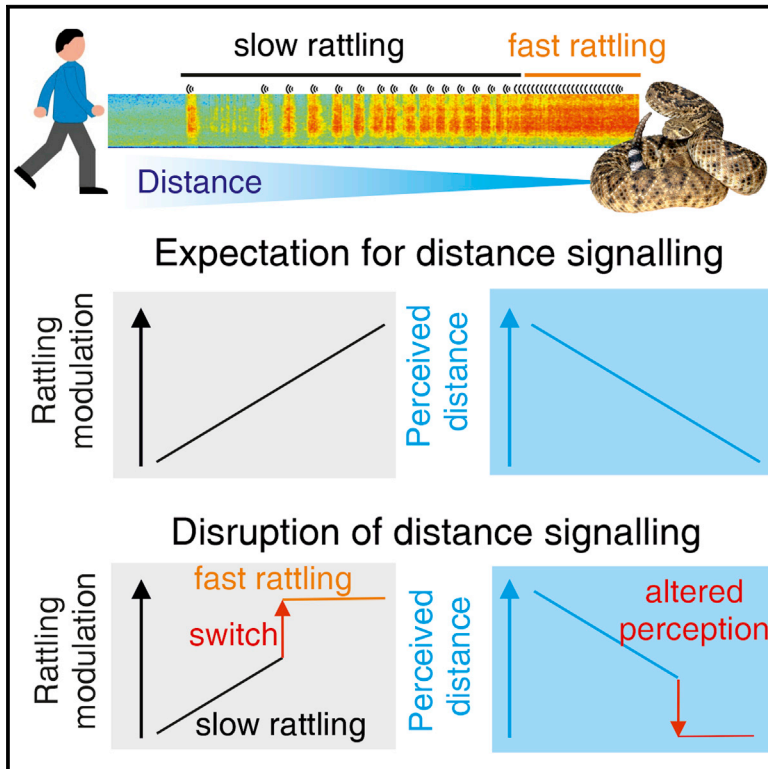# Current Biology

Frequency modulation of rattlesnake acoustic display affects acoustic distance perception in humans

Graphical abstract

Authors

Michael Forsthofer, Michael Schutte,
Harald Luksch, Tobias Kohl,
Lutz Wiegrebe, Boris P. Chagnaud

Correspondence

boris.chagnaud@uni-graz.at

In brief

Forsthofer et al. demonstrate that the rattling of rattlesnakes is used to convey information about the distance of an approaching subject to the snake and suggest that changes in rattling frequency fools the listener's auditory system in misjudging its distance to the snake.

Highlights

- Rattlesnake rattling conveys information about distance

- A jump in frequency range alters distance information

- Responses of human listeners were tested to this sound pattern in a VR

- Subjects underestimated distance to the virtual snake in the VR

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Frequency modulation of rattlesnake acoustic display affects acoustic distance perception in humans

Michael Forsthofer,[1,2,5] Michael Schutte,[1,2,5] Harald Luksch,[3] Tobias Kohl,[3,6] Lutz Wiegrebe,[1,6] and Boris P. Chagnaud[1,4,6,7,*]

[1]Department Biology II, Ludwig-Maximilians-University Munich, Großhaderner Str. 2, Planegg 82152, Germany
[2]Graduate School of Systemic Neurosciences, Ludwig-Maximilians-University Munich, Großhaderner Str. 2, Planegg 82152, Germany
[3]Chair of Zoology, Technical University of Munich, School of Life Sciences, Liesel-Beckmann-Str. 4, Freising 85354, Germany
[4]Institute for Biology, Karl-Franzens-University Graz, Universitätsplatz 2, Graz 8010, Austria
[5]These authors contributed equally
[6]These authors contributed equally
[7]Lead contact
*Correspondence: boris.chagnaud@uni-graz.at
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SUMMARY

The estimation of one's distance to a potential threat is essential for any animal's survival. Rattlesnakes inform about their presence by generating acoustic broadband rattling sounds.[1] Rattlesnakes generate their acoustic signals by clashing a series of keratinous segments onto each other, which are located at the tip of their tails.[1–3] Each tail shake results in a broadband sound pulse that merges into a continuous acoustic signal with fast-repeating tail shakes. This acoustic display is readily recognized by other animals[4,5] and serves as an aposematic threat and warning display, likely to avoid being preyed upon.[1,6] The spectral properties of the rattling sound[1,3] and its dependence on the morphology and size of the rattle have been investigated for decades[7–9] and carry relevant information for different receivers, including ground squirrels that encounter rattlesnakes regularly.[10,11] Combining visual looming stimuli with acoustic measurements, we show that rattlesnakes increase their rattling rate (up to about 40 Hz) with decreasing distance of a potential threat, reminiscent of the acoustic signals of sensors while parking a car. Rattlesnakes then abruptly switch to a higher and less variable rate of 60–100 Hz. In a virtual reality experiment, we show that this behavior systematically affects distance judgments by humans: the abrupt switch in rattling rate generates a sudden, strong percept of decreased distance which, together with the low-frequency rattling, acts as a remarkable interspecies communication signal.

RESULTS AND DISCUSSION

Our ability to convey information to our personal and work environment enables us to interact in the society. Communication is taking place when a signaler affects the behavior of a listener[12] and is thus not restricted to signaling within a species but also readily occurs across species. The acoustic display of rattlesnakes is one such striking example of interspecies communication. The probability of a snake to rattle and the acoustic properties of the rattling display depend on various factors, such as one's distance to a snake, body temperature, pregnancy, size of the snake, and on the amount of rattle segments.[2,7,9,13–17]

The acoustic display is, however, not a monotonous behavior. Several studies have described different amounts of variability in the rattling display within individuals in frequency[17,18] and amplitude modulation[3,19] and even abrupt changes from low to high rattling frequencies.[9,20] These studies have, however, not investigated the behavioral relevance of such variability or the instances where these occur and whether they provide the receiver with more information than just about the snake's presence. Such sound modulation could, for instance, be used to inform a potential threat about its relative distance to the snake, similar to how distance information from proximity sensors in the rear bumper of a car is encoded in the repetition rate of an acoustic signal.

Rattling in response to visual looming stimuli

Here, we tested the hypothesis that western diamondback rattlesnakes (*Crotalus atrox*)[21] actively vary their rattling behavior in response to distance changes of a potential threat. In a first experiment, we moved a human-like torso toward a stationary snake (Figure S1A). Snakes readily initiated their acoustic display, starting with sparse tail shakes that elicited distinct sound pulses (Figure S1B). With decreasing torso-snake distance, the frequency of individual sound pulses increased up to a frequency of about 40 Hz, which was followed by a sudden, sharp increase to a higher frequency range (60–100 Hz; Figure S1A, red arrow).

To avoid acoustic noise generated by the torso motion (motion artifact) and to gain better experimental control, a second experiment was devised: an approaching object was simulated using
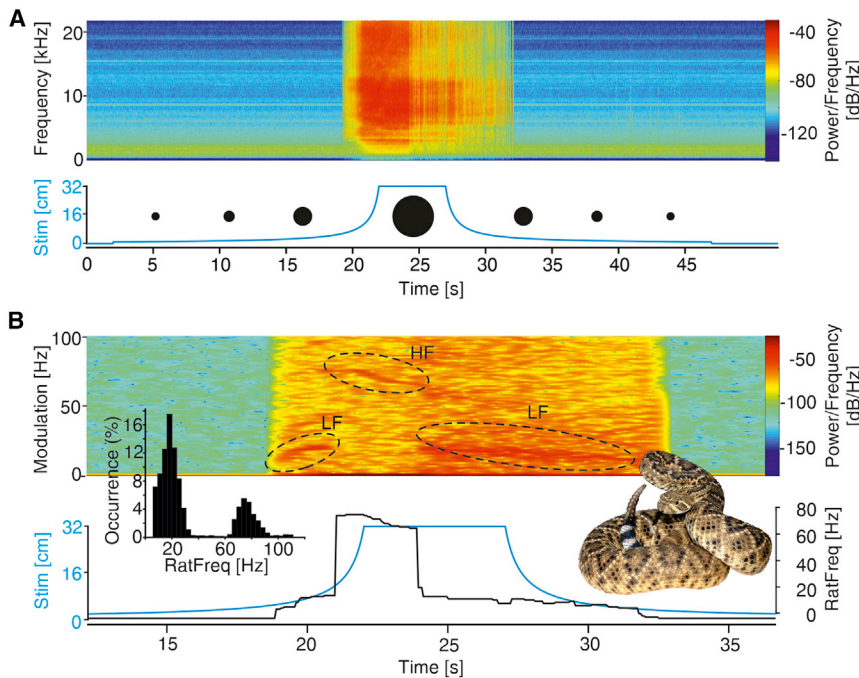
Figure 1. Acoustic properties of rattlesnake rattling

(A) Spectrogram of a recording of a rattling event (top) triggered by a looming stimulus (blue trace and black circles, bottom) using a constant approach velocity, resulting in a 1/distance increase in stimulus diameter. Black circles are for illustration purposes and not to scale.

(B) Modulation spectrogram (note different time axis) of rattling depicted in (A) and relationship between rattling modulation frequency (black line, right axis) and looming profile (blue line, left axis). Lower right inset: snake in striking pose with a raised rattle during rattling is shown. Lower left inset: histogram of the distribution of rattling modulation frequency (RatFreq) observed in all constant approach velocity experiments (n = 197 trials; N = 25 snakes) is shown. HF, high frequency; LF, low frequency.

See also Figures S1 and S2.

a visual looming stimulus consisting of a black disk that increased in size with a constant velocity profile (tested at four different velocities) by setting the diameter of the disk proportional to 1/x for a decreasing virtual object distance of x (Figures 1A and S2). Rattlesnakes readily responded to this looming stimulus with the acoustic display described above. Across trials (n = 197; N = 25 snakes), rattling rate showed a bimodal distribution (Figure 1B, inset), consisting of a low-frequency (LF) (<40 Hz) and a high-frequency (HF) (60–100 Hz) range. Interestingly, rattling rate in the LF mode linearly increased with the increasing visual stimulus, thus carrying information about the relative change of distance between an approaching animal and the snake. The slope of this LF mode change depended on stimulus velocity, with slower stimuli resulting in slower rate increases (Kruskal-Wallis; p = 0.036; χ^2 = 8.52; Figure 2A; Table S1). While individual snakes showed similar response patterns to multiple stimulus presentations, there was considerable variability in responses across snakes in terms of rattling duration and onset (Figure 3), a well-known feature in rattlesnakes.[22] The duration of rattling in the LF mode also depended on stimulus velocity, with faster stimuli resulting in shorter LF displays before switching to the HF (Kruskal-Wallis; p = 3.67 × 10^{-5}; χ^2 = 23.2; Figure 2B). In contrast, the HF component of the rattling was independent of the stimulus velocity in terms of both changes in rattling rate (Kruskal-Wallis; p = 0.98; χ^2 = 0.2; Figure 2C) and duration (Kruskal-Wallis; p = 0.125; χ^2 = 5.75; Figure 2D). While not significant, a trend between the stimulus velocity and the HF duration component is apparent. The HF acoustic display generally continued at a stable rate or slowly decreased over time, even when stimulus size was constant (average rate of −1.95 Hz/s at a medium stimulus velocity of 1.1 m/s; Table S1). Response latency also decreased on average with increasing approach velocity (p = 3.67 × 10^{-5}; χ^2 = 23.2; Figure 2E). After the approach phase of the stimulus (i.e., when the black disk grew in size), a

stationary phase of maximum stimulus size followed, until the black disk decreased in size, mimicking a stopping and a retreating motion of the object, respectively (Figures 1 and S2). In response, snakes generally left the HF rattling mode and changed back to the LF mode until the subsequent end of rattling (Video S1). In contrast to the rising phase of the stimulus, the rattlesnakes' responses to the stationary and retreating component were highly variable, with some snakes sustaining their HF rattling even until the stimulus had fully disappeared. Rattling was initiated independently of the stimulus size (Kruskal-Wallis; p = 0.036; χ^2 = 13.52; Figure 2F), indicating that snakes must have been able to interpret the different approach velocities.

Does rattling modulation depend on the approach profile?

To test whether the rattling rate depended not only on the looming stimulus velocity but also on the approaching profile, we altered the looming stimulus from a constant velocity to a decreasing velocity profile (Figures S2B and S2C). Compared to the constant velocity approach, snakes (n = 81 trials; N = 13 snakes) responded to this altered visual stimulus primarily with lower LF slopes, which, when compared to the constant approach velocity, was independent of approach duration (Kruskal-Wallis; p = 0.813; χ^2 = 0.41; Table S1; Figure S3). The HF mode neither correlated in terms of the slope nor the duration to stimulus velocity (Kruskal-Wallis; slope: p = 0.171, χ^2 = 3.53; duration: p = 0.93, χ^2 = 0.13). As the final stimulus size was identical between the constant and the declining velocity profiles, these results demonstrate that rattlesnakes adapt their rattling rate in response to the approach velocity of an object rather than its size.

Rattling modulation in a virtual reality environment

So why do the snakes switch to the HF mode instead of linearly increasing their rattling rate up until time of contact (which would more honestly advertise the relative distance between the approaching object and themselves)? We hypothesize that the
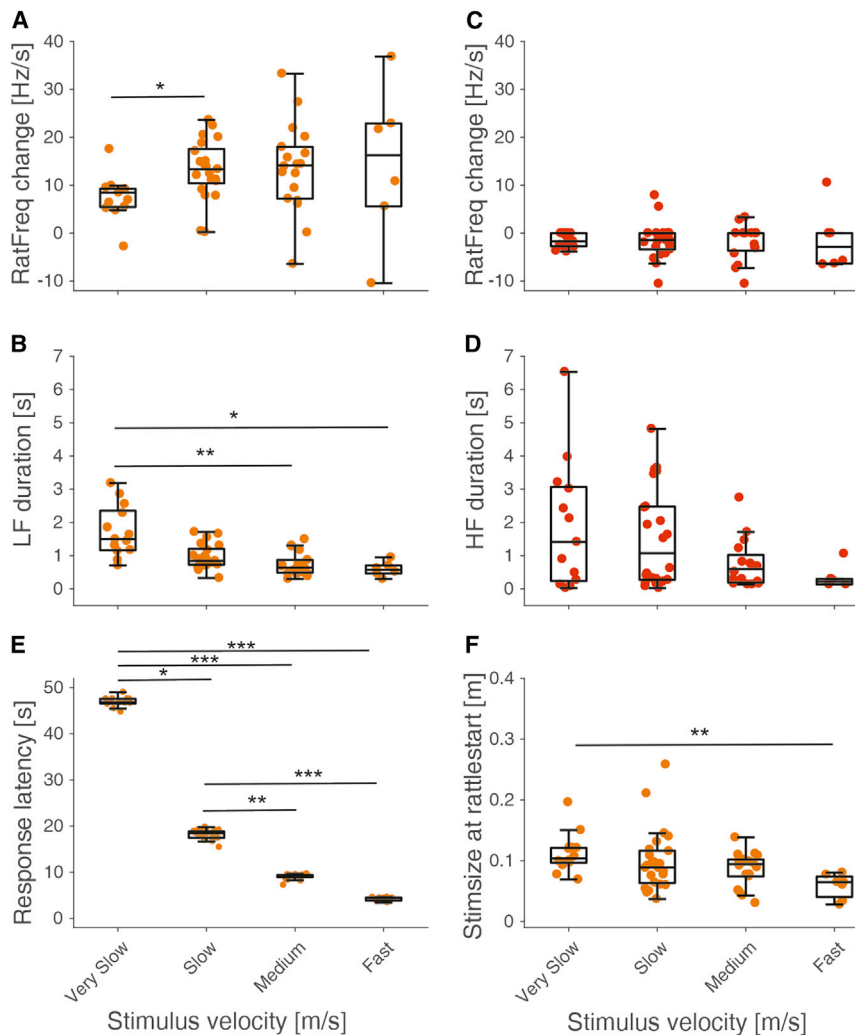
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Figure 2. Effect of constant approach velocity on low (LF, orange) and high (HF, red) modulation frequency modes of rattling responses

(A and B) Box and whisker plots of the LF rattling modulation frequency (RatFreq) changes (A) and LF mode duration (B) for different approach velocities until the shift to the HF mode. Both factors depend on stimulus velocity.

(C and D) HF rattling modulation frequency rate changes (C) and HF duration (D) are less variable and independent of approach velocity for the HF rattling mode.

(E and F) Response latency (E) depends on stimulus velocity, while the size of the looming stimulus at rattle onset is independent of stimulus velocity (F).

Significance levels (Tukey-Kramer post hoc test) are indicated by asterisks: $*p \leq 0.05$; $**p \leq 0.01$; and $***p \leq 0.001$. See also Figure S3, Table S1, and Videos S1 and S2.

sudden switch to HF mode could serve to create the perception in an approaching animal that contact with the snake is imminent, according to the previously established "rule" of the distance-dependent increase in rattle rate in the LF mode (Figures 4A and 4B). To test this hypothesis, we designed an audio-visual virtual environment in which naive human subjects (N = 11, 3 female and 8 male; age range: 20–36; mean: 23.9 years) were positioned on a chair and were virtually moved through a grass-land virtual reality (VR) environment (Videos S2 and S3) while approaching an invisible sound source (the "virtual snake"). This virtual snake emitted broadband sound pulses at either a constant (12 Hz) or at a rattling rate that depended on the listener-snake distance. These sounds were played back to the listener via a vertical loudspeaker array, with amplitude gains set dynamically to reflect geometric attenuation (closer snakes heard more loudly) and elevation (closer snakes heard from further below). Each trial randomly started at one of six distances (Figure 4C), and the listeners were asked to stop the automatic approach toward the sound source when they estimated the source to be 1 m away. In the distance-dependent rattling condition, the virtual snake was programmed to increase its rattling rate from 5 to 20 Hz for a distance decrease from 8 m to 4 m. When the

distance undercut 4 m, the virtual snake switched to a HF, distance-independent rattling of 70 Hz (Figure 4C). Thus, in trials where the starting distance was smaller or equal to 4 m ("short trials"), we compared stopping distances for a time-invariant low rattling frequency (12 Hz) and an equally time-invariant high rattling frequency of 70 Hz. We found a significant difference in the listeners' stopping distances between these two conditions, with LF rattling causing shorter stopping distances than HF rattling (Figure 4D; one-sided Mann-Whitney U test: p < 0.001; n = 412 trials). This indicates that the difference between rattling at 12 or

70 Hz by itself leads humans to significantly underestimate their distance to the virtual snake at the higher rattling rates, presumably due to an increase in perceived loudness.[23] In trials with a starting distance of more than 4 m ("long trials"), the stopping distances with distance-dependent rattling exhibited a clear bimodal distribution when compared to those with constant rattling, with a secondary mode at around 4 m, i.e., the time of the sudden rattling rate change (Figure 4E, orange arrow; medians 1.08 m versus 1.21 m; p < 0.001). This suggests that the sudden change from the LF to the HF range, in our VR experiments, indeed affected (whether intended or not) the human subjects to underestimate the distance to the virtual snake.

Our data show that the acoustic display of rattlesnakes, which has been interpreted for decades as a simple acoustic warning signal informing about the presence of the snake, is in fact a far more intricate interspecies communication signal. We suggest that the LF rattling mode informs the approaching subject in a predictive fashion about its approach toward the snake and that the sudden switch to the HF mode acts as a smart signal, fooling the listener about its actual distance to the sound source. The misinterpretation of distance by the listener (reported by our VR experiments) thereby creates a distance "safety margin." The
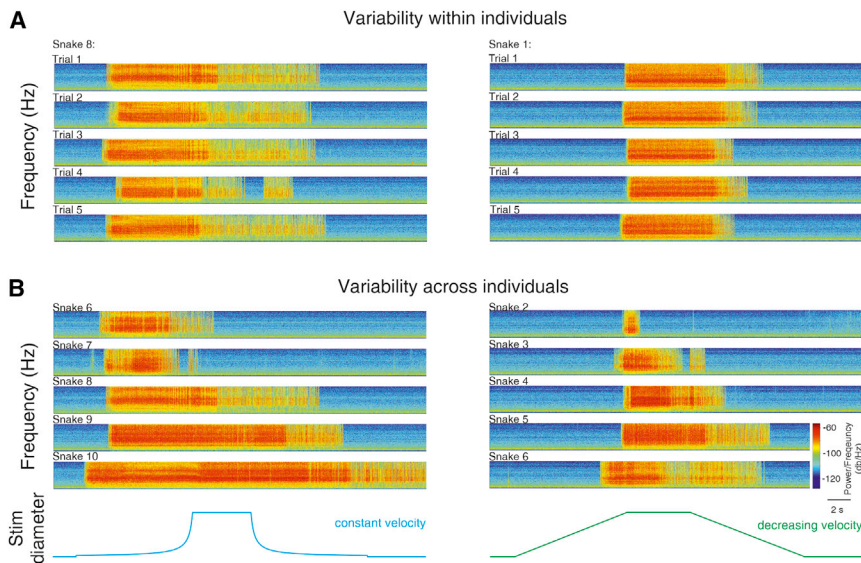
Figure 3. Response variability to looming stimuli across and within snakes

Spectrograms showing the variability of rattling responses to looming stimuli with constant (left) and decreasing (right) approach velocity across trials within a snake (A) and across snakes (B).

analyses in the looming experiments show that the sound level of single-rattle events is rather constant (Figures 1 and S1), but the snake adjusts the number of events, i.e., the rattling rate. While this does not change the physical peak level of the emitted sound, it potentially increases perceived loudness, due to the phenomenon of temporal integration. We compared loudness estimates for synthesized rattling sounds (as emitted by our virtual snake but from a stationary position) at 12 Hz versus 70 Hz using a well-established model for human loudness perception (and found that they differ by an approximate factor of 2).[31] While a full quantitative comparison of the true perceived loudness cannot be derived from such a model for the two types of time-varying stimuli that we actually presented to the subjects in the virtual reality experiment, this observation does suggest that their distance judgments may have been affected by such differences in loudness.

The human auditory system is biased toward perceiving sounds that increase in loudness as moving faster and getting closer, than sounds that become quieter.[32,33] The rattling behavior of the snakes could thus be interpreted as exploiting this bias by exaggerating the loudness increase beyond the purely physical intensification of the sound pressure at a listener's ears due to the approach. While the distance to the snake is not encoded in absolute values in the rattling display (different onset points of rattling and onset times of changes between the LF and the HF mode across snakes; see Figure 3), the relationship between approach velocity and rattling rate (e.g., Figures 1A and S1), however, suggests that the relative approach velocity and distance is encoded. This is enough to generate this unique auditory signal combination (at least in human subjects) which, as shown by our psychophysical experiment, acts as a highly effective interspecies communication system.

underestimation of sound distances outside of peri-personal space is a well-known tendency of human auditory distance perception in general.[24,25] The finding at hand can be interpreted as a particularly pronounced instance of this phenomenon, hinting at a possible adaptive advantage underlying this bias.

As rattlesnakes already inhabited northern America in the early-to-mid-Hemphillian (9–6 Ma),[26] rattling behavior evolved independently from the interaction with humans. Therefore, we hypothesize that similar changes in rattling behavior might lead to similar changes in behavior in other animals, as in our observations in humans. While rattling does not effectively deter all animals[27] and can even be exploited by other (highly adapted) species to assess the threat of a snake,[11] there is a consensus that it is being employed as an effective deterrent.[28] Interestingly, burrowing owls employ a similar sound, also with a deterrent effect on other animals.[29]

There are alternative interpretations of the effect of the shift in frequency range in the rattling behavior: the shift might simply lead to an increase in attention of the human subjects, similar to a startling response. If this is the case, it remains unclear why the snakes predictively change their rattling frequency to a moving object dependent on its velocity. A question remaining is to which aspect of the looming stimulus the snakes responded. As approach velocity and increasing visual stimulation (i.e., the diameter of the black disk) both depend on each other, we were not able to separate their contribution. Furthermore, while we have so far described the behavior of the rattlesnakes in terms of a relationship between rattling rate and object distance, it is also conceivable that the rattling rate is an intermediary parameter controlled by the snake to change the perceived loudness of the signal by its recipient. Extracting distance information from sound sources is generally a challenging task.[30] The primary distance cues that could be resolved by the listener in the virtual environment are the distance-dependent elevation cues (due to the snake being heard from a lower angle below the horizon as the listener gets closer) and the geometric attenuation of the rattling by the snake (both factors were included in our virtual acoustic environment). The latter cue is modified by the acoustic display of the snakes: our acoustic

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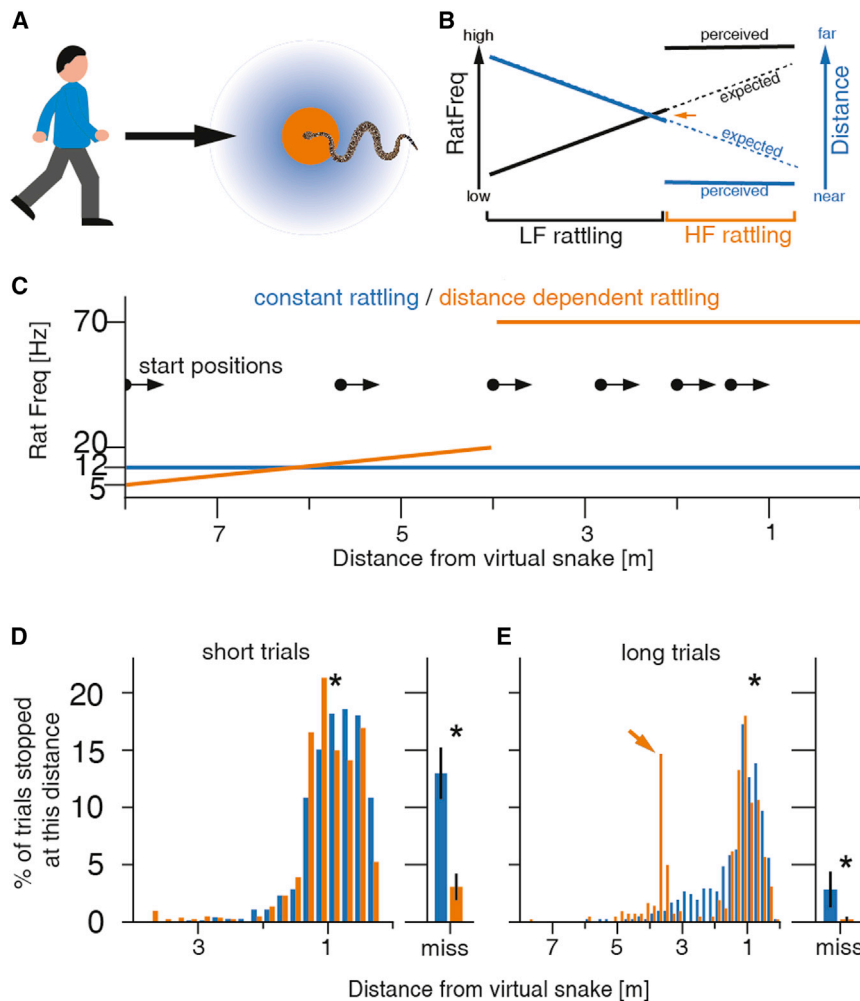
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Figure 4. Psychophysical experiments in a virtual reality environment reveal that adaptive rattling generates an underestimation of distance in human subjects

(A) Schematic drawing indicating the different modes in rattling, depending on distance to an approaching object: blue area, distance-dependent LF rattling; orange area, HF rattling.

(B) Schematic drawing of how a listener might predict the time course of the rattling frequency, compared to the rattling frequency they will actually experience, and how they would correspondingly predict their distance to the snake. Black lines indicate rattling modulation frequency, and blue lines indicate the perceived distance (expectation in dashed lines, respectively).

(C) Acoustic stimulation paradigm in the virtual environment: momentary rattling frequency computed from one of two modulation functions (constant versus adaptive) based on the distance between the virtual snake and the position of the listener in the virtual environment. The blue line shows a distance-independent, 12-Hz sound in the constant rattling frequency condition; the orange lines represent the adaptive rattling frequency condition with its gradual increase in LF mode up to 4 m and the jump to HF mode at 4 m.

(D and E) Histograms of virtual listener-snake distances at which the listeners stopped the trial because they perceived the virtual snake to be exactly 1 m away, in non-miss trials where the starting distance was less than or equal to (D) or greater than 4 m (E). Asterisks indicate significant differences in the distributions for the constant and adaptive rattling frequency conditions (p < 0.05 in one-sided Mann-Whitney U tests). To the right of (D) and (E), frequency of miss trials (trials which the listener did not stop before the virtual snake was at a distance of 0.2 m) in the respective trials is shown. Asterisks indicate significant differences in miss rate between the two conditions (p < 0.05 in one-sided Fisher's exact tests).

See also Figure S4 and Videos S2 and S3.

- ○ Real object stimulation
- ○ Visual stimulation
- ○ Recording of rattling sounds
- ○ Sound and video analysis
- ○ Modulation frequency analysis
- ○ Psychophysics
- ○ Procedure of psychophysical experiments
- ● QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2021.07.018.

A video abstract is available at https://doi.org/10.1016/j.cub.2021.07.018#mmc6.

AUTHOR CONTRIBUTIONS

B.P.C. planned and designed the study. M.F. and T.K. performed and M.F. analyzed the behavioral rattlesnake experiments. M.S. and L.W. designed, M.S. performed, and M.S. and L.W. analyzed the psychophysical experiments. B.P.C., M.F., and M.S. wrote the paper. M.F., M.S., H.L., T.K., and B.P.C. edited and proofread the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We worked to ensure sex balance in the selection of non-human subjects.

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KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Data to support findings	Harvard dataverse	https://doi.org/10.7910/DVN/1RTEJ7
Code	Harvard dataverse	https://doi.org/10.7910/DVN/1RTEJ7
Experimental models: Organisms/strains		
Crotalus atrox	TUM animal breeding facility-wild type	None existing
Homo sapiens	N/A	N/A
Software and algorithms		
MATLAB	Mathworks	RRID: SCR_001622
Psychophysics Toolbox	Mathworks	RRID: SCR_002881

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Boris P. Chagnaud (boris.chagnaud@uni-graz.at).

Materials availability
This study did not generate new unique reagents.

Data and code availability
The data that support the findings of this study is accessible at the Harvard Dataverse repository (https://doi.org/10.7910/DVN/1RTEJ7). All computer code used for data analysis is available at the Harvard Dataverse repository (https://doi.org/10.7910/DVN/1RTEJ7).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals
Experiments were performed on 30 juvenile (age: 1 and 2 years old) western diamondback rattlesnakes, *Crotalus atrox*,[21] of either sex (weight: 53-241 g; snout-vent-length: 37.7-69.9 cm) that were group-housed (two to three snakes per terrarium). Snakes were kept on a 12:12-hour day:night cycle at a temperature of 25–31°C with water *ad libitum* and were fed weekly with dead mice. All experimental animals were bred and kept at the Chair of Zoology of the Technical University of Munich, following the established guidelines for care and maintenance of venomous snakes.

During the course of experiments all snakes were kept solitarily. Snakes were not used for experiments the same day they were fed and were given at least one day to rest after each experimental session. For each session, individual snakes were transferred in a lightproof transport box from the animal facilities into the experimental setup (ambient temperature: 27-32°C). To motivate snakes to remain at a certain position, they were placed on an elevated platform (30x30 cm, height: 24.5 cm). A clay pot adjusted to the size of the snakes (diameter: 11 cm/27.5 cm) was provided as shelter. After being placed on the platform, snakes were allowed to acclimatize for 5 minutes before the shelter was removed and the experimental session started. Each session consisted of up to 5 trials with inter-trial intervals of 5 minutes.

Rattlesnake behavioral experiments were approved by the ethics committee of the chair of zoology TUM Freising (Protokoll Tier-schutzausschuss 28.02.19) after deliberation with the local government (Regierung von Oberbayern:Az. 2532.Vet_11-3-15).

Human subjects
Participants in the VR experiments were between 20 and 36 years old (mean 23.9). Eight of the eleven individuals were female and three were male. Human psychophysics procedures were approved by the ethics committee of the Faculty of Medicine, LMU Munich (project no. 18-327).

METHOD DETAILS

Real object stimulation

A Brüel & Kjaer Head and Torso Simulator (HATS, Type 4128-C) was mounted on a sled with Teflon runners, which was placed on a guide rail system positioned longitudinally to the experimental platform (Figure S1). A wire system, attached to the front and back ends of the sled and running along the guide rail, was used to manually move the HATS toward or away from the experimental platform. A distance sensor positioned at the back wall allowed to monitor the moved distance of the HATS.

After 2 s of pre-stimulus time, the HATS was manually pulled from a starting distance of 1.8 m toward the experimental platform until the snakes initiated HF rattling, down to a possible minimum distance of 0.25 m. Velocities were not constant due to the manually controlled movement and ranged between 0.07 and 0.35 m/s. Luminescent tubes were used as light sources.

Visual stimulation

Visual stimuli were back-projected by a projector (Mitsubishi, XD350U, Resolution: 1024x768 px, Image refresh rate: 60 Hz, *C. atrox* electroretinography temporal resolution: 36 Hz; Kohl and Young, 2011, Soc. Integr. Compar. Biol., abstract) onto a white screen located 35 cm in front of the center of the platform (Figure S2). Custom-written scripts (MATLAB, ver.7.11.0, Psychtoolbox) were used to generate visual looming stimuli which consisted of black disks with an increasing diameter over time (approach phase) after which the stimulus remained constant for 5 s (stationary phase). This constant phase was followed by a stimulus size decrease in a mirror-image fashion to the previous increase (retreat phase, Figure S2B). Two different visual stimulus paradigms were used: one mimicked an object approaching the snake and departing from it at a constant velocity, the other an object in which the approach velocity decreased over time (Figure S2C). For constant approaches, the diameter (d) of the black disk changed with approach duration (*t*) according to the function

$$d(t) = \frac{\Delta D}{x(t)}$$

where ΔD is the distance of the snake to the screen and x(*t*) the distance to the virtual approaching object. For constant approach speeds the object started at d = 3.2 cm (virtual distance: 11 m, visual angle: 1.47°) and increased to d = 32.5 cm (virtual distance: 0.32 m, visual angle: 49.13°, Figure S2). Four different approach durations were tested and velocity directly scaled with approach durations of 50 s (0.2 m/s), 20 s (0.5 m/s), 10 s (1.1 m/s) and 5 s (2.1 m/s). Decreasing approach velocity stimuli started at d = 0 cm and increased to a maximum d = 16.25 cm in the same time frames of 20 s, 10 s and 5 s. Thus, for both paradigms, 50, 20, 10 and 5 s approaches simulated a very slow, slow, medium and fast approach, respectively. Each snake was tested for up to four different velocities per experimental session, snakes that repeatedly left the experimental platform (N = 1) or did not or only rarely elicit rattling sound to the visual stimuli (N = 4) were not used in further experiments. Consequently, sample sizes differ across different stimulus presentations.

To test whether variation in acoustic responses toward different stimuli was caused by individual differences, we presented several snakes with 5 repetitions of one stimulus and compared the evoked responses to individual stimulations of five different animals. Multiple consecutive repetitions on one animal were done according to normal experimental procedure with 5 minutes inter-trial time (similar to Place and Abramson[22]) and one resting day between experiments.

Recording of rattling sounds

Rattling sounds were recorded with an electrostatic microphone (frequency range: 20 Hz–31.5 kHz; M215, MicW, Beijing, China) placed 11 cm above the plane of the experimental platform and at a lateral distance of 18 cm (Figure S2A), digitized at a rate of 44.1 kHz with an external soundcard (Profire 610, M-Audio, Cumberland, RI, USA) connected to a personal computer. Recordings were saved in the MATLAB MAT file format.

To assure that the snakes' visual field encompassed the screen in which the stimuli were presented, a video camera (Guppy, Allied Vision Technologies, frame rate 10 Hz) was placed above (distance 86 cm) the platform to monitor snake head orientation. Two infrared spotlights (Abus, TV6700, λ = 850 nm) suspended from the top were used to constantly illuminate the experimental platform.

The angle of the snake head was determined in the video frames just before, directly at, and just after stimulus onset. Only those recordings were analyzed in which the snake head was oriented toward the screen on which the visual stimuli were presented. An orientation of the snake's head directly toward the screen was set as a deviation angle α of 0° (Figure S2D). Only recordings with a deviation between −90° and 90° were analyzed. Snake head orientation toward the screen was measured post hoc. Sound and video acquisition was synchronized via MATLAB.

Sound and video analysis

Custom written software was used to analyze sound recordings (MATLAB). Spectrograms of the upper temporal envelope (calculated as a 200-fold downsampling of the absolute signal with a digital anti-aliasing filter) were generated (window: 128 samples, overlap: 95%, sample rate: 220.5 Hz, resulting bin size = 0.6 s), providing the power spectral density (PSD) of the down sampled signal. From the PSD a modulation spectrogram was generated to allow for extraction of the dominant modulation frequency within each bin. The resulting curve of the dominant modulation frequency represented the modulation frequency of the rattling sound: the snake's tail shake frequency (RatFreq). A high modulation power at 0 Hz due to the rectification of the signal, as well as powerful

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low frequency artifacts at 1.7 and 3.4 Hz in the modulation spectrogram could, however, mask the rattling frequency as the dominant modulation frequency. This led to the detection of rattling frequencies of 0, when in reality the snake was rattling. To limit detection of these false zero values during rattling, a lower cutoff value was set to remove modulation frequencies extraction below 5.17 Hz. Despite this cutoff, rattling could still be masked by low power modulation frequencies. We therefore excluded rattling sequences from further analysis that contained spontaneous drops of the rattling frequency to the lower analysis threshold during rattling. Since LF and HF sequences from one trial were analyzed independently, sample sizes for HF and LF sequences differ.

The sound level of the signal in decibels (relative to an arbitrary full-scale value) was calculated from the original signal (p) as

$$20 \times log_{10}p$$

with identical bin sizes to the modulation frequency extraction (0.6 s) without overlap between bins.

Modulation frequency analysis

Rattling sounds were characterized by a broadband component to single tail shakes (Figure S1B). The absolute frequency range of rattling sound pulses was not analyzed, as the rattling sound itself has been subject to multiple studies already. Our sampling rate was instead adjusted to cover the frequency ranges of the rattling sound containing the most power (2). Increasing tail flicking frequency led to a modulation of the spectrum. Several components of rattling sounds were analyzed: duration of sounds, the rate of frequency change and the time of an abrupt change in rattling modulation frequency, as well as the general distribution of rattling frequencies across trials.

LF rattling sequences were identified by searching for modulation frequencies that lay within the LF range (0-40 Hz) and directly preceded modulation frequencies in the HF range (> 60 Hz). Only the first LF rattling sequence per trial was analyzed. A linear regression was done through these sequences, from a frequency of zero preceding rattling initiation (to account for varying starting frequencies) and ending just before the shift to HF. The resulting regression coefficient served as the rate of RatFreq change. HF RatFreq sequences were determined similarly, starting at the beginning of HF rattling following LF rattling and ending before the first shift back below the lower HF limit or the end of the recording.

To assess the information contained in the rattling behavior elicited by an approaching object, only LF rattling during the approach phase of the stimulus was analyzed. Rattling durations were analyzed using the same start and end criteria used in RatFreq change analysis but were not limited to the end of the approach. In few cases snakes failed to elicit a HF mode (31.8%) or began rattling in HF mode with no preceding LF mode (4.9%). These recordings were also omitted from the analysis. Data was then pooled per velocity and significant outliers were identified (generalized extreme studentized deviate test) and removed.

Psychophysics

The human psychoacoustical experiments took place in an anechoic chamber with a 2x2 m^2 base and 2.2 m of height. The human subjects were individually seated on a chair facing a vertical array of five loudspeakers (Plus XS.2, CANTON Elektronik, Weilrod, Germany) at elevations of 0°, 12.5°, 25°, 37.5°, and 50° down, wore a Rift DK2 virtual reality head-mounted display (Oculus VR, Menlo Park, CA, US), and held a joystick in one hand.

The auditory stimuli were synthesized by repeating randomly generated individual rattling sounds at a rattling frequency that depended on the momentary virtual listener–snake distance and the trial condition (constant versus adaptive rattling frequency). The individual rattling sounds were made up of 20 identical linearly decaying sawtooth wave pulses (center frequency 8 kHz, 1 ms duration) which were randomly spaced in time according to an exponential distribution with a rate parameter of 6 ms. In order to ensure that these sounds are equivalent to the emissions of the true rattle in how well they can be localized, they were matched to the rattlesnake recordings in their spectral and temporal-envelope characteristics. The rattling frequency followed one of two functions of virtual listener–snake distance:

1) in the constant rattling frequency condition,

$$f_{const}(x) = 12 \; Hz$$

2) in the adaptive rattling frequency condition,

$$f_{adaptive}(x) = \begin{cases} 70 \; Hz, \; for \; x \leq 4 \; m \\ 35 \; Hz - 3.75 \; Hz/m \times x, for \; 4 \; m \; < \; x \leq 8 \; m \end{cases}$$

The stimuli were fed into a 24-channel audio interface (24I/O, MOTU, Cambridge, MA, US) that was connected to the speakers via a 12-channel power amplifier (CI9120, NAD Electronics International, Pickering, ON, CA).

The virtual reality visual stimulus was a binocular rendering of a dim, flat, grassy landscape, presented through the head-mounted display, in which subjects could look around freely by rotating their heads. A bright spot on the ground pointed out a distance of 1 m. There were no visual cues as to the location of the virtual snake. The auditory and visual stimuli were dynamic. A 1 m/s approaching motion of the listener toward the virtual snake was simulated acoustically by a decrease of geometric attenuation and of sound source elevation over time – by vector-base amplitude panning[34] between loudspeakers in the vertical array – and visually by the optic flow of a flight through the grassy landscape. Time-varying geometric attenuation (following the inverse-proportional law that links sound pressure to source distance) and elevation were sufficient to provide realistic cues for sound localization in depth because all other known cues,[35] arising principally from reverberation, atmospheric attenuation and near-field binaural effects, were inexistent or negligible in the simulated acoustic scene.

Procedure of psychophysical experiments

In two half-hour sessions performed at least one day before the main experiment, subjects were familiarized with the virtual audio-visual environment. We provided them with the same visual stimulation as in the main experiment and a similar auditory stimulation (500 ms on/250 ms off train of noise bursts, spectrally identical to the synthetic rattling pulses). In contrast to the main experiment, stimulus presentation automatically stopped when the virtual snake–listener distance reached 1 m, 1.41 m, 2 m, 2.83 m, 4 m, 5.66 m, or 8 m. The listeners "wore" a virtual headlamp and were asked to use it to point at the presumed location of the snake by moving their head. No feedback was given. The third session also lasted for approximately half an hour and constituted the main experiment. At the beginning of each trial, the subjects found themselves in silence and stationary in a new random location of the grassy landscape. After 0.5 s, sound and motion were turned on, until either a virtual snake–listener distance of 0.2 m was hit (miss trial) or the listener pressed a button on the joystick to indicate that they perceived the virtual snake to be 1 m away (non-miss trial). Acoustically, depending on trial condition, the momentary rattling frequency was calculated using either $f_{const}(x)$ or $f_{adaptive}(x)$. The starting distance from the virtual snake was either 1.41 m, 2 m, 2.83 m, 4 m, 5.66 m, or 8 m. The different rattling frequency conditions and starting distances were presented in a randomized order individual to each subject. Each pairing of trial condition and starting distance was measured 20 times. Data from all subjects were pooled for the main analysis. Data for individual subjects is reproduced in Figure S4.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data acquired from rattlesnake behavioral experiments (for both, the decreasing and the constant velocity approach paradigms) were statistically analyzed with a non-parametric Kruskal-Wallis test. The null hypothesis was that the rattle parameters in the test conditions followed the same distribution. If the null hypothesis was rejected, a follow up Tukey-Kramer pairwise comparison was performed to identify the test condition in which rattling behavior did not follow the same distribution.

Human psychophysical data were analyzed in two blocks of trials, based on whether the initial distance to the virtual snake was greater ("long trials") or less than ("short trials") 4 m. In each block, stopping distance data from the 11 subjects was pooled and, with a one-sided Mann–Whitney U test (N(constant) = 412, N(adaptive) = 423 for long trials; N(constant) = 738, N(adaptive) = 822 for short trials), tested for the null hypothesis that the distribution of stopping distances from the constant rattling frequency condition is stochastically greater or equal to the distribution of stopping distances from the adaptive rattling frequency condition. The proportion of trials in which subjects stopped at any point/did not stop at all (412/12 [constant] versus 423/1 [adaptive] in long trials, 738/110 versus 822/26 in short trials) were tested, again separately for the two blocks of trials, for the null hypothesis that they are independent of rattling condition with Fisher's exact test.