http://dx.doi.org/10.35630/2199-885X/2021/11/6.8

CORRECTION OF IMMOBILIZATION STRESS BY MELATONIN IN RATS UNDER CONDITIONS OF DARK DEPRIVATION

Received 6 October 2021;
Received in revised form 27 October 2021;
Accepted 29 October 2021

***Larisa Balykova*[1]✉, *Elena Moskaeva*[1], *Vera Inchina*[1], *Tatyana Tarasova*[1], *Larisa Mosina*[1], *Olga Ignatieva*[1], *Evgeniya Gvozdikova*[2], *Dalila Khaydar*[2], *Andrey Kuchuk*[2], *Igor Saushev*[1]**

[1] *National Research OgarevMordovia State University. Saransk;*
[2] *Peoples' Friendship University of Russia, Moscow, Russia*

✉ *doctor@is.mrsu.ru*

ABSTRACT — In this study, we have evaluated the effectiveness of melatonin in correcting stress in rats that occurred after dark deprivation and mobility restriction. The main group of animals (n=6) received melatonin in order to overcome stress. The obtained indicators for assessing the stress level (vertical activity, freezing, the number of boluses) in the main group of rats were compared with the indicators of the control (animals received placebo, n=6) and intact (n=6) groups. As a result, when the daylight lengthens, rats develop stress, which is manifested by a change in the activity of the animal. We have proved that the use of melatonin reduces hyperactivity in rats by 1.5 times ($p<0.01$) and the duration of freezing is reduced by ⅓ ($p<0.05$).
Conclusion: In animals treated with melatonin, physical and vegetative activity was significantly higher compared to the placebo group. Therefore, melatonin helps to overcome immobilization stress in rats under conditions of dark deprivation.

KEYWORDS — Stress, melatonin, dark deprivation, activity disorder, circadian rhythm, desynchronosys.

INTRODUCTION

Nowadays, a person's professional activity is often associated with the need to change time zones. Certain jobs require constant presence in conditions of round-the-clock illumination, especially for night shift workers [1]. Circadian disorders are also inherent in people living in the polar day zone, as well as those who make frequent business trips with a change of time zones. In such cases, desynchronization of the daily biorhythms of physiological functions develops in the human circadian system [2].

Desynchronosis is one of the most powerful stress factors for all body systems. With frequent occurrence and prolonged course, it can weaken the circadian organization of a person and lead to the development of pathological processes in a particular physiological system [3]. The development of desynchronosis is accompanied by a decrease in the night peak of melatonin levels, which occurs due to a violation of circadian rhythms. Therefore, the presence of the correct rhythm of melatonin production is a necessary condition for a healthy body. It is known that people experience stress when moving to work from the middle latitudes to the Far North due to a violation of the seasonal rhythm of melatonin secretion. This is confirmed by the facts of an increase in depressive states and alcoholism in this contingent.

Aim:
to evaluate the effectiveness of melatonin in the correction of immobilization stress in rats in a situation of dark deprivation.

METHODS

The object of the study were 18 sexually mature white rats weighing from 200 to 400 g, contained in standard vivarium conditions. All procedures with animals were performed according to the Guidelines for the Care and Use of Laboratory Animals. We divided all the animals into 3 equal groups: 1 group (main, n=6), 2 group (control, n=6), 1 group (intact, n=6).

The animals of groups 1 and 2 were in dark deprivation conditions for 10 days. Then we placed all the rats of these groups in immobilization chambers for one hour to create a model of a stress reaction. The animals of groups 1 and 2 were in stress mode for 10 days. At the same time, we administered melatonin at a dose of 1 mg/kg intramuscularly to all rats of group 1 during this period, and we gave an intramuscular injection of 0.9% NaCl solution as a placebo to animals of group 2.

The rats of the third group formed an intact group, we did not perform any manipulations with them and were kept on a rational diet throughout the entire time of the experiment.20 days after the end of the experiment, we evaluated the following indicators in all animals of the three groups: vertical activity (the rat taking a vertical position), freezing (freezing in place), the number of bolluses (the act of defecation). We took the sign of "high motor activity" in rats as normal and interpreted it as the absence of anxiety and stress in a particular animal.

Statistical processing was carried out with the calculation of arithmetic mean values (M) and their errors (m). The reliability of differences in the groups was calculated using the Mann-Whitney test. The differences were considered significant if $p<0.05$.

RESULTS

The indicators of motor activity, namely vertical activity, in the rats of the first group were 1.5 times lower compared to the intact group ($p<0.01$) (Fig. 1). This fact indicates that dark deprivation and impaired mobility leads to stress. However, we found that the vertical activity in the group of rats treated with melatonin was significantly higher by 22% compared to the intact second group ($p<0.05$) (Fig. 1). This means that anxiety melatonin helps to reduce stress levels and helps to neutralize anxiety disorders in the animal.

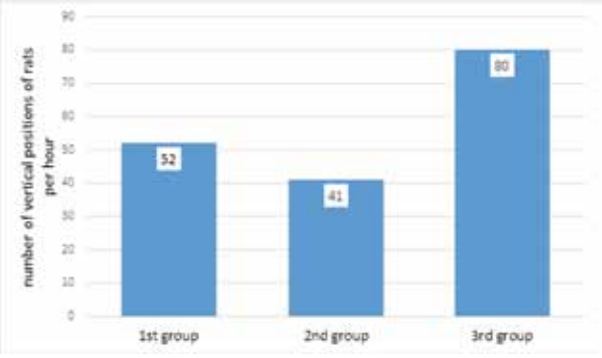

Fig. 1. *Vertical activity in rats of the studied groups*

Another indicator of stress is freezing (freezing of the rat without movement). We noticed that the duration of freezing during one hour of observation in the first and second groups of animals was more than ten times higher than in the intact animals (Fig. 2) ($p<0.001$). However, against the background of the use of melatonin, the activity of rats improves, and the duration of freezing decreases by 33% ($p<0.05$).

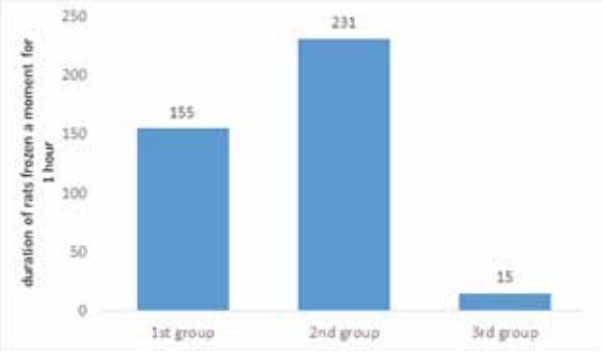

Fig. 2. *Indicators of freezing in rats under immobilization stress after dark deprivation*

The number of boluses reflects the state of the autonomic nervous system. Therefore, the more boluses there are, the more pronounced the stress reaction in the animal. The highest number of boluses per day was in the experimental group of rats receiving placebo — 24.1+1.7. This indicator had significant differences with the group of animals receiving melatonin — 16.5+2.3 ($p<0.05$). In the animals of the intact group, the number of boluses per day was minimal (7.8+1.1), but there were no significant differences with the first group($p>0.05$).

DISCUSSION

Circadian rhythms are important for the normal development and functioning of the nervous system. Violations of circadian rhythms suppress the function of the nervous system and lead to the formation of stress, which has been proven in animal models [4]. The systemic response to stress, aimed at eliminating or reducing stress, is accompanied by changes in behavioral, vegetative, motor, sensory, cognitive and other functions of the body [5].

It is known that melatonin contributes to the modulation of circadian rhythmicity. Melatonin is also involved in the regulation of blood pressure and the autonomic cardiovascular system, the regulation of the immune system, as well as in various physiological functions [6]. Melatonin synthesis and secretion are enhanced in the dark and suppressed by light. Low concentrations of melatonin are usually found in patients with neurological diseases and mental disorders [7]. Positive results of the use of melatonin were obtained in the correction of mental and behavioral disorders, such as attention deficit hyperactivity disorder. Melatonin has an effect on the organs of the gastrointestinal tract. Arendt J. et al. It was noted that melatonin protects the gastrointestinal tract from ulcers by reducing the secretion of hydrochloric acid and increasing the secretion of bicarbonate of the duodenal mucosa [1]. Melatonin also promotes the regeneration of the epithelium of the intestinal mucosa. These factors indicate the properties of melatonin to correct the state of the autonomic nervous system.

Our study demonstrates that animals that are in conditions of round-the-clock illumination and impaired mobility experience stress. This stress reaction can be successfully compensated by administering melatonin to animals. We noted that the vertical activity in the main group of rats improved by 1.3 times, the number of freezing decreased by 1.5 times, and violations of vegetative reactions were practically leveled.

CONCLUSION

Light desynchronosis leads to the development of stress and anxiety-depressive disorders. The restora-

tion of circadian rhythms helps to reduce stress, helps to maintain the balance of higher nervous activity, which contributes to the correction of behavior, mood and intellectual functions of an individual. We proved that animals treated with melatonin had significantly higher physical activity, and anxiety indicators were lower compared to the placebo group. This means that melatonin contributes to the successful overcoming of the consequences of induced stress, burdened with desynchronosis in rats.

REFERENCES

1. **Arendt J.** Melatonin: Countering Chaotic Time Cues. Front. Endocrinol. 2019;10:391. doi: 10.3389/fendo.2019.00391
2. **Walker WH, Walton JC, DeVries AC, Nelson RJ.** Circadian rhythm disruption and mental health. Transl Psychiatry. 2020;10(1):28. Published 2020 Jan 23. doi:10.1038/s41398-020-0694-0
3. **Dominoni DM, Borniger JC, Nelson RJ.** Light at night, clocks and health: from humans to wild organisms. Biol. Lett. 2016;12:20160015. doi: 10.1098/rsbl.2016.0015.
4. **Landgraf D, McCarthy MJ, Welsh DK.** BehavNeurosci. The role of the circadian clock in animal models of mood disorders. 2014; 128(3):344–59.
5. **Cardinali DP.** Melatonin: Clinical Perspectives in Neurodegeneration. Front. Endocrinol. 2019;10:480. doi: 10.3389/fendo.2019.00480
6. **Karatsoreos IN, Bhagat S, Bloss EB, Morrison JH, McEwen BS.** Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. Proc. Natl Acad. Sci. USA. 2011;108:1657–1662. doi: 10.1073/pnas.1018375108
7. **Bellivier F, Geoffroy PA, Etain B, Scott J.** Sleep- and circadian rhythm-associated pathways as therapeutic targets in bipolar disorder. Expert Opin. Ther. Targets. 2015;19:747–763. doi: 10.1517/14728222.2015.1018822