DOI 10.1007/s10517-023-05816-z

ECOLOGY

Analysis of Association of Ultradian Body Temperature Rhythms in Animals with Intensity of Fluctuations of Radioactive Decay of Natural ^{40}K Isotope

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 175, No. 1, pp. 100-106, January, 2023
Original article submitted October 11, 2022

The study monitored the long-term body temperature (BT) oscillations of C57BL/6 mice and outbred starlings (*Sturnus vulgaris*) to compare them with fluctuation in decay rate of radioactive natural ^{40}K isotope. The spectrum analysis revealed simultaneous changes of the predominant periods in BT spectra of the animals and those in fluctuation in ^{40}K decay rate. A positive correlation was established between BT dynamics and fluctuation in decay rate. The superposed epoch analysis revealed predominant coincidence of the moments of BT and fluctuation in ^{40}K decay rate. The novel data indicate association between BT ultradian rhythms with quasirhythmic variations of fluctuation in ^{40}K decay rate.

Key Words: *ultradian rhythms; body temperature; macrofluctuations; synchronizers; radioactive decay*

The ultradian rhythms of animal body temperature (BT) and the rest—activity circle are well known, and the intrinsic mechanisms of their formation are vigorously studied [1-3]. However, the physical factors, which synchronize the ultradian rhythms in mammals and birds are not reliably established. Some authors relate the ultradian rhythms in the tone of autonomic nervous system with heliogeophysical factors [4,5]. Previously we showed that the ultradian near 2-4-h BT rhythms in laboratory rodents depend on some external environmental heliogeophysical factor related to intensity of fluctuations in neutron count rate [6].

It is noteworthy that the coincidence of harmonics in BT power spectra of the animals, which are characterized by different intensity of metabolism, with those of natural oscillations of the Earth and the fluctuations of radioactive decay [7,8]. Some papers focus on the statistically significant oscillations in intensity of natural alpha-, beta-, and gamma-radiation [8-12]. At this, there are short-term deviations in the decay rate of radioactive isotopes, which nevertheless did not violate the law of their stable average levels. These fluctuations in radioactive decay can reflect the effect of some environmental factor on stability of the atoms [9-12]. It is also likely that the above rhythms can be detected in available radioactive isotope ^{40}K known to be inherent in the living organisms. Our aim was to compare the decay fluctuation intensity (FDR) of natural ^{40}K isotope with BT oscillations in cloned mice C57BL/6 characterized by polyphasic rhythms and with monophasic BT oscillations in common starlings.

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MATERIALS AND METHODS

The experiments were carried out on mature male C57BL/6 mice weighing 26-30 g (n=18) obtained from

Animal Breeding Department of A. N. Severtsov Institute of Ecology and Evolution. We also employed data on BT of common mature starlings (*Sturnus vulgaris*) caught in 2019-2020. The experiments were carried out in compliance to Directive 2010/63/EU of the European Parliament and of the Council (September 22, 2010; On the Protection of Animals Used for Scientific Purposes). The experimental protocols were approved by Bioethics Committee of the A. N. Severtsov Institute of Ecology and Evolution (Protocol No. 14, January 15, 2018).

When different animals are maintained in the same room, they synchronize their activities, so in our study, we placed the animals in three rooms with the distance of 1-2 km between them. Each room housed 6 mice with water and food *ad libitum*. They were kept 2 per plastic cage (40×14.5×24 cm) under moderate illumination with intensity of 200-300 lx supplied by a source with color temperature of 4200 K. This constant illumination was used for the following reasons. First, under these conditions, the circadian rhythms become "free-running" with the period of about 25 h, which eliminates possibility of accidental entrainment of biological and physical processes by geophysical 24-h periodicity. Second, the constant illumination does not affect the periods, but increases the amplitudes of the ultradian rhythms thereby enhancing significance of detection of these rhythms. The stock of food and water was sufficient to maintain normal supply for 20 days.

The starlings were kept under natural illumination in individual cages (60×40×35 cm) covered with a light cotton fabric. Every morning between 08.00 and 09.00, the food and water had been changed. The birds were fed with larvae of mealworm *Tenebrio molitor*.

BT was measured and recorded at the sampling rate of 1 min^{-1}. To this end, no later than 20 days prior to the onset of experiments, DTN4-28/TL4-28 sensor (EMBI RESERCH) was implanted intraperitoneally under intramuscular narcosis with Zoletil (5-7 mg/kg; Virbac Sante Animale). The data were stored in sensor's memory for 20 days; thereupon they were sampled by a non-contact way.

A natural radioactive isotope ^{40}K was the component of potassium sulfate (Buisky Chemical Plant). The decay rate of this isotope was measured with an Atom Fast 77100 scintillation detector (KB Radar) based on a CsI crystal (7×7×100 mm). To isolate the system from atmospheric gamma-quanta, the potassium sulfate packages and detector were placed in a plumbic box (15×15×15 cm) with the wall thickness of 12 mm. This screen attenuated the background scintillation rate by more than 10-fold. The sides of the detector placed in the center of the box were homogenously covered with potassium sulfate (4 kg). On the average, this set-up recorded about 2000 events/min.

The results were statistically processed with Statistica 7.0 (StatSoft, Inc.) software. Association between BT oscillations and ^{40}K decay was revealed with Pearson's correlation coefficients (r), superposed epoch analysis, and cross-correlation of Fourier power spectra. The intergroup difference was analyzed with Student's *t* test for normally distributed data; otherwise, Mann–Whitney *U* test was employed. Significance was assessed at $p<0.05$.

RESULTS

The spectral power of BT 1-min steps in animals and the rate of ^{40}K decay were calculated in daily intervals to analyze the association between biological and physical processes (Fig. 1). In these spectra, similar harmonics prevails, although in other day their periods could change but retain the coincidence of predominant periodicities in both processes. To confirm this regularity, we calculated the correlation coefficients between the spectra of BT and radioactive decay. For the periods ranging 20-400 min calculated without shift in the time scale, the prevailing number of observations was characterized by positive correlation r=0.13 (0.05; 0.23), while the relative shift of the records by 1-2 days decreased correlation to r=0.04 (-0.06; 0.08). There was a significant difference between the correlation coefficients calculated for simultaneous records of BT steps and the rate of ^{40}K decay, on the one hand, and between similar records calculated with a time shift, on the other hand (p=0.003). Thus, the study revealed a significant association between BT of the animals and the rate of radioactive decay. This association was not an accidental coincidence of the periods, which are characteristic of biological and physical processes, because in various time intervals, the different periodicities prevailed, although similar harmonics were observed in both processes.

To eliminate the circadian trend in BT dynamics, the 1-min-steps of this parameter were calculated. Assuming that the biotropic actions were produced by the changes in some environmental factor instead of its absolute value, we performed similar calculations for the rate of ^{40}K radioactive decay and obtained the plot of the modulus of 1-min steps of the decay rate (^{40}K FDR). Calculation of correlation between BT 1-min steps and ^{40}K FDR was performed after smoothing the curves by averaging with a 13-min sliding window. In various days, the correlation coefficients between ^{40}K FDR and mouse BT 1-min steps varied from 0.03 to 0.23, the corresponding values for the starlings being 0.07 and 0.39. These coefficients were always significant.

The correlation coefficients were larger for starlings than for mice. Probably, this difference can be

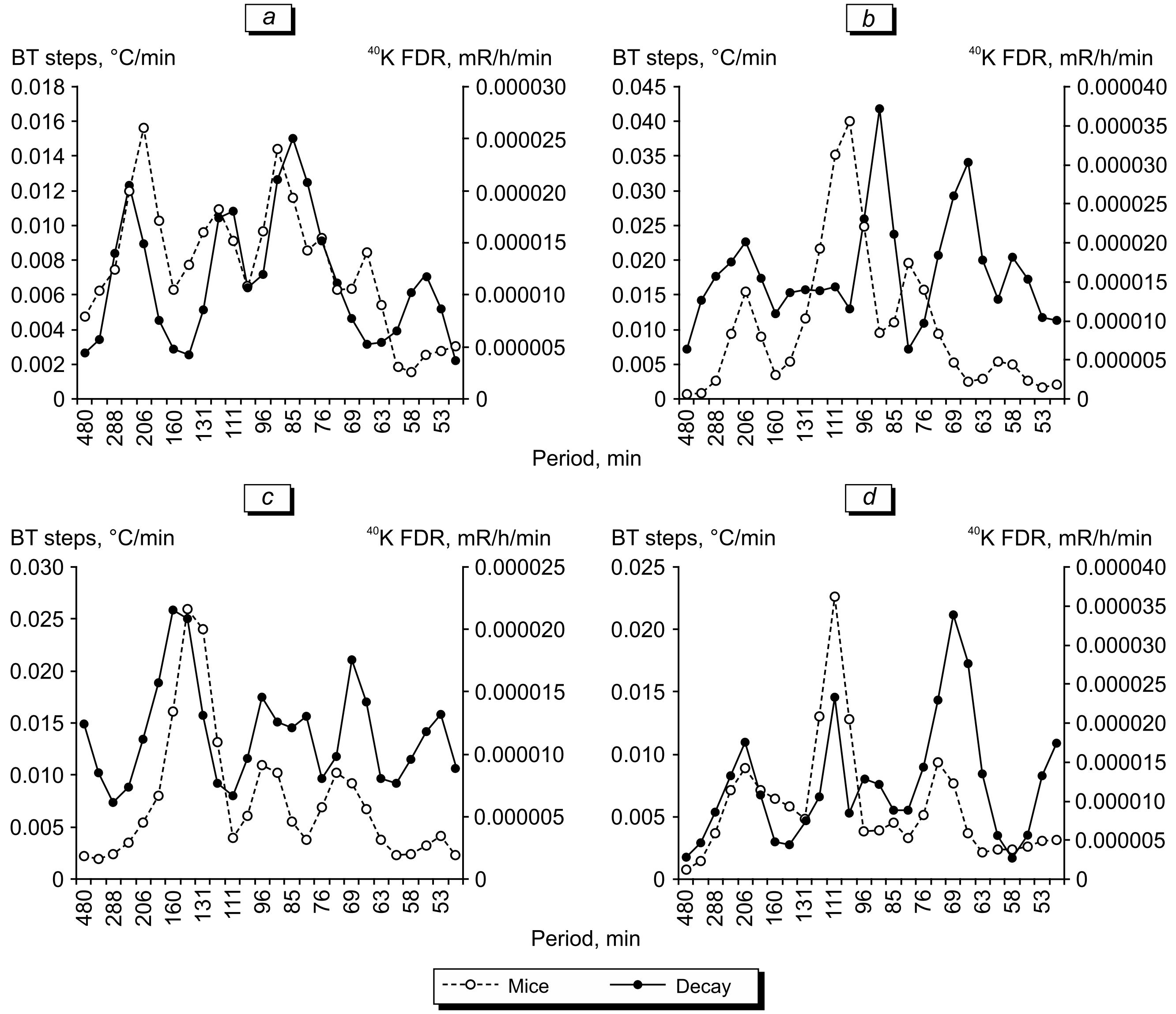

Fig. 1. The spectra of BT steps and of fluctuations in ^{40}K decay rate within the range of 50-480 min recorded on September 27, 2022 (*a*); September 28, 2022 (*b*); September 29, 2022 (*c*); and October 1, 2022 (*d*).

explained not by a greater sensitivity of birds to synchronizing environmental factor, but by the shorter cycles of their periodic activity. It is noteworthy that the starlings are diurnal: they are active during the day and sleep at night. In contrast, mice have 1-5 h periods in the sleep-wake cycle. Not all the intervals of rising ^{40}K FDR coincided with elevation of mouse BT, but all pronounced BT increments coincided with elevation of the decay rate (Fig. 2). Seemingly, a next-in-turn elevation of activity of the external factor does not excite an animal immediately after the active period, and *vice versa*, even a negligible increment of external factor activity occurring immediately prior to arousal dramatically elevates BT. This observation can explain the fact that the mice did not demonstrate especially high correlation coefficients. Therefore, the most convincing method to evaluate association between biological rhythms and the changes in environmental factor is superposed epoch analysis.

The distribution of ^{40}K FDR relatively to the moment of maximum BT increment (>0.02°C/min) in mice and starlings was calculated by the method of superposed epoch analysis. At first, we calculated these distributions for every day of experiment (Fig. 3, *a*); thereafter, the median was calculated over all the days of observations (Fig. 3, *b*, *c*). Importantly, ^{40}K FDR gradually increased to the moment of maximum BT increment in mice and starlings (zero point on abscissa). Previously, we analyzed association of BT with neutron count rate at the Earth's surface [6]. The intensity of

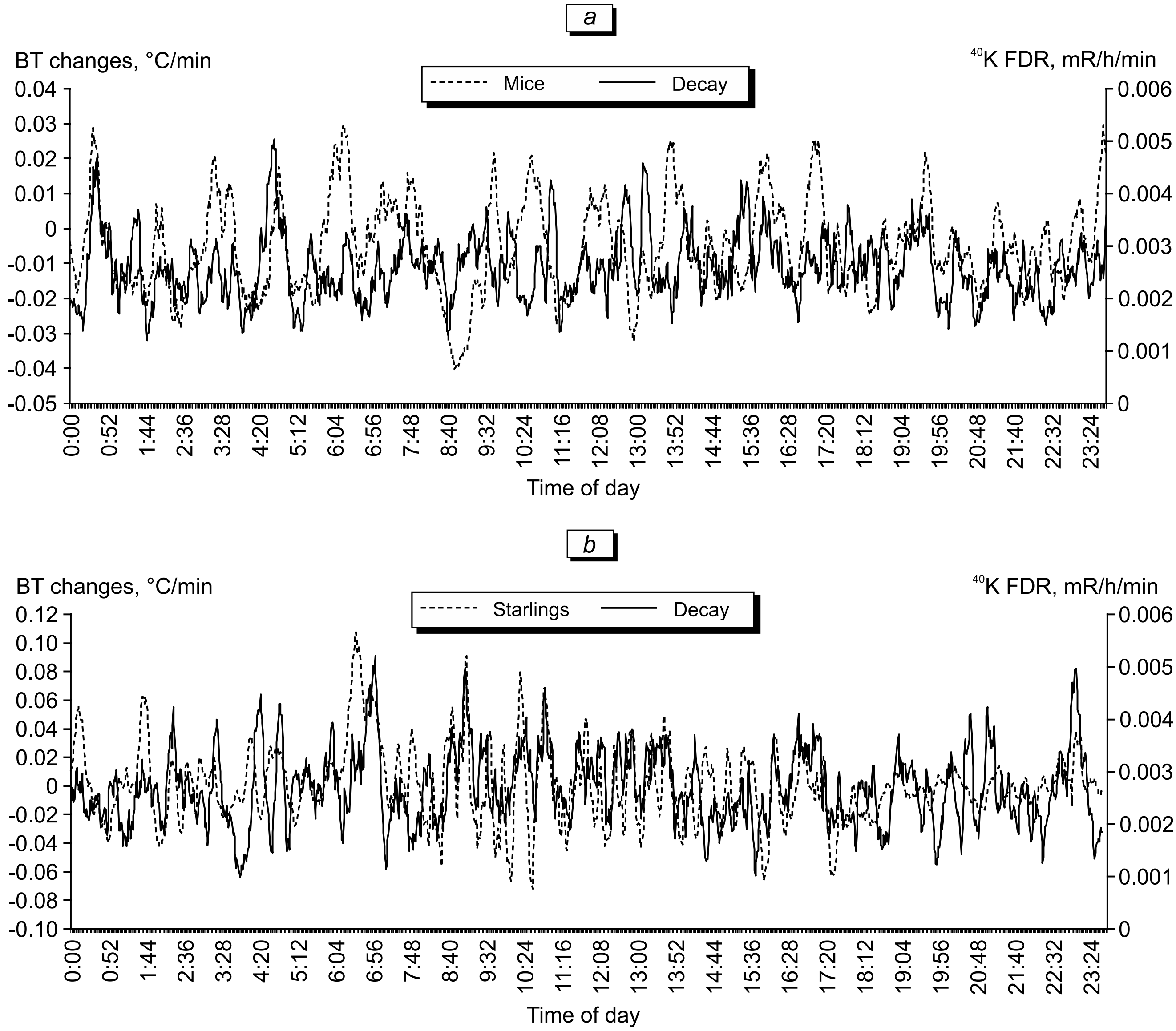

Fig. 2. Representative examples of one-minute-step changes in ^{40}K FDR and in BT of mice (*a*, *n*=18) and starlings (*b*, *n*=5): the median values recorded on September 27, 2022 and October 6, 2022, respectively.

neutron count rate fluctuations did not always attain expected maximum at minute 0 on abscissa, and statistical significance was achieved only in a large data sample. In contrast, every daily plot of ^{40}K FDR was positive near minute zero (Fig. 3, *a*). This fact attests to more pronounced association between ^{40}K FDR and BT in different animals in comparison with intensity of neutron count rate fluctuations. In experiments with mice and starlings, we observed greater ^{40}K FDR during the period of maximum daily increments of BT ($p<0.0001$). Thus, the periods of significant increment of BT are associated with elevation of ^{40}K FDR.

It is worthy to note that maintaining the mice under constant illumination is stressful, therefore these animals cannot be considered as intact. However, even under these conditions the animals demonstrated association between their physiological parameters and ^{40}K FDR, which confirms that this association does exist. To test possible effect of illumination regime on the examined association, we kept the starlings under natural illumination, so in this study we revealed association between BT and radioactive decay under various illumination regimes. The search for correlation between BT rhythms and ^{40}K FDR was performed separately for mice and starlings. Thus, the study was not focused on examination of general regularities: specifically, it did not compare the strength of revealed association in birds and mammals.

The established facts indicate association between ultradian BT rhythms in animals and quasirhythmic

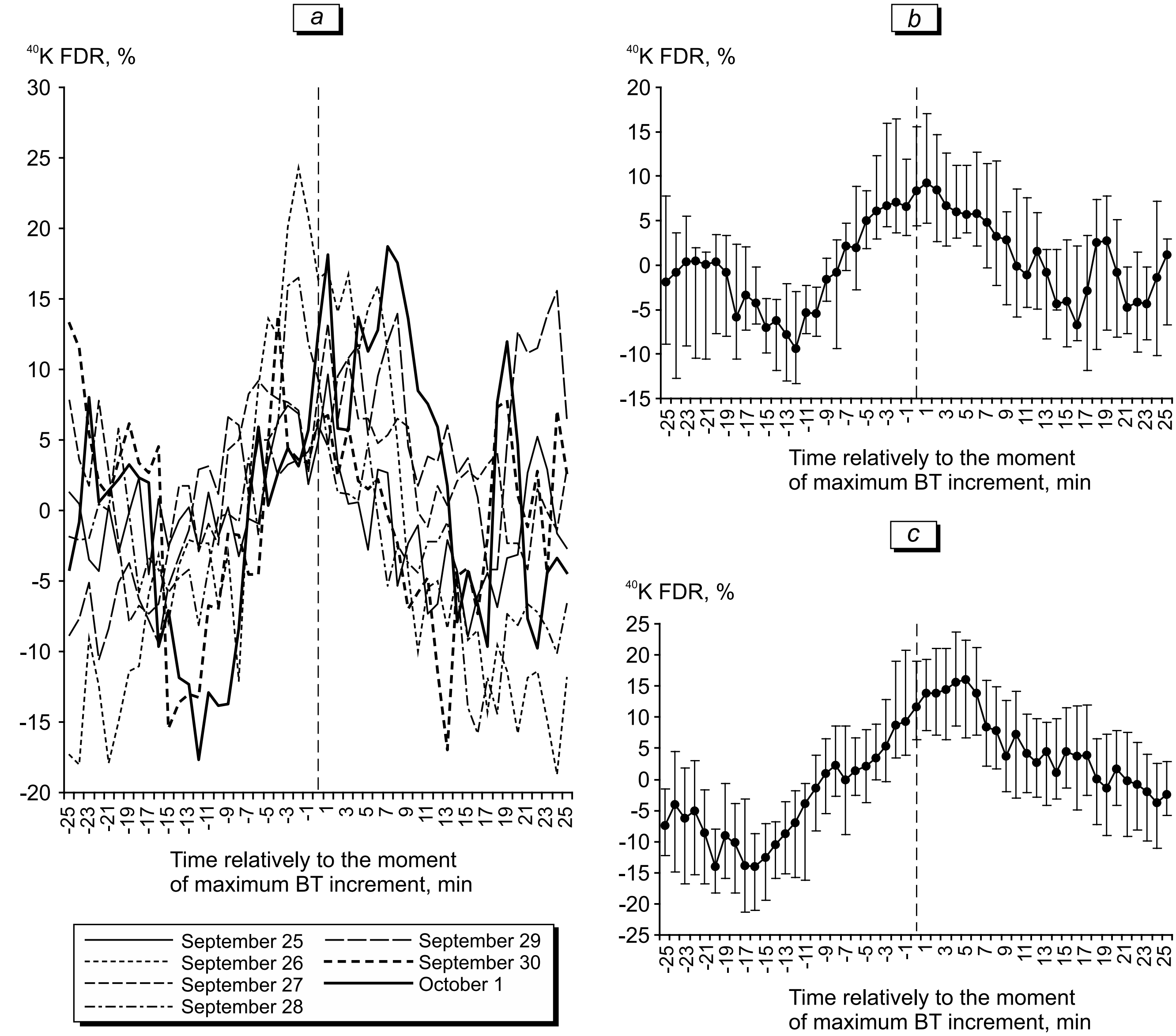

Fig. 3. Distribution of ^{40}K FDR relatively to the moment of maximum BT increment in mice and starlings calculated by the method of superposed epoch analysis. BT was averaged over 1-day intervals. *a*) An example of the changes in ^{40}K FDR relatively to the mean value obtain by superposed epoch analysis relatively to the moments of maximal BT 1-min-step function averaged over 1-day intervals; *b*, *c*) changes in ^{40}K FDR obtained by superposed epoch analysis relatively to BT maximums of mice (*b*) and starlings (*c*) calculated for the daily intervals for entire observation period from September 3 to October 10 (Me (Q1; Q3)).

changes in ^{40}K FDR. This natural isotope is contained in living organism being a source of intrinsic radioactivity. However, its spread is low (0.01%), and its specific radioactivity is small (^{40}K half-life is 1.2 billion years) so the physiological effect of this isotope should be negligible. Thus, the further studies should be focused on the search for the process underlying enhanced ^{40}K FDR as well as on examination of other physical targets of this unknown process. Specifically, these targets can be the water parameters known to have a similar spectrum in circahoralian range [13] or the mechanisms underlying the energy metabolism in cells [14,15].

The study was carried out within the framework of State Assignment No. 0089-2021-0004 for A. N. Severtsov Institute of Ecology and Evolution, Russian Academy of Science “Ecological and Evolutionary Aspects of Animal Behavior and Communication”.

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