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GENERAL BIOLOGY

Motor Activity and "Neotenic" Sleep in the Naked Mole Rat (*Heterocephalus glaber*) under Isolation

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Abstract– For the first time, continuous registration of motor activity and electroencephalogram for 40 days was carried out in four individuals of the naked mole rat (*Heterocephalus glaber*) in isolated conditions in the laboratory. A clear circadian rhythm of motor activity was found, with a gradual decrease during the night and an increase during the day, which remained both in the 12L/12D mode and in conditions of complete darkness. The rest states occupied, on average, about half the time of the day. There were both typical and atypical sleep periods, in which REM sleep episodes preceded NREM sleep periods. REM sleep percentage was unusually high (up to 50% of the total sleep time). During REM sleep episodes, a synchronized two-phase high-amplitude rhythm with a frequency of 12–16 Hz was recorded in the EEG. In addition, there were hard-to-identify periods of sleep, combining elements of both NREM and REM sleep. The sleep structure of naked mole rats resembles that of evolutionarily ancient species, as well as the "disorganized" sleep characteristic of the early stages of ontogenesis in altricial mammals.

Key words: sleep-wakefulness, circadian rhythm, motor activity, naked mole rat

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Naked (more precisely, hairless) mole rats (NMRs, *Heterocephalus glaber*) are one of the most amazing and mysterious land 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. At the colony, like in the bee swarm, only one female ("queen") of huge size reproduces, which is fertilized by one or more males. All other individuals are "workers", their secondary sexual characteristics are reduced and the gender is determined only by genotyping. NMRs do not get sick, do not age, have an exceedingly long lifespan, are resistant to hypoxia and hypercapnia, etc., and therefore attract close interest of representatives of various sciences [2–4]. The ecology, morphology, biochemistry, genetics and molecular biology of this species have been studied well [1, 3, 4], which cannot be said about its physiology. The latter is largely based only on isolated fragmentary observations [5–7]. Recently, we conducted a more detailed study of the rest-activity rhythm and body temperature in four representatives of this species, which are inside their colony in special laboratory conditions. An original type of circadian rhythm was discovered – with a high level of physical activity in the daytime, independent of illumination and combined, despite muscle activity, with a sharp *drop* in body temperature, down to room temperature (28°C), as if "in spite of" the second law of thermodynamics. After the cessation of motor activity, the body temperature quickly rose back to an average level of 33.5°C [8].

The aim of this work was to determine the previously unknown structure of the sleep-wakefulness cycle in naked mole rats placed in isolation.

MATERIALS AND METHODS

The NMRs came from a source at the Berlin Zoo and were not the first generation to grow up in captivity. These were adults, about three years old and weighing: males—37 and 46 g, females—44 and 45 g. The study was approved by the Bioethical Committee of the Severtsov Institute of RAS. The animals selected for experiments were removed from their "native" colonies and placed in individual Plexiglas boxes measuring 20 × 20 × 45 cm. A 3–5 cm layer of chips was used as bedding on the bottom of the

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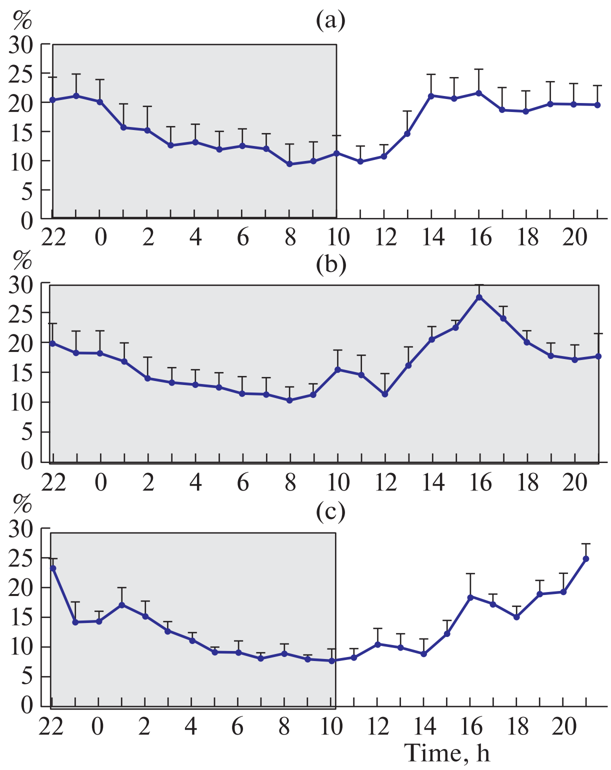

Fig. 1. A. Average hourly physical activity of four naked mole rats for 8 days (M ± S.E.M.). At 12L/12D light mode (lights on at 10AM and off at 10PM). The abscissa shows the time of day in hours. The ordinate is the accelerometer readings in %% (100% = 1g). **B**. The same, for 8 days in complete darkness. **C**. Same, 5 days after the return to the previous 12L/12D mode.

chambers. Adaptation to the conditions of confinement lasted 10 days at 12L/12D light/dark conditions. The bright white light was turned on at 10AM and turned off at 10PM. The animals were fed daily between 6PM and 8PM.

The operation was performed under general anesthesia (zoletil 35–40 mg/kg + meditin 7 mg/kg). Each animal was implanted with 5 electrodes for recording an electroencephalogram (EEG): 2 frontal, 2 parietal, and a reference one in the occipital bone. After the operation, the NMRs were placed back in their individual chambers. Each animal was permanently connected using a flexible cable to the input of a miniature 2-channel autonomous biopotential amplifier equipped with a 3D accelerometer and transmitting signals to a computer via Bluetooth channel. The design of the derivation makes it possible to record EEG without restricting the free movements of the animal, and the accelerometer responds even to its small movements. EEG was recorded with a sampling frequency of 250 Hz, and motor activity – with a frequency of 50 Hz. A visual scoring of the obtained EEG recordings off-line for 20-second epochs was carried out using a special program [9]. According to the generally accepted criteria for rodents, the states of wakefulness, NREM and REM sleep were scored.

Fig. 2. Sleeping naked mole rat during registration.

Registration of two EEG channels (fronto-parietal leads) and motor activity began immediately after the end of the operation and lasted continuously for 40 days. Registration was accompanied by video monitoring. In the first 3 weeks after the operation, the animals were kept at the same 12L/12D light/dark regime as during the adaptation period. Then, the animals were kept in complete darkness for 1 week. During the distribution of food to the animals, a dim red light was turned on between 6PM and 8PM. After a one-week period of stay in complete darkness, the animals were again transferred to the previous 12L/12D regime for one more week, until the end of the experiments.

RESULTS AND DISCUSSION

All four NMRs coped well with the transfer to the individual boxes. Figure 1 shows a clear circadian rhythm of animal motor activity, with its gradual decrease during the night and rise during the day, which was maintained both in the 12L/12D regime and in the complete darkness mode.

The behavior of animals during periods of activity consisted mainly of abrupt, quick movements: running around the chamber, eating, digging for bedding, imitation of digging corridors. The rest periods (Fig. 2) totaled (on average) about half the time of the day. The sleep cyclicity was poorly expressed. There were both “classic” periods of sleep with alternating episodes of NREM and REM sleep (Figs. 3–5), and episodes of atypical sleep, when periods of REM sleep preceded episodes of NREM sleep. Some episodes of NREM sleep were associated with muscle twitching. Episodes of REM sleep were observed both without twitching and with twitching—sometimes extremely violent. REM sleep percentage was unusually high (up to 50% of total sleep time). In the EEG, a synchronized two-phase rhythm was recorded, with the growing, sometimes huge amplitude, and a frequency of 12–16 Hz, 2 times higher than the usual regular theta rhythm char-

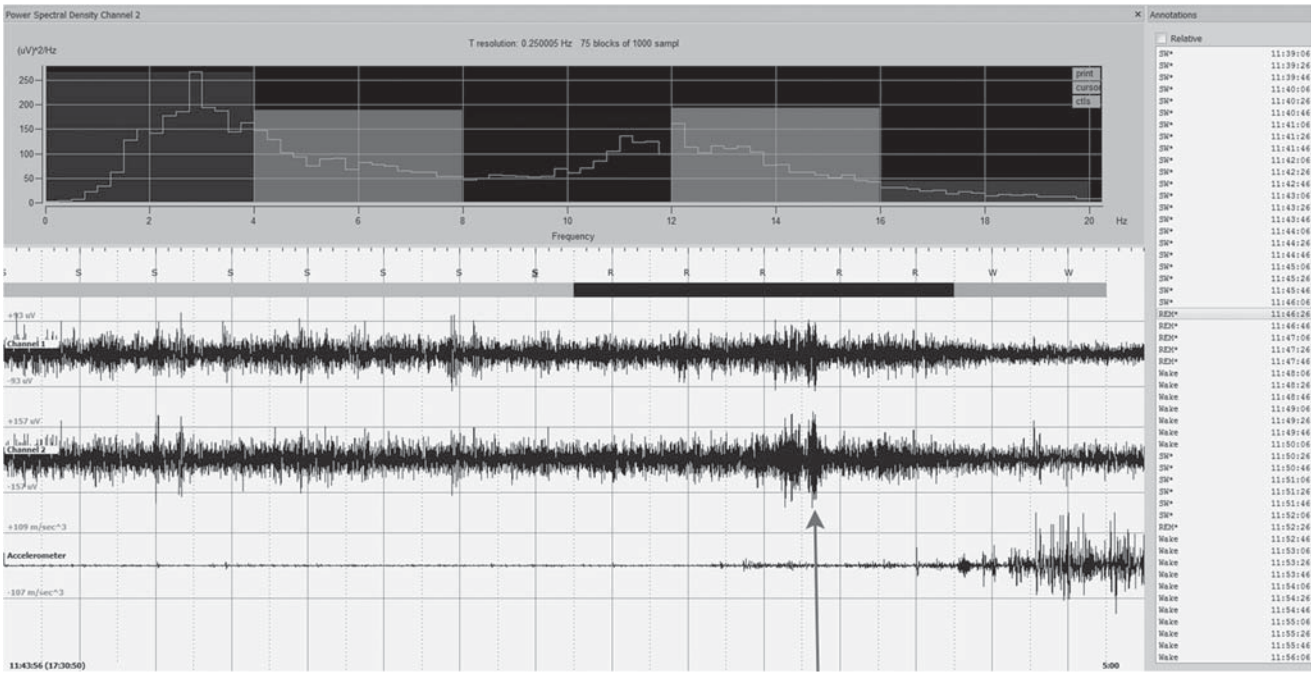

Fig. 3. A 5-minute fragment of a recording of a naked mole rat’s sleep (no. 8496♀), scored by 20-sec epochs. The lower part of the graph (light background) – registration of 2 EEG channels (frontal-occipital leads, Channel 1— left, Channel 2—right) and motor activity (Accelerometer). Vertical lines—time marks (20 s). Signal calibration—on the left (μV for EEG, m/sec^3 for 3D accelerometer). Above the EEG—scoring marks for 20 s epochs: S—slow wave (NREM) sleep (light gray stripe), R—REM sleep (black stripe), W—wakefulness (dark gray stripe). The upper graph (dark background) is the averaged spectral analysis of the 2nd EEG channel. The abscissa is the frequency (Hz). The ordinate is the signal power (μV^2/Hz). The column on the right is the result of scoring the current segment of the recording.

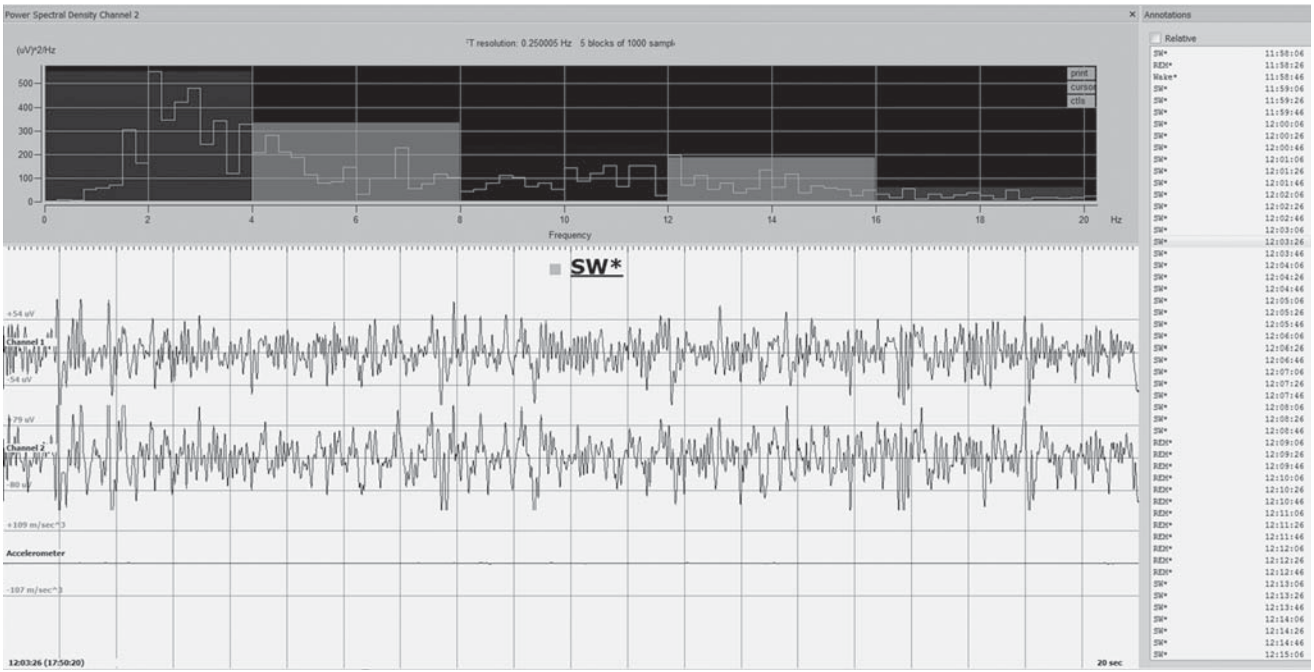

Fig. 4. 20-second episode of slow wave (NREM) sleep recording from the previous fragment. Other designations—as in Fig. 3.

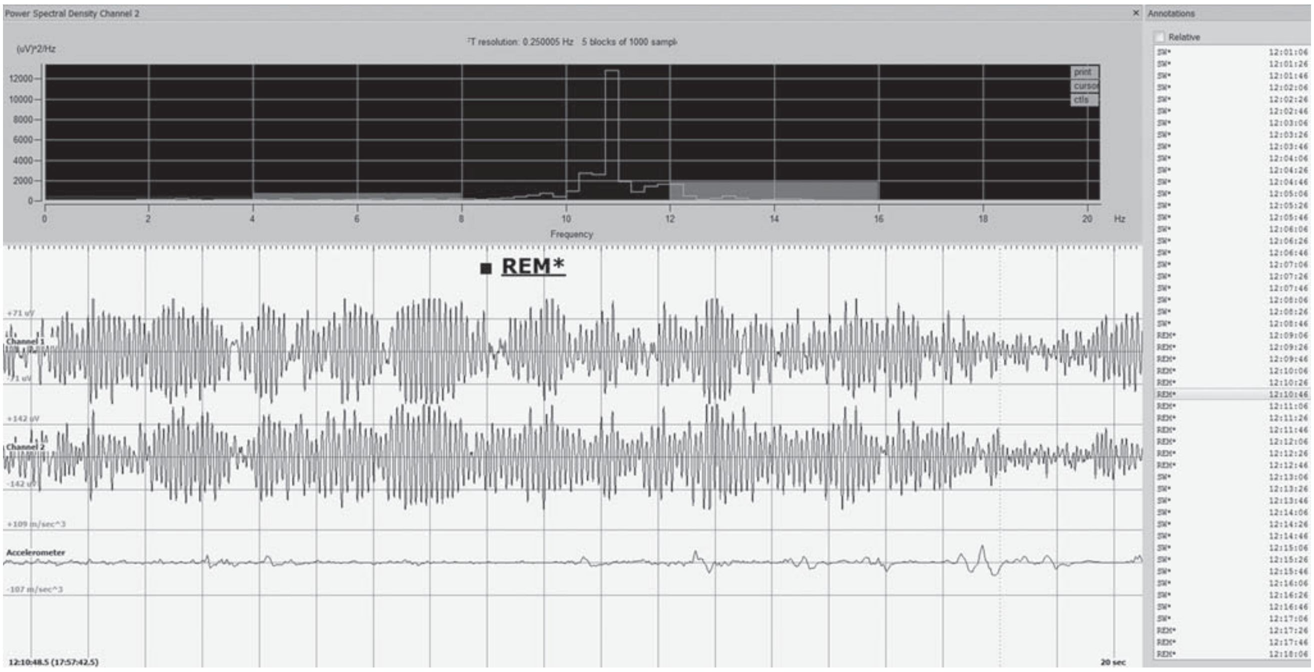

Fig. 5. The 20-second REM sleep recording episode indicated by the arrow in Fig. 3. Other designations—as in Fig. 3.

acteristic of REM sleep in rodents (6–8 Hz) (Fig. 5). Such electrical activity of the brain has not previously been described in any animal species. In addition, difficult to identify (the so-called "undifferentiated") periods of sleep were noted, combining elements of both NREM and REM sleep.

These features of sleep, namely: (1) poorly expressed cyclicity; (2) an extremely high percentage of REM sleep (more than in all currently studied species of adult mammals and birds); (2) episodes of REM sleep ahead of periods of NREM sleep; (3) muscle twitching not only during REM sleep, but also during NREM sleep; (4) the presence of "undifferentiated" sleep episodes – all these hallmarks sharply distinguish the sleep characteristics of NMRs from those of most species of mammals and birds studied so far [10–13]. Some of the above-mentioned signs of the sleep-wakefulness cycle were noted in such evolutionarily ancient species as the ostrich, platypus [14], armadillo [15, 16], ferret [17], and also resemble "disorganized" sleep characteristic of the early stages of postnatal ontogenesis in immature (altricial) mammals [18]. However, the totality of such characters forms a unique sleep structure of NMRs, which is not like any of all currently polysomnographically studied species (more than 100) of mammals. In work [3], a hypothesis was put forward that the main feature of the organism of NMRs neoteny, that is, the preservation of juvenile features in adulthood. The present study is in good agreement with this assumption.

There is no doubt that further careful study of the sleep-wakefulness cycle of naked mole rats is of considerable interest and should be carried out on a more representative sample of animals.

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