Available online at www.sciencedirect.com
ScienceDirect
Physiology & Behavior 93 (2008) 296–303
PHYSIOLOGY & BEHAVIOR

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

H. Szentgyörgyi, J. Kapusta, A. Marchlewska-Koj *

Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland

Received 2 January 2007; received in revised form 23 August 2007; accepted 3 September 2007

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.

* Corresponding author. Tel.: +48 12 664 52 07; fax: +48 12 664 67 12.
E-mail address: amkoj@eko.uj.edu.pl (A. Marchlewska-Koj).

0031-9384/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2007.09.015

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 thermo-higrometer (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