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ScienceDirect
Behavioural Brain Research 182 (2007) 208–222
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

# Situational factors, conditions and individual variables which can determine ultrasonic vocalizations in male adult Wistar rats

Rainer K.W. Schwarting *, Nikita Jegan, Markus Wöhr

*Experimental and Physiological Psychology, Philipps-University of Marburg, Gutenbergstr. 18, 35032 Marburg, Germany*

Received 11 September 2006; received in revised form 23 January 2007; accepted 31 January 2007
Available online 9 February 2007

**Abstract**

The fact that rats emit different types of ultrasonic vocalizations in a variety of contexts has received increasing experimental attention, since such calls might serve as indices of the animal's subjective state, and/or as social signals in various types of interactions with other rats. Here, we present two experiments in adult male Wistar rats where we tested several different situations and conditions with respect to the occurrence of high-frequency (50-kHz) and low-frequency (22-kHz) calls. These experiments showed that rats emitted high-frequency calls when tested singly in a housing cage, which was situated in a room with no other rats present. Calling did not habituate with repeated testing, and occurred in the animal's own home cage, or a fresh housing cage, and irrespective of whether the animal's motivational status was high or low, that is, irrespective of whether they were food-deprived or fed ad libitum. Furthermore, high- and low-frequency calls were observed when applying a standardized new tickling procedure, which provided evidence for effective types of tickling. Most, but not all, young adult rats still accepted this stimulation as play. Therefore, this procedure might be a useful method to elicit high-frequency calls in adult rats. Overall, substantial evidence for inter-individual variability and intra-individual stability in vocalization was provided both, within and between housing cage and tickle tests. This variability seems to depend at least partly on dispositions or traits, which can be gauged by specific screening tests, like measuring risk-assessment in the elevated plus-maze, since animals with more risk-assessment were more likely to emit high-frequency calls during cage and tickle tests. These findings are discussed with respect to the major hypotheses concerning the functional significance of ultrasonic vocalizations, namely the social/communicatory and the motivational/emotional hypothesis.


*Keywords:* Home cage; Motivation; Emotion; Tickling; Locomotor activity; Risk-assessment; Elevated plus-maze; Fear conditioning; Ultrasonic vocalization (USV)

## 1. Introduction

Since the middle of the last century it is known, that rats can emit ultrasonic vocalizations [1,44]. Also, distinct call types can be defined, the occurrence of which differs depending on the animal's age, its current status, and environment factors. Functionally, such calls may reflect specific motivational and affective states, and may serve as important social signals to conspecifics [14,39,59].

Adult and juvenile rats produce calls in two major ultrasonic ranges. On the basis of their sound frequency, these calls have been classified as low- and high-frequency vocalizations. Low-frequency vocalizations, often termed 22-kHz calls, are emitted when rats are exposed to predators [9], foot-shocks [2,13,27,35,65,69,76], during inter-male aggression [36,70], drug withdrawal [5,26], and handling [16,18]. Accordingly, it was assumed that these calls reflect a negative affective state akin to anxiety and depression [27,35,55,65].

High-frequency vocalizations, often termed 50-kHz calls, occur in several different contexts including juvenile play [37], and mating [4,53,74]. Furthermore, they are expressed during anticipation of positive consequences, like sex [8,40], play [37], food [19], electrical stimulation of mesolimbic pathways [19], and drugs of abuse [20,38,64,75]. Also, one can effectively induce 50-kHz calls via simulated play, that is, manually by an experienced human experimenter (termed "hetero-specific play" or "tickling") [21,46,47]. Besides tickling itself, even the presentation of cues associated with it are effective to elicit 50-kHz vocalizations in juvenile rats [21,46,47]. Therefore, it has been postulated that such calls "could index a positive and aroused emotional state akin to the appetitive excitement typically associated with reward seeking" (cited after [48, p. 464], see also

* Corresponding author. Tel.: +49 6421 28 23639; fax: +49 6421 28 23610.
*E-mail address:* schwarti@staff.uni-marburg.de (R.K.W. Schwarting).

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doi:10.1016/j.bbr.2007.01.029

[38,39]). Finally, Panksepp and Burgdorf [47] considered 50-kHz ultrasonic vocalizations as a rat homolog or antecedent of human laughter or joy.

Other evidence, however, shows that the occurrence of 50-kHz calls is apparently not solely dependent on reward. Rats, especially intruders, emit 50-kHz calls during aggressive encounters such as in resident-intruder tests [31,36,41, 52,54,56,60,63,69,70]. Rats also emit bursts of 50-kHz calls when entering an area associated with the potential presence of an aggressor [66,67]. Finally, 50-kHz calls were also detected in various experimental controls, for example, naive rats that were exposed singly to a test arena containing fresh bedding [17,40], or saline-injected rats in various drug studies [38,64,75]. These results indicate that 50-kHz vocalizations can occur in non-rewarding or possibly even aversive contexts as well. Indeed, Berridge [7] has questioned the interpretation that 50-kHz represent a rat homolog of human joy.

The present two experiments were performed to investigate such issues further, that is, we wanted to identify factors, which might be effective in eliciting 50-kHz calls in young adult male Wistar rats. In pilot work, we had observed that food-deprived rats spontaneously emit 50-kHz calls when being exposed singly to a housing cage, with no other cages next to them. In experiment I, we asked if such calling depends on features of the cage, namely whether it is the own, familiar home cage, or an identical but fresh one. In experiment II, we asked if such vocalization would also occur in satiated rats. In addition, we tested whether "tickling" may also be effective in case of our young adult rats, which we tested by applying a standardized new tickling procedure. Furthermore, we paid particular attention to inter-individual variability in ultrasonic calling. Such variability has repeatedly been reported before, both with respect to high- and low-frequency calls (e.g. [13,16,21,22,37,38,43,68,75,76]), and has also served to breed rat lines which differ in this behavioral feature [23]. We tested this aspect by correlating vocalizations between repeated tests of the same kind (e.g. tickling; retest–reliability), or between different tests (housing cage versus tickling). Furthermore, we correlated vocalizations with ongoing motor behavior and body weight, and with behavior in separate screening tests, namely activity box and elevated plus-maze. It is known that such screening tests are useful to gauge behavioral dispositions or traits, which can partly predict spontaneous and drug-induced behavior in a number of other tasks [25,28,42,58]. Therefore, we expected that these tests might identify behavioral measures, which can help to explain variability in ultrasonic calling. The usefulness of this approach recently has been demonstrated in the case of anxiety-related behavior in the plus-maze, which was related to 22-kHz calls in a fear-conditioning paradigm [13].

## 2. Materials and methods

### 2.1. Experiment I—spontaneous high-frequency calling

#### 2.1.1. Animals

In experiment I, 19 male Wistar rats were used which weighed 159–218 g at the beginning of the study. Until 2 days before the experiment, they were housed in group cages (four to five rats each; Macrolon type IV); thereafter, they were kept individually in type III cages (size: 378 mm × 217 mm × 180 mm plus high stainless steel covers) with Tapvei® peeled aspen bedding (indulab ag, Gams, Switzerland) in an animal room with a 12:12 h light/dark cycle (lights on 07:00–19:00 h). Until the period of behavioral testing, rat chow (Altromin, Lage, Germany) and tap water were provided ad libitum. Prior to testing, the rats were handled and gentled for several days in a non-standardized way.

#### 2.1.2. Behavioral testing—spontaneous calling in a housing cage test

The animals were randomly assigned to two groups: subjects in one group were tested in their home cages ($n = 9$), whereas those from the other group were tested in identical but fresh cages ($n = 10$). For testing, the cage of a given animal was removed from the cage array and was placed on a desk in the same room. Animals of the fresh cage group were removed manually from their home cage and placed into a clean cage (type III) with fresh bedding (Tapvei) and a clean stainless steel cover. Then, this cage was carried to the testing room, which was situated next door to the animal room. Animals of the home cage group were treated in the same way, except that they were placed back into their own cage instead of a new cage.

The testing room was equipped with an air-exchange system, which provided a constant flow of fresh air. In this room, where no other animal was present, the cage was placed on a desk which was surrounded by two curtains (illumination level inside: 2 lx) to visually separate the animal from the experimenter, who was supervising data acquisition. For monitoring of ultrasonic vocalizations, an UltraSoundGate Condenser Microphone CM 16 (Avisoft Bioacoustics, Berlin, Germany) was used which was sensitive to frequencies of 15–180 kHz, with a flat frequency response (±6 dB) between 25 and 140 kHz. This microphone was placed centrally about 10 cm above the cage cover. Additionally, a video camera (Digital Handycam DCR-TRV239E, Sony, Japan), which was connected to a DVD recorder, was placed on one of the longitudinal sides of the cage to monitor overt behavior. The animal remained in this situation for 15 min. Thereafter, it was brought back to the animal room. There, rats of the fresh cage group were placed back into their home cages. This test was performed on four consecutive days. Both groups were food-deprived during this period, that is, food was always removed in the evenings preceding a day of testing. They received their daily food access (Altromin, Lage, Germany, 3 h access) no sooner than 50 min after each testing to avoid temporal contingencies between housing cage tests and food availability.

#### 2.1.3. Behavioral analysis

Spontaneous behavioral activity during the cage tests was measured by observation of rearing and locomotor activity. Rearing was quantified as the number of times the animal reared on its hind legs. For locomotor activity, the cage was divided into two virtual halves, and the numbers of crosses between these two halves were counted. Both behavioral parameters were measured throughout the whole tests, which lasted 15 min each.

### 2.2. Experiment II—spontaneous calling, tickle-induced calling and individuality

#### 2.2.1. Animals and general procedure

Twenty male Wistar rats were used, which weighed 192–226 g at the start. All rats were treated in an identical manner. During days 1–12, they were kept in groups of five (type IV cages), and thereafter singly (type III cages). In contrast to experiment I, they always had unrestricted access to food. Also, it was decided to apply a standardized handling procedure, since manual interactions during the tickle tests played a major role in this experiment. The handling procedure consisted of the following components: stroking the rat from head to tail with the left or right hand (30 s each), stroking its flanks with either hand (20 s each), pushing the neck fur caudally with either hand (30 s each), holding the rat with both hands (1 × 30 s, 1 × 20 s), lifting it with either hand (10 s each), and lifting it with one hand and passing it to the other (2 × 30 s). The whole procedure lasted 5 min. It was applied on four consecutive days, and the order of its components was varied between days in a randomized way.

After handling (days 1–4), the rats underwent a standardized screening procedure. They were tested twice in an activity box (days 7–8; 10 min each), and twice in an elevated plus-maze (days 11–12; 5 min each). On days 15–18, they were tested in their home cage (housing cage test) in the ultrasonic laboratory