Ultrasonic Communication in Rats: Insights from Playback Studies

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Abstract

Mice and rats produce vocalizations in the ultrasonic range, i.e. clearly above the human frequency threshold and therefore not audible to humans. Such ultrasonic vocalizations (USVs) serve as situation-dependent affective signals and convey important communicative functions. Here, we tested whether different acoustic stimuli, including aversive 22-kHz USVs and appetitive 50-kHz USVs, elicit distinct behavioral response patterns that can be differentiated by the automated video tracking system EthoVision. Results show that playback of acoustic stimuli elicited distinct patterns of behavioral activity. While playback of aversive 22-kHz USVs were followed by behavioral inhibition, appetitive 50-kHz USVs induced an increase in locomotor activity, which is directed towards stimulus source. The present findings further support the conclusion that aversive 22-kHz USVs serve an alarm function to warn conspecifics about external danger and that 50-kHz USVs serve a social affiliative function as social contact calls. The distinct behavioral response patterns elicited can be measured by using the automated video tracking system EthoVision. This will allow high-throughput testing of social communicative behavior in rats.

Introduction

Mice and rats produce vocalizations in the ultrasonic range, i.e. clearly above the human frequency threshold and therefore not audible to humans. Such ultrasonic vocalizations (USVs) serve as situation-dependent affective signals [1; 2]. Rodent USVs have attracted considerable interest as it was suggested that they can serve as a direct measure of affect, i.e. of the animal's subjective emotional state. Furthermore, as rodent USVs convey important communicative information, measuring them offers the opportunity to study communication in rodent models of neuropsychiatric disorders characterized by social and communication deficits such as autism and schizophrenia. In the rat, three USV types are known: 1) 40-kHz USVs, which are emitted by pups when isolated from mother and littermates; 2) 22-kHz USVs, which are emitted by juvenile and adult rats in aversive situations such as fear-conditioning or social defeat; and 3) 50-kHz USVs, which are emitted by juvenile and adult rats in appetitive situations such as rough-and-tumble play, mating or when tickled playfully. In the mouse, similar call types exist with the exception of 22-kHz USVs, which are not present in mice.

In aversive situations, such as fear learning, 22-kHz USV production was found to be strongly correlated with freezing behavior, the most commonly used fear measure in rodents, and was shown to be highly dependent on the situation's aversiveness. Rats exposed to higher shock intensities emitted more aversive USVs than rats exposed to lower shock intensities during fear learning and testing, i.e. in absence of shock application when exposed to a formerly neutral stimulus that has been repeatedly paired with shocks [3]. In addition to situational factors, individual differences in disposition for anxiety-related behavior play an important role. Rats characterized as anxious based on their anxiety-related behavior on the elevated plus maze emitted more aversive USVs during fear learning than less anxious rats [4]. In contrast, in appetitive situations, such as rough-and-tumble play, 50-kHz USV production could serve as indicators of high welfare. When tickled playfully by an experienced human experimenter, appetitive USV production was found to be highly correlated with the level of newborn cells in the hippocampus, a brain area important for learning and memory as well as emotion and stress regulation. Rats displaying a depressive-like phenotype with low rates of appetitive USVs were characterized by low levels of newborn cells [5].

Behavioral, neuronal and pharmacological studies provide evidence that USVs serve communicative purposes. It is known for a long time that 40-kHz USVs elicit maternal search and retrieval behavior. More recently, however, it was demonstrated that also 22-kHz USVs and 50-kHz USVs induce call-specific behavioral

responses in the receiver [6]. While 22-kHz USVs induce freezing behavior, indicating an alarm function, 50-kHz USVs induce social approach behavior, supporting the notion that they serve as social contact calls. The opposite behavioral responses are paralleled by distinct patterns of brain activation [7]. While 22-kHz USVs induce activation in amygdala and periaqueductal gray, 50-kHz USVs are followed by activation in the nucleus accumbens. As indicated by subsequent pharmacological studies [8], social approach behavior in response to 50-kHz USVs depends on an intact opioid system.

Here, we tested whether different acoustic stimuli, including aversive 22-kHz USVs and appetitive 50-kHz USVs, elicit distinct behavioral response patterns that can be differentiated by an automated video tracking system (EthoVision, Noldus, Wageningen, The Netherlands).

Methods

Animals and housing: Male Wistar rats (HsdCpb:WU, Harlan-Winkelmann, Borchen, Germany) served as subjects. Animals were housed in groups on Tapvei peeled aspen bedding (indulab ag, Gams, Switzerland) in a Macrolon type IV cage (size: 378x217x180 mm, plus high stainless steel covers). Lab chow (Altromin, Lage, Germany) and water (0.0004% HClsolution) were available ad libitum. Animals were housed in an animal room with a 12:12 h light/dark cycle (lights on 7–19 h) where the environmental temperature was maintained between 20–25° Celsius. Prior to testing, all animals were handled for 3 days in a standardized way (5 min each day).

All experimental procedures were approved by the local government ethical committee (Regierungspräsidium Giessen, Germany; MR 20/35, Nr. 50/2010).

Experimental setting: Testing was performed on a radial maze of gray plastic with 8 arms (9.8x40.5 cm) extending radially from a central platform (diameter: 24 cm), which was elevated 52 cm above the floor (see Figure 1 & 2; for details see: [9]). Acoustic stimuli were presented through an ultrasonic speaker (ScanSpeak, Avisoft Bioacoustics, Berlin, Germany; see Figure 3) using an external sound card with a sampling rate of 192 kHz (Fire Wire Audio Capture FA-101, Edirol, London, UK) and a portable ultrasonic power amplifier with a frequency range of 1–125 kHz (Avisoft Bioacoustics). The loudspeaker had a frequency range of 1–120 kHz with a relatively flat frequency response (± 12 dB) between 15–80 kHz. It was placed 20 cm away from the end of one arm at a height of 52 cm above the floor. Testing was performed under red light (approximately 11 lux in the center of the maze and between 9 and 12 lux in the arms) in a testing room with no other rats present. All behavioral tests were conducted between 9–17 h. Prior to each test, behavioral equipment was cleaned using a 0.1 % acetic acid solution followed by drying.

Behavior was monitored by a video camera (Panasonic WV-BP 330/GE, Hamburg, Germany) from about 150 cm above the maze, which fed into DVD recorder (DVR-3100 S, Pioneer, Willich, Germany). Playback of acoustic stimuli were monitored by two UltraSoundGate Condenser Microphones (CM 16; Avisoft Bioacoustics; see Figure 3) placed 20 cm away from the maze at a height of 55 cm above the floor. One out of these two was placed next to the loudspeaker, i.e. in front of the three proximal arms, whereas the other one was placed vis-à-vis in front of the three distal arms. These microphones were sensitive to frequencies of 15-180 kHz with a flat frequency response (± 6 dB) between 25–140 kHz, and were connected via an Avisoft UltraSoundGate 416 USB Audio device (Avisoft Bioacoustics) to a personal computer, where acoustic data were displayed in real time by Avisoft RECORDER (version 2.7; Avisoft Bioacoustics), and were recorded with a sampling rate of 214,285 Hz in 16 bit format.

Acoustic stimuli: The following three acoustic stimuli were used: 1) 22-kHz USVs, 2) 50-kHz USVs, and background noise (see Figure 4). All stimuli were presented for 1 min with a sampling rate of 192 kHz in 16 bit format. USVs were presented at about 69 dB (measured from a distance of 40 cm), and noise was presented with about 50 dB, which corresponds to the background noise during playback of the other stimuli. The 22-kHz USVs had been recorded from a male Wistar rat after applications of foot-shocks (for setting and recording see: [3]). The 50-kHz USVs had been recorded from a male Wistar rat during exploration of a cage containing scents from a cage mate (for setting and recording see: [10]). The presentation was composed of a sequence of 3.5 s, which was repeated for 1 min, i.e. 17 times, to assure the presentation of a high number of frequency-modulated calls

Figure 1. Setup for playback experiments and red light device. Elevated eight arm radial maze equipped with two ultrasonic microphones and two loudspeakers (Avisoft Bioacoustics; Berlin Germany). The rat's behavioral responses during playback of ultrasonic stimuli are recorded with a video camera positioned above the elevated eight arm radial maze. Testing is performed under dim red light.

within a relatively short period of time. Since the two acoustic stimuli presented contained background noise, i.e. sounds, which occur when a rat is exploring an arena with bedding, background noise without calls was presented to control for its possible effects.

Experimental procedure: A given animal was placed onto the central platform of the radial maze, facing the arm opposite to the loudspeaker. After an initial phase of 15 min where no acoustic stimuli were presented (habituation), the rat was exposed to three presentations of acoustic stimuli for 1 min, each followed by an inter-stimulus-interval of 10 min.

Behavioral and acoustic analysis: Behavioral analysis was performed using an automated video tracking system (EthoVision, Noldus Information Technology, Wageningen, The Netherlands). Previously, the following parameters were obtained: 1) total distance travelled (cm), 2) number of arm entries into the three proximal or distal arms (n) and 3) the time spent thereon (6; 8). Here, the track profile was acquired. For the automated

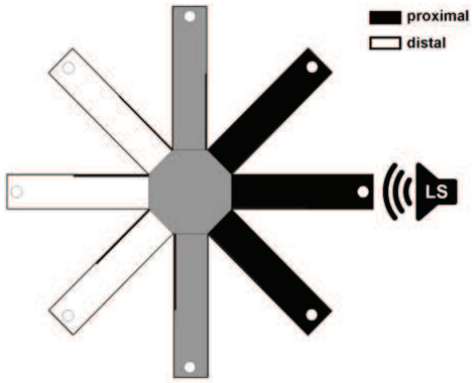

Figure 2. Schematic illustration of the elevated radial eight arm maze. Arms close to the loudspeaker are denoted as proximal, central arms as neutral and opposite arms as distal.

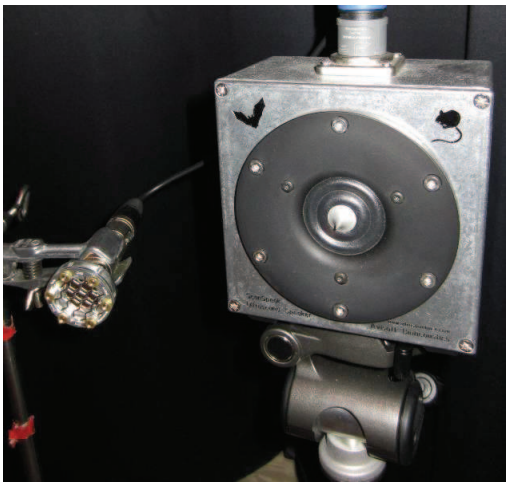

Figure 3. Ultrasonic loudspeaker for playback presentations and microphone for ultrasonic recording.

analysis, input filters were activated to avoid an over-estimation of locomotor activity due to head-movements: a minimal distance moved of 8 cm was used for the total distance travelled, whereas a minimal distance moved of 3 cm was used for the arm entries.

For acoustical analysis, recordings were transferred to SASLab Pro (version 4.38; Avisoft Bioacoustics) and a fast Fourier transform was conducted (512 FFT-length, 100 % frame, Hamming window and 75 % time window overlap). Correspondingly, the spectrograms were produced at 488 Hz of frequency resolution and 0.512 ms of time resolution.

Results

Playback of acoustic stimuli elicited distinct patterns of behavioral activity (see Figure 5). While playback of aversive 22-kHz USVs were followed by behavioral inhibition, appetitive 50-kHz USVs induced an increase in locomotor activity, which is directed towards stimulus source. Playback of noise led to an undirected increase in behavioral activity. The results show that different acoustic stimuli, including aversive 22-kHz USVs and appetitive 50-kHz USVs, elicit distinct behavioral response patterns that can be differentiated by an automated video tracking system (EthoVision).

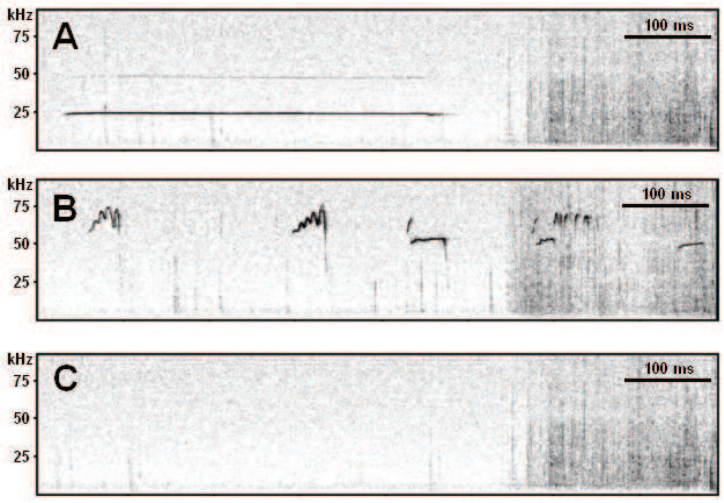

Figure 4. Exemplary spectrograms of the presented acoustic stimuli. A) 22-kHz USVs, B) 50-kHz USVs, and C) background noise.

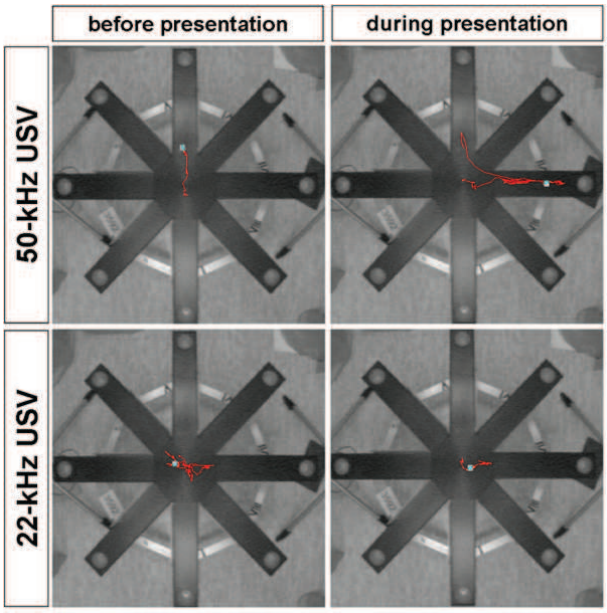

Figure 5. Exemplary patterns of behavioral activity measured by using EthoVision.

Discussion

In line with previous findings [6; 7; 8], it was found that aversive 22-kHz USVs and appetitive 50-kHz USVs induce call-specific behavioral responses in the receiver. The present findings further support the conclusion that aversive 22-kHz USVs serve an alarm function to warn conspecifics about external danger and that 50-kHz USVs serve a social affiliative function as social contact calls. Importantly, the present findings demonstrate that the behavioral response patterns elicited by aversive 22-kHz USVs and appetitive 50-kHz USVs can be measured by using an automated video tracking system (EthoVision). This will allow high-throughput testing of social communicative behavior in rats.

Social communicative deficits occur in a number of neuropsychiatric disorders, among them neurodevelopmental disorders such as autism and schizophrenia. Autism is characterized by aberrant reciprocal social interactions, deficits in social communication, and repetitive, stereotyped patterns of behaviors (DSM-IV-TR, 2000); and there are overlapping symptoms between autism and schizophrenia, particularly the negative symptoms such as poverty of speech and social withdrawal (DSM-IV-TR, 2000). To study underlying alterations in genes, neurotransmitter systems, neuronal plasticity, etc., the development of new rodent behavioral assays with face validity to social and communication deficits seen in neurodevelopmental disorders is crucial.

Since rats are social animals and rough-and-tumble play during adolescence has an important role for social development, separation from conspecifics during this phase is known to impair social behavior. Post-weaning social isolation in rats is a widely used animal model to induce behavioral effects and developmental changes in the nervous system related to symptoms in psychiatric disorders like schizophrenia [11; 12]. It is therefore of great interest to see whether the newly developed rat social communication assay is sensitive enough to detect deficits in social communication induced by juvenile social isolation.

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