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Whispering squirrels: Ultrasonic alarm signaling in a Sciurid rodent

Short of echolocation and the pursuit of prey by bats[1], the function of ultrasound (> 15 kHz) in animal communication is poorly understood[2]. Among rodents, only Murids are known to produce purely ultrasonic signals[3]; the function of these signals, however, remains unclear, as both the context for production (infant isolation and distress; sexual and predator encounters in adults) and the responses of recipients (maternal retrieval of infants; prolonged lordosis) vary greatly[3]. Here we report the first evidence of a purely ultrasonic signal produced by a Sciurid rodent and demonstrate for the first time in any animal group that an ultrasonic signal warns conspecifics of danger.

Ground-dwelling squirrels (family Sciuridae) produce audible (ca. 8 kHz) alarm vocalizations that warn others of danger. Call recipients benefit from enhanced predator detection, while callers benefit via kin selection[4]. While studying Richardson's ground squirrel (*Spermophilus richardsonii*) alarm communication[5], we noticed squirrels (10 of 181 individuals exposed to a model predator[5]) performing motor patterns consistent with alarm calling, but producing only faint sounds of rushing air. These "whisper calls," which have been observed in all of our study populations, contain pure ultrasonic frequencies near 50 kHz (Fig. 1a) and thus constitute an undescribed vocalization of Richardson's ground squirrels[6].

We recorded whisper calls from 15 free-living squirrels in 2003 (Supplementary Methods). The mean (± SE) sound pressure level of calls was 66.8 ± 2.1 dB at a mean (± SE) distance from the squirrel of 0.49 ± 0.02 m. The mean (± SE) duration and dominant frequency of the primary syllable were 225 ± 8 ms and 48.0 ± 2.3 kHz, respectively (Supplementary Information).

We examined call function by broadcasting whisper calls and 3 control calls (background noise; pure tone matching whisper call's dominant frequency; audible call) to free-living squirrels at a different site (Supplementary Methods). Receiver vigilance was scored and compared among treatments (Fig. 1b). Our results demonstrate that whisper calls warn nearby conspecifics of potential danger and that the dominant ultrasonic frequency is highly salient. Audible calls evoked a more pronounced response than did whisper calls, suggesting that whisper calls either convey less urgency than audible calls or that respondents react less conspicuously.

In addition to being inaudible to many rodent predators[3], ultrasound differs from audible sound in that it attenuates rapidly and is highly directional[7]. Though seemingly limiting as a warning signal, these properties may allow callers to selectively warn[8] philopatric kin[4] while remaining undetected by predators residing outside of the signal's active space. Although experiments addressing attenuation and directional propagation of whisper calls are necessary to determine whether whisper calling permits callers to remain cryptic and whether squirrels selectively beam calls to specific receivers, it is reasonable to assume that selection would favour the use of whisper calls under certain circumstances. It is clear that these calls function as anti-predator vocalizations and, given their spectral characteristics, both limit the audience

and reduce the probability that a predator will detect the signaler. All research complied with the guidelines set forth by the Canadian Council on Animal Care and was funded by the Natural Sciences and Engineering Research Council of Canada. We thank Glen Morris, John Page, and Bob Wrigley for facilitating this work.

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Supplementary Information accompanies the paper on **www.nature.com/nature.**

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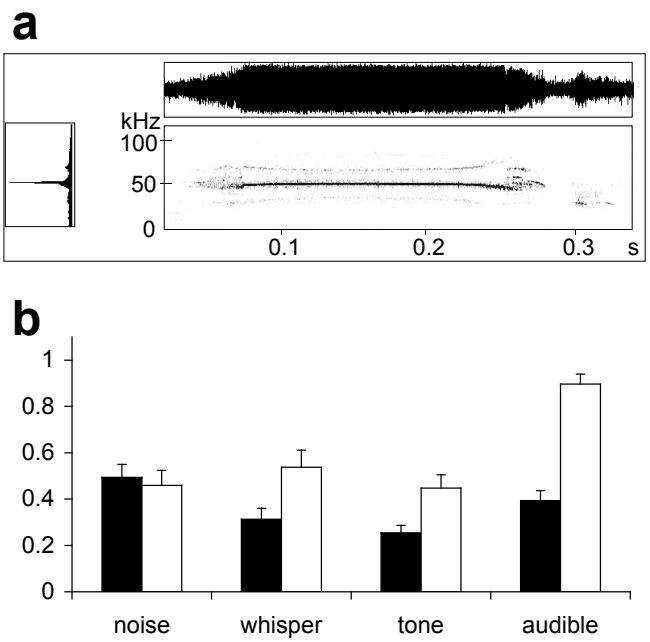

Figure 1 Richardson's ground squirrels' responses to ultrasonic 'whisper calls.' **a,** Spectrogram (bottom right) illustrates call duration (238 ms) and dominant frequency (51.6 kHz) of the primary syllable. Signal intensity is represented across the time axis by the density of the grayscale, while the intensity of individual frequencies (averaged across signal) is shown by the power spectrum (bottom left). The time-amplitude window (top) illustrates overall signal intensity relative to background noise. **b,** Proportion of time (± SE) that squirrels (n=19) devoted to vigilant behaviour before (black bars) and during the playback (open bars) of whisper calls and the 3 control calls.

Supplementary Methods (.doc MS Word: 28 KB)

Complete methods describing the recording and analysis of whisper calls, construction of whisper calls for playback, broadcast of whisper calls to call recipients, and quantification of squirrels' anti-predator behaviour in response to playback of calls.

Recording whisper calls

Alarm calls were recorded from marked Richardson's ground squirrels at Assiniboine Park (49.874º N, 97.243º W), Winnipeg, in 2003 using a portable ultrasound processor (PUSP: 224 kHz sampling rate, manual triggering) and a BAT detector (model U30) from UltraSound Advice. Squirrels typically commenced calling after re-emerging from an escape burrow and facing either the BAT detector (0.49 ± 0.02 m away, mean +/- SE) or the observer (4.27 ± 0.23 m away). Recorded whisper calls were printed, archived on minidisc (1:10 time-expansion ratio), and transferred into Avisoft-SASLab Plus (10:1 time-compression ratio) for spectral analysis (256 point FFT, Hamming window).

Describing whisper calls

Only one recording, selected for its high signal-to-noise ratio, was analyzed per individual, eliminating pseudoreplication[1]. If that recording contained multiple syllables (range: 1-5), only mean values were reported for that individual[1]. The measured parameters include: 1) duration of the primary syllable, 2) duration and frequency of a relatively short pulse of sound occasionally following the primary syllable, described for audible calls as a 'chuck,' 3) latency of

the chuck following the offset of the primary syllable, 4) dominant, or most intense, frequency, 5) minimum/maximum frequency and bandwidth of the dominant frequency, and 6) frequencies of the bands found immediately above (+1) and below (-1) the dominant band, as well as an additional band (+2) occasionally found above the (+1) band.

The sound pressure level of whisper calls was measured indirectly in a sound-proof chamber by replaying the signal into a Brüel and Kjær 0.25" microphone (distance from source matched to field conditions) attached to a type 2204 impulse precision sound level meter (response: hold). Whisper calls were broadcast with a Racal Store 4DS instrumentation recorder connected to an ultrasound amplifier (model S55) and loudspeaker (model S56) from UltraSound Advice. For each call measured, the playback volume was first calibrated by repeatedly re-recording the signal with the PUSP and BAT detector (distance from source matched to field conditions). The playback volume was adjusted so that the signal intensity on the PUSP (represented by the number of pixels on the spectrogram) matched the printout from the original field recording. This estimate is conservative because ultrasound is highly directional and the squirrel's angle with the microphone surface often deviated from normal in the field.

Construction of whisper calls for playback

For each caller, we used the syllable with the highest signal-to-noise ratio to construct one repeated whisper call containing 3 identical syllables separated by 4 s intersyllable silences. For each of the 15 callers, 3 additional calls were constructed that, in place of the 3 syllables, contained either: 1) background noise 2) a tone matching the amplitude and frequency of the

alarm call's dominant frequency, or 3) an audible call from an unfamiliar caller. Because the BAT detector used to record the ultrasonic signals is highly directional, all whisper calls and their associated tones were amplified to match the relative intensity of the whisper call with the highest signal-to-noise ratio. Background noise was amplified by the same factor as its corresponding whisper call. A total of 60 playbacks representing 4 treatments and 15 distinct callers were thus constructed and transferred back to minidisc for playback via the PUSP.

Playback of whisper calls

Each call, along with its 3 corresponding control calls, was broadcast in a dependent-groups design[2] to free-living, marked juvenile squirrels from a colony near Warren, Manitoba (50.170º N, 97.694º W) in 2003. The PUSP, amplifier, and speaker described above were used to broadcast ultrasonic playbacks, while audible calls were broadcast through an audible playback system[2]. Recipients' responses were videotaped and the exact time of playback was noted on the camera's time code. Due to the rapid attenuation and highly directional nature of ultrasound, trials were aborted if the subject did not remain within 8 m of the speaker or if its angle with the speaker exceeded 15º from normal.

Quantification of squirrels' responses

A squirrel's behaviour was characterized as non-vigilant when it was standing on 4 feet with its head below the horizontal plane. All other postures, including low vigilance (standing on 4 feet with its head elevated above the horizontal plane), slouch[3], and alert[3] were collectively considered vigilant behaviour. The proportionate duration of vigilant behaviour during the

playback period (corrected for the proportionate duration of vigilant behaviour during the 30 s prior to playback) were compared across treatment groups with paired sample t-tests.

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Supplementary Table 1 (.doc MS Word: 24 KB)

A complete description of Richardson's ground squirrel whisper calls, including the sound pressure level (dB), duration (ms), frequency (kHz), and bandwidth (kHz) of the dominant frequency, frequency of additional bands (kHz), and latency and duration of the chuck (ms). Refer to Supplementary Methods for a complete description of these parameters.

Table 1 **Description of whisper calls from 15 Richardson's ground squirrels**

Variable	$\overline{X}$ (± SE)	Range	n
Duration of 1º syllable (ms)	225 ± 8	174 - 290	15
Dominant frequency (kHz)	48.0 ± 2.3	27.2 - 62.8	15
Minimum (kHz)	43.5 ± 2.5	25.3 - 54.4	12*
Maximum (kHz)	51.4 ± 2.7	29.1 - 64.2	12*
Bandwidth (kHz)	7.9 ± 1.4	2.6 - 15.0	12*
Frequency (+1)	63.8 ± 3.6	51.6 - 83.4	9
Frequency (-1)	31.7 ± 3.3	16.9 - 45.9	9
Frequency (+2)	77.8 ± 18.8	59.1 - 96.6	2
Sound pressure level (dB)	66.8 ± 2.1	57.7 - 83.7	15
Chuck latency (ms)	20 ± 2	18 - 23	3
Chuck duration (ms)	28 ± 1	26 - 30	3
Chuck frequency (kHz)	31.6 ± 6.0	22.7 - 43.1	3

* Three individuals were excluded from the analysis because the limits of the dominant frequency band were not discernable.