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EXPERIMENTAL BIOLOGY

Association of Infradian Rhythms of Motor Activity, Concentration of Glucocorticoid Hormones, and One-Minute-Step Oscillations of Body Temperature with Intensity of Fluctuations of Secondary Cosmic Rays

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The study compared the daily mean intensity of one-minute-step fluctuations in intensity of the secondary cosmic rays reflected by neutron count rate and dynamics of body temperature, motor activity, as well as concentration of glucocorticoid hormones in birds and rodents. A positive correlation was established between body temperature oscillations and neutron count rate fluctuations. A similar correlation was observed between physical parameter (neutron count rate), on the one hand, and daily mean motor activity and concentration of glucocorticoid hormones in the animals. The periods and phases of these processes presented in synchronous time series coincided. The facts of simultaneous variations or disturbances of the periods in dynamics of biological and physical parameters attest to their relationships. The study concluded that the infradian rhythms with the periods of 3-5 days depend on some external environmental factor related to fluctuations in intensity of secondary cosmic rays.

Key Words: *infradian rhythm; body temperature; motor activity; glucocorticoid hormones; synchronizers*

Hypothesis on existence of heliogeophysical synchronizers of infradian rhythms was advanced by Franz Halberg [10,11] and other researchers [6,7]. Numerous behavioral and biochemical parameters describing the motor activity and the levels of glucocorticoid hormones or norepinephrine metabolites vary with near 4-day infradian rhythms presumably affecting the sympathetic-parasympathetic balance of autonomic nervous system (ANS) [1,2,12,13]. Oscillations of body temperature (BT) are also related to activation of the sympathetic branch of ANS [9].

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Previously we demonstrated that rhythmic oscillations of BT with the periods ranging 100-400 min correlate with intensity of fluctuations of the secondary cosmic rays assessed by neutron count rate (NCR) of neutron monitors [5]. Based on these data, we concluded that the biotropic action on ANS is exerted by oscillations with the periods smaller than 20 min, whereas the ultradian rhythms with longer periods result from the changes in intensity of oscillations namely in this frequency range. Logically, this view can be extrapolated for the infradian rhythms as well. Thus, the first task in this study was to perform the correlation analysis of one-minute-step oscillations of NCR and BT in mice and rats. The close correlation of these parameters would strengthen the

view on relationships between them in a broad range of periods.

Another finding was novel near 4-day infradian rhythm in the animal motor activity. At this, BT dynamics in laboratory rodents (but not birds) correlated with motor activity with r=0.5-0.8. Thus, one can expect association between daily mean motor activity of laboratory animals and daily mean one-minute-step fluctuations of NCR. Another important indicator of functional strength of an organism (and its motor activity in particular) is the level of glucocorticoid hormones assayed at the moment of its daily maximum.

This work was designed to examine association between intensity of one-minute-step fluctuations of the secondary cosmic rays, on the one hand, and the changes in BT, motor activity, and glucocorticoid hormones, on the other hand, within the infradian range of periods.

MATERIALS AND METHODS

The experiments were carried out on mature male Wistar rats weighing 280-340 g (n=20) and male C57BL/6 mice weighing 26-30 g (n=14) obtained from Animal Breeding Department Stolbovaya (Research Center of Biomedical Technologies, Federal Medical-Biological Agency of Russia). To reveal universal mechanism in the action of examined environmental factor on mammals and birds, we also used the males of common greenfinches Chloris chloris (n=8) caught 2-3 months before the study. The experiments were carried out in compliance to European Convention for Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The experimental protocols were approved by Bioethics Committee of Research Institute of Human Morphology.

The laboratory rodents were kept in plastic cages (2-3 subjects per cage) at 18-22°C and air humidity of 55-75% with water and food *ad libitum*. In the period from January 15 to March 10, 2021, the rats were maintained under natural illumination, whereas in the period from March 21 to April 24, 2021, they were exposed to constant illumination with intensity of about 1200 lx supplied by a source with the color temperature of 4200 K. The mice were kept under constant illumination with intensity of 400-500 lx. In experiments with natural illumination, the food, water, and bedding had been changed once per 5-7 days during evening activity of the animals whereas under the constant illumination, these procedures were performed at various daytime.

BT and motor activity were measured by DTN4-28/TL4-28 combined temperature/acceleration sensor with incorporated 3D accelerometer (EMBI RESERCH) implanted intraperitoneally under intramuscular narcosis with Zoletil (5-7 mg/kg; Virbac Sante Animale). The transducers were implanted no later than 2 weeks prior to the experiments. The sampling rate for BT and motor activity measurements was 1 min^{-1}. The data were accumulated in the sensor's memory for 20 days, thereupon they we read by wireless communication. Motor activity was summarized by one-minute-step function of acceleration vector reflecting the changes in motion and pose of the animals. Among other measures of motor activity assessed by 3D parameters of accelerometer and suggested by producer, this index correlated most closely with video recording data.

The study used our previous data on the levels of glucocorticoid hormones in rats and humans [1-3,5]. They were determined in blood drawn at the period of maximal hormonal level, which was 16.00-17.00 in rats and about 08.00 in humans. The examinees were men (n=8) aging 25-35 years without documented pathologies; they slept regularly from 22.00-23.00 to 07.00. The glucocorticoid hormones were assayed by ELISA: cortisol in humans and corticosterone in rats were measured using kits from DRG and IBL, respectively. In rats, the blood for corticosterone assay was drawn from the lateral tail vein under light ether narcosis. This procedure was performed immediately after the loss of posture and lasted for no more than 1 min. To avoid chronic stress due to repetitive blood drawing, a new group of rats was used every day for the assay.

To assess fluctuations of secondary cosmic rays, we used NCR data of IZMIRAN neutron monitor station (Troitsk, Moscow region, http://cr0.izmiran.ru/mosc) located at the distance of 40 km from the place of experiments. The magnitude of these fluctuations was quantified with the daily averaged modules of 1-min-steps of NCR.

The results were statistically processed with Statistica 7.0 (StatSoft, Inc.) software. Similarity in dynamics of examined biological and physical parameters was assessed by Spearman's correlation coefficient r calculated for the same time intervals. Significance was assessed at $p<0.05$.

RESULTS

In rats and mice, the changes of one-day-step function of daily mean of one-minute-step BT oscillations positively correlated with NCR fluctuations in all analyzed time intervals (Table 1). Despite the fact that the 4-day rhythmicity was typically observed in many analyzed time series, it is not universal. Actually, Figure 1 demonstrates BT oscillations and NCR fluctuations with 4- and 3-day periodicities but also the records without overt rhythmicity. The established fact

that predominance of a certain rhythm was observed simultaneously in physical and biological processes attests to their objective intimate association, which did not result from a casual coincidence of their periodicities. This phenomenon was mostly demonstrative in the interval (Fig. 1, *c*) where the positive correlation was revealed despite the absence of periodicity in both time series. Thus, the changes of one-day-step function of the daily mean of one-minute-step BT oscillations positively correlated with fluctuations in intensity of the secondary cosmic rays. During entire length of the study, the correlation coefficients of these physical and biological parameters for rats and mice were 0.31 (p=0.009) and 0.35 (p=0.002), respectively. It should be stressed that the analyzed functions are the moduli of the corresponding step-functions reflecting dispersion but not the current values of BT or NCR, whose mean values remained constant throughout the study. Thus, the positive correlation was established between the moduli of both step-functions and not between BT

TABLE 1. Correlation Coefficients for One-Day-Step Function of Daily Mean of One-Minute-Step BT Oscillations (n=6-10) and Similar Function of NCR Fluctuations in the Same Periods

Species	Period of data recording	Correlation coefficient	Mean for each species
Rats	January 15-February 1, 2021	r=0.60 (p=0.01)	r=0.31 (p=0.009)
	February 22-March 10, 2021	r=0.21 (p=0.40)	
	March 21-April 7, 2021	r=0.48 (p=0.05)	
	April 8-24, 2021	r=0.38 (p=0.13)	
Mice	December 4-29, 2020	r=0.16 (p=0.43)	r=0.35 (p=0.002)
	May 2-28, 2021	r=0.20 (p=0.33)	
	June 16-29, 2021	r=0.40 (p=0.15)	
	July 2-18, 2021	r=0.37 (p=0.14)	
	July 20-August 5, 2021	r=0.50 (p=0.03)	

TABLE 2. Correlation Coefficients for Parameters of Daily Changes in Total Motor Activity of Different Species (n=6-10) and Daily Mean of One-Minute-Step NCR Function in the Same Time Periods

Species	Period of data recording	Correlation coefficient	Mean for each species
Greenfinches	January 21-February 15, 2016	r=0.49 (p=0.005)	r=0.49 (p=0.005)
Mice	December 4-29, 2020	r=0.26 (p=0.23)	r=0.46 (p=0.001)
	May 2-May 28, 2021	r=0.22 (p=0.27)	
	June 16-29, 2021	r=0.37 (p=0.15)	
	July 2-18, 2021	r=0.40 (p=0.12)	
	July 20-August 5, 2021	r=0.45 (p=0.04)	

TABLE 3. Correlation Coefficients for Concentrations of Glucocorticoid Hormones (Corticosterone in Rats and Cortisol in Humans) and Daily Mean of One-Minute-Step NCR Function in the Same Time Periods

Species	Period of data recording	Correlation coefficient	Mean for each species
Rats	March 17-29, 2011	r=0.23 (p=0.43)	r=0.39 (p=0.0006)
	September 11-27, 2011	r=0.35 (p=0.16)	
	October 1-23, 2011	r=0.60 (p=0.001)	
	January 3-17, 2012	r=0.61 (p=0.014)	
	October 15-30, 2012	r=0.57 (p=0.02)	
Humans	September 3-30, 2008	r=0.57 (p=0.002)	r=0.42 (p=0.00004)
	June 2-22, 2010	r=0.35 (p=0.13)	
	September 29-October 16, 2010	r=0.27 (p=0.26)	
	December 2-28, 2011	r=0.55 (p=0.002)	

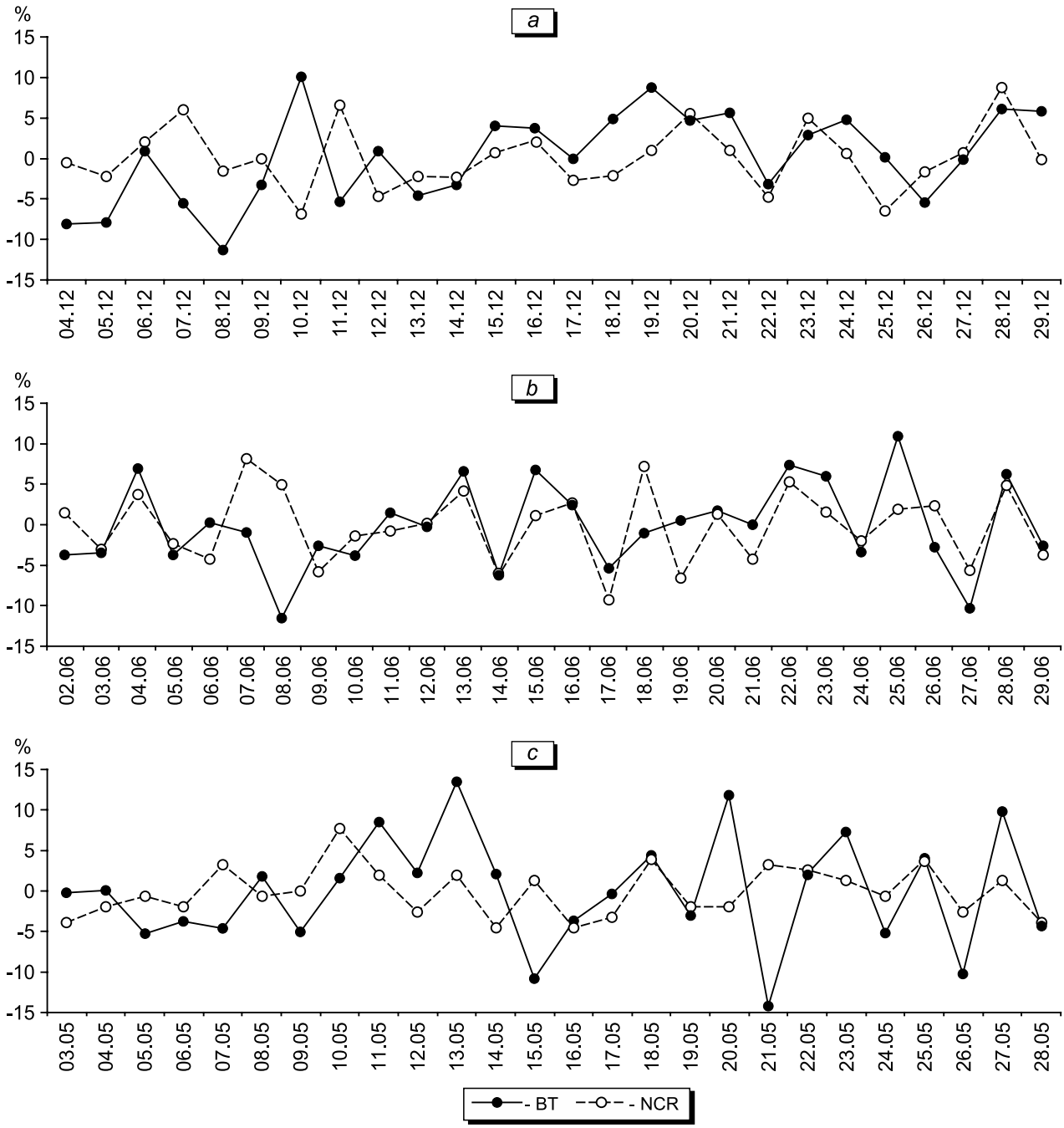

Fig. 1. Dynamics of one-day-step functions of daily mean of one-minute-step BT oscillations and NCR fluctuations. *a*) The period of near 4-day changes (December 4-29, 2020); *b*) the period of near 3-day oscillations (July 2-29, 2021); *c*) the period with no certain periodicity (May 3-28, 2021). The data are presented in percentage of deviation from the mean level in specified interval.

and NCR current values characterized by constant daily averages. In other words, association between biological and physical processes is underlain not by their current values (BT or NCR) but by their fluctuations within one-minute or possibly shorter range of periods.

Evidently, the dynamics of total daily motor activity and daily mean fluctuations of NCR analyzed in the same time period demonstrated the unidirectional changes. The 4-day rhythmicity was most frequently observed. The correlation coefficients for examined time series were positive and significant (Table 2) both for mice (r=0.46, p=0.001) and greenfinches (r=0.49, p=0.005). The established significant correlation between intensity of NCR fluctuations and oscillations of motor activity for mammals (mice) and birds (greenfinches) attests to common mechanism of biotropic

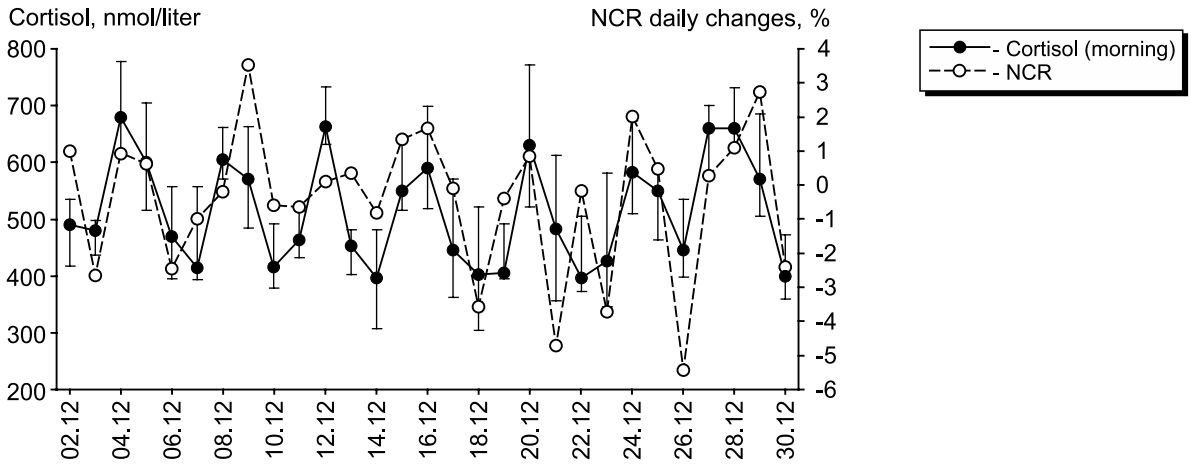

Fig. 2. Dynamics of morning cortisol concentration in blood serum of men monitored over many years and daily mean of NCR one-minute-step function in 2007, 2009, 2011, 2012, 2015, and 2016 years.

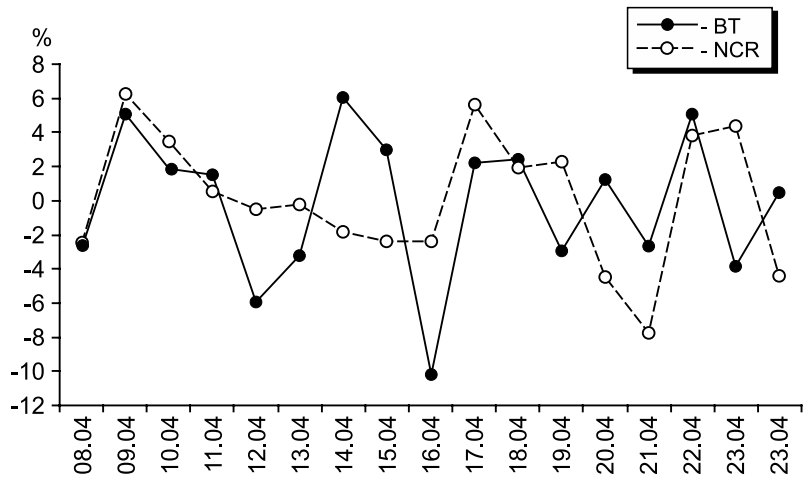

Fig. 3. Dynamics of daily mean of BT one-minute-step function in rats (n=8) and NCR fluctuations in the period of April 8-24, 2021. The parameters are given in percentage of deviation from the mean level in specified time interval.

action of examined environmental factor on these vertebral classes of animals.

Dynamics of glucocorticoid hormones in blood serum also positively correlated with the daily mean of one-minute-step fluctuations in intensity of the secondary cosmic rays (Table 3) both in rats (r=0.39, p=0.0006) and humans (r=0.42, p=0.00004). Thus, the biotropic action of examined physical factor is reflected by the level of glucocorticoid hormones.

In our previous study of variations of cortisol level in humans, we detected the most stable 4-day rhythm in December; importantly, the acrophases of this rhythm occurred at the same calendar dates [1,2]. Figure 2 shows an example illustrating coincidence of the phases of the 4-day rhythm in the level of glucocorticoid hormones and NCR fluctuations in the same years when the experiments were conducted. The phenomenon of appearance of the acrophases in both processes on the same calendar dates (with due account for the leap years) will be important in the search for the origin of NCR fluctuations. One of the possible explanations of these fluctuations is based on the hypothesis of anisotropy of the medium in which the Earth moves. Therefore, every year the planet visits the same place in the galactic space at the certain calendar date. This hypothesis was advanced by Simon Shnoll when he explained the origin of macroscopic fluctuations of various nature including oscillations in thermal neutron flux intensity at the surface of our planet [8].

The present data suggest that approximately 3-5-day infradian rhythms in motor activity and the level of glucocorticoid hormones depend on some environmental physical factor, which probably activates the sympathetic nervous system. However, one cannot exclude existence of an endogenous rhythm with near 4-day period. Probably, this endogenous oscillator has

a wide window to be captured by external synchronizer's rhythm. Actually, a regular peak of biological parameter with 4-day rhythmicity can be observed even during extremely rare event of a long-term period of stable fluctuations in cosmic ray intensity (Fig. 3).

It should be stressed that association of examined biological parameters is established with intensity of NCR fluctuations but not with NCR value, which indicates that the biological response (activation of sympathetic branch of ANS) is not determined by the flux of cosmic rays *per se*. Probably, intensity of NCR fluctuations is merely a marker of some presently unknown external biotropic factor. This factor can determine the intensity of NCR fluctuations. Alternatively and less likely, the fluctuations in intensity of galactic cosmic rays trigger the oscillatory processes in ionosphere, so the nature of examined factor can be geophysical.

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