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Ultrasonic vocalizations during male–female interaction in the mouse model of Down syndrome Ts65Dn

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HIGHLIGHTS

- We characterized ultrasonic vocalizations by the mouse model of Down syndrome Ts65Dn.
- Minimum and maximum peak frequencies of calls were generally lower than in controls.
- We found longer durations for many types of vocalizations compared to euploid mice.
- Ts65Dn produced a reduced number of complex vocalizations compared to euploid mice.

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ABSTRACT

Down syndrome (DS) is the leading cause of genetically defined intellectual disability. Although speech and language impairments are salient features of this disorder, the nature of these phenotypes and the degree to which they are exacerbated by concomitant oromotor dysfunction and/or hearing deficit are poorly understood. Mouse models like Ts65Dn, the most extensively used DS animal model, have been critical to understanding the genetic and developmental mechanisms that contribute to intellectual disability. In the present study, we characterized the properties of the ultrasonic vocalizations (USVs) emitted by Ts65Dn males during courtship episodes with female partners. USVs emitted by mice in this setting have been proposed to have some basic correlation to human speech. Data were collected and analyzed from 22 Ts65Dn mice and 22 of their euploid littermates. We found that both the minimum and maximum peak frequencies of Ts65Dn calls were lower than those produced by euploid mice, whereas the mean individual duration of "down" and "complex" syllable types was significantly longer. Peak, minimal and maximal, and the fundamental frequencies of short syllables generated by Ts65Dn mice were lower compared to those by euploid mice. Finally, Ts65Dn males made fewer multiple jumps calls during courtship and the mean total duration of their "arc", "u", and "complex" syllables was longer. We discuss the human correlates to these findings, their translational potential, and the limitations of this approach. To our knowledge, this is the first characterization of differences between adult Ts65Dn and euploid control mice with respect to USVs.

1. Introduction

Down syndrome (DS), the phenotypic consequence of the triplication of human chromosome 21 (HSA21) [36], is the most prevalent genetically defined cause of intellectual disability, with an incidence of 1 in 732 live births [9,58]. While individuals with DS maintain relatively high levels of social intelligence and procedural learning, they often display disproportionally impaired declarative memory [45]. In addition, children show particular difficulties in acquiring language [12,56]. The lack of linguistic development in children with DS seems to coincide with the beginning of an apparent developmental quotient (DQ) or intellectual quotient (IQ) decline during the first few years of life [71], suggesting that language deficiencies may be closely related to early cognitive impairment associated to this genetic disorder [12,56].

Toddlers with DS exhibit problems in assimilating all four basic components of language, i.e., phonology [17,55,66,67]; semantics [5,10,40]; syntax [13,18,29,34]; and pragmatics [1]. Deficits in each of these linguistic domains become readily salient as children and adolescents

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with DS often have poor speech intelligibility during conversation [65], stunted vocabulary growth, and reduced word production [5,40]. These shortcomings ultimately result in poor conversational or narrative skills, in which children and adults with DS show deficits in expressive language disproportionate to what would be expected from their general cognitive abilities and mental age [31,41].

Although, at first glance, the mouse may seem an unlikely model for the more cognitive, and hence "human" qualities of language, these animals nevertheless are able to emit ultrasonic vocalizations (USVs) at particular frequency ranges that then elicit ear twitches and vibrissa movements in conspecifics [8,53,57], and that are ideally suited to trigger strong cochlear microphonic responses [6] and peak electrical responses within the inferior colliculus [7]. Upon reaching the auditory cortex in the mouse brain, the signals additionally function as potent stimuli that can entrain cortical subfields to their particular frequencies over repeated introduction [38,39]. That is, like in the human brain, they can lead to neuroadaptations that will facilitate future detection. By and large, humans and mice also appear to use similar psychoacoustical mechanisms for the breakdown and perception of species-specific communicative sounds [21,23], and in both species, the recognition of these utterances is lateralized to the left hemisphere [20]. Further studies suggest that mice not only have the substrate to emit and process USVs, but that they also use these signals in a purposeful sense as adults to influence the behavior of conspecifics in agonistic settings and during courtship [48,51].

In the field of animal models for developmental and intellectual disabilities, the reduced level of calling and unusual calling patterns have been reported in mouse models of autism spectrum disorders [22,60,61]; Wohr et al., 2011. Such work inspired us to investigate USVs in the most widely used murine model for DS, the Ts65Dn mouse. Ts65Dn mice are trisomic for contiguous segments of mouse chromosome 16 highly homologous to the long arm of HSA21 ([16]; recently reviewed by Ref. [14]), and reproduce some of the most fundamental characteristics of DS involving abnormalities of the brain, as well as those in the craniofacial skeleton and audition [24,70].

To determine whether Ts65Dn mice show phenotypic characteristics similar to the dysfunctional articulatory processing seen in persons with DS, we recorded USVs from male euploid and Ts65Dn mice during "courtship" episodes with euploid female mice. Female-elicited USVs from male rodents are a very robust phenomenon that has been intensively studied for almost four decades. During social exploration and courtship of female rodents, males will emit unusually rich USV sequences that display characteristics of song with several syllable types organized into phrases and motifs with undulating or shift-like pitch changes, or sharp punctuations [28]. Here, we present our findings and discuss their human correlates, potential translational value, and the limitations of this approach.

2. Material and methods

2.1. Mice

We have used 22 Ts65Dn mice and 22 euploid littermates in this study. Their handling and care were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all experimental methods were approved by the University of Colorado Denver's Animal Care and Use Committee.

Ts65Dn mice were generated by repeated back-crossing of Ts65Dn females to C57BL/6JEiJ × C3Sn.BLiA-Pde6b+/D F1 hybrid males (as described by Ref. [15]) in colonies at the University of Colorado Anschutz Medical Campus or at The Jackson Laboratory. All experiments were performed at the University of Colorado Denver, during the authors' respective stints at that institution. Animals from the same litter were housed together by gender, and maintained on a 12:12 h light/dark cycle (lights off at 7:00 pm) with free access to food and water. All the mice were sexually naïve at the time of testing at 8–10 weeks of age.

2.2. Acoustic data acquisition, analysis, and classification of ultrasonic vocalizations

USVs were recorded during the early part of the dark or "active" phase once the mice had an hour's opportunity to register the change between the light and dark cycles (between 8:00 and 11:00 PM). This time of day has proven optimal for measuring USVs during affiliative behavioral interactions [49,68], and allowed us to evaluate USVs without disturbing the animal's circadian rhythms or sleep schedule. In general, the courtship assay involved: 1) a habituation process by which the male mice could acclimate to the testing environment; and 2) a 5-min session where an individual male mouse was paired with an individual female. Habituation reduces anxiety and stress during the USV recording session, directs the male's attention to the female, which should now be the most salient feature of interest, and increases the probability that the males will emit ultrasonic songs. Per habituation, all of the mice were progressively conditioned to the testing procedures over 3 days. On each of the first two nights, euploid and Ts65Dn males and euploid females were conditioned to being wheeled into the behavioral facility 1 h before lights out in the colony room. After adapting to the transition from the light phase to the dark for 60 min, cagemates were placed together under the recording microphone in empty acrylic cages and given time to explore the restricted space around a sound-attenuating chamber for 5–10 min. These "test cages" were positioned within the chamber about 10 in. directly underneath the microphone. At the end of the exploration period, the animals were returned to their home cages, kept in the behavioral facility overnight, and returned to the colony room the next day when the lights were on again. On the third night, all of the mice were further conditioned to being placed alone in the test cages for 5 min in preparation for being paired with a male/female partner the next evening.

On the fourth night (test day), each male mouse was paired with a different randomly-selected euploid female for 5 min. Clean cages with fresh bedding were used for each new pairing. Female mice were never used more than once and were placed in the testing cage first for 1–2 min before introducing the male. Interactions between male and female animals were left to unfold naturally. No mating attempts or aggressive behaviors were observed during these sessions.

Vocalizations were recorded over a 5-minute period using a pressure-field ¼-inch microphone (Type-4938, Bruel and Kjaer, Nærum, Denmark) with a functional range of 10 Hz to 100 kHz and a sensitivity of 1.6 mV/Pascal. The resulting electrical signal was pre-amplified (Falcon Range® 1/4-inch Microphone Preamplifier — Type 2670, Bruel and Kjaer), then processed by an instrumentation amplifier (Model 440; Brownlee, San Jose, CA). It was collected using a 16 bit analog-to-digital converter at a 200 kHz sampling rate (Digidata 1322A; Molecular Devices Corporation, Sunnyvale, California) driven by pClamp 9.2 software (Molecular Devices). An acoustic calibration of the system was performed by positioning a 1 kHz ± 0.1%, 94.0 dB ±0.2 dB Sound Level Calibrator (Type 4231, Bruel and Kjaer) in a clean test cage at the same relative position to the microphone where the animals would be placed. This calibration resulted in a final gain of 0.14812 V/dB; i.e., a full range of 135 dB peak-to-peak and a resolution of 0.002 dB/bit. Acquisition files were converted into audible.wav files using QuB (http://www.qub.buffalo.edu/wiki/index.php/Main_Page).

USVs were analyzed and categorized into syllable types using sound spectrograms (Avisoft Bioacoustics SasLab Pro software, Berlin, Germany, Software Version 5.2.06). Spectrograms were generated with a Fast Fourier Transform (FFT)-length of 512 and a Hamming style time window overlap of 50% (100% Frame). The spectrogram was produced at a frequency resolution of 391 Hz and a time resolution of 1.28 ms. A low cut-off frequency of 25 kHz was used to reduce background noise outside the relevant frequency band.

Analysis of USVs was performed blind to male genotype. Each syllable was classified as one of 9 waveform categories (Fig. 1) based on internal pitch change, length and shape, according to previously reported