PHYSIOLOGY

Ultrasonic Vocalization of Rats in Various Motivational and Emotional States

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We studied the specific features of ultrasonic vocalization of Wistar rats in various motivational and emotional states. No significant changes were found in ultrasonic vocalization of rats during experimental food motivation and after the satisfaction of food requirements. The state of thirst and satisfaction of water requirements in animals were associated with an increase in the mean frequency of ultrasound and dominance of ultrasonic waves of a higher frequency. The formation of a negative emotional state in rats after immobilization with simultaneous electrocutaneous stimulation was accompanied by a decrease in the total duration of ultrasonic vocalization and shift in the power spectrum of ultrasound towards the dominant frequencies of 20-30, 40-50, and 80-90 kHz. During the post-stress period, the maximum power of ultrasonic waves approached the baseline (30-40 kHz). Our results indicate that the formation of various motivational and emotional states in rats is characterized by specific patterns of ultrasonic vocalization. Therefore, the parameters of ultrasonic vocalization can serve as an objective criteria for the subjective state of a living organism.

Key Words: *ultrasonic vocalization; motivational and emotional states; rats*

Ultrasound (US) is a sound vibration of high frequency (from 20 kHz to 1 GHz) that cannot be perceived by the human ear. Over many years, much attention has been paid to the biological role of US. The first manuscripts on the presence of US waves in mammals, including the bat, were published in the 18th century. The studies devoted to the nature of acoustic location and frequency analysis of US signals in bats were first performed by G. W. Pierce and D. R. Griffin in 1938 [10]. In the follow-up period, it was experimentally proved that the frogs, dolphins, cetaceans, and insects can generate and perceive US.

The notions of the general biological significance of US have been extended in the past 20 years. Most scientists believe that ultrasound in animals serve as a mean of communication between specimens of the same species [9,11,13,15]. For example, the generation of US waves with a frequency of 30-90 kHz in rodents serve to communicate between the mother and offspring [4]. The exposure of mammals to physical or social factors can be accompanied by changes in US vocalization [1].

It can be hypothesized that US waves play an important informational role in living organisms. US vocalization in mammals probably contributes to the exchange of information about functional changes in the body, which develop under the influence of endogenous or exogenous factors. However, the informational value of US in animals during the formation

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of various motivational and emotional states is poorly understood. Little is known about the specific features of US wave generation by the same specimens under a successive change of dominant motivations that are associated with the major biological or social demands and accompanied by the development of various emotions. This work was designed to answer these questions.

MATERIALS AND METHODS

Experiments were performed on 20 male Wistar rats (body weight 237.6±8.4 g) at 10:00-16:00. The experiment was conducted in accordance with the "Rules of Studies on Experimental Animals" (approved by the Ethics Committee of the P. K. Anokhin Institute of Normal Physiology; protocol No. 1, 3.09.2005). The rats were housed in cages (5 animals per cage) under an artificial light/dark cycle (8.00-20.00, lightness; 20.00-8.00, darkness) and had free access to water and food.

US vocalization of rats was recorded during the formation of various motivational and emotional states:

- in the initial state (baseline);
- in the state of hunger (48-h food deprivation) and after the satisfaction of food requirements (free access to food for 1 h);
- in the state of thirst (48-h water deprivation) and after the satisfaction of water requirements (free access to water for 1 h);
- and in the state of stress (1-h immobilization in plastic tubes with simultaneous subthreshold electrocutaneous stimulation) and 1 h after the end of stress exposure [2].

US wave generation by each rat was recorded for 180 sec. To this end, we used a plastic cage (42×21 cm) surrounded by 25-cm walls. The cage was divided by a transparent plastic wall into 2 similar compartments. The rats were placed into each compartment. The animal in one compartment was in visual contact with the rat sitting in another compartment. This design of the chamber was chosen because according to published data US vocalization of increases in the presence of animals of the same species. Presentation of a predator (cat) to the group of laboratory rats was shown to be accompanied by an increased generation of US waves by these animals [3]. US waves were recorded by Sonotrack microphones (Mertis B.V.) placed at a distance of 20-25 cm form the head of animals. The signals were digitized and processed with Sonotrack software.

The results were subjected to statistical and analytical treatment. We calculated the total duration and mean frequency of US vocalization of animals in various motivational and emotional states. The power spectrum of ultrasound in various frequency ranges (20-100 kHz) was estimated at 10-kHz intervals. The significance of differences between these parameters was evaluated by the corresponding statistical methods (Statistica 10, Statsoft). The numerical data on the mean frequency and total duration of US vocalization in rats are presented as $M \pm m$.

RESULTS

In the initial state, the total duration of US vocalization of animals over 180 sec was 6311.3±1655.0 msec. The mean frequency of US waves was 61.4±3.0 kHz (Table 1). The power spectrum of US waves in rats appeared as follows: 20-30 kHz, 4.7%; 30-40 kHz, 12.7%; 40-50 kHz, 36.0%; 50-60 kHz, 17.3%; 60-70 kHz, 9.2%; 70-80 kHz, 8.0%; and 80-90 kHz, 12.1%. Hence, the power spectrum of US vocalization in intact animals was maximum in the range of 40-50 kHz (Fig. 1).

Food deprivation for 48 h was followed by the formation of food motivation. Under these conditions the total duration and mean frequency of US vocalization were 5580.6±1590.5 msec and 63.4±4.3 kHz, respectively (Table 1). The power spectrum of US vocalization in these animals was maximum in the range of 40-50 kHz (34.0%; Fig. 1, *a*). In one hour after the rats were returned to the home cages with a free access to food, the total duration and mean fre-

TABLE 1. Characteristics of US Vocalization of Rats in Various Motivational and Emotional States ($M \pm m$)

Conditions	Vocalization	
	total duration, msec	mean frequency, kHz
Initial state (baseline)	6311.3±1655.0	61.4±3.0
Food motivation (24-h food deprivation)	5580.6±1590.5	63.4±4.3
Satisfaction of food requirements	3728.3±1464.1	54.9±5.2
Drinking motivation (24 h water deprivation)	6458.9±2930.9	70.0±3.8*
Satisfaction of water requirements	6228.4±1509.1	72.7±1.8*
Stress state (immobilization and electrocutaneous stimulation)	4062.0±2705.8	60.5±5.0
Post-stress period (1 h after stress)	1307.8±458.1**	54.4±3.5

Note. *p<0.05 and **p<0.01 in comparison with the baseline.

quency of US vocalization tended to decrease by 1.5 and 1.2 times, respectively (as compared to hungry animals). The power spectrum of US waves in these rats was maximum in the range of 40-50 kHz (33.3%). Therefore, no significant changes were found in US vocalization of animals during food motivation and after the satisfaction of food requirements.

The total duration of US vocalization in rats after 48-h water deprivation practically did not differ from the baseline (6458.9±2930.9 msec). However, the state of thirst in animals was characterized by an increase in the mean frequency of US waves to 70.0±3.8 kHz ($p<0.05$ compared to the baseline; Table 1). The formation of drinking motivation in rats was accompanied by a shift in the power spectrum of US vocalization towards the dominant frequencies of 80-90 kHz (30.7%; Fig. 1, *b*). The power spectrum of US vocalization at a frequency of 40-50 kHz was 21% (*vs.* 36% in intact specimens). An experimental conflict situation in animals due to inability to satisfy water requirements for a long period was probably followed by the formation of a negative emotional state. It was manifested in a specific pattern of US vocalization. These changes persisted 1 h after the animals were returned to the home cages with *ad libitum* water supply. After the satisfaction of water requirements, the total duration and mean frequency of US vocalization in animals were 6228.4±1509.1 msec and 72.7±1.8 kHz, respectively. The dominant range of vocalization was 80-90 kHz (28.5%). These data are consistent with the results of previous studies. Increased generation of 18-24-kHz ultrasound by rats was observed not only during, but also after the exposure to a negative emotiogenic factor [3].

The formation of a negative emotional state in rats after immobilization and electrocutaneous stimulation was accompanied by a decrease in the total duration of US vocalization as compared to the baseline (Table 1). These changes were observed immediately and, particularly, 1 h after acute stress (by 1.5 and 4.8 [$p<0.01$] times, respectively). The mean frequency of US waves in stressed rats practically did not differ from the baseline. Acute emotional stress in animals was followed by changes in the distribution of dominant frequen-

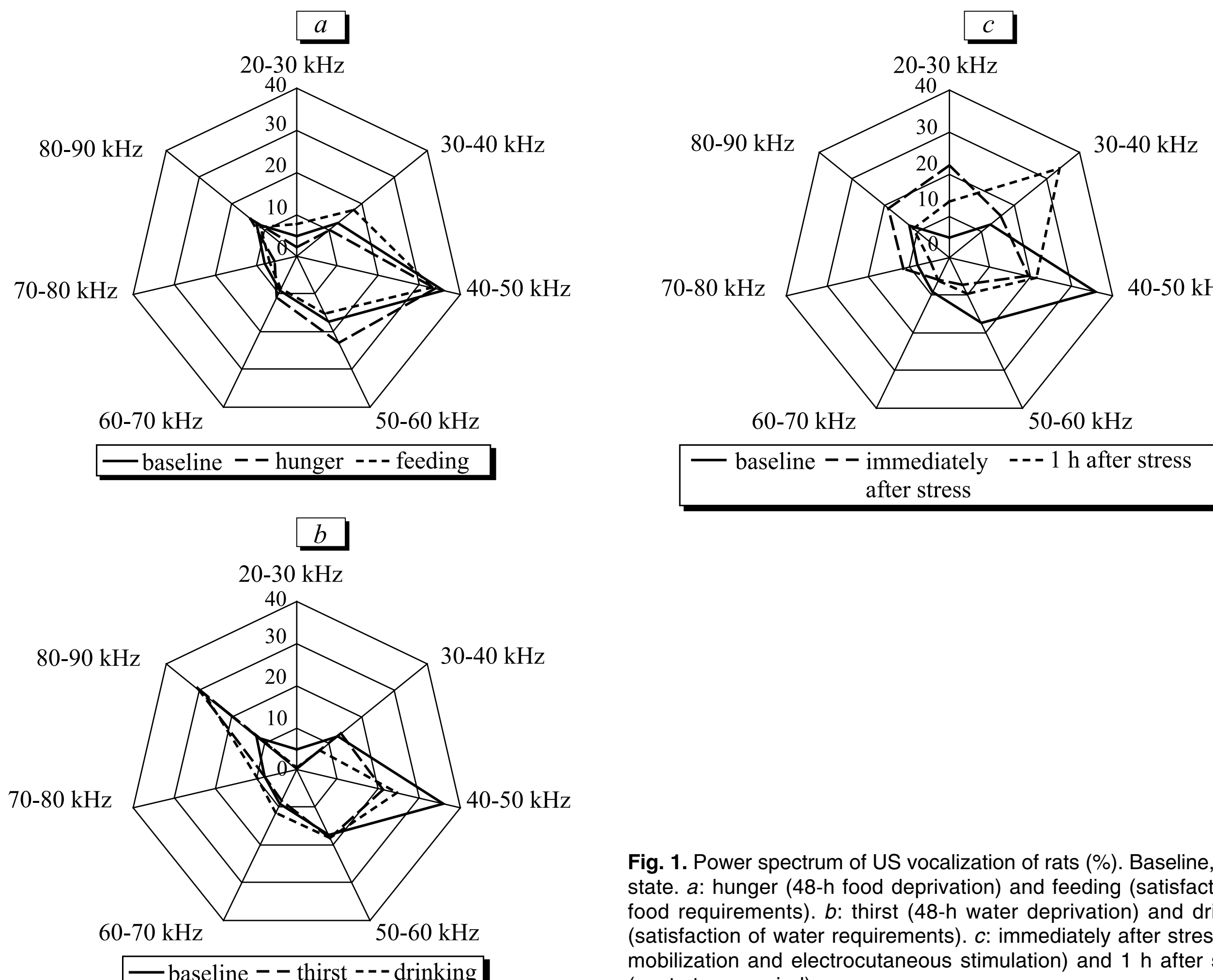

Fig. 1. Power spectrum of US vocalization of rats (%). Baseline, initial state. *a*: hunger (48-h food deprivation) and feeding (satisfaction of food requirements). *b*: thirst (48-h water deprivation) and drinking (satisfaction of water requirements). *c*: immediately after stress (immobilization and electrocutaneous stimulation) and 1 h after stress (post-stress period).

cies of US vocalization (Fig. 1, *c*). Stressed rats were characterized by a shift in the power spectrum of vocalization towards the dominant frequencies of 20-30 (22.0%), 40-50 (19.3%), and 80-90 kHz (18.8%). The power spectrum of other frequencies of US vocalization in animals immediately after stress exposure varied from 5.8 to 15.8%. One hour after emotional stress, we revealed a decrease in the power spectrum of US vocalization frequencies that appeared to be dominant immediately after stress (Fig. 1, *c*). During the post-stress period, the maximum power of US waves in animals approached the baseline (30-40 kHz, 33.9%).

There are contradictory data on the type of changes in US vocalization of mammals under the influence of adverse environmental factors. Some authors reported that US waves in rats during acute nociceptive stimulation reflect the emotional perception of pain by animals [5,6]. Other researchers showed that US vocalization remains practically unchanged in rats with chronic pain syndrome [7]. Studies on the models of visceral, inflammatory, and neuropathic pain in mice and rats revealed no relationship between US vocalization of animals and behavioral manifestations of the nociceptive response [14]. It was hypothesized that US vocalization does not serve as an objective behavioral criterion for the nociceptive response in rodents.

Opposite results were obtained in experiments on mammals exposed to the influence of negative emotiogenic factors. It was supposed that characteristics of US vocalization can serve as a criterion for the state of fear in animals [8]. Acoustic stimulation triggering startle response in rats is accompanied by changes in US wave generation. Acoustic stimulation was accompanied by a decrease in the duration of US vocalization and increase in the interpulse intervals. Under these conditions, the so-called "silent episodes" were observed (sudden and complete cessation of vocalization). These data are confirmed by the results of our study. We revealed that the formation of a stress state in rats after immobilization and electrocutaneous stimulation also results in a decrease in the total duration of US vocalization.

The observed changes in US vocalization during emotional stress supplement the results of previous studies. Published data show that adult rats demonstrate US vocalization with a frequency of 22 kHz under aversive conditions (*e.g.*, during unavoidable electrostimulation and acoustic stimulation) [12]. This vocalization was accompanied by the defense response and signaled a refractory, socially withdrawn, or helpless state. Brain structures involved in the mediation of anxiety-like behavior in mammals (*e.g.*, the dorsal periaqueductal gray and cortical areas) are also important for modulation of US waves. These data indicate that US vocalization of rats after the exposure to stress or nociceptive factors can serve as a quantitative measure of the emotional state of fear and anxiety in animals.

Our results and published data show that the formation of various motivational and emotional states in rats is characterized by the specific patterns of US vocalization. US vocalization in rats reflects the individual affective motivations that dominate in a certain period. The satisfaction of general biological requirements is also accompanied by emission of specific US waves in animals. These data illustrate the informational role of ultrasound in mammals. Therefore, the parameters of US vocalization can serve as one of the objective criteria for the subjective state of a living organism.

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