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COMPLEX SYSTEMS BIOPHYSICS

A Connection between Episodes of Normothermic Levels of Body Temperature in Animals during Hibernation and Secondary Cosmic Ray Fluctuation Intensity

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Abstract—Episodes of normothermic levels of body temperature over a short time during hibernation were compared in hedgehogs living in the Moscow region and ground squirrels inhabiting Yakutsk. The results of superposed epoch analysis showed that although the distance between the observation sites was about 5000 km and hedgehogs and ground squirrels lived in different places, there was a connection between episodes of normothermic levels of body temperature in the animals studied: the maximum number of short-term normothermia episodes in hedgehogs was observed on the same days that ground squirrels woke up. A 4-h biorhythm was established in the diurnal dynamics of wakefulness: the largest number of returns to normothermia was recorded both in hedgehogs and ground squirrels, according to local solar time within the time intervals 0–1 a.m, 4–5 a.m., 8–9 a.m., 12 a.m.–1 p.m., 4–5 p.m., and 8–9 p.m. It was shown that the episodes of normothermia were associated with the intensity fluctuations of secondary cosmic rays recorded by a neutron monitor. The noise intensity of silicon transistor and temperature sensors, which is small enough to be directly affected by extremely low variations in cosmic rays, correlated with the expressiveness of fluctuations in the neutron count. We believe that the magnitude of fluctuations in neutron monitor count rate is only a marker of a biotopic agent that has not yet been identified.

Keywords: hibernation, spontaneous arousal, external synchronizers, fluctuations in the neutron monitor count rate, mammals

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Quasi-rhythmic fluctuations of the Earth's magnetic field are thought to synchronize both infradian (period of 3–30 days) and ultradian (period shorter than 23 h) biological rhythms [1–6]. We have also found a connection between Pc5 geomagnetic pulsations and body temperature rhythms at the frequencies of 1.6, 2.3, and 3.1 mHz [7]. However, further experiments on passive shielding with permalloy, attenuating the general Earth's magnetic field by 35 times and by a minimum of 5 times in studied range, failed to reveal any changes in characteristics of body temperature rhythm within 4–20 min and the degree of synchronization between experimental and control groups of laboratory mice [8]. The dynamics of changes in the count rate of a neutron monitor (NM) was recommended as an alternative factor associated with body temperature oscillations in experimental animals, that is not inferior to the Earth's magnetic field in synchronization with the biological parameter studied [8]. It should be emphasized that the intensity of the atmospheric electric field is another potential biotopic factor associated with large-scale Pc5 pulsations of Earth's magnetic field that we have not yet analyzed.

We have previously compared the parameters of 100–400-minute body temperature oscillations in laboratory rats, reflecting the rest-activity rhythm in this period range, with the dynamics of secondary cosmic ray fluctuations at the Earth's surface assessed by NM count intensity [9]. The comparison of power spectra calculated with fast Fourier transform for synchronous and time-shifted body temperature oscillations and intensity of NM count rate fluctuations revealed a correlation only in synchronous measurements. Therefore, the hypothesis about accidental correspondence of this physical factor and biological parameter was invalid. The correspondence of the phases of the biological and physical parameters studied along with their spectral characteristics supports the assumption that increased intensity of neutron count fluctuations stimulates animal activity. Nevertheless, this fact does

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Abbreviations: NM, neutron monitor.

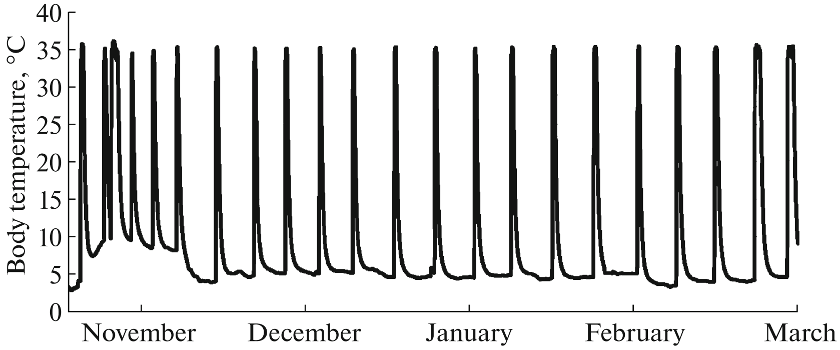

Fig.1. Example of body temperature dynamics in hedgehogs during hibernation in winter 2019–2020.

not prove direct action of NM fluctuation intensity on the organisms. NM fluctuation intensity can be considered only as a marker of a real biotopic quasi-rhythmic environmental factor.

In addition, a connection was found between the parameters of infradian biological rhythms and secondary cosmic ray fluctuation intensity [10]. Mean diurnal magnitude of 1-min body temperature oscillations of small mammals strongly positively correlated with NM fluctuations. Moreover, a positive correlation was found between the mean diurnal magnitude of 1-min fluctuations in NM count rate, the mean diurnal motor activity of animals, and the glucocorticoid level. Furthermore, the 4-day rhythm of the biological parameters analyzed and NM fluctuations predominates in November and December, whereas July is characterized by a 3-day rhythm. The synchrony of period fluctuations or disturbances in the dynamics of biological and physical parameters also indicates their connection.

Referring to the abovementioned studies, there is a connection between the dynamics of several biological processes associated with sympathetic activation and the intensity of NM count rate fluctuations in the period range from 4 min to 4 days.

A change in the balance between the sympathetic and parasympathetic tones is involved in the mechanism of waking from hibernation [11, 12]. Entry to hibernation is accompanied by the prevalence of parasympathetic tone, whereas the sympathetic system predominates in arousal. During winter sleep obligatory hibernators periodically wake up for short time periods at near-zero body temperature (Fig. 1). The state of hibernation is characterized by the depression of all vital functions, including brain cortical activity, metabolism, and hormone synthesis [13, 14]. The suppression of these functions during hibernation allows investigation of the up-regulation of the nervous system with environmental factors with little interference. The period between two sequential short-term arousals is inconsistent and species-specific, depends on metabolic intensity, external temperature, fat depots, the stage of the hibernation period, individual features, and other factors. [15]. A hibernating animal can be considered as a detector for external activating influences, though its sensitivity to them is inconsistent and depends on the time interval from the last arousal in addition to the aforementioned parameters. In other words, a hibernating hedgehog or ground squirrel is a detector with continually changing sensitivity. Nevertheless, a group of animals in a state of hibernation can be considered as a detector of environmental factors with averaged and relatively permanent sensitivity.

The aim of this study was to determine a possible connection between short-term episodes of wakefulness of hibernating animals and the intensity of secondary cosmic ray fluctuations.

MATERIALS AND METHODS

The white-breasted hedgehog *Erinaceus roumanicus*, Arctic ground squirrel *Spermophilus parryi*i, and long-tailed ground squirrel *Spermophilus undulatus* were used as subjects. The data on body temperature dynamics during hibernation were obtained from 32 hedgehogs (26 males and 6 females), from the winter of 2017–2018 (n = 7), 2018–2019 (n = 7), 2019–2020 (n = 14), and 2020–2021 (n = 4). The temperature curves for 25 ground squirrels were measured over the period from 2005 to 2020.

White-breasted hedgehogs were caught in Spassky district of Ryazan region during summer. In September the body weight of animals varied from 880 to 1520 g. Hedgehogs were fed with chicken mince mixed with raw eggs. Animals hibernated in conditions maximally imitating natural conditions. Artificial burrows were located in outdoor pens divided into sections. Burrows were constructed from holes 40 cm deep in the ground covered with a plastic bucket 90 cm long. Inside, the burrows were tightly packed with hay, the earth was loosened at the sides and covered with fir branches and later with snow.

Long-tailed ground hedgehogs were caught around Yakutsk in a 100 km radius. Arctic ground squirrels were caught in Batagay (Verkhoyansk Range) in the

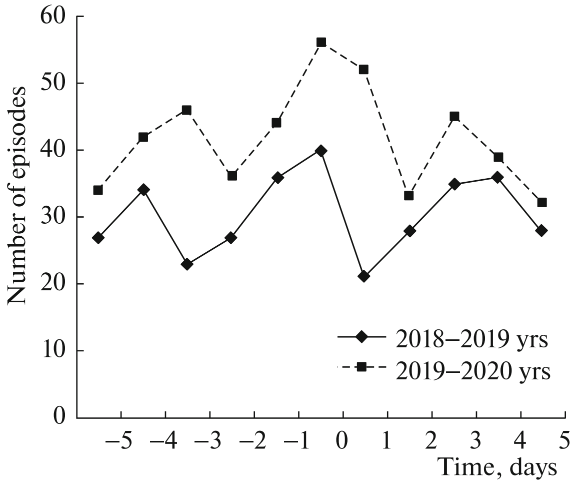

Fig. 2. Distribution of the number of normothermia episodes in hedgehogs (Moscow region) in reference to the dates of ground squirrel arousals (Yakutsk) during winter hibernation in 2018–2019 and 2019–2020 according to superposed epoch analysis.

vicinity of Yuttyakh cattle ranching. Before the beginning of the hibernation season ground squirrels were kept in individual terrarium cages in the vivarium of Institute for Biological Problems of Cryolithozone, Siberian Branch, Russian Academy of Sciences (SB RAS), at the territory of Yakutsk Botanical Garden. The ground squirrels were fed *ad libitu*m with grain, compound feed, sunflower seeds, and vegetables. In the middle of September animals were translocated to the basement of the vivarium with shortened daylight hours and temperature approximating the ground temperature in the corresponding season at the depth of wintering burrows for ground squirrels. From the end of December the temperature in the hibernation basement dropped to below-zero level and remained at minus 3–5°C throughout the hibernation season. In winter 2018–2019 the ground squirrels hibernated in the permafrost tunnel of Melnikov Permafrost Institute, SB RAS, at the depth of 16 m.

The hedgehog body temperature was measured intraperitoneally using DTN3–28 sensors (EMBI Research, Novosibirsk) with the test frequency of once in 20 min. Surgical intraperitoneal implantation of sensors was performed one month before the beginning of hibernation. Zoletil (Virbac Sante Animale, France) was injected intramuscularly at a dose of 15 mg/kg of body weight for anesthesia. Environmental temperature was controlled with the same sensor placed in an artificial empty hole.

Body temperature of long-tailed and Arctic ground squirrels was tracked using thermochrons, DS–1922 L temperature loggers surgically implanted in the peritoneum [15]. For experiment unification only young males of both species were enrolled.

To assess the magnitude of intensity fluctuations of cosmic rays, the data of neutron monitoring station in Pushkov Institute of Terrestrial Magnetism, Ionosphere and Radiowave Propagation (IZMIRAN, Troitsk, Moscow region, http://cr0.izmiran.ru/mosc) located 90 km away from the hedgehog hibernation site (Chernogolovka base) was used. The day-averaged index of 1-min change module of neutron count rate was accepted as the magnitude of secondary cosmic ray fluctuations, corrected for atmospheric pressure. A similar value was received by calculating the ratio of 1-min changes in NM count rate to total flow uncorrected for atmospheric pressure.

The dynamics of sampling noise was assessed using a P701A silicon transistor with a high level of flicker noise. The noise signal after amplification with the operational amplifier was transferred to full wave rectifier and bandpass filter of 0.1–1 Hz. Rectified voltage proportional to the noise amplitude in mentioned frequency bandwidth controlled the duration of interpulse intervals entering the computer recorder. Amplitude-frequency transduction for impulses registered by computer was performed using a varactor. The device was stored in a thermostat box at the temperature of 31°C. The data were kindly provided by A.G. Parkhomov.

The results were statistically processed with the help of Statistica 7.0 software (StatSoft In., United States). Spearman's correlation coefficient (r) was calculated in order to identify the similarities in the dynamics of the biological and physical parameters studied. Moreover, superposed epoch analysis was applied to detect the connection between inharmonic processes, when the values of the parameters of one process superimposed with relatively definite moments of another process. Power spectra of fluctuations were calculated with fast Fourier transform. Nonparametric Z-test for two proportions (SigmaStat) was applied to determine the statistical significance of differences between the number of normothermia cases in animals on a certain day and the mean level or between the adjacent values. The results were considered statistical at $p < 0.05$.

RESULTS

In winter 2018–2019 we conducted a parallel study of body temperature dynamics in hedgehogs of Moscow region and ground squirrels of Yakutsk hibernating 5000 km from each other. In order to assess the congruency of wakefulness episodes between hedgehogs and ground squirrels using superposed epoch analysis, the number of normothermia episodes was distributed in reference to the dates of ground squirrel arousals (Fig. 2). In winter 2018–2019 the dates of ground squirrel arousals coincided with the maximal number of hedgehog arousals (40 cases), whereas only 27–28 cases occurred 2 days before and after the moments of observation. Similar distribution was seen

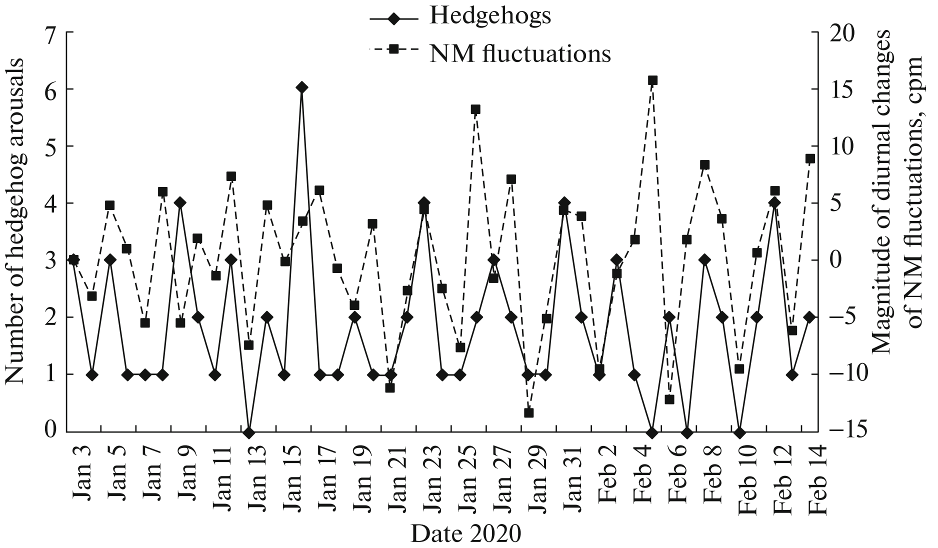

Fig. 3. Total number of episodes of hedgehog arousals in the group studied ($n = 14$) in accordance with the diurnal changes in mean diurnal intensity of NM fluctuations for the period from January 3 to February 14, 2020.

during the hibernation in 2019–2020: 56 cases of hedgehog arousals coincided with ground squirrel arousals and 33–36 episodes occurred 2 days before and after that moment. For two seasons in total the rate of correspondence between hedgehog and ground squirrel arousals statistically exceeded the rate of other events ($p < 0.001$ two proportion test). Three to 4 days before and after the peak were also associated with high number of arousals. This distribution was probably related to the phase correspondence of the near 4-day rhythm in hedgehogs and ground squirrels.

Figure 3 illustrates an example of wakefulness dynamics in hedgehogs according to the changes in NM fluctuation intensity. Our focus on the changes in physical factor rather than its absolute values was determined by the higher likelihood of reaction of the organism to that particular change in the environmental parameters. The distribution built with superposed epoch analysis of arousal episodes in hedgehogs is represented in Fig. 4 in reference to relatively abrupt diurnal changes in NM fluctuation intensities above 4 cpm. The vast majority of short-term episodes of wakefulness (109 cases) were detected on the day of sharp increase in NM fluctuation intensity, while only 68 cases occurred one to 2 days before and after such episodes. The comparison of the distribution using the test for two proportions showed statistical differences ($p < 0.001$). Therefore, there was a strong correlation between the increase in NM fluctuation intensity and hedgehog arousal. Moreover, the non-randomness of this process was confirmed by the similarity of distribution curves of hedgehog arousals for the periods between 2017–2019 and 2019–2021 ($r = 0.58$; $p = 0.057$). It is necessary to mention that the elevated number of awake animals was demonstrated using the epoch superposition method both for the distribution of hedgehog arousals compared to ground squirrels and to sharp increases in NM fluctuations three to 4 days before and after the moment of observation.

The distribution by day hours for the beginning of return to normothermia showed that in the first half of the day hedgehogs had a 4-h rhythm, whereas more prominent 4-h fluctuations in ground squirrels were seen in the second half of the day (Fig. 5). The distribution of moments when the body temperature of hedgehogs and ground squirrels started returning to normal in the 4-h rhythm revealed a total of 372 such

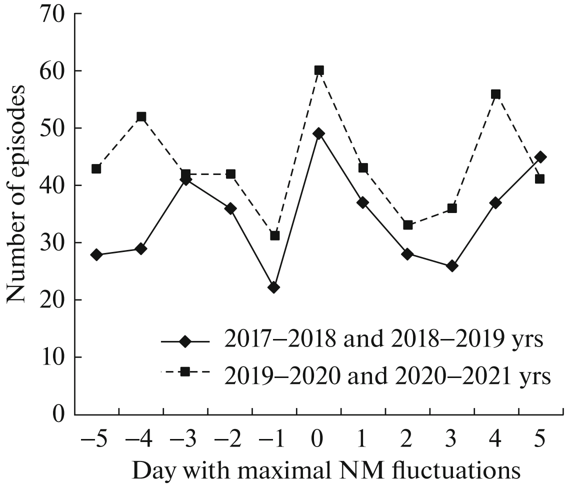

Fig. 4. The distribution of the number of normothermia episodes in hedgehogs in reference to sharp increases in diurnal values of NM fluctuation intensity above 4 cpm according to superposed epoch analysis.

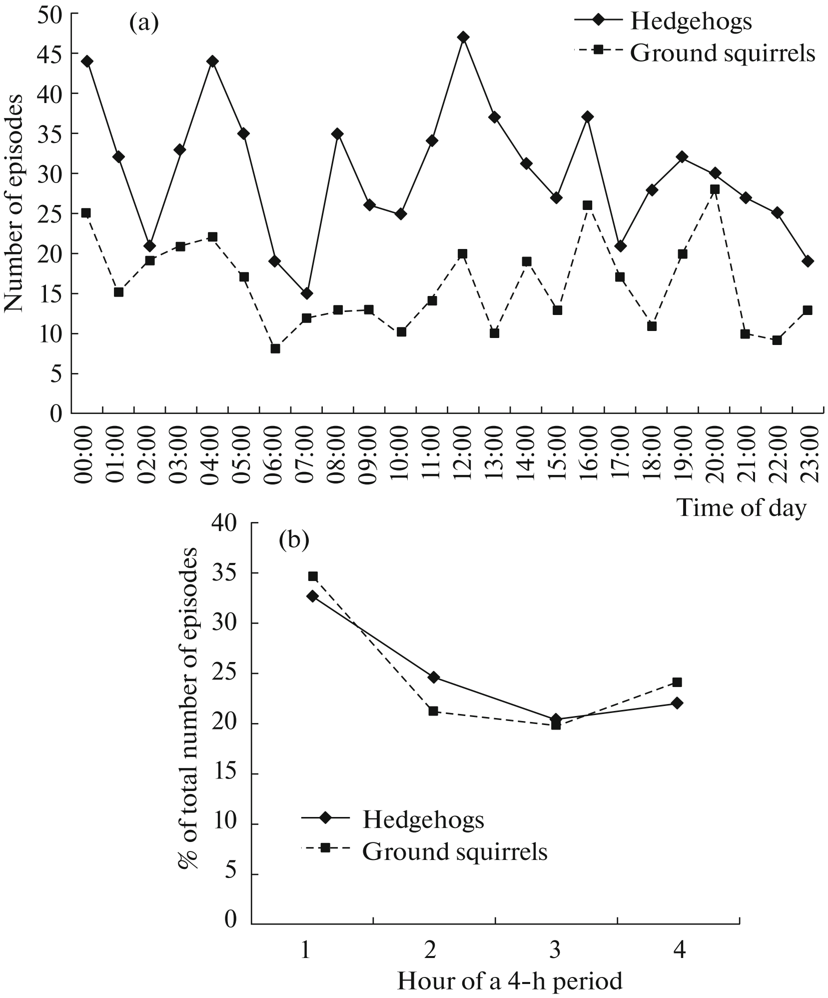

Fig. 5. Distribution of the number of normothermia episodes in hedgehogs and ground squirrel arousals by time of the day (a) and hours of a 4-h period (b). The intervals 0–1 a.m, 4–5 a.m., 8–9 a.m., 12 a.m.–1 p.m., 4–5 p.m., and 8–9 p.m. refer to the first hour; 1–2 a.m, 5–6 a.m., 9–10 a.m., 1–2 p.m., 5–6 p.m., and 9–10 p.m. refer to the second hour; 2–3 a.m, 6–7 a.m., 10–11 a.m., 2–3 p.m., 6–7 p.m., and 10–11 p.m. refer to the third hour; 3–4 a.m, 7–8 a.m., 11–12 a.m., 3–4 p.m., 7–8 p.m., and 11–12 p.m. refer to the fourth hour. The data are presented as percent of total arousal number.

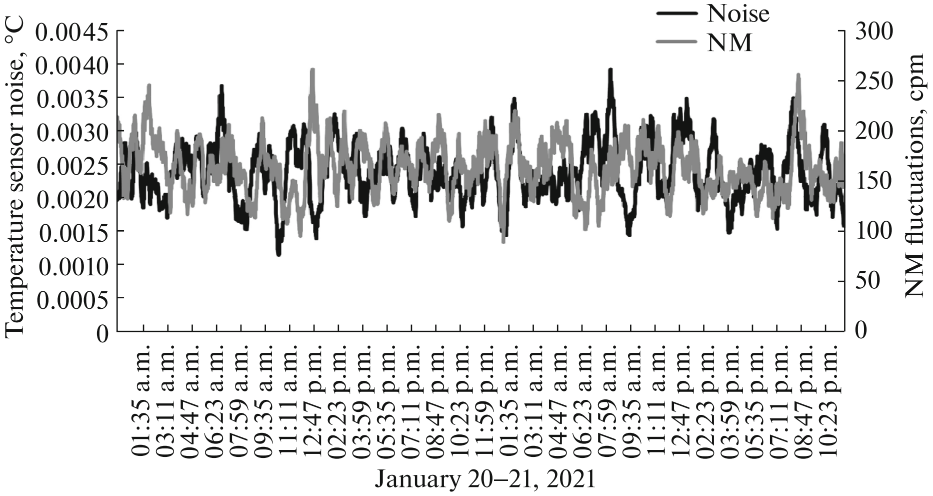

Fig. 6. The intensity dynamics of a module of 1-min changes in temperature sensor recordings averaged for nine sensors and the same parameter of NM averaged within a floating window of 30 min.

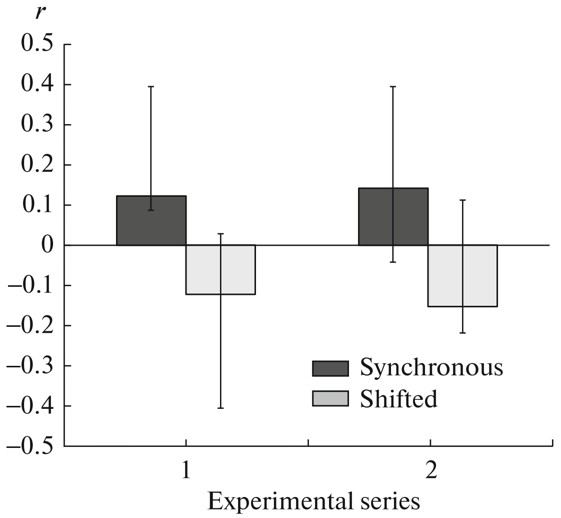

Fig. 7. Cross-correlation coefficient (*r*) of power spectra for temperature sensor noise intensities averaged for nine sensors and NM fluctuations within the period of 99–360 min. Data are presented as median and interquartile range of correlation coefficients calculated for 2-day intervals, when analyzing synchronous data and data shifted for 2 days. Series 1 from January 14 to January 25, 2021 (6 analyzed intervals); series 2 from February 25 to March 8, 2021 (6 analyzed intervals).

events at 12 a.m., 4 a.m., 8 a.m., 12 p.m., 4 p.m., and 8 p.m. by local solar time, and 249 episodes of normothermia were detected in the rest of the hours of a 4-h period (Fig. 5b). The differences between maximal and mean values were statistically significant (Z-test for two proportions: $p = 0.006$ and $p = 0.008$ for hedgehogs and ground squirrels, respectively). This implies that the dynamics of return to normothermia has a near 4-h rhythm both in ground squirrels and hedgehogs, which is dependent on local solar time.

DISCUSSION

We have demonstrated a direct connection between short-term episodes of wakefulness of ground squirrels in Yakutsk and hedgehogs in Moscow region. Considering that the distance between the experimental locations was around 5000 km, the synchronous arousal of animals is unlikely related to weather conditions such as fluctuations of atmospheric pressure or other local factors, but rather with global factors. Taking into account the different families of the animals studied and different adaptation strategies, including a different mean number of arousals, the detected phenomenon is not likely to be the result of chance correspondence of wakefulness rhythms.

Diurnal distribution of wakefulness episodes was characterized by the near 4-h rhythm, depending on local rather than global time. It is necessary to emphasize that the genes of the circadian system do not function during hibernation [16–18]. Therefore, this rhythm is not a circadian derivative. Furthermore, other authors found that ultradian activity rhythms of animals were relatively independent from the circadian system [19, 20]. Therefore, diurnal distribution of wakefulness rate should be considered to result from an external activating/irritant agent. It is to be noted that the sympathoadrenal system activates at these particular hours by local time in humans [21, 22].

Correspondence of the majority of arousal episodes to an abrupt increase in NM fluctuation intensity indicates its connection with an external factor causing the activation of the sympathetic nervous system and/or suppression of parasympathetic influences. However, in our opinion this phenomenon is unlikely associated with the direct influence of secondary cosmic rays on animals, even assuming active interaction with organism. The total flow of secondary cosmic rays approximates 10 000 events per m^2 per min and changes by 150 cpm on average. When calculated per body surface area of animals, the fluctuation intensity does not exceed 1.5 events within the total flow of 100 events per min.

The validity check of methods used in this study [7] for registering minor fluctuations of animal body temperature was performed with temperature sensors placed in a thermostat at the temperature of 37°C and a switched off refrigerator at room temperature. Noise-like fluctuations by 1–3 resolution units of the sensor were found in 1-min parameter changes of both cases, especially when the battery power of the sensor was reduced. These fluctuations were about ten times lower compared to the animal values, but the causative reason for them is intriguing. Figure 6 represents a fragment of intensity dynamics of 1-min temperature oscillations averaged for nine sensors and NM fluctuations averaged within a floating window of 30 min. Cross-correlation coefficients were calculated between the power spectra of detected temperature oscillations and NM fluctuation intensities for each of the 2-day experimental sections in the period range of 99–360 min. The results of these calculations for synchronous and 2-day-shifted time series are presented on Fig. 7. The correlation coefficients of spectra calculated for synchronous and 2-day-shifted data differed statistically ($p = 0.013$). This fact suggests a frequency dependence between the noise of the temperature sensors and real-time NM fluctuations, rather than chance correspondence of identical harmonics typical for both processes. Even if the whole sensor with an area of less than 0.5 cm^2 had detecting properties, a 1-min difference between the number of secondary cosmic particle hits would be 0.008 events. This means that the intensity of NM fluctuations is only a marker of an active agent similarly to the living objects.

Table 1. The connection between normothermic episodes. Calculated correlation coefficients between the changes in mean diurnal values of noise intensity of the silicon transistor and NM fluctuations

Study period	Correlation coefficient r	Statistical significance
November–December 2002	0.30	**0.023**
January–February 2003	0.09	0.58
March–April 2003	0.28	**0.035**
May–June 2003	0.14	0.36
July–August 2003	0.10	0.43
September–October 2003	0.31	**0.017**
November–December 2003	0.28	**0.030**
November 2002 – December 2003	0.22	**0.00005**

The temperature sensor is a sophisticated device consisting of a thermopile, an antenna for information transmission, a battery, an analog-to-digital converter, and other electronic elements. Therefore, we compared the noise intensity of a silicon transistor placed in a thermostat at 31°C with NM fluctuation intensity. The changes in mean diurnal values of noise intensity were analyzed for the transistor and NM fluctuations. Table 1 contains the computed data of correlation coefficient for two-month periods. In total for 14 months the correlation coefficient between diurnal changes in transistor noise and NM fluctuation intensity was 0.22, with significance of p = 0.00005. Interestingly, diurnal parameter changes had the strongest cross-correlation, unlike their absolute diurnal values, similarly to the biological parameters.

Ionizing radiation is the key unaccounted environmental parameter that affects the noise intensity in semiconductors. Two independent research groups discovered a near 4-day rhythm of radon degassing intensity [23, 24]. The fluctuation dynamics of natural radioactivity level should be experimentally proven and its intensity needs to be estimated to accept or reject a hypothesis on such a relationship. However, it is very unlikely that such small doses of radiation from surface radon could cause the observed effects both in the present study and our earlier works [7–10], where laboratory animals were kept on the ninth floor with lower radon concentration. The periods of rhythmic alterations in animal activity detectable in the fluctuations of various processes should always be considered, given the indirect correlation between the biological and physical processes. This refers to the Earth's natural oscillations, its magnetic pulsations, fluctuations of atmospheric electric intensity, microfluctuations of atmospheric pressure, fluctuations of thermal neutron flow, etc. [25].

In the absence of sufficient expertise, we did not review in detail the potential mechanisms of connection between NM fluctuation intensity, noise level of the devices, and the animal activity. Nevertheless, in our opinion, there are two general possibilities: (1) the fluctuations of secondary cosmic ray flow generate fluctuations of another biotopic environmental factor, potentially involving the ionization of the upper atmosphere; (2) provided that the NM device eliminates internal noise of such degree, there is an additional agent that determines both the intensity of the NM count rate fluctuations and noise in the sensors and affects animal activity itself.

The rhythmicity of biological parameter occurrence based on the local time adds to the search of a yet undetermined biotopic factor. We have previously demonstrated that the phases of activity rhythms emerged synchronously by local and not global time in two groups of mice isolated from the external light references of a day/night cycle using permanent illumination and distanced longitudinally (Moscow–Ulyanovsk direction) [26].

Further search for the reason causing such NM fluctuations or the agent provoked by NM fluctuations will allow external environmental factor that majorly influences the nervous system of animals to be defined, facilitating the next step towards the elaboration of non-pharmacological methods for managing various neurological diseases, including sleep disorders.

CONCLUSIONS

(1) Despite the substantial distance of 5000 km between the observation sites for assessing the body temperature dynamics in ground squirrels and hedgehogs, as well as their different families, the following connection was identified: the maximum number of episodes of short-term normothermia in hedgehogs was observed on the same days when ground squirrels woke up. This fact suggests a global external factor that provokes shifts in the balance of the autonomic nervous system towards the predomination of the sympathetic tone, which results in animal arousal.

(2) A connection was found between the episodes of short-term normothermia in hibernating animals and the increasing intensity of NM count rate fluctuations. In our opinion, the extremely low magnitude of secondary cosmic ray fluctuations does not allow this

factor to be considered a biotopic one. Probably, the intensity of NM fluctuations is only a marker for a biotopic environmental factor affecting the autonomic balance that is yet to be discovered.

(3) The positive correlation between the intensity of silicon transistor noise and NM fluctuations and the dependence of the near 4-hour diurnal rhythm of arousals according to local time provides a phenomenologically precise characteristic of the sought biotopic factor, which promotes further investigation.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interests.

Statement on the welfare of animals. All experiments were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasburg, 1986). Experiments were approved by the Ethics Committee of Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, no. 14, January 14, 2018.

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