EXPERIMENTAL PAPERS

Unusual Correlation between Rest–Activity and Body Temperature Rhythms in the Naked Mole Rat (*Heterocephalus glaber*) as Compared to Five Other Mammalian Species

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Abstract—Body temperature and locomotor activity were recorded over a month using intraperitoneal sensors in the following six mammalian species: naked mole rats (*Heterocephalus glaber*, 2♂, 2♀), C57Bl/6j mice (6♂), outbred white rats (4♂), Mongolian hamsters (*Allocricetulus curtatus*, 3♂), sables (*Martes zibellina*, 2♂, 2♀), forest ferrets (*Mustela putorius*, 2♂, 2♀). During experiments, the naked mole rats stayed in their colony under special laboratory conditions: 2 weeks in constant darkness (dim red light), 2 weeks under an alternating light–dark regime (12L/12D). Mice and rats were kept in individual chambers under standard laboratory conditions and a 12L/12D regime; sables and ferrets were kept in their outdoor enclosures under natural light conditions in autumn. All the rodents used in the experiments, except for mole rats, are nocturnal animals. Accordingly, all of them showed a clear circadian rest–activity rhythm: a high level of locomotor activity in the daytime and low at night. The body temperature rhythm showed a synphase correlation with the rest–activity rhythm: temperature rose at night and fell in the daytime ($0.9 < r < 1$). At the same time, in hamsters, an increase in locomotor activity at night, when the ambient temperature went down, was accompanied by a corresponding increase in the amplitude of body temperature elevations. Small predators, sables and ferrets, demonstrated a more smoothed circadian rhythm. In contrast to all these species, body temperature of mole rats dropped sharply by 3–5°C (down to 28°C) upon activation of their locomotor activity (usually in the morning and evening hours) and jumped (up to 36°C) at rest (in the night hours) both under constant darkness and 12L/12D regime ($-0.7 < r < -0.9$). Overall, these animals showed an original thermoregulatory system with a possible "set point" at about 33.5°C.

DOI: 10.1134/S0022093020050087

Keywords: rest–activity rhythm, body temperature, naked mole rat, mouse, rat, Mongolian hamster, sable, forest ferret

INTRODUCTION

Naked (more precisely, hairless) mole rats (*Heterocephalus glaber*, NMR) are one of the most amazing and mysterious terrestrial mammals [1−3]. They live in the arid zones of North-East Africa (Ethiopia, Kenya, Somalia), live underground in large colonies of several tens or even hundreds of animals. Moreover, they, like bees, breed only one female of huge size, which is fertilized by one or more males. All other individuals are "workers," their secondary sexual characteristics are reduced and sex is determined only by genotyping. NMRs do not get sick, do not age, have a very long lifespan, are resistant to hypoxia and hypercapnia, etc., and therefore attract the keen interest of representatives of various scientific fields [2−6]. Ecology, morphology, biochemistry, genetics and molecular biology of this species have been studied well [1, 3, 5−7], which cannot be said about its physiology. The latter is largely based on isolated fragmentary observations [8] and even "legends." So, it is believed that naked mole rats are not able to maintain their body temperature, although no one has really studied this [9, 10]. The circadian rhythms of this animal are also very poorly studied [11, 12]. By automatic analysis of two-year video recordings of the behavior of two colonies of mole rats located in the laboratory in transparent labyrinths, it was shown that, in general, the colony lacks a circadian rhythm of activity−rest, both in constant darkness and in 12L/12D mode. This means that either it is really absent in an individual, or individual rhythms are not in phase with each other. However, within the colony there is a synchronization of activity−rest rhythms, because when most members of the colony are awake, they interfere with sleep for all other individuals. Working individuals spend 45−70% of the time of the day in a state of behavioral rest and sleep, and the breeding female, the most active members of each colony, 30−40% [11].

In another work, very extensive and difficult to set out, the authors studied both individual rhythms of activity when running in a wheel, and the rhythm of the colony as a whole, by registering the number of intersections of each animal at a specific place in the maze where the recorder was located. Very significant inter-individual discrepancies were found; nevertheless, it was confirmed that in the vast majority of colony members (25 of 29), indeed, the circadian rhythm of activity−rest is absent, but when animals were isolated, it was detected in 13 of 23 individuals, with an increase in the level of activity in the dark ("nocturnal") period in the chamber [12].

In both studies, only indirect, behavioral methods for determining circadian rhythms were used, which cannot give a definitive answer to the question of the presence and nature of such rhythms in individuals of NMRs. In this work, we set ourselves the goal of directly answering the question: are the circadian rhythms of activity−rest and body temperature of these animals different from similar parameters of other mammals?

MATERIALS AND RESEARCH METHODS

For this purpose, we implanted intraperitoneally through a small incision (under light anesthesia: naked mole rats—isoflurane, others—Zoletil 35−40 mg / kg, i/m) the ECOLOGGERs (miniature stand-alone temperature and motor activity sensors, EMBI RESERCH LLC, Novosibirsk) to the following animals: naked mole rats (2 males, 2 females), laboratory mice of the C57Bl/6j line (6 males), laboratory white rats (4 males), Mongolian hamsters (*Allocricetulus curtatus*, 3 males), sables (*Martes zibellina*, 2 males, 2 females) and forest ferrets (*Mustela putorius*, 2 males, 2 females). The incision was sutured, the animals were given an antibiotic. The study was conducted in accordance with the principles of Basel declarations and approved by the bioethical committee at the Severtsov Institute.

Body temperature was measured in centigrade, and motor activity was measured in fractions of G, the deviation of the sensor's acceleration at the time of measurement from the acceleration of gravity (i.e., if the animal falls at the time of measurement, then the motor activity indicator = 1). Sensors made measurements every minute and averaged them over the period set by the user. In this study, this period was 10 minutes. In this mode, the battery charge is enough for about 6 weeks.

The NMRs came from the Berlin Zoo and were

not the first generation to grow up in captivity. These were adult individuals, about three years old and weighing: males—34 and 47 g, females—42 and 52 g. After the operation, the NMRs were put back into their colony, which was kept in a separate laboratory room in special labyrinths made of transparent Plexiglass cylinders, with a diameter of 25 cm and a height of 22−30 cm, interconnected by a system of Plexiglass tubes with a diameter of 5 Ч 4 cm. These labyrinths simulate tunnels and habitats of naked mole rats in nature. In the room in which the labyrinths were located, a stable microclimate was maintained: temperature 27 ± 1.5°C, humidity 50 ± 15%. Wooden chips were used as a bedding. Every day, from 18:00 to 20:00, the animals were provided with a feed mixture consisting of vegetables (sweet potato, carrots, kohlrabi, celery root), fruits (apples, pears) and granulated autoclavable compound feed, at the rate of 6 grams per 1 individual per day. NMRs do not consume water.

During the first two weeks, all the NMRs were kept in "constant darkness"; illumination with a weak red light (20 lx) was turned on only during feed distribution, at 18−20 h. Then, for two weeks, the animals were in day−night conditions: 12 h (from 10 to 22 h)—bright white light (200 lux), 12 h (from 22 to 10 h)—weak red (12L/12D). After a month, the experimental animals were again removed from their colony (all animals in the colony are microchipped), and under mild anesthesia, the sensors were removed through a small incision in the skin of the abdomen. The wound was sewn up, the animals were given an antibiotic, and they were put back into their colony. Data from the Ecologgers were read using a special device.

After the operation, mice and rats were placed in individual chambers kept in a separate room isolated from external influences, with a light cycle of 12L/12D, a temperature of 22−24°C, and free access to water and food. After a month, the sensors were removed as described above. A similar procedure was applied to all other animals. In the winter, the hamsters were placed in individual chambers located in a special refrigeration unit, where the animals underwent adaptation for a month at room temperature (24 ± 1°C) and 12L/12D light mode, and then the temperature in the chamber was gradually reduced to 4 ± 1°C, at 1°C per day. The illumination period was also gradually reduced—from 12 (from 9 to 21 h) to 2 (from 14 to 16 h) hours a day, for half an hour per day. Mongolian hamsters are optional hibernators, and only part of the animals fall into torpor. For the purposes of this study, we selected those animals (3 males) that *did not* go into hibernation. Sables and ferrets were kept in extensive enclosures in semi-natural conditions in a forest on the territory of the Chernogolovka biological station in the autumn.

The obtained body temperature and motor activity curves were subjected to mathematical procedures—averaging, standardization, and (for sables and ferrets) exponential smoothing (with a coefficient of 0.3) with the determination of the correlation coefficient between them.

RESULTS AND DISCUSSION

The presented illustrations demonstrate not only fragments of native curves, but also the results of their processing. All investigated rodents, except for mole rats, are nocturnal animals. Accordingly, as laboratory animals in isolation and artificial light, they showed well-known [13] clear circadian rhythms: a high level of motor activity in the dark, "night" period of the day, and low in the bright, "daytime" mode. The rhythm of body temperature was synchronous and in phase with the rhythm of activity−rest: rises at night (up to 39.5°C) and falls—in the daytime (up to 34.5°C) (Figs. 1a−1f).

Motor activity dynamics is largely related to the intensity of muscle heat production—this was especially pronounced in experiments with hamsters (Figs. 2a−2c). As can be seen from the presented record, a gradual decrease in the external temperature led to a significant increase in motor activity in the period corresponding to the "night" during adaptation. This was combined with a similar increase in body temperature at night (up to 39.2°C). In other words, the lower the ambient temperature, the more the hamster would run in its habitual night time (despite the fact that the light period was shrinking all the time) and warmed up more, which allowed him to keep warm during the daytime rest period. The correla-
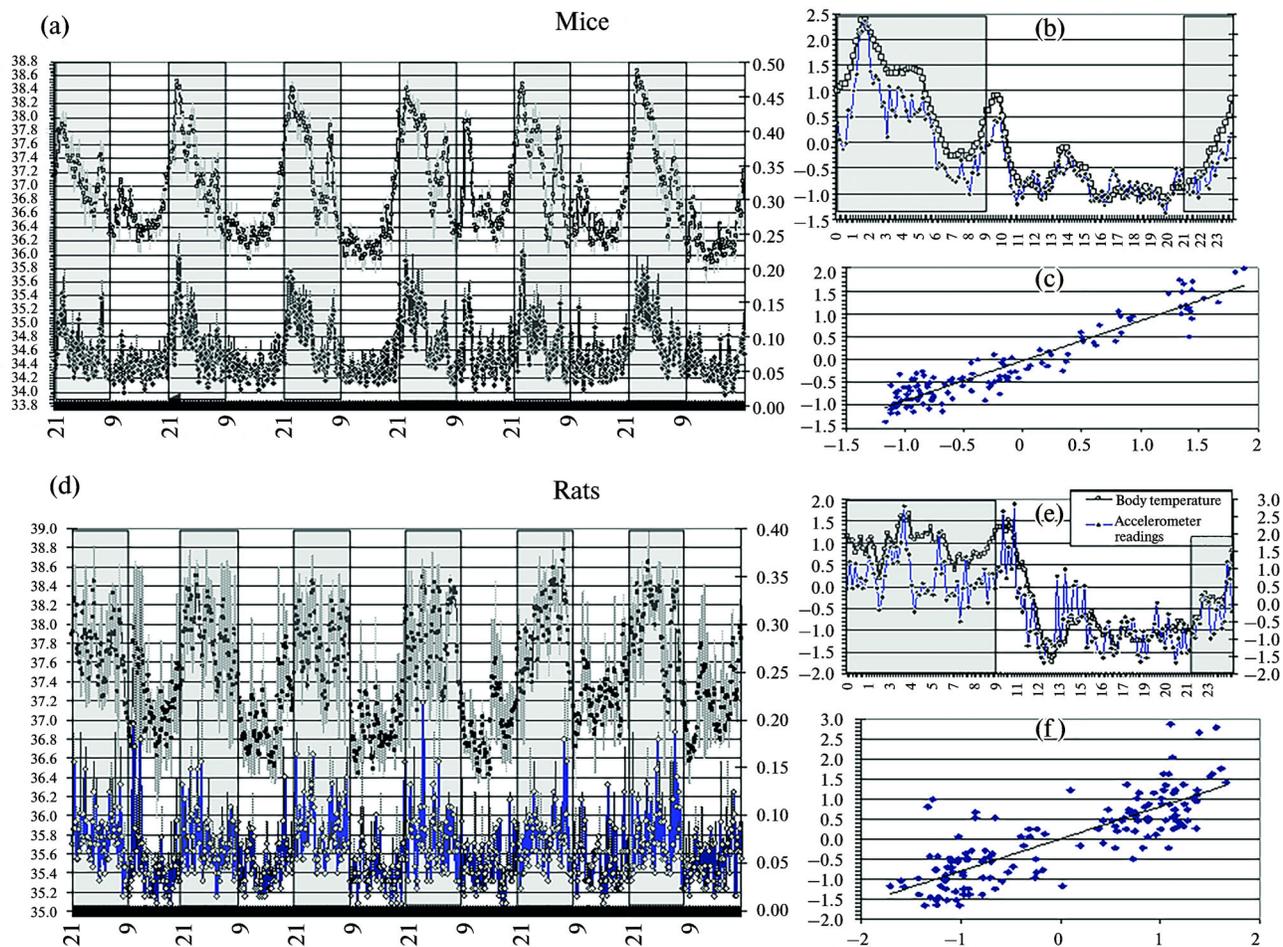
Fig. 1. (a) Averaged dynamics of body temperature (upper curve) and locomotor activity (lower curve) in 6 mice: 6-day fragment of a monthly record, $M \pm SEM$. Shaded areas—night periods in a chamber (21:00−9:00). *Abscissa*: the time of day. *Left ordinate*: temperature of the abdominal cavity (˚C). *Right ordinate*: accelerometer readings (1 = 1g). (b) Averaged standardized curves of the daily dynamics of body temperature and locomotor activity over the entire period of the experiment. *Abscissa*: the time of day; *both ordinates*: deviations from the average values of both parameters. (c) Scatter plot of body temperature dependency (*abscissa*) on locomotor activity (*ordinate*). Both axes—deviations of the parameters from their average values. The sloping line shows the trend ($0.9 < r < 1$; $p < 0.001$). (d)−(f). Same as in (a)−(c), for 4 rats. It is evident that increases in locomotor activity in mice and rats (mainly at night) are accompanied by body temperature elevations up to 38.5˚C, while rest or dormancy periods (mainly in the daytime) are accompanied by decreases in body temperature down to 36−36.5˚C. These fluctuations are synchronized by turning the light on and off.

tion coefficient between motor activity and bode temperature in all the rodents studied was close to unity ($0.9 < r < 1$; $p < 0.001$).

In small predators, sables and ferrets, the circadian rhythm looked less pronounced and less dependent on the light cycle, partly because they were influenced by a variety of olfactory and sound stimuli coming to them from the surrounding forest (Figs. 3a−3f). The circadian curves of ferrets indicate a slight predominance of night activity, and sables—rather in the evening. The correlation between both recorded indices on the native curves was practically absent. However, a detailed mathematical analysis of the entire recording period using the method of exponential smoothing revealed the internal periodicity of circadian curves with a main acrophase of about 20 h, which apparently reflects the period of feeding the animals. A rather high correlation was found for both smoothed circadian curves ($0.7 < r < 0.8$). The weakly expressed nature of the circadian rhythm of activity−rest in the European ferret (*Mustela furo*),
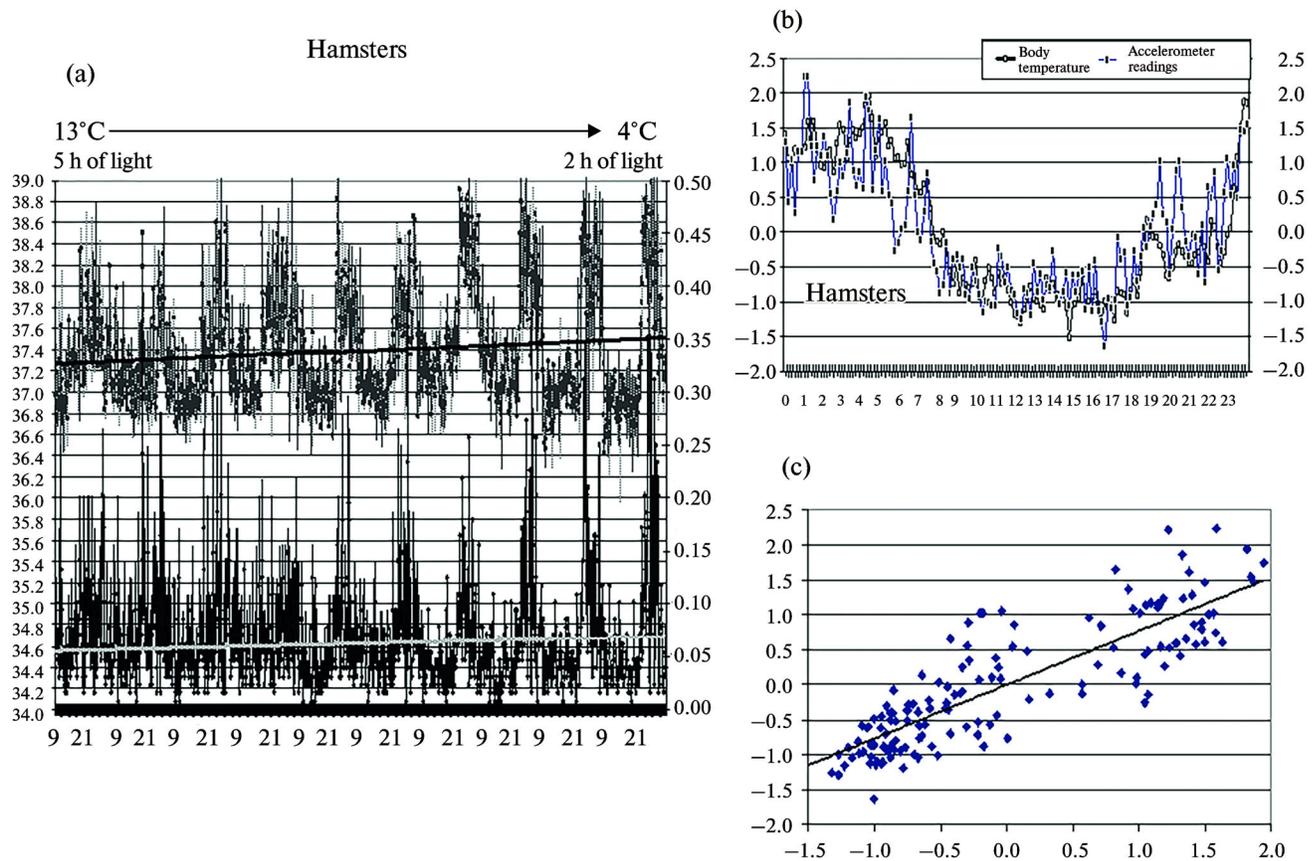
Fig. 2. (a) Averaged 10-day fragment of a 40-day record of body temperature (upper curve, $M \pm SEM$) and locomotor activity (lower curve) in three Mongolian hamsters (*Allocricetulus curtatus*) during the experiment on lowering the ambient temperature. Axial distributions—as in Fig. 1a. Animals were pre-adapted to an ambient temperature of 24°C and a 12L/12D cycle (light from 9 a.m. to 9 p.m.). During the experiment, the temperature in the chamber was lowered by 1°C a day for 20 days, i.e. down to 4°C. At the same time, the illumination period was shortened by 30 min a day, i.e. down to 2 h (14:00−16:00). On this graph fragment, the chamber temperature was 13°C in its left part and 4°C in the right; the illumination period was 5 h (0:30−15:30) in the left part and 2 h in the right. This is indicated by a horizontal arrow at the top of the graph. It is evident that a decrease in the external temperature is accompanied by an increase in locomotor activity and, accordingly, an increase in body temperature elevations (trends are shown). It is also evident that all these elevations, both of temperature and locomotor activity, occur during the nocturnal period, to which all the animals are accustomed, despite a gradual shortening of the real light period in the chamber. (b), (c) Same as in Figs. 1b, 1c ($0.9 < r < 1$; $p < 0.001$).

in contrast to the pronounced rhythm in the golden hamster (*Mesocricetus auratus*), was previously indicated by other authors [14].

A striking contrast with all these mammals was demonstrated by naked mole rats. As can be seen from Fig. 4a−4e, body temperature in them sharply *fell* by 3−5°C with increasing motor activity, almost to the level of the environment (up to 28°C) and *jumped* to 36°C at rest. Moreover, all animals clearly showed the presence of thermoregulation, trying to maintain body temperature at 33.5°C (apparently close to the "set point") compared to 37−37.5°C in mice, rats and hamsters,

and 38−38.5°C in sables and ferrets. The thermograms demonstrated clearly indicate the existence of an original system for maintaining temperature homeostasis in naked mole rats. Such a paradoxical "negative" relationship between motor activity and body temperature in naked mole rats was first noted in [8], but never was previously systematically studied. How mole NMRs manage to cool dramatically with increased muscle activity, as if "contrary to" the second law of thermodynamics, remains completely mysterious.

The negative correlation between the motor activity and body temperature of the NMRs was
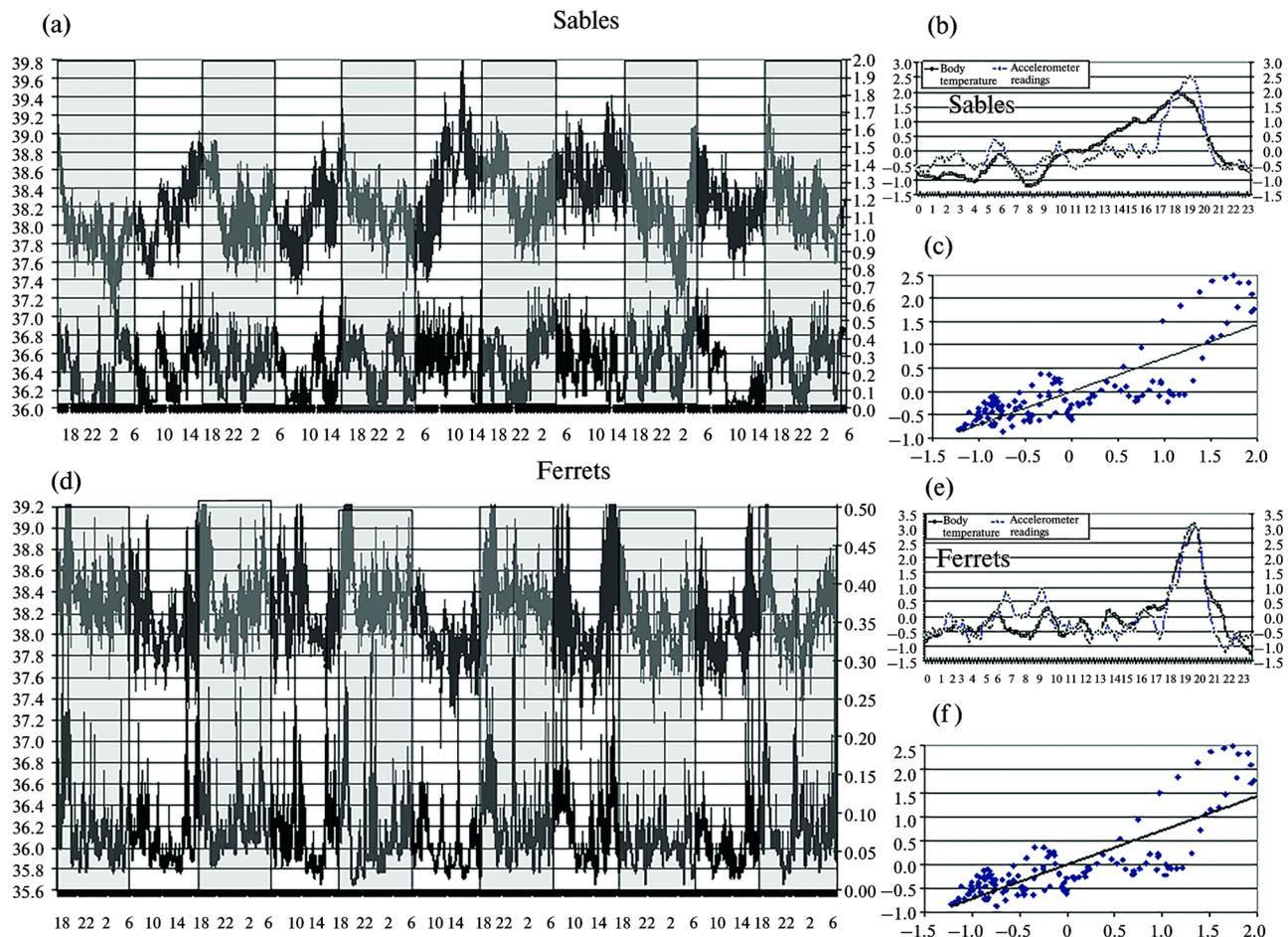
Fig. 3. (a) Same as in Figs. 1a−1c, for 4 sables. (b), (c) Same as in Figs. 1b, 1c, but the curves are additionally subjected to a mathematical smoothing procedure. (d)−(f). The same for 4 ferrets ($0.7 < r < 0.8$; $p < 0.001$).

very high ($-0.8 < r < -0.9$), mainly with two circadian activity peaks (and, correspondingly, the minimum body temperature values): in the morning and evening hours. Moreover, this rhythm did not depend on the light cycle: it was preserved both in the conditions of "constant darkness" and in the 12L/12D light mode. Apparently, the NMRs somehow sense the presence of people—through the vibrations, noise, odors, etc. that penetrate them (despite the measures taken to isolate them), and this presence bothers them. Then the first peak of activity (10−12 h) is probably associated with the appearance of people in the Institute building, and the second (18−20 h)—with employees entering the room, where the labyrinths are located, and feed distribution. At night, when the building is empty, NMRs are mostly in a state of behavioral rest.

CONCLUSION

Thus, the present study in rodents showed that mice, rats and Mongolian hamsters in laboratory conditions are pronounced nocturnal animals, and their nocturnal peak of motor activity is combined with a rise in body temperature, and to a large extent determines it. Small predators living in the natural environment, sable and ferrets, show a weakly expressed circadian rhythm, which is not clearly associated with a change in illumination.

Naked mole rats demonstrate a completely different type of circadian rhythm—with a high level of motor ativity in the daytime, independent of illumination and combined, despite muscle activity, with a sharp drop in body temperature, up to room temperature. Upon termination of motor activity, body temperature quickly rises back to
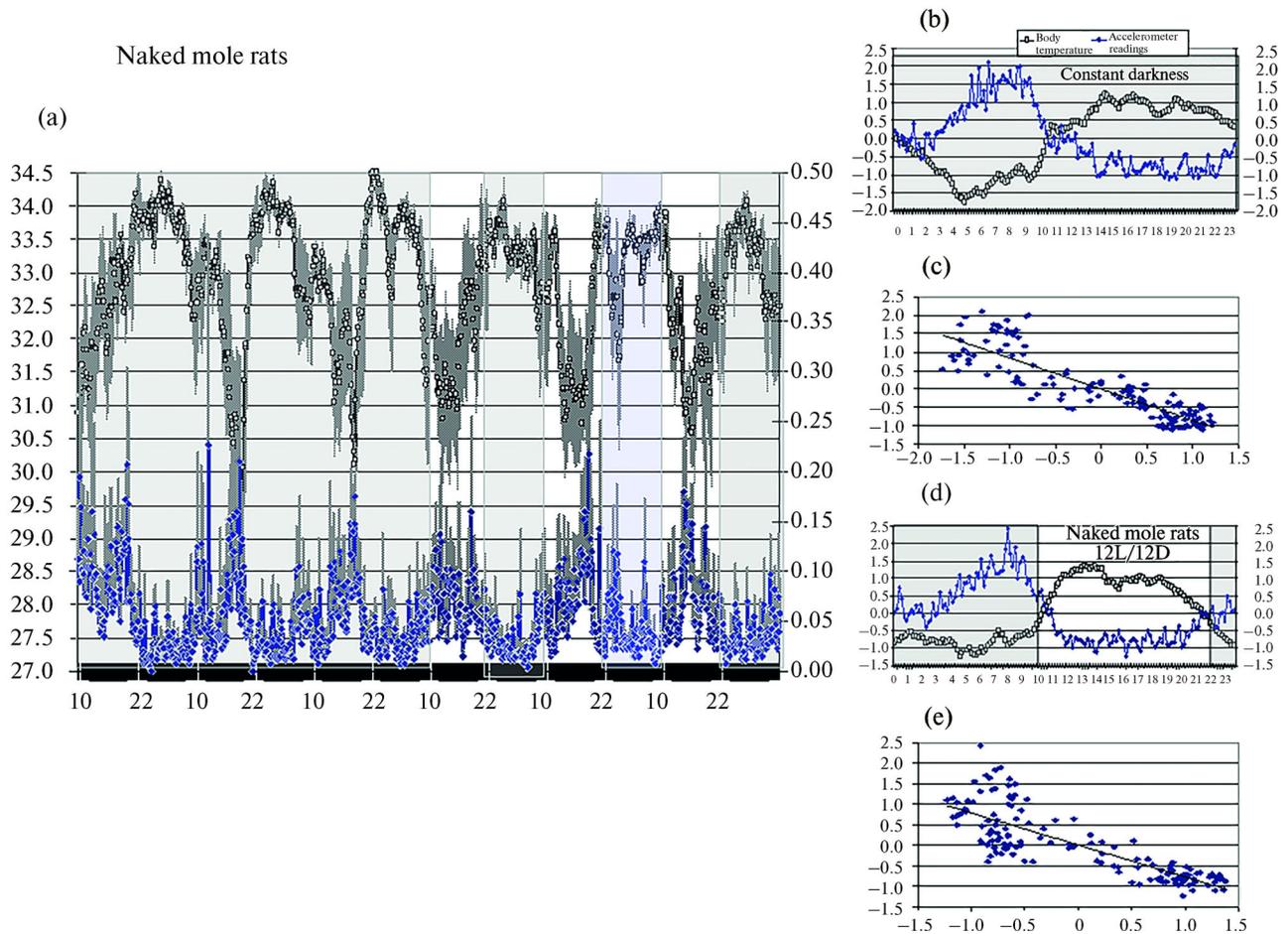
Fig. 4. Naked mole rats, $n = 4$. (a) Same as in Fig. 1a. The left half of the Figure—3-day recording under conditions of constant darkness. The right half of the Figure—3-day recording after switching to a 12L/12D regime. (b) Same as in Fig. 1b in total darkness. (c) Same as in Fig. 1c in total darkness ($-0.8 < r < -0.9$; $p < 0.001$). (d), (e) Same as in (b) and (c) under a 12L/12D regime. It is evident that body temperature drops sharply (down to 29−31°C) when locomotor activity rises, mainly in the daytime. When locomotor activity decreases at night, body temperature quickly reverts to normal (for these animals) values of 33−34°C. It is also evident that turning the light on and off has a little effect on circadian rhythms of locomotor activity and body temperature, which appear to be synchronized by the other external factors, e.g., such as the appearance of people coming to feed animals.

the average level, apparently close to the "set point". In general, our results are another evidence of the unique biological characteristics developed by this species of mammals during its evolution. There is no doubt that the study of the physiological characteristics of naked mole rats will bring many more surprises.

CONTRIBUTION OF AUTHORS

Designing the experiment was the responsibility of V.M. Kovalzon and M.Yu. Vysokikh; data collection was performed by V.M. Kovalzon, O.A. Averina, A.A. Petrin and M.Yu. Vysokikh, data processing—by V.M. Kovalzon and V.A. Minkov; the manuscript was written and edited by V.M. Kovalzon.

REFERENCES

1. Browe, B.M., Vice, E.N., and Park, T.J., Naked mole-rats: blind, naked, and feeling no pain, *Anat. Rec.*, 2020, vol. 303(1), pp. 77−88.

2. Kupferschmidt, K., Forever young? Naked mole rats may know the secret, *Science*, 2018, vol. 359(6375), pp. 506−507.

3. Skulachev, V.P., Holtze, S., Vyssokikh, M.Y., Bakeeva, L.E., Skulachev, M.V., Markov, A.V., Hildebrandt, T.B., and Sadovnichii, V.A., Neoteny, prolongation of youth: from naked mole rats to "naked apes" (humans), *Physiol. Rev.*, 2017, vol. 97, pp. 699−720.

4. Buffenstein, R., Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species, *J. Comp. Physiol. B*, 2008, vol. 178, pp. 439−445.

5. Clayson, M.S., Devereaux, M.E.M., and Pamenter, M.E., Neurokinin-1 receptor activation is sufficient to restore the hypercapnic ventilatory response in the substance P-deficient naked mole-rat, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2020. doi: 10.1152/ajpregu.00251.2019

6. Schuhmacher, L.-N., Husson, Z., Smith, E. St. John, The naked mole-rat as an animal model in biomedical research: current perspectives, *Open Access Animal Physiol.*, 2015, vol. 7, pp. 137−148.

7. Kim, E.B., Fang, X., Fushan, A.A., Huang, Z., Lobanov, A.V., Han, L., Marino, S.M., Sun, X., Turanov, A.A., Yang, P., Yim, S.H., Zhao, X., Kasaikina, M.V., Stoletzki, N., Peng, C., Polak, P., Xiong, Z., Kiezun, A., Zhu, Y., Chen, Y., Kryukov, G.V., Zhang, Q., Peshkin, L., Yang, L., Bronson, R.T., Buffenstein, R., Wang, B., Han, C., Li, Q., Chen, L., Zhao, W., Sunyaev, S.R., Park, T.J., Zhang, G., Wang, J., and Gladyshev, V.N., Genome sequencing reveals insights into physiology and longevity of the naked mole rat, *Nature*, 2011, vol. 479, pp. 223−227.

8. Herold, N., Spray, S., Horn, T., and Henriksen, S.J., Measurements of behavior in the naked mole-rat after intraperitoneal implantation of a radio-telemetry system, *J. Neurosci. Meth.*, 1998, vol. 81, pp. 151−158.

9. Buffenstein, R. and Yahav, S., Is the naked mole-rat *Heterocephalus glaber* an endothermic yet poikilothermic mammal? *J. Therm. Biol.*, 1991, vol. 16(4), pp. 227−232.

10. Yahav, S. and Buffenstein, R., Huddling behavior facilitates homeothermy in the naked mole rat *Heterocephalus glaber*, *Physiol. Zool.*, 1991, vol. 64(3), pp. 871−884.

11. Davis-Walton, J., Sleep arrhythmia in the eusocial naked mole-rat, *Naturwiss.*, 1994, vol. 81, 272−275.

12. Riccio, A.P. and Goldman, B.D., Circadian rhythms of locomotor activity in naked mole-rats (*Heterocephalus glaber*), *Physiol. Behav.*, 2000, vol. 71, pp. 1−13.

13. Benstaali, C., Mailloux, A., Bogdan, A., Auzéby, A., and Touitou, Y., Circadian rhythms of body temperature and motor activity in rodents. Their relationships with the light-dark cycle, *Life Sci.*, 2001, vol. 68, pp. 2645−2656.

14. Stockman, R., Albers, H.E., and Baum, M.J., Activity in the ferret: oestradiol effects and circadian rhythms, *Anim. Behav.*, 1985, vol. 33, pp. 150−154.