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Comparative analysis of the effect of experimental models of light desynchronosis on the platelet aggregation activity

© O.V. Zlobina, V.F. Kirichuk, S.S. Pakhomii¶, A.N. Ivanov, E.S. Terekhina, N.V. Shlyapnikov, I.O. Bugaeva

Saratov State Medical University, Saratov, Russia

¶ e-mail: spakhomy03@gmail.com

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The effect of different models of artificial light exposure on the aggregation activity of platelets of white male rats using a continuous Light-Light (LL) lighting model and models with alternating Light-Dark (12:10) and Light-Dark (18:6) light and dark modes was studied. Analysis of platelet aggregation activity was performed with a computerized 230LA „Biola“ aggregation analyzer using the method of V.A. Gabbasov. It was found that under light exposure in the body of laboratory animals there is an increase of platelet aggregation activity accompanied by an increase in the parameters of the weighted average radius curve and the parameters of the light transmittance curve. Alteration of circadian rhythms in the form of impaired photoperiodism leads to an increase in platelet aggregation activity and provokes the development of microcirculatory disorders. The most conspicuous changes in the microcirculatory bloodstream by day 10 of the experiment are formed with the use of the Light-Dark (18:6) model.

Keywords: platelet aggregation, circadian rhythms, light exposure, photoperiodism disturbance, microcirculatory disorders.

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Introduction

A forced exposure to artificial lighting at night is increasingly changing the lifestyle of modern people. The relevance of this problem is especially high for people with irregular work schedules, working overtime and in night shifts, as well as for those who regularly change time zones [1,2]. Uneven and unnatural alternation of sleep and wake cycles can provoke both structural and functional changes in the human body, significantly increasing the risk of endocrine and cardiovascular pathology [3–6].

Light desynchronosis, characterized by a disturbance of circadian rhythms, is one of the most important stressors. With the development of a stress reaction in the blood, a decrease in the concentration of melatonin and an increase in the titer of adrenocorticotropic hormone and catecholamines are observed, which leads to the launch of a cascade of microcirculation system reactions [7,8]. In the microcirculatory bloodstream, a vasospasm occurs and the phenomenon of blood flow centralization develops. A decrease in the level of melatonin in the blood leads to an increase in the functional activity of platelets and microcirculation and clotting disorders. A stress-induced breakdown of autoregulatory and adaptive mechanisms can lead to circulatory disorders, first of all in the vessels of the microcirculatory bloodstream [9–15].

The duration of light exposure period, the type of lighting mode and its power have a direct effect on the severity of changes in the microcirculatory bloodstream. In a series of experiments on laboratory animals, it has been shown that simulation of the continuous artificial lighting regime (the Light-Light model) leads to an increase in platelet aggregation activity in the vessels of the microcirculatory bloodstream and the development of the most conspicuous changes in parenchymal organs. The use of the Light-Dark (12:12) mode, which implies the presence of dim lighting at night, led to sleep disturbances in rats [1]. In the study of [16,17], it has been found that with a prolonged exposure (over 4–6 weeks) to the Light-Dark (10:10) lighting mode with 10 h period of light and 10 h darkness, metabolic disorders develop in the body of laboratory mice, leading to an increase in body weight and an increase in the concentration of insulin and triglycerides in the blood plasma.

However, aspects of the effect of extended photoperiod using different lighting alternation modes of the Light-Dark model on the platelet aggregation activity, as well as comparison of such changes with the 24-hour lighting model, remain unstudied so far. The purpose of this study was a comparative analysis of the severity of the effect of various modes of continuous and alternating artificial lighting (Light-Light, Light-Dark 12:10 and Light-Dark 18:6 models) on the aggregation activity of platelets in male rats in the experiment.

Materials and methods

The experimental study was carried out on the basis of the central research laboratory of the Razumovsky Saratov State Medical University and the laboratory of hemostasis of the Chuevsky Department of Normal Physiology. The

experiment was carried out using 48 white mongrel male rats with a body weight of 250 g in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and the „International Guiding principles for Biomedical Research Involving Animals“ (2012), and with approval of the Ethics Committee of the Razumovsky Saratov State Medical University FSFEI HE of the Ministry of Health of the Russian Federation (minutes № 4 dated 06.12.2016).

Laboratory animals were divided into 4 groups: three experimental groups, one reference group, 12 animals each. Experimental animals were subjected to round-the-clock artificial light exposure in the Light-Light (24L:0D) mode, as well as in alternating light and dark modes using the Light-Dark (12:10) and Light-Dark (18:6) models for 10 days. The Light-Ligh model assumes continuous artificial light exposure with an intensity of 300 lk during daylight hours and 500 lk during the dark time of the day. Light-Dark modes assume 18 h or 12 h continuous light exposure with a light intensity of 500 lk and 6 h or 10 h duration of the dark mode, respectively‘[18]. The experiment was carried out during the fall period. Animals of the reference group were in the natural photoperiod conditions for 10 days. All groups of experimental animals had the same free access to water and food.

The functional activity of platelets was studied according to the method of Z.A. Gabbasov et al. [19] using a computerized 230LA „Biola“ two-channel aggregation laser analyzer not later than 3 h from the moment of blood sampling. Blood sampling was performed by puncture from the right parts of the heart in animals withdrawn from the experiment by intramuscular injection of a combination of Telazol (Zoetis Inc, USA) at a dose of 0.1 ml/kg and Xylanit (Nita-Farm, Russia) at a dose of 0.1 ml/kg . A 0.2% solution of sodium citrate in a ratio of 9:1 was used as an anticoagulant. The laser analyzer of aggregation investigates the process of platelet aggregation on the basis of recorded changes in the light transmission of platelet-rich plasma (the turbidimetric method) and fluctuations in the light transmission of plasma caused by a random change in the number and size of platelets and their aggregates in a thin laser optical channel. The relative dispersion of such fluctuations is proportional to the average aggregate size and is used to study the aggregation kinetics. The instrument was calibrated for each animal by recording the light transmission of platelet-poor plasma (the result was taken as 100%) and platelet-rich plasma with the addition of 10 μl 100 mM EDTA solution (the data obtained were evaluated as 0). The weighted average radius of platelet-rich plasma was taken as 1 arbitrary unit. Platelet aggregation in platelet-rich plasma with a volume of 300 μl was measured under the conditions of temperature control at 37°C in the aggregometer working cell and a stirring speed of 800 rpm. The platelet aggregation process was recorded on the basis of the data of the light transmission curve and the curve of the average weighted radius, displayed on the screen of a computer monitor connected to the aggregometer [20].

The dynamics of changes in the platelet aggregation activity was assessed using the parameters of the weighted average platelet radius curve and the light transmission curve. The ADP solution (by NPO „RENAM“, Russia) with a concentration of 2.5 mmol/ml was used as an inducer of the platelet aggregation.

The data were statistically processed using the „STATISTICA 10.0“ software package (StatSoftr, USA). In case of difference of values distribution in a sample from the normal distribution, median and quartiles were calculated. When assessing the significance of differences, the Mann-Whitney U-test was used. Changes were considered statistically significant when $p < 0.05$.

Results and discussion

On the 10th day of the experiment, in all models of light exposure, an increase in the parameters of the weighted average radius curve was observed compared to the reference group (Fig. 1–4).

The most severe changes were observed in groups with the LD 18:6 lighting mode: the maximum size of formed platelet aggregates was 10.8 arb. units [9.1;12.9], the maximum rate of formation of the largest platelet aggregates was 19.4 arb. units [15.9;24.9], which was 75% higher than that in the reference group.

In the LL model, there was a 15% increase in the time to reach the maximum size of platelet aggregates and a 8% increase in the time to reach the maximum rate of formation of platelet aggregates compared with the reference group. In the group with the LD 12:10 mode modeling, a trend towards increase in the parameters of the weighted average radius curve was also found, however, no significant changes were found compared to the reference group.

Among the parameters of the light transmission curve, the largest reliable increase in parameters was noted in the LD 12:10 group (Fig. 5–8). In this group, the maximum degree of aggregation was 67.4% [64.3;71.7], the maximum rate of aggregation was 91.4% [89.8; 101], which exceeded the parameters in the reference group by 56% and 65%, respectively.

The LL model also shows a significant increase in parameters; there is an increase in the maximum rate of aggregation by 42%, the time to reach the maximum degree of aggregation is increased by 12%, as well as a trend towards an increase in the maximum degree of aggregation by 34% is observed. In the LD 18:6 model, there is only a moderate increase in the curve parameters. Time to reach the maximum aggregation rate did not change significantly in all models compared to the reference values.

The results of the performed study indicate the negative effect of light desynchronosis on the aggregation activity of platelets. It has been experimentally established that

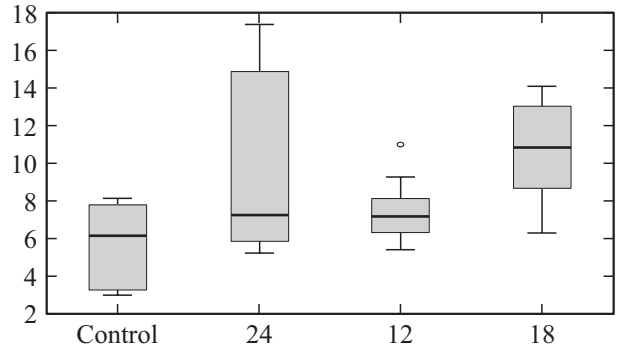

Figure 1. Maximum size of platelet aggregates formed, arb. units, for the reference group and groups exposed to light for 24, 12, 18 h.

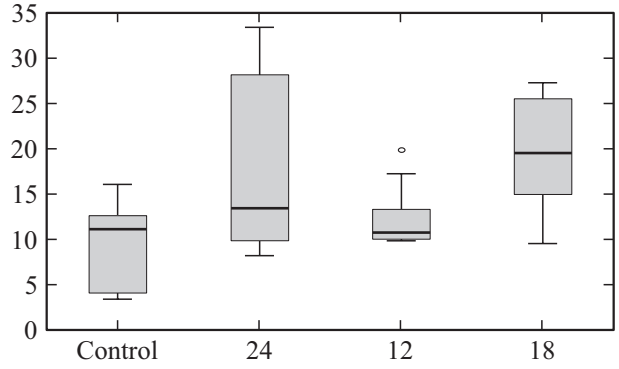

Figure 2. Maximum rate of formation of the largest platelet aggregates, arb. units, for the reference group and groups exposed to light for 24, 12, 18 h.

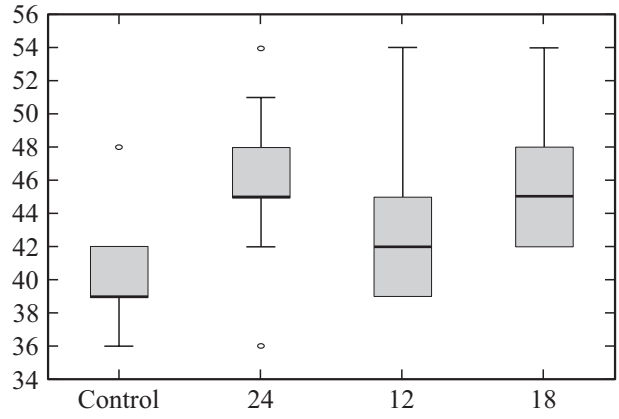

Figure 3. Time to reach the maximum size of formed platelet aggregates, s, for the reference group and groups exposed to light for 24, 12, 18 h.

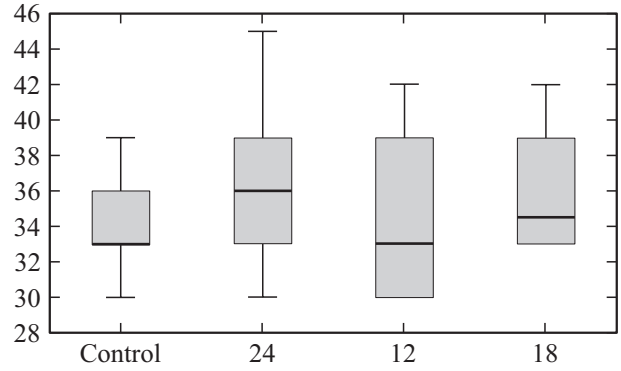

Figure 4. Time to reach the maximum rate of formation of the largest platelet aggregates, s, for the reference group and groups exposed to light for 24, 12. 18 h.

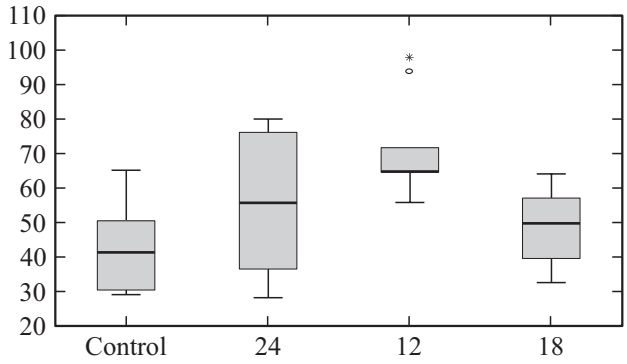

Figure 5. Maximum degree of aggregation, %, for the reference group and groups exposed to light for 24, 12, 18 h.

prolonged exposure of laboratory rats to disturbed photoperiodism provokes the development of stress reactions and activation of proaggregant processes [21,22].

Platelet aggregation is a complex process, in the development of which a variety of proaggregant (thrombin, thromboxane A2, epinephrine, collagen, and others) and antiaggregant (nitrogen oxide, prostacyclins) factors, as well as platelet glycoprotein receptors, play their roles. The most important is the balance between vascular endothelial prostacyclin and platelet thromboxane A2. With an increase in aggregation activity, a shift towards thromboxane A2 occurs [21,23]. The development of this condition is possible as a result of stress reactions in response to a prolonged light exposure. The key link in the development of stress response is melatonin — one of the main regulatory hormones of circadian rhythms. A number of studies have shown that the level of melatonin concentration in tissues depends directly on the level of light exposure during sleep. For example, light exposure with an intensity of 500 lk provokes a disturbance of the hormone synthesis by pinealocytes of the epiphysis in laboratory animals [23]. Insufficient production of melatonin entails a disturbance of the organism's biorhythm pattern and the development of a stress response.

Activation of the sympathoadrenal system in response to a stress triggers a large cascade of proaggregant phenomena in the organism. An increase in the level of catecholamines and the subsequent development of an oxidative stress in the organism provokes not only destabilization of platelet membranes, which contributes to their activation and an

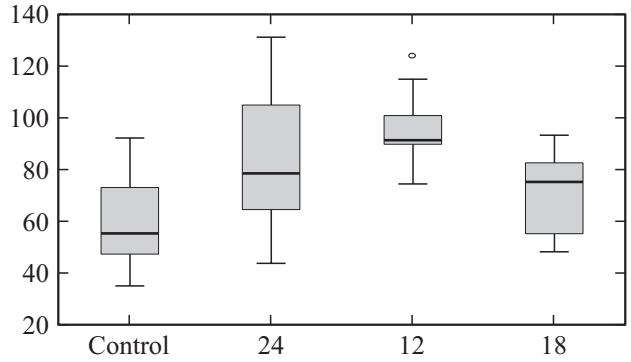

Figure 6. Maximum aggregation rate, % min, for the reference group and groups exposed to light for 24, 12, 18 h.

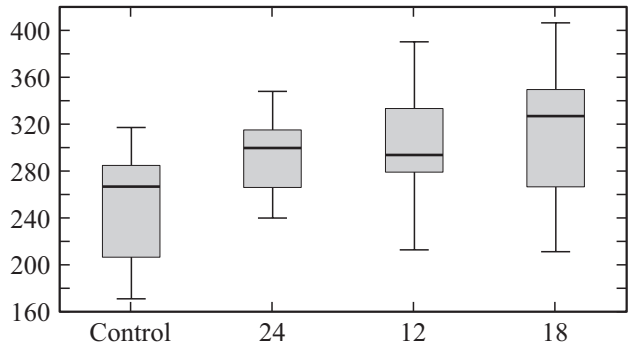

Figure 7. Time to reach the maximum degree of aggregation, s, for the reference group and groups exposed to light for 24, 12, 18 h.

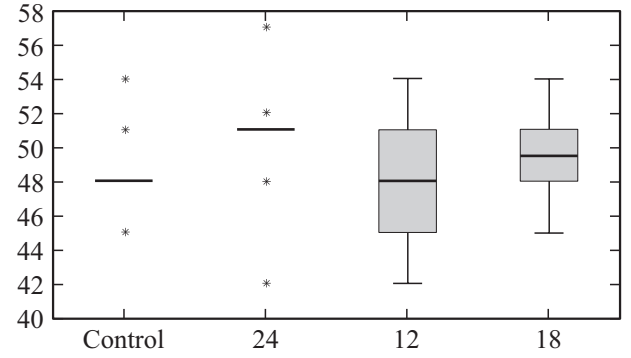

Figure 8. Time to reach the maximum aggregation rate, s, for the reference group and groups exposed to light for 24, 12, 18 h.

increase in the concentration of thromboxane A2, but also the release of a number of proinflammatory cytokines, such as IL-1β. This, in turn, promotes the development of a low-intensity inflammation of the vascular endothelium with the release of a number of vasoconstrictor and proaggregant substances. An increase in the adhesive and aggregative activity of platelets can lead to thrombosis in capillary vessels with the development of ischemia and subsequent tissue necrosis, which is one of the key causes of cerebrovascular pathology and myocardial infarction [24,25].

On the 10th day of the experiment, all models showed changes in platelet activity towards hyperaggregation. The greatest increase in the proaggregant ability of platelets is observed in rats in the LD 18:6 model according to the weighted average radius curve, however, in terms of the light transmission curve, the most significant increase is observed in animals of the LD 12:10 model. Due to the fact that the method of light transmission fluctuations has a higher sensitivity than the Born method, after all it should be concluded that there are more severe disorders of platelet aggregation in rats of the 18:6 model because of more conspicuous processes of urgent adaptation and subsequent depletion of the organism [15,22,26].

Conclusion

The results of the experiment on keeping rats under artificial lighting conditions using the LL, LD 12:10 and LD 18:6 models allow concluding that light deprivation triggers a stress response in the organism and provokes an increase in platelet activity, leading to the development of microcirculatory bloodstream pathology. In animals on the 10th day of exposure to light desynchronosis, a significant increase in platelet activity was noted, which is indicative of the development of processes of urgent adaptation of the organism. The most severe changes are observed in the group with the LD 18:6 lighting mode modeling. Thus, the exposure to continuous lighting and disturbed photoperiodism conditions is a powerful stressor that causes an increase in platelet aggregation activity and provokes the development of microcirculatory bloodstream pathology.

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Conflict of interest

The authors declare that they have no conflict of interest.

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