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Possible External Factors Determining Ultradian (4-20-min) Rhythms of Body Temperature

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The study examined the effect of passive magnetic shielding on the magnitude of rhythmic oscillations of body temperature (BT) with 4-20 min periods in mice and their correlation with similar oscillations in unshielded control group. A magnetic permalloy screen that 35-fold attenuates the total geomagnetic field and decreased the undulations of magnetic field with the periods of few minutes by 5 times, produced no effect on the mean amplitude of BT oscillations within the same period range, their spectral power, and the cross-spectral density of examined rhythms in comparison with the control (unshielded) mice. Thus, either the mice possess a very sensitive magnetic sensory system or there exists an external non-magnetic factor affecting rhythmicity of BT. The study advanced intensity of thermal neutron radiation near the Earth's surface known to reflect the flow of accelerated particles generated by the secondary cosmic rays as the external factor, which strongly correlates with BT rhythms revealed by cross-spectrum analysis.

Key Words: *ultradian rhythms; body temperature; geomagnetic field; thermal neutron radiation*

The rhythmic changes are typical of most processes in the animate and inanimate nature. At this, the seasonal (circannual) and circadian biological rhythms are rather well examined. In contrast, the ultradian rhythms (those with the period of few minutes included) are mostly unclear, and their functional role is obscure [5-9].

Previously, we demonstrated that dynamics of the changes in body temperature (BT) with the periods of 10-60 min reflects the influence of some external environmental factor [4]. This phenomenon was indicated by synchronicity in the rhythmic changes of intraperitoneal temperature not only between the animals of the same species but also between the diverse species of mammals and birds, which were isolated from each other and maintained under different illumination regime [3]. Based on the facts that the spectral maxima of geomagnetic undulations (GMU) and BT oscillations are most probably observed at the frequencies of 1.6, 2.3, and 3.1 mHz, and the mean square of the distance between spectral maxima of simultaneous oscillations of BT and GMU is smaller than that for stochastic intervals, a hypothesis was advanced that namely GMU synchronize the examined biological rhythms.

To test this hypothesis, magnetic shielding of experimental animals is needed. There are important features of such shielding. It is a common knowledge that passive shielding with the use of permalloy compositions is an effective way to suppress the high-frequency GMU. Actually, in our experiments the

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permalloy screen attenuated GMU with the periods ranging 6-20 min by 5.5 times. However, to decrease GMU in Pc5 range, an active magnetic shielding is necessary, which employs the automatically controlled compensating solenoids to counterbalance the external magnetic field and its undulations. To implement this approach and construct a screen to counterbalance the field smaller than 0.1 nT, an extremely sensitive low-noise system is needed, which we could not find on the market.

Thus, the present study employed passive magnetic shielding to examine its effect on pronouncedness of BT oscillations with the periods in 4-20-min range and their coherency in control and screened mice.

MATERIALS AND METHODS

The experiments with magnetic shielding were carried out on male C57BL/6 mice (n=8) weighing 26-28 g obtained from Stolbovaya Animal Breeding Department (Scientific Center of Biomedical Technologies, Federal Medical-Biological Agency of Russia). They were housed 4 mice per cage. The experimental mice (n=4) were shielded magnetically during April 24-29. The control mice (n=4) were placed in a similar system made of cardboard. The analyzed period was April 27-29, which started after the mice had adapted to the novel environment. Both groups were isolated from each other by housing them at the distance of about 1 km.

Special experiments focused on relationship between BT and environmental factor(s) were carried out on another group of mice (n=10) during May 14-31, 2020. These mice were randomized into 2 equal groups, one of which was exposed to constant artificial illumination, while other group was maintained under natural light. This experimental paradigm was chosen to eliminate possible synchronizing effect of natural illumination on ultradian rhythms.

The data on geomagnetic activity were obtained from the nearest geophysical observatory located in Borok (INTERMAGNET, https://www.intermagnet.org/data-donnee/download-eng.php#view). The data on neutron radiation (NR) were obtained from Moscow neutron monitor (http://cr0.izmiran.ru/mosc), *i.e.* near location of the mice.

The body temperature (BT) was measured every minute employing DTN4-28/TL4-28 transducers (EMBI RESERCH) implanted intraperitoneally 20 days prior to experiments under intramuscular narcosis with zoletil (5-7 mg/kg, Virbac Sante Animale). BT medians in the groups were used to calculate the spectral parameters.

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 data were processed statistically using Statistica 7.0 software (StatSoft, Inc.). BT rhythms were revealed with fast Fourier transform. Similarity of the spectra was assessed with Spearmen's correlation coefficient. To assess consistency of BT rhythmic changes in control and experimental mice, the cross-spectral density and the magnitude squared coherence (MSC) were calculated. The data were summarized as Me (Q_1-Q_3). Significance of intergroup difference was estimated with nonparametric Mann—Whitney U test at p<0.05.

RESULTS

The mean value of BT changes observed within the periods of a few minutes in experimental magnetically shielded mice was 0.064 (0.060-0.069)°C, while in the control (unshielded) group it was 0.067 (0.061-0.68)°C, so this parameter did not significantly differ in both groups (p=0.79). Thus, the 35-fold decrease of total GMU intensity and a 5-fold drop of this intensity in the period range of 4-20 min did not affect BT changes in mice.

Figure 1 shows the spectra of BT in control and experimental mice calculated for the data obtained prior to magnetically shielding of the mice and in 3 days after this shielding. Before shielding, the correlation between BT spectral density in control and experimental mice was r=0.24 (p=0.002), while after shielding of experimental mice it became r=0.21 (p=0.008). Thus, in both cases the spectra of control and experimental groups correlated significantly. It should be stressed that despite the fact that BT rhythmicity was characterized with certain most frequently observed spectral set of frequencies, collation of spectra recorded in various days showed that significance of their correlation was small or altogether absent, which attests once more to existence of some external environmental factor determining the formation of these spectra.

The values of cross-spectral density, which reflected coherence of rhythmical changes in both groups of mice prior to magnetic shielding of experimental mice and during such shielding were similar r=0.20 (p=0.015, Fig. 2, *a*). MSC values for control and experimental mice (assessed prior to magnetic shielding and during it) did not differ in absolute value and indicated the positive correlation between BT in both groups (r=0.16, p=0.046, Fig. 2, *b*). Thus, the employed magnetic screen produced no effect on the coherence of rhythmic changes in BT between both groups in the period range of 4-20 min. It was logical to suggest that a decrease in GMU amplitude would be reflected by diminished amplitude of BT

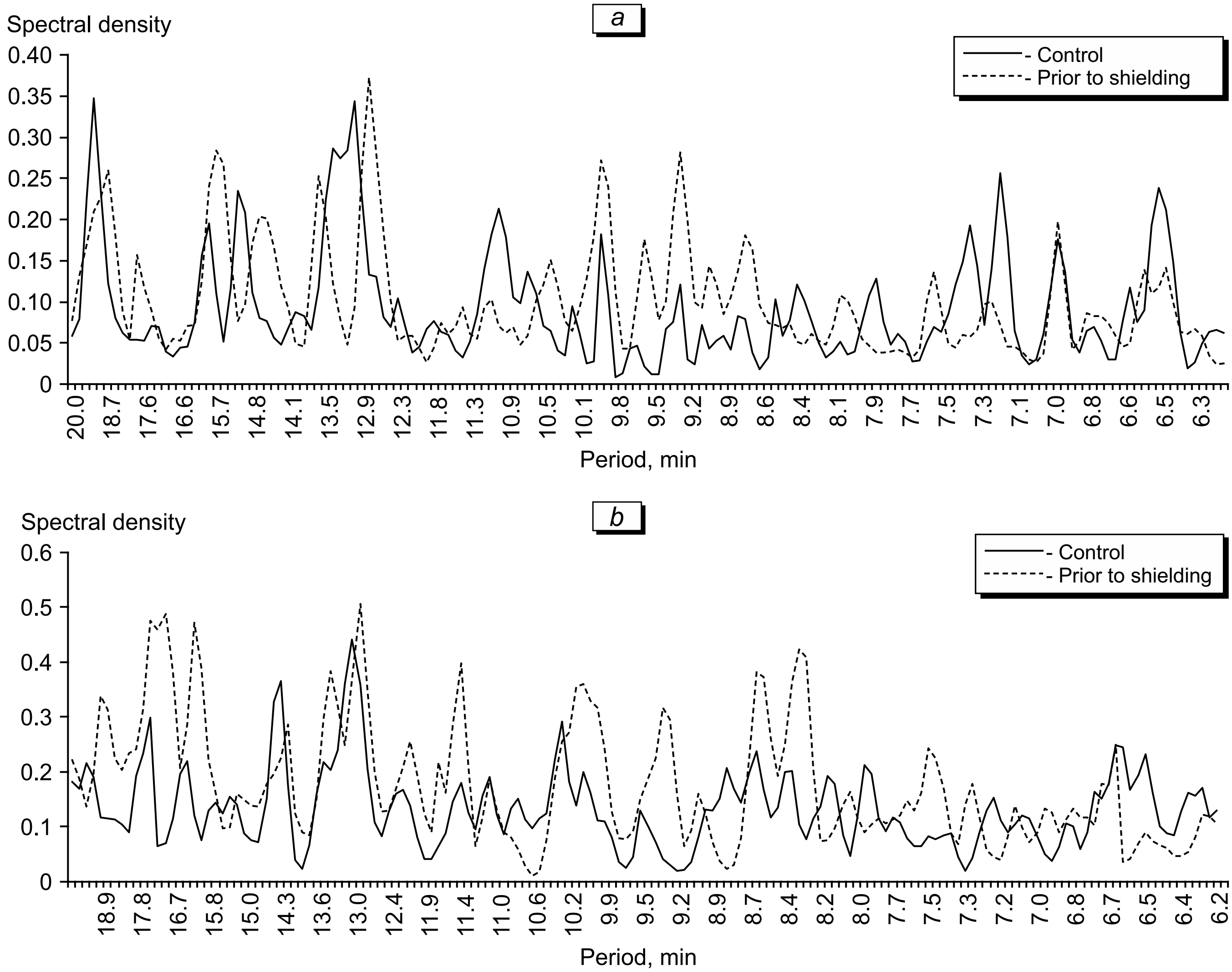

Fig. 1. Spectra of BT oscillations with the periods of few minuntes in two groups of mice prior to magnetic shielding (*a*) and after a 3-day-long stay in magnetically shielded case (*b*).

oscillations provided the latter were induced directly by this external (*i.e.*, magnetic) stimulus. At least, elimination (shielding) of this external factor would lead to mismatch between GMU and BT oscillations if the biological rhythms are synchronized by GMU. However, the experiments did not corroborate both hypotheses. Thus, either the animals can respond to low-intensity GMU (<1 nT), which could not be eliminated by the employed screen, and moreover, these animals were characterized with a nonlinear dependence of BT oscillations on GMU amplitude, or the factor determining the BT rhythms is not magnetic.

A reasonable alternative to GMU as a factor affecting BT can be intensity of the secondary cosmic rays at the Earth's surface reported by neutron monitoring. Some researchers hypothesized on possibility of biotropic action of background charged particles and neutrons at the Earth's surface [1,2]. Actually, the spectrum of their intensity is similar to that of BT oscillations [4]. To assess the degree of concordance between the rhythmic variations of examined parameters, we calculated the cross-spectral density of BT oscillations in a minute time scale with GMU and background variations of NR (Fig. 3, *a*). The correlation coefficient between the corresponding variations (r=0.45, p<0.0001) indicates a close association between both environmental factors and mouse BT. MSC between BT oscillations in both groups on the one hand, and that between BT oscillations and background NR, on the other hand (Fig. 3, *b*) were comparable, while dynamics of this parameter in both groups was similar (r=0.16, p=0.026). In contrats, association revealed by MSC between BT in both groups on the one hand, and between the spectra of BT oscillations and GMU, on the other hand, was not

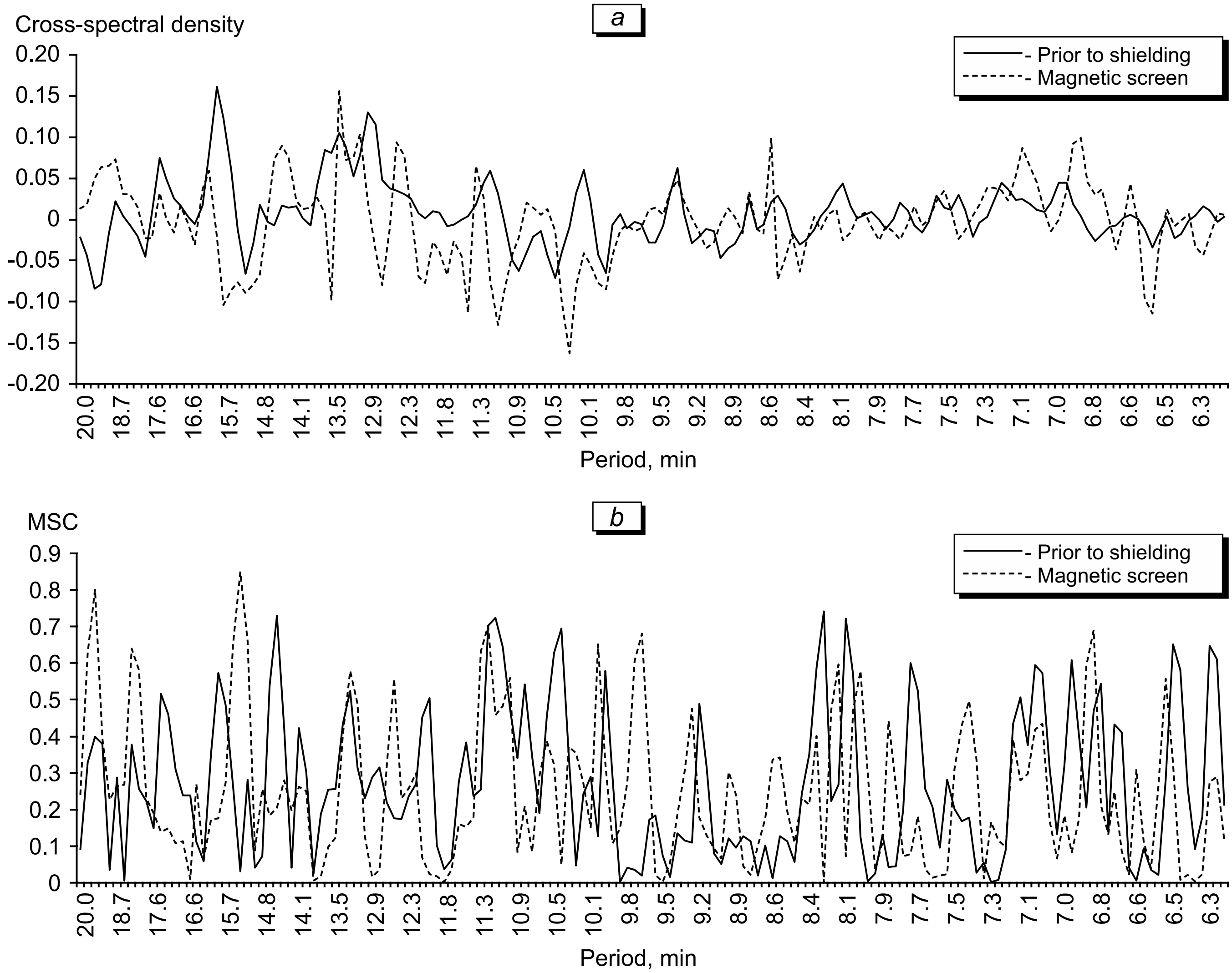

Fig. 2. Association of BT oscillations between control and experimental mice prior to magnetic shielding and during magnetic shielding of experimental group analyzed with cross-spectral density (*a*) and MSC (*b*).

so much pronounced (r=0.13, p=0.036), and it was observed only in the low-frequency range of 8-20 min (Fig. 3, *c*).

The present data attest to advisability of experiments with shielding of mice against the background radiation of secondary cosmic rays and thermal neutrons of various energy at the Earth's surface. It should be stressed that association between BT oscillations and GMU in a time scale of few minutes is pronounced, so hypothesis on relationship between BT and the geomagnetic pulsations in Pc5 frequency range cannot be rejected unless no experiments on active shielding of magnetic field eliminating GMU greater than 0.1 nT are conducted.

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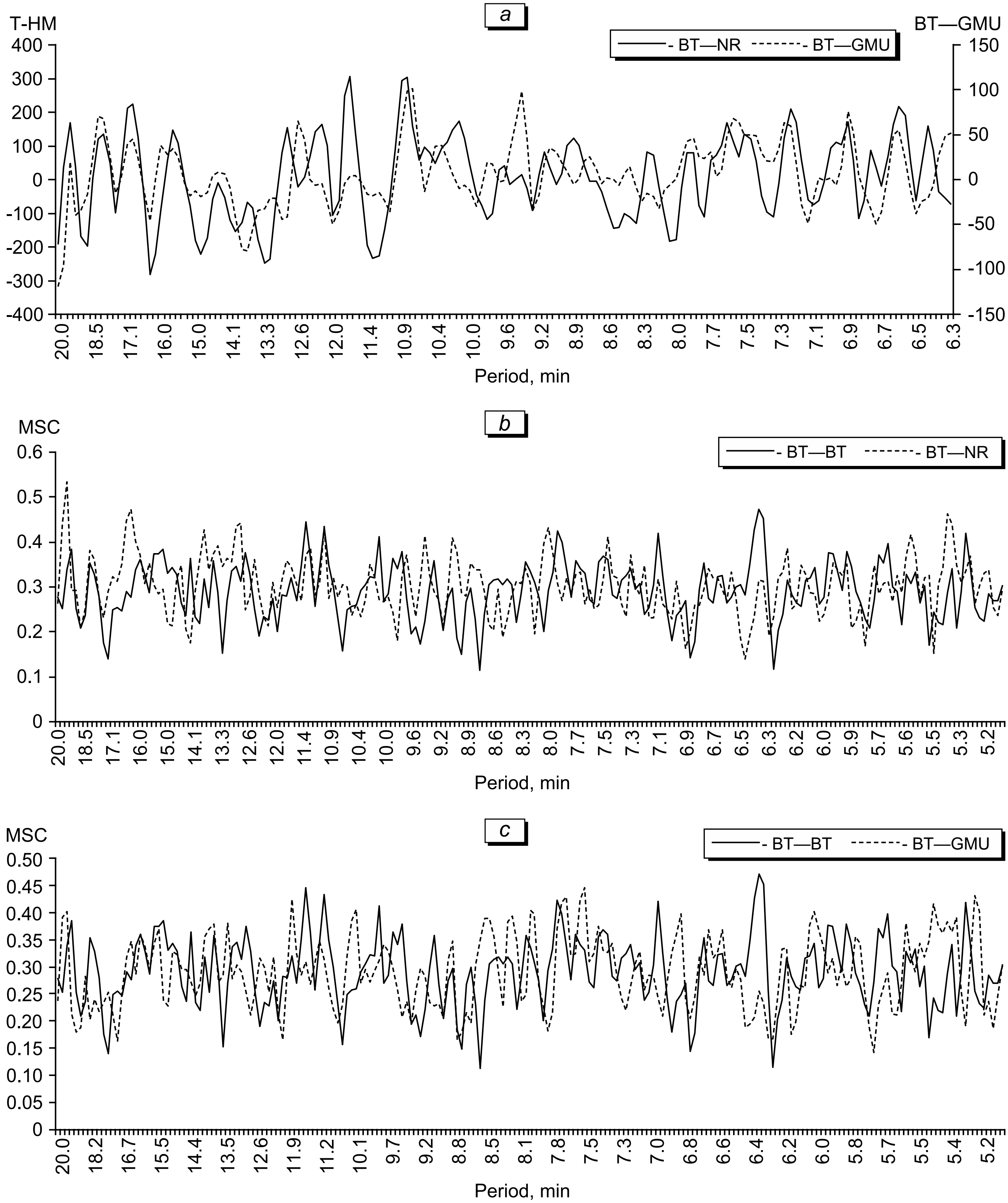

Fig. 3. Association between BT oscilaltions, GMU, and NR variations with the periods of few minuntes. *a*) Cross-spectral density of BT oscillations and NR variations and that of BT oscillations and GMU; *b*) MSC between BT oscilaltions in two isolated groups of mice (BT—BT), and between BT oscilaltions in both groups and NR variations (BT—NR) averaged over the period of May 14-31, 2020; *c*) MSC between BT oscilaltions in two isolated groups of mice (BT-BT), and between BT oscilaltions in both groups and GMU (BT—GMU) averaged over the period of May 14-31, 2020.

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