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Ultrasonic vocalization in juvenile and adult male rats: A comparison among stocks

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ABSTRACT

Ultrasonic vocalizations (USV) are widely studied in mice and rats, and in case of rats, the bulk of empirical evidence is based on outbred rats, which in most studies belong to either Long Evans, Sprague-Dawley or Wistar stocks. It is known that these stocks can differ in terms of specific brain variables and also behaviorally, but there is only few evidence so far showing whether these stocks behave in similar or substantially different ways in paradigms which are often used to study USV. Therefore, we have started a larger series of comparative studies, where we analyzed different classes of USV in rats from these three stocks spanning from pups to adults. Here, we report our findings in juvenile and adult male Long Evans, Sprague-Dawley and Wistar rats, which we tested as juveniles for appetitive 50-kHz calls during a so-called cage test or when being tickled by an experimenter, and later as adults for 22-kHz calls in a fear conditioning paradigm. In general, all three stocks showed the expected USV responses, indicating that they are all feasible for this kind of research. In detail, however, there were various quantitative differences between stocks both, in terms of specific USV features (like call rates, call durations etc.) as well as visible behavior, like spontaneous locomotor activity and shock-induced immobility. These findings are discussed in the context of the relevant, but somewhat equivocal literature on these stocks, including factors which might contribute to such variability, like breeding, housing, or details of the given test.

1. Introduction

Neurobehavioral research in outbred rats mainly relies on the study of Sprague-Dawley (SD), Wistar (WU), and Long-Evans (LE) rats, that is, stocks which are basically domesticated descendants of *Rattus norvegicus* [34]. These stocks have been found to differ in terms of anatomy/physiology (e.g. [28,37]), pharmacology (e.g. [10,12,20,21]), and also behaviorally. Here, differences were obtained in measures of anxiety [28], fear-conditioning [14,16,43], defensive reactions to predator odors [36], stress reactivity [11], open-field behavior [50], novelty-seeking [43], social play behavior [18,20,21], spatial learning [16], object discrimination [2], or prepulse inhibition [11,27,39].

In contrast, stock differences have not yet received considerable attention with respect to rat ultrasonic vocalizations (USV), although USV has become a powerful tool in basic research on emotion, motivation, and social communication, and in models of human disorders and diseases, like autism, schizophrenia, anxiety, depression, addiction or Parkinson's disease. Such USV can be differentiated into different call classes, namely so-called 40-kHz calls of pups, and 22- and 50-kHz calls of juvenile and adult rats. Importantly, the calls are related to negative (40-kHz, 22-kHz) or positive affective (50-kHz) states, and their emission is known to be dependent on several factors, especially age, experience, expectancy, context, or individual dispositions (for reviews see [4,17,46]). Regarding genetic background, most rat USV work has been done in outbred stocks, and dependent on the given lab either WU, SD, or LE rats have been used most often. Despite the wealth of evidence on USV in these stocks, and except for some reported differences in adult animals [8,14,20,21,43], and pups [29] little is known about possible USV differences between stocks, for example in response to tickling (for review see [19]), which is surprising since stocks, as outlined above, seem to differ substantially at several behavioral and physical levels.

We have therefore decided to perform a series of studies where we investigated various USV classes in WU, SD and LE rats, and there either as pups, juveniles, or adult subjects. Recently [32], we showed that LE, SD and WU pups tested in isolation differed in various call features, like call numbers, peak frequency and frequency modulation, for example, that male LE and WU pups tested in isolation emitted more 40-kHz calls than SD rats, or that WU rats emitted calls with higher frequency modulation. Here, we extended this research to juvenile and adult rats, applying tests which are effective for the induction of either appetitive 50-kHz or aversive 22-kHz calls. Again, we asked whether LE, SD and WU rats differed in these tasks in various USV measures and/or in terms of visible behavior, like locomotion or immobility. For 50-kHz calls, we

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used the so-called cage test and we also tickled them. In the cage test [23,33], a rat is tested singly in a clean cage test with fresh bedding. In this condition, rats emit moderate rates of 50-kHz calls, which usually have flat or simple shapes. These calls are thought to serve as a social calls, which are emitted to enhance the likelihood with other rats [45]. Call rates in such a test can differ considerably between subjects, but individual call rates correlate positively with repeated testing [31,33]; hence, these differences probably reflect individuality. Therefore, we and others often use such a cage test to screen rats before assigning them for possible treatment groups, for example in psychopharmacological studies (e.g. [26]). Tickling (also termed hetero-specific play; [23,25]) was used since this manual procedure typically leads to high rates of 50-kHz calls which mostly have trill-like shapes and which are thought to reflect the rat's pleasure. Finally, and as a measure of aversive acoustic signaling, the three stocks underwent a fear-conditioning procedure routinely used in our lab [3,47], which is highly effective to induce 22-kHz calls in response to the CS-US combinations (tone/shock), but also when subsequently exposing such rats to context and tone alone.

2. Methods

2.1. Animals and general procedure

All subjects were born in the laboratory. For that purpose, pregnant dams had been purchased from either Harlan (Borchen, Germany) in case of WU rats, or Janvier (Le Genest Saint Isle, France) in case of SD and LE rats. These dams (3 per stock) arrived in the laboratory on E14 and delivered their pups around 7 days after arrival. To avoid effects of litter size [52], each litter was planned to consist of five pups. Therefore, females and surplus males were removed from the nest on PND 0 or 1. Each litter was kept on Tapvei peeled aspen bedding (indulab ag, Gams, Switzerland) in Macrolon type-IV cages (550 × 330 × 200 mm, with high stainless steel covers) in a climate-controlled cabinet (UNI-PROTECT, Ehret, Emmendingen, Germany, where the environmental temperature was maintained at 22 ± 1° Celsius (humidity: 53–67%), and with a 12:12 h light/dark cycle (lights on at 07:00 am). Lab chow (Altromin, Lage, Germany) and water (tap water with 0.0004% HCl) were available ad libitum.

On PND 21, the juvenile rats were weaned, but remained in their group cages. The cage test (test details see below) was performed on PND 26–27, preceded by three days of handling (about 5 min/animal each day). The tickle test was performed on PND 30–34, and fear conditioning was tested on PND 77–79, again preceded by a 5 min handling phase on PND 76. All animal protocols were approved by the ethics committee of the local government (Regierungspräsidium Gießen; TVA MR 20/35, Nr. 12/2007).

2.2. Cage test

The animals were tested in clean type-III Makrolon cages (378 × 217 × 180 mm) containing a layer (about 2 cm thick) of fresh bedding. For testing (10 min), the given rat was removed from its group cage and placed into the test cage covered by a steel grid cover. Then, the cage was carried to the adjacent ultrasonic lab, where it was placed on a small testing desk. No other rat was present in that lab, which was illuminated by white light of about 2 lx. The ultrasonic microphone was mounted centrally at about 35 cm above the cage floor. Additionally, a video camera connected with a DVD recorder was positioned at a longitudinal side of the cage to record visible behaviour, which was evaluated offline by observation of rearing and locomotor activity (according to [33]). 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 number of times the rat crossed this line was counted. After testing, the animal was brought back to the animal room and placed back into its group cage. Tests were always done with fresh cages (and bedding) and were repeated on two consecutive days, with rat testing order changed randomly between days.

2.3. Tickle test

This test was also performed in clean type-III Makrolon cages that contained fresh bedding. All rats were tickled on five consecutive days, with their testing order changing randomly from day to day. For testing, a given rat was removed from its group cage and placed into the testing cage without a cover. Then, the cage was carried to the dimly-lit adjacent ultrasonic lab, where it was placed on a small testing desk, and where no other rat was present. The ultrasonic microphone was mounted centrally at about 35 cm above the cage floor. The experimenter manipulated the rat with the right hand following a standardized procedure lasting 10 min. This procedure contained different components, namely “neck tickle”, “belly tickle”, “push and drill”, “hand chase” and “flip over” (for details see [23,33]). Each component lasted 30 s; furthermore, six 30-s breaks were interspersed at 0, 60, 150, 300, 420 and 570 s. During these breaks, the experimenter's hand remained passively inside the cage. After testing, the animal was brought back to the animal room and placed into its group cage. USV measures were only taken on the 5th day of this procedure.

2.4. Fear conditioning

The fear conditioning test was performed in a standard shock chamber (33.5 cm × 35 cm × 38 cm) made of gray plastic walls. The roof and one wall were made of transparent plastic to allow video observation. A tone, which served as the CS, was provided by a loudspeaker (diameter: 7.5 cm) mounted into one wall 30 cm above the floor. This floor was made of stainless steel rods (diameter: 5 mm) spaced 1 cm apart. The chamber was situated within an isolation cubicle (51 cm × 71 cm × 51 cm, Coulbourn Instruments USA) equipped with 2 LED spots providing around 40 lx (Conrad Electronics) and a video camera (CCD Camera-model s/w; Conrad electronics) connected to a recorder. The tone, a 3-kHz sinewave tone (generated with: GoldWave Digital Audio Editor) was presented for 20 s, and as the USC, a 0.5 mA scrambled shock (Med Associates, Standalone shocker) was used. This shock was administered during the last 500 ms of the tone. Testing was performed on 3 consecutive days. On the first day (termed habituation), each rat was placed into the chamber for 11 min without tone or shock. On the next day (termed conditioning day), each animal was again placed into the chamber for 11 min. After an initial phase of 3 min where no tone or shock was given, the rat was exposed to six CS/UCS pairings, each followed by an inter-stimulus interval (ISI) of 60 s. On the third day (termed testing day), the rat was again placed into the shock chamber for 11 min. After an initial phase of 3 min, the tone was presented six times for 20 s each. Behavior was measured in terms of immobility, i.e. suppression of all somatic motility except of respiratory moves, rearing and grooming. Also, audible calls in response to shock on the conditioning day were counted from the spectrograms (see below) as a measure of pain sensitivity [49].

2.5. Recording and analysis of ultrasonic vocalization

An UltraSoundGate Condenser Microphone (CM 16) sensitive to frequencies between 10 and 120 kHz with a flat frequency response between 15 and 30 kHz (± 6 dB) and between 40 and 70 kHz (± 12 dB) was used. It was connected via an Avisoft UltraSoundGate 416 USB audio device (Avisoft Bioacoustics, Berlin, Germany) to a personal computer, where acoustic data were displayed in real time by Avisoft Recorder (Avisoft Bioacoustics), which recorded with a sampling rate of 214,285 Hz in 16 bit format. For acoustical analysis, recordings were transferred to SASLab Pro (version 4.3; and 4.52; Avisoft Bioacoustics) and a fast Fourier transform was conducted (512 FFT-length, 100% frame, Hamming window and 75% time window