

# Potential effects of anthropogenic noise on echolocation behavior in horseshoe bats

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**Abbreviations:** BFN, band pass-filtered noise; BFN20, band pass-filtered noise centered at 20 kHz and ranging from 10 to 30 kHz; CF, constant frequency; RF, resting frequency

We previously reported that band-pass filtered noise (BFN, bandwidth 20 kHz) affected the echolocation behavior of horseshoe bats in different ways depending on which frequencies within the bats' hearing range BFN was centered. We found that call amplitudes only increased when BFN was centered on the dominant frequency of the bats' calls. In contrast, call frequencies were shifted for all BFN stimuli centered on or below the dominant frequency of echolocation calls including when BFN was centered at 20 kHz (BFN20), which is far below the range of the bats' echolocation call frequencies. In the present study we focused on masking effects of BFN20 and used it as a model stimulus for anthropogenic noise to investigate how this noise affected call parameters of echolocation calls, such as their frequency, amplitude, duration and rate. We show that only call frequencies shifted in response to masking BFN20, whereas all other call parameters tested exhibited no noise-related changes. Our findings provide an empirical basis to quantitatively predict the impact of anthropogenic noise on echolocation behavior in bats. They also emphasize the need for a better understanding of the impact of anthropogenic noise on bioacoustic communication and orientation systems in general.

Animal communication signals face the challenge of being masked by ambient noise. In acoustic communication, several mechanisms evolved in numerous vertebrate species to overcome masking noise, such as increasing call amplitude,<sup>1-8</sup> frequency,<sup>4,5,9</sup> duration<sup>4,6</sup> or rate.<sup>10,11</sup> One source for environmental noise that severely affects avian song behavior is anthropogenic noise.<sup>12</sup> Several bird species sing their songs louder,<sup>13,14</sup> at a higher pitch<sup>14-16</sup> and less complex<sup>16</sup> when they are exposed to urban noise. If and how anthropogenic noise affects echolocation behavior in bats is as of now poorly understood. Two recent studies revealed that traffic noise significantly deteriorated foraging behavior in greater mouse-eared bats (*Myotis myotis*), a gleaner bat that passively locates its prey by listening to the rustling sounds produced by it.<sup>17,18</sup> These bats forage for prey less frequently,<sup>17</sup> their hunting performance was significantly decreased and their search times increased in areas where traffic noise was prominent.<sup>18</sup> It remains unclear, however, whether this change in foraging behavior was based on masking effects or a distraction by the ambient noise.

Previously, we reported that bandpass-filtered noise (BFN, bandwidth 20 kHz) significantly affected echolocation behavior in horseshoe bats and that the effects on call amplitudes and frequencies differed depending on which frequency bands of the bat's hearing range were masked by BFN.<sup>19</sup> We showed that

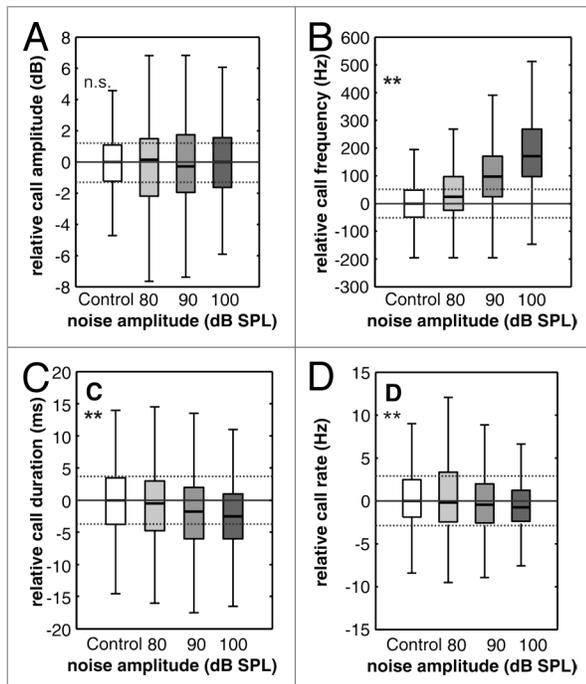
call amplitudes increased only when BFN was centered on the dominant frequency component of the bats' echolocation calls. In contrast, the frequencies of echolocation calls rose for many more masking conditions, indicating that different neuronal mechanisms were underlying amplitude and frequency changes, respectively. Interestingly, call frequencies also increased when BFN was centered at frequencies far below the bats' range of echolocation call frequencies: When BFN was centered at 20 kHz (BFN20), thus masking frequencies between 10 and 30 kHz. This frequency band, however, is present in several anthropogenic noise sources such as traffic noise at highways,<sup>17,18</sup> urban noise<sup>20</sup> and noise produced by wind turbines.<sup>21</sup> In the present report, we investigated the potential effect of anthropogenic noise on echolocation behavior by analyzing how BFN20 affected several call parameters such as frequency, amplitude, duration and rate during echolocation of Greater horseshoe bats, *Rhinolophus ferrumequinum*.

We recorded 11,825 echolocation pulses from three horseshoe bats. All calls were emitted at rest with (5598 calls) and without masking BFN20 (6227 calls; see Materials and Methods for further details). Horseshoe bats emit echolocation calls that are characterized by a long constant frequency (CF) component and the CF frequency emitted while the bat is perched ("at rest") is

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**Figure 1.** Changes in call parameters in response to masking with different BFN20 amplitudes compared with the control condition (no BFN20); data averaged for all three bats. (A) Call amplitude, (B) call frequency, (C) call duration, (D) call rate. Medians: horizontal lines inside boxes; first and third quartiles: upper and lower margins of boxes, respectively; 5% and 95% quantile: small horizontal bars above and below boxes, respectively. Dotted lines indicate the thresholds of the population effect sizes [57 Hz for (A), 1.1 dB for (B), 3.7 ms for (C) and 2.9 Hz for (D)].

called the resting frequency (RF).<sup>22,23</sup> RFs of the bats without masking were stable at  $69.9 \pm 0.05$  kHz,  $74.7 \pm 0.05$  kHz and  $75.9 \pm 0.05$  kHz in the three bats. The bats' echolocation calls uttered without masking had median durations of 37, 41 and 44 ms, emitted at call rates of 8.1, 7.1 and 11.8 Hz, respectively.

As reported previously,<sup>19</sup> call amplitudes were not affected by BFN20 ( $p > 0.1$ ,  $df = 3$ ,  $X^2 = 5.18$ , Kruskal-Wallis test; Fig. 1A), whereas call frequencies increased significantly in response to the masking BFN20. Louder noise, i.e., larger BFN20 amplitudes, caused higher rises in call frequencies up to a maximum of 171 Hz at a BFN20 amplitude of 100 dB SPL ( $p < 0.001$ ,  $df = 3$ ,  $X^2 = 2809.3$ , Kruskal-Wallis test; Fig. 1B). Based on the population effect size,<sup>24</sup> we found that shifts in call frequency differed from the control condition for BFN20 amplitudes of 90 and 100 dB SPL, but not for 80 dB SPL (threshold: 57 Hz). Call durations were significantly shortened in response to masking by BFN20 with shorter values for higher BFN20 amplitudes [maximum reduction by 2.5 ms ( $p < 0.001$ ,  $df = 3$ ,  $X^2 = 297.4$ , Kruskal-Wallis test; Fig. 1C)]. Similarly, call rates significantly decreased for louder masking noise, reaching a maximum of 0.7 Hz for BFN20 amplitudes of 100 dB SPL ( $p < 0.001$ ,  $df = 3$ ,  $X^2 = 72.8$ , Kruskal-Wallis test; Fig. 1D). Nevertheless, changes in both call duration and rate did not differ from the control condition based

on the population effect size<sup>22</sup> [threshold: 3.7 ms (duration), 2.9 Hz (rate)].

The data presented here therefore demonstrate that echolocation behavior in horseshoe bats is also significantly altered by low-frequency noise ranging from 10 to 30 kHz, thus masking frequencies far below the range relevant for echolocation. This low-frequency band, however, is present in several anthropogenic noise sources.<sup>17,18,20,21</sup> Our findings suggest that noise-related deterioration in echolocation performance, which have recently been found in recent studies<sup>17,18</sup> might be due to a direct effect of anthropogenic noise on the echolocation behavior rather than a general distraction of the bats' attention to the echolocation tasks.

Interestingly, we found that only the call frequencies but not any of the other call parameters were affected by the low-frequency masking noise. In horseshoe bats, the RF is maintained with high accuracy (standard deviation  $\pm 50$  Hz in the present study) to keep the frequency of the returning echoes constant and within the bats' best range of hearing. Therefore, the auditory system of horseshoe bats is equipped with a filter mechanism that is narrowly tuned to each bat's individual RF. This so called "auditory fovea"<sup>25,26</sup> is based upon an increased number of receptor cells in the cochlea as well as higher-order auditory neurons that are very particularly tuned to frequencies around RF.<sup>27,28</sup> Hence, the increases in call frequencies that we observed in response to masking by BFN20 (as well as in our previous study)<sup>19</sup> may cause the corresponding echo to drop out of the auditory fovea. This could impede the echolocation performance of horseshoe bats and therefore, negatively impact their foraging behavior. Recently developed telemetry techniques that allow one to record echolocation call parameters in freely behaving bats "on board"<sup>29,30</sup> may aid in tackling the question whether noise-dependent shifts in call frequency indeed affect the echolocation performance in freely flying bats.

## Materials and Methods

We used three Greater Horseshoe Bats, *Rhinolophus ferrumequinum*, two males and one female, collected in the People's Republic of China. All procedures were in accordance with NIH guidelines for experiments involving vertebrate animals and were approved by UCLA's Animal Research Committee.

Echolocation pulses were played back with a 4 ms-delay and a playback attenuation of 30 dB relative to the intensity of the echolocation pulses. In the control condition, we presented these "echo mimics" through an ultrasonic loudspeaker under free-field conditions and monitored the bats' call parameters. In the present study, we focused on changes in several echolocation call parameters in response to masking the echo mimics with 20 kHz noise stimuli that were centered around 20 kHz (BFN20), thus masking the frequency range between 10 and 30 kHz. For the masking experiments, the bats were acoustically stimulated with continuous BFN20 that was produced by digitally band pass filtering broadband noise to a bandwidth of 20 kHz with steep roll-off (bandwidth of BFN20 at  $-10$  dB:  $< 21$  kHz). BFN20 stimuli

were presented via a second ultrasonic loudspeaker (in addition to the one used for the echo mimics).

The frequency band of BFN20 is far below that used for echolocation in horseshoe bats but is present in low-frequency ambient, especially anthropogenic noise, for which BFN20 therefore served as a model. BFN20 stimuli were presented at amplitudes of 80, 90 and 100 dB SPL.

During experiments, the bats were mildly restrained in a foam sandwich. Signals were digitized with 16-bit resolution and 200 kHz sample rate. Custom-made software (MATLAB, Mathworks) was used to measure call frequency, amplitude, duration and rate. Please see our recent study for more details.<sup>19</sup>

We used Kruskal Wallis tests to test for significant differences in the call frequency, amplitude, duration and rate between the control condition and masking with BFN20. Differences in distributions were considered significant, if the probability of

error was less than 5%. To evaluate the relevance of the statistical significance for changes in call parameters, we calculated the population effect sizes<sup>24</sup> (defined as 0.8 times of the SD to determine large effects) as performed previously.<sup>19</sup> To reduce the effect of individual differences, we normalized all data for each bat relative to their respective median call parameter values emitted without noise present prior to all analyses.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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