

# Effects of Echo Intensity on Doppler-Shift Compensation Behavior in Horseshoe Bats

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**Smotherman, Michael and Walter Metzner.** Effects of echo intensity on Doppler-shift compensation behavior in horseshoe bats. *J Neurophysiol* 89: 814–821, 2003; 10.1152/jn.00246.2002. Echolocating horseshoe bats respond to flight-speed induced shifts in echo frequency by adjusting the frequency of subsequent calls. Under natural conditions, Doppler effects may force the frequency of a returning echo several kilohertz above the original emission frequency. By lowering subsequent call frequencies, the bat can return echo frequencies to within a narrow spectral bandwidth to which its highly specialized auditory system is most sensitive. While Doppler-shift compensation (DSC) behavior specifically refers to frequency compensation, other parameters of the returning echo, such as delay, duration, and interaural time and intensity differences have been shown to influence DSC performance. Understanding the nature of these influences has already led to a better appreciation of the neurophysiology of DSC. Here we provide a quantitative analysis of the effects of a prominent feature of the returning echo, its intensity, on DSC performance in horseshoe bats. Although DSC performance generally tolerates echo attenuation up to approximately 40 dB relative to the outgoing emission intensity, a systematic decline in DSC performance can be observed over this range. Generally, the effects of echo attenuation are characterized by a reduction in 1) the overall amount of compensation relative to the size of the shift in echo frequency and 2) the rate at which the bat responds to perceived echo shifts. These effects appear to be the consequence of a systematic shift in the range of echo frequencies capable of inducing DSC behavior. In particular, the reference frequency (the minimum shift in echo frequency that will elicit DSC behavior) appears to be highly sensitive to echo intensity. Every 10-dB reduction in echo intensity shifts the reference upward nearly 250 Hz. Our results indicate that, even at the highest intensity levels, relatively minor changes in echo intensity critically influence frequency compensation during normal DSC. We conclude with a discussion of how these results might impact echolocation behavior of horseshoe bats under natural and experimental conditions.

## INTRODUCTION

Two groups of echolocating bats, horseshoe bats and the mustached bat, emit a call characterized by its prolonged constant-frequency (CF) component. Although their calls also includes a brief initial rise in frequency and a concluding brief and rapid drop in frequency (the FM component), these bats rely heavily on the information contained in the CF portion of the returning echo to navigate through the dense vegetation in which they hunt for prey (Neuweiler

1990; Neuweiler et al. 1987; Novick and Vaisnys 1964; Schnitzler 1968, 1970). Accordingly, the auditory system of these bats is sharply tuned to a narrow spectrum just above the frequency of the emitted call (Neuweiler 1970; Suga et al. 1975; Suga and Jen 1976, 1977). Flight speed induces Doppler-shifts into the frequency of the returning echo and can thereby push this bandwidth of critical information beyond the region of best auditory sensitivity (or “auditory fovea”) (Schuller and Pollak 1979). To correct for Doppler-shifts and ensure that ensuing echoes fall within the auditory fovea, horseshoe bats adjust the frequency of subsequent calls accordingly.

This “Doppler-shift compensation” behavior, or DSC, significantly enhances echolocation performance in their natural habitat (Neuweiler 1990; Neuweiler et al. 1987; Novick 1977). However, several acoustic parameters must be met for an echo to evoke DSC: the timing of the returning echo relative to the outgoing call and the direction in space from which the echo returns to the bat both appear to serve as gating mechanisms for the behavior (Neumann and Schuller 1991; Schuller 1977; Schuller and Suga 1976b). The effects of yet another acoustic parameter, echo intensity, have been implied but not yet systematically analyzed. It was previously reported that DSC was relatively immune to changes in echo intensity (Schuller 1974) and, indeed, apparently normal DSC has been reported to occur under limited conditions when echoes have been attenuated by as much as 40 dB relative to the emitted call intensity (Behrend et al. 1999). What is unclear, however, is whether small changes in echo intensity lead to any identifiable changes in the dynamic properties of DSC, such as its speed, sensitivity, or acuity. Such changes might reveal important clues about the underlying neural mechanisms. We undertook a quantitative analysis of the effects of echo playback attenuation on DSC behavior in the Greater Horseshoe Bat, *Rhinolophus ferrumequinum*. We found significant systematic changes in the way these bats responded to frequency-shifted echoes when presented at different sound intensities. Specifically, echo intensity influenced the relationship between echo frequencies and the magnitude of subsequent changes in call frequency during DSC. The results suggest that echo intensity is indeed an important parameter for the accurate performance of DSC by horseshoe bats.

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## METHODS

Five Greater Horseshoe Bats, *R. ferrumequinum*, both males and females collected in the Peoples Republic of China, were used in this study. All experiments conformed to National Institutes of Health guidelines for the care and use of animals and were approved by the local IACUC. The bats were restrained in a foam sandwich but could freely move their head. Frequency-shifted echo playbacks (= echo mimics) were generated as described previously (Metzner et al. 2002; Schuller et al. 1974). Briefly, vocalizations were captured by a ¼-in. Brüel and Kjaer (type 4135) microphone placed 15 cm ahead of the bat's nostrils. These calls were then electronically frequency-shifted (positive or negative shifts  $\leq 5$  kHz) by a **double-heterodyning technique (custom design modified** after Schuller et al. 1974) and played back to the bat under free-field conditions after a 4-ms delay through an ultrasonic loudspeaker positioned on either side of the bat approximately 20° laterally and 15 cm from the bats left or right pinna. Within a frequency range of 71 to 85 kHz the playback system (including loudspeaker) had a frequency response of  $\pm 3$  dB and all harmonic distortions for pure tone signals present in the playback occurred at intensities below 60 dB SPL. The playback was initiated by customized software on a PC. The parameters of the imposed frequency shift were preset manually with a signal generator, whereby the bat could be exposed to either 1) long or short rectangular changes in playback frequency, 2) continuous sinusoidally modulated shifts in playback frequency, or 3) triangularly modulated shifts in playback frequency. The modulation rate, polarity, and peak magnitude of the frequency shift could be predesignated for each experimental protocol. At 0 dB attenuation the playback system was calibrated to produce a playback signal from the speaker equal in intensity (at the bat) to the recorded call intensity at the microphone placed 15 cm directly ahead of the bat. The remaining 15-cm traveling distance contributed roughly 6 dB of added attenuation, thus our unattenuated playback should have been approximately  $-6$  dB relative to the emitted call *at the bat*. Cross talk between the speaker and microphone was minimized by a piece of sound-insulating foam placed between the microphone and loudspeaker and projecting 5 cm toward the bat. All sound levels are given relative to the intensity of the preceding call. Playback attenuation applied in steps of 10 dB was performed electronically. All experiments were performed in an anechoic chamber. Some minor natural echoes emanating from the experimental apparatus were present during the experiments, the potential significance of which is considered in the discussion. A totally anechoic condition can only be achieved with dichotic stimulation (headphones). However, no evidence suggests that natural echoes occurring under free-field conditions impair or alter the DSC performance (Behrend et al. 1999) relative to the headphone condition. Head movements can induce small Doppler-effects in the recorded call frequency, which may contribute to some of the observed variability, but since these contributions are small relative to the magnitude of the electronic frequency shifts presented, and since they are presumed to occur equally under all experimental conditions, head restraint was deemed unnecessary.

Frequency analysis of the echolocation calls was performed using the dominant second harmonic of the CF component, which was transformed via a custom-made frequency-to-voltage converter and then digitized and stored on VHS tape (model 3000A, A.R. Vetter, Rebersberg, PA) and analyzed off-line using the **software suite Signal (V 3.1, Engineering Design, Belmont MA)**. Frequency measurements after digitization were accurate to within  $\pm 48$  Hz or  $\pm 0.06\%$ . Call frequencies reported here are the maximum frequencies of the dominant second harmonic component of each call's CF portion as determined in Signal (Metzner et al. 2002). On a second channel of the VHS, call intensity was recorded after being transformed by an AC/DC converter. Call intensities varied from approximately 92 to 112 dB SPL (SPL re 20  $\mu$ Pa) between experiments. Plots and curve fits were generated using the software SigmaPlot 2000 v. 6.0 (SPSS

Science, Chicago IL). For statistical comparisons, either a Student's *t*-test or a Mann-Whitney rank sum test was used to establish significant differences in call parameters between data sets. For each of the figures shown here, call frequencies are given relative to each bat's resting call frequency (or RF). RF was determined experimentally by recording  $\geq 60$  s of calls both at the beginning and at the end of each recording session. RF values used for analysis were specific to each recording session. Normally RF did not change over the length of a recording session. A decline in call intensity and repetition rate was associated with length of experimental sessions, therefore most recording sessions were limited to  $< 20$  min per day. Each of the bats described here underwent 6 to 10 experimental recording sessions over a period of approximately 2 mo. One bat (RF12) was tested at least twice a week for more than 4 mo, during which time its resting frequency and DSC performance were notably consistent. The term "reference frequency" is defined as the mean amplitude of the under-compensation exhibited by a bat during DSC (Schuller et al. 1974): for example, if a bat typically lowered its call frequency 800 Hz in response to 1 kHz increase in echo frequency, the reference frequency would correspond to a value 200 Hz above the original call frequency. Conversely, the term "threshold," as used here, refers to the observation that the same bat would only begin to perform DSC once a change in echo frequency exceeded 200 Hz.

## RESULTS

In this study we sought to characterize the overall effects of playback intensity on DSC performance. For this purpose we analyzed first the effects of echo intensity on the resting and reference frequencies by quantifying changes in compensation "depth" (how much the bat raised/lowered its call frequency relative to shifts in playback frequency). Next we examined the rate at which DSC happened in response to both brief and prolonged rectangular changes in playback frequency. Finally, we show evidence for a change in the frequency threshold for eliciting DSC.

*Intensity effects on RF and on compensation depth*

At the beginning of each experiment,  $\geq 60$  s of continuous echolocation calls were recorded first without and then in the presence of electronic playback. Generally there was no significant difference in RF in the presence (playback frequency = RF) or absence of playback. Resting frequencies varied between bats from 77.1 to 78.6 kHz. The mean of the SDs in call frequency for five bats recorded while actively calling for 100 s in the absence of playback was  $251 \pm 65$  Hz.

As demonstrated previously (Schuller et al. 1974), when a CF shift was added to the echo-mimic playback, the bats continued to compensate for the perceived frequency difference (dF) for as long as the dF was present in the playback. Typical responses to a range of these CF shifts presented at different playback intensities are shown in Fig. 1. At 0 dB attenuation the bat maintained full compensation for positive dFs by lowering its call frequency to the point where the final echo frequency fell within the range of the reference frequency: for example a +2 kHz dF added to the playback elicits a mean change in call frequency of  $-1.825 \pm 0.095$  kHz relative to RF (Fig. 1A, bottom gray box at far left). As echo attenuation was increased, the overall depth of compensation decreased for all dFs. The responses to playback attenuated by 20 dB or more were always significantly different ( $P < 0.01$ ) from the responses elicited by unattenuated playback at the

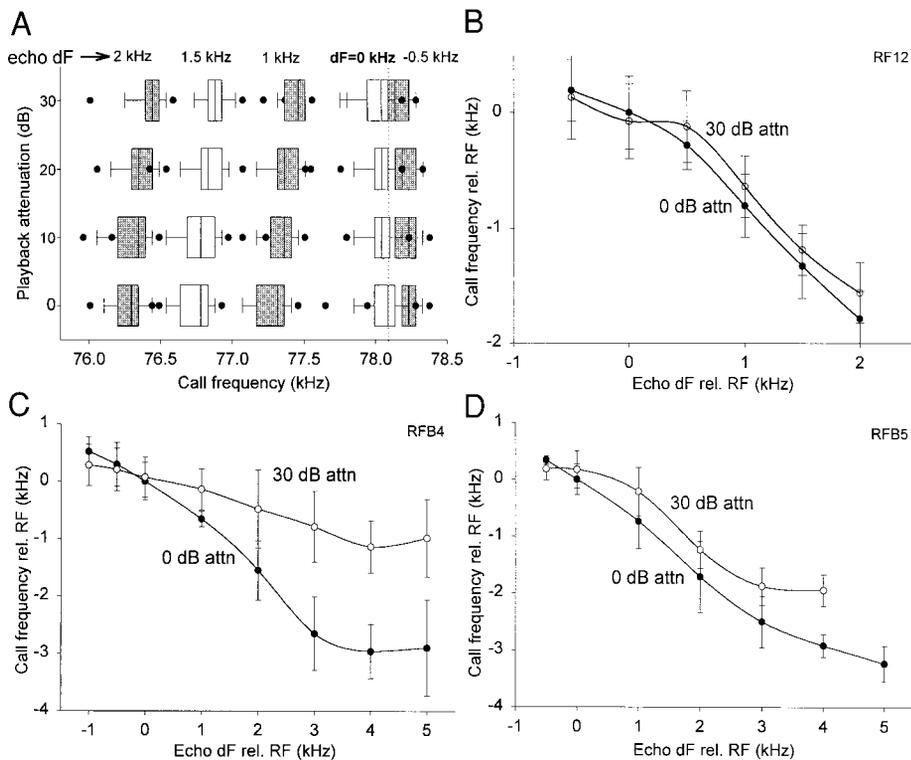


FIG. 1. A: set of 20 box plots of call frequencies recorded during 60-s presentations of single continuous shifts in playback frequency presented at different playback intensities. The imposed echo frequency shifts ranging from  $-0.5$  to  $2.0$  kHz are listed along the top axis and correspond to the alternating sets of white or gray box plots. Sample sizes for the individual box plots vary from 389 to 827 calls. For each box, the midline represents the median call frequency, whiskers are SD, and the points are 5 and 95% outliers. Dotted line denotes resting call frequency (RF) measured at maximum playback intensity. B: same bat as in A, the data representing 0 and 30 dB playback attenuation are plotted for comparison. Here echo frequency shifts (Echo dF) are given relative to RF. C and D: same format as in B but with two different bats tested over a broader range of imposed playback frequency shifts.

same dF. Stimuli presented at  $-10$  dB produced results intermediate to the effects of 0 and  $-20$  dB but were not always significantly different from either of those data sets. The responses of three bats to multiple dFs presented at two different echo intensities are given in Fig. 1.

Although bats typically do not show large increases in call frequency when presented with negative dFs, small but significant increases do occur (Metzner et al. 2002; Schnitzler 1968 1973; Schuller et al. 1974). For example, one bat used in this study (RF12, Fig. 1, A and B) raised its mean call frequency from  $78.356 \pm 0.136$  to  $78.664 \pm 0.135$  kHz, a difference of 308 Hz, in response to a  $-1$  kHz dF at 0 dB attenuation. However larger negative shifts in echo frequency did not elicit significantly greater increases in the mean call frequency. This may reflect upper frequency limits inherent to the laryngeal mechanisms that control calling (Novick and Griffin 1961; Schuller and Suga 1976a; Schuller and Rübtsamen 1981). This apparent asymmetry in DSC performance complicates the study of the DSC response to negative echo frequency shifts. A detailed analysis of the effects of playback intensity on the response to negative echoes has been presented elsewhere (Metzner et al. 2002). Here we found some evidence that responses to negative dFs were sensitive to echo intensity when the bat was pressed to call above RF. When responding to negative shifts in the frequency of the playback echo mimic, the mean increase in call frequency above RF achieved by all five bats tested was always greater as playback intensity was increased (see for example Fig. 1A, gray boxes on far right).

It has been shown that mustached bats swung on a pendulum will compensate for changes in echo intensity as well as frequency during DSC performance (Kobler et al. 1985), but they do not perform the same intensity compensation in response to electronic playback manipulations such as were used in this study. The specific reason for this discrepancy is un-

known, but it appears to be true for horseshoe bats as well. We have recorded horseshoe bats actively changing call intensity while being swung on a pendulum in a manner similar to the behavior reported for mustached bats, although we cannot yet say whether this actually reflects true intensity compensation behavior. We have not observed horseshoe bats making adjustments in call intensity when responding to artificial playback. For the data sets presented in Fig. 1, a detailed analysis of call intensity was performed to address whether horseshoe bats were actively compensating for electronic playback attenuation by increasing call intensity. However, in our artificial playback experiments, call intensity was found to vary by  $<3$  dB during the course of any one experiment and did not covary with playback intensity. Other parameters of the echolocation call, including duration and call structure, were also examined and were found not to vary significantly when responding to different playback intensities (data not shown).

#### *Influences of echo intensity on the response to rectangular changes in playback frequency*

Consistent with the above results, we found that the maximum compensation depth achieved during the response to rectangular changes in echo frequency was also diminished with greater playback attenuation. Figure 2A illustrates how a 30-dB attenuation affected a bat's response to a series of 5-s-long 2.2-kHz rectangular increases in echo frequency compared with rectangular changes delivered with no attenuation. Although the reduction in the maximum compensation depth was small, there was a dramatic effect on the time course of the compensation. This is quantified in Fig. 2B, which shows the average effects of increasing echo attenuation on the rate (represented by time constants) of lowering call frequency. When comparing the first three or four calls of the response at

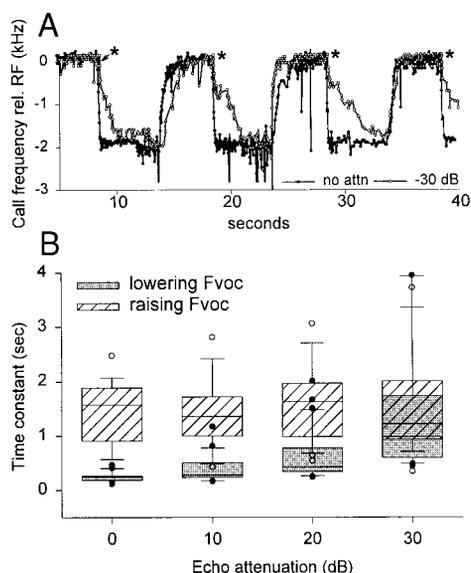


FIG. 2. *A*: a calling bat is presented with a series of 5-s-long, 2.2-kHz rectangular echo frequency modulations, during which the bat lowers its call frequency at step onset and subsequently raises call frequency at step offset. Call frequencies are plotted relative to median value emitted at rest (RF), which in this case was 78.02 kHz (bat Rfb4). The 2 traces show the response to the same protocol played back at 0 (filled circles) and then 30 dB (open circles) attenuation. \* denote initial responses referred to in the text. *B*: box plots of time constants measured for the responses to step-onset (gray boxes) and step-offset (striped boxes) evoked at 4 different levels of playback intensity. Single-exponential curves were fit to each sequence of calls recorded during which the bat lowered its call frequency, and the time constants to several such fits were collected to generate box plots at each intensity. Call rates did not vary significantly with playback intensity: for the lowering of call frequency the mean call rates were  $9.2 \pm 3.3$  Hz (0 attn),  $9.0 \pm 2.5$  Hz ( $-10$  dB),  $8.3 \pm 2.0$  Hz ( $-20$  dB), and  $8.7 \pm 1.6$  Hz ( $-30$  dB) and during the raising of call frequency the mean call rates were  $9.3 \pm 2.8$  Hz (0 attn),  $8.4 \pm 2.9$  Hz ( $-10$  dB),  $8.2 \pm 2.1$  Hz ( $-20$  dB), and  $8.3 \pm 2.8$  Hz ( $-30$  dB). For all box plots  $n = 23$  to 27.

$-30$  dB (Fig. 2*A*, open circles) to a similar time period for 0 dB attenuation (Fig. 2*A*, closed circles), it can be seen that the *initial* response to the rectangular change appeared similarly fast in either case. The effects of echo attenuation seemed to appear midway through the time course of the full down response. Echo playback with no attenuation induced a rapid and smooth lowering of call frequency almost to the level of complete compensation, while increasing attenuation led to slower and more erratic changes in call frequency. This is quantified in Fig. 2*B* as an increase in the variability in the time constants as well as the median values. In Fig. 2*A* it can also be observed that, while echo attenuation had consistent effects on the lowering of call frequency, its effects on the subsequent raising of call frequency were much more inconsistent. In this study we found no significant effect of playback attenuation on the time course of raising call frequency, although Metzner et al. (2002) have reported evidence that the return to RF during DSC is indeed sensitive to echo intensity under certain experimental conditions.

#### Investigating the initial DSC response by using very brief frequency shifts

In the previous section we pointed to evidence suggesting that the initiation of DSC may not be sensitive to playback intensity (refer to Fig. 2*A*, asterisks). To investigate this fur-

ther, very brief rectangular changes in echo frequency were used to highlight the effects of playback intensity on the initial response (Fig. 3*A*). In these experiments, the imposed dF is only presented for 1 s. The length of time the bat is actually exposed to frequency-shifted echoes will depend in part on when and how often the bat calls during that 1 s. Many of these brief responses (Fig. 3*A*) can be collected and averaged in a short period of time and were found to be generally very consistent. Under this protocol, the raising of call frequency immediately following termination of the brief frequency shift occurs entirely in the absence of playback and so was not investigated here. The average compensation depth and the rate at which that compensation occurred ( $dF_{VOC}/dt$ ) for varying dFs are shown in Fig. 3, *B* and *C*, respectively. For large dFs ( $>3$  kHz) compensation was routinely incomplete even at the maximum playback intensity. For example, the bat for which responses are shown in Fig. 3*B* typically achieved full com-

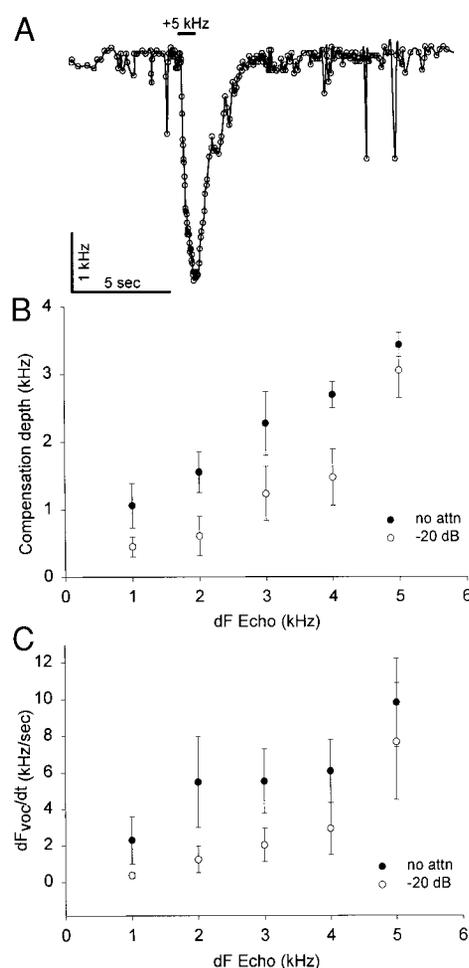


FIG. 3. Doppler-shift compensation (DSC) responses to 1-s shifts in playback. These brief responses are useful for investigating the effects of playback intensity on the initial slope of the response (asterisks in Fig. 2). *A*: single DSC response (solid line) to a 1-s 5-kHz dF (indicated as a bar above trace). Clear circles mark individual calls. *B*: maximum compensation depth (mean  $\pm$  SD) of DSC evoked at maximum playback intensity (no attn) and with 20 dB attenuation for playback dFs ranging from 1 to 5 kHz. *C*: for the same data as in *B*, the slope of the initial response (or rate at which the bat lowered its call frequency;  $dF_{VOC}/dt$ ) was calculated and plotted for no and 20 dB attenuation. For each point in *B* and *C*,  $n = 12$ ; Each mean value collected at  $-20$  dB was significantly lower ( $P \leq 0.01$ ) than the equivalent value collected without attenuation. Bat RF12.

compensation for the 1 kHz dF within the 1-s window, but relative compensation depth declined as dF increased. Because of this, we believe that brief responses can be used to measure DSC under circumstances in which the system can be assumed to be performing at its maximum speed. We thereby sought to explore the effects of playback intensity on the upper limits of DSC performance. A 20-dB attenuation caused a significant ( $P \leq 0.01$ , see Fig. 3 legend) reduction in the compensation depth induced by any of the 1-s playback dFs (Fig. 3B). The observed reduction in compensation depth could be due to both changes in  $dF_{VOC}/dt$  and/or an absolute change in the compensation depth independent of time (i.e., a change in the reference frequency). We found that the initial rate at which the call frequency was lowered was significantly slower at all dFs tested at this intensity. Changes in  $dF_{VOC}/dt$  could result from either differences in call rate (number of calls per second) or differences in the mean frequency change occurring between succeeding calls. For the data shown in Fig. 3 we measured the mean intercall intervals during the downward changes in call frequency for all impulse responses at 0 and -20 dB. We observed a small, technically nonsignificant decrease in call rate at the reduced playback intensity: the mean intercall intervals during the initial responses were 1) for no attenuation,  $65.8 \pm 28.8$  ms and 2) for -20 dB,  $83.5 \pm 35.7$  ms ( $P = 0.06$ ). In similar experiments conducted on three other bats we also found no significant changes in the initial call rate associated with attenuation of the playback. For all four bats studied in this way, a 20-dB attenuation resulted in an average decrease of the resulting compensation depth by  $42.3 \pm 17.5\%$  for all dFs and an average decrease of  $51.0 \pm 24.2\%$  in  $dF_{VOC}/dt$  for all dFs. The effects of playback attenuation were significant ( $P \leq 0.05$ ) at every dF. We interpret these observations as to suggest that the observed changes in maximum compensation depth and the rate of change are primarily due to differences in the average magnitude of single changes in frequency between succeeding calls during the entire downward component of the response, although minor changes in call rate may also contribute to the observed effects.

#### Influence of playback intensity on the threshold for eliciting DSC

Doppler-shift compensation behavior is defined in part by an inherent "reference frequency" (Schnitzler 1968; Schuller et al.

1974). This refers to both 1) the minimum positive change in the echo frequency necessary to evoke a compensatory change in call frequency and 2) the remaining uncompensated frequency difference of the returning echo with active compensation engaged (i.e., a consistent undercompensation level). When a bat is presented with a slowly increasing shift in playback frequency, the bat will start to lower its call frequency only after the dF has surpassed this threshold (reference frequency) and will subsequently lower its call frequency equal to any additional perceived dF. Using a triangular function for presenting slowly increasing echo frequency shifts, we investigated the effects of playback intensity on this threshold value (Fig. 4) and on the subsequent rate of change in call frequencies. Bats were presented with slow ( $\approx 333$  Hz/s) positive dF triangles initiated from RF and lasting 10 s. After a complete cycle was obtained, DSC threshold was determined by first fitting a straight line to the bat's lowering call frequency and extrapolating backward to the point in time where the bat first began to lower its call frequency. This time was then used as a reference point to estimate the value of the echo dF at DSC onset (illustrated in Fig. 4A). In general, as playback attenuation was increased, the bats consistently required larger playback dFs to initiate a lowering of call frequency. Once threshold was surpassed,  $dF_{VOC}/dt$  was directly correlated with the rate of change in the echo dF triangle. We did not observe a significant difference in the slope of the change in call frequency at different playback intensities; for the data in Fig. 4, the mean of the slopes ( $dF_{VOC}/dt$ ) were  $295 \pm 35$  Hz/s (no attenuation),  $297 \pm 44$  Hz/s (-10 dB),  $338 \pm 138$  Hz/s (-20 dB), and  $343 \pm 94$  Hz/s (-30 dB), but the differences were not significant ( $n = 8$ ,  $P = 0.516$ ). Figure 4B plots the mean effects of playback intensity on DSC threshold for four bats. One bat (RF20) exhibited a substantially greater frequency threshold than the other bats at any playback intensity and would not respond to any playback attenuated  $>20$  dB relative to the emitted call intensity.

#### Intensity-dependent changes in the DSC responses to sinusoidal manipulations of the echo frequency

If playback frequency is slowly increased and then decreased in a sinusoidal manner, horseshoe bats will slowly lower and then raise their call frequency in a similarly sinusoidal manner. We can define the rate at which playback

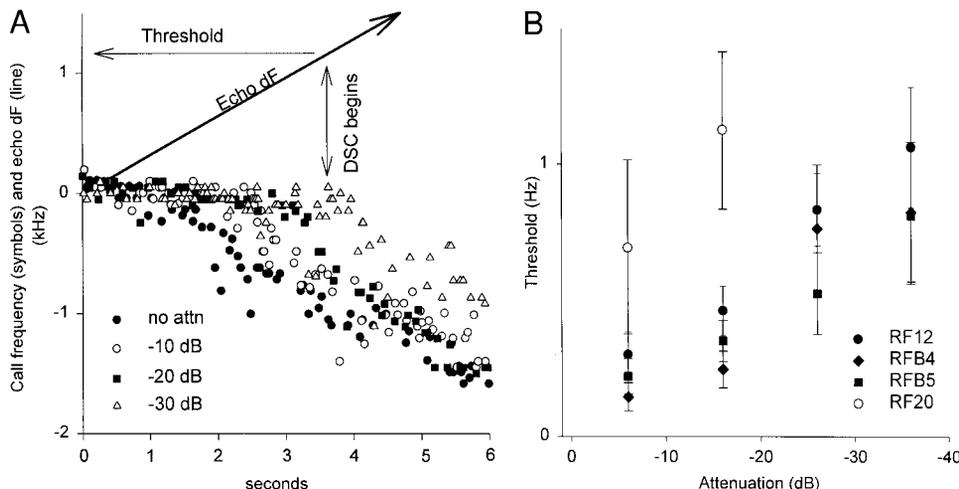


FIG. 4. A method for measuring the frequency threshold for DSC at different playback intensities. *A*: playback frequency is increased linearly to determine the minimum change in echo frequency necessary to elicit DSC. A series of typical DSC responses by one bat (RF12) to the same dF ramp presented at 4 different playback intensities is shown. The method of estimating the threshold from the time at which the bat begins to compensate is demonstrated for the lowest playback intensity (open triangles). *B*: here the means ( $\pm$ SD) of the threshold (or reference frequency) estimated as in *A* are plotted for 4 bats at four different playback intensities. Bat RF20 would not respond to playback attenuated  $>20$  dB. Data include a correction for an additional 6 dB attenuation resulting from the distance between the speaker and the bat (see METHODS).

frequency is raised and lowered as the *modulation frequency* (Fig. 5A). If modulation frequency is slow enough, then the compensation depth achieved by the bat during a single cycle will closely reflect the maximum change in playback frequency imposed. However, as modulation frequency is progressively increased, the relative change in call frequency will become progressively less. A plot of the relative amplitude of the DSC response over a series of modulation frequencies has been referred to as an “amplitude response curve” (Schuller et al. 1975). DSC amplitude response curves were determined as described previously by measuring the peak-to-peak amplitude of the sinusoidal changes in call frequency during responses to a progressive series of different modulation rates, calculating the amplitude of the response relative to the maximum playback dF for each modulation frequency, and plotting the results as relative attenuation. Such plots are useful for characterizing

the filtering properties of a feedback system such as DSC (Schuller et al. 1975). Analyzed in this way, DSC was previously shown to exhibit properties similar to a digital filter (Schuller 1986; Schuller et al. 1975). We found that reducing playback intensity degraded the DSC response to sinusoidal shifts in playback frequency in a predictable way (Fig. 5B). Analysis of the amplitude response curves collected at different playback intensities revealed that the effects of echo attenuation caused a decrease in the amplitude of the response at all modulation frequencies. The shift in the relative response amplitudes at very slow modulation rates corresponds well to the observed changes in the threshold (Fig. 4) and steady-state compensation levels (Fig. 1), because at lower playback intensities the bats were observed to begin compensating later in the cycle and ultimately exhibited a corresponding reduction in the peak amplitude of the response (i.e., compensation depth in

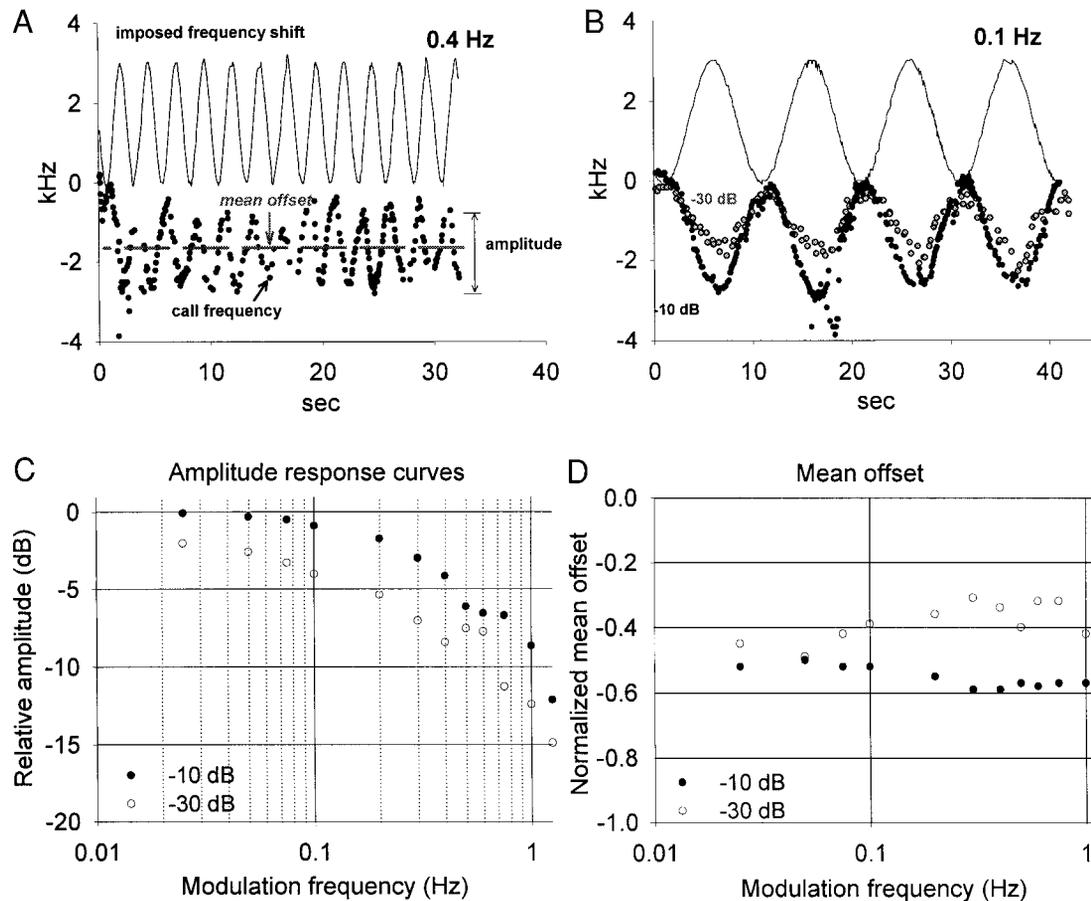


FIG. 5. Effects of playback intensity on the frequency–response properties of DSC performance. *A*: sinusoidal manipulations of playback frequency cause sinusoidal changes in the bats call frequency. The ability of the bat to follow sinusoidal changes in playback frequency depends on the peak amplitude of imposed frequency shift (set to 3 kHz here) and the modulation rate at which the playback frequency changes (here 0.4 Hz, or alternatively a sequence of 2.5-s periods of cyclic shifts in playback frequency; solid line in *A*). DSC performance over a broad range of modulation frequencies is quantified by measuring the mean *amplitude* of the peak-to-peak changes in call frequency during DSC and relating these to the maximum peak-to-peak change in playback frequency. *Mean offset* is defined as the midline of the peak-to-peak responses during several successive cycles of sinusoidal DSC performance. *B*: DSC is degraded when playback intensity is attenuated: here an additional 20 dB of attenuation lowered the amplitude of the response, and with greater attenuation the mean offset is closer to the original resting frequency (0 = RF). *C*: amplitude response curves measured at 10 and 30 dB relative playback attenuation (bat RF12). Sinusoidal frequency shifts  $\leq 3$  kHz were played back at modulation rates varying from 0.025 to 1.25 Hz. The mean peak-to-peak response amplitude was measured for either 10 cycles or, for the very slow modulations, a minimum 100 s of DSC performance. *Relative amplitude* is defined as the mean response amplitude relative to the maximum amplitude of the imposed frequency shift, plotted here in dB scale: 0 dB corresponds to a perfect 1-to-1 relationship while  $-6$  dB would correspond to an approximate 50% decrease in response amplitude relative to the maximum change in echo frequency. *D*: from the same data as *C*, the mean offset was measured at  $-10$  and  $-30$  dB.

Fig. 1). The effect became slightly greater as modulation frequency increased up to approximately 0.1 Hz, after which the downward slope in the amplitude response curve appeared similar at both playback intensities.

Another important measure of the response to sinusoidal changes in playback frequency is the *mean offset*. In these experiments, only positive shifts in playback frequency were presented to the bat: during a single cycle the imposed frequency shift varied from 0 to 3 kHz and back. As a result, perfect compensation would result in the bat lowering and raising its call frequency by 3 kHz, and the mean offset would be 1.5 kHz, or a normalized mean offset of 0.5 (1.5/3.0 kHz). This offset will reflect the net performance of the system as call frequency is both lowered and then raised in sequence; a mean offset of 0.5 is predicted if both the lowering and raising phases occur with similar time courses. However, we have shown previously that the lowering and raising of call frequency do not follow identical paths and may have different temporal limitations (Metzner et al. 2002). With loud playback intensity, increasing modulation frequency resulted in a bias toward more negative mean offsets, apparently because the raising of call frequency is constrained to a slower maximum speed than the lowering of call frequency (Metzner et al. 2002). The reasons for this are not known. Figure 5D plots the measured mean offset during DSC plotted versus modulation frequency. In Fig. 5C, playback attenuation led to a reduction in the relative response amplitude at all modulation frequencies, and we can see that this was accompanied by a positive shift in the normalized mean offset (Fig. 5, B and D). Had playback intensity affected both the raising and lowering of call frequency equally, the mean offset could have remained unchanged despite either similar or more dramatic reductions in the amplitude response curves. Therefore, based on the upward shift observed in Fig. 5D, we must conclude that reductions in playback intensity affect the lowering of call frequency more than the subsequent raising of call frequency.

## DISCUSSION

The principle conclusion from this study is that DSC is highly sensitive to echo intensity. As playback intensity was decreased, the maximum compensation depth diminished just as the magnitude of the shift in echo frequency required for eliciting DSC increased. This shift in *threshold* resulted directly in greater degrees of undercompensation, created greater phase lags during the response to triangular or sinusoidal frequency modulations, and led to more erratic time courses during the response to rectangular modulations of echo frequency. That DSC should be so sensitive to echo intensity is surprising because DSC is a behavior expected to occur reliably in flight despite constant natural fluctuations in echo intensity. The horseshoe bat audiogram reveals thresholds below 0 dB SPL (Long and Schnitzler 1975) for the frequency range studied here. Yet the DSC system requires echo intensities roughly 50–60 dB greater than the auditory thresholds for this range of frequencies (Behrend et al. 1999; Neumann and Schuller 1991; Schuller et al. 1974). This discrepancy might indicate a role for an echo's loudness as a cue to its relevance for DSC.

The reference frequency was first defined as a target echo frequency to be maintained during DSC (Schnitzler 1968 1973;

Schuller et al. 1974; Simmons 1974). Such a distinction was necessary since compensating bats were never observed to return the echo frequency to their actual resting frequency. A strong correlation between the value of the reference frequency and measurements of the threshold for eliciting DSC led to the conclusion that the two values are likely to be linked by a common underlying mechanism (Schnitzler 1973; Schuller et al. 1974). Similar measurements pointed to the possibility that the reference frequency represented a point of greatest auditory sensitivity and that the goal of the DSC system was to maintain the frequency of the returning echo at or near this point of greatest sensitivity (Schuller et al. 1974; Schuller and Pollak 1979). However, from our results, we can now conclude that this would only be true in principle for the loudest echoes, since the DSC threshold for fainter echoes, even those returning from large objects a mere 1–2 meters away, would probably fall as much as a kilohertz above the acoustic fovea. Our results are consistent with the assumption that the threshold for eliciting DSC and the reference frequency are products of the same phenomenon. It also remains that the purpose of DSC is to maintain the frequency of the returning echoes at or near a region of greatest auditory sensitivity. However, the evidence that the reference frequency is labile and depends almost linearly (Fig. 4B) on relative playback intensity suggests that the reference frequency should be thought of *not* as a target frequency or an internal reference, but rather as an indication of the acuity by which the underlying DSC neural circuitry can detect and compensate for minimal shifts in echo frequency. Interestingly, further examination of Fig. 4B can offer this possibility: extrapolating back to the origin one could conclude that, if playback intensity perfectly matched the outgoing emission intensity, the DSC threshold in a perfectly hearing bat would be close to zero! At least two possibilities emerge from this scenario: 1) both the DSC system and that which establishes the call frequency at rest are calibrated to the same internal reference or 2) the DSC system is calibrated directly to the parameters of the outgoing call.

Is it possible that masking effects arising from natural echoes emanating from the small electronic equipment present, namely the speaker and microphone, could contribute to the effects of echo attenuation? We believe this is not a significant issue since Behrend et al. (1999) reported essentially identical sound level thresholds for eliciting DSC under either free-field or closed-field (headphones) conditions. Nevertheless, DSC is known to be sensitive to masking effects, and the results shown here are similar to some of the effects of noise masking (Neumann and Schuller 1991). Since interference by noise is believed to degrade frequency discrimination performance by bats (Engelstätter et al. 1980; Neumann and Schuller 1991), it follows that the results reported here may reflect a similar degradation in frequency discrimination.

The sensitivity of DSC to the intensity of the returning echo must not create serious problems for horseshoe bats under natural conditions, since they obviously continue to echolocate successfully despite facing undoubtedly large natural fluctuations in echo intensity every day. The reasons for this may be fairly straightforward. First, DSC is known to be biased toward nearer objects rather than more distant objects (Schnitzler 1968; Schuller 1977), and especially so to those objects located directly in front of the bat (Behrend et al. 1999). Normally, the intensity of an echo will depend on the distance of an object

from the bat. The results presented here suggest that a bat would most likely compensate far less for an echo returning from an object farther away than to an object located immediately in front of it. Furthermore, as the bat approaches an object, echo intensity would normally be expected to increase, and concurrently the sensitivity and accuracy of the compensation system should improve. Similarly, echoes returning from the sides of the bat will be less intense and contain less Doppler-effects, making them less likely to trigger DSC. In this way the sensitivity to echo intensity could be viewed as a space filter for compensation behavior. Alternatively, it may also prove to be true that horseshoe bats manage to avoid broad fluctuations in the intensity of the returning echoes by continuously adjusting the intensity of the returning echo. Kobler et al. (1985) reported that Doppler-shift-compensating mustached bats adjusted the intensity of outgoing call emissions when swung on a pendulum, lowering and subsequently raising call intensity as they swung toward and then away from a target. The purpose of intensity compensation was presumed to be maximizing the intensity of the returning echo while avoiding overstimulation of the cochlea, which could lead to unwanted signal distortion. It is certainly possible that intensity compensation also could enhance DSC performance in horseshoe bats by reducing large changes in echo intensity during flight. There may be other reasons why intensity sensitivity has not been rigorously selected against in the Doppler-shift compensation neural circuitry. However, we hope that this may provide an important tool for further studies into the neurophysiology of Doppler-shift compensation behavior in bats.

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