



Echo feedback mediates noise-induced vocal modifications in flying bats

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Abstract

Diverse animal taxa are capable of rapidly modifying vocalizations to mitigate interference from environmental noise. Echolocating bats, for example, must frequently perform sonar tasks in the presence of interfering sounds. Numerous studies have documented sound production flexibility in echolocating bats; however, it remains unknown whether noise-induced vocal modifications (NIVMs) mitigate interference effects on echoes or calls. In this study, we leverage echo level compensation behavior of echolocating bats to answer this question. Using a microphone array, we recorded echolocation calls of *Hipposideros pratti* trained to approach and land on a perch in the laboratory under quiet and noise conditions. We found that *H. pratti* exhibited echo level compensation behavior during approaching flights, which depended critically on distance to the landing perch. Broadcast noise delayed and affected the rate of echo level compensation in *H. pratti*. Moreover, *H. pratti* increased vocalization amplitude, i.e., exhibited the Lombard effect, while also adjusting call duration and bandwidth with increasing noise levels. Quantitative analyses of the data show that *H. pratti* relies on echo feedback, not vocal feedback, to adjust signals in the presence of noise. These findings provide compelling evidence that NIVMs in echolocating animals and non-echolocating animals operate through different mechanisms.

Keywords Environmental noise · Audio-vocal integration · Echo level compensation · Lombard effect · Vocal feedback

Introduction

Sound signals are used by a wide range of animals, from chorusing insects to trumpeting elephants, for communicative purposes (Bradbury and Vehrencamp 2011). Similarly, humans rely heavily on speech for communication and social interaction (Lieberman 1984; Fitch 2000). Acoustic communication often takes place in the presence of environmental noise that may originate from either abiotic or biotic sources

(Brumm 2013). Understanding noise mitigation strategies in acoustic communication across the animal kingdom has implications for theory and applications in biology, medicine, and engineering.

Animals have evolved diverse adaptations in call production and hearing to minimize interference of environmental noise on acoustic communication. A striking example is the ultrasonic calling of torrent frogs in Huangshan Hot Springs, China, where animals encounter ultrasonic noise from local fast flowing streams (Feng et al. 2006; Shen et al. 2008). Most frogs produce low frequency communication calls and typically only hear sound frequencies below five kHz (Capranica 1978; Gerhardt 1986). Dr. Albert Feng and his colleagues made the remarkable discovery that torrent frogs have evolved ultrasonic vocalizations and hearing to communicate in the presence environmental noise. Feng was lead author on the groundbreaking publication of this research in *Nature* in 2006 (Feng et al. 2006), and we dedicate our article to his memory.

Diverse species exhibit rapid modifications to the structure of sound signals in response to environmental noise (Brumm and Slabbekoorn 2005; Hotchkiss and Parks 2013;

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Luo et al. 2018). When background noise spectrally overlaps with acoustic communication signals, nearly all vertebrates produce sounds of higher amplitude, an audio-vocal phenomenon widely known as the Lombard effect (Brumm and Zollinger 2011). Noise-induced vocal amplitude increases are often accompanied by modifications to other sound parameters, such as an increase in duration and repetition rate, which serve to boost signal detection (Hotchkiss and Parks 2013). It is noteworthy that the magnitude of noise-induced vocal modifications (NIVMs) grows larger with increasing background noise level, suggesting that animals reference the signal-to-noise ratio (SNR) to make call adjustments (Luo et al. 2018). When environmental noise does not spectrally overlap acoustic communication signals, animals show a much weaker, or even no Lombard effect (Tressler and Smotherman 2009; Hage et al. 2013; Luo et al. 2015; Lu et al. 2020). Thus, SNR in the spectral range of calls seems to drive vocal modifications that mitigate noise interference.

Bats are a diverse group of mammals, consisting of > 1,400 recognized species, all of which rely heavily on acoustic signals for orientation and social communication (Griffin 1958; Busnel and Fish 1980; Popper and Fay 1995; Simmons et al. 1998; Schnitzler et al. 2003; Thomas et al. 2004; Denzinger and Schnitzler 2013; Fenton and Simmons 2014). Most species of bats have evolved the ability to echolocate, which they use to navigate in three-dimensional (3D) space and find food. One remarkable feature of bat echolocation is the adaptive changes in signal design, driven by behavioral tasks (Moss and Surlykke 2010; Jones et al. 2021). For example, echolocating bats actively control the spectro-temporal features of echolocation calls during foraging and obstacle avoidance (Griffin et al. 1960; Chiu et al. 2009; Moss et al. 2011).

There is evidence that bats exhibit changes in call features in response to echo feedback (Moss and Surlykke 2010; Wohlgenuth et al. 2016). During approaching flights, echolocating bats not only produce calls of gradually reduced duration and interval, but they also decrease the amplitude of emitted calls. A reduction in call amplitude contributes to amplitude stabilization of echoes received at the bat's ears (Kobler et al. 1985; Boonman and Jones 2002; Koblitz et al. 2011; Stidsholt et al. 2020), which may complement range-dependent changes in hearing sensitivity (Kick and Simmons 1984; Hartley 1992).

Two recent studies have demonstrated the crucial role of vocal feedback in the control of sonar call parameters in bats. When vocal feedback and echo feedback were selectively perturbed during sonar prey tracking, echolocating big brown bats made compensatory frequency adjustments in response to vocal feedback perturbation, but not to echo feedback perturbation (Luo and Moss 2017). Furthermore, dynamic adjustments in sonar call frequency by the great roundleaf bat can be accurately predicted by the same

computational model for human speech, underscoring the essential role of vocal feedback in bat sonar call control (Wang et al. 2022).

Here, we explore the question whether echo feedback mediates vocal changes in the presence of environmental noise. In this study, we first examine the effects of noise broadcast on echo level compensation (ELC) behavior of *Hipposideros pratti* during approaching flights. Then, we directly test the hypothesis that NIVMs are driven by auditory responses to echoes, as opposed to vocalizations, by leveraging the ELC behavior of the hipposiderid bat. ELC is characterized by the active reduction of call amplitude to stabilize the level of echo returns. Specifically, we analyzed acoustic measurements to determine if echolocating hipposiderid bats rely on the SNR of emitted vocalizations or the SNR of echo returns to guide NIVMs.

Methods

Animals

Four adult *H. pratti*, two males and two females, participated in the experiment. Bats were wild-caught with a hand net during the daytime in a cave in Xianning County, Hubei Province. Bats were housed in a custom-made metal meshed cage (40×40×40 cm), placed in shelves of a room with a regulated air temperature of around 24 °C, relative humidity of around 60%, and a reversed light regime of 12 h darkness and 12 h light. Bats had ad libitum access to water and food. All experimental procedures were approved the Institutional Animal Care and Use Committee of the Central China Normal University.

Experimental setup

Experiments took place in a large room (6.5×5×2.3 m, length×width×height), with walls and ceiling covered by 8-cm thick acoustic foam and the floor was covered by a nylon blanket to reduce echoes and reverberations (Fig. 1A). A landing perch (20×20 cm) hung about 0.9 m down from the ceiling and at approximately 0.75 m in front of a microphone array. Between the landing perch and the microphone array, two one-inch diameter loudspeakers (XT25SC40-04, Tympany and Peerless, Denmark), separated by ~ 10 cm, were mounted on two horizontal bars extended from the perch. The loudspeakers were 25 cm away and at the same height of the center of the landing perch. Each loudspeaker was driven by a power amplifier (ATA101, Aigtech, China). The microphone array consisted of 9 broadband ultrasonic microphones (NEUmic, Ultra Sound Advice, UK), arranged in a “+” configuration. Each microphone was 50 cm away from its nearest neighbor. The accurate 3D position of each

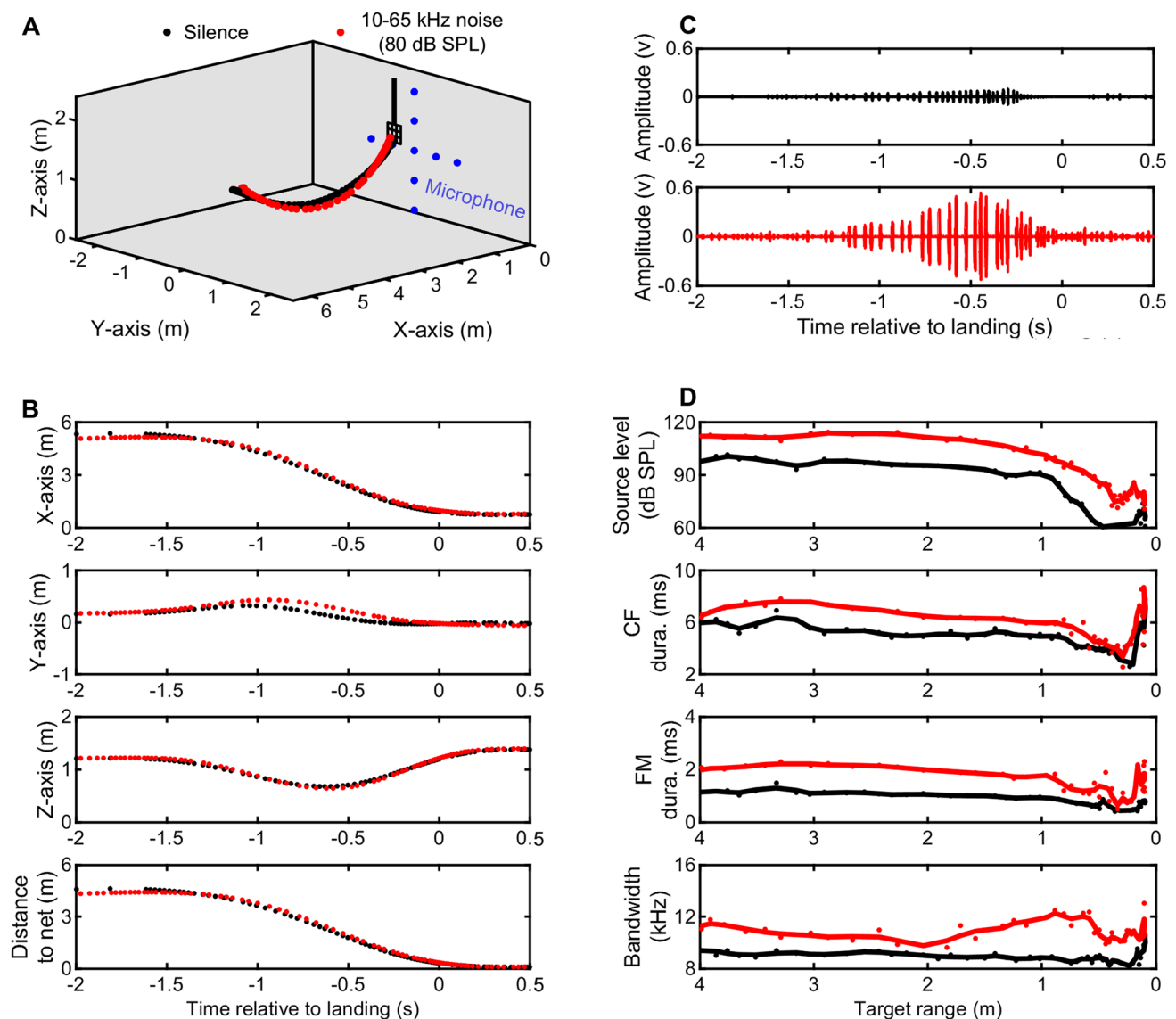


Fig. 1 Examples of the 3D flight path and echolocation behavior of a *Hipposideros pratti* during approach flights in the laboratory. **A** Two reconstructed flight paths (a silence, i.e., no noise control trial and a noise trial) based on an array of 9 microphones of a bat approaching a landing net. **B** Flight trajectories of two trials in the *x*-, *y*-, and *z*-dimension, as well as the estimated distance between the bat and the net across time. Time 0 is the vocal landing time based on call rate, call amplitude, and call duration (see **Methods**). **C** Waveforms of the

recorded echolocation calls from the central microphone during two trials. **D** Estimated source level (referenced to 10 cm in front of bat), the duration of the CF and FM components, and the bandwidth of the FM component of the emitted calls of the bat, as a function of the distance to the landing net during two trials. Source level was estimated based on the central microphone recording after accounting for the distance-related transmission loss and atmospheric attenuation

microphone was measured with a ruler to a precision of about 1 cm. Microphones were parallel to each other and pointed toward the approaching bat.

Sound recording and playback

H. pratti were trained to fly from one side of the room, over a distance of approximately 4.5 m, to a landing perch at the other side of the room. All the bats were well trained to fly

a consistent path to the landing perch (Luo et al. 2022). In addition to the silence (no noise) control condition, each bat was recorded under three noise level conditions of 40 dB, 60 dB, and 80 dB SPL RMS (root mean square) and under three bandwidths. Noise levels were measured with a calibration microphone placed 50 cm in front of the loudspeaker. Validation tests confirmed that the actual noise levels during the experiments differed by < 2 dB from the specified noise levels. White noise was bandpass filtered as

follows: 10~65 kHz, 35~65 kHz, and 10~45 kHz. The first two noise bands overlap with the dominant second harmonic of *H. pratti*'s echolocation calls, while the third noise band does not (see insets of Fig. 2). Both sound recording and noise playback were controlled by a custom-written MATLAB script (SoundMexPro Toolbox) that interfaced with a multi-channel soundcard (RME Fireface 802, Germany) at a sampling rate of 192 kHz with a 24 bits resolution.

In each trial, audio recordings and noise broadcasts ran continuously for 8 s. During this time, a bat flew from the hand of one experimenter to land on the perch. The entire

flight duration was about 1.5 s. The order of noise conditions (including the silence control) was pseudo-randomly determined each day for each bat. Typically, every experimental condition was repeated 20 times over multiple days for each animal subject. The sensitivity of the playback system was measured with a 1/4-inch calibration microphone (46BF, GRAS, Denmark) and a sound calibrator (521 SPL Calibrator, ACO, USA). Compensatory impulse response of each playback channel was designed to achieve a flat frequency response (± 1 dB) using a data acquisition card (PXIe 6358, National Instruments, Austin, TX, USA) at a rate of

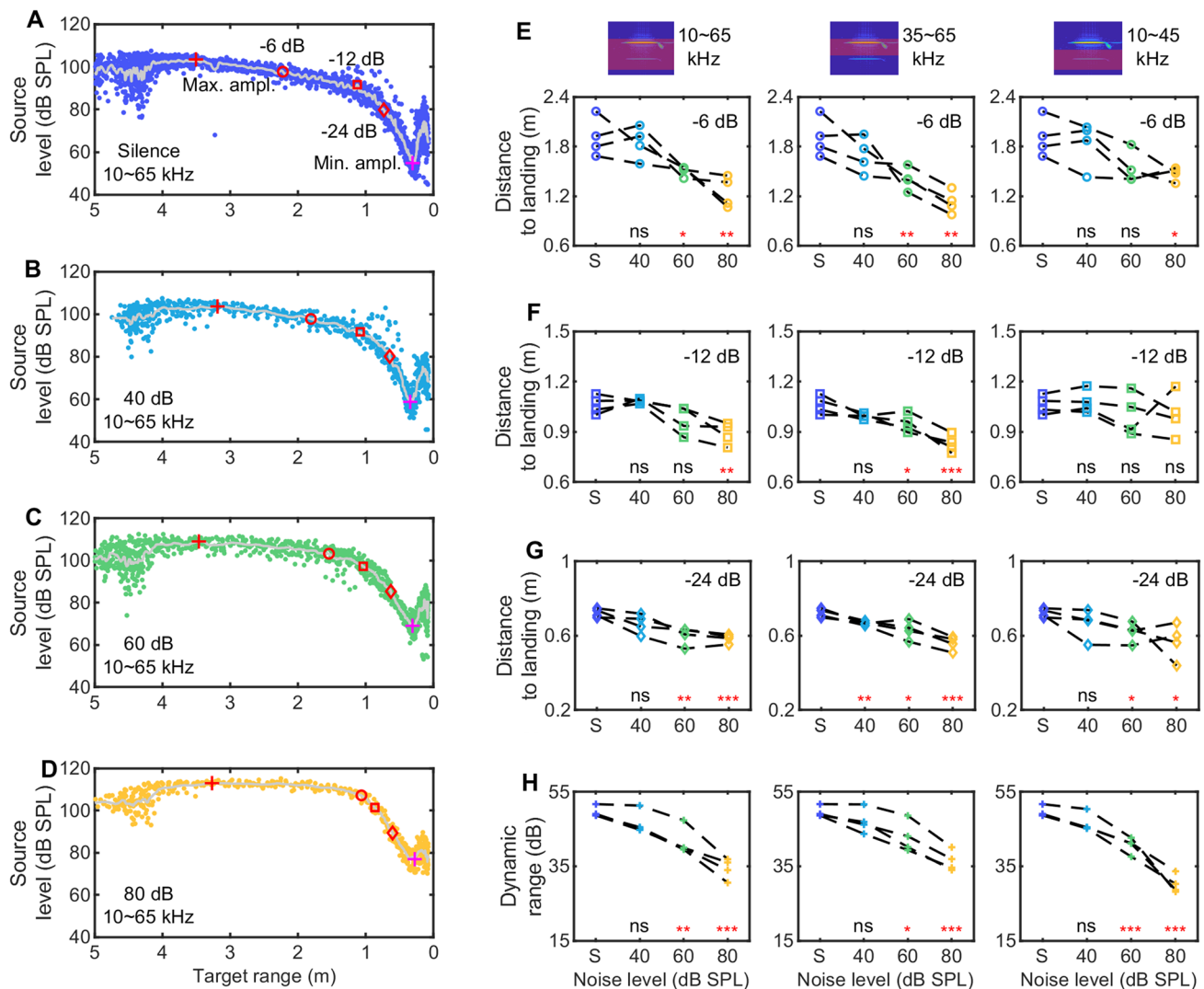


Fig. 2 Environmental noise delays the echo level compensation behavior in *Hipposideros pratti*. **A–D** Source level of one example individual of *H. pratti* as a function of distance to landing. Red and pink crosses mark the maximum and minimum source level; Circle, square, and diamond mark the source level point at the -6 dB, -12 dB, and -24 dB relative to the maximum, estimated from the smoothed amplitude curve with 25 data points (Gray curves). The noise levels were silence (no noise) control, 40 dB, 60 dB, and 80 dB

SPL for these conditions. The bandwidth of environmental noise was 10~65 kHz. **E–G** The estimated distance to landing for source level point at -6 dB, -12 dB, and -24 dB relative to the maximum across noise levels. **H** The dynamic range of source level (amplitude different between the maxima (red cross) and minima (pink cross)) across noise levels. For **E–H**, each data point within a group represents one individual. ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

250 kHz. Then, these compensatory impulse responses were down-sampled to 192 kHz to match the sampling rate of the audio interface. General details for sound recording and playback can be found in previous studies (Luo et al. 2015; Lu et al. 2020).

3D path reconstruction of flying bats

The 3D location of *H. pratti* along its flight path was reconstructed at the time of each sonar call using the time-of-arrival-differences (TOAD) picked up by the microphone array. We first identified all calls using the audio channel of the central microphone in the array, i.e., the microphone at the intersection of the horizontal and vertical microphones. For each identified call, we located the corresponding calls from other microphone channels and computed the time delays between paired channels using cross-correlation. Then, we triangulated the bat location with the TOAD measurements. The localization accuracy of this method is affected by the number of microphones picking up the signal, the SNR of the signal at each microphone, signal spectro-temporal features, and the configuration of the sensor array (Madsen and Wahlberg 2007). In our experiments, we only reconstructed 3D bat position when at least four microphones picked up calls with a minimum SNR of 8 dB. We improved bat localization accuracy by setting the limits of the searching range algorithm in the 3D domain (D'Errico 2022), taking into account the size of the flight room. Further, we automatically detected and excluded outliers of the location data with a moving median method. Then, we applied the Cubic Smoothing Spline to the raw bat location data to get a smoothed version of the flight path. Lastly, we calibrated the accuracy of the 3D reconstruction using the location of the landing perch. As illustrated in Fig. 1B, the reconstructed bat location precisely matched with the 3D location of the landing perch at the end of the two trials.

Call analysis

We measured multiple features of the echolocation calls and here we focus on four signal parameters: call amplitude, duration of the CF component, duration of the FM component, and the FM bandwidth. All signal parameters were estimated using sound recordings from the central microphone of the array that typically had the highest SNR. Calls were batch-processed with custom-written scripts in MATLAB, which were validated in several previous studies (Lu et al. 2020; Luo et al. 2022; Wang et al. 2022). The quality of the sound analysis scripts was manually checked graphically by displaying the waveform, spectrogram, and power spectrum for randomly selected sound files. In addition, we paid special attention to the calls of lower SNRs, such as those emitted by bats during the strong ELC period,

shortly before landing. We observed no systematic errors in signal parameter measurement.

Source level estimation

The source level of the echolocation calls of *H. pratti* during the approaching flights were estimated by compensating for the distance-related transmission loss from

$$SL = ML + TL$$

where SL is the (apparent) source level of the emitted calls as if measured at 10 cm in front of the bat nostrils; ML is the measured amplitude level at the central microphone of the array; TL is the transmission loss across the distance from bat location to the microphone. All three variables have the unit of dB. TL is estimated from

$$TL = 20 \times \log_{10} \left(\frac{d}{d_{\text{ref}}} \right) + \alpha \times (d - d_{\text{ref}})$$

where d is the bat-to-microphone distance in meter, d_{ref} was set to 0.1 m, and α is the frequency-specific atmospheric attenuation. In this experiment, we calculated atmospheric attenuation based on sound frequency of 58 kHz (mean dominant call frequency during flight), 24 °C, and 60% relative humidity.

Statistics

Statistical analyses were performed using the Statistical and Machine Learning Toolbox in MATLAB. We used *t-test* to compare the distance of ELC between noise levels as shown in Fig. 2E–H. We fitted a logarithmic function in the form $SL = a \times \log_{10}(r) + b$ to assess the relationship between source level and target range (r), using the Least Square method. Then we estimated the coefficient a and its 95% confidence intervals. Then we compared the coefficient a between noise levels as in Fig. 3E–G. When the 95% confidence intervals of one group did not include the mean of the other group, these two groups were considered to be statistically different and was denoted by $P < 0.05$. We built linear mixed models to investigate the effects of noise level on each of the four signal parameters of source level, duration of the CF component, duration of the FM component, and the FM bandwidth. We did this for each of the three selected phases (Phase I, II, and III as shown in Fig. 4A) separately. In this mixed model, the received noise level was set as fixed effect, the identity of the bat and the identity of the file were set as random effects. The received noise level was estimated by accounting for the distance-related transmission loss as explained above for source level estimation. Then, we compared the signal parameter between phases using the confidence interval method.

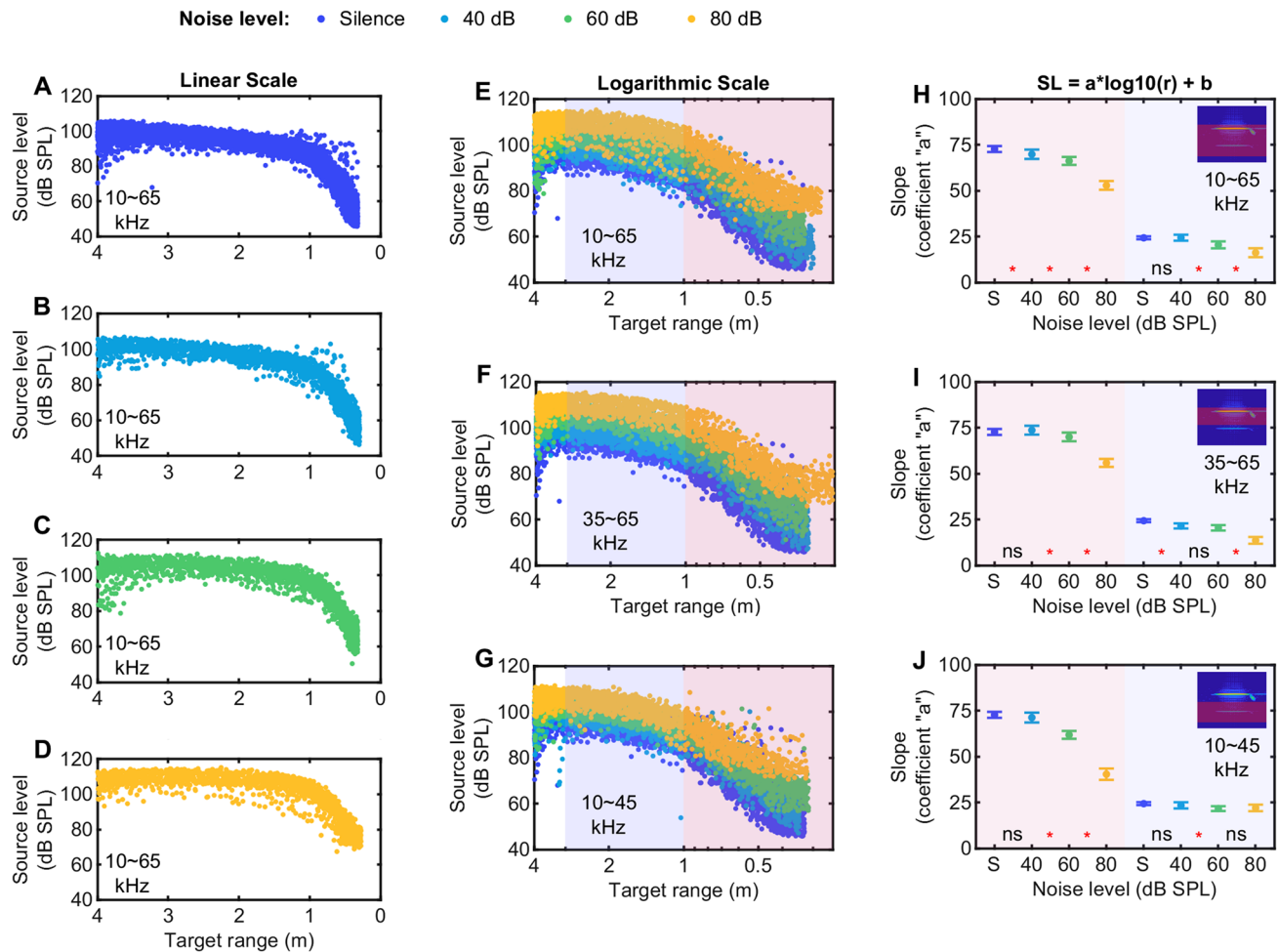


Fig. 3 Environmental noise affects the slope of echo level compensation behavior in *H. pratti*. **A–D** Source level of *H. pratti* pooled for all four individuals as a function of target range (distance to landing). The bandwidth of environmental noise was 10–65 kHz. **E–G** Superimposed source level of *H. pratti* across noise levels for three noise types. **H–J** The slope of echo level compensation, estimated for

two different target ranges (< 1 m and $1 \sim 3$ m) in silence (no noise) control condition, and in three noise level conditions, for three noise types. Data are plotted as mean and 95% confidence intervals. Statistical significance between two neighboring conditions is shown at the bottom (*, $P < 0.05$; ns, $P > 0.05$)

Results

Overall flight and echolocation behavior of *H. pratti* during approaching flights

Two representative flight trials from a single bat, one silence control and one in the 80 dB SPL 10–65 kHz noise condition, are illustrated in Fig. 1A. Figure 1B shows the detailed trajectory of the x -, y -, and z - dimension of these two flight trials, as well as the distance to landing, across time. These two examples show that it took approximately 1.5 s for the bat to perform a flight trial. While the bat flew close to the central line of the y -axis and was thus facing the central microphone of the array with a defined 3D coordinate of [0.2, 0, 1.1] (x -, y -, and z -axis, in meter),

it lowered its flight height at most by approximately half a meter during the approaching flights.

Figure 1C shows the waveforms of these two example trials with recordings from the central microphone. Figure 1D shows the source level, the duration of the CF component, duration of the FM component, and the bandwidth of the FM component of these two trials as a function of the distance to landing. Source level was estimated from the central microphone recording after compensating for the transmission loss (see **Methods** for details). Call data from these two trials suggest that *H. pratti* modulated several call parameters while approaching the landing net in noise. The source level data show that the bat lowered the amplitude of the emitted calls shortly before landing, i.e., exhibiting ELC behavior.

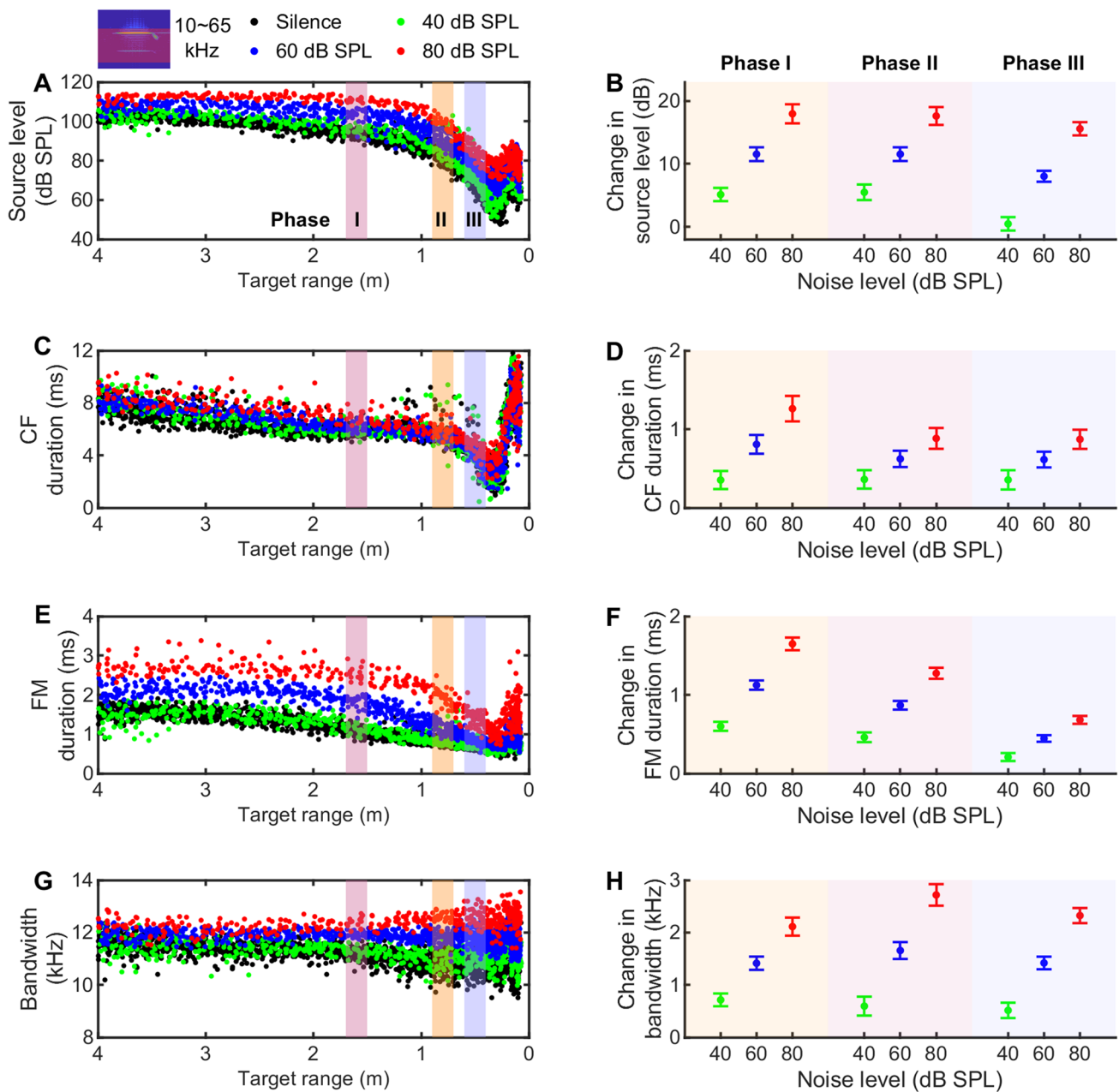


Fig. 4 Noise-induced vocal modifications in *Hipposideros pratti* in the phases prior to and during echo level compensation in the 10–65 kHz noise conditions. **A–D** Distance-dependent echolocation behavior of *H. pratti* in 10–65 kHz bandpass filtered noise that overlaps with both the first and the second harmonic of the echolocation calls. The four conditions were silence (no noise) control, and noise at 40, 60, and 80 dB SPL relative to 20 μ Pa. The left column

of panels shows data from one bat (Hp036); the right column of panels shows the predicted mean $\pm$ 95% confidence intervals for three selected phases by linear mixed models using data from all four bats. Compared to the first selected phase prior to echo level compensation) bats produced calls of reduced amplitude in the second and third phases during the echo level compensation

Environmental noise affects the ELC behavior of flying *H. pratti*

Echo level compensation (ELC) behavior of *H. pratti* during approaching flights becomes evident by aligning the source level data across the trials. Figure 2A–D show the source

levels of one *H. pratti* during the approaching flights in the silence control, 40 dB, 60 dB, and 80 dB SPL noise conditions. The frequency range of the noise conditions was 10 to 65 kHz, which thus covered the first two harmonics of the echolocation calls of *H. pratti* (see the inset above Fig. 2E). We found that this bat exhibited ELC behavior in both the

silence control and noise conditions. A large drop in the source level of the emitted calls occurred during the last meter before landing. To address the question whether noise playback affects the ELC behavior of *H. pratti*, we compared the start distance of ELC, i.e., target range at which the source level of the bat decreased by 6 dB (red circle marker), 12 dB (red square marker), and 24 dB (red diamond marker) from the maxima (red cross marker) between the silence control and noise conditions. In addition, we compared the dynamic range of source level between the silence control and noise conditions. The dynamic range of source level was defined as the difference in source level between the maxima (red cross marker) and minima (pink cross marker) during the approaching flights.

We found that the noise level affected the distance at which bats lowered source level when they approached the landing perch. The distance at which the bat initiated ELC was significantly shorter in the highest noise level of 80 dB SPL than in the silence control for all three measured source level points of -6 dB, -12 dB, and -24 dB (Left column of panels, Fig. 2E–G; *T-test* between the blue group and the yellow group, all $P < 0.05$). In other words, the bats delayed the ELC and were closer to the landing perch when they lowered the source level by the same amount in the 80 dB SPL noise condition than in the silence control condition. For the 60 dB SPL noise condition, the distance of ELC was significantly shorter for the -6 dB and -24 dB source level points, but not for the -12 dB source level point. There was no difference in the distance to landing between the silence control and the 40 dB SPL noise condition for any of the three source level points. Similarly, the dynamic ranges were smaller in the 60 dB and 80 dB SPL noise conditions than in the control (Left panel, Fig. 2H). In particular, the bats reduced the dynamic range from 49.4 ± 1.5 dB in the silence control condition to 34.4 ± 2.8 dB in the 80 dB SPL condition.

We also assessed the effects of the 35~65 kHz and 10~45 kHz noise types on the ELC behavior of *H. pratti*. For the 35~65 kHz noise, the distance of ELC was significantly shorter in all noise conditions than in the silence control condition, except for the -6 dB source level point at the 40 dB SPL noise condition (Middle column of panels, Fig. 2E–G). Similarly, the dynamic ranges of the source level of the bats were smaller in the 60 dB and 80 dB SPL noise conditions than in the control (Middle panel, Fig. 2H). These data suggest that the 35~65 kHz noise type has more consistent effects on the ELC behavior than the 10~65 kHz type. For the 10~45 kHz type, the noise affected the distance of ELC for 2 out of 9 noise conditions (Right column of panels, 2E–G). However, the dynamic ranges of the source level were similarly reduced in the 60 dB and 80 dB noise conditions of the 10~45 kHz type, as in the other two types of noise.

Next, we assessed the effects of noise playback on the slope of ELC behavior, i.e., the rate of echo level adjustment over distance to the landing perch. Figure 3A–D show the source level of emitted calls as a function of target range on the linear scale. Figure 3E–G show the superimposed source levels of emitted calls across noise levels for the three noise types, as a function of landing perch range on a logarithmic scale. As the slope of ELC appears range dependent, where the slope of ELC function is steeper at closer ranges, we fit the logarithmic function for the short range (< 1 m, pink background) and long range (1~3 m, blue background) respectively. We found that for all three noise types, the slopes of ELC function were approximately three times greater for the short range than the long range (Fig. 3H–J). Moreover, at the short range, the slope of ELC function decreased with increasing level of noise for all three noise types (Confidence interval comparison, $P < 0.05$), except for the comparison between the control and 40 dB SPL noise condition in the 35~65 kHz noise type (Fig. 3I, Confidence interval comparison, $P > 0.05$).

Echo feedback, not vocal feedback, mediates NIVMs of flying *H. pratti*

As shown in Fig. 2A and H *H. pratti* decreased the source level by approximately 50 dB during the approaching flights in the silence control condition. A large drop in source level occurred in the last meter distance before landing (Fig. 2A–D). To determine whether *H. pratti* relies on the SNR of emitted calls or the SNR of echo returns to guide the signal adjustments in noise, we compared the NIVMs between three distance-windows in which the bats exhibited ELC of varying degrees: weak (Phase I), medium (Phase II), and strong (Phase III) (Fig. 4A). As a result, the SNRs of emitted vocalizations were highest in Phase I, but lowest in Phase III. By contrast, the SNRs of echo returns were probably similar across these three phases due to the ELC behavior. If bats rely on the SNR of emitted calls to adjust call amplitude, the increase in source level would be largest in Phase III and smallest in Phase I. If bats rely on the SNR of echo returns to adjust call amplitude, the increase in source level would be similar in all three phases.

Because the two loudspeakers were mounted close to the landing perch, the received noise levels by *H. pratti* were different across the three phases due to the transmission loss of sound energy in air. To account for the uneven noise levels received by *H. pratti* at different phases (target ranges), we built linear mixed models and predicted the changes in source level for noise levels of 40 dB, 60 dB and 80 dB SPL, relative to the control condition. We found that for each phase, *H. pratti* increased the amplitude of the emitted calls with increasing level of noise playback, exhibiting a clear Lombard effect. *H. pratti* increased the amplitude of emitted calls by

3.2 ± 0.2 dB, 3.0 ± 0.2 dB, and 3.8 ± 0.2 dB, per 10 dB increase in noise level from 40 dB SPL to 80 dB SPL for Phase I, Phase II, and Phase III, respectively. The increase in source level was similar at all three noise levels of 40 dB, 60 dB, and 80 dB SPL between Phase I and Phase II (Fig. 4B; Confidence interval comparisons, all $P > 0.05$). The increase in source level was even smaller at all three noise levels in Phase III than in Phase I and Phase II (Fig. 4B; Confidence interval comparisons, all $P < 0.05$). These results show that *H. pratti* appears to have relied on the SNR of echo returns (i.e., echo feedback), instead of the SNR of emitted vocalizations (i.e., vocal feedback), to adjust call amplitude.

To further test whether *H. pratti* also relies on echo feedback to adjust other vocal parameters in noise, we performed similar analyses on the duration of CF component, duration of the FM component, and FM bandwidth (Fig. 4C–H). Like source level, *H. pratti* produced calls of longer duration in both the CF and FM components, and of larger bandwidth, with an increase in noise level. The increase in the duration of CF component in the 60 dB and 80 dB noise conditions was smaller in Phase II and Phase III, than in Phase I (Fig. 4D; Confidence interval comparisons, $P < 0.05$). The increase in the duration of FM component in all three noise levels became progressively smaller from Phase I to Phase III (Fig. 4F; Confidence interval comparisons, $P < 0.05$). The increase in the bandwidth of FM component in the 60 dB and 80 dB noise conditions was greater in Phase II and Phase III, than in Phase I (Fig. 4H; Confidence interval comparisons, $P < 0.05$). The call duration data suggest that the bats did not rely on vocal feedback to guide call adjustments. Although the FM bandwidth data suggest some contribution of vocal feedback to the bat's adaptive vocal behavior, there were no statistical differences in the increase in FM bandwidth between Phase II and Phase III (Fig. 4H; Confidence interval comparisons, $P > 0.05$).

Lastly, we compared data from the 35–65 kHz and 10–45 kHz noise conditions to further assess the role of vocal feedback vs. echo feedback in mediating NIVMs. As shown in Fig. S1, the NIVMs in these two noise conditions were very similar to those in the 10–65 kHz noise conditions (Fig. 4). In the 10–45 kHz noise condition, the NIVMs were similar to other two types of noise, except for the CF duration (Fig. S2). The CF duration was longer across all noise conditions in the three approaching flight phases. Together, these data suggest that *H. pratti* makes signal adjustments in noise to maintain constant echo level during the landing task.

Discussion

Noise-induced vocal modifications (NIVMs) are exhibited by a wide range of animal taxa, including species that have evolved echolocation. Although the question whether

NIVMs are different between echolocating and non-echolocating species has been raised by several researchers (Tressler and Smotherman 2009; Hotchkin and Parks 2013), this question has until now remained unaddressed. In this study, we leveraged the ELC behavior of echolocating animals and showed that (1) environmental noise affects the ELC behavior of *H. pratti*; and (2) NIVMs in *H. pratti* are tied to echo feedback, not vocal feedback. These two lines of evidence suggest that NIVMs in echolocating animals and non-echolocating animals may rely on different mechanisms.

Environmental noise affects echo level compensation behavior

Some bats, and some toothed whales, exhibit range-dependent source level adjustments, whereby they decrease sonar signal level while closing in on a target (Kobler et al. 1985; Au and Benoit-Bird 2003; Nørum et al. 2012; Ladegaard et al. 2019). If echolocating animals do not reduce the source level when approaching a target, the received echo level would increase by more than 70 dB over a change in target range of 5 m (Nørum et al. 2012; Stidsholt et al. 2020). It is noteworthy that the numeric relationship between source level and target range varied from < 1 dB to about 30 dB per halving of target range in different studies (Stidsholt et al. 2020), which may be due to differences in species, tasks, or target range involved in analysis. Importantly, source level is not the only contributing factor, as features of the physical stimulus may not map directly to the perceived stimulus. Indeed, several studies reported range-dependent changes in echo detection and perceived loudness in bats performing behavioral tasks (Kick and Simmons 1984; Hartley 1992; Moss and Schnitzler 1995; Smotherman and Bakshi 2019).

In our study, we found consistent effects of environmental noise on the ELC behavior in *H. pratti*. Environmental noise not only delayed the onset of echo level compensation, but also flattened the ELC function. Both effects were more pronounced when bandpass noise spectra overlapped the second harmonic of the echolocation calls of *H. pratti*. Both effects were more pronounced at higher noise level conditions. Thus, the effects of environmental noise on ELC behavior can be attributed to the noise-induced increases in call amplitude, i.e., the Lombard effect. *H. pratti* showed greater increases in source levels at shorter ranges, when the received noise level was higher, due to distance-related attenuation of sound energy. This also explains the reduced dynamic range of the bat's call level during approaching flights. However, there were also differences between the effects of 10–65 kHz and 35–65 kHz noise broadcasts on the ELC behavior. These differences may be related to the difference in the spectral power density (dB/Hz) of the

effective frequency band of the noise. Since we fixed the total noise level, the spectral power density was lower in the 10 ~ 65 kHz than in the 35 ~ 65 kHz condition.

We found that the rate of change of ELC was range dependent. This result is in line with findings from another study that quantified ELC behavior of two Hipposiderid bat species (*H. armiger* and *H. pratti*), from onboard microphone recordings taken from bats approaching targets of variable sizes under quiet conditions (Stidsholt et al. 2020). Many previous studies on ELC behavior in echolocating bats have reported a relatively shallow slope of compensation change over distance, around -6 dB per halving target range. By contrast, we found in the silence (no noise control) condition that *H. pratti* decreased source level at a rate of approximately -22 dB per halving target range when within 1 m of the landing perch. At landing perch ranges of 1 ~ 3 m, the rate of ELC was only -8 dB per halving of distance. Together, these data suggest that the slopes of ELC functions have been underestimated in many studies that did not include source level data when bats were < 1 m from a landing perch or target.

It is worth noting that the present study did not account for the beam direction (aim) when estimating the source level of echolocation calls. Estimated source level of bat sonar calls can be strongly affected by the angle between the directed beam aim and the microphone, because sonar beams of echolocating bats are directional (Hartley and Suthers 1987, 1989; Ghose and Moss 2003; Surlykke et al. 2009; Kounitsky et al. 2015). During the final stage of landing (e.g., < 1 m within the target), *H. pratti* gradually raised the flight height by approximately half a meter (Fig. 1B). This behavior potentially caused an increase in the bat-to-microphone angle that can lead to an underestimation of call source level. Based on the spatial configuration of the landing perch and the central microphone that was used to estimate source level, the maximum difference in the bat-to-microphone angle between the long range (1 ~ 3 m) and short range (< 1 m) was about 25°. This estimated angle approximates the half amplitude directionality (-6 dB) of many bat sonar beams (Jakobsen et al. 2013), but is much smaller than the 50° ~ 70° horizontal beam directionality in a closely related Hipposiderid bat (*H. terasensis*) (Hiryu et al. 2006). Assuming a gradually (linearly) increased underestimation of source level from 0 to 6 dB during the last meter of landing, the slope of the ELC function would change from -22 dB to -18 dB per halving of target range, which is thus still much steeper than the -8 dB per halving of target range at distances of 1 ~ 3 m. Further study of the range dependence of echo level compensation may reveal the precise function of this adaptive behavior in echolocating animals.

Comparison of NIVMs across species

For animals that do not use echolocation, it is straightforward to infer that noise-induced changes in SNR directly mediate vocal modifications. By contrast, it is not clear whether NIVMs in echolocating animals is mediated by auditory responses to sonar vocalizations or echoes. To date, most studies on NIVMs in echolocating bats have been conducted on stationary animals that were not directly engaged in echolocation tasks. Past studies have documented clear NIVMs, from the Lombard effect to adjustments of signal duration, call repetition rate and other parameters. Studies of bat sonar call adjustments in noise have left open the question whether vocal feedback or echo feedback mediates NIVMs in animals tracking prey, discriminating target distance or landing (Tressler and Smotherman 2009; Luo et al. 2017, 2022; Luo and Moss 2017; Simmons 2017; Pedersen et al. 2022).

In the current study, we leveraged the ELC behavior of *H. pratti* during approaching flights and determined that echo feedback, not vocal feedback, mediates NIVMs. To our knowledge, this is the first evidence showing that NIVMs operate differently in animals that echolocate from those that do not echolocate. This also highlights the importance of the signals an animal listens to in guiding behavioral adjustments. More broadly, we propose that noise encountered by echolocating bats may be one of the driving forces of vocal flexibility in these animals. Yet, it is noteworthy that our data do not rule out the potential contribution of vocal feedback in mediating NIVMs in echolocating bats. As shown in Fig. S2, *H. pratti* also exhibited strong NIVMs when noise only masked the first harmonic of the echolocation calls. Inferred from neurophysiological data, Suga (2015) posited that the first harmonic of the CF-FM bat, *Pteronotus parnellii*, is used to register call onset, while the second and the higher harmonics are used to register echo properties. Lastly, our finding raises several open questions. For example, do echolocating bats always rely on echo feedback to adjust signals in noise? Are there tasks in which bats rely on vocal feedback to adjust call production in noise? By selectively perturbing vocal feedback and echo feedback, the distinct contributions of auditory feedback from transmitted and received signals can be determined (Luo and Moss 2017; Wang et al. 2022). Considering that echolocation is an active sensing system that always involves signal production and reception, both vocal feedback and echo feedback should be carefully analyzed for a deeper understanding of the mechanisms underlying biosonar.

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Authors contributions JL designed the study; ML and JL performed the study; JL and ML analyzed the data; JL and CFM wrote the paper.

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Declarations

Conflict of interest The authors declare no competing conflicts of interests.

Ethical approval Capture, housing, and behavioral studies were approved by the Institutional Animal Care and Use Committee of the Central China Normal University.

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