



# Echolocating bats rely on audiovocal feedback to adapt sonar signal design

Jinhong Luo<sup>a,1</sup> and Cynthia F. Moss<sup>a</sup>

<sup>a</sup>Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD 21218

Edited by Terrence J. Sejnowski, Salk Institute for Biological Studies, La Jolla, CA, and approved September 1, 2017 (received for review July 3, 2017)

**Many species of bat emit acoustic signals and use information carried by echoes reflecting from nearby objects to navigate and forage. It is widely documented that echolocating bats adjust the features of sonar calls in response to echo feedback; however, it remains unknown whether audiovocal feedback contributes to sonar call design. Audiovocal feedback refers to the monitoring of one's own vocalizations during call production and has been intensively studied in nonecholocating animals. Audiovocal feedback not only is a necessary component of vocal learning but also guides the control of the spectro-temporal structure of vocalizations. Here, we show that audiovocal feedback is directly involved in the echolocating bat's control of sonar call features. As big brown bats tracked targets from a stationary position, we played acoustic jamming signals, simulating calls of another bat, timed to selectively perturb audiovocal feedback or echo feedback. We found that the bats exhibited the largest call-frequency adjustments when the jamming signals occurred during vocal production. By contrast, bats did not show sonar call-frequency adjustments when the jamming signals coincided with the arrival of target echoes. Furthermore, bats rapidly adapted sonar call design in the first vocalization following the jamming signal, revealing a response latency in the range of 66 to 94 ms. Thus, bats, like songbirds and humans, rely on audiovocal feedback to structure sonar signal design.**

echolocation | jamming avoidance response | sensorimotor integration | speech | vocal production

Echolocating animals represent the direction and distance of objects by producing ultrasonic signals and processing information carried by echo returns (1, 2). Many species of echolocating bat adjust sonar call design in response to acoustic information carried by echo returns. Indeed, echo feedback lays the foundation for spatial perception by sonar (3–6). Surprisingly, no study to date has directly investigated the potential contribution of audiovocal feedback to the bat's active control of sonar call features. Audiovocal feedback is the process of listening to one's own vocalizations during sound production (Fig. 1A). Mounting evidence shows that diverse groups of animals, along with humans, rely on audiovocal feedback to control the features of ongoing vocalizations (7–9). For example, birds (10) and humans (8) adjust their vocal frequency (e.g., formant or pitch) in response to altered auditory feedback.

The observation that many echolocating bat species often fly in very large groups has raised the question of how an individual bat sorts its vocalizations and echoes from a cacophony of sounds produced by conspecifics. Research on this topic has stimulated some recent controversy in the field. On one hand, there are reports that bats adjust the frequency of their individual calls to reduce the acoustic interference of echolocation signals produced by neighboring conspecifics, a behavior referred to as jamming avoidance response (JAR) (11–16). On the other hand, some recent publications assert that changes in a bat's echolocation call features in the presence of conspecifics can be explained entirely by its vocal reaction to echo returns from physical objects, i.e., bats in close proximity (17, 18), not JAR. Regardless of the interpretation of past research on bat acoustic interactions, what remains elusive is the question of whether echolocating bats rely

on audiovocal feedback or on echo feedback to adjust their sonar calls in response to interfering sounds. Studies of free-flying bats cannot shed light on this question as the directional aim, timing, and characteristics of interfering signals cannot be experimentally controlled. Here, we overcome this challenge by engaging stationary bats in a sonar prey-tracking task that permits precise control over the timing and frequency characteristics of acoustic jamming signals. We show that the big brown bat, *Eptesicus fuscus*, exhibits the largest sonar call-frequency adjustments when jamming signals occur during call production. By contrast, bats do not change sonar call frequency when the jamming signals coincide with the arrival of target echoes. Thus, our study demonstrates that bats, like songbirds and humans, rely on audiovocal feedback to structure their call design.

## Results

We trained four big brown *E. fuscus* bats (three males and one female) to rest on a platform and track approaching tethered insects (i.e., prey targets) by echolocation (Fig. 1B and Movie S1). The prey targets were controlled by a motorized pulley system and traveled a distance of 3 m in ~2.5 s in each trial. The bat-target distance and the arrival time of the target echoes to the bat over the time course of a trial are shown in Fig. S1. The bat's echolocation calls were recorded by an array of 13 microphones (Fig. 1B, blue circles), covering a range of about –65 to +65° horizontally and –30 to +45° vertically. An additional microphone was used as an analog trigger for broadcasting acoustic jamming signals (Fig. 1B, green circle) from a loudspeaker located at a 15-cm distance to the left side of the bat (Fig. 1B, red square). Using the analog triggering technique, we were able to place the jamming signals at time delays greater than ~0.6 ms relative to the onset of the bat's echolocation calls. Thus, we can selectively perturb the sound production or echo reception

## Significance

Echolocating animals are well known for their capability to extract rich information about the environment from echo returns. However, past research has not determined whether audiovocal feedback contributes to sonar call design. Audiovocal feedback is the process whereby an animal listens to the sounds it is producing and is used by both nonecholocating animals and humans to control their ongoing vocalizations. Here, we show that echolocating bats rely on audiovocal feedback, instead of echo feedback, to adapt their sonar call design in response to acoustic jamming signals. Our findings demonstrate that bats, like birds and humans, not only use audiovocal feedback to fine-tune the features of their calls, but also do so on a rapid timescale.

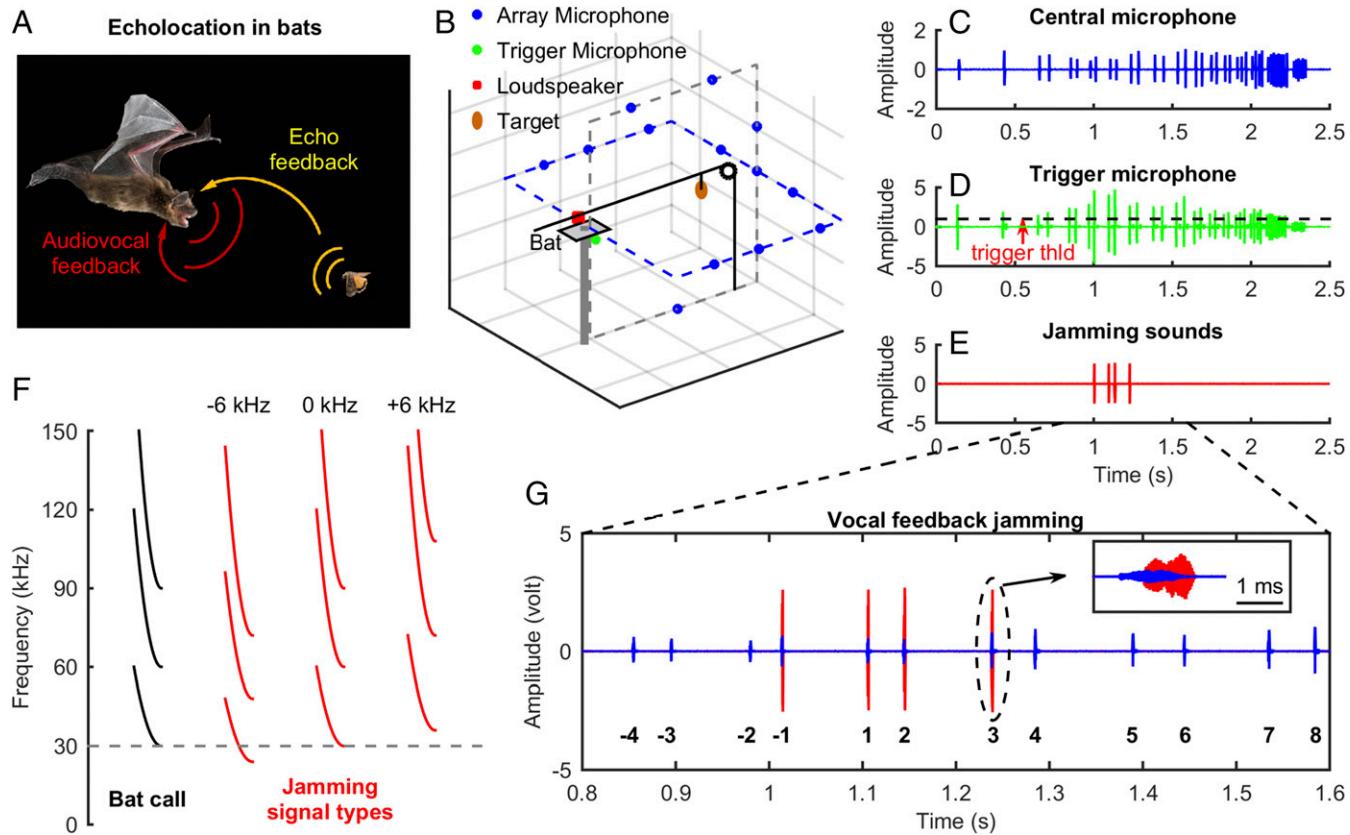
Author contributions: J.L. and C.F.M. designed research; J.L. performed research; J.L. analyzed data; and J.L. and C.F.M. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

<sup>1</sup>To whom correspondence should be addressed. Email: jluo18@jhu.edu.

This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1711892114/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1711892114/-DCSupplemental).



**Fig. 1.** Experimental setup, behavioral task, and jamming signals. (A) Illustration of two potential auditory processes in echolocation. Audiovocal feedback is the auditory process of listening to one's own vocalization during sound production. Echo feedback refers to the process of listening to sonar returns from objects in the environment. (B) Bats were trained to rest on a platform and to track approaching tethered insects by echolocation. In the middle of the tracking task, jamming signals were broadcast from a loudspeaker (red square) 15 cm away from the bat. The bat's echolocation calls were recorded by a trigger microphone (green circle) and an array of 13 microphones (blue circle). (C) An example of the echolocation behavior of a bat tracking the approaching tethered insects through a trial. (D) The sound recording from the trigger microphone of the same trial as shown in C. The dashed line indicates the amplitude threshold for broadcasting the jamming signals. In addition to the amplitude threshold, a time threshold was implemented so that the jamming signals were only presented to the bat 1 s after a trial began. (E) Jamming signals recorded directly from an analog input channel. (F) The jamming signal types designed for each individual bat. (G) An example of the jamming trial in which the jamming signals occurred during the vocal production process. For each trial, four calls before the jamming (call -4 ~ call -1), four calls during the jamming (call 1 ~ call 4), and four calls after the jamming (call 5 ~ call 8) were consecutively numbered and analyzed. The photo in A is credited to Brock Fenton and Ben Falk.

process. An example of a big brown bat's sonar tracking behavior is shown in Fig. 1C, a sonar call recording from the trigger microphone is shown in Fig. 1D, and a recording of the acoustic jamming signals is shown in Fig. 1E.

A few days before introducing the acoustic jamming signals, we made echolocation call recordings in silence from each bat and designed the jamming signals based on these baseline audio recordings. For each individual bat, we created three types of acoustic jamming signals that were frequency-modulated sweeps containing three harmonics (Fig. 1F) simulating echolocation calls of a big brown bat. We referred to the three types of jamming signals as -6-kHz type, 0-kHz type, and +6-kHz type. The -6-kHz and +6-kHz types were pitch-shifted versions of the 0-kHz type by a magnitude of 6 kHz down or up, respectively. For each perturbation trial, we presented four jamming signals consecutively after four sequential echolocation calls. Jamming signals were presented in the middle of a target tracking trial, ~1 s following the trial start time. Fig. 1G shows a vocal production jamming trial in which all four jamming signals occurred within 1 ms after the bat produced its echolocation calls. By digitally delaying the jamming signals after the analog trigger, we broadcast the jamming signals at varying delays relative to echolocation call onset. Below we report the effects of jamming signals on the end frequency of the echolocation calls produced by bats engaged in the

tracking task; this was measured as the frequency 20 dB down from the peak frequency of the first harmonic (Fig. S2 A–D). We focus on end frequency because this is the parameter that has been shown to be sensitive to jamming signals in *E. fuscus* and is most robust to directional changes in the bat's head aim that influence acoustic measurements of sonar calls (15).

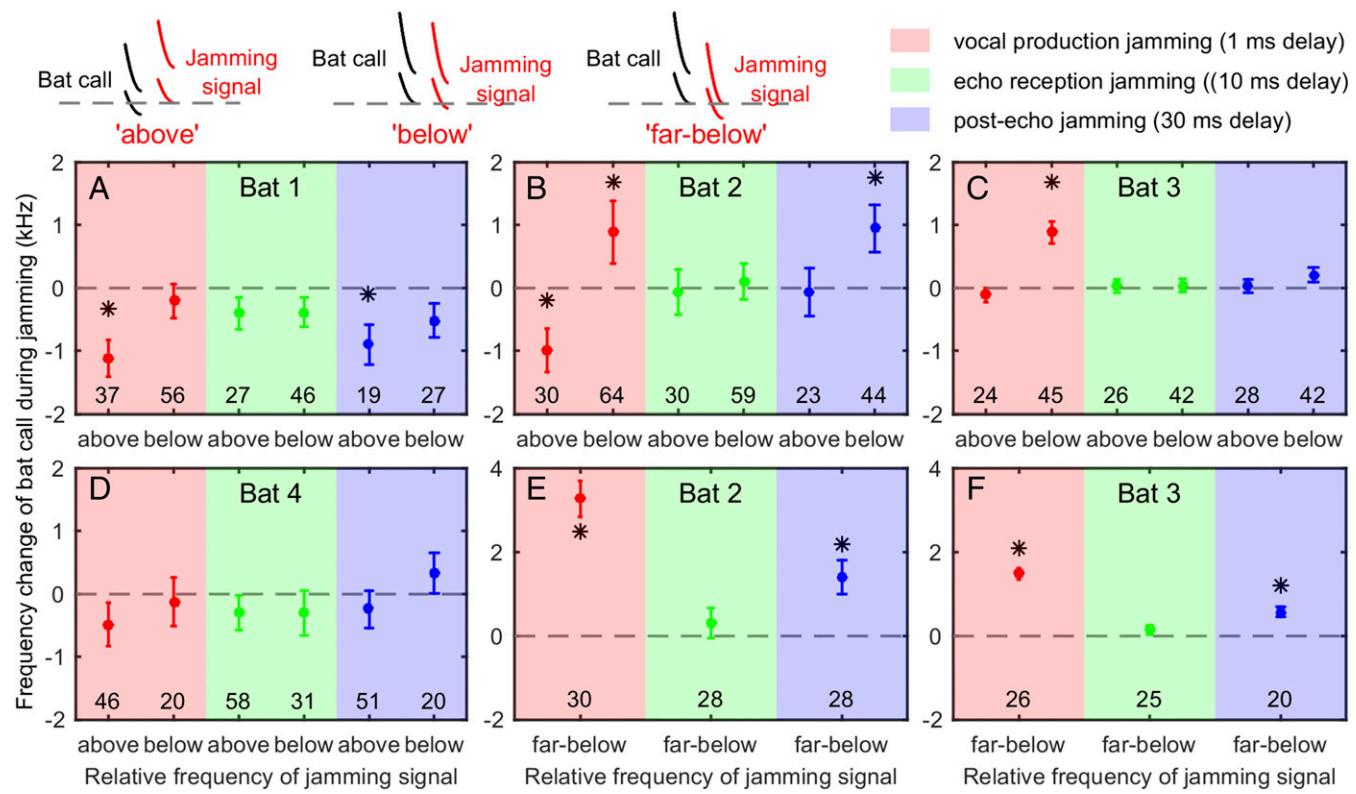
**Experiment 1: Effect of Acoustic Jamming Signal Frequency.** We broadcast each type of jamming signal at three different time delays relative to call production: 1, 10, and 30 ms. Together with a silence control, in which there was an “empty” sound file with all samples set to zero, there were 10 treatments in total. The echolocation call duration of each test subject, based on the four calls that were unperturbed in the silence control condition, were  $1.47 \pm 0.2$  ms,  $1.86 \pm 0.29$  ms,  $3.36 \pm 0.28$  ms, and  $1.97 \pm 0.39$  ms for bats 1–4, respectively (mean  $\pm$  SD). For each bat, jamming signals broadcast at a 1-ms delay overlapped sonar call production. Jamming signals broadcast at a 10-ms delay occurred very close to echo reception (Fig. S1). The actual time delays when the bats received echoes from the prey targets for the four perturbed calls were  $9.6 \pm 1.1$  ms,  $9.96 \pm 1.86$  ms,  $9.53 \pm 1.24$  ms, and  $9.54 \pm 1.1$  ms, respectively (mean  $\pm$  SD). Jamming signals broadcast at a 30-ms delay occurred after target echo reception and before the production of a subsequent echolocation call, i.e., occurred in a

postecho window. Because the end frequency of the echolocation calls was controlled by the bat, each jamming sound type resulted in a range of actual frequency separations between the bat's sonar vocalizations and the jamming signals (Fig. S2E). Thus, data analysis was performed on the actual frequency separation between each bat call and the jamming signal, instead of the preset jamming signal types. We calculated the actual frequency separation before jamming by taking the frequency difference between call -1 and the jamming signal. Call -1 represented the call immediately before the jamming signals.

The JAR hypothesis states that animals should increase their call frequency when they are jammed by low-frequency signals and decrease their call frequency when they are jammed by high-frequency signals. In the first analysis, based on the actual frequency separation between sonar vocalizations and acoustic jamming signals, we separated the trials as follows: the "above" condition in which the end frequency of the jamming signals was above the end frequency of bat calls and the "below" condition in which the end frequency of the jamming signals was below the end frequency of bat calls (Fig. 2 and Fig. S2E). We discovered that bats adjusted the end frequency of their calls only in particular conditions (Fig. 2). By comparing the end frequency of the four calls directly after acoustic jamming (call 1 to call 4, Fig. 1G) to the corresponding four calls in the silence control trials, we found that frequency adjustments of bat 1, bat 2, and bat 3 (male subjects) occurred only in sound production and postecho perturbation conditions. By contrast, no frequency adjustment was found in

the echo reception jamming conditions. For bat 4 (female subject), there was no change in end frequency across any of the six jamming conditions. Examination of the direction of call-frequency adjustments revealed that, when the jamming signals were above the initial call frequency, bats decreased the call frequency during jamming; when the jamming signals were below the initial call frequency, bats increased the call frequency during jamming. Thus, sonar vocal adjustments in these subjects enlarged the frequency separation between bat echolocation calls and acoustic jamming signals.

Moreover, we observed that, for the bats that increased the call frequency in the audiovocal feedback jamming condition (Fig. 2B and C), the magnitude of the frequency increase was larger when the analysis was limited to trials in which the jamming signals were at least 5 kHz below bat call frequency before jamming (referred to as the "far-below" condition, Fig. 2E and F). A comparison of the magnitude of the frequency adjustments for the same jamming signals presented during vocal production, echo reception, and postecho window revealed that the call-frequency adjustment was greatest when the jamming signals occurred during vocal production, and there was no call-frequency adjustment when the jamming signals occurred during echo reception (Movie S1). A moderate call adjustment occurred in the postecho window. For example, in the far-below condition, bat 1 increased the end frequency by 3.3 kHz in the vocal production jamming treatment (two-sample Kolmogorov-Smirnov test,  $P < 0.0001$ ), 1.4 kHz in the postecho jamming treatment (two-sample Kolmogorov-Smirnov



**Fig. 2.** Frequency adjustments of bats in the presence of jamming signals of varying frequency and time delays relative to echolocation call production. Vocal production jamming occurred when the acoustic jamming signals were presented at a time delay of 1 ms, echo reception jamming occurred at a time delay of 10 ms, and postecho jamming occurred at a time delay of 30 ms. (A–D) Trials were grouped into two conditions: the above condition in which the jamming signals were above bat call frequency and the below condition in which the jamming signals were below bat call frequency. (E and F) Results based on a subgroup of trials in the below condition in which the jamming signals were at least 5 kHz below the bat call frequency before jamming. Data were plotted as mean and 95% confidence interval and were based on the four calls during jamming (i.e., call 1 ~ call 4 in Fig. 1G) from each trial. Each asterisk above a condition indicates a statistical significance between the condition and silence control (plotted with silence control type I in which the silence trials randomly alternate with treatment trials during an experimental day). The results hold for silence control type II in which only silence trials were presented to bats during an experimental day. The number of trials for each condition (i.e., the sample size) is shown at the bottom of each panel.

test,  $P < 0.0001$ ), and 0.3 kHz in the echo reception jamming treatment (two-sample Kolmogorov–Smirnov test,  $P > 0.05$ ), respectively.

**Experiment 2: Effect of Acoustic Jamming Signal Delay.** To better understand the effect of the timing of jamming signals on vocal adjustments, we conducted a second experiment in which we broadcast jamming signals at three time delays relative to call onset: 1, 5, and 20 ms, along with a silence control, using the jamming signal type that elicited a bat's largest adjustments in call frequency in the first experiment. Specifically, we used the +6 kHz acoustic jamming signal type for bat 1, and the -6 kHz acoustic jamming signal type for bat 2 and bat 3. Bat 4, the only female in this study, was not included in this experiment, as it showed no JAR in experiment 1. Given the single female in this study, we cannot test whether there are gender differences in the JAR of big brown bats. We found that bats showed much weaker or no call adjustments when the jamming signals arrived at either 5-ms or 20-ms delays after vocal onset (Fig. 3). Again the bats showed the greatest call adjustments when the jamming signals occurred at a 1-ms delay, which perturbed the vocal production process, reproducing the finding from the first experiment.

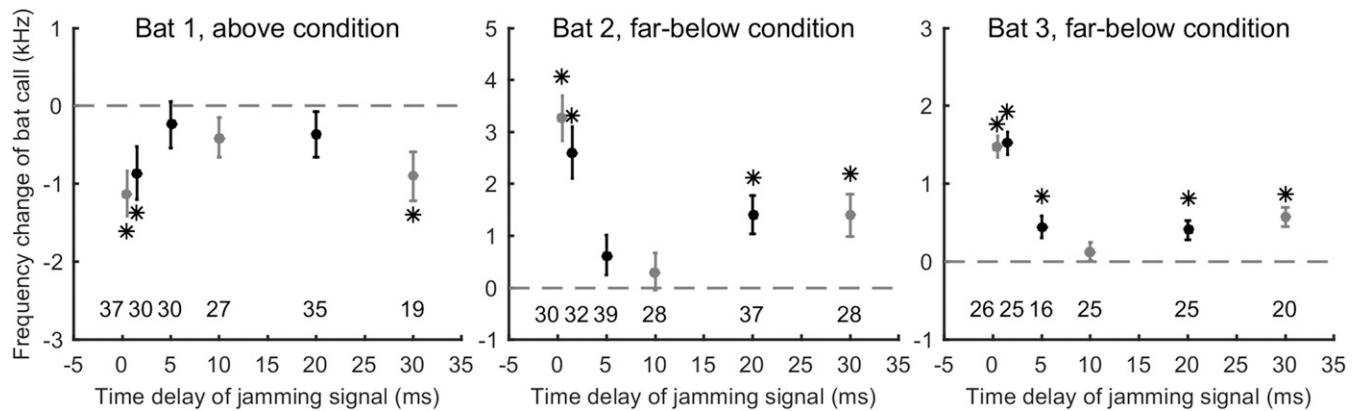
**Audiovocal Adjustments On a Rapid Timescale.** We further analyzed sonar vocal adjustments on a call-by-call basis using the data from the first experiment. Specifically, for each trial, we compared the bat's sonar call end frequency between the jamming condition and the silence control for 12 echolocation calls, including 4 calls before jamming, 4 calls during jamming, and 4 subsequent calls after jamming (Fig. 1G). We found that bats showed sonar vocal adjustments in the first call after the jamming signal (Fig. 4), which corresponded to a response latency of  $66 \pm 20$  ms (bat 1, mean  $\pm$  SD),  $105 \pm 40$  ms (bat 2, mean  $\pm$  SD),  $90 \pm 29$  ms (bat 2, mean  $\pm$  SD), and  $94 \pm 31$  ms (bat 3, mean  $\pm$  SD). Before the jamming signal presentation, there was little difference in call end frequency across calls (two-sample Kolmogorov–Smirnov test, all  $P > 0.05$ ). During jamming, bats shifted the end frequency of their sonar calls in the opposite direction of the jamming signals (two-sample Kolmogorov–Smirnov test, all  $P < 0.05$ ). After jamming signals ended, the call

frequency of the jamming conditions gradually returned to the baseline level of silence control ([Movie S1](#)).

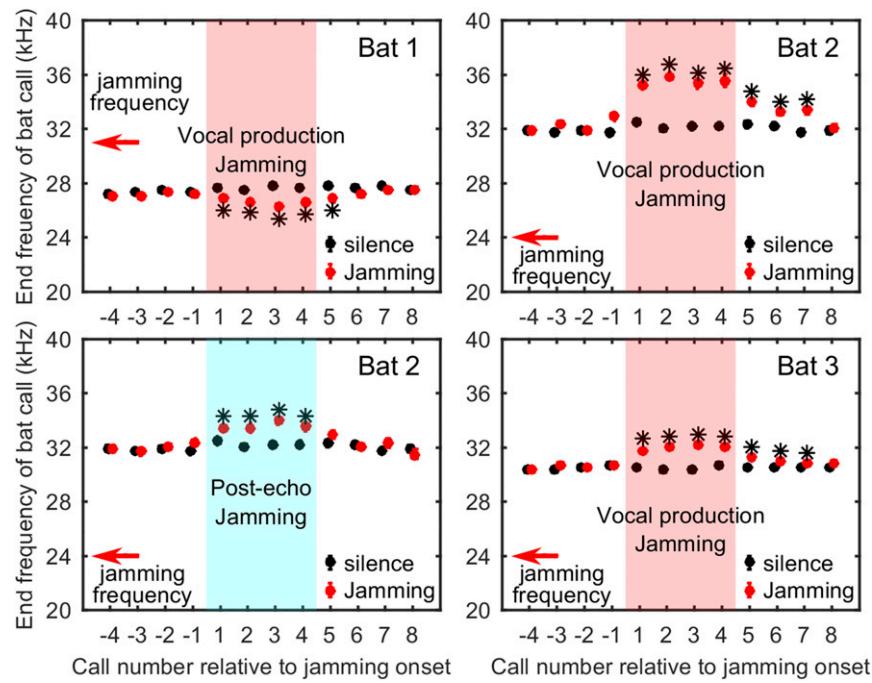
## Discussion

There is a large literature on adaptive sonar call adjustments by bats in response to ambient sounds that range from vocalizations of other bats (12, 14, 19, 20) to computer-generated noise (21–25). Physiologically, it has also been shown that the acoustic parameters of bat sonar calls induced by electrical stimulation of the midbrain became more stable when the bat received additional acoustic stimulation, demonstrating the role of auditory feedback in bat vocal production control (26). Moreover, decades of research have contributed to data showing that echolocating bats rely on echo returns, or echo feedback, to adapt their echolocation call structure. Specifically, bats that produce frequency-modulated (FM) calls show the largest adjustments in the duration and repetition rate of signals with changes in sonar target distance (2, 27). Thus, it follows that adaptive sonar call adjustments by bats in response to ambient sounds depend on echo feedback. Our results, however, show that big brown bats do not adjust the end frequency of their echolocation calls when acoustic jamming signals coincide with the reception of target echoes. Instead, big brown bats showed the greatest adjustments in echolocation call frequency when jamming signals coincided with sonar vocal production. These results suggest that the big brown bat's adjustments of sonar call frequency in response to acoustic jamming signals are based on audiovocal feedback, instead of echo feedback.

Why do big brown bats rely on audiovocal feedback, instead of echo feedback to adjust their sonar call frequency in the presence of jamming signals? We propose the following explanation. Compared with audiovocal feedback, echo feedback is much weaker and more variable, particularly in spectral characteristics, which creates a higher threshold for active vocal adjustment. After a sonar call is emitted, it first travels in air to strike an object and then reflects back to the bat. Due to spherical spreading losses, atmospheric attenuation, and interference patterns created by multiple reflecting surfaces of natural targets, echoes are spectrally modulated and weaker than transmissions (28, 29). Furthermore, when bats forage close to obstacles, each echolocation call results in a stream of echo returns that can mask the target echo (30).



**Fig. 3.** Frequency adjustments of bats in the presence of jamming signals of varying time delays relative to echolocation call production. Data are shown for experiments 1 and 2. Experiment 1 investigated the influence of acoustic jamming signal frequency at 1-, 10-, and 30-ms delays from vocal onset. Experiment 2 investigated the influence of jamming signals at 1-, 5-, and 20-ms delays from vocal onset, using the jamming signal type that induced the largest call-frequency adjustments of individual bats tested in experiment 1. Data in gray are replotted from experiment 1, as shown in Fig. 2 A, E, and F, and data in black are from experiment 2. The above condition refers to the trials in which the end frequency of the jamming signals was above that of the bat calls. The far-below condition refers to the trials in which the end frequency of the jamming signals was more than 5 kHz below that of the bat calls (see Fig. 2 for illustration). Data were plotted as mean and 95% confidence interval and were based on the four calls during jamming (i.e., call 1 ~ call 4 in Fig. 1G) from each trial. Each asterisk above a condition indicates a statistical significance between the condition and silence control. The number of trials for each condition (i.e., the sample size) is shown at the bottom of each panel.



**Fig. 4.** Bats exhibited the jamming avoidance in the first call after the jamming signals. Data were plotted as mean and 95% confidence interval, including 12 calls for each trial: 4 calls before the jamming, 4 calls during the jamming, and 4 calls after the jamming. The asterisk associated with each data point indicates a statistical significance between the treatment condition and the silence control (silence control type I in which the silence trials randomly alternate with treatment trials during an experimental day). The number of trials for each condition (i.e., the sample size) was 37 for bat 1, 30 and 28 for bat 2, and 26 for bat 3.

Collectively, these factors may reduce the bat's weighting of frequency information extracted from target echoes. Hence, big brown bats may not rely on echo feedback to adapt sonar call spectral structure in response to jamming signals.

In addition to sonar call adjustments in the audiovocal feedback jamming condition, big brown bats also made small but significant frequency adjustments in the postecho jamming condition. At present, we do not have a solid explanation for this phenomenon. We speculate that, after receiving the target echo, the bat could start to plan for the parameters (including frequency) of a subsequent call. Following the principles of the motor control theory, which successfully accounts for a wide range of motor behaviors such as visually guided arm movement (31) and speech production (32), we hypothesize that call-frequency adjustment in the postecho period reflects motor planning. The motor control theory posits that motor control is based on a comparison of sensory prediction generated from an internal forward model with direct sensory feedback, and sensory feedback is then used to train and update the internal model. Specifically, when there was a mismatch in the call frequency between the planned call and the acoustic jamming signals, bats made adaptive adjustments accordingly. Similarly, the gradual return of the call frequency after the jamming signals (Fig. 4) might indicate an update of the internal model after the bat listened to repeated jamming signals, which fits into the role of sensory feedback in training and updating the internal model (32). These ideas remain to be tested in future research.

At present, it remains an open question whether the importance of close temporal coupling between vocal onset and perturbation stimuli to the JAR of the big brown bat generalizes to other animal species. Hage et al. (23) tested the effect of echo intensity on vocal control in the greater horseshoe bat, *Rhinolophus ferrumequinum*, while jammed by 20-kHz bandpass filtered noise centered on the resting frequency of the bat's echolocation call. In their study, simulated target echoes of different intensity were presented to the bats at a delay of ~5 ms relative to the bat's vocal onset. The echolocation calls of greater horseshoe bats are

typically >40 ~ 50 ms long. They found that the bats produced slightly more intense calls when receiving the weakest echoes, but they did not observe any effect of echo intensity on echolocation call frequency. Interestingly, when simulated echoes of shifted frequency (either increased or decreased) were presented to the horseshoe bats at the same time delay of ~5 ms, these animals made compensatory frequency adjustments (3). Our data show that big brown bats made the largest frequency adjustments when the jamming signals occurred at a delay of 1 ms relative to vocal onset, but made little frequency adjustment when the jamming signals occurred at a delay of 5 ms. This suggests that the time window of audiovocal feedback may not go beyond the duration of vocal production. Thus, acoustic signals occurring at a 5-ms delay fall outside an audiovocal feedback window for big brown bats that produce echolocation calls of <3 ms in duration but fall within an audiovocal feedback window for greater horseshoe bats that produce echolocation calls typically >40 ~ 50 ms in duration. Furthermore, there are no comparable data from humans or other animals in which altered auditory feedback was presented to the subjects at varying time delays beyond the length of a single vocalization. Two relevant studies in humans have presented altered pitch feedback at different delays, but all feedback was within the sustained vowel production process (33, 34). Moreover, vocalizations of both humans and songbirds are more than an order of magnitude longer in duration than FM bat echolocation calls, which limits analysis of audiovocal feedback adjustments at a high temporal resolution.

Another important finding from this study is that bats showed vocal adjustments in the first call after the jamming sound, corresponding to a response latency in the range of 66 to 94 ms. This estimate is thus more than two times shorter than an earlier estimate of the JAR latency in the Brazilian free-tailed bat, *Tadarida brasiliensis* (13). Vocal response latency has been repeatedly measured in humans participating in vocal feedback perturbation tasks. In brief, the human vocal response latency is ~100 ~ 150 ms for pitch feedback perturbation (8, 35), ~160 ms for formant feedback perturbation (36), and ~160 ~ 170 ms for sound-level feedback perturbation

(37, 38). The vocal adjustments of bats in this study are even faster than the vocal compensation reported for pitch feedback perturbation in humans, which suggests that evolutionary specializations for echolocation might also contribute to a faster audiovocal integration system compared with nonecholocating animals. Geberl et al. (39) showed that the Daubenton's bat (*Myotis daubentonii*) responded to the removal of the insect prey during the terminal catching phase by adjusting its echolocation call behavior, which resulted in a response latency of around 80–90 ms. In the current study, the mean response latency of 66 ms associated with the vocal adjustments of one bat approximates that of the auditory-laryngeal reflex, in which clicks elicit vocal frequency adjustments with a latency of ~50 ms (40). In another study, Luo et al. (41) reported that it takes a mere 30 ms for the big brown bat to increase its vocal intensity in response to broadcast noise. Thus, echolocating bats offer a valuable opportunity to investigate mechanisms of audiovocal integration on a rapid timescale.

The question of whether and how bats accurately represent the parameters of their sonar vocalizations has been considered for almost three decades (42). Studies of brain mechanisms of echolocation have primarily investigated neural responses to artificial pulse-echo pairs in passively listening animals, disregarding the crucial link between vocal production and echo reception. Multi-unit recordings in the superior colliculus of the freely echolocating big brown bat, however, have revealed premotor activity with each sonar vocalization, with a long-lead burst that preceded calls by ~8–40 ms and a short-lead burst that preceded calls by ~2–5 ms, which could provide an efferent copy of sonar call timing (43).

- Griffin DR (1944) Echolocation by blind men, bats and radar. *Science* 100:589–590.
- Griffin D (1958) *Listening in the Dark: The Acoustic Orientation of Bats and Men* (Yale Univ Press, New Haven, CT).
- Metzner W, Zhang S, Smotherman M (2002) Doppler-shift compensation behavior in horseshoe bats revisited: Auditory feedback controls both a decrease and an increase in call frequency. *J Exp Biol* 205:1607–1616.
- Schnitzler H-U (1968) Die Uhrsall-Ortslauten der Hufeisen-Fledermaeuse (Chiroptera-Rhinolophidae) in verschiedenen Orientierungssituationen. *Z Vgl Physiol* 57:376–408.
- Wheeler AR, et al. (2016) Echolocating big brown bats, *Eptesicus fuscus*, modulate pulse intervals to overcome range ambiguity in cluttered surroundings. *Front Behav Neurosci* 10:125.
- Hiryu S, Bates ME, Simmons JA, Riquimaroux H (2010) FM echolocating bats shift frequencies to avoid broadcast-echo ambiguity in clutter. *Proc Natl Acad Sci USA* 107:7048–7053.
- Brainard MS, Doupe AJ (2000) Auditory feedback in learning and maintenance of vocal behaviour. *Nat Rev Neurosci* 1:31–40.
- Burnett TA, Freedland MB, Larson CR, Hain TC (1998) Voice F0 responses to manipulations in pitch feedback. *J Acoust Soc Am* 103:3153–3161.
- Lee BS (1950) Effects of delayed speech feedback. *J Acoust Soc Am* 22:824–826.
- Sober SJ, Brainard MS (2009) Adult birdsong is actively maintained by error correction. *Nat Neurosci* 12:927–931.
- Habersetzer J (1981) Adaptive echolocation sounds in the bat Rhinopoma hardwickei. *J Comp Physiol A* 144:559–566.
- Ulanovsky N, Fenton MB, Tsoar A, Korine C (2004) Dynamics of jamming avoidance in echolocating bats. *Proc Biol Sci* 271:1467–1475.
- Gillam EH, Ulanovsky N, McCracken GF (2007) Rapid jamming avoidance in biosonar. *Proc Biol Sci* 274:651–660.
- Chiu C, Xian W, Moss CF (2009) Adaptive echolocation behavior in bats for the analysis of auditory scenes. *J Exp Biol* 212:1392–1404.
- Bates ME, Stamper SA, Simmons JA (2008) Jamming avoidance response of big brown bats in target detection. *J Exp Biol* 211:106–113.
- Takahashi E, et al. (2014) Adaptive changes in echolocation sounds by *Pipistrellus abramus* in response to artificial jamming sounds. *J Exp Biol* 217:2885–2891.
- Cvikel N, et al. (2015) On-board recordings reveal no jamming avoidance in wild bats. *Proc Biol Sci* 282:20142274.
- Götze S, Kobitz JC, Denzinger A, Schnitzler H-U (2016) No evidence for spectral jamming avoidance in echolocation behavior of foraging pipistrelle bats. *Sci Rep* 6:30978.
- Jarvis J, Jackson W, Smotherman M (2013) Groups of bats improve sonar efficiency through mutual suppression of pulse emissions. *Front Physiol* 4:140.
- Chiu C, Xian W, Moss CF (2008) Flying in silence: Echolocating bats cease vocalizing to avoid sonar jamming. *Proc Natl Acad Sci USA* 105:13116–13121.
- Griffin DR, McCue JGG, Grinnell AD (1963) The resistance of bats to jamming. *J Exp Zool* 152:229–250.
- Simmons JA, et al. (1978) Echolocation by free-tailed bats (*Tadarida*). *J Comp Physiol A* 125:291–299.
- Hage SR, Jiang T, Berquist SW, Feng J, Metzner W (2013) Ambient noise induces independent shifts in call frequency and amplitude within the Lombard effect in echolocating bats. *Proc Natl Acad Sci USA* 110:4063–4068.
- Luo J, Goerlitz HR, Brumm H, Wiegreb L (2015) Linking the sender to the receiver: Vocal adjustments by bats to maintain signal detection in noise. *Sci Rep* 5:18556.
- Luo J, Lingner A, Firzlaff U, Wiegreb L (2017) The Lombard effect emerges early in young bats: Implications for the development of audio-vocal integration. *J Exp Biol* 220:1032–1037.
- Suga N, Simmons JA, Shimoza T (1974) Neurophysiological studies on echolocation systems in awake bats producing CF-FM orientation sounds. *J Exp Biol* 61:379–399.
- Moss CF, Chiu C, Surlyke A (2011) Adaptive vocal behavior drives perception by echolocation in bats. *Curr Opin Neurobiol* 21:645–652.
- Luo J, Kosek J, Zsebők S, Siemers BM, Goerlitz HR (2013) Global warming alters sound transmission: Differential impact on the prey detection ability of echolocating bats. *J R Soc Interface* 11:20130961.
- Lawrence BD, Simmons JA (1982) Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *J Acoust Soc Am* 71:585–590.
- Ulanovsky N, Moss CF (2008) What the bat's voice tells the bat's brain. *Proc Natl Acad Sci USA* 105:8491–8498.
- Shadmehr R, Krakauer JW (2008) A computational neuroanatomy for motor control. *Exp Brain Res* 185:359–381.
- Hickok G, Houde J, Rong F (2011) Sensorimotor integration in speech processing: Computational basis and neural organization. *Neuron* 69:407–422.
- Hain TC, Burnett TA, Larson CR, Kiran S (2001) Effects of delayed auditory feedback (DAF) on the pitch-shift reflex. *J Acoust Soc Am* 109:2146–2152.
- Behroozmand R, Liu H, Larson CR (2011) Time-dependent neural processing of auditory feedback during voice pitch error detection. *J Cogn Neurosci* 23:1205–1217.
- Chen SH, Liu H, Xu Y, Larson CR (2007) Voice F0 responses to pitch-shifted voice feedback during English speech. *J Acoust Soc Am* 121:1157–1163.
- Tourville JA, Reilly KJ, Guenther FH (2008) Neural mechanisms underlying auditory feedback control of speech. *Neuroimage* 39:1429–1443.
- Heinks-Maldonado TH, Houde JF (2005) Compensatory responses to brief perturbations of speech amplitude. *Acoust Res Lett Online* 6:131–137.
- Bauer JJ, Mittal J, Larson CR, Hain TC (2006) Vocal responses to unanticipated perturbations in voice loudness feedback: An automatic mechanism for stabilizing voice amplitude. *J Acoust Soc Am* 119:2363–2371.
- Geberl C, Brinklow S, Wiegreb L, Surlyke A (2015) Fast sensory-motor reactions in echolocating bats to sudden changes during the final buzz and prey intercept. *Proc Natl Acad Sci USA* 112:4122–4127.
- Sapir S, McClean MD, Larson CR (1983) Human laryngeal responses to auditory stimulation. *J Acoust Soc Am* 73:315–321.
- Luo J, Kothari NB, Moss CF (2017) Sensorimotor integration on a rapid time scale. *Proc Natl Acad Sci USA* 114:6605–6610.
- Neuweiler G (1990) Auditory adaptations for prey capture in echolocating bats. *Physiol Rev* 70:615–641.
- Sinha SR, Moss CF (2007) Vocal premotor activity in the superior colliculus. *J Neurosci* 27:98–110.
- Wohlgemuth MJ, Luo J, Moss CF (2016) Three-dimensional auditory localization in the echolocating bat. *Curr Opin Neurobiol* 41:78–86.

Behavioral research on echolocation has largely focused on the role of echo feedback in vocal adaptations. Here, we demonstrate that audiovocal feedback is involved in adaptive sonar call design in the big brown bat. The current experiment provides direct evidence of the role of audiovocal feedback in representing the features of echolocation calls in bats. Thus, echolocating big brown bats, like songbirds and humans, rely on audiovocal feedback to adjust their call features, and audiovocal feedback might be a direct precursor for the more specialized adaptive sonar behaviors observed in echolocating animals (14, 27, 44).

## Materials and Methods

Experimental procedures were approved by Johns Hopkins University Institutional Animal Care and Use Committee. Detailed methods on behavioral testing procedures, acoustic broadcast stimuli, echolocation call recordings, and data analysis are presented in *SI Materials and Methods*.

**ACKNOWLEDGMENTS.** We thank Melville Wohlgemuth for suggestions during animal training, Ninad Kothari for help during data collection, and James Garmon for engineering support. Two anonymous reviewers provided thoughtful comments that improved the manuscript. This project is funded by the National Science Foundation (Grants IOS-1010193 and IOS-1460149); the Human Frontiers Science Program (Grant RGP0040); the Office of Naval Research (Grant N00014-12-1-0339) and the Air Force Office of Scientific Research (Grant FA9550-14-1-039) (to C.F.M.); and by a long-term postdoctoral fellowship from the Human Frontier Science Program (LT000279/2016-L) (to J.L.). C.F.M. worked on this manuscript while participating at the Kavli Institute for Theoretical Physics workshop The Physics of Hearing: From Neurobiology to Information Theory and Back and was supported in part by the National Science Foundation under Grant NSF PHY11-25915.

# Supporting Information

Luo and Moss 10.1073/pnas.1711892114

## SI Materials and Methods

Four adult *E. fuscus* big brown bats (three males, bats 1–3, and one female, bat 4)—a species that produces frequency-modulated signals for echolocation—were trained to rest on a platform and track approaching tethered insects (i.e., prey targets) by sonar (Fig. 1B and Movie S1). The prey targets were tethered to a motorized pulley system, and its speed was precisely controlled by a customized MATLAB program. In this experiment, for each trial the prey targets accelerated at a speed of ~2.8 m/s from a 3-m distance away from the bat, traveled at a constant speed of ~1.5 m/s for the time window 0.5 ~ 1.9 s, and finally decelerated at a speed of ~2.1 m/s. The bat-target distance over the time course of a trial is shown in Fig. S1. The start and end of the motor system mark the start and end of a trial, and a trial lasts about 2.5 s.

We recorded the echolocation calls of the bats with an array of 13 microphones (DX500; Petterson Elektroniks, with the horn on) that covers a horizontal space of about -65 to +65° and a vertical space of about -30 to +45° (Fig. 1B, indicated by blue circles). An additional microphone placed beneath the platform (Fig. 1B, indicated by a green circle) was used to trigger the playback system to broadcast jamming signals from a customized electrostatic loudspeaker powered by a wideband amplifier (Model 7500; Krohn-Hite) placed to the left of the bat at a 15-cm distance (Fig. 1B, indicated by a red square). The diaphragm of the loudspeaker was 1 cm in diameter. Systematic measurements showed that the border between near-field and far-field was within 10 cm from the loudspeaker. Thus, at a 15-cm distance, the bat was located in the far-field of the loudspeaker. An example of the sonar tracking behavior of the bats is shown in Fig. 1C, the recording from the trigger microphone is shown in Fig. 1D, and the recording of the jamming signals is shown in Fig. 1E. Before this experiment, we made sound recordings from each bat in silence and used these recordings to guide the design of the jamming signals. For each bat, we constructed three types of individualized jamming sounds, namely -6-kHz type, 0-kHz type, and +6 kHz. Both -6-kHz and +6-kHz jamming sound types were pitch-shifted versions of the 0-kHz type by a magnitude of 6 kHz downward and upward, respectively.

Sound recordings were digitized at a sampling rate of 250 kHz, and the jamming signals were generated at a sampling rate of 1 MHz. Each array microphone was directed to the platform using a laser beam. We localized the spatial position of the array microphones by playing and recording chirp sequences (5- to 25-kHz FM sweeps) through a loudspeaker placed at different locations. The exact location of the speaker was recorded by a motion tracking system containing three high-speed cameras (MX T40; Vicon Motion Systems). The distance between the platform and each microphone was confirmed by direct measurements from a laser distance meter (GLR 500; Bosch). We ensured a flat frequency response of the playback system for the frequency range of 20–100 kHz ( $\pm 1$  dB) by digitally compensating for the uneven frequency response of the loudspeaker with its compensatory impulse response. The compensatory impulse response of the loudspeaker was designed using the Maximum Length Sequence method. We broadcast the jamming signals at amplitudes of 80 dB SPL (sound pressure level; root mean square amplitude, relative to 20  $\mu$ Pa). Since bat 4 was disturbed by the jamming signals, as indicated by flying off the platform much more often than the other bats, and showed no sign of adapting over more than a week of tests, we turned down the playback amplitude to 70 dB SPL, which restored the normal behavior of this subject almost immediately. Bat 4 was thus tested with the jamming

signals broadcast at 70 dB SPL. The amplitude of the playbacks was determined with a measurement microphone (Model 7016, 1/4 inch Condenser microphone; ACO Pacific with protection grid removed), which was calibrated using a Brüel & Kjær, type 4231 calibrator.

To selectively perturb the vocal feedback or echo feedback process, we employed the triggered playback technique that works as follows: When an echolocation call is emitted from the bat's mouth, the call travels about a 5-cm distance to reach the trigger microphone; the amplitude of the call is assessed by the data acquisition system (National Instruments, PXIe 8135, with two data acquisition cards PXIe 6358 consisting of 16 analog input channels and 8 analog output channels) and compared with the trigger threshold in real time; the playback system is activated after a specified digital delay to broadcast the stored jamming sound or a silenced sound file if the trigger threshold is met; and, finally, the jamming sound or the silence sound file travels about a 15-cm distance to reach the ear of the bat. The total time delay of the playback system is determined by the distances that the sounds (both call and the jamming sound) travel in air, the processing time of the program and the hardware, the digital delay introduced by the experimenter, and the interaction between the trigger threshold and the waveform shape of the bat's calls. By placing both the trigger microphone and the loudspeaker close to the bat, we were able to achieve a minimum delay of <1 ms (Fig. 1G). To achieve a precise synchronization of the sound recording and playback system, we split the playback signal from the analog output channel into two copies: one copy went to the loudspeaker and the other copy went to one of the analog input channels via a direct wire connection.

Two experiments were conducted in this study. In experiment 1, we broadcast the three types of jamming signals over three delays: 1 ms (with zero digital delays), 10 ms, and 30 ms. Together with a silence control, there were 10 treatments in total. After we analyzed the data from the first experiment, we conducted a second experiment on three bats. Specifically, in experiment 2 we tested the conditions in which the bats showed the largest vocal adjustments in call frequency (bats 1–3). The second experiment collected data from two more time delays at 5 ms and 20 ms. In addition, we repeated the 1-ms delay condition in which the bats showed the greatest vocal adjustments and included a silence control. For all experiments, the order of presenting the treatments was pseudorandomized and the experimenter was not aware of the exact condition during data collection. Bats were rewarded with a mealworm after a trial. On average, each bat performed about 30 trials per experimental day and was tested 6 d per week.

In addition to the silence control trials included among other treatment trials with jamming signals during the test (silence control type I), we also recorded the echolocation calls of the bats only in silence conditions both before and after the first experiment. These trials thus served as an additional silence control (silence control type II).

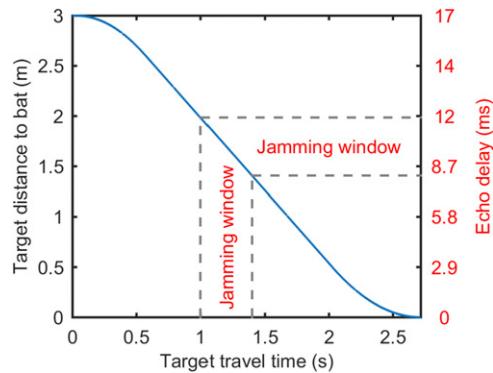
Echolocation calls were analyzed in MATLAB (R2015a; MathWorks) with custom-written programs, based on code used by Luo et al. (24, 25). We first accounted for the frequency response of the microphones by filtering the recorded calls with each microphone's compensatory impulse response (32nd order finite impulse response filter) and high-pass-filtered all recordings at 10 kHz. Because of the spectral notch of the microphones at around 75 kHz, we low-pass-filtered the recordings at 70 kHz. This is also a practical reason to limit our frequency analysis to the first harmonic of the call that ranges from ~55 kHz to 20 kHz

(excluding the terminal buzz phase). Subsequently, we identified echolocation calls from the microphone that was directed toward the bat (central microphone, 0-degree azimuth and elevation), from which each echolocation call was cut with a fixed time window of 5 ms before the call and 5 ms after the call. Then we estimated the call duration ( $-20$  dB relative to the peak amplitude) and the root mean square amplitude over the duration. Through cross-correlation, calls in all other channels were readily available for analysis after compensating for the frequency- and distance-specific transmission loss. The quality of the sound analysis program was manually checked by displaying the waveform and spectrogram at varying stages of the analysis and confirmed its high quality.

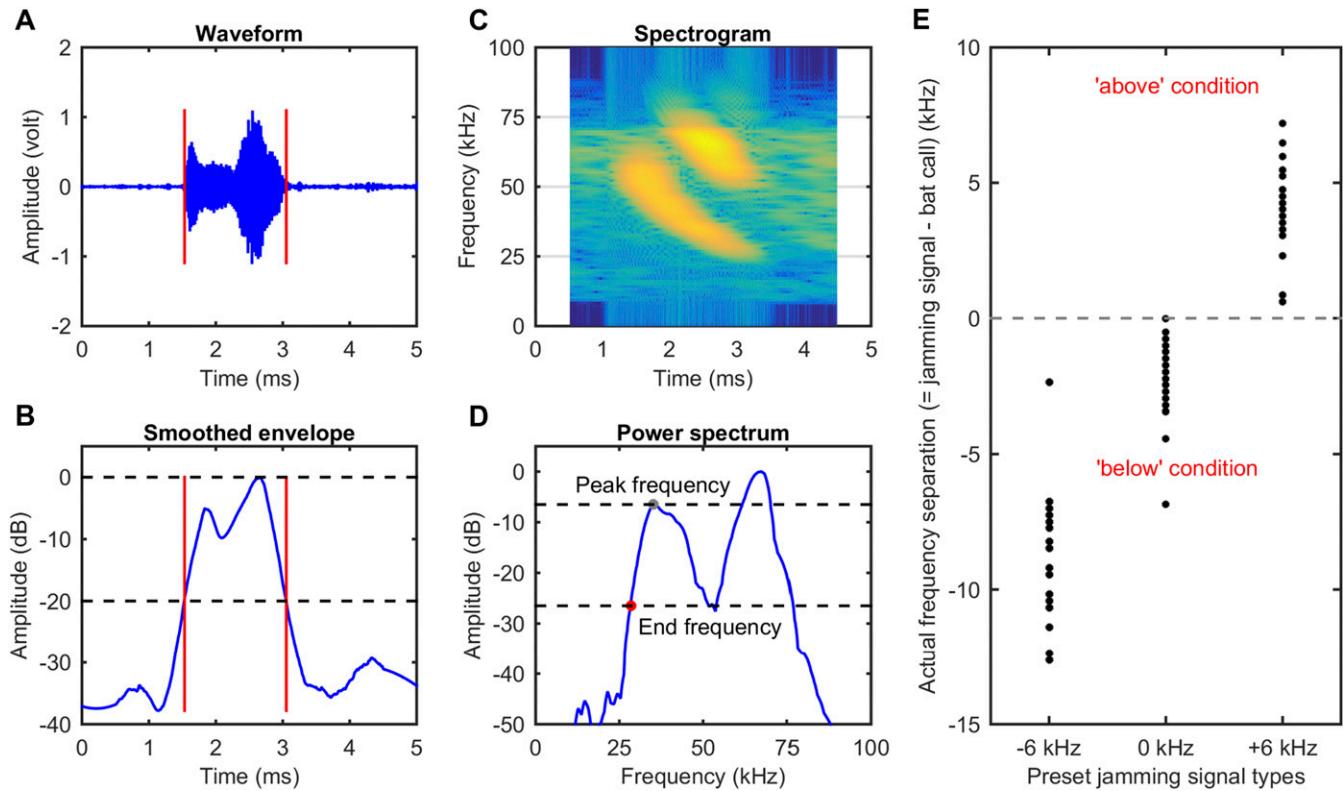
Frequency estimations were based on the call that had the maximum root mean square amplitude among the 13 array mi-

crophones so that the influence of the changes in the calling direction of the bat on signal parameter estimation is minimized. End frequency was estimated as the frequency component of  $-20$  dB amplitude relative to the peak frequency of the first harmonic (Fig. S2D). We focused on the end frequency of the echolocation calls because a previous study had shown that big brown bats adjust the end frequency of their sonar calls in response to jamming signals (15).

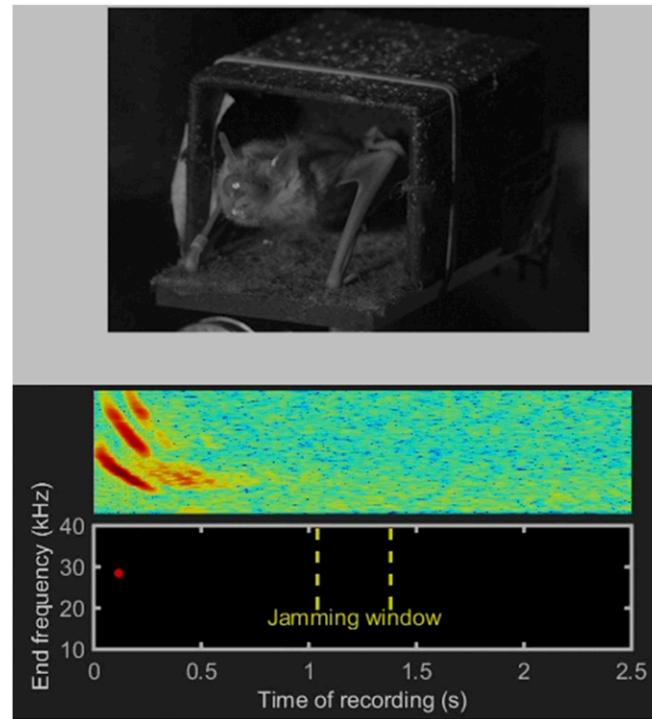
When comparisons were performed for two treatments or conditions, the nonparametric two-sample Kolmogorov-Smirnov test was used to calculate the  $P$  value in MATLAB. In addition, all statistical tests were performed with two types of silence control (see earlier section within *SI Materials and Methods* for description) and only when the conclusion held for both control types did we consider it to be statistically significant.



**Fig. S1.** The relationship between the target distance to the bat, the elapsed time during a trial, and the target echo arrival time at the bat. Jamming signals occurred between 1 s and 1.4 s after a trial onset, and thus it took about 10 ms for the bat to receive its echo from the target.



**Fig. S2.** Sound analysis routine and grouping of trials based on the actual frequency separation between bat call and jamming signal. For each echolocation call, the call samples (between the two horizontal red lines in A) were first extracted from the continuous recording sequence, based on the smoothed envelope shown in B. Fast Fourier Transformation was then used to compute the spectrogram (C) and power spectrum (D). From the power spectrum, the end frequency of the calls (red dot) was measured as the frequency 20 dB down from the peak frequency (gray dot) from the first harmonic. (E) Trials were separated into two groups based on the actual frequency separation between bat call and the jamming signal.



**Movie S1.** Call-frequency control of a bat in response to acoustic jamming signals. (*Top*) High-speed video filming the behavior of the bat. (*Middle*) The spectrogram of the echolocation calls. (*Bottom*) End frequency of the echolocation calls. High-speed video was recorded at a frame rate of 250 Hz, and the calls were recorded at a sampling rate of 250 kHz. Here, both video and sound recordings were slowed down by a factor of 10 to make the ultrasonic calls audible to human ears.

[Movie S1](#)