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Ultrasonic calls of bank vole pups isolated and exposed to cold or to nest odor

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Abstract

Bank vole pups produce ultrasounds when isolated from the nest, as other rodents do. The present study was intended to elucidate the possible interaction between the social stress of isolation from the mother and the physical stress of low ambient temperature during the nesting period. Although bank vole pups removed from nests and monitored at nest temperature vocalized at high frequency, the number and duration of signals increased at lower ambient temperature. In the tested voles it appears that exposure to cold was the most important stimulus of vocalization during the preweaning period. This effect can be enhanced by prolonged isolation from the mother, manifested as longer duration of calls. Moreover, vocalization was reduced not only by the odor from the home nest, but also by exposure to bedding of an alien lactating bank vole or even a lactating mouse fed the same diet. This suggests that the olfactory signals affecting the ultrasonic vocalization of bank vole pups probably are a mixture of volatile metabolites related to the physiological status and diet of rodent females. The reported experiments provide convincing evidence that the vocalization of bank vole pups is affected by isolation from the mother, by ambient temperature, and by olfactory signals released by lactating rodent females.

Keywords: Olfactory signals; Rodent; Social stress; Ultrasonic vocalization; Vole

1. Introduction

During the first weeks after birth, infant rodents depend on the mother for her feeding and body warmth. Isolation from the nest and exposure to low ambient temperature influences the behavior of pups; this is manifested as emission of ultrasounds. Behavioral observations and neurophysiological investigations indicate that ultrasounds are perceived by adult rodents and elicit behavioral reactions in them [1]. The auditory cortex area in the mouse brain has been established by electrophysiological mapping; perception of the ultrasonic field has been found to be localized in this part of the brain [2]. Ultrasonic calls produced by infants can be perceived by lactating female rats, and mothers are attracted to the source of the signals. High-frequency calls stimulate prolactin release [3,4] and promote typical maternal behavior: nest building [5], searching for pups, retrieving [6–11] and licking of pups [12].

The results of various experiments summarized and discussed by Smith and Sales [13] show that low ambient temperature, among the different stimuli that elicit ultrasonic calling in newborn rodents, is the most important factor stimulating ultrasounds, but those studies also demonstrate the importance of other factors affecting the ultrasonic vocalization of pups, such as tactile stimulation or olfactory signals.

Most information concerning ultrasonic vocalizations of pups has come from experiments on laboratory animals such as rats or mice. Here we investigated ultrasound responses to stressful conditions during postnatal development in the bank vole (*Clethrionomys glareolus*). In nature, bank voles inhabit almost all of Europe and the western part of Asia, where they live mainly in forests and feed on fruits, seeds and animal food such as larvae (for a review, see Ref. [14]). Field studies on the ecology and population dynamics of bank voles were carried out in the early 1960s. This species breeds seasonally and produces 3–4 litters per year (for reviews, see Refs [15,16]). Bank voles are relatively easy to breed in the laboratory; when reared in stable conditions, they reproduce throughout the year.

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Similarly to other *Microtidae*, *C. glareolus*, is characterized by provoked ovulation induced by mating; ovulation occurs 6 to 14 h after coitus [17]. Under laboratory conditions, ovulation also occurs in females without copulation when they are exposed to males present in the same cage but separated from the female by a net. Although copulation does not occur, tactile, olfactory, visual and auditory stimuli are exchanged between the animals [18]. Adult females are sensitive to male pheromones, as manifested by increased numbers of Graafian follicles in the presence of male urine [19].

Social interaction between adult bank voles is based on the olfactory system [20,21] and acoustic signals. During social encounters, both the female and the male emit audible sounds and also ultrasounds with a fundamental frequency of about 28 kHz [22]. Earlier we found that the olfactory system is not essential for reproduction of sexually experienced bank voles: males bulbectomized after the first mating copulated when paired with receptive females [23]. Also, when nine sexually experienced bank vole females were bulbectomized and paired with males after weaning of the first litter, six of them gave birth and weaned healthy pups [24]. Since bulbectomized females nurse the pups, it is clear that signals other than olfactory stimuli must be involved in communication between mothers and their young. It is well documented that bank vole pups produce ultrasounds when they are isolated from the nest [25,26].

The work reported here focuses on the reactions of pups to isolation from the mother and littermates, and to exposure to low temperature. Their behavioral responses were assessed in terms of the number of their calls and the duration and frequency of signals emitted during the nesting period. Two levels of isolation stress were applied: bank vole pups were isolated from the mother for 5 or 120 min before testing. We also wanted to know whether the reaction to that stress could be modified by nest odor. The protocol was designed to evaluate vocal behavior in response to situations imposed on bank vole pups during their nesting period.

2. Materials and methods

2.1. Animals

The experiments used bank voles (*C. glareolus*) and CBA mice (*Mus musculus*) reared in the Institute of Environmental Sciences, Jagiellonian University in Krakow. The animals were kept in polyethylene cages (42 × 26 × 17 cm) at 18–20 °C and 60–70% humidity under a 14 h photoperiod (lights on at 06:00). Standard pelleted chow (Labofeed H, Feeds & Concentrates) and water were provided *ad libitum*. Wood shavings were used as bedding material and changed weekly.

Adult 2–3-month-old bank voles were paired (one female per male). Pregnant females were separated from the males and kept one per cage. The day of the neonates' birth was defined as day 0 of life, and the bank vole pups stayed with the mother until testing. Nest temperature was measured with a thermohigrometer (Abatronic, type AB 06912); 33–35 °C was recorded with the tip of the cord inserted into nests with 3–5-day-old pups hooded by the mother.

2.2. General procedures

The animals in their home cages were transferred from the colony room to the laboratory about 24 h before testing, and the tests were performed between 10:00 and 14:00. A single pup was taken from its home cage, placed in a 5 cm diameter glass dish and immediately located on a heating pad at 35–37 °C for 3–5 min. After this period the glass dish with the pup was transferred to an acoustically isolated chamber (72 × 72 × 75 cm). The temperature in the chamber during the test was maintained at 35–37 °C or 18–20 °C. Only 1 or 2 pups from the same litter were used per experimental group, and each pup was tested only once. After the test each pup was weighed.

2.3. Ontogenic development and ultrasonic vocalization

The vocalization of 192 pups from 97 litters between days 2 and 18 of life was tested in the absence of bedding. The pups were divided into the following three age groups: first week of life (2, 4 and 6 days old; pups naked or with very poorly developed pelage, movements limited to the nest); second week of life (8, 10 and 12 days old; improved pelage, opening of eyes between 10 and 12 days of life, moving actively around the nest area); and third week of life (14, 16 and 18 days old; well-developed pelage, very active, moving and exploring the territory outside the nest). Ultrasonic vocalization was monitored as described below.

2.4. Effect of short or prolonged isolation of pups on ultrasonic vocalization

Ultrasonic vocalization was assessed in 5–6-day-old bank voles weighing 3.2–5.3 g. The animals were left undisturbed with the mother, or else the mother was taken from the litter and the pups were kept in home cage but warm (33–37 °C) with a lamp (Day-GloA21/150 W for terraria) for 2 h before testing. Each pup was checked for the presence of milk in the stomach (at 5–6 days, bank voles are sparsely furred and the milk band in the stomach is visible). The 46 pups from 25 litters kept with the mothers showed stomachs filled with milk, while the 49 animals from 25 litters isolated from the mothers had empty stomachs. Ultrasonic vocalizations of single pups were monitored in an acoustically isolated chamber at 35–37 °C or 18–20 °C as described below.

2.5. Effect of bedding on ultrasonic vocalization

Tests were performed on 5–6-day-old bank voles weighing 3.1–4.7 g. In total, 214 pups from 109 litters were randomly exposed to familiar or unfamiliar odors. Ultrasounds were monitored in the absence of bedding or in the presence of clean bedding (wood shavings), home bedding, bedding from the cage of an alien bank vole nursing 5-day-old pups, or bedding from the cage of a CBA mouse nursing 5-day-old pups. For each test, 6 to 9 different nests were used as bedding donors.

An individual pup was removed from its home cage, placed in a 5 cm diameter glass dish and kept on a heating pad at 35–37 °C for 3–5 min. After this period, approximately 2 cm^3 of the tested sample was placed on a dish next to the pup and the animal had direct contact with the tested samples. The container with the pup was transferred to an acoustically isolated chamber, and vocalization was monitored at 35–37 °C or 18–20 °C.

2.6. Analysis of vocalization

The vocalization of the pup was monitored and recorded for 1 min with an ultrasound receiver suspended 5 cm above the tested animal. The microphone was connected to an ultrasound detector (type S30, QMC, UK) set to ultrasonic range (20–110 kHz) and coupled to a cassette recorder (Sony, HX PRO). For assessment of the pup's development, behavior in the home cage was observed before the animal was taken for experiments, and pelage condition, ear development and eye development were described after the vocalization test.

Recorded sounds were analyzed with Canary v.1.2 (Cornell Bioacoustic Workstation, Cornell University, U.S.A.). The number of ultrasounds emitted during 1 min sessions was counted. The first five pulses of each test (or fewer if not enough calls were produced) were averaged for estimation of the duration and frequency of calls.

2.7. Statistical analysis

Statistical calculations used the means of the values recorded from each tested nest. One or two pups per nest were used. Transformed data were analyzed by the Kruskall–Wallis test for comparison of the effects of age and temperature on the number of signals emitted by the pups. Two-way ANOVA was used for the remaining experimental groups, followed, where necessary, by one-way ANOVA or post-hoc pair-wise comparisons using Tukey's test (SAS v. 8.2—SAS Institute Inc., Cary, NC, U.S.A.).

3. Results

3.1. Effect of isolation and ambient temperature on ultrasonic vocalization of bank vole pups during postnatal development

Bank vole neonates are poikilothermic at birth and completely dependent on the mother. They stay in the nest until developed at 17–18 days of life. Visible pelage appears around day 4 of life and slowly improves during the following days. As described in the Materials and methods, morphological development is accompanied by increased behavioral activity. During the first week, bank voles show little mobility; their movement is limited to the nest. After the eyes open at the end of the second week, the animals move around the nest area, and during the third week they start exploring territory outside the nest.

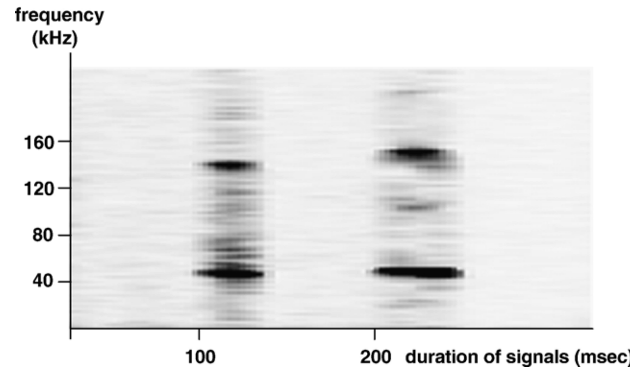

Fig. 1. Spectrogram of ultrasounds emitted by 5-day-old bank vole isolated from the nest and tested at 18°–20 °C during 1 min.

Table 1 summarizes the number of bank vole pups tested and vocalizing at high frequency during the nesting period. The spectral frequencies of calls (Fig. 1) emitted by 2–18-day-old pups tested at 35–37 °C or 18–20 °C ambient temperature were similar, with the fundamental frequency ranging from 25 to 65 kHz.

The animals were divided into three age groups, and ultrasonic calls were compared between 1-, 2- and 3-week-old bank voles. Vocalization of bank voles isolated from the nest depended on their age and on ambient temperature (Fig. 2a). As indicated by the percentage of nests with vocalizing pups, more animals produced ultrasounds when tested at 18–20 °C ambient temperature than at 35–37 °C.

The number of calls emitted by the young (Fig. 2b) and the duration of pulses (Fig. 2c) were influenced by the age of the animals and by ambient temperature during the test. Voles in the first and second weeks of life vocalized at 35–37 °C; 3-week-old pups tested in the same conditions did not produce

Table 1
Vocalization and body weight (g) of bank voles during preweaning period

Age		35–37 °C			18–20 °C		
In weeks	In days	No. of tested litters (pups)	Body mass of tested pups (g)	No. of vocalizing litters (pups)	No. of tested litters (pups)	Body mass of tested pups (g)	No. of vocalizing litters (pups)
1st	2	6 (12)	2.54±0.066	5 (8)	6 (12)	2.68±0.130	6 (11)
	4	6 (12)	3.88±0.101	2 (3)	6 (12)	3.42±0.174	6 (11)
	6	6 (12)	4.63±0.278	3 (4)	6 (12)	3.96±0.137	6 (10)
2nd	8	4 (8)	5.86±0.267	1 (2)	6 (12)	5.08±0.219	4 (6)
	10	6 (12)	5.44±0.188	3 (4)	6 (12)	6.34±0.144	6 (9)
	12	6 (12)	6.54±0.107	2 (2)	6 (12)	6.67±0.245	5 (8)
3rd	14	7 (11)	7.45±0.370	0 (0)	6 (12)	7.13±0.290	6 (9)
	16	4 (8)	7.38±0.193	0 (0)	3 (6)	8.12±0.462	2 (2)
	18	4 (8)	7.70±0.328	0 (0)	3 (6)	9.83±0.367	0 (0)

Number of litters, number of pups (in parenthesis) tested and vocalized at 35°–37 °C or 18°–20 °C during 1 min (mean±SE).

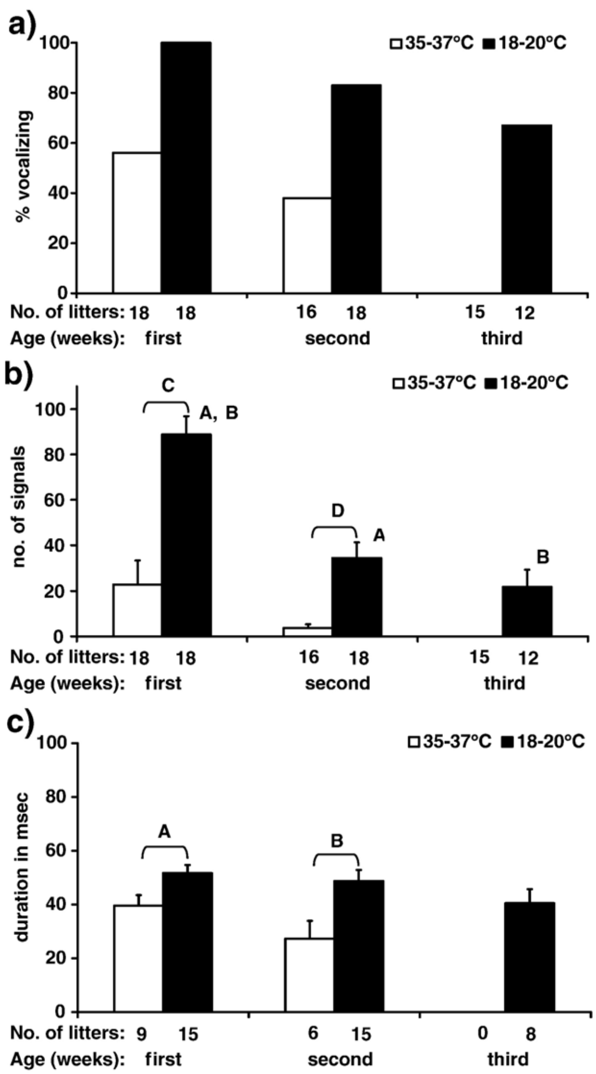

Fig. 2. Ultrasonic vocalization of 1-, 2- and 3-week-old bank voles isolated from the mother and tested at 35°–37 °C or 18°–20 °C during 1 min. Mean bearing the same letter differ significantly. a) Percentage of litters with ultrasonic vocalized pups. b) Number of ultrasonic calls produced by pups. A, B, C — $p<0.0001$; D — $p<0.01$. c) Duration (ms) of ultrasonic calls produced by pups. A — $p<0.05$; B — $p<0.01$.

ultrasonic calls (Fig. 2a). The mean number of signals declined with the age of the pups, but there was no statistically significant difference between 1- and 2-week-old pups [$H(1, 34)=2.13$, $p=0.14$]. Pups isolated from the nest and tested at 18–20 °C ambient temperature emitted ultrasounds during the first, second and third weeks of life. The number of ultrasonic signals declined with age [$H(2, 48)=23.30$, $p<0.0001$]. We found differences between 1- and 2-week-old [$H(1, 36)=16.67$, $p<0.0001$] and between 1- and 3-week-old week-old pups [$H(1, 30)=15.71$, $p<0.0001$] but not between 2- and 3-week-old animals [$H(1, 30)=1.47$, $p=0.22$].

Comparison of the number of calls emitted by same-age bank voles tested at 18–20 °C or 35–37 °C (Fig. 2b) showed that low ambient temperature increased vocalization in 1-week-old [$H(1, 36)=16.07$ $p<0.0001$] and 2-week-old [$H(1, 36)=10.36$, $p<0.005$] animals.

The duration of calls was influenced by ambient temperature (Fig. 2 c). Bank voles of the same age emitted longer calls at 18–20 °C than at 35–37 °C. That difference was significant for 1-week-old [$F(1, 22)=5.97$ $p<0.05$] and 2-week-old animals [$F(1, 19)=8.34$, $p<0.01$].

3.2. Effects of short or prolonged isolation from the mother on ultrasonic vocalization of 5–6-day-old pups tested at 35–37 °C or 18–20 °C

Like other rodents, young bank voles are fed regularly by the mother. Pups kept for 2 h in the home cage at 35–37 °C but without the mother had no visible white milk band in the stomach. The fundamental frequency of recorded calls ranged from 39 to 50 kHz.

Vocalization after prolonged isolation was compared with the production of ultrasounds by pups separated from the mother for 5 min. To test the interactive effect of prolonged isolation and cooling on the ultrasonic vocalization of 5–6-day-old bank voles, the animals were monitored at 35–37 °C or 18–20 °C. Table 2 summarizes the results on vocalization at high frequency after short-term and prolonged isolation from the mother at those ambient temperatures. The percentage of vocalizing animals did not depend on ambient temperature or on the duration of isolation from the mother (Fig. 3a), but the number of calls increased at low temperature. The results presented in Fig. 3b and analyzed by two-way ANOVA show that the voles produced more calls at 18–20 °C than at 35–37 °C [$F(1, 48)=32.72$, $p<0.0001$]. There was no effect of time isolated from the mother (5 or 120 min) on the number of calls [$F(1, 48)=0.93$, $p=0.34$], and no interaction between these two treatment factors [$F(1, 48)=0.84$, $p=0.36$]. The pups produced fewer ultrasounds at 35–37 °C than at 18–20 °C, both after 5 min [$F(1, 23)=12.12$, $p<0.005$] and after l20 min isolation [$F(1, 23)=21.56$, $p<0.0001$].

The duration of pulses produced by 5–6-day-old vole pups (Fig. 4c) isolated from the mother for 5 or 120 min, monitored at 18–20 °C or at 35–37 °C, was compared by two-way ANOVA. Pulse duration was affected by low temperature [$F(1, 64)=10.27$, $p<0.005$] and prolonged isolation [$F(1, 64)=7.25$, $p<0.01$]. There was significant interaction between these two factors [$F(1, 64)=9.99$, $p<0.005$]. Pups tested at 18–20 °C produced longer pulses after 120 min isolation than after 5 min isolation [$F(1, 46)=29.40$, $p<0.0001$], and also longer pulses

Table 2
Vocalization of 5–6 day-old bank voles isolated from the mother for 5 or 120 min

Temperature:	35°–37 °C		18°–20 °C	
Isolation	No. of tested litters (pups)	No. of vocalizing litters (pups)	No. of tested litters (pups)	No. of vocalizing litters (pups)
5 min	11 (22)	7 (7)	14 (24)	13 (21)
120 min	12 (23)	11 (11)	13 (26)	12 (24)

Number of litters, number of pups (in parentheses) tested and vocalized at 35°–37 °C or 18°–20 °C during 1 min.

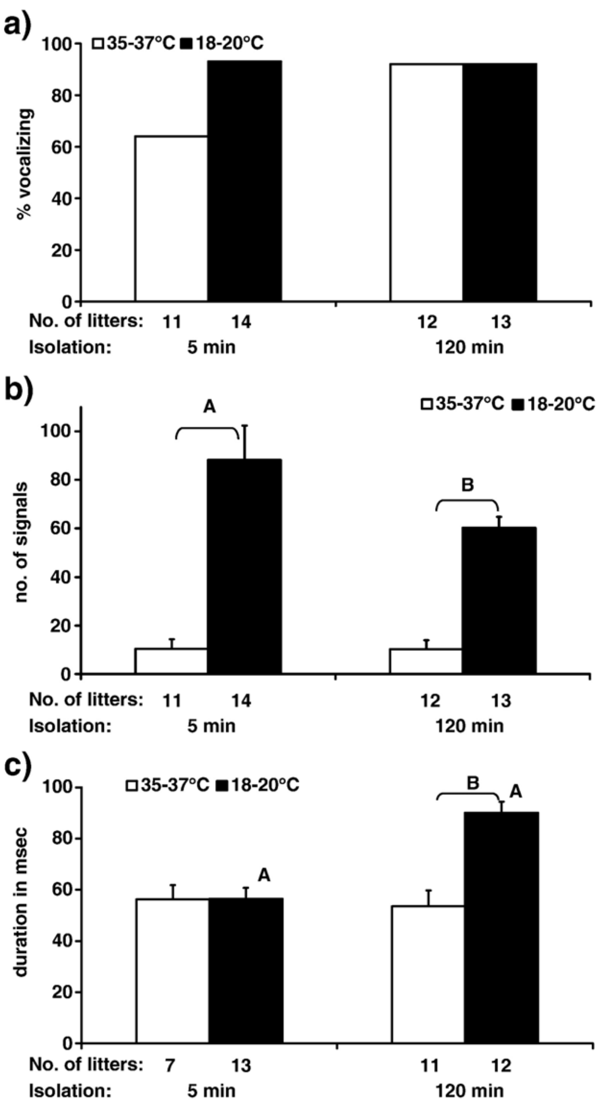

Fig. 3. Ultrasonic vocalization of 5– 6 day-old bank voles isolated from the mother for 5 or 120 min and tested at 35°–37 °C or 18°–20 °C during 1 min. Mean bearing the same letter differ significant. a) Percentage of litters with ultrasonic vocalized pups. b) Number of ultrasonic calls produced by pups. A — $p<0.01$; B — $p<0.01$. c) Duration (ms) of ultrasonic calls produced by pups. A, B — $p<0.0001$.

than pups isolated for 120 min and tested at 35–37 °C [F(1, 36)=22.17, $p<0.0001$].

3.3. Effect of nest odors on ultrasonic vocalization of 5–6-day-old pups isolated and tested at 35–37 °C or 18–20 °C

Bank vole pups monitored during 1 min tests in the presence of different beddings at 35–37 °C or 18–20 °C produced calls (Table 3) at similar fundamental frequencies (range 40–53 kHz), but the percentage of vocalizing animals was higher during testing at 18–20 °C than at nest temperature (Fig. 4a). Analysis by two-way ANOVA showed that the number of calls (Fig. 4b) was also affected by ambient temperature [F(1, 107)=76.71, $p<0.0001$] but not by differences in bedding [F(4, 104) 0.39, $p=0.81$] or by the interaction between these two treatment factors [F(4, 107)=0.43, $p=0.78$]. Pups produced more calls at 18–20 °C than at 35–37 °C when tested on a clean plastic dish [F(1, 20),=6.58, $p<0.05$], in the presence of clean bedding [F(1, 21)=9.28, $p<0.01$], home bedding [F(1, 21)=30.45, $p<0.0001$], alien bank vole nest bedding [F(1, 19)=25.32, $p<0.0001$] or mouse bedding [F(1, 18)=24.67, $p<0.0001$].

The duration of calls produced by pups in the presence of different beddings was measured at 35–37 °C and 18–20 °C ambient temperature (Fig. 4 c). Two-way ANOVA indicated that call duration was not affected by temperature [F(1, 109)=3.109, $p=0.08$] but was influenced by nest odor [F(1, 106)=3.02, $p<0.05$]. The interaction between these two factors was not statistically significant [F(1, 109)=0.71, $p=0.58$]. The presence and type of bedding influenced the duration of pulses produced by pups at 18–20 °C [F(1, 82)=2.76, $p=0.05$]. Tukey's test confirmed that pups exposed to the home nest produced

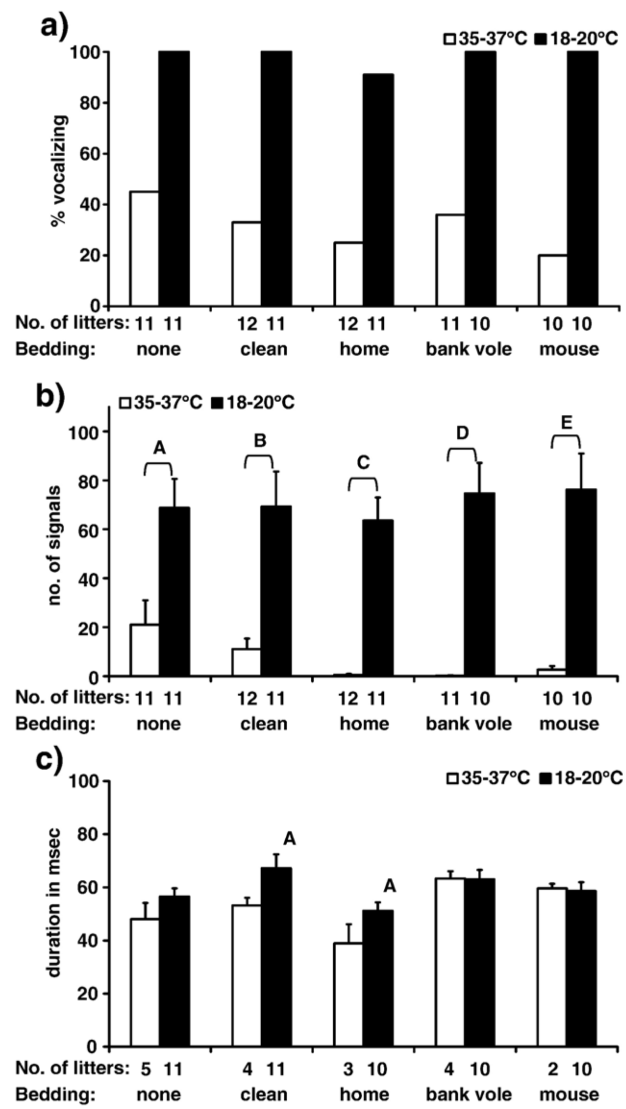

Fig. 4. Ultrasonic vocalization of 5–6 day-old bank voles isolated from the mother and tested without bedding or in presence of clean, home nest, bank vole nest or mouse nest bedding at 35°–37 °C or 18°–20 °C during 1 min. Mean bearing the same letter differ significantly. a) Percentage of litters with ultrasonic vocalized pups. b) Number of ultrasonic calls produced by pups. A, B, C, D, E — $p<0.05$. c) Duration (ms) of ultrasonic calls produced by pups. A — $p<0.05$.

Table 3
Vocalization of 5–6 day-old bank voles isolated from the mother and tested without bedding or in the presence of clean, home nest, bank vole nest or mouse's nest bedding

Temperature	35°–37 °C		18°–20 °C	
Presented bedding	No. of tested litters (pups)	No. of vocalizing litters (pups)	No. of tested litters (pups)	No. of vocalizing litters (pups)
None	11 (22)	5 (7)	11 (22)	11 (18)
Clean	12 (24)	4 (8)	11 (22)	11 (18)
Home	12 (22)	3 (3)	11 (21)	10 (18)
Bank vole' nest	11 (22)	4 (4)	10 (20)	10 (20)
Mouse's nest	10 (19)	2 (2)	10 (20)	10 (17)

Number of litters, number of pups (in parentheses) tested and vocalized at 35°–37 °C or 18°–20 °C during 1 min.

significantly shorter calls those monitored in the presence of clean bedding ($p<0.05$).

4. Discussion

During the first 2–3 weeks after birth, the infant rodent depends on the warmth of the mother and on the presence of siblings in the same nest. Pups removed from the nest are stressed both by lower ambient temperature and by the absence of the mother, and they emit ultrasounds. Bank vole pups monitored in the nest with the mother present do not produce ultrasounds (data not shown), unlike those removed from the nest and tested at 18–20 °C or even at 35–37 °C ambient temperature. Our experimental protocol was designed to elucidate the possible interaction between the social stress of isolation from the nest and the physical stress of low ambient temperature.

In these experiments the number of calls was higher when the animals were tested at room temperature than at nest temperature. The temperature measured directly in nests of 3–5-day-old pups hooded by the mother in a cage was around 33–35 °C, and the temperature in the soundproof chamber we used (35–37 °C) was close to the thermal conditions of a bank vole nest. Pups removed from nests and monitored at nest temperature vocalized at high frequency, but the number and duration of the signals increased significantly at lower ambient temperature. The reaction of bank vole pups to stress, expressed as the number of calls, was highest during the first week of life and decreased during the next two weeks.

Studies using pups of other rodents, including laboratory mice [27], rats [28,29], several species of *Microtidae* [30–33], Mongolian gerbils [34] and California mice [35] indicate that individual species differ considerably in the number and sound frequency of emitted ultrasounds, but the general pattern of vocalization during the nesting period is similar. A difference between sexes has been recorded in *Peromyscus californicus* pups. In California mice, females vocalized more than males [36], but no difference between sexes was found for *C. glareolus* pups [37].

Ultrasonic vocalization starts during the first week of life, and usually disappears before weaning. Studies on rats selected for high and low vocalization [38–40], and similar studies in mice [41–43], show that the reaction of young to isolation from the nest, expressed as high-frequency calls, depends on the genotype of the animals but can be modified by environmental factors.

The social system of the species also has a certain influence on the reaction of pups to isolation from a nest. Pups of prairie voles, which are characterized by biparenatal care, emit more ultrasounds than pups of montane voles, a typical promiscuous species [44,45]. The difference in vocalization between the two species is not related to their ability to keep warm after removal from the nest, since pups of both species show a similar drop of body temperature when isolated from the mother. However, they exhibit a different pattern of vocalization during the nesting period [46].

Our results demonstrate that bank vole pups' ultrasound calls are influenced by prolonged absence of the mother from the nest. Separating 5–6-day-old animals from the mother for 2 h before testing increased the percentage of vocalizing pups monitored at 35–37 °C. The ones tested at 18–20 °C produced significantly longer ultrasound calls than those kept away from the mother for only 5 min before testing. Separating bank vole pups from the mother for 2 h did not affect their physical condition, but there was no milk in the pups' stomachs. The lack of milk may possibly stimulate the gastric interoceptive sensory system, and that could affect behavior, including ultrasonic vocalization.

In many species of rodents the number of calls decreased when the animals were exposed to the nest odor [47,48]. Brunelli et al. [49] found that if rat pups reared by both parents were isolated and subsequently exposed to their anesthetized mother or father, vocalization was inhibited by the presence of either parent. Myers et al. [50] reported that the number of calls and the average amplitude of ultrasounds were increased following brief interaction with the mother during an initial isolation period. This implies that social interaction between the mother and pups affects the ultrasonic vocalization of pups isolated from the nest.

In promiscuous species such as the rat, the number of signals emitted by young decreased in the presence of male bedding, and this was interpreted as antipredator behavior [51–53]. However, mouse pups produce significantly more calls after exposure to urine from infanticidal males than in the presence of urine from non-infanticidal males [54]. Inbred mice are able to distinguish the odor of their own genotype, and can discriminate the sex of adult animals. Pups of CBA mice produced shorter ultrasounds in the presence of CBA females, and longer signals after exposure to C57 BL males [55].

Blumberg and co-workers [56,57] interpret the ultrasonic vocalization of young rodents as a reaction to the thermal consequences of isolation from the mother and littermates, and regard these vocalizations as an acoustic by-product of the physiological action that maintains cardiopulmonary homeostasis. They demonstrated that the brainstem's neural circuits are adequate for the production of ultrasounds, but also suggested that forebrain mechanisms modulate the vocal response to cold stress in postnatal day 8 pups [58]. Moreover, there is abundant evidence that the ultrasonic response of pups to isolation from the nest is modified by different social factors, including olfactory stimuli: the odor of the home nest or of adult conspecifics [41,44,47,51,59–61]. Newborn rodents have a well-developed olfactory system which begins to be functional at the end of gestation; the sensory capabilities of this system are developed during the first days after birth [62–65]. Olfactory perception in infant rats has been assessed on the basis of physiological parameters such as the heart rate-orienting response; starting from postnatal day 4, pups showed discrimination between novel olfactory signals, and habituation to odor [66,67].

As discussed above, most information about the influence of social factors on ultrasonic vocalization of pups has been obtained from studies with laboratory animals, mostly inbred strains. Our experiments used bank voles from a highly heterozygous colony (67–69 generations), introduced to the laboratory in 1976 and maintained according to a system described by Green [68]. Working on the same colony, Kapusta et al. [26] showed that bank vole pups isolated from the nest and exposed to home bedding at room temperature produced fewer and shorter calls than when tested in the absence of bedding. In the present study we found that bank vole pups exposed to home bedding emitted shorter calls than in the presence of clean bedding when tested at 18–20 °C, but the decrease of vocalization in the presence of nest beddings was more pronounced when the pups were tested at 35–37 °C. Fewer calls were recorded not only in response to home bedding, but also in response to the nest bedding of an alien lactating bank vole, or even a lactating laboratory mouse fed the same diet (Fig. 4b). No statistically significant differences between beddings were found, but the influence of nest odor from lactating females cannot be excluded. Porter and Doane [69] showed that *Acomys cahirinus* pups do not discriminate between the odors of the mother and lactating laboratory mice maintained on the same diet.

Neurophysiological studies on such laboratory animals as mice and rats indicate that ultrasounds are produced as an effect of physiological alterations induced by cooling, and thus accompany increased thermogenesis. Moreover, abundant data support the view that olfactory cues play an important role in enhancing or inhibiting ultrasonic vocalization. Our present results from experiments with bank voles, as well as numerous data from the literature on ultrasonic vocalization of infant rodents, indicate that generation of ultrasounds during cooling is a common feature of poikilothermic rodent newborns. Modulation of ultrasound emission by olfactory signals, such as nest odor, has been documented and confirmed by our work. Bank vole pups do not respond to the presence of a virgin bank vole female or a male of the same species [26] but show reduced vocalization in the presence of an alien lactating bank vole or even a lactating laboratory mouse fed with the same diet, so it is reasonable to suggest that the olfactory signals are a mixture of compounds related to the physiological status of females and to their diet. Although the mechanisms of generation of high-frequency sounds are not yet fully understood, there is no doubt that ultrasound signals are an important element of the interaction between progeny and parents; they may benefit survival during the early postnatal development of rodents.

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