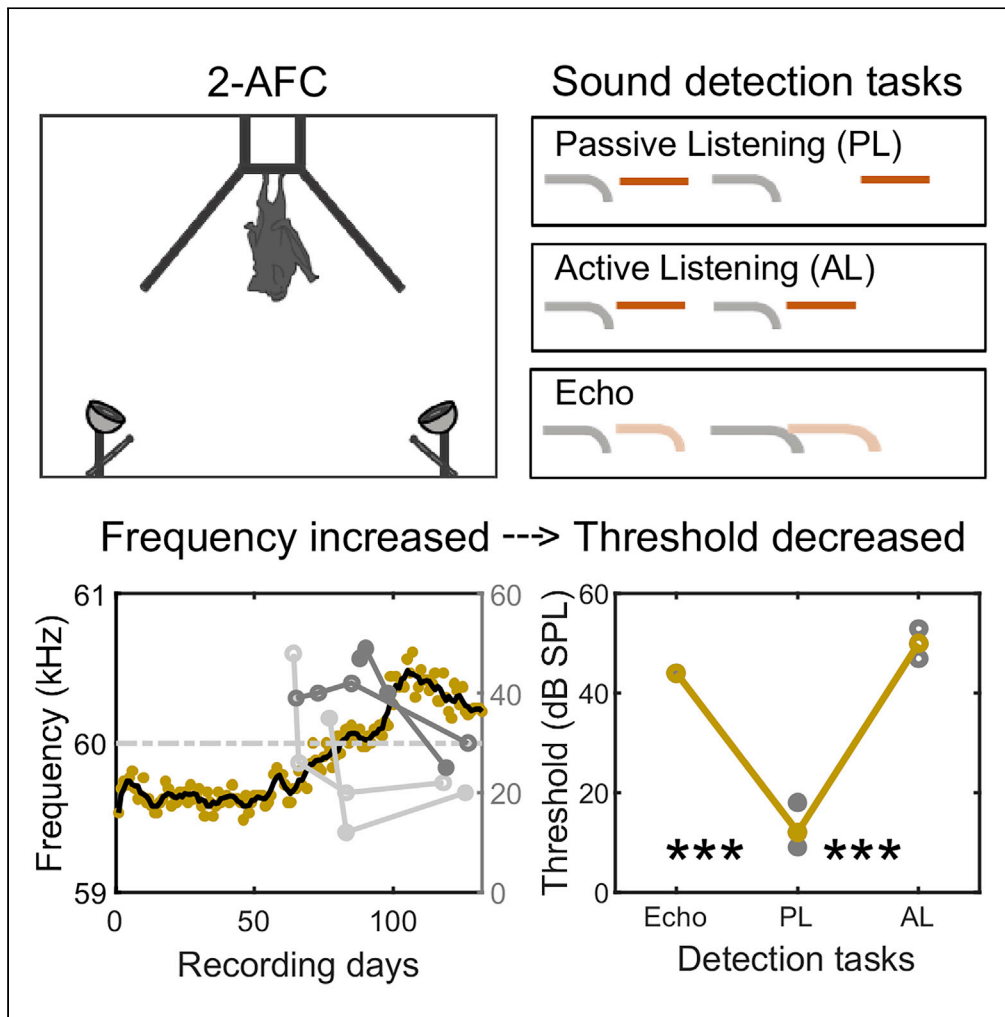


Article

Perceptual hearing sensitivity during vocal production



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Highlights

Vocal production strongly affects the perceptual hearing sensitivity of bats

Forward masking explains the reduced hearing sensitivity during vocalization

Long-term vocal plasticity enables bats to overcome self-generated auditory masking



Article

Perceptual hearing sensitivity during vocal production

Huan Ye¹ and Jinhong Luo^{1,2,*}

SUMMARY

Vocalization, such as speaking, inevitably generates sensory feedback that can cause self-generated masking. However, perceptual hearing sensitivity during vocal production is poorly understood. Using an adaptive psychophysical method, we measured the perceptual hearing sensitivity of an echolocating bat, *Hipposideros pratti*, in a passive listening (PL) task to detect pure tones, an active listening (AL) task to detect pure tones triggered by its vocalization, and a phantom echo task. We found that hanging *H. pratti* had the best hearing sensitivity of approximately 0 dB sound pressure level (SPL) in the PL task but much lower hearing sensitivity (nearly 40 dB worse) in the echo task. In the AL task, all bats gradually increased call frequency by 0.8–1.1 kHz, which improved their hearing sensitivity by 25–29 dB. This study underscores the need for studying the sensory capability of subjects engaged in active behaviors.

INTRODUCTION

Sensory processing in the brain rarely takes place without self-generated movements. For instance, eye and head movements frequently occur in visual processing,¹ palpation is inherently linked to tactile sensing,² and sniffing is a natural behavior of olfaction.³ One effect of self-generated movements concerns the compromised ability in processing sensory stimuli from external sources, i.e., causing self-generated masking.^{4–6} Self-generated masking can be extremely severe for active-sensing animals that rely on self-generated energy to probe the environment.

Echolocating bats use self-generated sounds for orientation and foraging, acting as a biological model for active sensing.^{7,8} Echolocating bats require high hearing sensitivity for analyzing echo returns merely milliseconds after call production.^{9–12} The auditory processing of echo returns in echolocating bats is thus vulnerable to self-generated masking from call production.¹¹ Considerable efforts have been made to understand the effects of self-generated auditory masking on subsequent echo processing in echolocating bats, using neurophysiological or psychophysical methods.^{11,13} Puzzling, these two methods mostly reached contradictory conclusions. While neurophysiological investigations suggested that vocal production minimally decreases, and may even increase, bats' hearing sensitivity for detecting echo returns,^{14–18} psychophysical data consistently revealed much worse perceptual hearing sensitivity in echo tasks that require active call production than in passive listening (PL) tasks routinely measured with pure tones.^{13,19,20}

It is noteworthy that caveats exist in the perceptual hearing sensitivity comparisons between the PL and echo tasks in echolocating bats. First, pure tones are distinct from echo signals in spectrotemporal properties, and typically the duration of pure tones used in PL tasks was many times longer than that of signals used in echo tasks. It is well known that the detection threshold decreases with increasing durations for brief signals due to the fundamental auditory process of temporal summation.^{21–23} Second, the detection threshold for the PL and echo tasks was typically measured with different experimental setups or paradigms by different researchers and were not from the same individual animals. It has been suggested that the configuration of the experimental setup can directly affect the detection threshold measurements of bats.^{13,24,25} Thus, it remains unclear whether and how vocal production affects the perceptual hearing sensitivity of echolocating bats.

RESULTS AND DISCUSSION

The Pratt's roundleaf bat, *Hipposideros pratti*, is an old-world bat that emits echolocation consisting of a relatively long constant frequency (CF) component, followed by a brief frequency-modulated (FM)

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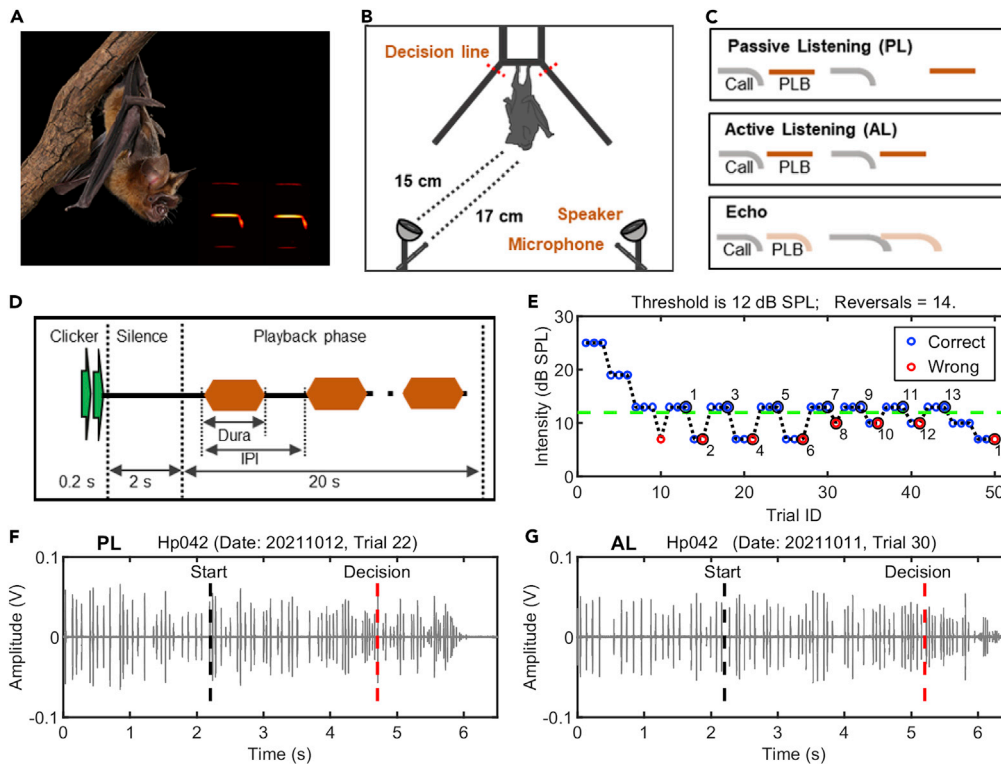


Figure 1. Measuring the Detection Threshold of *H. pratti* in Three Detection Tasks with a 2-AFC Paradigm

(A) An image of the *H. pratti* bat and the spectrogram of its typical echolocation calls.
 (B) The 2-AFC psychophysical paradigm. 2-AFC, two-alternative forced-choice.
 (C) Illustrations of three sound-detection tasks, including the passive listening (PL) task to detect a sequence of pure tones randomly (top panel), the active listening (AL) task to detect pure tones triggered by their vocalization at a fixed time delay (middle panel), and an echo task to detect virtual echoes at a fixed time delay (bottom panel). PLB, playback.
 (D) Illustration of the stimulus sequence that included a clicker sound (0.2 s), followed by 2 s of silence, and the target signals afterward in each trial. IPI, interpulse interval; Dura, duration.
 (E) An example of the detection threshold measurement with the transformed up-down method and the midrun criterion. SPL, sound pressure level.
 (F and G) Two examples of call recordings of an *H. pratti* bat in a PL trial and an AL trial.

component (Figure 1A). *H. pratti* live in large colonies, with hundreds of individuals sometimes observed to roost in the same cave. These traits make *H. pratti* a suitable animal model to understand the effects of, and countermeasures against, self-generated auditory masking. In this study, we measured the hearing sensitivity of hanging *H. pratti* in three sound-detection tasks, using a two-alternative forced-choice psychophysical paradigm (Figures 1A–1C). Hearing sensitivity was measured for the same individual in the PL, active listening (AL), and echo tasks using the same experimental setup. Each bat was first trained for the PL task, then the AL task, and lastly the echo task (Figure 1C; Video S1). The signals to be detected by the bats, herein referred to as target signals, were pure tones of the same frequency and duration in the PL and AL tasks. The only difference between these two tasks was the temporal relationship between target signals and emitted calls. While pure tone broadcasts were not related to the emitted calls in the PL task (Figure 1C, top panel), they were synchronized to the emitted calls at a fixed time delay in the AL task (Figure 1C, middle panel). In the echo task, digitally generated virtual echoes from the emitted calls were returned to the bat at a fixed time delay (Figure 1C, bottom panel). In all tasks, a trial started with the broadcast of a clicker sound (0.2 s), followed by 2 s of silence, and then by target signals (Figure 1D). In both PL and AL tasks, the intensity of target signals was automatically adjusted by the program based on the performance of the bat in previous trials (Figure 1E), following a three-down, one-up algorithm (see STAR methods). This transformed up-down method estimates the detection threshold of 79.4% performance rate.²⁶ In the echo task, the intensity of the echoes was under the bat's active control. We predetermined the attenuation factor (gain) of the echoes relative to the emitted calls on each investigation day and

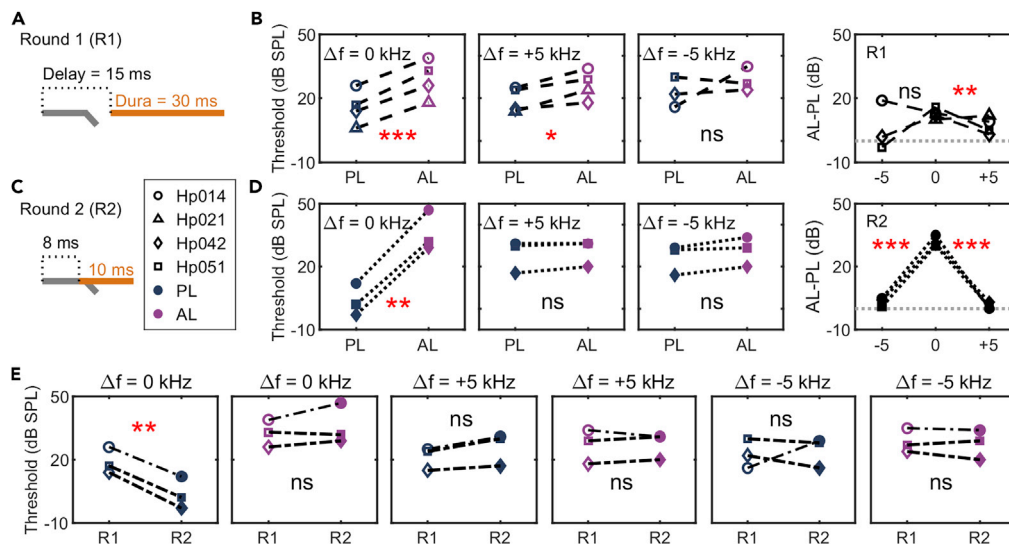


Figure 2. Forward Masking Explains Differences in Hearing Sensitivity of *H. pratti* Between the PL and AL Tasks

(A and C) Schematic illustrations of the parameter relationship between the target signals and emitted calls for the first round (A) and second round (C) of tests.

(B and D) Comparisons of the detection thresholds of individual bats between the PL and AL tasks for the 0-kHz (frequency-matched), +5-kHz, and -5-kHz conditions for the first-round (B) and second-round (D) tests.

(E) Comparisons of the absolute detection thresholds of the bats between the round 1 and round 2 tests.

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$.

calculated the actual intensity of the echoes returned to the bat and the corresponding performance rate offline. From the performance rates of 6 days that surrounded the 79.4% target success rate, the detection threshold in the echo task was estimated for each bat. In total, three bats learned to conduct all three detection tasks. A fourth bat participated in four days of the data collection in the PL and AL tasks before dropping out due to an accidental arm injury. The vocalizations of the bat in each trial were recorded (Figures 1F and 1G). Note, *H. pratti* consistently emitted echolocation calls in all three detection tasks, although there is no need for them to vocalize in the PL task.

Forward Masking Explains the Reduced Hearing Sensitivity of *H. pratti* in the AL Task

In the first round of hearing sensitivity measurements, we first matched the frequency of the target signals to each bat's peak call frequency (Figure 2A). The duration of the target signals for both tasks was 30 ms. For the AL task, the time delay of the target signals relative to the onset of emitted calls was 15 ms. We found that the detection thresholds of *H. pratti* were on average 13.3 ± 1.9 dB higher (from 12 to 16 dB in four bats) in the AL task than those in the PL task (Figure 2B; paired t-test, $p < 0.001$).

Considering that the AL task differed from the PL task only by the temporal relationship between the target signals and the emitted calls and bats only made minor adjustments to call rate, call amplitude, and call duration between the PL and AL tasks (Figure S1), we hypothesized that the reduced hearing sensitivity of *H. pratti* in the AL task is due to the forward masking effect from self-generated calls.^{19,27} To test the forward masking hypothesis, we measured bats' detection thresholds with target signals of frequencies that were either 5 kHz above or 5 kHz below the emitted call frequency. If the reduced hearing sensitivity of *H. pratti* in the AL task was due to auditory masking from self-generated calls, hearing sensitivity in the ± 5 -kHz conditions would be markedly improved compared to the 0-kHz condition because the effect of auditory masking decreases with increasing frequency differences between the masker and the signal.^{22,28,29} Our data largely support the auditory masking hypothesis (Figure 2B). Specifically, the threshold difference between the AL and PL tasks significantly decreased from 13.3 ± 1.9 dB in the frequency-matched condition to 6.5 ± 3.1 dB in the +5-kHz condition (Figure 2B; Paired t-test, $p < 0.01$). In the -5-kHz condition, the threshold difference between the AL and PL tasks decreased in two bats, but not in the third bat, revealing no overall statistical difference (Figure 2B; Paired t-test, $p > 0.05$).

To further test the forward masking hypothesis, we conducted a second round of hearing sensitivity measurements with target signals of reduced-duration (from 30 ms to 10 ms) broadcast at a reduced time delay (from 15 ms to 8 ms) (Figure 2C). As shown in Figure 2D, the data support the auditory forward masking hypothesis. The threshold difference between the AL and PL tasks in the frequency-matched condition averaged 32.3 ± 2.5 dB but decreased to 1.3 ± 1.5 dB in the +5-kHz condition and increased to 3.3 ± 2.1 dB in the -5-kHz condition (Figure 2D; Paired t-test, both $p < 0.001$).

However, there are two puzzling observations when comparing the absolute detection thresholds of *H. pratti* between the two rounds of measurements. First, in the PL task, *H. pratti* had on average 15.3 ± 1.5 dB lower detection threshold when detecting target signals of 10-ms duration than when detecting target signals of 30-ms duration (Figure 2E, first panel to the left; Paired t-test, $p < 0.01$). This result is unexpected, as the detection threshold typically decreases with increasing signal duration due to temporal summation.^{21–23} Nonetheless, an absolute hearing sensitivity of approximately 0 dB sound pressure level (SPL) in *H. pratti* is comparable to that of other echolocating bat species.¹³ Before the current study, absolute hearing sensitivity information was available only for one CF-FM bat, *Rhinolophus ferrumequinum*, which was measured with classical conditioning of the heart rate response.³⁰ Second, in the AL task, we did not find a higher detection threshold when bats listened to target signals broadcast at a shorter time delay (Figure 2E, second panel to the left; Paired t-test, $p = 0.329$). This result is equally unexpected in light of the auditory masking hypothesis that predicts a stronger masking effect for a shorter time interval between the masker and the signal.^{18,27,29,31,32} By contrast, detection thresholds did not differ between the two rounds of measurements for either the +5-kHz or the -5-kHz condition in either task (Figure 2E, third to sixth panels from the left; Paired t-test, all $p > 0.05$). Altogether, it seems that the *H. pratti* has improved its hearing sensitivity over time in the frequency-matched condition for both tasks.

Long-Term Vocal Plasticity Enables Adult Bats to Overcome Self-generated Auditory Masking

We continued to measure the detection thresholds of *H. pratti* in the frequency-matched condition again in both the PL and AL tasks for more days. For each bat, we plotted the threshold estimates in the frequency-matched condition across time (Figure 3A). We found that, for each bat and task, there is a clear trend of improved hearing sensitivity over a testing period of two to three months (dates for the first and last measurements are indicated at the top of Figure 3A). The overall effect of days of testing on hearing sensitivity was significant for both tasks (partial correlation; AL task, $r = -0.6$, $p < 0.001$; PL task, $r = -0.5$, $p < 0.05$). Compared to the first day of threshold measurement, the three *H. pratti* improved their hearing sensitivity by 25, 29, and 25 dB in the AL task and by 36, 22, and 15 dB in the PL task over two to three months. The hearing sensitivity improvement was similar between the AL and PL tasks (Paired t-test, $p = 0.789$).

In theory, echolocating bats can reduce auditory masking from self-generated calls by adjusting several aspects of their vocal behavior (Figure 3B). They can shift the spectral energy of emitted calls away from the target signal to enlarge the frequency difference, and they can decrease the duration and the intensity of emitted calls.^{22,33} For the PL task, bats can additionally reduce auditory forward masking by calling less frequently. Next, we examined these possibilities with statistical models, in which the effects of one factor can be assessed while controlling for the effects of other factors.

The optimized statistical models (generalized linear model [GLM]) indicated that, among the four candidate strategies of vocal adjustments, shifting the spectral energy of emitted calls away from the target signals (i.e., enlarging frequency difference) was effective in improving hearing sensitivity in both the AL and PL tasks (Figure 3C; adjusted *R*-Square were 0.7 and 0.6; estimated coefficients were -20.5 and -14.9 per unit frequency difference in kHz, $p < 0.01$ and $p < 0.05$, respectively), while call duration, call amplitude, and interpulse interval (call rate) did not explain the absolute hearing sensitivity (Figure S2; GLMs, all $p > 0.05$). Similarly, frequency difference accounted for the relative detection thresholds (Figure 3D; adjusted *R*-Square were 0.4 and 0.2; estimated coefficients were -20.5 and -14.9 per unit frequency difference in kHz, $p < 0.01$ and $p < 0.05$). These data show that bats can better detect faint sounds after call production if the emitted calls are higher in frequency than the target signals.

Call frequency of some CF-FM bats, such as *R. ferrumequinum* and *Hipposideros terasensis*, can vary considerably across days, weeks, or years.^{34,35} To further clarify that *H. pratti* are indeed actively shifting their call frequency upward to reduce the effects of forward masking on detecting target signals, rather

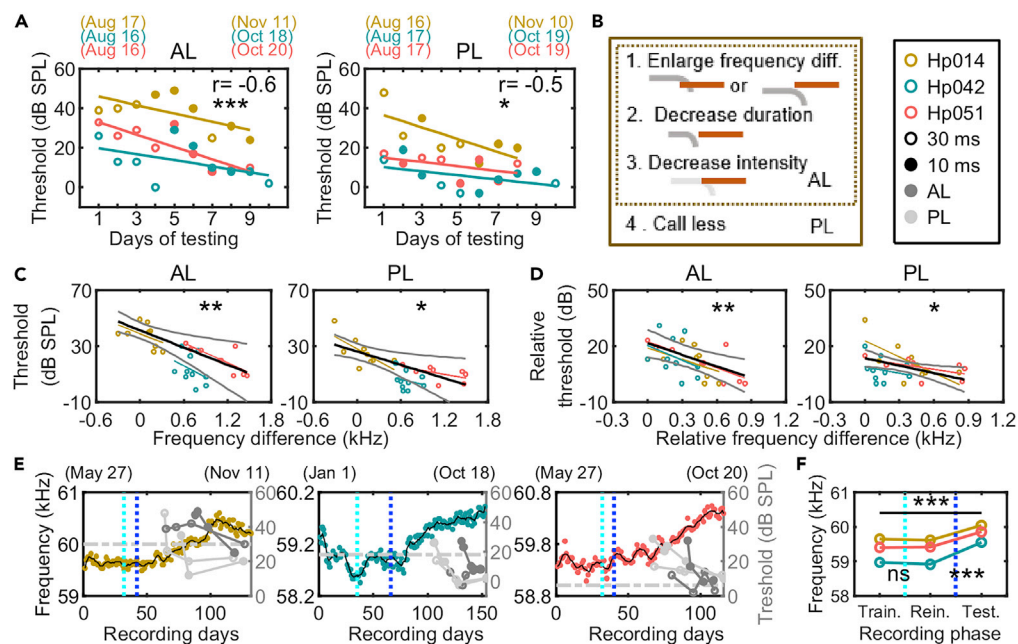


Figure 3. Long-Term Vocal Plasticity Enables Adult Bats to Overcome Self-generated Auditory Masking

(A) Changes of thresholds with the days of testing in the AL and PL tasks. The dates for the first and last measurements are shown at the top for each bat (color-coded)

(B) Illustration of four forms of vocal adjustments that can reduce auditory forward masking from self-generated calls, including enlarging the frequency difference between emitted calls and target signals, decreasing the duration or the intensity of emitted calls, and calling less frequently for the PL task.

(C and D) The relationship between the frequency difference (between target signals and emitted calls) and detection thresholds—(C) for absolute threshold and (D) for relative threshold—and in the AL and PL tasks. Lines in black (mean) and gray (95% confidence interval) are the predicted results from the generalized linear model.

(E) Daily median call frequency (left y-axis) and the threshold (right y-axis) of individual bats across recording days. The dashed lines in cyan and blue divided the recording days into three phases: the training phase, the reinforcement phase, and the testing phase. The black line in each panel shows the smoothed (3-point average) call frequency. The dates for the first and last measurements are shown at the top.

(F) Pairwise comparison of call frequency between the three phases of experiments. Train., the training phase; Rein., the reinforcement phase; Test., the testing phase. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$.

than a result of natural call frequency fluctuations, we plotted the median call frequency as a function of the date of recording for each bat (Figure 3E). These call frequency data spanned a period of five to six months and included recordings from the training phase, the reinforcement phase when bats learned the task but before detection thresholds were measured, and the testing phase when detection thresholds were measured. We found that a systematic increase in call frequency occurred only after the start of the testing phase (Figure 3F). The maximum frequency increases since the start of the testing phase were 0.8, 1.0, and 1.1 kHz for the three bats. Data from the fourth bat, whose detection thresholds were measured only at the beginning of the testing phase before dropping out, did not show an increase in call frequency in the end (Figure S3). When similar analyses were conducted for signal duration, signal amplitude, and call rate, we did not find evidence for task-driven vocal plasticity (Figure S4).

Actively shifting call frequency above, instead of below, the target signals is probably more effective in reducing forward masking. On the one hand, given the same amount of frequency separation between the masker and the signal, masking is more effective when the frequency of the masker is below the signal, a phenomenon typically referred to as the upward spread of masking.²² On the other hand, it may be due to the asymmetric sensitivity of the hearing of *H. pratti* for detecting sound signals above and below their dominant CF. Audiograms from other CF-FM bats, including *Hipposideros speoris* and *Hipposideros bicolor*,³⁶ and *R. ferrumequinum*³⁰ all show that these bats are less sensitive to sound frequencies above their dominant CF than to sound frequencies below it.

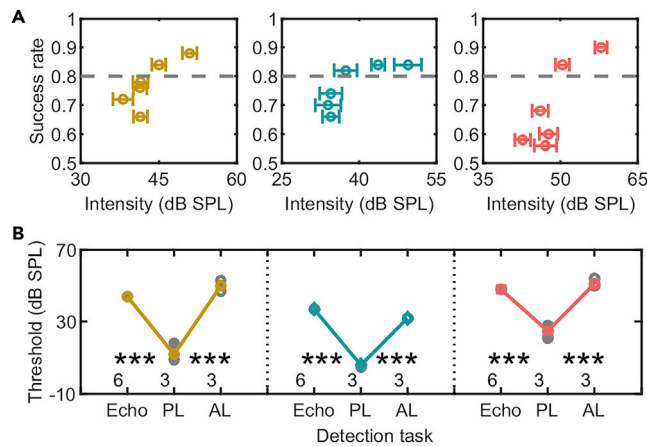


Figure 4. Reduced Hearing Sensitivity of *H. pratti* in an Echo Task

(A) Estimation of the detection thresholds of individual bats in the echo task from six success rates surrounding the 80% performance. Echo amplitude was shown as mean and SD (error bar).

(B) Comparisons of the absolute detection thresholds of individual bats between the echo, PL, and AL tasks. Numbers at the bottom indicate the times of repeated estimates. *** $p < 0.001$.

It is noteworthy that the process of increasing the call frequency is slow for *H. pratti*. It took 26, 26, and 40 days for the three bats to increase the median call frequency by 0.2 kHz, which is approximately the standard variation of the call frequency of *H. pratti* within a test session. Echolocating bats are renowned for their short-term vocal plasticity. They not only dynamically adjust the structure of echolocation signals to ongoing navigation and foraging tasks^{37–39} but also rapidly modify signal features to cope with interfering sounds.^{40–44} By contrast, little is known regarding the ability to adaptively adjust signal parameters at a longer timescale, which is relevant to long-term learning.^{45,46} We suggest that the slow vocal adaptation to auditory masking in *H. pratti* may offer an opportunity for future studies to address exciting topics such as vocal learning.

Call Production Reduces the Hearing Sensitivity of *H. pratti* for an Echo Task

Lastly, we address whether forward masking from self-generated calls revealed in the AL task affects *H. pratti* in detecting echo returns. As shown in Figure 1A, the natural echolocation signals of *H. pratti* contain both CF and FM components, which is different from the pure tones used in the AL task. Moreover, adjusting the emitted calls by bats would have a direct effect on echo returns in a natural echolocation task, which is lacking in the AL task. To gain insights, we trained all three bats for a third task to detect virtual echoes at a time delay of 15 ms, which would be returned by an object from a distance of 2.6 m. After individual bats have reached an 85% performance rate, we measured their detection thresholds corresponding to an 80% overall success rate (Figure 4A). We found that the detection thresholds of the bats in the echo task were similar to those in the AL task (Figure 4B; independent t-test, $p > 0.05$) but significantly higher than those in the PL task (Figure 4B; independent t-test, $p < 0.001$). These data indicate that hanging (perching) *H. pratti* indeed face a similar degree of auditory masking from self-generated calls during echolocation tasks.

Our data indicate that the hearing sensitivity of *H. pratti* can be seriously compromised by forward masking for a prolonged time window beyond 15 ms. These data are consistent with neuronal data showing that in both *H. pratti* and *Hipposideros armiger*, the recovery cycle of many auditory neurons in the auditory midbrain inferior colliculus is longer than 20 ms when assessed with simulated pulse-echo pairs of the same frequency.^{29,47} By contrast, both neural and psychophysical data showed that the effects of forward masking on the hearing sensitivity of FM bats are typically limited to a time window of a few milliseconds after call emission.^{19,20,24,27} The absolute detection thresholds for detecting echoes at a 15-ms delay, corresponding to a target range of approximately 2.6 m, were between 37 dB SPL and 48 dB SPL in the three tested *H. pratti*. Whether this degree of hearing sensitivity is sufficient for hanging *H. pratti* to detect small targets, whether hearing sensitivity in hanging *H. pratti* is dramatically improved when detecting fluttering insects, and whether hearing sensitivity is completely different in flying *H. pratti* are important questions for future research. For the latter two behavioral contexts, it is likely that hearing sensitivity for the echo detections in *H. pratti*, as well as in other CF-FM bats, will be significantly better.

Conclusion

This study shows that perceptual hearing sensitivity measured from conventional PL paradigms can be substantially off from that during vocalization. Nevertheless, *H. pratti* managed to find a way to improve their hearing sensitivity in the AL task using the ability of long-term vocal plasticity. Long-term vocal plasticity is the core element for vocal production learning, a rare trait in mammals. Considering the natural coupling of sensory processing and motor control, this study underscores the need for understanding the sensory capability of animals, as well as humans, engaged in active behaviors.

Limitations of the Study

One key question that cannot be addressed by the current study is whether the hearing sensitivity of hanging *H. pratti* measured in the echo task reflects the hearing sensitivity during active prey detection. Similar to other CF-FM bats, *H. pratti* are potentially more sensitive at detecting fluttering insects. Moreover, CF-FM bats lower their emitted call frequency while approaching targets, exhibiting the Doppler-shift compensation, which creates a frequency difference between the emitted calls and echo returns. It is conceivable that CF-FM bats may have a greater sensitivity of hearing for echo returns during flight. Lastly, due to the limited number of tested animals involved in this study and their unknown ages (bats were wild-caught), we were not able to examine the effects of sex or age on hearing sensitivity.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.105435>.

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AUTHOR CONTRIBUTIONS

J.L. and H.Y. designed the study. H.Y. collected the data. H.Y. and J.L. analyzed the data. J.L. and H.Y. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
Pratt's roundleaf bat <i>Hipposideros pratti</i>	A cave in Xianning County, Hubei Province, China	N/A
Software and algorithms		
MATLAB R2019b	MathWorks	RRID: SCR_001622
Custom-written setup app	This paper	https://doi.org/10.17632/c8x3wxz97c.1
SoundMexPro toolbox	Hörzentrum Oldenburg	https://soundmexpro.hz-ol.de/index.php?page=3
Other		
2-AFC setup	This paper	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Jinhong Luo (jluo@ccnu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The datasets supporting the current study have not been deposited in a public repository because of their large size, but are available from the corresponding author on request.
- The original code has been deposited at Mendeley and is publicly available as of the date of publication. DOIs are listed in the [key resources table](#).
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Four adult *H. pratti* (3 males and 1 female) were trained to perform signal detection tasks in a two-alternative forced-choice (2-AFC) setup. All bats were wild-caught with a hand net during the daytime at a Cave in Xianning County, Hubei Province, China, in August 2020. Bats were housed in social groups of two to four, in custom-made cages (40 × 40 × 40 cm), placed in a room with a regulated air temperature of 24 ± 2°C (mean ± SD), relative humidity 71 ± 5% (mean ± SD), and a reversed light regime of 12 h darkness and 12 h light. Bats had free access to water in roost cages. Bats participated in the experiment typically 6 days a week, during which they received a piece of mealworm (larvae of *Tenebrio Molitor*) as a reward for each correct trial on the platform. When no experiment was conducted, bats received a reduced amount of food in the roost cage. The body masses of the bats were carefully maintained between 45 and 60 g throughout the experiments. As the bats were wild-caught, we cannot determine their specific age except to confirm that they were adult. Capture, housing, and behavioral studies were approved by the Institutional Animal Care and Use Committee of the Central China Normal University.

METHOD DETAILS

Behavioral setup

The 2-AFC psychophysical setup for measuring the hearing threshold of *H. pratti* was configured as an isosceles trapezoid without the lower base side (Figure 1B). The upper base side was 10 cm long and served as the starting position for *H. pratti* in each trial. The two arms were 30 cm long and were separated by

approximately 45° when viewed from the bat's position. A 1-inch sized electrostatic loudspeaker (ES1, TDT, USA) and a 1/4-inch sized measurement microphone (7016, ACO, USA) were mounted at the end of each arm of the setup and were about 15 and 17 cm away from the head of the freely hanging bat. The setup was hung from the ceiling of a test room (5 × 4 × 2.2 m, length × width × height) and was at approximately 1.8 m height above the floor. The walls and ceiling of the test room were covered by acoustic foam, and the floor was covered by nylon blankets, to reduce echo interference. Similarly, the setup was wrapped with 1 mm thick velvet felt to reduce the strength of echoes received by the bat from the setup.

During the test, the bat was physically separated from the experimenter by a black curtain hanging from the ceiling. The experimenter monitored and rated the online performance of the bat with the aid of a small IR camera (Mini, Batian'an, China) mounted from the ceiling. For most trials, we videotaped the performance of the bat during the experiment with a second IR camera (FDR-AX700, SONY, Japan) at a sampling rate of 25 Hz, aided by an external IR light source (3022HW, Maixiu, China). The second camera and the IR light source were both mounted on a tripod standing on the floor and were located approximately 3 m away from the bat.

Sound recording and playback

In all experimental trials, we recorded the echolocation calls of *H. pratti* using a multiple-channel audio interface with four channels of microphone pre-amplifiers (Fireface 802, RME, Germany). Echolocation signals were picked up by both microphones, amplified by approximately 40 dB, and digitized at a sampling rate of 192 kHz before saving into the hard drive of the desk computer. Sound stimuli were generated online, converted into analog signals with the same audio interface, amplified by a two-channel power amplifier (ED1, TDT, USA), and broadcast via two ultrasonic electrostatic speakers (ES1, TDT, USA). We compensated for the uneven frequency response of the playback system by adjusting the digital voltage of the output signal. The frequency response of the playback system (Figure S5) was measured with a 1/4-inch free-field calibration microphone (46BF-1, GRAS, Denmark) at a 15 cm distance (the same bat-speaker distance) with pure tones between 5 and 90 kHz, with a frequency resolution of 100 Hz for 55 to 65 kHz frequency range and a frequency resolution of 1 kHz for other frequency ranges. The sensitivity of the calibration microphone system was measured by a sound calibrator that outputs a 1 kHz tone of 94 dB SPL level (Type 521, ACO, USA). Sound recording and stimulus presentation were both achieved with a custom-written app, using the SoundMexPro toolbox (Hörzentrum Oldenburg, Germany) in MATLAB (R2019b, MathWorks, USA). A screenshot of the app is shown in Figure S6.

Sound detection tasks

Hearing sensitivity, or detection threshold, measured as the lowest intensity of the target signals which results in an 80% performance rate in the bat, was estimated for the same individual of *H. pratti* ($n = 3$) participating in three sound detection tasks, i.e., the PL, AL, and Echo tasks, using a 2-AFC psychophysical paradigm (Figure 1C). A fourth *H. pratti* learned the PL and AL task, and contributed to the data, before being excluded from further tests due to accidental arm injury. For all three sound detection tasks, sound stimuli were broadcast from one of the two loudspeakers that were selected according to a predetermined pseudo-random schedule.⁴⁸ The hanging bat was required to move toward the arm with the sound stimuli to indicate its decision. Each correct response was rewarded with a piece of mealworm offered in forceps and each incorrect response was followed by a brief timeout. The stimulus sequence of a trial was shown in Figure 1D, including a 0.2 s clicker sound to get the attention of the bat and a 2 s silence period, and the target stimuli afterward. If the bat did not make indicate its decision after 20 s, the trial was terminated and the bat's response was rated as incorrect.

Because the PL and AL tasks were designed to quantify the potential influence of vocal production on hearing sensitivity, auditory stimuli of the same signal frequency and duration were used for both tasks. The only difference between the PL and AL tasks was the timing relationship between the acoustic stimuli and the emitted calls of the bat. In the PL task, the acoustic stimuli of the same intensity were randomly broadcast to the bat until the trial ended, whereas in the AL task the acoustic stimuli were broadcast to the bat at a fixed time delay relative to the onset of each emitted call. Pure tones, with 10% ramping at both the start and the end were the acoustic stimuli. Initially, we measured the detection threshold of *H. pratti* using 30 ms pure tones for both tasks and the time delay was set to 15 ms in the AL task (Round 1, Figure 2A). Then, we measured bats' detection threshold with 10 ms pure tones and the time delay was reduced to 8 ms in the AL task (Round 2, Figure 2C). For the short delay (8 ms) condition, the calls and the playbacks typically

overlapped in time. However, there were typically some frequency differences between the calls and the stimuli, due to the natural variations of the calls from one to another and the bats also increased their call frequency slowly to go beyond the stimuli (as shown in Figure 3E). Thus, amplitude modulations (or spectral modulations) by overlapping two signals rarely occurred. For the PL task, the average interval between consecutive pure tones was 50 ± 10 ms (mean \pm SD). Previous studies on bats' perceptual hearing sensitivity typically used stimuli longer than 50 ms.^{30,49} Following this tradition, we used stimuli of 30 ms duration for the first round of tests. Considering the inter-call-interval (~ 50 ms) and call duration (10–15 ms) of *H. pratti*, and the input-output delay of the system and atmospheric transmitting (~ 6 ms), 30 ms stimulus duration is the maximum that we can choose to avoid temporal overlapping between the stimuli and the subsequent call. For both tasks, the frequency of the pure tones was firstly matched to the population median of the dominant CF of the echolocation signals of each *H. pratti* from all training days before the start of data collection. For the four individuals, the measured median peak call frequency was 59.6, 59.5, 58.8, and 59.4 kHz, and the frequency of the target signals was set to 60, 60, 59, and 59 kHz.

For the Echo task, virtual echoes were broadcast to the bat at a time delay of 15 ms relative to the emitted calls of the bat, which correspond to a target range of 2.6 m for a real target. Virtual echoes were created electronically online from a copy of the bandpass-filtered (cutoff frequencies: 40 and 70 kHz) echolocation calls emitted by the bat. We additionally measured the detection thresholds of bats in the PL and AL tasks to compare the hearing sensitivity across the three tasks. In these PL and AL tasks, the call frequency of the target signals was once again from the five habituation trials of the same date during the threshold measurement and the signal duration was matched to the median CF duration of the calls to a precision of 0.1 ms. The frequency-match precision was 50 Hz.

Animal training

Four *H. pratti* were first trained for the PL task and then the AL task. Three of the four *H. pratti* were subsequently trained for the Echo task. For the PL task, bats were first trained to hang at the start position without flying off and then were trained to move on the two arms of the platform. Then we introduced the training stimuli, i.e., high-intensity pure tone stimuli of ~ 80 dB SPL, from one of the two loudspeakers, and the bat was required to associate the stimuli with a food reward. To control for the possibility that training stimuli may affect subsequent hearing sensitivity estimation, different individuals were assigned to one of the four training stimulus frequencies of 15, 35, 55, and 75 kHz. The duration of the training tones was 400 ms. When the success rates were above 85% for three consecutive days, the bat was considered to have learned the PL task. Then, we started the reinforcement phase in which we gradually shortened the signal duration to 10 ms, adjusted the frequency of training pure tones in small steps until converging to the target signals, and reduced signal intensity to get an initial understanding of the rough detection threshold of the bats. During the last few days of the reinforcement phase, we introduced the adaptive Up-Down paradigm to the bats. We started to train the bat for the AL task until the bat achieved a success rate above 85% for three consecutive days. Bats were trained for the Echo task after completing the PL and AL tasks.

Detection threshold measurement

To estimate the detection threshold of *H. pratti* in the PL and AL task, we adopted the transformed Up-Down method,²⁶ in which the signal intensity was decreased by a step size after three consecutive correct trials and increased by a step size after an incorrect trial (Figure 1E). The estimated detection threshold by the three-down, one-up algorithm corresponds to a 79.4% performance.²⁶ For each testing day, 50 trials were run for each bat, which typically took about 1 h. The step size was 6 dB for the first 30 trials and 3 dB for the remaining 20 trials. As suggested by Levitt (1971), the precision of threshold estimation may be increased by systematically decreasing step size during the test. Before starting the 50 trials test, 6 habituation trials were run to check the motivational status of the bat for each testing day, in which the intensity of the target signals was set well above the threshold. Following previous studies,^{50–52} we estimated the detection threshold of the bat based on the mid-run estimates from the performance data. One example of the estimated detection threshold is shown in Figure 1E. The reversals (filled with black circles) were defined as the trial of intensity at peaks and troughs. Following recommendations,⁵³ we excluded the first reversal and took the median of all reversals as the detection threshold. Further, we compared the performance curves of the same bat between the PL and AL tasks and did find a quality difference in the threshold estimates (Figure S7), ruling out the possibility that differences in the detection threshold of the bats were caused by a methodological bias.

In the Echo task, the amplitude of the echoes was related linearly to the amplitude of emitted calls and controlled by the bat directly, and thus is different from the PL and AL tasks in which the absolute amplitude of the playback stimuli can be specified. To estimate the detection threshold of *H. pratti* in the Echo task, we adjusted the gain of the playback system on different test days and estimated the average amplitude of the played echoes when the bat showed an overall success rate of 80% (Figure 4A). Specifically, we collected success rates of each bat on 6 consecutive days with some success rates above and others below 80%, from which the average echo amplitude corresponding to 80% success rate was taken as the echo detection threshold.

To minimize the possible effects of factors other than the task on detection threshold estimation, we collected the detection threshold data in a pseudo-random and matched manner. Whenever the detection thresholds were to be compared between groups, we estimated these detection thresholds on successive days. The detection threshold of which group to be estimated first was pseudo-randomly determined and differed across individual bats.

QUANTIFICATION AND STATISTICAL ANALYSIS

Call analysis

Echolocation calls were batch-processed with custom-written MATLAB scripts (R2019b, Mathworks, USA), which have been described in a previous study.⁵⁴ For each recording file, candidate calls were detected (with an amplitude threshold) and cut out, and the call of a higher peak amplitude from the two recording channels was used for further spectro-temporal analyses. In this study, we estimated the call frequency as the frequency point of the maximum spectral energy from the second harmonic, with an FFT size of 8192 which resulted in a frequency resolution of 30 Hz. Call amplitude referred to the peak amplitude of the calls. Call duration was the time difference between the onset and offset of the calls, which were the time points when the call samples of 30 dB lower amplitude relative to the peak. Inter-pulse-interval (IPI) was the time difference between the onsets of two neighboring calls. Quality of sound analysis was manually checked for randomly selected recordings as a routine by displaying the waveform, power spectrum, and spectrogram graphically (Figure S8). Intensive manual checking confirmed the quality of sound analysis.

Statistics

All statistical analyses were performed with the Statistical and Machine Learning Toolbox of MATLAB (R2019b, Mathworks, USA). To compare the detection threshold of the bats between conditions, we used Paired t-test ('ttest' function). To examine the relationship between call parameters and detection threshold, we built linear models ('fitlm' function), with the frequency difference between the calls and playback stimuli, call duration, call amplitude, IPI, and bat identity (categorical) as predictor variables and detection threshold as the response variable. We started with a full model including all the fixed effects of the predictor variables and performed automatic model selection based on the statistical significance of each predictor variable ('step' function). The final model was in the form of 'threshold ~1 + batid + frequency + duration + IPI'. Detailed information on the models is shown in Figure S9. The significance levels were set at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, $p > 0.05$.