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Amplitude of One-Minute Fluctuations of Secondary Cosmic Rays as a Marker of Environmental Factor Determining Ultradian Rhythms in Body Temperature of Laboratory Rats

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The study examined association between oscillations of body temperature of laboratory Wistar rats maintained under constant illumination with the amplitude of fluctuations of secondary cosmic rays reported by neutron count rate provided by neutron monitors and geomagnetic undulations. In contrast to geomagnetic undulations, neutron count rate variations and body temperature oscillations in rats assessed by spectrum analysis of the corresponding step functions at 1-min intervals demonstrated almost permanent variations with the periods ranging from 100 to 400 min. Under conditions of constant illumination inducing changes in the period of circadian rhythm and predominance of the ultradian rhythms, an association between neutron count rate fluctuations and body temperature oscillations was observed perpetually during the day- and nighttime.

Key Words: *ultradian rhythms; body temperature; secondary cosmic rays; neutron monitoring; exogenous rhythm*

The rhythmic nature of seemingly spontaneous daily activity of animals is a common knowledge [6-10,14]. Many researchers agree with the view that such activity reflects the intrinsic endogenous rhythms of biological organisms unrelated to exogenous environmental factors [6-10]. In contrast, we demonstrated that oscillations of body temperature (BT) in small mammals and birds with the periods ranging from 4 to 20 min are synchronous in various species characterized by different intensity of metabolism, which reflects the effect of an exogenous factor on their autonomic nervous systems [2].

It is firmly established that the heliogeophysical factors can affect autonomic nervous systems activity [5,12,13,15]. Previously we demonstrated that BT oscillations in animals are synchronized with Pc5 geomagnetic undulations (GMU) [3]. Although the latter are observed regularly, they are not stable in contrast to similar oscillations of BT that occur far more frequently. Thus, our data cannot be explained only by synchronization of endogenous rhythms with GMU. The absence of correlation between the magnitude of biological effect and GMU amplitude also casts doubt on hypothesis that namely this factor is the biotropic one.

In addition to Pc5 GMU, the intensity of the secondary cosmic rays (SCR) recorded with neutron monitors and reported by neutron count rate (NCR) is characterized with periodicities, which are similar to those of BT oscillations, but in contrast to GMU, they are observed permanently [4].

Examination of a number of parameters of geomagnetic environment harvested an integral ULF index describing GMU intensity within the range of 2-10 mHz, which closely correlates with elevations of BP [11] known to be controlled by sympathetic system. Importantly, dispersion of activity of the aquarium fishes maintained under constant illumination correlated only with SCR intensity but not with

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other analyzed heliogeophysical factors [1]. Probably, manifestation of this phenomenon namely under constant illumination can be explained by hypothesis that alternating light-dark regimen is a stronger stimulus in comparison with other environmental factors.

Thus, it can be concluded that oscillations in the period range of 4-20 min exert the biotropic effect on autonomic nervous systems, while slower biological ultradian rhythms are formed due to the changes in intensity of oscillations namely within this period range.

This work was designed to analyze associations between ultradian BT rhythms with the periods ranging 100-400 min on the one hand and *1*) GMU and 2) NCR fluctuations, on the other hand, all of which were assessed with corresponding 1-min interval step functions.

MATERIALS AND METHODS

The experiments were carried out on mature male Wistar rats (*n*=20) obtained from Animal Breeding Department "Stolbovaya" (Scientific Center of Biomedical Technologies, Federal Medical-Biological Agency of Russia). The study had been 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 the Research Institute of Human Morphology. The rats were kept 2-3 per plastic cage (60×18.5×38 cm) at 18-22°C and air humidity of 55-75% with water and food *ad libitum*. The rats were exposed to constant illumination of 1200-1500 lx supplied by a source with the color temperature of 4200 K. Food, water, and bedding were changed once per 5-7 days at various daytime.

BT was measured with DTN3-28 transducers (EMBI RESERCH) implanted intraperitoneally under intramuscular narcosis with Zoletil (5-7 mg/kg; Virbac Sante Animale). The transducers were implanted no later than 3 weeks prior to experiments. The sampling rates of BT measurements were 0.5 min^{-1} in the period from April 22 to May 14 in 2019 (*n*=6) and from May 23 to June 8, 2019 (*n*=6), and 1 min^{-1} in the period from March 21 to April 24 in 2021 (*n*=8), respectively.

The GMU data were taken from INTERMAGNET (International Real-time Magnetic Observatory Network in Borok, Moscow Region; https://www.intermagnet.org/data-donnee/download-eng.php#view), which was the nearest observatory at location of experimental rats. The neutron monitoring data were obtained directly for the place of experiments (Moscow, http://cr0.izmiran.ru/mosc). The magnitude of SRC fluctuations was assessed using the modules of 1-min-interval steps of NCR averaged over 1 h with a sliding window.

The results were statistically processed with Statistica 7.0 (StatSoft, Inc.) software. The rhythmic components in examined processes were revealed with fast Fourier transform. Similarity of power spectra of examined functions were assessed with Spearman's cross-correlation coefficient *r*. Significance of the coincidence of BT maxima and maximal NCR fluctuations was assessed with *z*-test for two samples (SigmaStat) at $p<0.05$.

RESULTS

Figure 1 shows simultaneous time profiles (median values) of BT in a group of rats (*n*=8) kept under constant illumination, NCR (1-min step function), and GMU. Evidently, almost all BT increments were associated with elevations in NCR (Fig. 1, *a*). In contrast, not all fluctuations in NCR correlated with similar changes in BT. This observation is consistent with the view on activating biotropic action of the changes in SCR: when the sleeping animals are not ready for waking, they are immune against activating action of exogenous factor, and any arousing is caused by an increase in its intensity. It is important that GMU are observed far rarely than the fluctuations in NCR, and the absence of GMU is not accompanied with a drop in the amplitude of ultradian BT oscillations (Fig. 1, *b*).

We calculated the power spectra of BT oscillations and NCR variations for 3-day-long intervals. Despite the absence of certain harmonics in summarized spectra, different harmonics dominated in various 3-day-long intervals. It is noteworthy that these temporally dominating harmonics were identical in the time profiles of BT and NCR (Fig. 2). To characterize their similarity, we calculated the cross-correlation coefficients for the spectral densities of biological (BT) and physical (NCR) parameters in each 3-day-long interval. Table 1 shows correlation between BT oscillations in the group of rats and NCR step function reporting variations in SCR. In all cases, the correlation coefficients were positive and ranged 0.2-0.6; at this, significance surpassed $p=0.05$ in 92% cases. Most frequently (in 75% cases), BT power spectrum calculated for a 3-day-long interval did not significantly correlate with that of NCR calculated for another interval of the same duration. This fact indicates association between examined parameters, which cannot be a casual one. If a biological object and a physical factor had similar rhythms, collation of their spectra obtained in various periods would also detect significant correlations. The present data showed that BT rhythms reflecting activity of the animals are determined by intensity of NCR fluctuations.

Figure 3, *a* shows ultradian time profile of BT in a group of rats (*n*=6) maintained under constant illu-

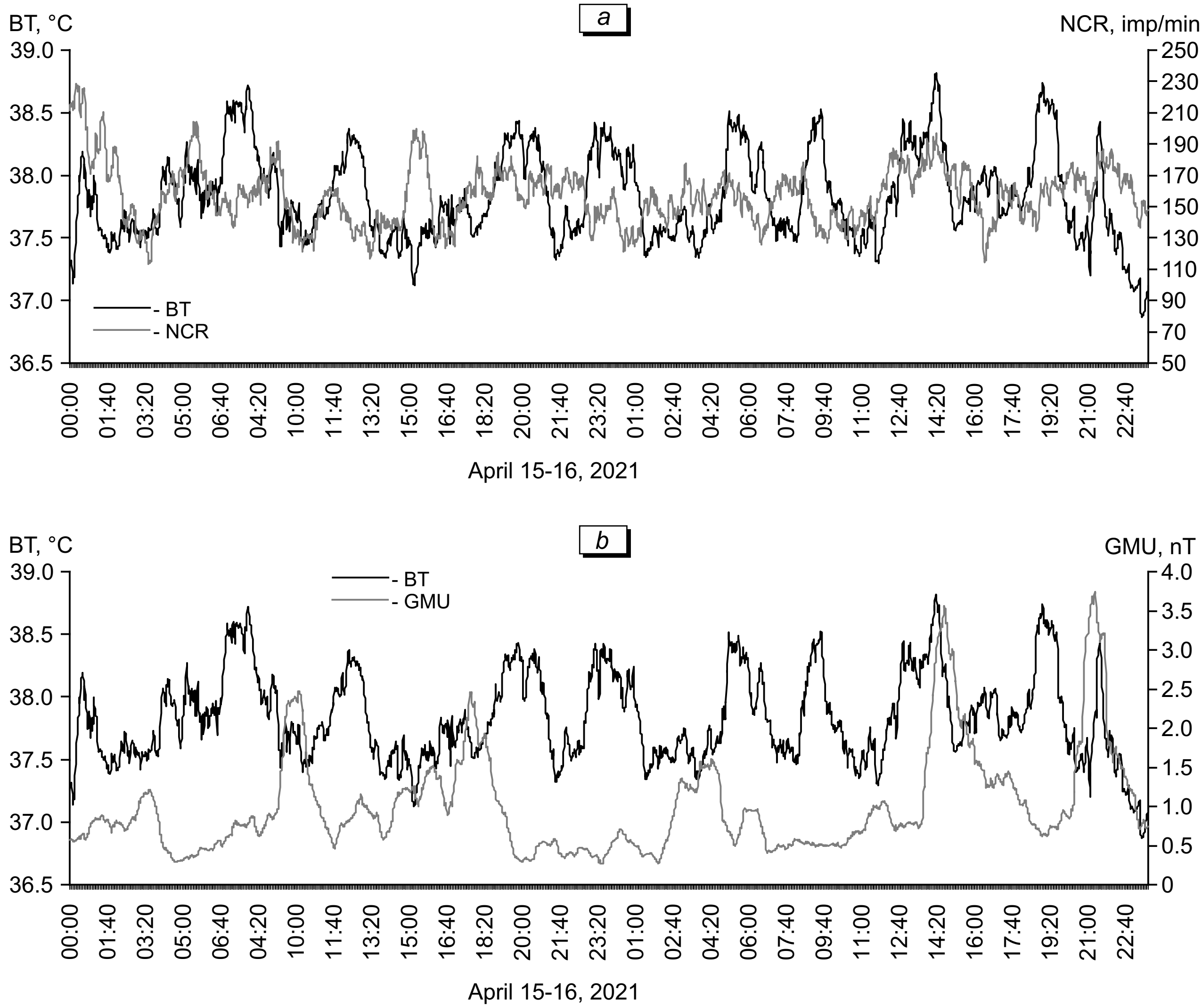

Fig. 1. Time profile of BT (median value) of a group of rats (n=8) kept under constant illumination in comparison with the time course of NCR acquired with sampling rate of 1 min and averaged with the sliding window over 60 min (*a*) and the time profile of GMU (*b*).

mination averages over a certain observation period. Correlation between this curve and the step function of NCR calculated for 1-min intervals and summarized for the same observation period was r=0.23 (p<0.001). Similarly, the correlation coefficient calculated for another observation period (Fig. 3, *b*) was also significant (r=0.19; p<0.001). The correlation between BT oscillations and NCR fluctuations increased at the midnight as well as in 4, 8, 12, 16, and 20 h. Thus, the previously revealed ultradian BT rhythms in rats with the period about 4 h corresponded to fluctuations in SCR detected with neutron monitors.

The moments of time corresponding to maxima of NCR step function calculated for 1-min intervals and that of median BT in a group of rats indicate association between both parameters. Actually, in 76% cases (n=406) the phase of BT rhythm coincided with that of NCR with significance of p<0.001.

Thus, under constant illumination known to increase the period of circadian rhythm and to decrease its amplitude in parallel with increasing the amplitude of ultradian rhythms, there is permanent association between elevation of SRC fluctuations and BT increments.

Evidently, the amplitude of fluctuations of SCR reported by neutron monitors is very small to determine the examined biological effect. Actually, the amplitude of fluctuations of NCR step function calculated for 1-min intervals is merely 1-3%, which means that only 2-6 events per minute can affect an animal with the size of a rat. Such small number of atomic events seems to be insufficient to produce any physiological effect even if thermal neutrons actively interact with biological tissues. Probably, intensity of NCR fluctuations is only a marker of some physical factors affecting the living organisms. To reveal the mode of action

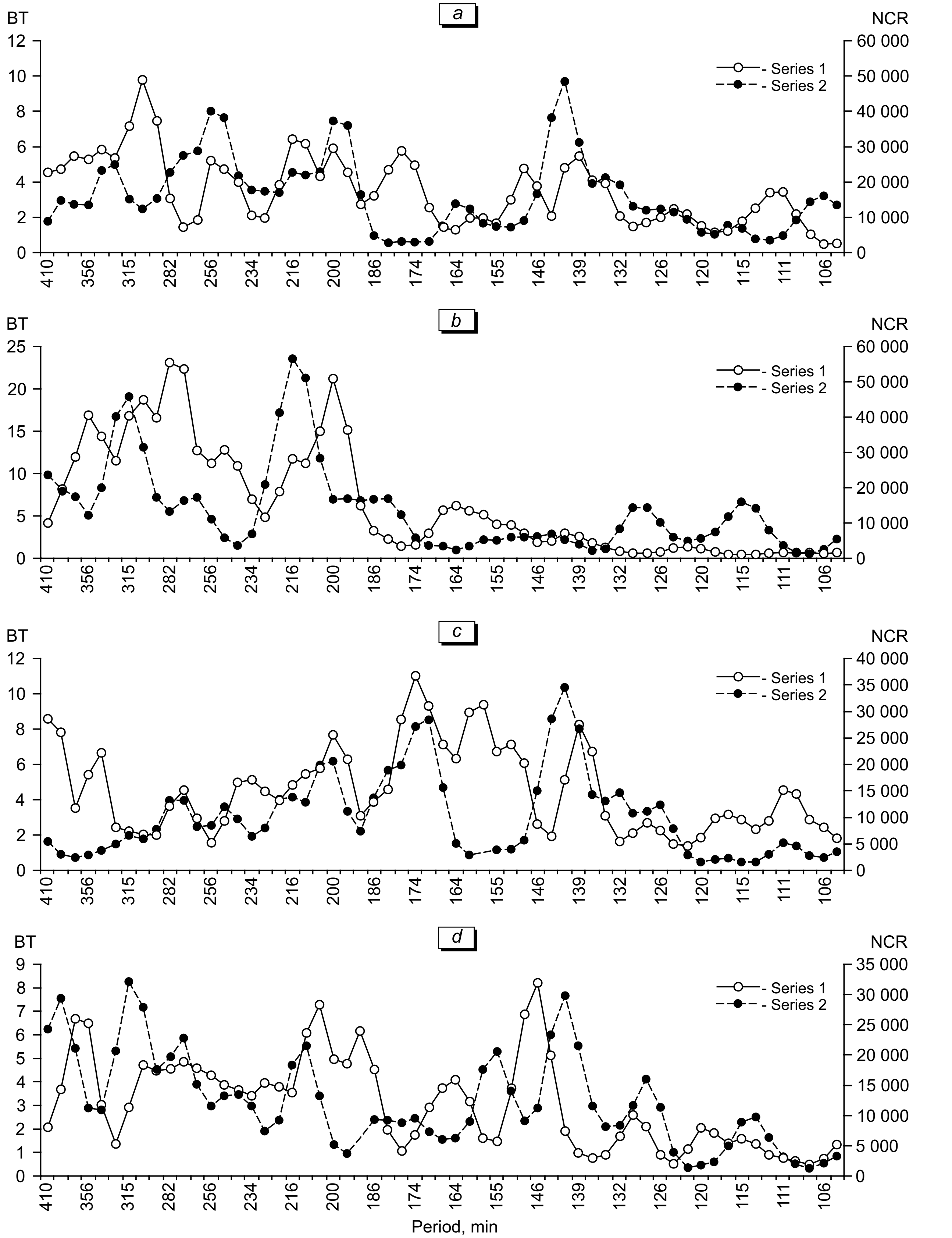

Fig. 2. Spectral densities of BT (median value) of a group of rats (*n*=8) and 1-min interval NCR Step Function calculated for 3-day-long periods in 2021. *a*) April 6-8, *b*) April 12-14, *c*) April 16-17, *d*) April 18-20.

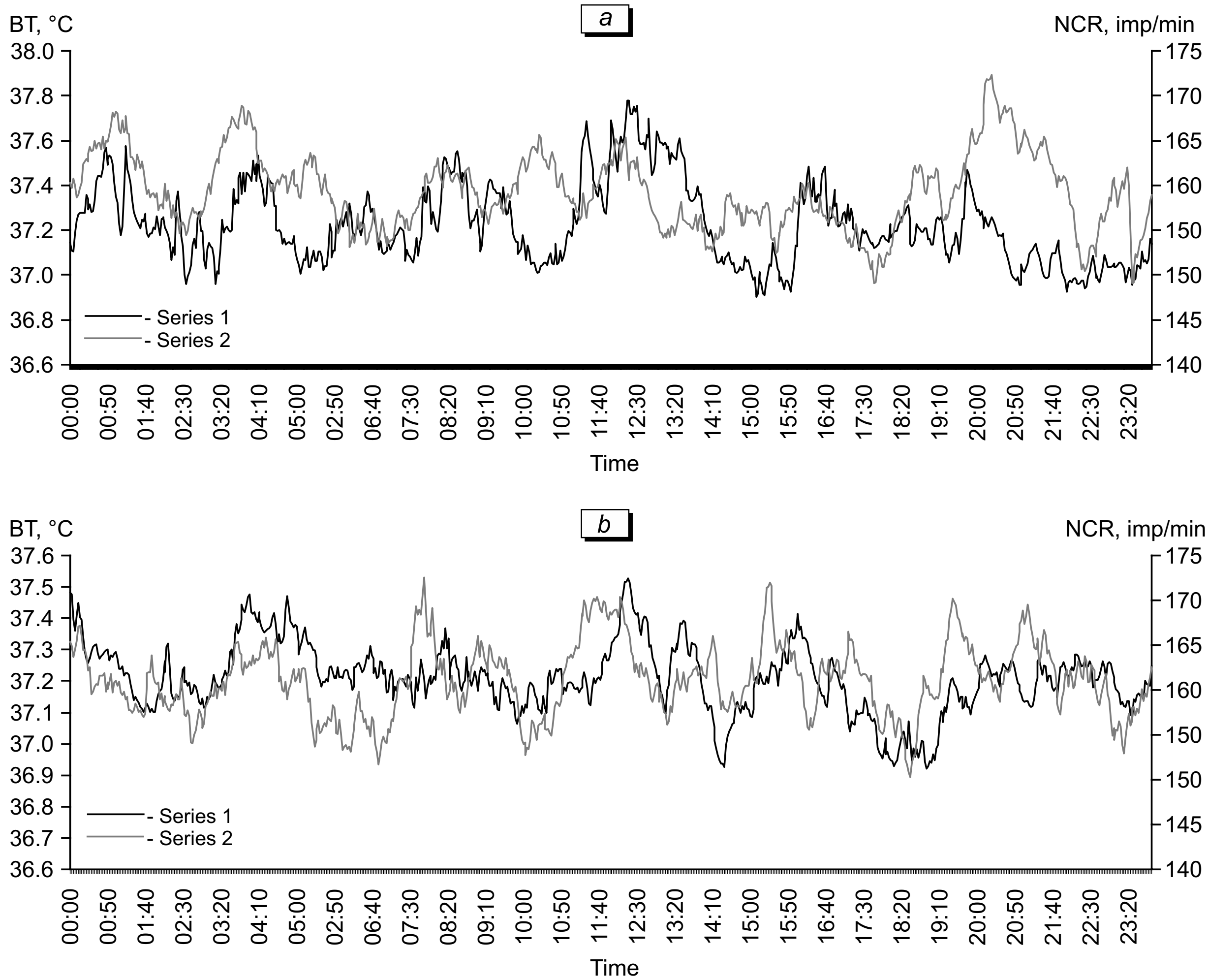

Fig. 3. Ultradian oscillations of BT (median value) of a group of rats (*n*=6) kept under constant illumination and NCR fluctuations assessed with 1-min-interval steps, which were summarized for the periods of April 22—May 14 (*a*) and May 23-June 8 (*b*) in 2019.

TABLE 1. Cross-Correlation between Rat BT Spectrum and Spectra of 1-min-Interval Steps of NCR Averaged over 1 h with a Sliding Window Obtained without Shift and with a Shift for the Next Time Interval

Time interval	Without temporal shift	With temporal shift
March, 21-23	*r*=0.33; *p*=0.009	*r*=0.15; *p*=0.272
March, 24-26	*r*=0.50; *p*<0.001	*r*=0.42; *p*<0.001
March, 27-29	*r*=0.46; *p*<0.001	*r*=0.24; *p*=0.063
March, 30 — April, 1	*r*=0.48; *p*<0.001	*r*=0.13; *p*=0.299
April, 2-4	*r*=0.29; *p*=0.027	*r*=0.17; *p*=0.191
April, 5-7	*r*=0.43; *p*<0.001	*r*=0.23; *p*=0.089
April, 8-10	*r*=0.27; *p*=0.035	*r*=0.46; *p*<0.001
April, 11-13	*r*=0.38; *p*=0.003	*r*=-0.10; *p*=0.421
April, 14-16	*r*=0.48; *p*<0.001	*r*=0.24; *p*=0.063
April, 17-19	*r*=0.22; *p*=0.09	*r*=0.13; *p*=0.299
April, 20-22	*r*=0.36; *p*=0.005	*r*=0.37; *p*=0.003
April, 23-25	*r*=0.37; *p*=0.003	*r*=-0.18; *p*=0.185

of this geophysical factor on BT, further experiments should be conducted with the changes of corresponding environmental parameters.

Thus, this study showed that rhythmic oscillations of BT in rats with the period ranging 100-400 min positively correlate with the amplitude of NCR variations assessed with step functions calculated for 1-min intervals.

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