


# Acoustic complexity of pup isolation calls in Mongolian hamsters: 3-frequency phenomena and chaos

Marina V. Rutovskaya<sup>a</sup>, Ilya A. Volodin<sup>a,b,\*</sup> , Natalia Y. Feoktistova<sup>c</sup>, Alexey V. Surov<sup>c</sup>,  
Anna V. Gureeva<sup>c</sup>, and Elena V. Volodina<sup>a</sup>

<sup>a</sup>Department of Behaviour and Behavioural Ecology of Mammals, A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow 119071, Russia

<sup>b</sup>Department of Vertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Moscow 119234, Russia

<sup>c</sup>Department of Comparative Ethology and Biocommunication, A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow 119071, Russia

\*Address correspondence to Ilya A. Volodin. E-mail: [volodinsvoc@gmail.com](mailto:volodinsvoc@gmail.com)

Handling editor: Zhi-Yun Jia

## Abstract

Studying pup isolation calls of wild rodents provides background for developing new early-life animal models for biomedical research and drug testing. This study discovered a highly complex acoustic phenotype of pup isolation calls in 4–5-day-old Mongolian hamsters *Allocrietulus curtatus*. We analyzed the acoustic structure of 5,010 isolation calls emitted in the broad range of frequencies (sonic, below 20 kHz, and ultrasonic, from 20 to 128 kHz) by 23 pups during 2-min isolation test trials, 1 trial per pup. In addition, we measured 5 body size parameters and the body weight of each pup. The calls could contain up to 3 independent fundamental frequencies in their spectra, the low (f0), the medium (g0), and the high (h0), or purely consisted of chaos in which the fundamental frequency could not be tracked. By presence/absence of the 3 fundamental frequencies or their combinations and chaos, we classified calls into 6 distinctive categories (low-frequency [LF]-f0, LF-chaos, high-frequency [HF]-g0, HF-h0, HF-g0 + h0, and HF-chaos) and estimated the relative abundance of calls in each category. Between categories, we compared acoustic parameters and estimated their relationship with pup body size index. We discuss the results of this study with data on the acoustics of pup isolation calls reported for other species of rodents. We conclude that such high complexity of Mongolian hamster pup isolation calls is unusual for rodents. Decreased acoustic complexity serves as a good indicator of autism spectrum disorders in knockout mouse models, which makes knockout hamster models prospective new wild animal model of neurodevelopmental disorders.

**Key words:** audible and ultrasonic vocalization, Cricetinae, nonlinear phenomena, pup isolation calls, rodents, wild animal models.

Vocalizations of laboratory rodent pups serve as indicators for modeling diseases of humans and for drug testing (Scattoni et al. 2009, 2018; Gulia et al. 2014; Wöhr 2014; Zeskind et al. 2014; Riede et al. 2015; Esposito et al. 2017; Hülsmann et al. 2019; Klenova et al. 2021a). The most commonly used mouse and rat pup models of neurodevelopmental disorders (Scattoni et al. 2008; Grimsley et al. 2013; Brudzynski 2015, 2021; Duvauchelle et al. 2018; Granon et al. 2018; Shekel et al. 2021), however, are not exhaustive in terms of resolving the entire spectrum of biomedical problems (Yurlova et al. 2020; Klenova et al. 2021b). Thus, identifying new wild rodent models for medical research remains a worthwhile task (Hashimoto et al. 2001; Rutovskaya 2020; Yurlova et al. 2020; Warren et al. 2022; Chen et al. 2023).

Cricetids may prove to be promising biomedical models involving vocalization, because they maintain a high constancy of the acoustic parameters for many generations in captivity. This high constancy was demonstrated for example, for laboratory golden hamsters *Mesocricetus auratus* (Schneider and Fritzsche 2011) and may be inferred from comparison between wild and laboratory-reared California

mice *Peromyscus californicus* (Kalcounis-Rueppell et al. 2010). At the same time, laboratory rat strains selected for call rate, display progressive changes of laryngeal morphology in a series of generations (Lesch et al. 2021) which may affect the results of biomedical tests. More importantly, rats of inbred strains may suddenly change the behavioral phenotype they were selected for (Alekhina et al. 2016).

Mechanisms of adult cricetids sound production have been thoroughly investigated (Pasch et al. 2017; Riede et al. 2017, 2022; Riede and Pasch 2020). These mechanisms involve voice-based and whistle-based sound production, similar to the mechanisms found in murids (Riede 2011, 2013; Riede et al. 2020; Darwaiz et al. 2022; Håkansson et al. 2022). While “voice-based” calls are produced with vibration of the vocal folds, the “whistle-based” calls are produced by an aerodynamic whistle mechanism based on airflow vorticities in the vocal tract (Mahrt et al. 2016; Riede et al. 2017, 2022; Håkansson et al. 2022). Both voice and whistle-based calls can be sonic (below 20 kHz) or ultrasonic (above 20 kHz) (Fernández-Vargas et al. 2022; Riede et al. 2022). For different call types, adult cricetids may use different production

mechanisms (Pasch et al. 2017; Fernández-Vargas et al. 2022; Riede et al. 2022). For the same call types, cricetid pups use the same production mechanisms as adults (Riede et al. 2022).

Acoustic complexity has been studied in many rodent taxa, including mice, rats, hamsters, voles, Neotomine mice, and gerbils (Grimsley et al. 2011; Fernández-Vargas and Johnston 2015; Zaytseva et al. 2019; Yurlova et al. 2020; Klenova et al. 2021a; Kozhevnikova et al. 2021; Dymkaya et al. 2022). A broad range of frequencies, from human-audible to ultrasonic, was also reported for pup isolation calls of golden, Djungarian *Phodopus sungorus*, Campbell *P. campbelli*, and Chinese *Cricetulus griseus* hamsters (Hashimoto et al. 2001; Schneider and Fritzsche 2011; Piastolov et al. 2023). Measurements of peak frequency suggest the presence of 3 independent fundamental frequencies in calls of white-throated woodrats *Neotoma albigula* (Kobrina et al. 2022). Pup and adult Key Largo woodrats *Neotoma floridana smalli* also produce low-frequency (LF) squeaks (with mean frequency of 1.8 kHz), mid-frequency squeaks (with mean frequency of 10.3 kHz), and ultrasonic squeaks (with mean frequency of 39.1 kHz) (Soltis et al. 2012). Mid-frequency (12–18 kHz) calls were noted in adult laboratory mice during restraint, along with audible LF and ultrasonic calls (Grimsley et al. 2016).

The most important traits of acoustic complexity are percentage and diversity of nonlinear vocal phenomena: frequency jumps, deterministic chaos, subharmonics, and biphonation (Wilden et al. 1998; Fitch et al. 2002; Stoeger et al. 2012; Volodin et al. 2018; Sibiryakova et al. 2021). Among 5–10-day-old pups of different rodent species, percentages of ultrasonic calls with nonlinear phenomena vary from nearly zero, as in some species of gerbils (Kozhevnikova et al. 2021) to 30–40% in mice and voles (Grimsley et al. 2011; Yurlova et al. 2020).

Abundant nonlinear phenomena in rodent vocalizations predetermine terminology used for the calls and their parts. For calls with noisy segments, the broadly used term “deterministic chaos” comes from the application of chaos theory in nonlinear dynamic systems to the work of sound source in mammals and birds. The deterministic chaos represents non-random noise which is characterized by a sudden onset and the occurrence of harmonic windows and traces of subharmonics (Riede et al. 2022). The term “deterministic chaos” was introduced by publications of Wilden et al. (1998) for mammals and Zollinger et al. (2008) for birds and then extended for rodent voice-based calls, as audible squeaks of domestic mice (Finton et al. 2017) and those calls of cricetids which are produced by the vocal folds in the ultrasonic range of frequencies (Riede et al. 2022). For the noisy segments in the ultrasonic calls produced by whistle mechanism, the terminology has yet to be established.

For rodent ultrasonic calls with frequency jumps, the whole calls are sometimes termed syllables and their parts are termed notes. This terminology was proposed for describing ultrasonic calls with 1 or a few frequency jumps which are numerous in the courting songs of male domestic mice (Holy and Guo 2005; Arriaga et al. 2012; Arriaga and Jarvis 2013). Furthermore, the use of this term was extended to ultrasonic calls of other species of rodents, as for example, Neotropical singing mice of the genus *Scotinomys* (Campbell et al. 2014), fat-tailed gerbils *Pachyuromys duprasi* (Zaytseva et al. 2019), yellow steppe lemmings *Eolagurus luteus* (Yurlova et al.

2020), and voles of the genus *Lasiopodomys* (Dymkaya et al. 2022).

In the wild, vocalizations of hamster pups have not been studied. In captivity, hamster pups vocalize in response to cooling when isolated from the nest (e.g., Hashimoto et al. 2001; Piastolov et al. 2023), provoking parental retrieval behavior (Schneider and Fritzsche 2011). Pup isolation tests can easily be standardized and conducted in laboratory conditions. They are thus widely used for studying sonic and ultrasonic communication of rodent pups including mice, rats, gerbils, voles, and hamsters (Okon 1971; Hofer et al. 2002; Shair et al. 2003; Scattoni et al. 2009; Yurlova et al. 2020; Kozhevnikova et al. 2021; Piastolov et al. 2023).

In contrast to pups, adult hamsters do not vocalize in captivity when they are alone (Keesom et al. 2015), although they produce some calls when alone in the wild (Briggs and Kalcounis-Rueppell 2011; Petric and Kalcounis-Rueppell 2013). Captive adult hamsters vocalize during aggressive (Frank and Johnston 1981; Keesom et al. 2015; Rendon et al. 2015) and sexual interactions (Floody et al. 1977; Floody and Pfaff 1977a; Cherry 1989; Pierce et al. 1989; Fernández-Vargas and Johnston 2015) and respond to playbacks of synthesized ultrasonic calls (Floody and Pfaff 1977b; Floody 2018).

For Mongolian hamsters *Allocricetulus curtatus*, there are no data on the acoustic structure of adult or pup calls. For adult hamsters of other species, the acoustic structure of the ultrasonic calls was investigated in the sexual context for golden hamsters (Floody and Pfaff 1977a; Fernández-Vargas and Johnston 2015) and during peaceful and aggressive interactions with same-sex and other-sex partners for Eversmann hamsters *Allocricetulus evermanni* and gray hamsters *Cricetulus migratorius* (Kapusta et al. 2006). Ultrasonic calls of adult golden hamsters (with the average maximum peak frequency of about 54 kHz) varied by contours of fundamental frequency and note composition (Fernández-Vargas and Johnston 2015). Adult Eversmann hamsters produce ultrasonic calls with descending contour in the range of 25 to 60 kHz in fundamental frequency and from 90 to 136 ms in duration (Kapusta et al. 2006). Adult gray hamsters produced ultrasonic calls with various contours, including the U-shaped one, in the range of 15 to 60 kHz in fundamental frequency and from 118 to 210 ms in duration (Kapusta et al. 2006).

Hamster pup calls produced during pup isolation tests were investigated for Djungarian hamsters (Hashimoto et al. 2001; Piastolov et al. 2023), Chinese hamsters (Hashimoto et al. 2001), golden hamsters (Hashimoto et al. 2001; Schneider and Fritzsche 2011) and Campbell hamsters (Piastolov et al. 2023). Three-day-old Djungarian hamsters produced ultrasonic calls with a maximum fundamental frequency of 45–50 kHz, peak frequency of 42–45 kHz, and call duration of 130–150 ms, with chevron-like and wave-like contours (Hashimoto et al. 2001). Comparative interspecies analysis of pup sonic and ultrasonic isolation calls in golden, Djungarian, and Chinese hamsters showed that calls were produced within approximately the same frequency ranges and did not differ in peak frequency, but differed between species in contour shapes, depth of fundamental frequency modulation, call duration and bandwidth (Hashimoto et al. 2001).

The Mongolian hamster is an average-sized rodent (adult body mass of 50–70 g), inhabiting dry steppes and semideserts of Central Asia (Feoktistova et al. 2013). Sustainable captive populations of this species can be kept for many generations

(Gureeva et al. 2015, 2021). In captivity, parental pairs live together and jointly raise offspring (Feoktistova et al. 2013, 2019). The aim of this study was to conduct phenotyping of prethermal pup sonic and ultrasonic calls of Mongolian hamsters as a background for developing a potential new wild biomedical vocal model of emotional dysfunction on a wild cricetid rodent species.

## Materials and Methods

### Study site and subjects

Audio recordings of pup isolation calls of Mongolian hamsters *Allochromatus curtatus* (Allen 1925) were collected at the Joint Usage Center “Live collection of wild species of mammals” at the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Science (the biological station “Tchernogolovka”), Moscow Region, Russia, from 28 June 2020 to 31 July 2020. This laboratory population was established in 2009 from 10 (5 male and 5 female) individual Mongolian hamsters originating from a natural population in Eastern Siberia, Tuva Republic (surroundings of Tere-Khol lake, 50°38'1"N, 97°24'28"E), Russia (Gureeva et al. 2015).

Parental pairs were kept in 24 × 54 × 12 cm plastic cages with wire-mesh lid, with sawdust bedding and hay as nest material; food (oats and sunflower grains, carrot, apples, cabbage, and beet) and water were available *ad libitum*. Before parturition, males were separated from pregnant females. Pregnant females were checked 3 times a week for the appearance of a litter, and birth dates and numbers of pups per litter were recorded. The day of birth was considered day zero of pup life. The number of pups per litter varied from 2 to 6 (mean ± SD 4.3 ± 1.6 pups). Subjects were 26 pups (15 males and 11 females), aged 4–5 days old, from 6 litters delivered from 6 parental pairs. Subject pups were sexed at 20–25 days old, based on visible testicles in males or vagina in females and separated from their mothers at 1 month of age.

### Call recording

Pup isolation calls were recorded in the daytime, in a room where only the recorded pup was present, at room temperature 18–19 °C. Before the start of test trials, a female was separated from its litter to a separate cage and the home cage with the focal litter was placed near a heater, which provided a comfortable temperature at which pups stop vocalizing. Pups were recorded singly when isolated in an open from above plastic cylinder 17 cm diameter and 13 cm high. Audio recording lasted 2 min. Then a hand-held pup was measured using a digital caliper DEKO GJ61 (DEKO Tools Co., Malaysia, Kuala Lumpur) with 0.1 mm precision for head length (from tip of nose to occiput), body length (from tip of nose to anus), tail length (from tip of tail to anus), foot length (from tip of middle digit to heel), and forearm length (from tip of middle digit to elbow). After measurements, a focal pup was weighed on digital scale CAS MWP-3000 B (CAS Corporation, Seoul, Korea) with 0.1 g precision and then placed in a nonhome plastic cage 24 × 54 × 12 cm near the heater. All pups of the recorded litter were tested 1 by 1 and returned together to their home cage; a mother was then also returned to the home cage. Before each test trial, the experimental plastic cylinder was cleaned with water, dried with rubbing by clean cotton napkin, and then rubbed with cotton washed with 40% ethanol, because a higher concentration of ethanol may affect rodent behavior (Lopez-Salesansky et al. 2021).

During each test trial (1 per pup), we continuously recorded pup LF calls (range of recording from 40 Hz to 24 kHz, 48 kHz sampling frequency, 16-bit resolution) using a Zoom-H1 digital recorder with built-in microphone (Zoom Corp., Tokyo, Japan) and simultaneously recorded pup high-frequency calls (HF calls, range of recording from 6 kHz to 128 kHz, 256 kHz sampling frequency, 16-bit resolution) using an Echo Meter Touch 2 PRO (Wildlife Acoustics, Inc., Maynard, MA, USA) attached to a smartphone. The microphones were mounted at 25 cm over the table in the focal pup area, providing a high signal-to-noise ratio during recording. Recordings of each test trial were stored as 2 separate wav-files, 1 for the LF and 1 for the HF calls.

### Call samples

Of the 26 pups tested during the 2-min test trials (1 trial per individual), 20 pups provided LF calls for analysis, 23 pups provided HF calls and 3 individuals were silent. For counting percentages of different call categories (see Results), we used all recorded calls, including the calls of poor quality. For acoustic analyses, we selected calls within files, taking only those calls which had a high signal-to-noise ratio and were not damaged by noise.

We created 4 different call samples, 2 samples for LF calls (1 for counting percentages of different categories of LF calls and another for detailed acoustic analyses of call structure within categories), and 2 samples for HF calls (analogously, 1 for counting percentages of different categories of HF calls and another for detailed acoustic analyses of call structure within categories).

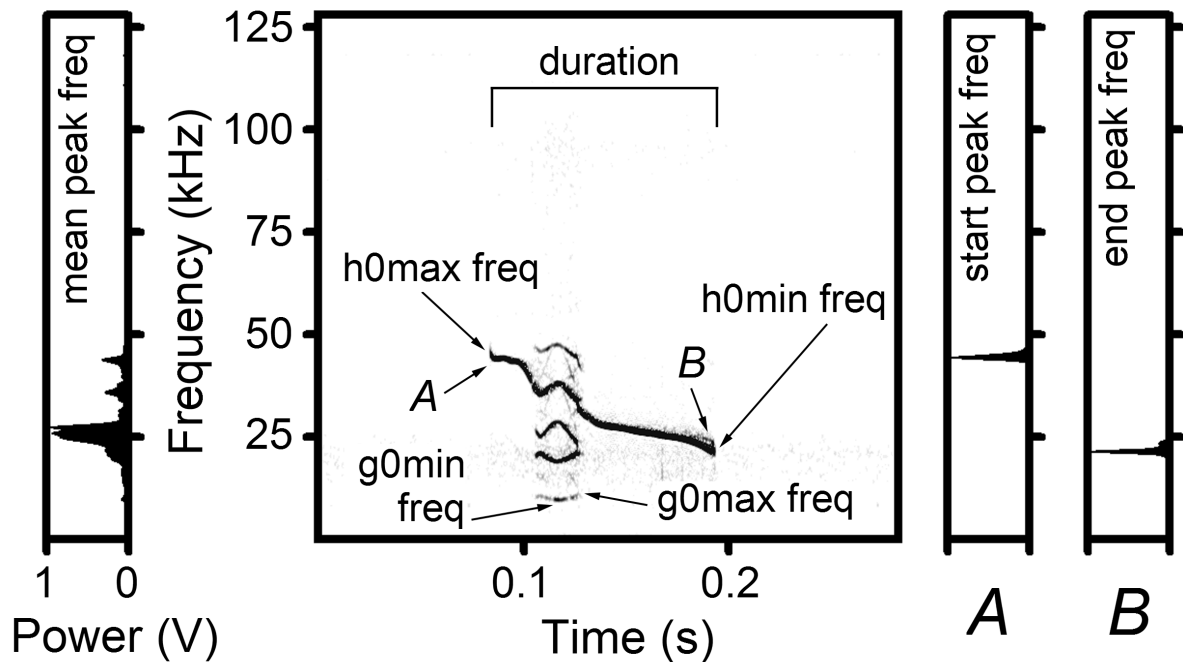
The call sample for counting percentages of different categories of LF calls included all LF calls of 20 pups emitted during test trials (from 12 to 134 LF calls per individual, 1,404 LF calls in total). The call sample for counting percentages of different categories of HF calls included all HF calls of 23 pups emitted during test trials (from 12 to 287 HF calls per individual, 3,606 HF calls in total).

The call sample for acoustic analysis of LF calls included 354 LF calls from 18 pups (20 LF calls per individual from 17 pups and 14 LF calls from the 18th pup); 2 pups did not provide enough calls for analysis (<5 measurable calls). Sample for acoustic analysis of HF calls included 1,192 HF calls from 22 individuals (from 18 to 86 HF calls per pup, in average 54.2 ± 15.7 HF calls per pup), 1 pup did not provide enough calls for analysis (<5 measurable calls).

### Call analysis

Acoustic analyses were carried out using spectrographic software Avisoft-SASLab Pro, v. 5.2.13 (Avisoft Bioacoustics, Berlin, Germany). Spectrograms were created using a Hamming window (Fast Fourier Transform [FFT] length 1,024, frame 50%, overlap 87.5%, frequency resolution was 47 Hz for LF calls and 250 Hz for HF calls, time resolution was 2.7 ms for LF calls and 0.5 ms for HF calls).

Using the semi-automatic option of Avisoft, for either LF ( $n = 354$ ) or HF calls ( $n = 1,192$ ), we measured call duration, the peak frequency averaged over the entire call (mean peak freq), and also the peak frequency at the beginning point of a call contour (start peak freq) and at the end point of call contour (end peak freq) (Figure 1). In cases where the call contained 1 or 2 independent fundamental frequencies, we additionally measured, in the spectrogram window, with reticule cursor, the maximum (max freq) and minimum (min



**Figure 1** Measured parameters for HF pup Mongolian hamster isolation call. Spectrogram (middle), mean power spectrum (left), and 2 single power spectra (right). Designations: duration—call duration; mean peak freq—the peak frequency averaged over the entire call; start peak freq—the peak frequency at the beginning point of a call; end peak freq—the peak frequency at the end point of call; g0max freq—the maximum of the medium fundamental frequency; g0min freq—the minimum of the medium fundamental frequency; h0max freq—the maximum of the high fundamental frequency; h0min freq—the minimum of the high fundamental frequency; A and B—the start and end points of call where the single power spectra were taken. The spectrogram was created at 256 kHz sampling frequency, FFT length 1,024, Hamming window, frame 50%, overlap 87.5%.

freq) values of each fundamental frequency band. We calculated the depth of frequency modulation (depth freq) as the difference between the maximum and minimum values of the fundamental frequency.

Each LF call ( $n = 1,404$ ) and each HF call ( $n = 3,606$ ) was checked for the presence of nonlinear vocal phenomena (see Results): subharmonics, chaos, frequency jumps, and biphonations (Wilden et al. 1998; Yurlova et al. 2020; Kozhevnikova et al. 2021). Subharmonics were noted when frequency bands of 1/2 or 1/3 of the fundamental frequency were present in the call spectrum. Chaos was noted when a chaotic segment (sometimes with residual fundamental frequency) was present in call spectrum. We only noted the presence of chaos and/or subharmonics, if the duration of call fragments containing these nonlinear phenomena comprised at least 10% of the entire call duration (Yurlova et al. 2020; Kozhevnikova et al. 2021). We also noted the presence of frequency jumps, when the fundamental frequency increased or decreased abruptly for  $\geq 1$  kHz (for LF calls) or for  $\geq 10$  kHz (for HF calls). Additionally, each HF call ( $n = 3,606$ ) was checked for the presence of short broadband clicks spanning the audible through the ultrasonic range of frequencies (Groszer et al. 2008; Gaub et al. 2010; Klenova et al. 2021a).

Biphonations could only be noted in HF calls, because the recorder for HF calls (Echo Meter Touch 2 PRO) cut off all frequencies lower than 6 kHz. As we made audio recordings of LF and HF calls independently with 2 recorders (Zoom-H1 and Echo Meter Touch 2 PRO, respectively), we could not check the calls for presence of biphonations between LF and HF calls. For HF calls, biphonation was noted when 2 independent fundamental frequencies and their combinatory

frequency bands were present in the call spectrum (Yurlova et al. 2020).

### Statistical analyses

Statistical analyses were carried out with STATISTICA, v. 13 (StatSoft, Tulsa, OK, USA). Descriptive statistics were indicated as mean  $\pm$  SD, all tests were 2-tailed and differences were considered significant whenever  $P < 0.05$ . We used 2-way ANOVA with Tukey HSD (Honest significant difference) *post hoc* for estimating the effects of call category on the acoustic parameters, with call category included as a fixed factor and pup individual identity (ID) as a random factor. We used a repeated measures (r-m) ANOVA controlled for individuality, to compare the mean values of the acoustic parameters of the medium (g0) and the high (h0) fundamental frequencies within and between HF calls of different categories with 1 or 2 fundamental frequencies. We used principal component analysis (PCA) to estimate the degrees of correlation between body size parameters and for calculating the body size index on the basis of these parameters. We used the Pearson correlation with Bonferroni corrections for multiple testing with body size index as a proxy of body size to estimate the effect of body size on the acoustics of calls of different categories.

We used a standard procedure of discriminant function analysis (DFA) to calculate the probability of the assignment of calls to the correct category. We used Wilks' Lambda to estimate how strongly acoustic parameters contribute to the discrimination of individuals. To validate our DFA results, we calculated the chance values of correct assignment of calls to call category by applying a randomization test for misclassification probability in discriminant analysis (Solow 1990).



The values by chance were calculated from DFAs performed on 1,000 randomized permutations on the data sets (Solow 1990; Mundry and Sommer 2007).

## Results

### Call categories

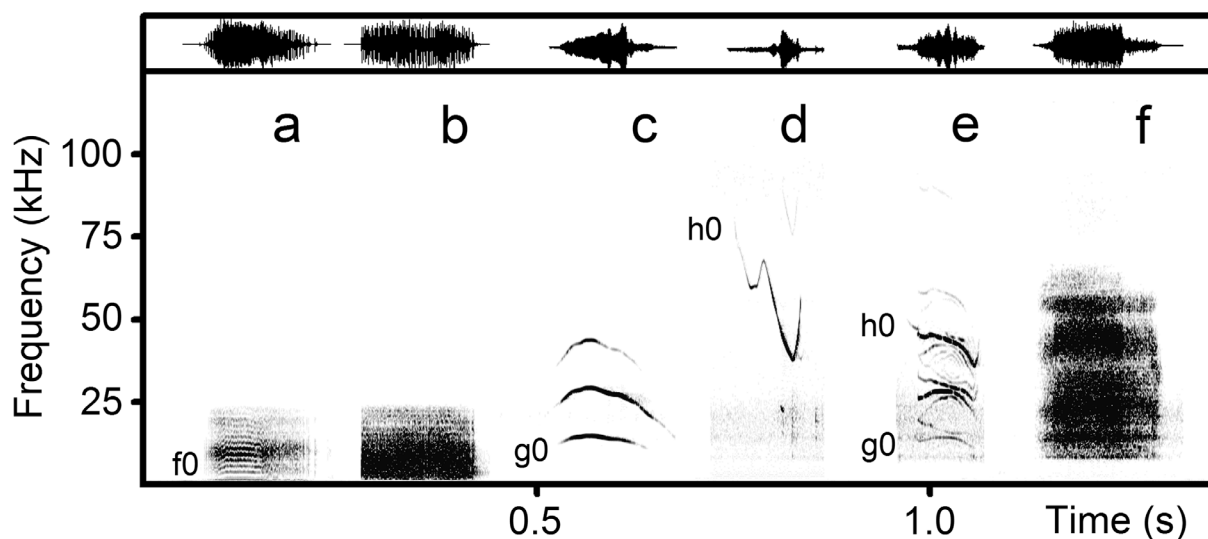
Mongolian hamster pup isolation calls displayed a high complexity of the acoustic structure, resulting from 3 independent fundamental frequencies in call spectra. The LF calls could contain the low fundamental frequency ( $f_0$ ), while HF calls could contain the medium fundamental frequency ( $g_0$ ) and the high fundamental frequency ( $h_0$ ), either alone or together (Figure 2). Both LF calls and HF calls contained calls in which the fundamental frequency could not be tracked in chaos (Figure 2). By presence/absence of fundamental frequency ( $f_0$ ,  $g_0$ ,  $h_0$ , or their combination) and chaos, we classified all calls to 6 categories (Figure 2): LF- $f_0$  calls—monophonic calls with a visible low fundamental frequency ( $f_0$ ) band in the call spectrum; LF-chaos calls—calls in which the low fundamental frequency ( $f_0$ ) could not be tracked in the chaos; HF- $g_0$  calls—monophonic calls with a visible medium fundamental frequency  $g_0$  band; HF- $h_0$  calls—monophonic calls with a visible high fundamental frequency  $h_0$  band; HF- $g_0$  +  $h_0$  calls—calls with 2 visible fundamental frequencies ( $g_0$  and  $h_0$ ); HF-chaos calls—HF chaotic calls, in which the fundamental frequency band could not be tracked in the chaos.

In total, 23 pup callers emitted, during the 2-min isolation trials (1 per pup), 5,010 isolation calls, 1,404 (28.02%) LF calls, and 3,606 (71.98%) HF calls (Figure 3). Of the LF calls, 659 (13.15%) were LF- $f_0$  calls and 745 (14.87%) were LF-chaos calls. Of the HF calls, 1,059 (21.14%) were HF- $g_0$  calls, 1,020 (20.36%) were HF- $h_0$  calls, 846 (16.89%) were HF- $g_0$  +  $h_0$  calls, and 681 (13.59%) were HF-chaos calls (Figure 3).

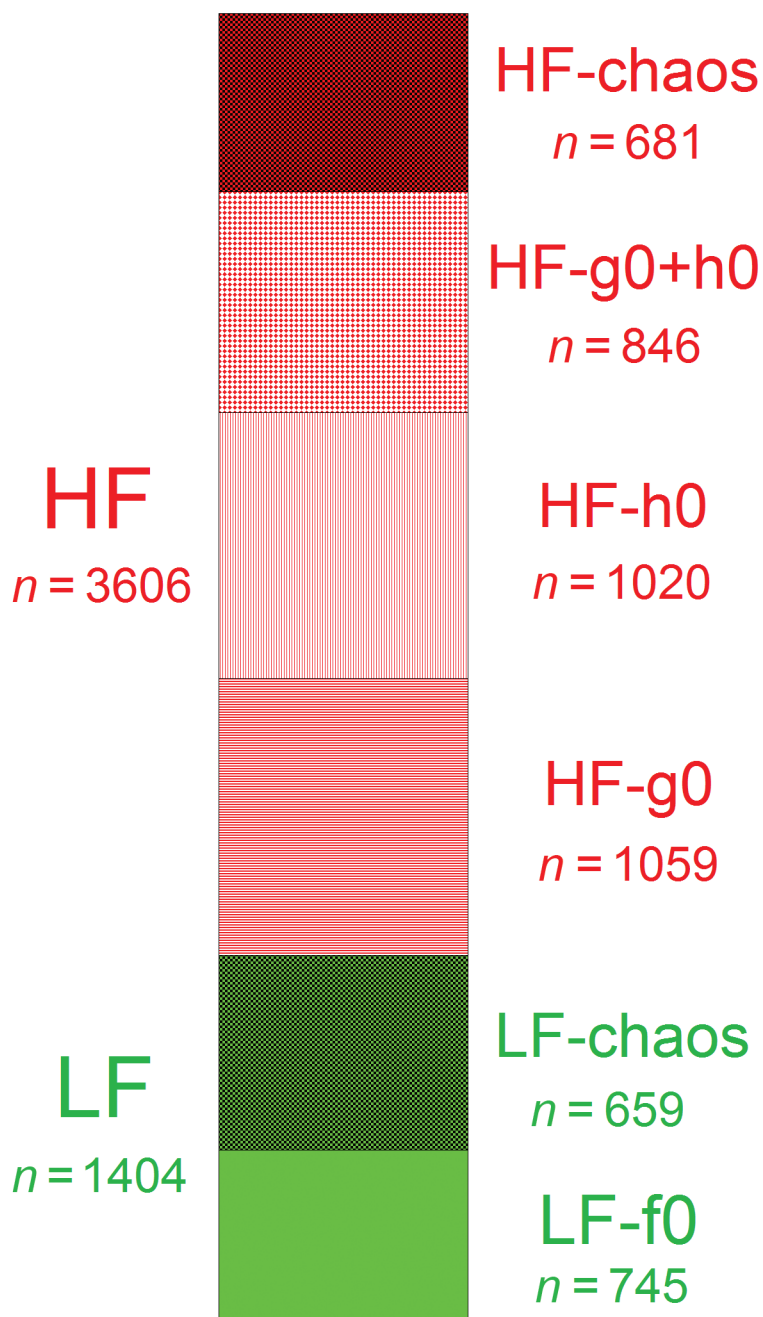
### Acoustic comparison between monophonic call categories

Two-way ANOVA revealed that all acoustic parameters of pup isolation calls containing only 1 fundamental frequency (LF- $f_0$ , HF- $g_0$ , and HF- $h_0$ ) differed significantly between calls of the 3 categories (Table 1). Tukey HSD *post hoc* revealed significant differences for all pairwise comparisons of the acoustics between different categories. Call duration was the longest for LF- $f_0$ , intermediate for HF- $g_0$ , and the shortest for HF- $h_0$  calls. The values of fundamental and peak frequencies and of depth of frequency modulation were the smallest for LF- $f_0$  calls, intermediate for HF- $g_0$  calls, and the largest for HF- $h_0$  calls (Table 1).

Calls with the low fundamental frequency alone (LF- $f_0$  calls) are calls lying in the audible range of frequencies, with greater duration compared to other call categories with 1 fundamental frequency. The values of  $f_0$ max ranged from 0.52 to 4.64 kHz and values of mean peak freq ranged from 2.06 to 14.06 kHz, both within the audible range of frequencies (below 20 kHz) (Figure 4). Calls with the medium fundamental frequency alone (HF- $g_0$  calls) were located at the border of the audible and ultrasonic (over 20 kHz) frequency ranges, with values of  $g_0$ max ranging from 8.0 to 31.70 kHz and values of mean peak freq ranging from 8.0 to 37.0 kHz. The HF- $g_0$  calls often had, in addition to the fundamental frequency band, readily visible harmonics, and peak frequency could lie on a frequency band of one of those harmonics (Figure 4). Calls with the high fundamental frequency (HF- $h_0$  calls) were entirely within the ultrasonic range, with values of  $h_0$ max ranging from 24.75 to 109.25 kHz, however, the mean peak freq values were lower and ranged from 7.70 to 86.50 kHz, primarily because of presence of nonlinear vocal phenomena in the calls. The HF- $h_0$  calls commonly lacked harmonics and were therefore represented by a single frequency band, usually strongly modulated and decreasing from the beginning to the end of a call (Figure 4).



**Figure 2** Spectrograms illustrating categories of LF and HF calls of 4–5-day-old pup Mongolian hamsters. Normalized waveforms above the spectrograms show the lack of clipping in the calls. (a) LF- $f_0$ , call with visible low fundamental frequency  $f_0$ ; (b) LF-chaos, LF chaotic call, in which the fundamental frequency band cannot be tracked; (c) HF- $g_0$ , call with visible medium fundamental frequency  $g_0$ ; (d) HF- $h_0$ , call with visible high fundamental frequency  $h_0$ ; (e) HF- $g_0$  +  $h_0$ , call with 2 visible fundamental frequencies  $g_0$  and  $h_0$ ; (f) HF-chaos, HF chaotic call, in which the fundamental frequency band cannot be tracked. Spectrograms were created using sampling frequency 256 kHz, Hamming window, FFT 1024 points, frame 50%, overlap 93.75%.



**Figure 3** Numbers of pup isolation calls of 2 categories of LF and 4 categories of HF calls: LF-f0—calls with visible low fundamental frequency f0; LF-chaos—LF chaotic calls; HF-g0—calls with visible medium fundamental frequency g0; HF-h0—calls with visible high fundamental frequency h0; HF-g0 + h0—calls with 2 visible fundamental frequencies g0 and h0; HF-chaos—HF chaotic calls.

We conducted DFA for acoustic differences between the 3 call categories with 1 fundamental frequency, with inclusion of values of the 4 acoustic parameters: duration, max freq, min freq, and mean peak freq of each of 892 calls within these 3 categories. The DFA results confirmed ANOVA results regarding good discriminability of calls between the 3 categories (Figure 5). The average percent of correct assignment of the calls to category was 91.6%, which was significantly higher than the level expected by chance of  $35.8 \pm 1.0\%$ , min = 31.9%, max = 39.6% (permutation test, 1,000 permutations,  $P < 0.001$ ). In order of decreasing importance, the max

freq and duration were mainly responsible for discrimination of call categories. Discriminant root 1 (eigenvalue = 3.55) was highly correlated with the max freq ( $r = 0.95$ ), and discriminant root 2 (eigenvalue = 0.25) was highly correlated with duration ( $r = 0.81$ ).

#### Acoustic comparison between monophonic and 2-frequency call categories

Duration of HF-g0 + h0 calls with 2 fundamental frequencies ( $0.098 \pm 0.031$  s) exceeded (2-way ANOVA,  $F_{2,1019} =$

**Table 1** Values (mean  $\pm$  SD) of acoustic parameters for 3 call categories with 1 fundamental frequency and 2-way ANOVA results for their comparison. Call category was included as a fixed factor and animal ID as a random factor. Designations: LF-f0—calls with visible low fundamental frequency f0 band; HF-g0—calls with visible medium fundamental frequency g0 band; HF-h0—calls with visible high fundamental frequency h0 band; duration—call duration; max freq—the maximum value of the fundamental frequency; min freq—the minimum value of the fundamental frequency; depth freq—the depth of frequency modulation; mean peak freq—the mean peak frequency over entire call; start peak freq—peak frequency at the beginning point of a call; end peak freq—peak frequency at the end point of a call;  $N$  = number of animals;  $n$  = number of calls

Acoustic parameter	LF-f0 calls $N = 17, n = 162$	HF-g0 calls $N = 21, n = 362$	HF-h0 calls $N = 20, n = 368$	ANOVA
Duration (s)	0.152 $\pm$ 0.056	0.086 $\pm$ 0.040	0.077 $\pm$ 0.036	$F_{2,868} = 237.3; P < 0.001$
Max freq (kHz)	2.19 $\pm$ 1.01	18.02 $\pm$ 5.25	52.56 $\pm$ 16.74	$F_{2,868} = 1490.2; P < 0.001$
Min freq (kHz)	1.66 $\pm$ 0.96	12.81 $\pm$ 4.17	35.27 $\pm$ 12.84	$F_{2,868} = 1141.3; P < 0.001$
Depth freq (kHz)	0.54 $\pm$ 0.33	5.21 $\pm$ 3.08	17.30 $\pm$ 11.36	$F_{2,868} = 322.8; P < 0.001$
Mean peak freq (kHz)	7.49 $\pm$ 3.58	20.11 $\pm$ 5.77	34.24 $\pm$ 14.08	$F_{2,868} = 487.6; P < 0.001$
Start peak freq (kHz)	7.33 $\pm$ 3.78	20.88 $\pm$ 8.37	42.34 $\pm$ 22.06	$F_{2,868} = 308.7; P < 0.001$
End peak freq (kHz)	5.36 $\pm$ 3.49	19.58 $\pm$ 7.51	36.22 $\pm$ 15.48	$F_{2,868} = 511.1; P < 0.001$

25.6;  $P < 0.001$ ) the duration of the monophonic HF-g0 calls (0.086  $\pm$  0.040 s) and the duration of the monophonic HF-h0 calls (0.077  $\pm$  0.036 s). Mean peak freq for the HF-g0 + h0 calls (26.27  $\pm$  10.77 kHz) was higher ( $F_{2,1019} = 139.4; P < 0.001$ ) than those of HF-g0 calls (20.11  $\pm$  5.77 kHz) and lower than in HF-h0 calls (34.24  $\pm$  14.08 kHz).

The g0max was higher in the monophonic HF-g0 calls (18.12  $\pm$  3.96 kHz) than in 2-frequency HF-g0 + h0 calls (15.73  $\pm$  2.61 kHz) of the same individual (r-m ANOVA,  $F_{1,17} = 8.05, P = 0.01$ ) (Figure 6). The g0min did not differ between the monophonic HF-g0 calls (12.89  $\pm$  3.04 kHz) and HF-g0 + h0 calls (12.43  $\pm$  2.20 kHz) of the same individual (r-m ANOVA,  $F_{1,17} = 0.45, P = 0.51$ ) (Figure 6).

The h0max did not differ between the monophonic HF-h0 calls (51.85  $\pm$  11.08 kHz) and 2-frequency HF-g0 + h0 calls (50.12  $\pm$  12.04 kHz) of the same individual (r-m ANOVA,  $F_{1,17} = 1.04, P = 0.32$ ) (Figure 7). The h0min did not differ between the monophonic HF-h0 calls (34.55  $\pm$  8.99 kHz) and HF-g0 + h0 calls (32.94  $\pm$  8.10 kHz) of the same individual (r-m ANOVA,  $F_{1,17} = 2.13, P = 0.16$ ) (Figure 7).

Values of g0max were always lower than the values of h0min of the same individual, in comparing monophonic HF-g0 calls and HF-h0 calls (r-m ANOVA,  $F_{1,17} = 38.44, P < 0.001$ ), and in comparing the fundamental frequencies within 2-frequency HF-g0 + h0 calls (r-m ANOVA,  $F_{1,17} = 92.94, P < 0.001$ ) (Figure 8).

### Acoustic comparison between 2 chaotic call categories

Two-way ANOVA showed that all acoustic parameters of pup isolation calls differed between the LF chaotic calls (LF-chaos) and HF chaotic calls (HF-chaos) (Table 2). The LF-chaos calls had a longer duration and lower peak frequencies compared to HF-chaos calls (Table 2).

The LF-chaos calls are the calls lying within the audible range of frequencies, entirely chaotic, in which the fundamental frequency cannot be tracked. The values of LF-chaos calls mean peak freq ranged from 1.31 to 14.01 kHz, entirely within the audible range of frequencies (Figure 4). The values of acoustic parameters of LF-chaos calls (Table 2) were similar to those of LF-f0 calls (Table 1). Call duration ( $F_{1,335} = 0.83; P = 0.36$ ), start peak freq ( $F_{1,335} = 0.59; P = 0.44$ ) and end peak freq ( $F_{1,335} = 0.62; P = 0.43$ ) did not differ between LF-chaos calls and LF-f0 calls, but the values of the mean

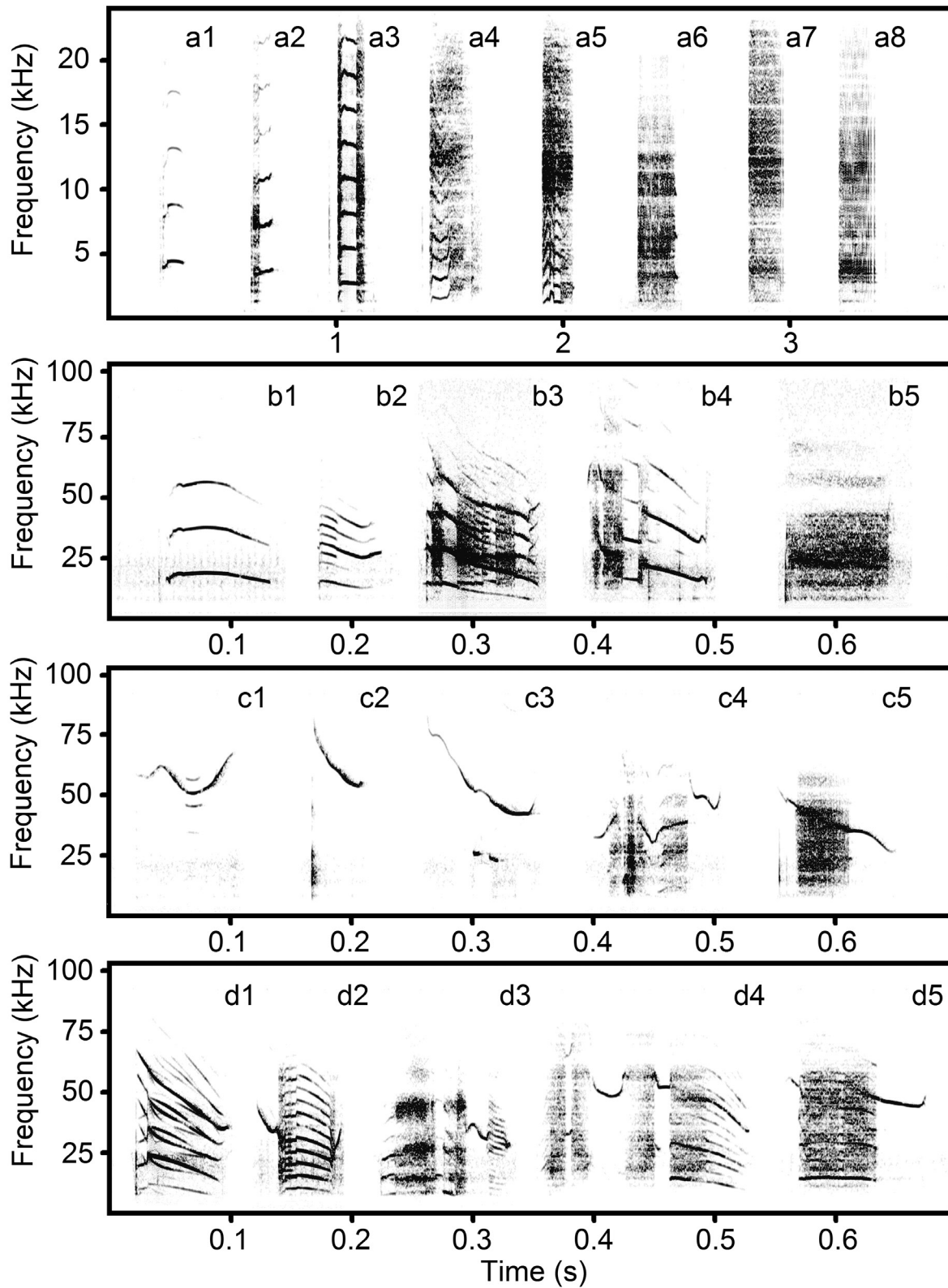
peak freq were higher ( $F_{1,335} = 6.37; P = 0.012$ ) in LF-chaos calls compared to LF-f0 calls. The HF-chaos calls lie primarily in the ultrasonic range of frequencies, the values of the mean peak freq ranged from 12.70 to 42.20 kHz (Figure 4). Because in these calls, the fundamental frequency could not be tracked, we could not determine, which of these fundamental frequencies, g0 or h0 was masked with chaos.

We conducted DFA for acoustic differences between the 2 call categories with chaos. We included in DFA the values of 4 acoustic parameters: duration, mean peak freq, start peak freq, and end peak freq of each of the 341 calls of 2 categories. The DFA confirmed ANOVA results regarding good discriminability of chaotic calls between the 2 categories. The average value of correct assignment to call category was 99.7% (only 1 call was mistakenly assigned to the incorrect category), which was significantly higher than the level expected by chance of 52.6  $\pm$  2.2%, min = 48.2%, max = 61.3% (permutation test, 1,000 permutations,  $P < 0.001$ ). In order of decreasing importance, the mean peak freq and duration were mainly responsible for discrimination of call categories.

### Percentages of nonlinear phenomena in calls of different categories

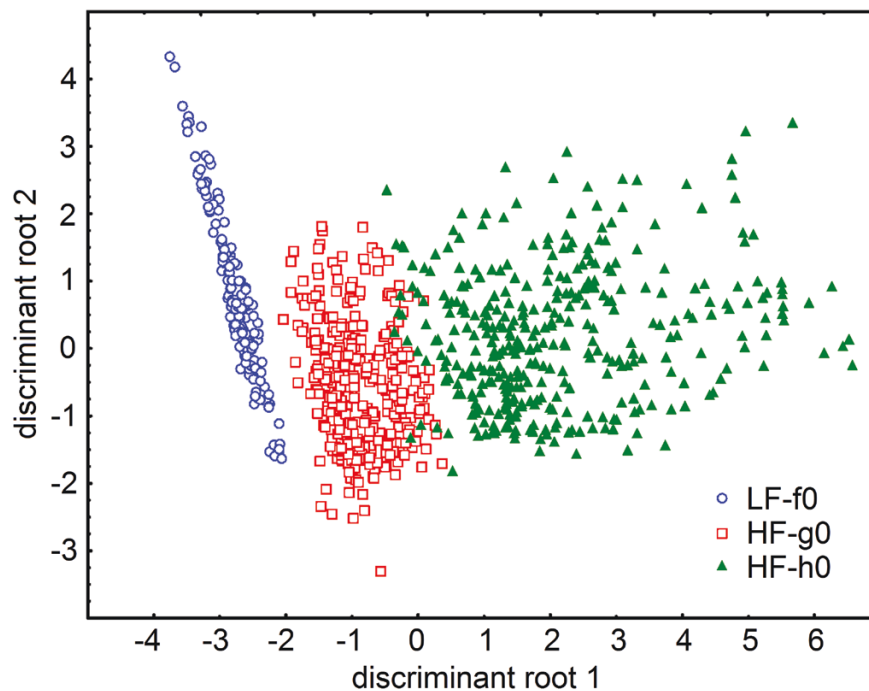
Nonlinear phenomena occurred within all 6 call categories, in 1,393 (99.2%) of 1,404 LF calls, and in 2,788 (77.3%) of 3,606 HF calls (Figure 4). The most frequent nonlinear phenomenon was chaos, which was present in 1,391 (99.1%) of the LF calls and in 2,434 (67.5%) of the HF calls. The enormously large percentage of calls with chaos could be subdivided into 2 call categories in which chaos entirely masked the fundamental frequency: LF-chaos calls and HF-chaos calls. Subharmonics were detected in 23 (1.6%) of the LF calls and in 857 (23.8%) of the HF calls; biphonations were not detected in LF calls but were found in 481 (13.3%) HF calls; frequency jumps were the most rare nonlinear phenomenon and were present in as little as 3 (0.2%) LF calls and in 51 (1.4%) HF calls. Some calls contained more than 1 nonlinear phenomenon; nonlinear phenomena were absent in 11 (0.8%) LF calls and in 818 (22.7%) HF calls. Broadband clicks were detected in 916 (25.4%) HF calls.

The occurrence of nonlinear phenomena and clicks in calls of the 6 call categories is illustrated by Figure 9. While frequency jump was the rarest nonlinear phenomenon, chaos was the most frequent nonlinear phenomenon in any call



**Figure 4** Spectrograms illustrating diversity and nonlinear vocal phenomena of LF and HF calls of 4–5-day-old pup Mongolian hamsters. (a1) LF-f0 call lacking nonlinear phenomena; (a2) LF-f0 call with chaos at the beginning; (a3) LF-f0 call with chaos at the beginning and at the end; (a4) LF-f0 call with chaos at the end; (a5) LF-f0 call with chaos and frequency jump; (a6) LF-chaos call; (a7) LF-chaos call; (a8) LF-chaos call; (b1) HF-g0 call lacking nonlinear phenomena; (b2) HF-g0 call with subharmonics; (b3) HF-g0 call with chaos and biphonation; (b4) HF-g0 call with chaos and frequency jump; (b5) HF-chaos call; (c1) HF-h0 call with biphonation; (c2) HF-h0 call with a broadband click at the beginning; (c3) HF-h0 call with subharmonics; (c4) HF-h0 call with chaos, subharmonics and frequency jump; (c5) HF-h0 call with chaos; (d1) HF-g0 + h0 call, g0 and h0 together throughout a call creating biphonation; (d2) HF-g0 + h0 call, h0 at the beginning and at the end, g0 in the middle of a call; (d3) HF-g0 + h0 call with chaos and biphonation; (d4) HF-g0 + h0 call with chaos and subharmonics; (d5) HF-g0 + h0 call with chaos. Spectrograms were created using sampling frequency 48 kHz (for LF calls) and 256 kHz (for HF calls), Hamming window, FFT 1,024 points, frame 50%, overlap 87.5%.





**Figure 5** Scatterplot showing separation produced by the first 2 discriminant roots of 892 pup isolation calls of Mongolian hamster to 3 call categories with DFA. Designations of call categories: LF-f0—calls with visible low fundamental frequency f0; HF-g0—calls with visible medium fundamental frequency g0; HF-h0—calls with visible high fundamental frequency h0.

category. Calls without nonlinear phenomena were rare; the largest percent of calls without nonlinear phenomena (48.0%) was detected in HF-h0 calls (Figure 9).

### Body size relationship with 3 call fundamental frequencies

The 4–5-day-old pup Mongolian hamsters had no fur, but their skin was already pigmented by growing hairs. Hindleg and forepaw digits were still fused, pinnae were well separated but ear passages closed, eyes were still covered by a layer of skin. Pups locomotory movements involved crawling. Pup body mass ( $n = 26$ ) was on average of  $4.4 \pm 0.3$  g, body length  $43.6 \pm 0.7$  mm, head length of  $16.5 \pm 0.4$  mm, forearm length of  $13.4 \pm 0.4$  mm, foot length of  $7.6 \pm 0.2$  mm, and tail length of  $7.1 \pm 0.3$  mm.

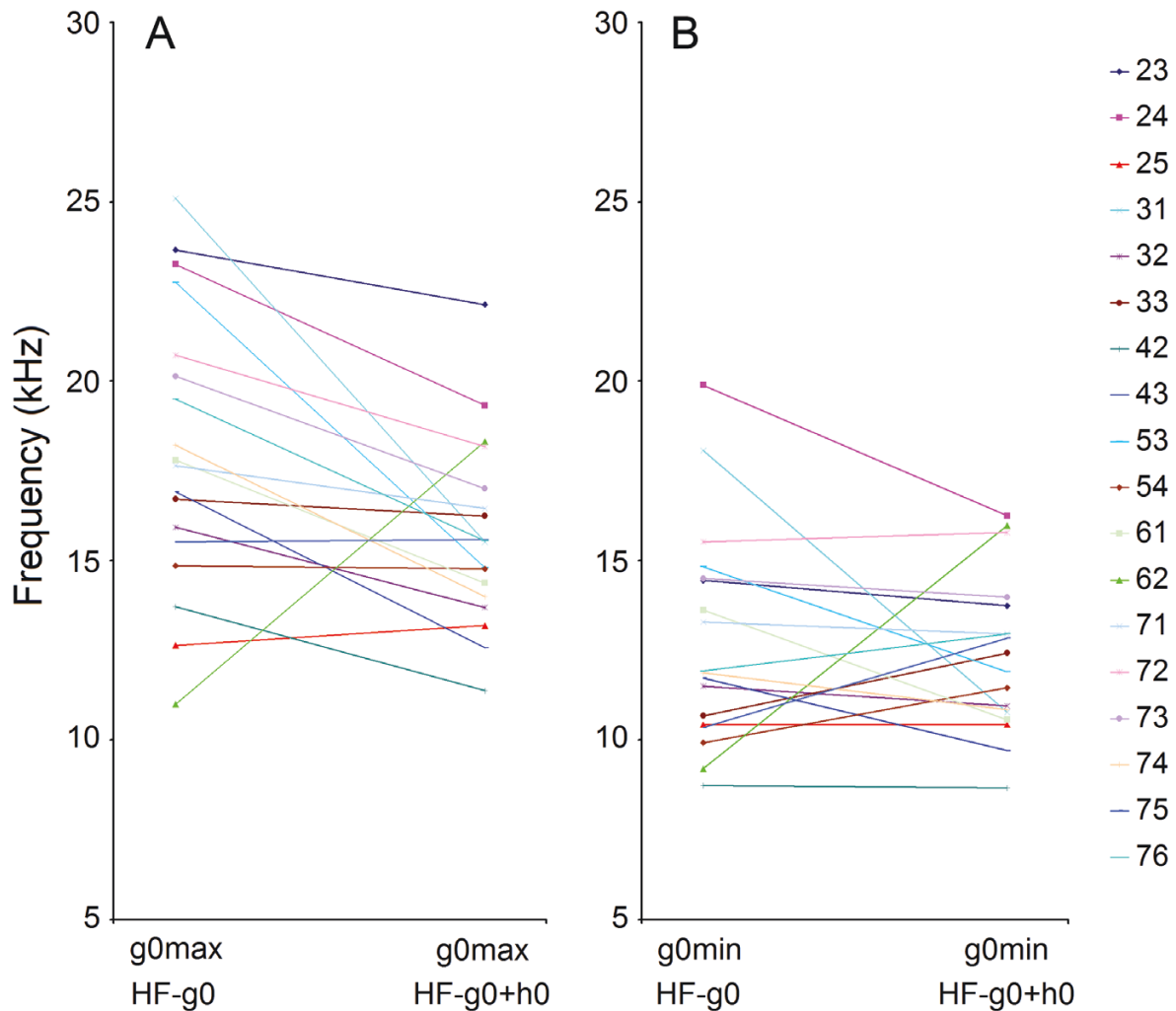
For calculating body size index for estimating the effect of body size on the acoustic parameters, we included all 6 measured parameters of body size: body mass, and the lengths of body, head, forearm, foot, and tail. We calculated body size index for 22 of 23 pup callers (data for the 23rd pup were lost), for which the measurements of fundamental frequency were made for calls of different categories. All these body parameters strongly correlated with the first PCA factor, with correlation coefficients ranging from 0.73 to 0.97 (Table 3). The first PCA factor accounted for 81.75% of variation, so we used the values of the first PCA factor as a generalizing body size index for each of the 22 pups.

Pup body size index (first PCA factor) only significantly positively correlated (after Bonferroni correction) with mean peak frequency of LF-f0 calls (Table 4). The maximum and minimum fundamental frequencies of calls of all the 3 call categories with fundamental frequency and the mean peak frequencies of HF-g0 calls and HF-h0 calls did not show any relationship with body size index (Table 4).

### Discussion

This study investigated, for the first time, pup isolation calls in 4–5-day-old Mongolian hamsters. The calls were produced in both sonic and ultrasonic frequency ranges. We identified 6 call categories: LF-f0, LF-chaos, HF-g0, HF-h0, HF-g0 + h0, and HF-chaos (Figures 2 and 3). Pup isolation calls of Mongolian hamsters may contain 3 independent fundamental frequencies, the low f0, the medium g0, and the high h0 (Figures 2 and 4). In 2 categories of calls (fully audible LF-chaos calls and partially ultrasonic HF-chaos calls) the fundamental frequency band could not be tracked in chaos (Figures 2 and 4). In addition, calls of Mongolian hamster pups had high percentages of nonlinear vocal phenomena: frequency jumps, chaos, subharmonics, and biphonations (Figure 9). Such extremely high structural diversity of pup isolation calls is unusual for rodents and can be used for creating new animal models in biomedicine. It is likely that this model will display a more detectable decrease of acoustic complexity, because mouse and rat models of human neural disorders display calls of less complex structure than in intact control strains (Scattoni et al. 2008, 2018; McMurray et al. 2013; Gulia et al. 2014; Duvauchelle et al. 2018; Shekel et al. 2021).

The percentage of calls with nonlinear phenomena (99.2% for 1,404 LF calls and 77.3% for 3,606 HF calls) and the diversity of nonlinear phenomena were higher than in other hamsters (Hashimoto et al. 2001; Schneider and Fritzsche 2011; Piastolov et al. 2023). Most (67.5%) HF pup isolation calls of Mongolian hamsters contained chaos (Figure 9). Partially ultrasonic broadband calls with frequency bandwidth from 10 to 60 kHz, reminiscent of the HF-chaos calls of pup Mongolian hamsters, were reported for 3–12-day-old pup golden hamsters (Schneider and Fritzsche 2011). Similar in the acoustic structure broadband



**Figure 6** Plots illustrating the differences in (A)  $g0_{max}$  between the monophonic HF-g0 calls and 2-frequency HF-g0 + h0 calls; (B)  $g0_{min}$  between the monophonic HF-g0 calls and 2-frequency HF-g0 + h0 calls. Numbers indicate the IDs of pup callers.

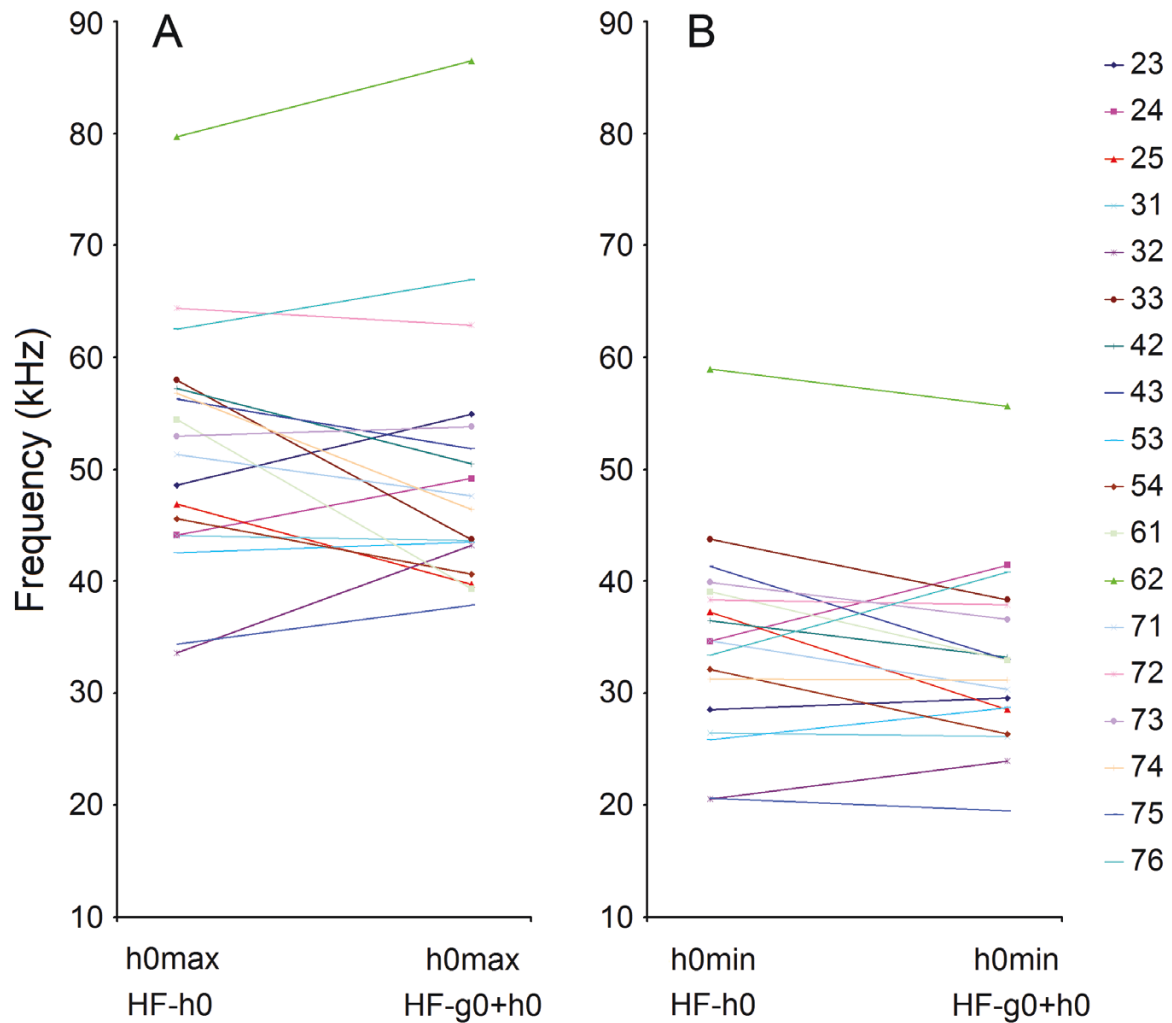
audible-through-ultrasonic noisy rasp calls with bandwidth from 9 to 58 kHz were described for pup and adult Key Largo woodrats (Soltis et al. 2012) and white-throated woodrats (Kobrina et al. 2022). Chaos is abundant in the broadband ultrasonic calls of the *calsyntenin2* deficient knockout pup mouse model of autism (Klenova et al. 2021a). Broadband ultrasonic calls were detected in 58.3% of individual autistic mice pups compared to 15.9% in the control (nonautistic) mouse strain (Klenova et al. 2021a). In addition, published spectrograms indicate that adult golden hamsters also produce very complex ultrasonic calls with chaos, however, the authors do not provide the percentage of these calls (Floody and Pfaff 1977a; Fernández-Vargas and Johnston 2015). At the same time, chaos was missing in all of the 4,000 examined ultrasonic calls in 4–8-day-old Campbell and Djungarian hamsters (Piastolov et al. 2023).

Subharmonics and biphonations were detected in 23.8% and 13.3% of HF calls of Mongolian hamster pups, respectively (Figure 9). Among pups of other hamster species, these nonlinear phenomena were less abundant. Subharmonics were detected in 8.5% of ultrasonic calls in Campbell hamster pups and in 4.2% of ultrasonic calls in Djungarian hamster

pups (Piastolov et al. 2023). Biphonations were rare in these 2 hamster species, occurring on only in 0.2–0.7% of pup ultrasonic calls (Piastolov et al. 2023).

Frequency jumps were most rare nonlinear phenomena of pup Mongolian hamsters and were detected in 1.4% of HF calls (Figure 9). In contrast, frequency jumps of 10 or more kHz occurred in 24.6% in Djungarian and 30.1% in Campbell hamster pup ultrasonic calls (Piastolov et al. 2023). In adult Djungarian hamsters, frequency jumps occurred in 34.6% of ultrasonic calls (Keesom et al. 2015). In adult golden hamsters, frequency jumps of 10 or more kHz were detected in 12% of female and in 26% of male ultrasonic calls (Fernández-Vargas and Johnston 2015) and frequency jumps of 5 kHz and more were detected in 70% of female and in 18% of male ultrasonic calls (Floody and Pfaff 1977a).

The most likely production mechanism for pup isolation calls of Mongolian hamsters with low fundamental frequency ( $f_0$ , of 1.7–2.2 kHz, Table 1) is vibration of the vocal folds. For cricetids, production of the voice-based LF calls was confirmed by experiments in light gases for the long-distance advertisement calls (with fundamental frequency of 11–14 kHz) and agonistic barks (about 8 kHz) of adult grasshopper



**Figure 7** Plots illustrating the differences in (A)  $h0_{max}$  between the monophonic HF-h0 calls and 2-frequency HF-g0 + h0 calls; (B)  $h0_{min}$  between the monophonic HF-h0 calls and 2-frequency HF-g0 + h0 calls. Numbers indicate the IDs of pup callers.

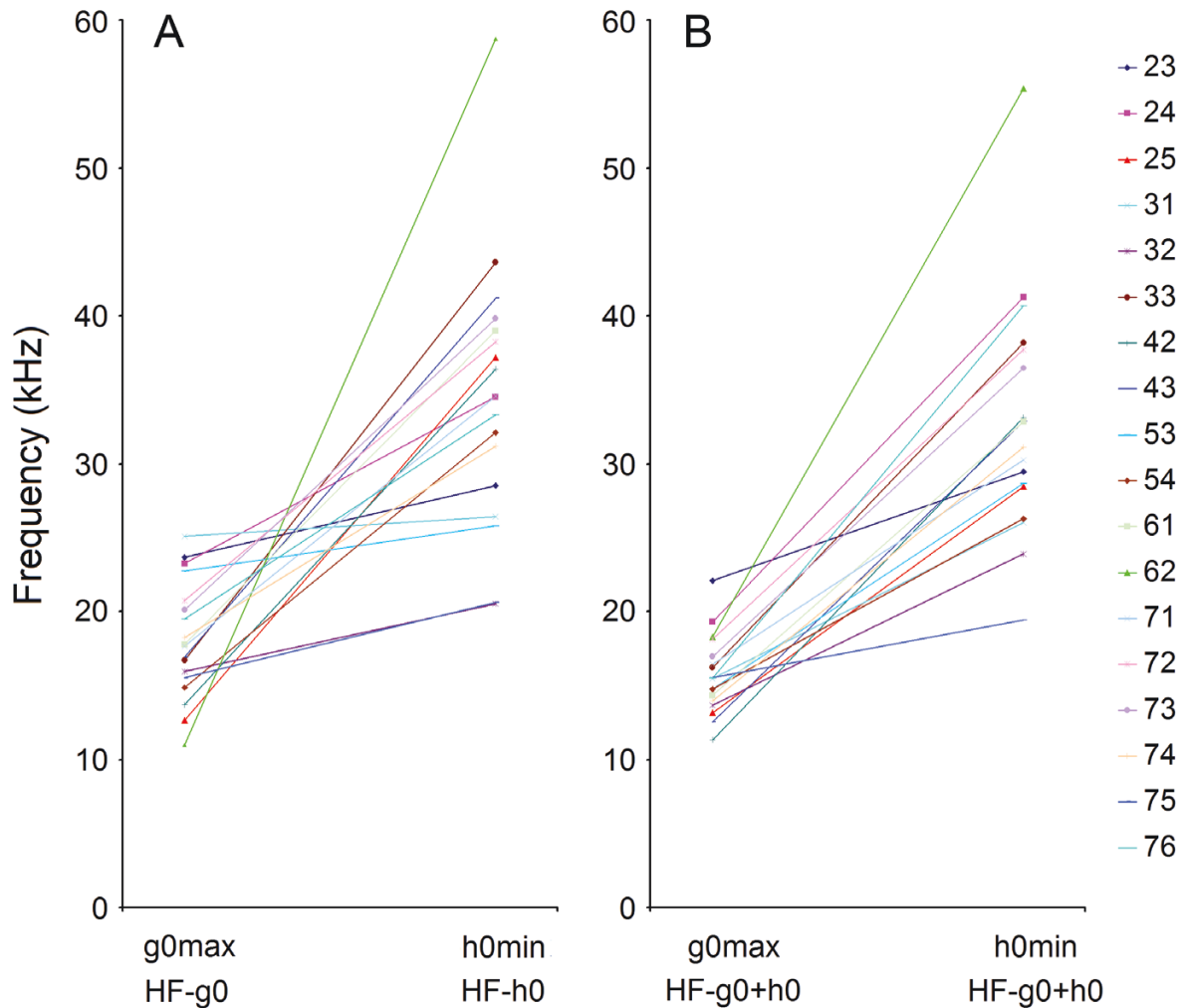
mice (genus *Onychomys*) (Pasch et al. 2017) and for the barks (6–10 kHz) of adult *Peromyscus* deer mice (Riede et al. 2022).

The high fundamental frequency ( $h0$ , of 35.3–52.6 kHz, Table 1) in calls of pup Mongolian hamsters is probably produced by the whistle-based mechanism. For cricetids, this mechanism was confirmed experimentally for close-distant mating down-sweeps and trills (of 40–55 kHz) of grasshopper mice (Pasch et al. 2017) and for sweeps (of 30–80 kHz) of adult deer mice (Riede et al. 2022). The same mechanism might be expected for production of pup ultrasonic isolation calls with descending contour (from 120 to 60 kHz) by pup deer mice *Peromyscus maniculatus* (Smith 1972).

However, for pup isolation calls of Mongolian hamsters with medium-frequency ( $g0$ , of 12.8–18.0 kHz, Table 1), the sound production mechanism is not clear and must be discovered by physiological experiments. At this range of frequencies, Neotominae cricetids produce both whistle-based and voice-based calls. For instance, northern pygmy mice *Baiomys taylori* use the whistle mechanism for producing their long-distance songs, consisting of calls with descending contours, starting at about 40 kHz and ending at about 18

kHz (Riede and Pasch 2020). At the same time, in deer mice, both sustainable calls of adults (with fundamental frequency of 12–19 kHz) and pup isolation calls (with fundamental frequency of 18–22 kHz) were voice-based calls (Riede et al. 2022).

Pup Mongolian hamsters could produce the 2 fundamental frequencies  $g0$  and  $h0$  within a call, either 1 after another (as frequency jump) or simultaneously (as biphonation); the simultaneously produced fundamentals could interact, creating the combinatory frequency bands (Figure 4). Biphonic calls with 2 ultrasonic fundamental frequencies, 1 about 40–50 kHz, and the second about 90–125 kHz, were also reported in 2–5-day-old pup *Lasiopodomys voles* (Dymkaya et al. 2022), 1–8-day-old yellow steppe lemming *Eolagurus luteus* pups (Yurlova et al. 2020), and 6–10-day-old *Meriones* gerbil pups (Kozhevnikova et al. 2021). The highest percentage of ultrasonic calls containing biphonations (34% calls) was found in 1–4-day-old yellow steppe lemming pups (Yurlova et al. 2020). In contrast, in adult cricetids, calls with 2 ultrasonic fundamental frequencies are lacking (Pasch et al. 2017; Riede et al. 2022). Thus, findings 2 simultaneously produced high



**Figure 8** Plots illustrating the differences in (A) g0max and h0min of the monophonic HF-g0 and HF-h0 calls; (B) g0max and h0min of 2-frequency HF-g0 + h0 calls. Numbers indicate the individual identities of pup callers.

**Table 2** Values (mean  $\pm$  SD) of pup isolation calls with chaos, and the results of 2-way ANOVA for differences between call categories. Call category was included as a fixed factor and animal ID as a random factor. Designations: LF-chaos—LF chaotic call; HF-chaos—HF chaotic calls; duration—call duration; mean peak freq—the mean peak frequency over entire call; start peak freq—peak frequency at the beginning point of a call; end peak freq—peak frequency at the end point of a call;  $N$  = number of subject pups;  $n$  = number of calls

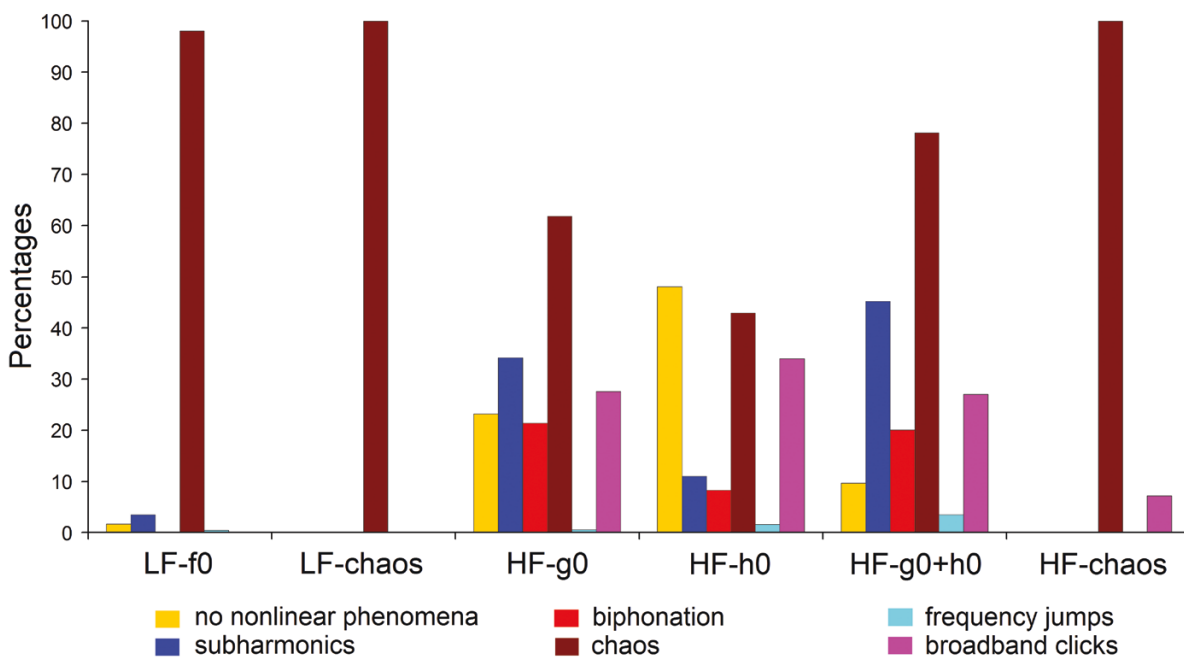
Acoustic parameter	LF-chaos calls $N = 17, n = 192$	HF-chaos calls $N = 14, n = 149$	ANOVA
Duration (s)	$0.151 \pm 0.032$	$0.081 \pm 0.034$	$F_{1,319} = 92.2; P < 0.001$
Mean peak freq (kHz)	$8.00 \pm 3.71$	$22.63 \pm 4.45$	$F_{1,319} = 464.2; P < 0.001$
Start peak freq (kHz)	$7.81 \pm 3.73$	$23.44 \pm 8.07$	$F_{1,319} = 241.9; P < 0.001$
End peak freq (kHz)	$5.53 \pm 3.70$	$24.35 \pm 8.37$	$F_{1,319} = 352.5; P < 0.001$

fundamental frequencies in spectra of rodent pup ultrasonic isolation calls suggests that the mechanisms underlying production of ultrasonic calls in pups can be more complex than those described for adults.

The production mechanism of chaos in voice-based calls of Mongolian hamster pups, with low fundamental frequency  $f_0$  might be related to the irregular vibrations of the dynamic system of the 2 vocal folds, resulting in deterministic chaos

(Herzel et al. 1994; Wilden et al. 1998; Fitch et al. 2002). At the same time, in the whistle-based calls of Mongolian hamster pups, with high fundamental frequency  $h_0$ , chaos arises in the vocal tract from airflow-induced noise (Mahrt et al. 2016; Håkansson et al. 2022). So, it can be termed turbulent chaos. For chaos in pup isolation calls of Mongolian hamsters with medium-frequency  $g_0$ , the production mechanism is not clear.





**Figure 9** The occurrence of nonlinear phenomena and clicks in 6 categories of Mongolian hamster pup isolation calls for the total sample of 1,404 LF and 3,606 HF calls. For each call category, the total percent sum is not equal to 100%, as some calls contained more than 1 nonlinear phenomenon. Designations: LF-f0—calls with visible low fundamental frequency f0; LF-chaos—LF chaotic calls; HF-g0—calls with visible medium fundamental frequency g0; HF-h0—calls with visible high fundamental frequency h0; HF-g0 + h0—calls with 2 visible fundamental frequencies g0 and h0; HF-chaos—HF chaotic calls.

**Table 3** Correlation coefficients between 6 body size parameters and PCA factors, eigenvalues and percent variance, described by each PCA factor

Body size parameter	PCA factor 1	PCA factor 2	PCA factor 3	PCA factor 4	PCA factor 5	PCA factor 6
Body mass	-0.974	0.080	0.018	-0.059	0.143	-0.147
Body length	-0.910	0.001	0.376	-0.117	-0.129	0.007
Head length	-0.918	0.297	0.127	0.191	0.092	0.090
Forearm length	-0.946	0.029	-0.228	0.142	-0.168	-0.062
Foot length	-0.928	0.127	-0.277	-0.193	0.014	0.092
Tail length	-0.728	-0.681	-0.004	0.046	0.053	0.037
Eigenvalue	4.405	0.575	0.287	0.113	0.077	0.043
Percent variance	81.75%	9.59%	4.78%	1.88%	1.28%	0.72%

**Table 4** Pearson’s correlation coefficients between body size index and the acoustic parameters of 3 call categories containing fundamental frequency. Threshold for significant values after Bonferroni correction is  $P < 0.017$  (highlighted in bold). Designations: LF-f0—calls with visible low fundamental frequency f0; HF-g0—calls with visible medium fundamental frequency g0; HF-h0—calls with visible high fundamental frequency h0; max freq—maximum value of fundamental frequency; min freq—minimum value of fundamental frequency; mean peak freq—the mean peak frequency over entire call;  $N$ —number of animals

Call category	$N$	Max freq	Min freq	Mean peak freq
LF-f0	17	$r = -0.08, P = 0.76$	$r = -0.09, P = 0.74$	$r = 0.77, P < 0.001$
HF-g0	21	$r = -0.01, P = 0.95$	$r = 0.10, P = 0.65$	$r = -0.44, P = 0.046$
HF-h0	20	$r = 0.29, P = 0.22$	$r = 0.20, P = 0.39$	$r = 0.14, P = 0.56$

**Acknowledgments**

We thank Andrey Popov and Ilya Popov for technical support. We thank 2 anonymous reviewers and James Hare for their constructive comments and advices. We thank Stephen Pollard for consulting regarding language and style.

**Authors’ Contributions**

M.R., N.F., A.S. and A.G. conceived the study and provided animals for the study. M.R. collected the data. M.R., I.V., and E.V. analyzed the data and prepared the draft of the manuscript. I.V. carried out the statistical analyses. All authors

contributed critically to the drafts and gave final approval for publication.

## Funding

This study was not supported by any foundation.

## Conflict of Interest

The authors declare no conflict of interest.

## Ethical Statement

The authors adhered to the “Guidelines for the treatment of animals in behavioral research and teaching” (Anim. Behav., 2020, 159, I-XI) and the legal requirements of Russia pertaining to the protection of animal welfare (State Standard 3 53434-2009). The experimental procedure was approved by the Committee of Bio-ethics of Severtsov Institute of Ecology and Evolution, protocol # 38 (2020).

## References

- Alekshina TA, Palchikova NA, Kozhemyakina RV, Prokudina OI, 2016. The signs of destabilization in behavioral and somatovegetative parameters in rats selected for catatonia. *Russ J Genet* 6:798–803.
- Allen GM, 1925. Hamsters collected by the American Museum Asiatic expeditions. *Am Mus Novit* 179:3.
- Arriaga G, Jarvis ED, 2013. Mouse vocal communication system: Are ultrasounds learned or innate? *Brain Lang* 124:96–116.
- Arriaga G, Zhou EP, Jarvis ED, 2012. Of mice, birds, and men: The mouse ultrasonic song system has some features similar to humans and song-learning birds. *PLoS ONE* 7:e46610.
- Briggs JR, Kalcounis-Rueppell MC, 2011. Similar acoustic structure and behavioural context of vocalizations produced by male and female California mice in the wild. *Anim Behav* 82:1263–1273.
- Brudzynski SM, 2015. Pharmacology of ultrasonic vocalizations in adult rats: Significance, call classification and neural substrate. *Curr Neuropharmacol* 13:180–192.
- Brudzynski SM, 2021. Biological functions of rat ultrasonic vocalizations, arousal mechanisms, and call initiation. *Brain Sci* 11:605.
- Campbell P, Pasch B, Warren AL, Phelps SM, 2014. Vocal ontogeny in Neotropical singing mice (*Scotinomys*). *PLoS ONE* 9:e113628.
- Chen Y, Xiang Z, Su Q, Qin J, Liu Q, 2023. Vocal signals with different social or non-social contexts in two wild rodent species (*Mus caroli* and *Rattus losea*). *Anim Cogn* 26:963–972. doi:10.1007/s10071-023-01745-6.
- Cherry JA, 1989. Ultrasonic vocalizations by male hamsters: Parameters of calling and effects of playbacks on female behaviour. *Anim Behav* 38:138–153.
- Darwaiz T, Pasch B, Riede T, 2022. Postnatal remodeling of the laryngeal airway removes body size dependency of spectral features for ultrasonic whistling in laboratory mice. *J Zool* 318:114–126.
- Duvauchelle CL, Maddox WT, Reno JM, Thakore N, Mittal N et al., 2018. Alcohol-preferring rats and 22-kHz negative-affect ultrasonic vocalizations. In: Brudzynski SM, editor. *Handbook of Ultrasonic Vocalization, v. 25. A Window into the Emotional Brain*. London: Elsevier, Academic Press, 401–411.
- Dymskaya MM, Volodin IA, Smorkatcheva AV, Vasilieva NA, Volodina EV, 2022. Audible, but not ultrasonic, calls reflect surface-dwelling or subterranean specialization in pup and adult Brandt’s and mandarin voles. *Behav Ecol Sociobiol* 76:106.
- Esposito G, Hiroi N, Scattoni ML, 2017. Cry, baby, cry: expression of distress as a biomarker and modulator in autism spectrum disorder. *Int J Neuropsychopharmacol* 20:498–503.
- Feoktistova NY, Kropotkina MV, Potashnikova EV, Gureeva AV, Kuznetsova EV et al., 2019. Speciation in allopatric species of the hamster subfamily Cricetinae (Rodentia, Cricetidae). *Biol Bull Rev* 9:230–242.
- Feoktistova NY, Naidenko SV, Surov AV, Menchinskii EM, 2013. Ecological and physiological characteristics of seasonal biology of the Mongolian hamster *Allocricetulus curtatus* Allan 1940 (Cricetinae, Rodentia). *Russ J Ecol* 44:56–59.
- Fernández-Vargas M, Johnston RE, 2015. Ultrasonic vocalizations in golden hamsters *Mesocricetus auratus* reveal modest sex differences and nonlinear signals of sexual motivation. *PLoS ONE* 10:e0116789.
- Fernández-Vargas M, Riede T, Pasch B, 2022. Mechanisms and constraints underlying acoustic variation in rodents. *Anim Behav* 184:135–147.
- Finton CJ, Keesom SM, Hood KE, Hurley LM, 2017. What’s in a squeak? Female vocal signals predict the sexual behaviour of male house mice during courtship. *Anim Behav* 126:163–175.
- Fitch WT, Neubauer J, Herzel H, 2002. Calls out of chaos: The adaptive significance of nonlinear phenomena in mammalian vocal production. *Anim Behav* 63:407–418.
- Floody OR, 2018. Ultrasonic communication in hamsters. In: Brudzynski SM, editor. *Handbook of Ultrasonic Vocalization, v. 25. A Window into the Emotional Brain*. London: Elsevier, Academic Press, 197–206.
- Floody OR, Pfaff DW, 1977a. Communication among hamsters by high-frequency acoustic signals: I. Physical characteristics of hamster calls. *J Comp Physiol Psychol* 91:794–806.
- Floody OR, Pfaff DW, 1977b. Communication among hamsters by high-frequency acoustic signals: III. Response evoked by natural and synthetic ultrasounds. *J Comp Physiol Psychol* 91:820–829.
- Floody OR, Pfaff DW, Lewis CD, 1977. Communication among hamsters by high-frequency acoustic signals: II. Determinants of calling by females and males. *J Comp Physiol Psychol* 91:807–819.
- Frank DH, Johnston RE, 1981. Determinants of scent marking and ultrasonic calling by female Turkish hamsters *Mesocricetus brandti*. *Behav Neural Biol* 33:514–518.
- Gaub S, Groszer M, Fisher SE, Ehret G, 2010. The structure of innate vocalizations in Foxp2-deficient mouse pups. *Genes Brain Behav* 9:390–401.
- Granon S, Faure A, Chauveau F, Cressant A, Ey E, 2018. Why should my mouse call me? Acoustic communication in mouse models of social disorders: ultrasonic vocalizations as an index of emotional and motivational states. In: Brudzynski SM, editor. *Handbook of Ultrasonic Vocalization, v. 25. A Window into the Emotional Brain*. London: Elsevier, Academic Press, 423–431.
- Grimley J, Gadziola M, Wenstrup JJ, 2013. Automated classification of mouse pup isolation syllables: From cluster analysis to an Excel based “mouse pup syllable classification calculator”. *Front Behav Neurosci* 6:89.
- Grimley JMS, Monaghan JJM, Wenstrup JJ, 2011. Development of social vocalizations in mice. *PLoS ONE* 6:e17460.
- Grimley JMS, Sheth S, Vallabh N, Grimley CA, Bhattal J et al., 2016. Contextual modulation of vocal behavior in mouse: Newly identified 12 kHz “mid-frequency” vocalization emitted during restraint. *Front Behav Neurosci* 10:38.
- Groszer M, Keays DA, Deacon RMJ, de Bono JP, Prasad-Mulcare S et al., 2008. Impaired synaptic plasticity and motor learning in mice with a point mutation implicated in human speech deficits. *Curr Biol* 18:354–362.
- Gulia KK, Patel N, Radhakrishnan A, Kumar VM, 2014. Reduction in ultrasonic vocalizations in pups born to rapid eye movement sleep restricted mothers in rat model. *PLoS One* 9:e84948.
- Gureeva AV, Feoktistova NY, Matveevsky SN, Kolomiets OL, Surov AV, 2015. Speciation of Eversman and Mongolian hamsters (*Allocricetulus*, Cricetinae): Experimental hybridization. *Zool Zh* 94:614–620 [in Russian].
- Gureeva AV, Lebedev VS, Feoktistova NY, Surov AV, 2021. Geographical variability of the craniological characters in Eversmann’s hamsters and the taxonomic structure of the genus *Allocricetulus* (Cricetidae). *Biol Bull Russ Acad Sci* 48:1380–1388.

- Håkansson J, Jiang W, Xue Q, Zheng X, Ding M et al., 2022. Aerodynamics and motor control of ultrasonic vocalizations for social communication in mice and rats. *BMC Biol* 20:3.
- Hashimoto H, Saito TR, Moritani N, Komeda K, Takahashi KW, 2001. Comparative study on isolation calls emitted from hamster pups. *Exp Anim* 50:313–318.
- Herzel H, Berry D, Titze IR, Saleh S, 1994. Analysis of vocal disorders with methods from nonlinear dynamics. *J Speech Hearing Res* 37:1008–1019.
- Hofer MA, Shair HN, Brunelli SA, 2002. Ultrasonic vocalizations in rat and mouse pups. *Curr Protoc Neurosci* 8:8–14.
- Holy TE, Guo Z, 2005. Ultrasonic songs of male mice. *PLoS Biol* 3:e386.
- Hülsmann S, Oke Y, Mesuret G, Latal AT, Fortuna MG et al., 2019. The postnatal development of ultrasonic vocalization-associated breathing is altered in glycine transporter 2-deficient mice. *J Physiol* 597:173–191.
- Kalcounis-Rueppell MC, Petric R, Briggs JR, Carney C, Marshall MM et al., 2010. Differences in ultrasonic vocalizations between wild and laboratory California mice *Peromyscus californicus*. *PLoS ONE* 5:e9705.
- Kapusta J, Szentgyörgyi H, Surov A, Ryurikov G, 2006. Vocalization of two palaeartic species of hamster: eversmann hamster *Allocrietulus eversmanni* and grey hamster *Cricetulus migratorius*. *Bioacoustics* 15:315–330.
- Keesom SM, Rendon NM, Demas GE, Hurley LM, 2015. Vocal behaviour during aggressive encounters between Siberian hamsters *Phodopus sungorus*. *Anim Behav* 102:85–93.
- Klenova AV, Volodin IA, Ilchenko OG, Volodina EV, 2021b. Discomfort-related changes of call rate and acoustic variables of ultrasonic vocalizations in adult yellow steppe lemmings *Eolagurus luteus*. *Sci Rep* 11:14969.
- Klenova AV, Volodin IA, Volodina EV, Ranneva SV, Amstislavskaya TG et al., 2021a. Vocal and physical phenotypes of calyntenin2 knockout mouse pups model early-life symptoms of the autism spectrum disorder. *Behav Brain Res* 412: 113430.
- Kobrina A, Letowt ME, Pasch B, 2022. Vocal repertoire and auditory sensitivity of white-throated woodrats *Neotoma albigula*. *J Comp Psychol* 137:116–128.
- Kozhevnikova JD, Volodin IA, Zaytseva AS, Ilchenko OG, Volodina EV, 2021. Pup ultrasonic isolation calls of six gerbil species and the relationship between acoustic traits and body size. *R Soc Open Sci* 8:201558.
- Lesch R, Schwaha T, Orozco A, Shilling M, Brunelli S et al., 2021. Selection on vocal output affects laryngeal morphology in rats. *J Anat* 238: 1179–1190.
- Lopez-Salesansky N, Wells DJ, Chancellor N, Whitfield L, Burn CC, 2021. Handling mice using gloves sprayed with alcohol-based hand sanitiser: Acute effects on mouse behaviour. *Anim Technol Welf* 20:11–20.
- Mahrt E, Agarwal A, Perkel D, Portfors C, Elemans CPH, 2016. Mice produce ultrasonic vocalizations by intra-laryngeal planar impinging jets. *Curr Biol* 26:R865–R881.
- McMurray MS, Zeskind PS, Meiners SM, Garber KA, Tien H et al., 2013. Effect of prenatal cocaine on early postnatal thermoregulation and ultrasonic vocalization production. *Front Psychol* 4:882.
- Mundry R, Sommer C, 2007. Discriminant function analysis with non-independent data: consequences and an alternative. *Anim Behav* 74:965–976.
- Okon EE, 1971. The temperature relations of vocalization in infant Golden hamsters and Wistar rats. *J Zool* 164:227–237.
- Pasch B, Tokuda IT, Riede T, 2017. Grasshopper mice employ distinct vocal production mechanisms in different social contexts. *Proc R Soc B* 284:20171158.
- Petric R, Kalcounis-Rueppell MC, 2013. Female and male adult brush mice *Peromyscus boylii* use ultrasonic vocalizations in the wild. *Behaviour* 150:1747–1766.
- Piastolov SV, Volodin IA, Vasilieva NY, Khrushchova AM, Shekarova ON et al., 2023. Crying ultra-different: dwarf hamster hybrids inherit properties of pup ultrasonic calls beyond the ranges of parental species. *Behav Process* 211: 104917.
- Pierce J, Sawrey DK, Dewsbury DA, 1989. A comparative study of rodent ultrasonic vocalizations during copulation. *Behav Neural Biol* 51:211–221.
- Rendon NM, Keesom SM, Amadi C, Hurley LM, Demas GE, 2015. Vocalizations convey sex, seasonal phenotype, and aggression in a seasonal mammal. *Physiol Behav* 152:143–150.
- Riede T, 2011. Subglottal pressure, tracheal airflow, and intrinsic laryngeal muscle activity during rat ultrasound vocalization. *J Neurophysiol* 106:2580–2592.
- Riede T, 2013. Stereotypic laryngeal and respiratory motor patterns generate different call types in rat ultrasound vocalization. *J Exp Zool A* 319:213–224.
- Riede T, Borgard HL, Pasch B, 2017. Laryngeal airway reconstruction indicates that rodent ultrasonic vocalizations are produced by an edge-tone mechanism. *R Soc Open Sci* 4:170976.
- Riede T, Kobrina A, Bone L, Darwaiz T, Pasch B, 2022. Mechanisms of sound production in deer mice (*Peromyscus* spp.). *J Exp Biol* 225:jeb243695.
- Riede T, Pasch B, 2020. Pygmy mouse songs reveal anatomical innovations underlying acoustic signal elaboration in rodents. *J Exp Biol* 223:jeb223925–jeb.223925.
- Riede T, Schaefer CP, Stein A, 2020. The role of deep breaths in ultrasonic vocal production of Sprague-Dawley rats. *J Neurophysiol* 123:966–979.
- Riede T, Zhao Y, LeDoux MS, 2015. Vocal development in dystonic rats. *Physiol Rep* 3:e12350.
- Rutovskaya MV, 2020. Acoustic communication in four species of subgenus *Alexandromys* (Rodentia, Cricetidae). *Russ J Theriol* 19:21–36.
- Scattoni ML, Crawley J, Ricceri L, 2009. Ultrasonic vocalizations: A tool for behavioural phenotyping of mouse models of neurodevelopmental disorders. *Neurosci Biobehav Rev* 33:508–515.
- Scattoni ML, Gandhi SU, Ricceri L, Crawley JN, 2008. Unusual repertoire of vocalizations in the BTBR T+tf/J mouse model of autism. *PLoS One* 3:e3067.
- Scattoni ML, Michetti C, Ricceri L, 2018. Rodent vocalization studies in animal models of the autism spectrum disorder. In: Brudzynski SM, editor. *Handbook of Ultrasonic Vocalization, v. 25. A Window into the Emotional Brain*. London: Elsevier, Academic Press, 445–456.
- Schneider N, Fritzsche P, 2011. Isolation calls and retrieving behaviour in laboratory and wild-derived golden hamsters: No sign for inbreeding depression. *Mamm Biol* 76:549–554.
- Shair HN, Brunelli SA, Masmela JR, Hofer MA, 2003. Social, thermal, and temporal influences on isolation-induced and maternally potentiated ultrasonic vocalizations of rat pups. *Dev Psychobiol* 42:206–222.
- Shekel I, Giladi S, Raykin E, Weiner M, Chalifa-Caspi V et al., 2021. Isolation-induced ultrasonic vocalization in environmental and genetic mice models of autism. *Front Neurosci* 15: 769670.
- Sibiryakova OV, Volodin IA, Volodina EV, 2021. Polyphony of domestic dog whines and vocal cues to body size. *Curr Zool* 67:165–176.
- Smith JC, 1972. Sound production by infant *Peromyscus maniculatus* (Rodentia: Myomorpha). *J Zool* 168:369–379.
- Solow AR, 1990. A randomization test for misclassification probability in discriminant analysis. *Ecology* 71:2379–2382.
- Soltis J, Alligood CA, Blowers TE, Savage A, 2012. The vocal repertoire of the Key Largo woodrat *Neotoma floridana smalli*. *J Acoust Soc Am* 132:3550–3558.
- Stoeger AS, Baotic A, Li D, Charlton BD, 2012. Acoustic features indicate arousal in infant giant panda vocalisations. *Ethology* 118:896–905.
- Volodin IA, Matrosova VA, Frey R, Kozhevnikova JD, Isaeva IL et al., 2018. Altai pika *Ochotona alpine* alarm calls: individual acoustic variation and the phenomenon of call-synchronous ear folding behavior. *Sci Nat* 105:40.

- Warren MR, Campbell D, Borie AM, Ford CL, Dharani AM et al., 2022. Maturation of social-vocal communication in prairie vole *Microtus ochrogaster* pups. *Front Behav Neurosci* 15: 814200.
- Wilden I, Herzel H, Peters G, Tembrock G, 1998. Subharmonics, biphonation, and deterministic chaos in mammal vocalization. *Bioacoustics* 9:171–196.
- Wöhr M, 2014. Ultrasonic vocalizations in Shank mouse models for autism spectrum disorders: Detailed spectrographic analyses and developmental profiles. *Neurosci Biobehav Rev* 43:199–212.
- Yurlova DD, Volodin IA, Ilchenko OG, Volodina EV, 2020. Rapid development of mature vocal patterns of ultrasonic calls in a fast-growing rodent, the yellow steppe lemming *Eolagurus luteus*. *PLoS ONE* 15:e0228892.
- Zaytseva AS, Volodin IA, Ilchenko OG, Volodina EV, 2019. Ultrasonic vocalization of pup and adult fat-tailed gerbils *Pachyuromys duprasi*. *PLoS One* 14:e0219749.
- Zeskind PS, McMurray MS, Cox Lippard ET, Grewen KM, Garber KA et al., 2014. Translational analysis of effects of prenatal cocaine exposure on human infant cries and rat pup ultrasonic vocalizations. *PLoS ONE* 9:e110349.
- Zollinger SA, Riede T, Suthers RA, 2008. Two-voice complexity from a single side of the syrinx in northern mockingbird *Mimus polyglottos* vocalizations. *J Exp Biol* 211:1978–1991.