Ultrasonic Communication in Mouse Models of Autism

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Abstract

Autism is a complex neurodevelopmental disorder characterized by 1) aberrant reciprocal social interactions, 2) deficits in social communication, and 3) repetitive, stereotyped patterns of behaviors, along with narrow restricted interests. Designing mouse behavioral assays with face validity to the three core symptoms of autism in humans is a daunting challenge for behavioral neuroscientists. Mice produce vocalizations in the ultrasonic range, i.e. clearly above the human frequency threshold and therefore not audible to humans. Such ultrasonic vocalizations serve as situation-dependent affective signals and convey important communicative information**.** Measuring the emission of ultrasonic vocalizations and the behavioral responses to ultrasonic vocalizations in playback experiments provides therefore an unique tool to study communication in mice. This is of high relevance for mouse models of autism, since delayed language and poor communication skills are fundamental to the diagnosis of autism.

Introduction

Autism is a complex neurodevelopmental disorder characterized by 1) aberrant reciprocal social interactions, 2) deficits in social communication, and 3) repetitive, stereotyped patterns of behaviors, along with narrow restricted interests (DSM-IV-TR, 2000). While the causes of autism remain unknown, a strong genetic component in the etiology of autism is indicated by extraordinarily high [1-3]. Genome-wide and pathway-based association studies led to the identification of more than hundred autism candidate genes [4-6]. Considerable efforts are made to better understand the genetic causes of autism. The generation of genetically modified mice is a powerful tool to study the physiological functions of such candidate genes. Mouse models with homologous mutations, e.g. Shank3 knockout mice, have revealed behavioral phenotypes relevant to the three autism core symptoms [7,8].

Designing mouse behavioral assays with face validity to the three core symptoms of autism in humans is a daunting challenge for behavioral neuroscientists. While reliable tests are available to assess the first core symptom, social deficits, and third core symptom, repetitive behavior, in mice, it is particularly difficult to measure communication deficits in mice [9].

Mice produce vocalizations in the ultrasonic range, i.e. clearly above the human frequency threshold and therefore not audible to humans. Such ultrasonic vocalizations serve as situation-dependent affective signals [10,11]. Currently, three types of ultrasonic vocalizations are known in mice. Their occurrence is dependent on the animal's age. 1) Isolation-induced ultrasonic vocalizations are emitted by mouse pups during the first two weeks of life when separated from mother and littermates [12]. Isolation-induced ultrasonic vocalizations were the first purely ultrasonic signals reported to be produced by mice. They were discovered by Zippelius and Schleidt in 1956. Zippelius and Schleidt suggested that isolation-induced ultrasonic vocalizations serve communicative purposes, since they have observed that mothers leave the nest to retrieve vocalizing pups scattered outside the nest, whereas no retrieval behavior was seen in response to pups that have been anesthetized and hence did not emit isolation-induced ultrasonic vocalizations. Later, their communicative function was confirmed by playback experiments [13-15]. 2) Interaction-induced ultrasonic vocalizations occur during social investigation in juvenile mice aged three to four weeks [16]. Because their occurrence is positively associated with social investigation behavior, it was suggested that interaction-induced ultrasonic vocalizations help to maintain social contact [16]. 3) Female-induced ultrasonic vocalizations are uttered by adult male mice when exposed to females [17] or female urine [18]. As shown in devocalization and playback studies, female-

induced ultrasonic vocalizations serve an important communicative function as well, namely to attract females [19,20].

Measuring the emission of ultrasonic vocalizations and the behavioral responses to ultrasonic vocalizations in playback experiments provides therefore an unique tool to study communication in mice. This is of high relevance for mouse models of autism, since delayed language and poor communication skills are fundamental to the diagnosis of autism.

Methods

Isolation-induced ultrasonic vocalizations in pups: For the induction of isolation-induced ultrasonic vocalizations, pups are separated from mother and littermates under room temperature for 10 minutes during the first two weeks of life. Pups are removed individually from the nest at random and gently placed into an isolation box made of plastic (an example is shown in Figure 1; [21]) or an isolation container made of glass, containing clean bedding material [22,23]. Emission of ultrasonic vocalizations is monitored by an UltraSoundGate Condenser Microphone CM 16 (Avisoft Bioacoustics, Berlin, Germany) placed in the roof of the sound attenuating box. The microphone is connected via an UltraSoundGate 116 USB audio device (Avisoft Bioacoustics) to a personal computer, where acoustic data are recorded with a sampling rate of 250,000 Hz in 16 bit format by Avisoft RECORDER (version 2.97; Avisoft Bioacoustics). The microphone that is used for recording is sensitive to frequencies of 15-180 kHz with a flat frequency response (±6 dB) between 25-140 kHz.

Interaction-induced ultrasonic vocalizations in juveniles: For the induction of interaction-induced ultrasonic vocalizations pairs of mice are allowed to socially interact after a short period of social deprivation. For acoustic recording, the same equipment can be used as for isolation-induced ultrasonic vocalizations (Avisoft Bioacoustics).

Female-induced ultrasonic vocalizations in adult males: For the induction of female-induced ultrasonic vocalizations adult male mice are exposed to a drop of female urine. To elicit high rates of ultrasonic vocalizations female urine needs to be fresh [24]. Also, male mice with female experience emit more ultrasonic

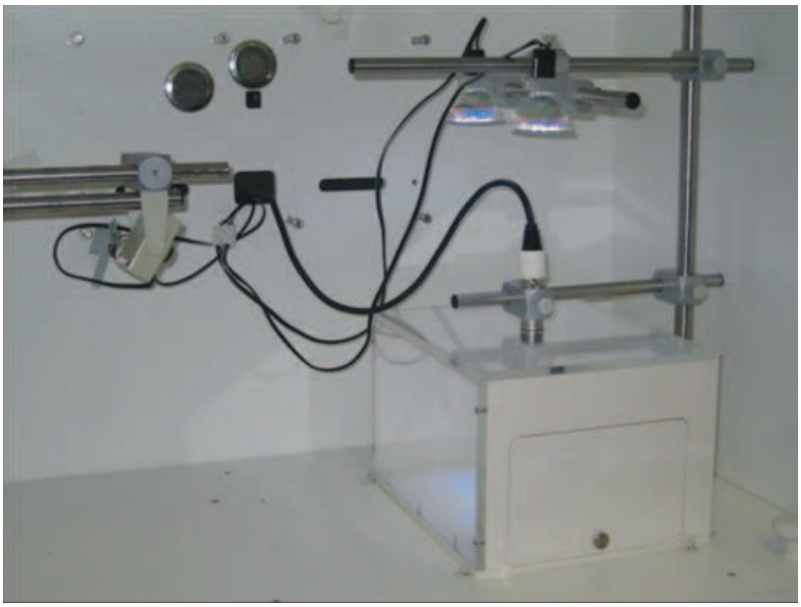

Figure 1. **Setup for measuring isolation-induced ultrasonic vocalizations in rodent pups.** The roof and one wall are made of transparent plastic to allow video observation during the test. The isolation box is placed in a sound attenuating isolation cubicle (51x71x51 cm; Coulbourn Instruments, Allentown, USA) equipped with 2 white-light LED spots (63 lux, Conrad Electronic GmbH, Hirschau, Germany) and a black/white CCD camera (Conrad Electronic GmbH) connected to a DVD recorder (DVR-3100 S, Pioneer, Willich, Germany).

vocalizations than male mice without such an experience [24]. For acoustic recording, the same equipment can be used as for isolation-induced ultrasonic vocalizations (Avisoft Bioacoustics).

Acoustical analysis: For acoustical analysis, recordings are transferred to Avisoft SASLab Pro (version 4.50; Avisoft Bioacoustics) and a fast Fourier transform is conducted (512 FFT length, 100 % frame, Hamming window and 75 % time window overlap [21]). Correspondingly, the spectrograms are produced at 488 Hz of frequency resolution and 0.512 ms of time resolution. Call detection is provided by an automatic threshold-based algorithm (amplitude threshold: -40 dB) and a hold-time mechanism (hold time: 10 ms). A high-pass filter of 20 kHz is used to reduce background noise outside the relevant frequency band to 0 dB. The accuracy of call detection by the software is verified manually by an experienced user. When necessary, missed calls are marked by hand to be included in the automatic parameter analysis. Total number of ultrasonic vocalizations is calculated for the entire session and in 60 s time bins, to visualize the time course of the ultrasonic vocalization response. Additional parameters include peak frequency and peak amplitude, i.e. loudness, which are derived from the average spectrum of the entire call, are determined automatically. Peak amplitude is defined as the point with the highest energy within the spectrum. Peak frequency is defined as the frequency at the location of the peak amplitude within the spectrum. In addition, the extent of frequency modulation, i.e. the difference between the lowest and the highest peak frequency within each call, is measured automatically. Temporal parameters include latency to start calling, total calling time, and call duration.

Results

Results obtained provide an in-depth characterization of auditory communication in mice throughout life. Exemplary spectrograms are shown (see Figure 2).

Discussion

BTBR T+tf/J mice are currently among the most commonly used mouse models of autism [9]. In a series of experiments it was shown that all three types of ultrasonic vocalizations are affected in this mouse model. As

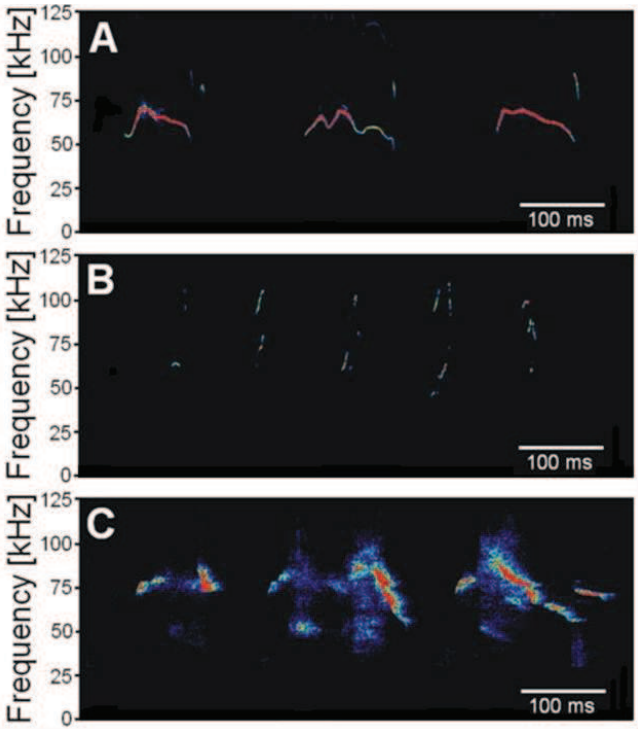

Figure 2. **Exemplary spectrograms of mouse ultrasonic vocalizations.** A) Isolation-induced ultrasonic vocalizations; B) Interaction-induced ultrasonic vocalizations; C) Female-induced ultrasonic vocalizations.

pups, BTBR T+tf/J mice display an untypical repertoire of isolation-induced ultrasonic vocalizations [25]. As juveniles, reduced social interaction behavior is paralleled by a reduction in interaction-induced ultrasonic vocalizations [26]. As adults, male BTBR T+tf/J mice displayed minimal ultrasonic vocalization responses to female urine obtained from both BTBR T+tf/J and C57BL/6J, a standard mouse strain showing high levels of social behavior [27]. The lack of female-induced ultrasonic vocalizations was associated with untypically low scent marking, a measure of olfactory communication [27]. This indicates that the BTBR T+tf/J mouse model of autism incorporates phenotypes relevant to the second diagnostic symptom of autism, communication deficits, along with its strong behavioral phenotypes relevant to the first and third diagnostic symptoms, impairments in social interactions and high levels of repetitive behavior [24].

Candidate genes for autism include genes encoding for the SHANK family of synaptic scaffolding proteins. Mutations in *SHANK3* and deletions containing *SHANK3* have been detected in several autistic individuals [28]. Despite behavioral phenotypes with relevance to the first and third diagnostic symptoms of autism, ultrasonic communication behavior was found to be only mildly impaired in Shank3 knockout mice [7,8]. Shank1 is a related scaffolding protein within the postsynaptic density, promoting the morphological and functional maturation of synapses. When tested for isolation-induced USVs as pups, Shank1 null mutants emitted fewer USVs as compared to wild-type littermates; and as adults in response to female urine, the USV production by Shank1 null mutant males was characterized by an unusual time pattern [23]. In addition, Shank1 null mutant males displayed lower levels of scent marking in proximity to the female urine spot [23].

Together, these data support the relevance of ultrasonic vocalizations for mouse models of neuropsychiatric disorders characterized by social deficits, such as autism. Importantly, the automated assessment of ultrasonic vocalizations at different time points during mouse development will allow high-throughput testing of social communicative behavior in mice.

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