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**CHANGES IN THE EMOTIONAL STATE OF MALE AND FEMALE RATS AFTER PERFORMING THE “OPEN FIELD” AND “ELEVATED PLUS MAZE” TESTS****Abror Shakirovich Kasymov**

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Uzbekistan<https://doi.org/10.5281/zenodo.17539359>**ARTICLE INFO**Received: 26th October 2025Accepted: 30th October 2025Online: 31st October 2025**KEYWORDS***Melatonin, emotional state, increased locomotor activity, sports pharmacology.***ABSTRACT**

This article presents the results of evaluating the effects of melatonin on the emotional state of male and female rats using the “Open Field” and “Elevated Plus Maze” methods over a 20-day period. On days 9 and 19, changes in blood biochemical parameters were observed with the first method. For the second method, assessments were performed on days 10 and 20 from the start of the study.

RELEVANCE. Professional sports involve prolonged, extremely intense physical exertion. An athlete's physical condition depends on the balanced function of regulatory systems that ensure maximum adaptive capacity to emotional and physical stress [4]. From a medical perspective, melatonin is attracting increasing attention due to scientific evidence of its potential therapeutic use and safety profile. Limited data exist on its effects on emotional state depending on sex, based on studies in laboratory animals, including rats and mice. Melatonin is involved in regulating metabolism, the immune system, and emotional state. Animal research has shown that melatonin can influence emotional behavior in a sex-dependent manner, and some studies have demonstrated its anxiolytic effects in both sexes [2,3].

Overall, studies on the influence of melatonin on emotional state by sex in laboratory animals show mixed results. Further research on various models, including human studies, is needed for more precise and generalized conclusions. Developing new approaches in sports pharmacology, particularly evidence-based use of permitted medications with consideration of gender differences, can help target adaptive mechanisms to restore and enhance athletes' emotional and physical activity.

PURPOSE OF THE STUDY. To investigate the effects of melatonin on emotional state and physical activity in relation to gender differences under stress and physical load in animal experiments.

Materials and Methods. Experiments were conducted using standard methods described in the literature on outbred albino rats of both sexes (male and female) weighing 180–200 g, in compliance with bioethical principles according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [1,7]. Emotional state was assessed using the Open Field (OF) and Elevated Plus Maze (EPM) tests, the latter featuring open and closed arms [5,6,8].



The test substance was a dietary supplement—melatonin (INN: melatonin) 5 mg fast-dissolving tablets (NOW Foods, USA). The experiment included four groups (n = 6 each):

1. Group 1 (male control): received purified water throughout, exposed to emotional and physical stress.
2. Group 2 (female control): same as Group 1.
3. Group 3 (male experimental): received melatonin at 10 mg/kg, exposed to emotional and physical stress.
4. Group 4 (female experimental): same as Group 3.

Melatonin was administered intragastrically one hour before physical loading. Statistical analysis was performed using the Student's t-test for independent samples; differences were considered significant at $p < 0.05$.

RESULTS. Initially, the males of the control group, which received purified water, were subjected to the open field test, during which observation and square counting were performed. Subsequently, the females of the control group, which likewise received purified water, were examined. The third and fourth groups consisted of males and females, respectively, that received melatonin intragastrically at a dose of 10 mg/kg and were similarly exposed to emotional stimulation in the open field model. In all groups, the horizontal locomotor activity of rats of both sexes was recorded. Experimental evaluation of rats' emotional state was conducted on days 9 and 19. Horizontal locomotor activity—running across different paths, including circling—was recorded. Movement was defined as the rat crossing from one marked sector of the arena to another with all four paws.

The arena floor was divided into three rows of sectors of equal area, and one crossed sector was taken as a unit of movement during the visual recording of behavior. Given that the rat's body length may at times approximate the length of a sector base, the issue of registering locomotion was addressed as follows: if the animal was located within the boundaries of a single sector (with all four paws) and then moved into an adjacent sector (its hind paws crossing the dividing line), this was counted as one sector crossing. Each experimental rat was placed in the center of the arena, and its behavior was observed for 5 minutes. Instances in which the rat entered a new square with both forepaws were recorded. After the 5-minute observation period, the animal was returned to its home cage [7].

Groups	Females (Mean ± SD)	Males (Mean ± SD)
Control group	24.00 (22.64 ± 25.36)	25.17 (25.64 ± 18.36)
Melatonin Day 9	32.33 (30.26 ± 34.40)	28.50 (26.42 ± 30.58)
Melatonin Day 19	42.00 (39.99 ± 44.01)	35.83 (34.71 ± 36.95)

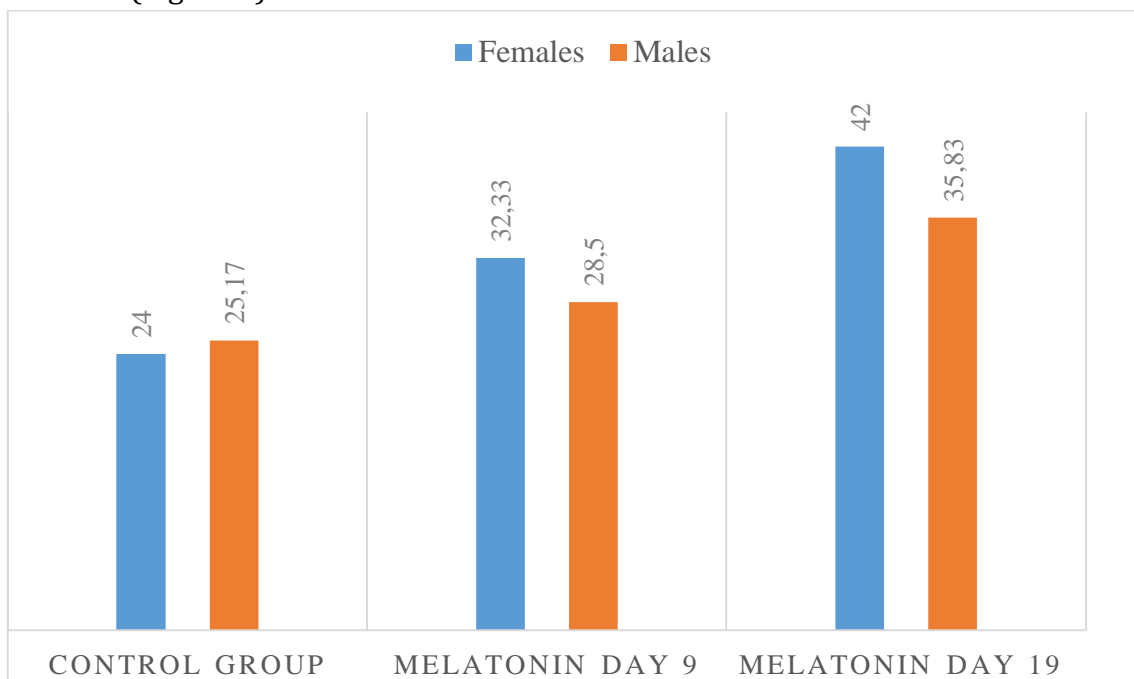
On day 9, female experimental rats crossed an average of 32.33 squares, while males crossed 28.5. By day 19, females receiving melatonin crossed 42 squares on average, compared with 35.83 for males. In the control groups on day 19, females crossed 24 squares and males 25.17 (table 1).

Table 1. Open Field Test Results



When comparing the control and experimental groups on day 9, females in the melatonin-treated group crossed 34.7% more squares, and males crossed 21.5% more squares, than their counterparts in the control group receiving purified water. On day 19, the difference in square crossings in the melatonin-treated group was even greater, with females exceeding the control group by 75% and males by 42%, respectively.

When analyzing the ratio of females to males within the groups, on day 9 females crossed 13.4% more peripheral squares, and on day 19 after melatonin administration this difference increased to 17.2% compared with males. Thus, it can be concluded that females exhibited, on average, higher locomotor activity in the Open Field test compared with males (Figure 1).



Note: $p < 0.05$ – data are statistically significant compared with the control group.

The number of rats in each group (both sexes) was 6. Emotional state was also assessed on days 10 and 20 using the Elevated Plus Maze, which measures anxiety levels by the preference for open versus closed arms and behaviors such as risk assessment.

For the experimental study, four groups were formed, each consisting of six animals.

The Elevated Plus Maze apparatus, designed to assess rodent behavior under conditions of variable stressogenicity (through the free choice of comfortable environments), allowed for the evaluation of: the level of anxiety (based on preference for darkness/light, fear of heights, and the intensity and dynamics of “head-dipping” behavior); symptoms of neurological deficit; and habituation. Initially, the males of the control group, which received purified water, were subjected to the Elevated Plus Maze test. Subsequently, the females of the control group, also receiving purified water, were tested. The third and fourth groups consisted of males and females, respectively, that received melatonin intragastrically at a dose of 10 mg/kg and were likewise exposed to emotional stimulation in the Elevated Plus Maze model. Immediately prior to testing the



animals of the control and experimental groups, a rat from the “zero” group was placed in the Elevated Plus Maze (EPM) and allowed to freely explore the apparatus for 5 minutes. Under standard testing conditions (closed arms dimly lit, open arms illuminated), control animals generally avoided entering the open arms, and in cases when they did, they remained there only for a short period of time [6]. Subsequently, each animal was placed in the center of the EPM facing an open arm.

The results of the experimental study of emotional state using the Elevated Plus Maze demonstrated that on day 10 following melatonin administration, females in the experimental group exhibited on average 0.8 more arm entries and spent 27 seconds longer in the arms compared with the control group that received purified water. In males of the experimental group, the number of arm entries on day 10 was on average 0.2 higher, and the time spent was 23.8 seconds longer than in the control group receiving purified water. On day 20 after melatonin administration, females in the experimental group showed on average 0.6 more arm entries and spent 26.4 seconds longer in the arms compared with the control group. In males, the experimental group demonstrated 1.2 more arm entries and 30.8 seconds longer time spent in the arms relative to the control group receiving purified water (Table 2).

Table 2. Elevated Plus Maze Results

Groups	Control	Melatonin Day 10	Melatonin Day 20
Females - Visits	2.60 (1.99 ± 3.21)	3.40 (2.41 ± 4.39)	3.20 (2.70 ± 3.70)
Females - Time (s)	138.40 (122.49 ± 154.31)	165.00 (150.52 ± 179.48)	164.80 (153.74 ± 175.86)
Males - Visits	2.40 (1.79 ± 3.01)	2.60 (1.99 ± 3.21)	3.60 (2.99 ± 4.21)
Males - Time (s)	137.80 (125.30 ± 150.30)	161.60 (147.54 ± 175.66)	168.60 (156.83 ± 180.37)

When comparing the control and experimental groups on day 10, females in the melatonin-treated group exhibited on average 23.6% more arm entries and 16.1% longer time spent in the arms than females in the control group. In males, the experimental group showed 7.6% more arm entries and 16.7% longer time spent compared with the control group.

On day 20, females in the melatonin-treated group demonstrated 18.75% more arm entries and 16% longer time spent in the arms compared with the control group. In males, the experimental group exhibited 33.3% more arm entries and 18.2% longer time spent relative to the control group.

When comparing females to males within the groups, the Elevated Plus Maze results showed that on day 10 after melatonin administration, females had 23.5% more arm entries and spent 2% longer in the arms compared with males. By day 20, the number of arm entries in females was 12.5% higher, while the time spent in the arms was 2.3% shorter than in males (Figure 2).

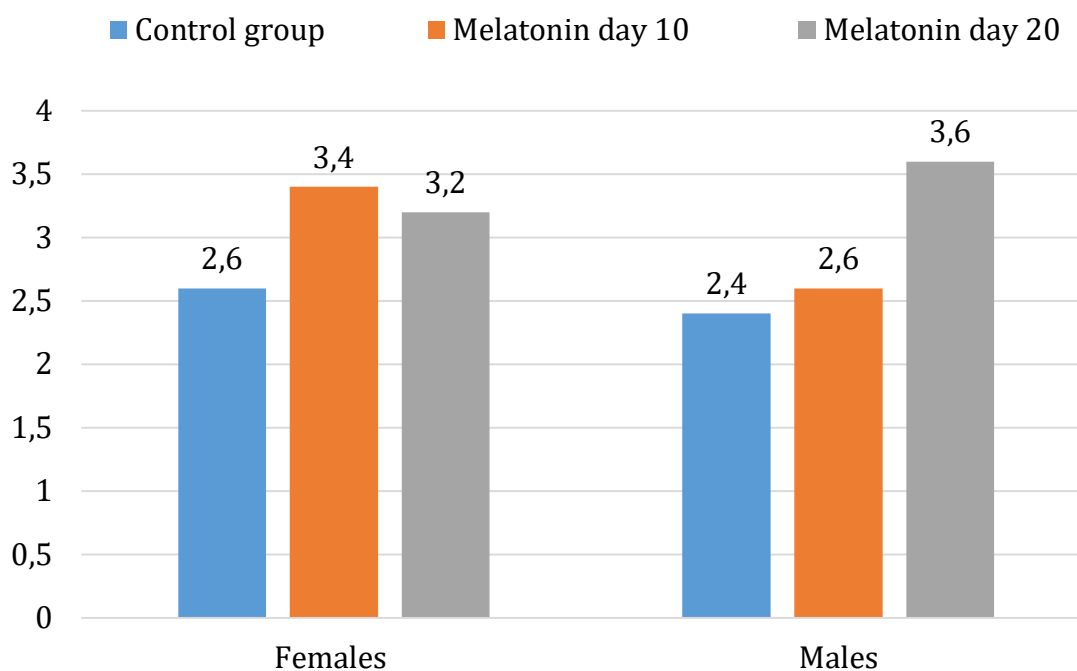


Figure 2.1. Number of arm entries in females and males

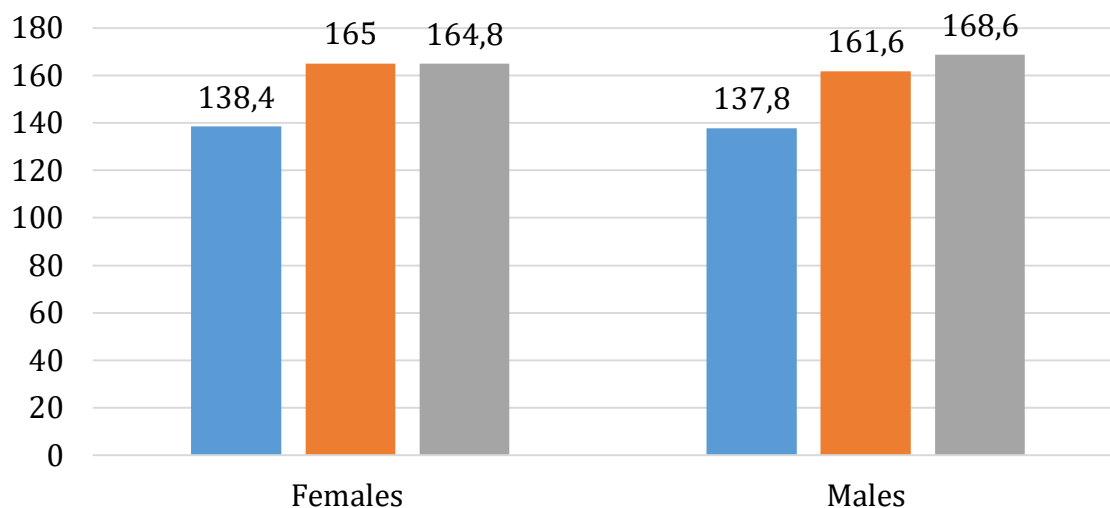


Figure 2.2. Time spent in the arms by females and males

Thus, melatonin administration exerted a more pronounced excitatory effect on females than on males, as females demonstrated, on average, greater locomotor activity in the Elevated Plus Maze test. Thus, melatonin administration exerted a more pronounced excitatory effect on females than on males, as females demonstrated, on average, greater locomotor activity in the Elevated Plus Maze test.

CONCLUSIONS



1. Administration of melatonin at 10 mg/kg increases locomotor activity in the Open Field test compared with controls, with females showing 3.8 % higher activity than males.
2. Prolonged melatonin administration (10 mg/kg for 20 days) in the Elevated Plus Maze produces stronger anxiolytic effects in females than in males, as evidenced by greater locomotor activity.

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