MODULATING THE EFFECT OF MELATONIN ON BEHAVIORAL RESPONSES OF RATS IN AN EXPERIMENTAL MODEL OF INFLAMMATION

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Abstract. The problem of the influence of inflammation on the processes of higher nervous activity is still relevant. In particular, it implies an analysis of the safety and effectiveness of biological regulators' effects with neurotropic and immunomodulatory potential. The study examined the effects of Salmonella tiphy LPS on behaviour and cognitive function in adult male Wistar rats with different levels of melatoninergic system activity, using various tests. It was found that daily injection of LPS to rats of the group A (50 μg/kg, i.p.) for 10 days leads to the formation of a special pattern of behavior with a low level of tentative research activity in the presence of increased anxiety. The rats of the group B received combined exposure to LPS (50 μg/kg, i.p.) and melatonin (5 mg/kg, orally) distinguished a higher level of various variants of motor and research activity in conditions of relatively low anxiety in contrast to the rats of the group A. The paper discusses the features and mechanisms of the formation of "painful" behavior in an experimental model of inflammation, depending on the level of activation of the components of the melatoninergic system.

Keywords: behavior, lipolysaccharide, inflammation, rats.

List of Abbreviations
LPS – lipolysaccharide

Introduction

Currently, the processes of inflammation at the level of the central nervous system structures are considered as significant triggers of disorder in the functional activity of neurons and various types of glial cells. There is evidence that neuroinflammation leads to impaired activity of monoaminergic, GABAergic and other neurotransmitter systems (Park *et al*., 2020; Felger & Treadway, 2017; Yan *et al.*, 2015; Mravec *et. al*., 2014; Kamata *et al*., 2000; Shuto *et al.,* 1997). Under conditions of neuroinflammation, microglia are activated and the production of pro-inflammatory cytokines can disrupt the coordinating functions of the brain and has a neurotoxic effect (Cheng *et al*., 2021; Viviani *et al*., 2014; Hegazy *et al*., 2015). The active and prolonged action of pro-inflammatory cytokines leads to the emergence of «painful» behavior with the reduction of motor activity, cognitive impairment, and emotional-motivational disfunction (Benatti *et al*., 2016). The data of experimental biology and clinical observations, demonstrate that various neurodegenerative diseases are associated with the process of neuroinflammation (Leng & Edison, 2021; Kaur *et al*., 2019). There are evidences of neuroinflammation being observed in patients with *COVID-19* with violation of mental functions presented during the period of the disease and in a longer period of time – *post-Covid-19 syndrome* (Tang *et al.*, 2021; Kempuraj *et al*., 2020).

Currently, the preclinical studies identified the importance of researching the effect of the biological modulators on the prevention and reduction of impaired brain functions and behaviors associated with neuroinflammation conditions of various origins. Taking this into consideration, the pineal hormone melatonin attracts the attention of researchers. At present, the effects of melatonin in the circadian rhythm regulation system are well studied and documented in the literature (Vasey *et al*., 2021). In addition, its regulatory effect on cardio (Imenshahidi *et al*., 2020), digestive (Motilva *et al.,* 2001) and other systems has been established. The anxiolytic, antidepressant (Xu *et al*., 2019; Emet *et al.*, 2016) and immunomodulatory (Kvetnoy *et al*., 2022; Nabavi *et al*., 2019; Hardeland, 2018; Arushanyan & Naumov, 2013; Carrillo-Vico *et*

al., 2003) effects of melatonin are shown. Such a pleiotropic nature of the melatonin action is determined by its lipophilicity and the effect on various types of receptors: membrane, cytosolic, nuclear (Ng *et al*., 2017). The general protective effect of melatonin is related to its high antioxidant activity (Reiter *et al.*, 2018). The modulating effect of melatonin on brain function and behavior in conditions of inflammation remains debatable and poorly understood.

The purpose of the current research was to study the influence of an experimental model of inflammation (the effect of *Salmonella tiphy* LPS) on the behavioral responses in adult rats in various test settings, depending on the activity of the melatoninergic system.

Materials and Methods

The study was performed on 45 male Wistar rats weighing 275 ± 27 g. The animals were kept 5 individuals per cage. Sterile sawdust was used as bedding. The rats received standard granulated complete feed for rodents and purified tap water without restriction. The animals were kept in controlled environments: air temperature – 18-22 °C, relative humidity – 60-70%, lighting regime – 12/12. The conditions for feeding, housing and handling of animals were in accordance with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, Strasbourg, 1986). The protocol of the experiment was approved by the Bioethics Committee of the Biological Faculty of Samara University.

Animals were divided into three groups. The rats of the experimental group A (n = 15) were daily injected with a solution of *Salmonella typhi* LPS (50 μg/kg, i.p.; N.F. Gamaleya NIIEM). The injections were given for 10 consecutive days in the morning. The rats of group B (n = 15) also received similar injections of LPS solution together with melatonin suspension (5 mg/kg in a volume of 0.5 ml, per os; Sigma) for 10 days. The rats of the control group (n = 15) received injections of 1 ml of sterile saline for 10 days. To eliminate the effect of oral exposure, the rats of the experimental group A and the control group additionally received purified tap water (0.5 ml, per os).

Animal behavior was studied in special setups for small rodents (Openscience). In the open field, the following indicators were analyzed: horizontal motor activity (by the number of crossed sectors), vertical motor activity (by the number of rearing up on the hind legs), anxiety level (by the number of acts of anxious grooming and exits to the central sector). The testing time in the open field was 3 minutes.

In the elevated cruciform maze, the time spent by rats in the open and closed arms of the apparatus, as well as horizontal motor activity by the number of crossed sectors in the open heats, was recorded. The behavior of rats in the cross maze was observed for 3 minutes. An increased time the rats spent in closed arms was designated as a sign of a high level of anxiety.

The Barnes maze was used to study spatial navigation and spatial memory. For this purpose, the time spent by rats in the true shelter was determined during the first, second, and third testing. The extrapolation escape test examined the time required for rats to escape from an active stressor (being in water within a narrow cylinder). Avoiding a stressful situation was defined as diving under the edges of the cylinder and exiting the rat into open water.

The behavior of rats was video-recorded for subsequent analysis of behavioral responses in various tests. Behavioral reactions of rats of each group were observed in the initial state, on the first, third, fifth and tenth days of the experiment.

Data were presented as the means ± SEM. Differences in the withdrawal threshold between the groups were analyzed using one- or two-way analysis of variance followed by either the Bonferroni test. The commercial software SigmaStat version 12.5 was used to calculate statistical significance. $P < 0.05$ was considered significant.

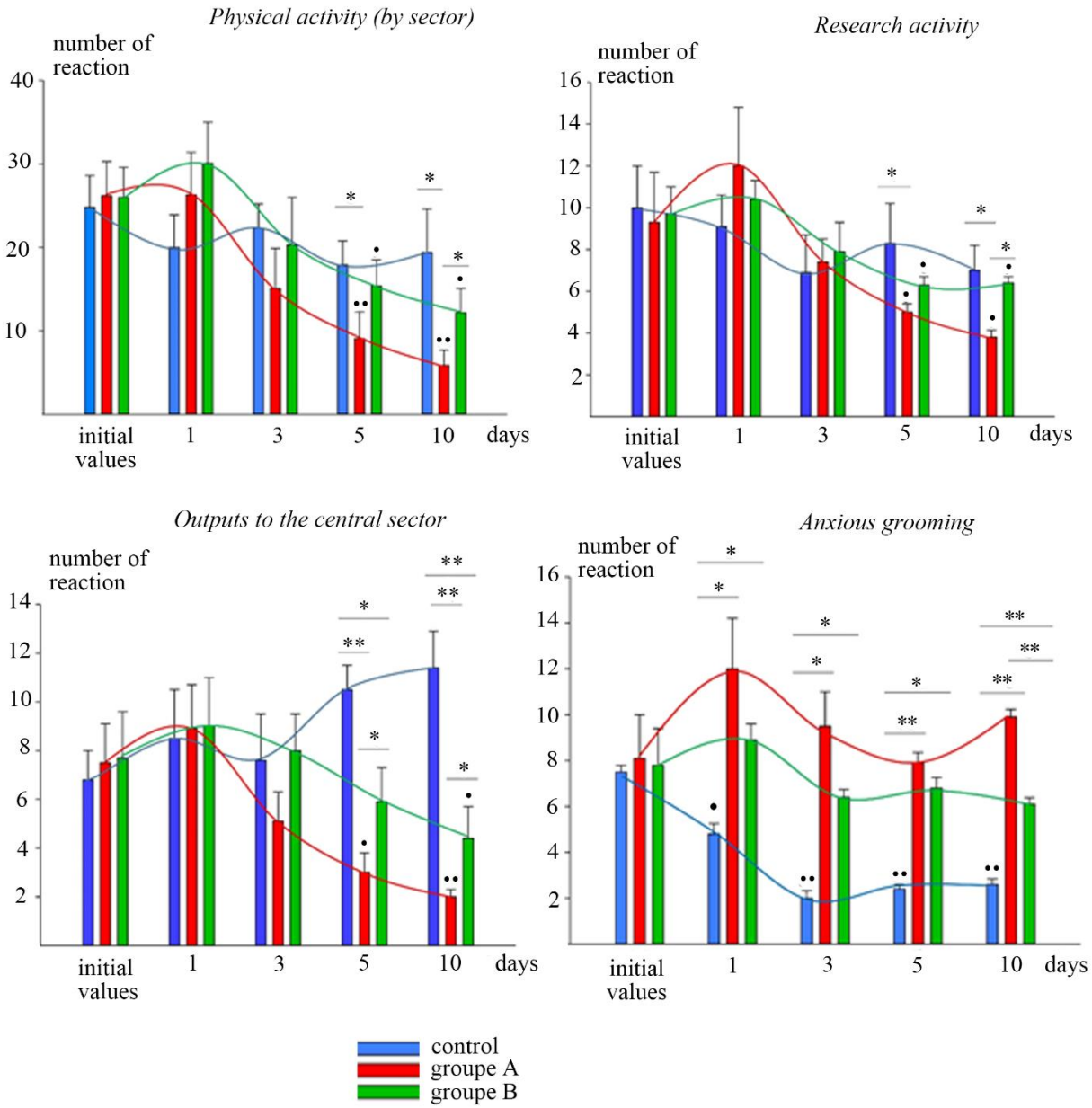

Fig. 1. Effect of melatonin on behavioural responses of rats in the open field in LPS-induced inflammation · – P < 0.05 vs pre values, ·· – P < 0.01 vs pre values, * – P < 0.05 vs to different groups, ** – P < 0.01 vs to different groups

Results

The study found that the effect of LPS had a modulating effect on the behavior of rats in various test settings. In the open field, the rats of the group A under the influence of LPS, demonstrated a tendency to decrease motor and exploratory activity (Fig. 1). Significant differences in the pattern of behavior of rats group A and group B were established on the 10 day of the experiment. In group A rats, motor activity decreased by 76% and research activity by 56% compared to the initial values. In group A, along with a deficit in motor and exploratory activity, there was a pronounced reduction in exits to the central sector and an increase in anxious grooming compared to the initial level. In the rats of group B in the open field, more active orienting-exploratory behavior was observed. In addition, the behavior of the group B rats was characterized by a greater number of

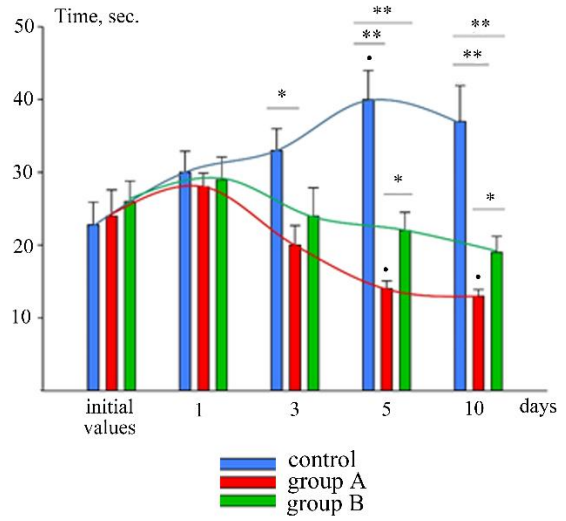

Fig. 2. Effect of melatonin on behavioural responses of rats in the elevated cruciform maze in LPS-induced inflammation (time spent in open heats)
· – P < 0.05 vs pre values, * – P < 0.05 vs to different groups, ** – P < 0.01 vs to different groups

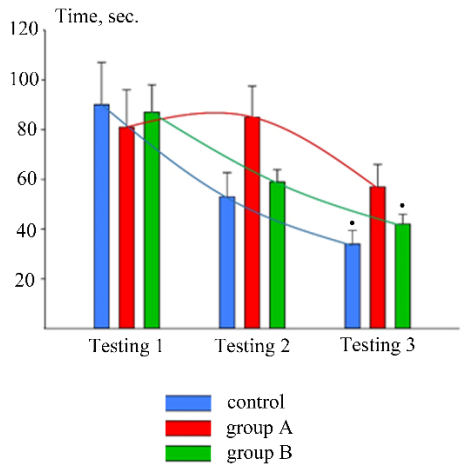

Fig. 3. Effect of melatonin on behavioural responses of rats in the Barnes maze in LPS-induced inflammation (time to find the true refuge on 10 day of the experiment)
· – P < 0.05 vs pre values

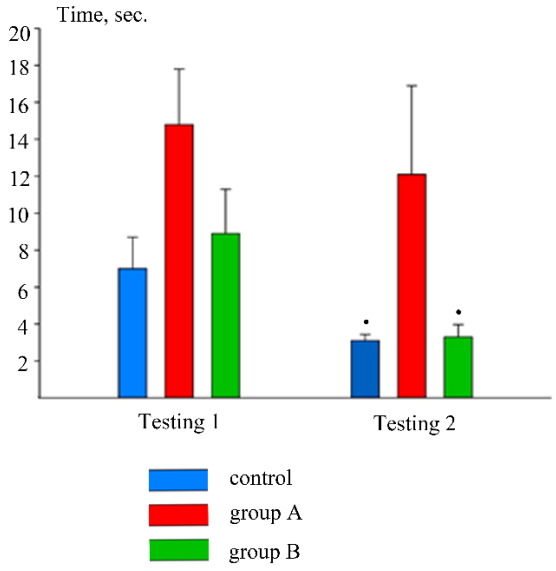

Fig. 4. Effect of melatonin on behavioural responses of rats in the extrapolative escape task in LPS-induced inflammation (time to get out of stressful situation on 10 day of the experiment)
· – P < 0.05 vs pre values

exits to the central sector and lower anxiety grooming.

The results obtained in the elevated cruciform maze prove the lower level of anxiety in the rats of the group B (Fig. 2). The impact of LPS on the rats of group A led to a reduction in the time spent in the open heats and a decrease in motor activity in them. On the fifth and 10 days of the experiment, the rats of the group B spent more time in the open heats and demonstrated the higher level of motor activity associated with crossing the sectors in the open heats of the maze compared to the rats in group A.

The results of the analysis of the behavior of rats in the Barnes maze indicate a negative effect of LPS on spatial memory associated with the ability to remember finding a true refuge (Fig. 3). The rats of the control group and group

B on the 10 day of the experiment found a true refuge faster during repeated testing (testing 3). In the control group, the search time decreased by 57%, in the group B – by 53%. The group A rats exposed only to LPS did not demonstrate a reduction in the time spent searching for a true refuge during all periods of the experiment.

In the extrapolative escape task, the features of the stress behavior of rats in a water space bounded by a narrow cylinder were proved (Fig. 4). On the 10 day of the experiment, repeated testing revealed a reduction in the time of getting rid of a stressful situation in the control group by 58%, in group B – by 66%. Group A rats spent more time on the reaction of diving under the edges of the cylinder to get rid of a stressful situation. Learning to overcome a stressful situation in group A rats in this test was not observed throughout the experiment.

Discussion

Repeated treatment in a relatively high concentration of bacterial LPS is a classic way of modeling systemic inflammation among rodents (Fritz *et al*., 2020; Kirsten & Bernardi, 2017; Catorce & Gevorkian, 2016; Muccigrosso *et al*., 2016). The mechanism of triggering the inflammatory reaction as a result of LPS exposure is associated with the specific activation of Toll-like receptors and the beginning of a complex intracellular cascade that provides the synthesis of pro-inflammatory cytokines (Kumar, 2019; Takeda & Akira, 2001).

There is evidence of the ability of pro-inflammatory cytokines to penetrate the blood-brain barrier (Barton *et al*., 2019; Rochfort & Cummins, 2015) and have various effects (from regulatory to toxic) on brain structures (Patterson, 2015). In addition, prolonged exposure to high levels of inflammatory factors can lead to damage to the blood-brain barrier. In this case, a more active penetration of both LPS and pro-inflammatory cytokines into the brain structures should be expected.

Microglia plays an important role in triggering and maintaining neuro-inflammation (Conti *et al.*, 2020; Skaper *et al.*, 2017). These immune cells have membrane Toll-receptors for interacting with LPS, trif and regulatory proteins for activating the expression of pro-inflammatory cytokine genes. The action of LPS and peripheral pro-inflammatory cytokines trapped in the brain focus microglia towards the synthesis of central pro-inflammatory cytokines (Rodriguez-Gomez *et al*., 2020). There is evidence of the ability of astrocytes and oligodendrocytes to also be involved in the process of neuro-inflammation and produce pro-inflammatory cytokines (Li *et al.*, 2021; Hanke & Kielian, 2011).

Neuro-inflammation leads to the formation of «painful» behavior with a expressed lack of research activity, cognitive deficits and impaired ability to find a quick way out of a stressful situation. This behavior may be accompanied by symptoms of depression.

There is data that central inflammation and the action of pro-inflammatory cytokines disrupt the functioning of various neurotransmitter systems, which are important in the regulation of behavior and cognitive functions (Felger & Treadway, 2017; Yan *et al.*, 2015; Felger *et al.*, 2013; Dowell *et al.*, 2006; Kitagami *et al.*, 2003; Kamata *et al*., 2000). Apparently, the experimental model of inflammation used in our study leads to the formation of such a «painful» pattern of behavior with a deficit of motor, research activity and impaired cognitive functions (Benatti *et al*., 2016).

The combined effect of bacterial LPS and melatonin maintained a higher level of adaptive behavior of group B rats. The rats of this group, in comparison with group A, showed signs of "painful" behavior to a lesser extent. In the open field, exposure to melatonin increased motor and research activity. Group B rats receiving melatonin were more active in the central sector of the open field and the open hoses of the elevated cruciform maze. These results indicate a relatively low level of anxiety in rats treated with melatonin under inflammatory conditions. In the Barnes maze and the extrapolation escape test, group B rats showed more developed cognitive abilities related to spatial memory and overcoming a stressful situation.

The effect of melatonin on the behavior of rats under inflammatory conditions researched in our study may be associated with the neurotropic and immunomodulatory effects of this

hormone. This conclusion is confirmed by data on the presence of MT-receptors in brain structures that are critical for the formation of certain patterns of behavior in various environmental conditions (Xu *et al*., 2019). The importance of melatonin for ensuring the normal functioning of the neurotransmitter systems of the brain is shown (Zhang *et al.*, 2021; Xu *et al*., 2019; Ouakki *et al*., 2013). Studies have described the anti-anxiety and antidepressant effects of melatonin (Satyanarayanan *et al*., 2018; Emet *et al.*, 2016). Melatonin contributes to reshape gut microbiota and improves inflammatory processes in the hippocampus (Lv *et al*., 2020). It is necessary to take into account the high antioxidant activity of melatonin, which can have a neuroprotective effect in conditions of neuro-inflammation and activation of oxidative stress mechanisms (Reiter *et al.*, 2018; Arushanyan, 2012).

Recently, the ability of melatonin to act as an immune buffer has been actively discussed. This important functional role is provided by the ability of melatonin to have a modulating effect on the mechanisms of innate and adaptive immunity. Depending on the concentration and type of MT-receptors, melatonin can cause both pro-inflammatory and anti-inflammatory effects (Kvetnoy *et al*., 2022; Nabavi *et al*., 2019; Hardeland, 2018).

Thus, reduced expression of "painful" behavior in inflammation may be associated with the neurotropic and immunomodulatory effects of melatonin. In the future, it is necessary to study in more detail the contribution of certain types of MT-receptors to the realization of the behavioral effects of melatonin in conditions of central inflammation. The study of behavior in conditions of inflammation against the background of impaired functioning of the circadian rhythm regulation system and melatonin deficiency is also relevant. Such model experiments will make it possible to clarify the value of melatonin for the regulation of behavior in the conditions of this pathology. Undoubtedly, this will be an important condition for the creation of effective melatonin-based remedies for the correction of impaired brain functions in inflammation of various origins.

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