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Research Paper

Early life energy drink exposure impairs social and cognitive behaviour in female rats before and after pregnancy

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ABSTRACT

This study aimed to investigate the effects of early life energy drink consumption on social interaction, locomotor activity, and learning in female rats prep-regnancy and postpartum period. Twenty-seven 21-day-old Wistar albino rats were divided into three groups (n=9): control (CG, tap water), EG1 (10 % Energy Drink-ED), and EG2 (20 % Energy Drink-ED). The 63-day experiment comprised two phases. Data were analysed via one-way ANOVA and Tukey's post hoc test. No significant difference in feed consumption was found, but fluid intake was higher in ED groups. EG2 showed the greatest weight gain. Locomotor activity was higher pre-pregnancy than postpartum. In social tests, EG1 and EG2 preferred familiar individuals pre-pregnancy, whereas CG preferred unfamiliar ones. Postpartum, all groups showed increased unfamiliar preference. In the Barnes Maze, CG reached the target hole fastest and with fewest errors; EG2 performed worst ($p < 0.05$). These results indicate that chronic, dose-dependent energy drink consumption increases fluid intake and body weight, causes stress-like behaviours, and negatively affects learning and memory. Furthermore, these findings will contribute to the implementation of preventive public health measures, thereby helping to prevent obesity, diabetes and mental health disorders associated with excessive energy drink consumption. Communicating these results to manufacturers and consumers may support evidence-based regulatory measures, particularly for adolescents and women of reproductive age.

1. Introduction

Eating habits are essential behaviors for sustaining life. These habits are influenced by genetic, demographic, socio-economic, and cultural factors. The agricultural revolution increased the consumption of meat and high-fat dairy products, while the industrial revolution increased the production and consumption of refined grains, sugars, and vegetable oils [1,2]. In addition, lifestyle changes in Westernized societies in recent years have led to the emergence of a Western-style (WD-style) diet. This diet is poor in fiber, vitamins, and minerals and includes processed foods, fast food, convenience foods, snacks, and sugary carbonated beverages [3].

This new way of eating has led to a significant rise in the prevalence of obesity, metabolic syndrome, fatty liver disease, developmental delays, infertility, and various chronic diseases [4]. This shift has affected not only solid food consumption patterns but also the quantity and variety of liquids consumed in daily diets.

In recent years, the variety and availability of energy drinks containing caffeine and other active substances have increased significantly. Their consumption is widespread among children and adolescents. However, excessive and prolonged intake of these beverages from an early age is associated with serious health risks, raising growing concern [5]. The underlying social concern arises from their adverse effects on the nervous and cardiovascular systems, as well as their potential for addiction.

Energy drinks contain high levels of caffeine and other added substances. Excessive consumption of these compounds can overstimulate the adrenergic system, leading to central nervous system effects such as seizures, cerebral vasculopathy, and manic psychosis [6]. Moreover, high caffeine intake may cause symptoms including restlessness,

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anxiety, insomnia, and tremors [7]. Richards and Smith (2016) reported in their study that chronic energy drink consumption is associated with anxiety, stress, and depression [8]. Similarly, animal studies have indicated that prolonged energy drink consumption is linked to reduced motor activity, increased anxiety-like behaviors, and alterations in body weight [9,10].

Individuals respond differently to energy drinks depending on their developmental stage. In this context, childhood, adolescence, and pregnancy represent particularly critical periods. Childhood and adolescence are stages during which physical, psychological, and social maturation occurs and when cognitive development and learning are most intense. Pregnancy is a unique physiological and emotional process in a woman's life. The emotions experienced during this period are profound and distinctive; however, it also entails specific challenges and risks. Both positive and negative experiences during pregnancy can directly impact the mother and the developing fetus. In the literature, mental health disorders are among the most frequently reported adverse experiences during pregnancy. These conditions may lead to prolonged effects in the fetus, including delayed motor development and impaired social interaction [11].

The underlying causes of mental health issues during pregnancy are not attributable solely to physiological changes and hormonal fluctuations in the body, but also involve the individual's pre-pregnancy psychological state [12]. Numerous studies have shown that women with pre-existing mental health conditions such as depression or anxiety are more likely to experience a worsening of symptoms during pregnancy, as well as an elevated risk of developing new mental health disorders [13,14].

Additionally, some studies have indicated that harmful pre-pregnancy habits such as alcohol, tobacco, or stimulant use may increase the risk of depression and anxiety and are associated with psychological and physiological complications during pregnancy [15]. Among these, alcohol and tobacco are the most prominent. In recent years, energy drink consumption has also become increasingly widespread, with a notable rise particularly during sensitive developmental periods such as childhood, adolescence, and pregnancy. Our literature review revealed that studies on the effects of energy drinks are predominantly limited to metabolic outcomes; in contrast, research focusing on learning, memory, and, in particular, the behavioural profiles of females both before and after pregnancy is very limited. To bridge this critical gap, the present study was designed in accordance with the imperative for animal models that demonstrate robust translational validity to human physiological and behavioural outcomes.

In the study, using an experimental animal model established from recently weaned female rats, the effect of early-life energy drink consumption on social interaction, locomotor activity, and learning both pre-pregnancy and postpartum rats was investigated.

2. Materials and methods

2.1. Experimental procedure, animals

The study was conducted at the Animal Experimentation Unit, Faculty of Medicine, Ahi Evran University, Kırşehir. The rats used in this study were bred at the Animal Experimentation Unit of Kırşehir Ahi Evran University Faculty of Medicine and were selected from the same age group of offspring born to unselected breeding stock. They were housed together under standard conditions. Those included in the experimental groups constituted approximately 60 % of these offspring. Twenty-seven 21-day-old female Wistar albino rats were used. Throughout the study, the rats were housed under controlled environmental conditions: temperature (22 ± 2°C), relative humidity (65 %), and a 12:12 h light/dark cycle (lights on from 08:00 to 08:00 p.m.). Rats were not removed from their home cages except for behavioural testing.

Rats were fed ad libitum with standard rodent chow (Optima; 24 % crude protein, 3.94 % crude cellulose, 5.08 % crude fat, 8.8 % crude ash, 1.44 % lysine, 0.61 % methionine, 1.14 % calcium, 0.89 % phosphorus, 0.28 % sodium; Lot No. 58931, Turkey). The control group received tap water ad libitum, while the experimental groups were provided with tap water containing 10 % or 20 % energy drink ad libitum.

Prior to commencement of the experiments, the animal use protocol was approved by the Kırşehir Ahi Evran University Local Ethics Committee on Animal Experiments (Approval No: 15-02; dated 23/07/2025). All procedures were carried out in accordance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments). Furthermore, all experimental procedures involving live animals complied with the Regulation on the Welfare and Protection of Animals Used for Experimental and Other Scientific Purposes, issued by the Ministry of Food, Agriculture and Forestry of the Republic of Türkiye [16].

2.2. Energy drink composition and administration procedure

In the present study, a commercially available sugar-free energy drink (ED), Red Bull Sugarfree® (250 mL cans), was used. The sugar-free formulation was selected to eliminate potential confounding effects associated with carbohydrate intake. Furthermore, the ease of implementation and the growing preference for low-calorie, sugar-free caffeinated energy drinks have contributed to the development of this idea. Therefore, we selected the sugar-free Red Bull energy drink for testing [17]. As per the manufacturer's label, the Red Bull Sugarfree® ED used contained the following ingredients per litre; caffeine: 150 mg/L, taurine: 800 mg/L, vitamins: niacin 8 mg/100 mL, pantothenic acid 2 mg/100 mL, vitamin B_6 2 mg/100 mL, vitamin B_{12} 2 μg/100 mL, additives: xanthan gum (thickener), natural and artificial flavourings, colourants: plain caramel, riboflavin, phenylalanine. In the study, the energy drink (ED) was administered by mixing it into the drinking water to avoid irritation from its taste and odor, to reduce the risk of adverse effects such as aspiration pneumonia associated with oral gavage, and to minimize stress related to the delivery procedure [18].

Inter-species dose extrapolation has been used to determine the application dose of energy drinks, based on studies reported in the literature [19]. Dosing was extrapolated from typical human consumption: assuming a 65 kg adult consumes either one (250 mL) or two (500 mL) cans daily, equivalent to approximately 10 % and 20 %, respectively, of the estimated total daily fluid intake. Accordingly, the experimental groups received drinking water containing 10 % (v/v) or 20 % (v/v) ED [20].

2.3. Study groups

The study included three groups: a control group (CG) receiving fluid (tap water), Experimental Group 1 (EG1) receiving a 10 % ED solution, and Experimental Group 2 (EG2) receiving a 20 % ED solution. Based on similar published studies, a power analysis was conducted using G*Power and the MINITAB statistical software package [21]. This analysis determined that a minimum of nine animals per group was required to achieve adequate statistical power, resulting in a total of 27 animals [21,22]. All animals had ad libitum access to food and their fluid throughout the study period.

2.4. Experimental procedure

The study used 21-day-old female rats. The rats were placed in groups of three in nine breeding cages (425 × 265 × 150/800 cm^2), with three rats per cage. As shown in Fig. 1 the study was conducted in two sequential phases, spanning postnatal days 1–34 and 35–63, for a total duration of 63 days. In the first part of the experiment, the Open-Field Test and Social Maze Test were performed to assess locomotor activity, socialisation, and social preference in the rats. Spatial learning, learning ability, and memory were evaluated using the Barnes Maze Test. In parallel, Body Weight, Fluid Consumption, and Feed Consumption were monitored regularly to calculate group-wise changes in

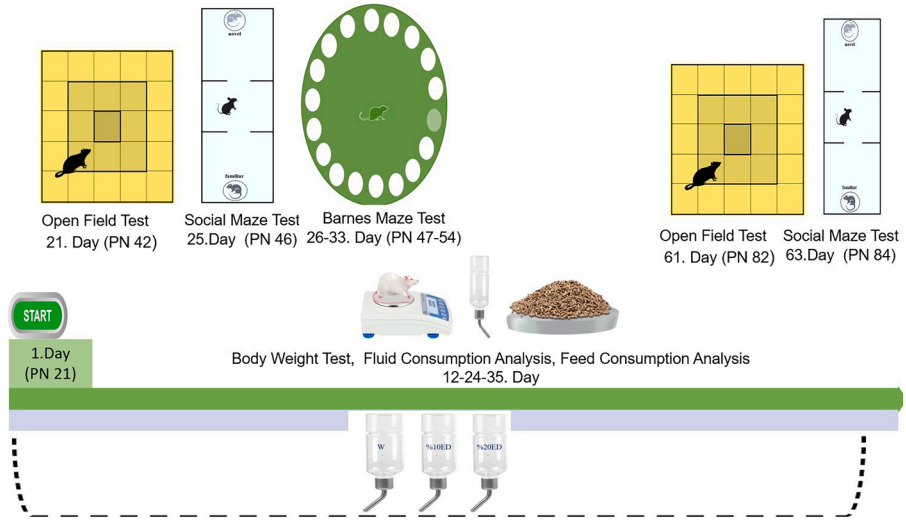

Fig. 1. Experimental procedure. Rat study groups were given energy drinks for 63 days. To determine the effect of the intervention on social and cognitive behaviors, an open field test was conducted on days 21 and 61, a social maze test on days 25 and 63, and a Barnes maze test on days 26 and 33. Measurements were taken on days 12, 24, and 35 to evaluate feed and fluid consumption and live weight gain. (PN: postnatal).

body weight, and feed and fluid intake. In the second part of the experiment, rats from each group were mated to induce pregnancy. For this purpose, female rats were housed with breeding males in mating cages at a ratio of 2 females to 1 male for 6 days. At the end of this period, the females were regrouped into sets of three and placed into nine breeding cages (425 × 265 × 150/800 cm^2), where they remained until the end of the study. Births occurred on days 54-56 of the study in these cages. To determine the locomotor activity, socialization, and social preferences of the mothers who gave birth, the Open Field Test was administered on day 61 of the study, and the Social Maze Test was administered on day 63.

2.5. Analyses

2.5.1. Physical analyses

2.5.1.1. Fluid and feed consumption analysis. Feed and fluid intake was measured on days 12, 24, and 35 of the study in 3 cages per group, totaling 9 cages, by calculating the difference between the initial and remaining amounts. This value was divided by the number of days to determine the average daily consumption per cage. Additionally, the weights of the rats in each cage were measured on days 12, 24, and 35 to determine the total weight of the rats in the cage. Subsequently, absolute and relative consumption values for each cage were calculated using the following formulas, and daily feed consumption was expressed as g/100 g body weight (BW) and daily fluid consumption as mL/100 g BW.

$$Feed\,consumption\,amount = \frac{Average\,daily\,feed\,consumption\,in\,the\,cage}{Body\,weight\,value\,in\,the\,cage} x100$$

$$Fluid\ consumption\ amount = \frac{Average\ daily\ fluid\ consumption\ in\ the\ cage}{Body\ weight\ value\ in\ the\ cage} x100$$

2.5.1.2. Body weight measurement. Changes in body weight between days 1, 12, 24 and 35 were recorded for each rat during routine cage cleaning, conducted, using a calibrated laboratory balance.

2.5.2. Behavioural analyses

The Open-Field Test, Social Maze Test, and Barnes Maze Test were administered to rats (n=5) in their experimental groups to assess the effects of ED consumption on locomotor behaviour, social preference, and learning. Rats' movements on the test apparatuses were recorded using a digital video camera positioned above each arena. For automated tracking and analysis, the Python programming language was employed [23]. Using a custom Python-based script, trajectory plots were generated from the recorded movement paths, and the total distance travelled (walking score) was computed for each rat. All behavioural tests were conducted in a dedicated testing room within the same facility, maintained under standard environmental conditions (temperature: 22 ± 2°C; humidity: 65 %; 12:12 h light/dark cycle). Additionally, all behavioral tests were conducted during the light phase of the light/dark cycle.

2.5.2.1. Open-field test. For this test, a 100 × 100 × 30 cm Open-Field Test platform was used. First, the rats were brought into the room containing the Open Field Test platform and kept in this environment for 60 minutes before being placed on the test platform. To ensure that the rats voluntarily chose to spend time in the center, they were placed in the corner instead of the center. Their movements were then recorded by camera for 5 minutes. At the end of each experiment, the test platform

was washed with 70 % ethanol. At the end of the test, the rats' trajectories on the platform (The trajectory of the open-field test, Total Distance Travelled (TDT) and Number of Entries and Exits from the Centre (NEEC), Time Spent in the Centre (TSC) and Number of vertical exploration behaviours (NVEB)) were determined for use in assessing locomotor activity [24].

2.5.2.2. Social maze test. This test is defined as the tendency to spend time with a novel conspecific rather than a familiar one. The procedure was designed to assess, in the same cohort of female rats, sociability and social novelty preference toward familiar peers during adolescent rats following prolonged ED consumption, and sociability and social novelty preference toward their own offspring after these same rats underwent parturition. An adapted version of the three-chambered apparatus for sociability and social novelty preference testing, as described by Aydemir and Ergün (2025), was used [25]. The day before testing, each rat was placed in the empty Social Maze Test apparatus for 3 minutes to allow habituation. One hour prior to the test, the subject rat was transferred to the testing room and remained there until the conclusion of the procedure. In the experiment, the familiar rat was the cage mate/own offspring of the rat undergoing the test. As unfamiliar rat, rats from other groups present in the same environment during the study period/stranger offspring were used. The unfamiliar rats selected for the study were used equally from the two groups outside the tested group. During the experiment, the familiar rat was placed in the wire mesh compartment on the right side of the platform, while the unfamiliar rat was placed in the wire mesh on the left side.

The subject rat was then placed in the central chamber, and its behaviour was recorded by camera for 3 minutes. After each trial, the apparatus and enclosures were cleaned with 70 % ethanol. For each experimental group, the time spent in each chamber, the distance travelled, and the mean velocity were calculated. The effects of ED consumption on sociability and social novelty preference were determined using the following formulas:

PFI (Preference for familiar individuals) = TSF/TT x 100

PUI (Preference for unfamiliar individuals) = TSU/TT x 100

(TSF: Time spent with familiar individuals, TSU: Time spent with unfamiliar individuals, TT: Total time)

OOP (Own offspring preference) = TSOO/TT x 100

FOP (Foreign offspring preference) = TSFO/TT x 100

(TSOO: Time spent with own offspring, TSFO: Time spent with foreign offspring, TT: Total time)

2.5.2.3. Spatial learning and memory assessment (barnes maze test). In the study, a modified version of the Barnes Maze Test platform, as used by Peris et al. (2024), was employed to assess the effect of ED consumption on spatial learning and memory [26]. For this purpose, the following parameters were recorded for rats on the maze platform: time to find the target hole, distance to find the target hole, velocity to find the target hole, and total number of errors. As shown in Fig. 2 the modified Barnes Maze platform has a diameter of 108 cm, with 18 holes (each 10 cm in diameter) spaced evenly around the perimeter and an escape box positioned beneath one of the holes. In addition, the waterproof floor is black and features four visual cues of distinct shapes and colours, positioned on each of the four cardinal sides.

In the Barnes maze used in the experiment, the escape box was painted a darker shade of black than the platform floor to enhance its visibility to the rats. The perimeter of the platform was enclosed with white opaque screens to ensure that the animals attended exclusively to the distal visual cues. Light intensity at the centre of the platform was maintained at 1000 lx [27]. An auditory stimulus, a whistle sound of approximately 80 dB, was used motivate escape behaviour.

All trials were conducted between 08:00 and 02:00, p.m., during the light phase of the light/dark cycle. After each rat's trial, the maze surface was cleaned with 70 % ethanol. Prior to the experiment, rats were habituated to the apparatus by being placed on the platform for 3 minutes, followed by 1 minute inside the escape box [28–30]. The experiment was conducted in three phases: the training phase, the first probe trial, and the final probe trial.

Stage 1 (training test) (TT): The training phase was conducted over four consecutive days. At the start of each trial, the rat was placed in the centre of the platform within a transparent start box for 10 seconds. The box was then lifted, and the rat was allowed to freely explore the maze for up to 3 minutes to locate the escape hole and enter the escape box. If the rat successfully entered the escape box, the trial was terminated, and the animal was permitted to remain inside for 1 minute. If the rat failed to locate the escape box within the 3-minute period, it was gently guided to the target location [31].

Stage 2 (first research attempt) (FR): On the fifth day of testing, the first research attempt was conducted to evaluate short-term memory retention. For this purpose, a single trial was performed [32]. Rats were placed in the centre of the test maze and allowed to explore the platform and locate the escape hole for 3 minutes.

Stage 3 (second research attempt) (SR): A second trial was conducted 48 hours after the first to assess long-term memory retention. Prior to the trial, the platform was rotated by 180° [26].

2.5.2.4. Evaluation of behavioural analyses. In this study, the behavioral activities of the rats were recorded and evaluated using a video camera (XIAOMI REDMI NOTE 10®) placed at the center of the behavioral platforms. To ensure representativeness, behavioural tests were administered to more than 50 % of the rats (n=5). For this purpose, the

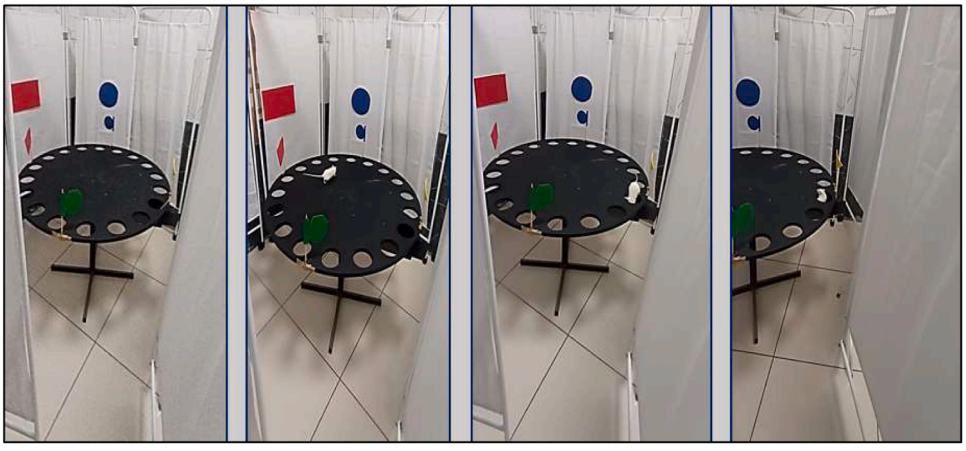

Fig. 2. The Barnes maze platform. To help the rats find their way out of the maze, extra-maze cues in different colors are mounted on the white curtain backdrop surrounding the maze.

Python programming language, widely used in image processing, was used to track the rats' movements. Using Python's OpenCV library, object tracking was performed by analyzing the input video frames step by step, and the rat's position was updated at the end of each frame. The rat's tracking process within frames was achieved with the CSRT (Discriminative Correlation Filter with Channel and Spatial Reliability) algorithm. This algorithm ensures high accuracy despite minor image changes [23]. In each frame, the rat's center coordinates were calculated, creating a movement path, and these path lines were reflected in the video. The frames processed throughout the process were both displayed simultaneously on the screen and saved as an output video, preparing for the next step. In the next step, the total distance walked by the rat (walking score) was converted to centimeters using the path image generated by the rat's movement using a program calculated solely in pixels. To this end, the input image in RGB format was converted to HSV color space using the OpenCV library, and this conversion enabled the filtering of path lines representing the rat's movements. Appropriate HSV ranges were defined for each color, and masking was performed on the image based on these ranges. This allowed the path formed after the walk to be separated from the raw image. After this process, a list was created by sequentially taking the coordinates of each pixel on the resulting route, and the length of each step was calculated by applying the Euclidean distance between consecutive points using this coordinate set. As a result of this calculation, a pixel-based walk score was obtained directly from the digital image. In the next step, the aim was to identify the path formed by the rat's movement from the digital image and export it in a format with a transparent background. For this purpose, the input image in RGB format was converted to HSV color space using the OpenCV library and thresholded according to the path color to create a mask. The resulting masks were used to separate the path color pixels. Subsequently, a new four-channel (RGBA) output image was created using the mask as the alpha channel. This resulted in the final image being opaque only for the pixels containing the path traces, while all other regions were fully transparent. By viewing these images, differences in the behavioral activity of the rats between the groups were determined.

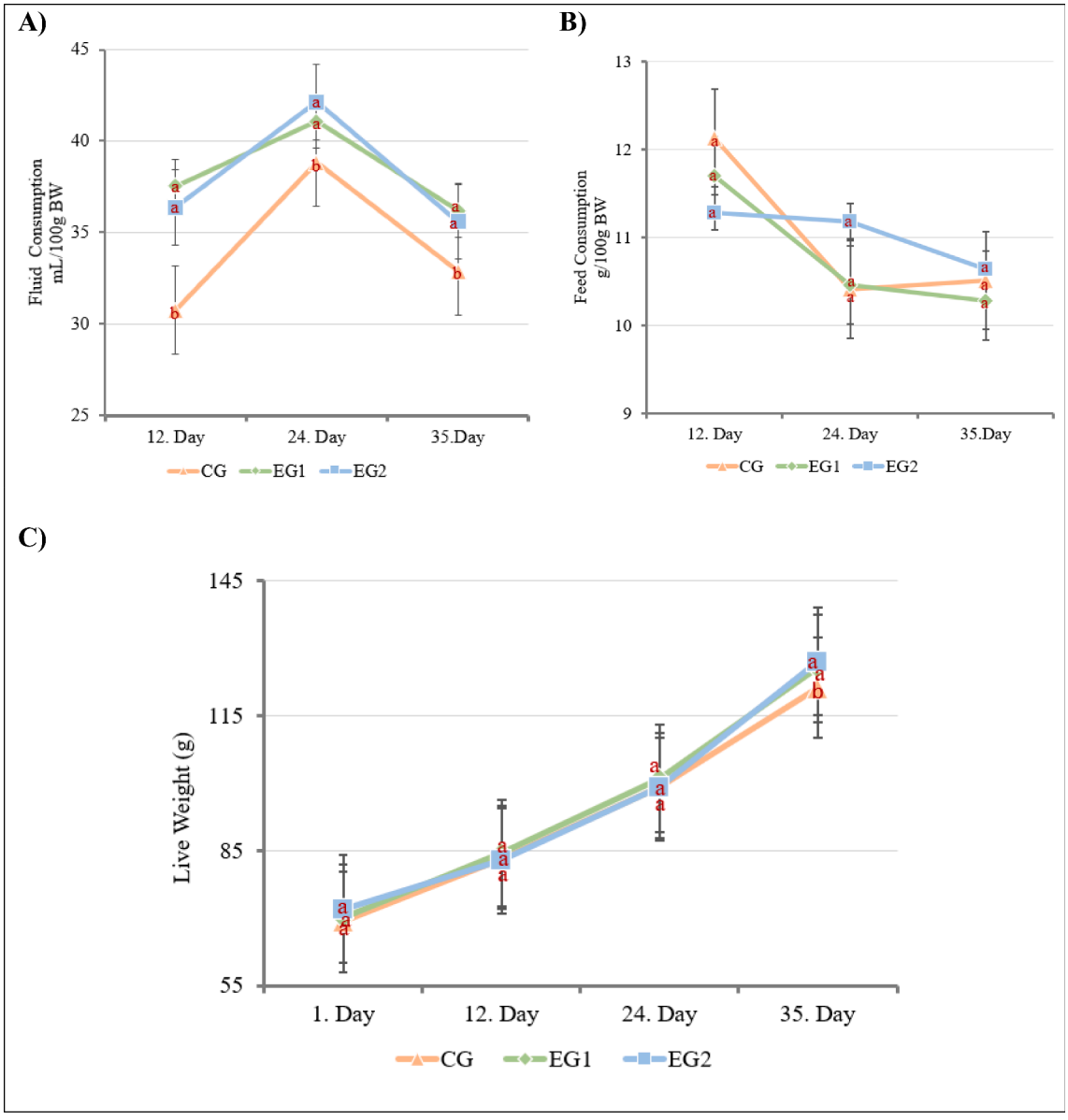

Fig. 3. Groups' feed and fluid consumption amounts and live weight gain. CG, Control group. EG1, Group consuming 10 % energy drink. EG2, Group consuming 20 % energy drink. Differences between means denoted by the same letter are not significant at the $p<0.05$ level.

2.6. Statistical analyses

Data were analysed using SPSS Statistics version 22 V. The effects of energy drink consumption on selected behavioural and physiological parameters in rats were first assessed using one-way analysis of variance (ANOVA). Where ANOVA revealed significant differences ($p < 0.05$), Tukey test was applied to identify which group(s) differed significantly. The threshold for statistical significance was set at $p < 0.05$.

3. Results

No deaths or other pathological problems were observed in any rats during the study period. Therefore, no rats were removed from the experiment. Feed and fluid consumption amounts and live weight gain rates were calculated for each group on days 12, 24, and 35. Feed intake showed no statistically significant differences between groups on day 12 [F(2,6) = 1.44, p = 0.308], day 24 [F(2,6) = 2.88, p = 0.133], and day 35 [F(2,6) = 1.16, p = 0.370] (Fig. 3A). Fluid consumption differed significantly between groups on day 12 [F(2,6) = 92.27, p = 0.001], day 24 [F(2,6) = 5.27, p = 0.048], and day 35 [F(2,6) = 22.16, p = 0.002] (Fig. 3B). Groups receiving energy supplementation showed higher intake. In the study, there were no significant differences in live weight gains between groups on day 1 [F(2,6) = 0.48, p = 0.636], while there was a difference on day 12 [F(2,6) = 4.00, p = 0.050], but it was small. Significant differences between groups were observed on day 24 [F(2,6) = 7.54, $p = 0.023$] and day 35 [F(2,6) = 34.97, p = 0.001] (Fig. 3C). The largest increase was recorded in EG2.

Behavioral assessments in the study were conducted by blinded researchers. As shown in Fig. 4 in the study, an open-field test was administered twice to the rats in each group to assess the effects of energy drink consumption on locomotor activity: once pre-pregnancy and once postpartum. To this end, the rats' movement paths on the platform during the test, specifically, the trajectory of the open-field test (Fig. 4A), total distance travelled (TDT) (Fig. 4B), number of entries and exits from the centre (NEEC) (Fig. 4C), time spent in the centre (TSC) (Fig. 4D), and number of vertical exploration behaviours (NVEB) (Fig. 4E), were recorded and analysed. Overall, locomotor activity was higher during the pre-pregnancy testing period compared with the postpartum period.

In the study, during the pre-pregnancy period, the total distance travelled (TDT) on the open-field test platform was similar between the control group (CG) and EG1, whereas EG2 differed significantly from both [F (2,12) = 14.40, p = 0.02]. In the postpartum period, EG1 and EG2 showed similar TDT values, but both differed significantly from the control group [F (2,12) = 4.86, p = 0.001].

In the study, the number of entries and exits from the center (NEEC) of rats on the platform differed significantly between groups both in the pre-pregnancy period [F(2,12) = 6.61, p = 0.03] and in the postpartum period [F(2,12) = 54.76, p = 0.001]. Similarly, the time spent in the center (TSC) varied significantly among groups before pregnancy [F (2,12) = 42.63, p = 0.001] and after birth [F(2,12) = 22.15, p = 0.002]. The number of vertical exploration behaviors (NVEB) (Fig. 4E) was also assessed, revealing significant group differences in both the pre-pregnancy [F(2,12) = 34.06, p = 0.001] and postpartum periods [F (2,12) = 28.03, p = 0.001]. Moreover, EG1 exhibited higher NVEB counts than the other groups during both the pre-pregnancy [F(2,12) = 14.40, p = 0.02] and postpartum [F(2,12) = 4.86, p = 0.001] phases.

As shown in Fig. 5 the socialisation and social novelty preferences of rats were assessed pre-pregnancy and postpartum. During the pre-pregnancy period, significant differences were observed between

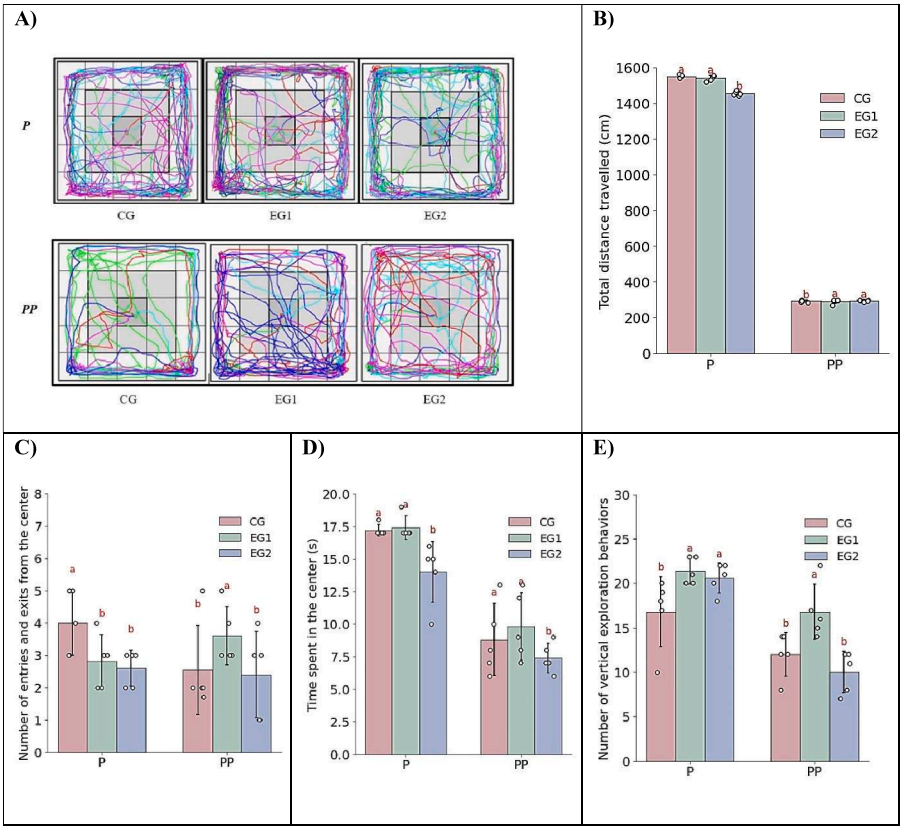

Fig. 4. Open field activation values. A, The trajectory of the open-field test. Each color represents the path followed by a rat within the group. B, Total Distance Travelled (TDT). C, Number of Entries and Exits from the Center (NEEC). D, Time Spent in the Center (TSC). E, Number of vertical exploration behaviors (NVEB). P, Pre-pregnancy. PP, Postpartum. CG, Control group. EG1, Group consuming 10 % energy drink. EG2, Group consuming 20 % energy drink. Differences between means denoted by the same letter are not significant at the p<0.05 level.

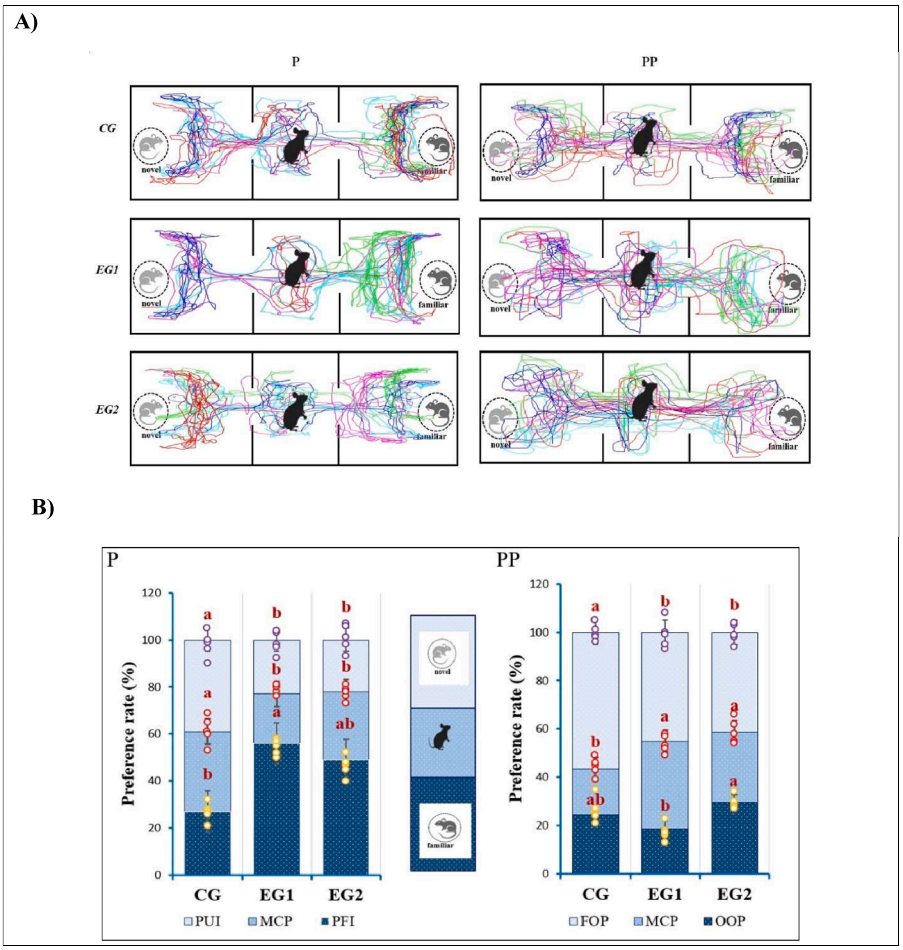

Fig. 5. Social preference test scores. A, The trajectory of the social maze test. Each color represents the path followed by a rat within the group. B, Sociability and social novelty preference. P, Pre-pregnancy. PP, Postpartum. PFI, Preference for familiar individuals. PUI, Preference for unfamiliar individuals. MCP, Middle chamber preference. OOP, Own offspring preference. FOP, Foreign offspring preference. CG, Control group. EG1, Group consuming 10 % energy drink. EG2, Group consuming 20 % energy drink. Differences between means denoted by the same letter are not significant at the $p < 0.05$ level.

groups in the time spent with familiar individuals [F (2,12) = 33.72, p = 0.01], unfamiliar individuals [F(2,12) = 8.01, p = 0.02], and in the middle area [F(2,12) = 7.43, p = 0.02]. During this phase, both EG1 and EG2 showed a preference for familiar individuals, whereas the CG preferred unfamiliar individuals. In the postpartum period, all groups exhibited an increased preference for unfamiliar individuals. Significant between group differences were found in the time spent with familiar individuals [F(2,12) = 8.71, p = 0.01], unfamiliar individuals [F(2,12) = 11.67, p = 0.009], and in the middle area [F(2,12) = 15.57, p = 0.004]. Moreover, the CG displayed the strongest interest in unfamiliar offspring. Each line represents the trajectory of one rat within each colour coded treatment group. This pattern may be explained by the possibility that energy drink consumption exerts a positive influence on the mother–offspring bond.

In the study, during the test period, the routes taken by rats on the test platform (Fig. 6A), primarily to assess spatial learning and memory, were recorded. Additionally, the time to find the target hole (Fig. 6B), the distance to find the target hole it (Fig. 6C), the velocity to find the target hole (Fig. 6D), and the total number of errors (Fig. 6E) were determined.

During the Training Test (TT) phase, rats progressively reduced the time taken to reach the target hole across repeated trials, made fewer errors, and showed overall performance improvement indicating learning over successive days. On the first day, CG and EG1 showed similar times to reach the target. However, compared with the other groups, EG2 took significantly longer to reach the target hole; on day 1 [F (2,12) = 46.46, p = 0.001], day 2 [F (2,12) = 60.48, p = 0.001], day 3 [F (2,12) = 7.31, p = 0.02], day 4 [F (2,12) = 281.09, p = 0.001], day 5 [F (2,12) = 105.79, p = 0.001] and day 7 [F (2,12) = 46.90, p = 0.001]. This difference persisted until the final day of the training tests.

Throughout the training test, the total distance travelled by the rats on the platform was measured. Significant differences between groups were observed on day 1 [F(2,12) = 11.52, p = 0.009], day 2 [F(2,12) = 60.04, p = 0.001], day 3 [F(2,12) = 73.92, p = 0.001], day 4 [F(2,12) = 11.74, p = 0.008], day 5 [F(2,12) = 28.49, p = 0.001], and day 7 [F (2,12) = 17.09, p = 0.003]. Initially, the distance required to reach the target hole was high, but it gradually decreased over successive days, reflecting improved spatial learning. The highest distance values were recorded on day 1 in EG1, day 2 in EG2, day 3 in the CG, and on the final day in EG2.

In the study, the movement speeds of rats on the test platform were measured. Significant differences were observed on day 1 [F(2,12) = 150.96, p = 0.001], day 2 [F(2,12) = 43.22, p = 0.001], day 3 [F(2,12) = 32.72, p = 0.001], day 4 [F(2,12) = 6.53, p = 0.03], day 5 [F(2,12) = 45.37, p = 0.001], and day 7 [F(2,12) = 97.82, p = 0.001]. Rats consuming energy drinks located the target hole at speeds that decreased in inverse proportion to the dose consumed. Additionally, the total number of errors made during the training tests was assessed. On the 2nd day of the study [F (2,12) = 4.30, p = 0.06] and on the 4th day [F (2,12) = 4.24, p = 0.07] the differences between the groups were insignificant, while on the 1st day of the study [F (2,12) = 8.13, p = 0.02], on the 3rd day [F (2,12) = 11.37, p = 0.009], day 5 [F (2,12) =

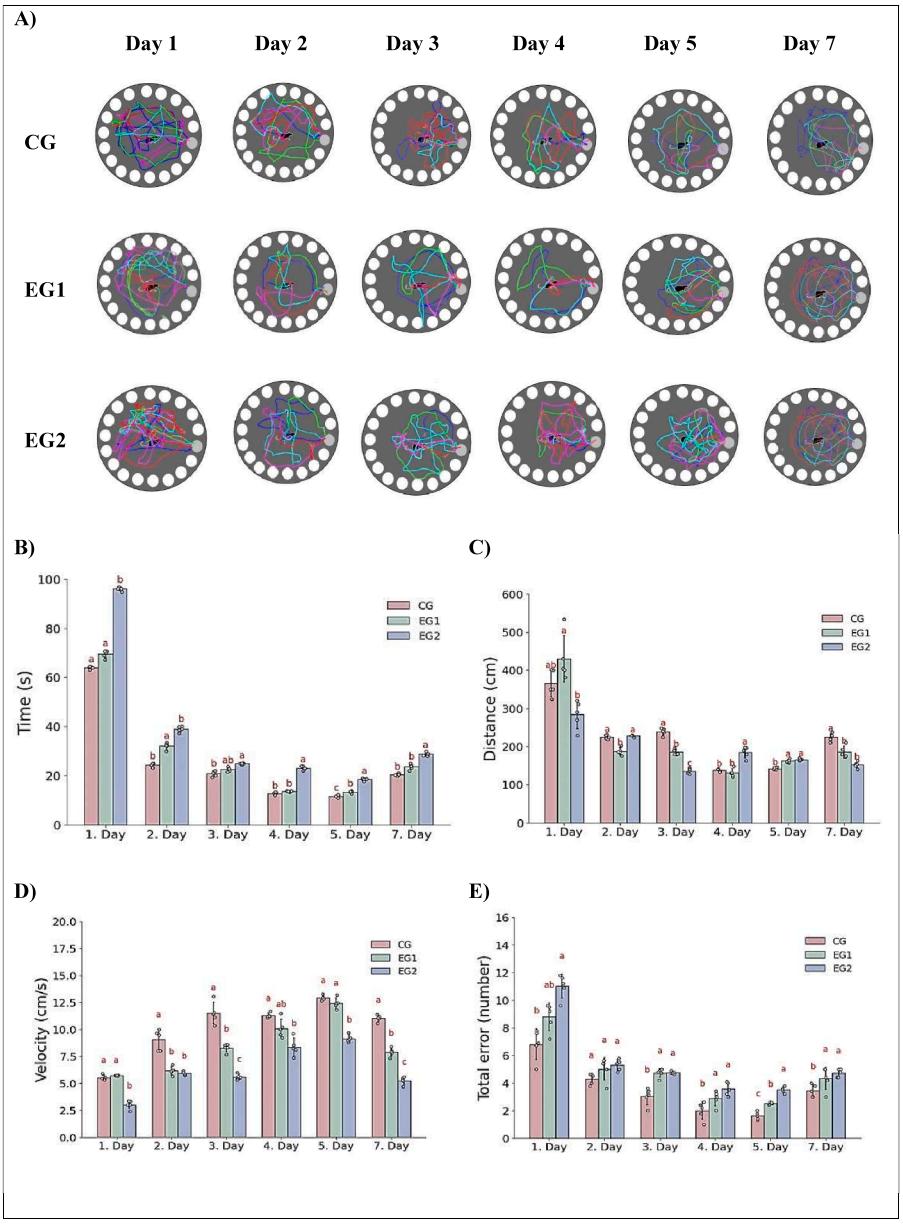

Fig. 6. Detection of spatial learning and memory. A, The trajectory of the Barnes maze performance test. Each box in the diagram represents a trial conducted for a group in turn. The gray circle in the arena indicates the location of the escape box. Each color represents the path followed by a rat within the group. B, Time to find the target hole. C, Distance to find the target hole. D, Velocity to find the target hole. E, Total number of errors. CG, Control group. EG1, Group consuming 10 % energy drink. EG2, Group consuming 20 % energy drink. Differences between means denoted by the same letter are not significant at the $p<0.05$ level.

47.74, $p = 0.001$], and day 7 [F (2,12) = 12.50, $p = 0.007$] ($p < 0.05$).

Following the initial research trial, the second phase of the test, the rats' time to locate the target hole, distance to find the target hole, velocity to find the target hole, and total number of errors were evaluated. Statistically significant differences between the groups were observed for all these parameters. The CG reached the target hole in the shortest time and over the shortest distance, whereas EG2 took the longest time and travelled the greatest distance. In terms of movement speed, CG was the fastest group and EG2 the slowest. Similarly, EG2 made the highest number of errors, while CG made the fewest during this trial.

In the second research trial, the rats' time to find the target hole, distance to find the target hole, velocity to find the target hole, and total number of errors were assessed. Statistically significant differences between the groups were observed for all these parameters. The CG located the target hole in the shortest time and with the fewest errors, whereas EG2 took the longest time and committed the most errors in reaching the target.

4. Discussion

The present study aimed to investigate the effects of early life energy drink consumption on social interaction, locomotor activity, and learning in female rats, both pre-pregnancy and postpartum. Energy drinks are now the most widely consumed beverage globally after bottled water [33]. Their use is especially prevalent among children and adolescents; one study reported that over half of children worldwide consume these beverages regularly [34]. This rising consumption, coupled with the trend of use beginning at increasingly younger ages, has prompted warnings from numerous medical associations due to potential health risks [35]. Although originally marketed to enhance

physical and cognitive performance, evidence indicates that excessive intake of energy drinks can lead to adverse physical and psychological effects, including cardiovascular issues, headaches, gastrointestinal discomfort, stress, anxiety, hyperactivity, and insomnia [36,37].

Anxiety and stress are among the most significant factors affecting the quality of life and well-being of living organisms. In stressful situations, living beings may deviate from their typical behaviour and display altered patterns of activity. These behavioural and physiological changes serve as indicators of stress levels. Various behavioural tests have been developed to assess such responses, one of the most common being the open field test. In this test, the time an animal spends in the centre of the arena is considered inversely related to its anxiety level: a longer duration in the centre suggests lower stress, whereas a shorter duration reflects higher anxiety and stress [38].

In the study, rats in both the pre-pregnancy and postpartum energy drink treated groups visited the central area of the open field arena less frequently and spent less time there compared with the control group. This suggests that prolonged energy drink consumption may induce stress or anxiety in rats, leading them to perceive the centre, the more exposed and potentially threatening zone, as unsafe, and thus avoid it. This behavioural change may be linked to the high caffeine content of energy drinks. Caffeine acts as an antagonist at adenosine receptors, and this blockade is primarily responsible for its stimulatory effects [39]. Furthermore, caffeine exerts a psychostimulant effect on the central nervous system and, when consumed in excess, has been shown to induce anxiety in both humans and rodents [40,41].

Similar studies have reported that long term energy drink consumption can increase anxiety and depression like behaviours in rodents and impair general behavioural responses [42,43]. In the present study, prolonged energy drink intake was also associated with a reduction in the total distance travelled during the postpartum period, suggesting a decline in the animals' overall exploratory motivation. However, the opposite pattern emerged during the pre-pregnancy period: the distance covered by groups consuming energy drinks increased during this period. This difference observed between groups in terms of total distance walked is consistent with the behavioural pattern determined by the number of centre visits and time spent at the centre. Moreover, group differences in number of vertical exploration behaviours further support the notion that energy drink consumption may initially induce stress in the animals, with this stress influencing both horizontal and vertical exploratory activities.

In a similar study, energy drink consumption in rats was found to reduce the number of centre visits, total distance travelled, and vertical exploration behaviours in the open field test in a dose-dependent manner compared with the control group, suggesting an anxiogenic effect [44]. Our findings are consistent with these results. Human studies have also shown that energy drink consumption significantly increases the risk of insomnia, irritability, and hyperactivity. These adverse effects appear to intensify with higher consumption frequency, larger serving sizes, or when energy drinks are combined with other stimulants such as alcohol [45]. Conversely, some research indicates that specific doses of caffeine containing energy drinks may enhance athletic performance, for example, improving running performance by approximately 2 % [46]. Nevertheless, given the potential short term and long term health risks, excessive consumption of these products should be avoided [47]. It has been reported that energy drink consumption pre-pregnancy is associated with an increased risk of pregnancy-induced hypertension [48]. Similarly, caffeine intake during pregnancy has been linked to a higher risk of fetal growth restriction in pregnant women [49]. Moreover, the use of energy drinks during pregnancy is not recommended, as even a minimal risk of adverse effects could result in irreversible consequences [50].

Numerous factors influence social novelty preference in living organisms, including reduced general sociability, impaired perception of social cues, and a diminished rewarding value of novel social interactions [51]. In present study, the fact that rats in the experimental groups actively investigated a stranger they encountered for the first time indicates intact social perception abilities. This suggests that energy drink consumption during adolescence and adulthood does not appear to impair social development in rats. In our experimental design, familiarity was defined as "prior cohabitation experience" and was tested using two distinct social stimuli. The results revealed that adolescent rats in the CG preferred the unfamiliar individual, whereas those in the energy drink treated groups showed a preference for the familiar one. Although previous studies on social bonding in rats have yielded inconsistent results, numerous studies have reported that rats exhibit different behaviours towards familiar individuals compared to unfamiliar ones [52,53]. Early life olfactory bonds formed with the mother are known to be critical for healthy social development, and positive early social experiences can have lasting beneficial effects on subsequent behavioural and emotional development [25]. Studies on these olfactory bonds between mother and offspring have generally been conducted using material based experimental setups [54,55].

In our study, the preferences of mother rats for their own pups versus foreign pups were examined. The findings showed that mothers in EG2 exhibited the strongest preference for their own offspring, whereas those in EG1 showed the weakest preference. Previous research indicates that philopatric female mammals tend to direct their social preferences toward kin [56], a pattern also observed in rats. While the mother offspring bond is typically strong and enduring, social bonds among adult rats, particularly unfamiliar, are generally weaker [57]. Motherhood is a distinctive biological and behavioural state. During this period, a mother's locomotor activity is influenced by physiological and hormonal changes, emotional bonding with her pups, and neurochemical adaptations. Notably, the hormone oxytocin is secreted in large amounts during lactation, and numerous studies have demonstrated that it reduces locomotor activity in rats [58,59].

The daily diet of rats can significantly influence their learning, memory, and cognitive performance. Romero-Delgado et al., (2021) found that female rats fed a high sucrose diet exhibited impaired long term memory [60]. Similarly, Fedorova et al. (2009) demonstrated that a deficiency in n-3 fatty acids led to deficits in spatial learning, as assessed using the Barnes maze test [61]. In contrast, Chen and Hu (2023) reported that intranasal administration of creatine enhanced performance in the Barnes maze, suggesting a potential cognitive benefit [22].

In present study, conducted using the Barnes maze test platform, long term energy drink consumption was found to negatively affect learning and long term memory performance in female rats. This cognitive impairment is likely linked to primary ingredients of energy drinks: caffeine (1,3,7-trimethylxanthine) and taurine (2-aminoethylsulfonic acid). Caffeine acts as an antagonist at A1 and A2 adenosine receptors in the central nervous system [6]. Furthermore, it has been reported that caffeine alone can improve cognitive functions such as memory and concentration [62]. Taurine, meanwhile, plays several key physiological roles, including neuromodulation, neuroprotection, osmoregulation, maintenance of cell membrane stability, and regulation of intracellular calcium. In rats, taurine has been shown to interact with N-methyl-D-aspartate receptors, which are critical for synaptic plasticity and memory formation [63,64]. Taken together, these findings suggest that the cognitive impact of energy drink constituents is complex and potentially bidirectional, capable of both beneficial and detrimental effects, depending on factors such as dose, duration of consumption, and interactions between ingredients.

In a study on laboratory animals, the combination of caffeine and taurine, both present in high concentrations in energy drinks, was reported to enhance memory and attention, indicating a cognitive improving effect [65]. However, contrasting findings have emerged from other research. Lee et al. (2020) observed that long term energy drink consumption during adolescence impaired learning and memory in rats [42]. Similarly, Bawazir (2017) reported that chronic intake of energy drinks reduced cognitive performance and disrupted behavioural

responses in rodents [66]. Human studies also show mixed results. Among school aged children, higher energy drink consumption has been associated with lower academic achievement. Conversely, a study on university students found that energy drink intake significantly improved secondary memory, attention speed, and overall mental performance [67].

Similarly, energy drinks have been reported to exert beneficial effects on cognitive function and mood in sleep deprived individuals [68]. The findings of our study align with those of [66] and Lee et al [42], who observed impairments in learning, memory, and behaviour following chronic energy drink consumption. However, our results contrast with the positive cognitive outcomes reported by [65,67,68]. These inconsistencies may arise not only from differences in study design, dose, or population but also from the complex interactions among the various ingredients in energy drinks beyond just caffeine and taurine.

Studies in humans have shown that energy drinks can enhance memory and attention in a döşe dependent manner [69], and improve response accuracy and processing speed in tasks involving working and episodic memory [70]. Additionally, acute high dose consumption has been associated with improved performance under mental stress, including better short term memory, verbal fluency, and attention [71]. However, other findings are more nuanced. For instance, Evans et al. (2021) observed an initial increase in pattern comparison speed and reduced errors in psychomotor vigilance tests following energy drink consumption, but no significant improvement in higher-order cognitive functions, such as selective attention or cognitive flexibility, during later testing phases [72]. These conflicting results underscore that the cognitive effects of energy drinks are not uniform and may depend on several factors, including dose, duration of use, individual variability, specific formulation, life stage, and potential interactions among ingredients.

This study has several limitations that should be considered when interpreting the findings. The use of an animal model, specifically female rats, limits the direct generalisability of the results to humans, as metabolic, behavioural, and hormonal responses may differ across species. The inclusion of only female animals, while justified by the focus on reproductive and maternal outcomes, precludes any conclusions regarding potential sex-specific effects of energy drink consumption. Although behavioural changes were thoroughly assessed, the absence of direct hormonal measurements (e.g., oestrogen, progesterone, or oxytocin levels) prevents a mechanistic understanding of the observed effects on maternal behaviour and cognition.

In conclusion, this study conducted using an experimental animal model, comprehensively investigated the effects of chronic energy drink consumption initiated at an early age in female rats, reflecting the fact that children, adolescents, and young adults are the primary consumers of these beverages. The findings indicate that such consumption may adversely affect learning, spatial memory, and cognitive performance, as well as influence behavioural parameters including social interaction, emotional state, and locomotor activity during both pre-pregnancy and postpartum periods. The results also suggest that early and consistent consumption of energy drinks may interact with daily stress factors and potentially exacerbate impairments in social learning and memory processes. Because stress affects emotional, cognitive, behavioral, and physiological activities and the learning process in living beings. Stress hinders normal behavior and normal thinking activities in young individuals and, depending on the severity of increasing stress factors, causes the learning process to deteriorate or even be completely blocked. Given the complex formulation of energy drinks, future research employing differently designed, controlled studies is needed to fully elucidate the roles and interactions of their key constituents, especially caffeine and taurine, as well as other ingredients, in shaping neurobehavioural outcomes.

Ethics approval and consent to participate

Prior to commencement of the experiments, the animal use protocol was approved by the Ahi Evran University Local Ethics Committee on Animal Experiments (Approval No: 15-02; dated 23/07/2025). All procedures were carried out in accordance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments). Furthermore, all experimental procedures involving live animals complied with the Regulation on the Welfare and Protection of Animals Used for Experimental and Other Scientific Purposes, issued by the Ministry of Food, Agriculture and Forestry of the Republic of Turkey

Informed consent

Informed consent was not obtained because the current study did not involve human subjects.

Consent to publish

Not applicable.

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Data availability

The data and analyses from this study will be made available by the authors upon reasonable request for academic and scientific review purposes. Data sharing will be conducted in accordance with legal requirements and ethical principles regarding the protection of personal data.

CRediT authorship contribution statement

Demirel Ergün: Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Halime Aydemir:** Writing – review & editing, Writing – original draft, Software, Resources. **Fatma Ergün:** Writing – review & editing, Writing – original draft, Software. **Tolga Tunçel:** Resources, Formal analysis.

Declaration of competing interest

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

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