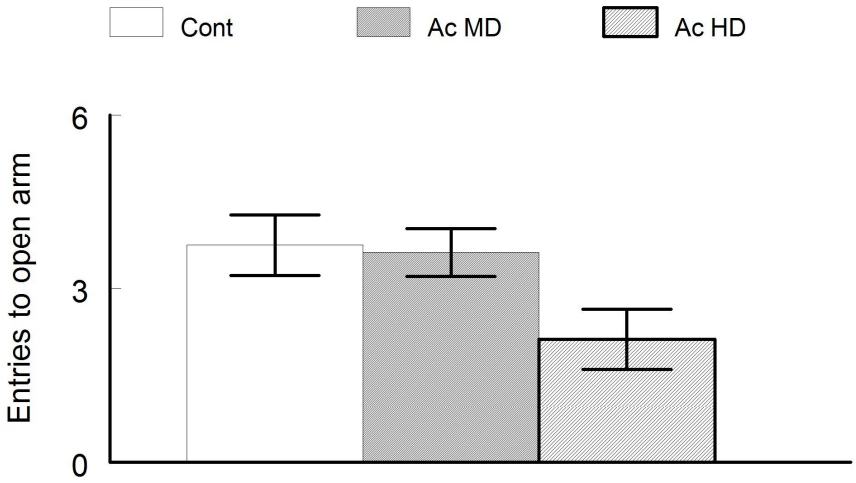

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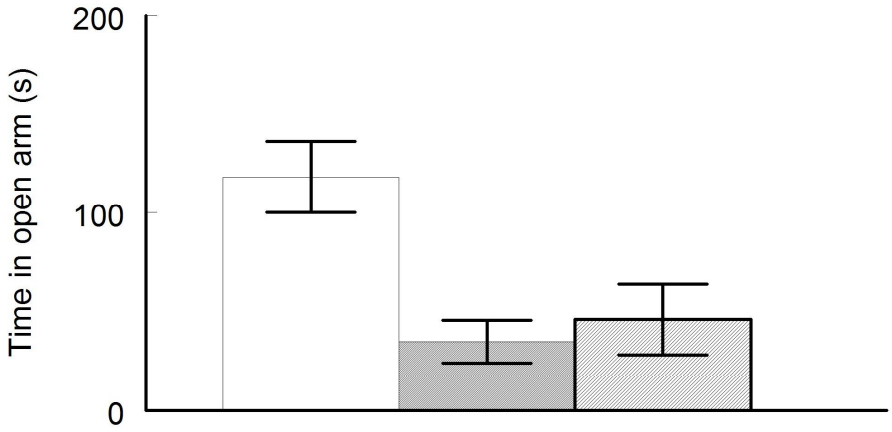

B

Figure 3. (A) and (B). Elevated Plus Maze test to assess anxiety. Although Ac MD group (3.6 ± 0.42 s) entered the open arm more frequent than the Ac HD group (2.13 ± 0.52) (Fig 3A), it spent the least amount of time (34.9 ± 10.8) (Fig 3B). Overall, the caffeine-treated mice were more anxious than the control group.

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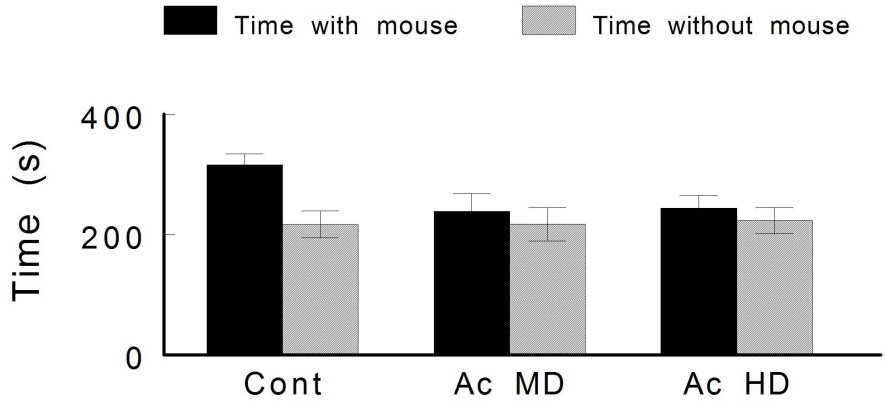

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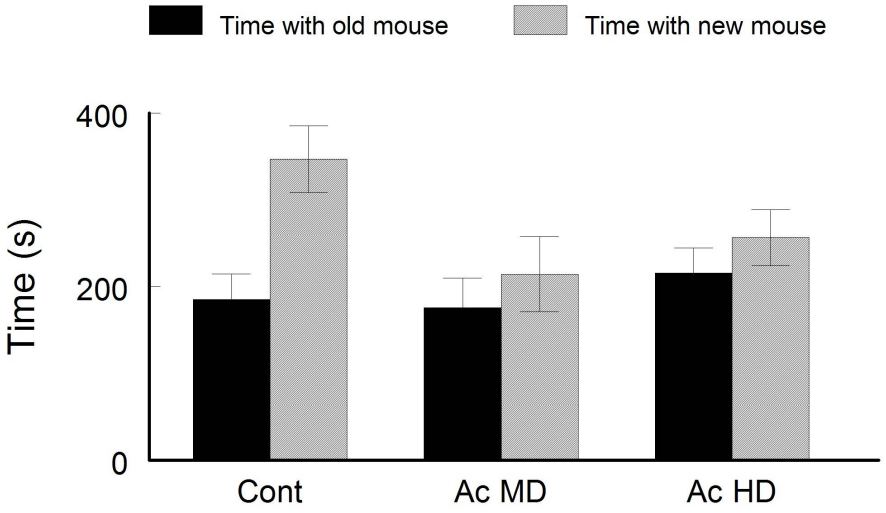

B

Figure 4. Three Chambers test to assess sociability and preference for social novelty. (a) Sociability (Session 1: Time spent in the chamber with the mouse versus chamber without the mouse). (b). Preference for social novelty (Session 2: Time spent in the chamber with the old mouse versus chamber with the novel mouse). Control animals showed normal sociability and preference for social novelty. Caffeine treated mice showed decreased sociability and preference for social novelty.