Doppler-shift compensation behavior in horseshoe bats revisited: auditory feedback controls both a decrease and an increase in call frequency

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Summary

Among mammals, echolocation in bats illustrates the vital role of proper audio-vocal feedback control particularly well. Bats adjust the temporal, spectral and intensity parameters of their echolocation calls depending on the characteristics of the returning echo signal. The mechanism of audio-vocal integration in both mammals and birds is, however, still largely unknown. Here, we present behavioral evidence suggesting a novel audio-vocal control mechanism in echolocating horseshoe bats (*Rhinolophus ferrumequinum*). These bats compensate for even subtle frequency shifts in the echo caused by flight-induced Doppler effects by adjusting the frequency of their echolocation calls. Under natural conditions, when approaching background targets, the bats usually encounter only positive Doppler shifts. Hence, we commonly believed that, during this Doppler-shift compensation behavior, horseshoe bats use auditory feedback to compensate only for these increases in echo frequency (=positive shifts) by actively lowering their call frequency below the resting frequency (the call frequency emitted when not flying and not experiencing Doppler shifts). Re-investigation of the Doppler-shift compensation behavior, however, shows that decreasing echo frequencies (=negative shifts) are involved as well: auditory feedback from frequencies below the resting frequency, when presented at similar suprathreshold intensity levels as higher echo frequencies, cause the bat's call frequency to increase above the resting frequency. However, compensation for negative shifts is less complete than for positive shifts (22 % *versus* 95 %), probably because of biomechanical restrictions in the larynx of bats. Therefore, Doppler-shift compensation behavior involves a quite different neural substrate and audio-vocal control mechanism from those previously assumed. The behavioral results are no longer consistent with solely inhibitory feedback originating from frequencies above the resting frequency. Instead, we propose that auditory feedback follows an antagonistic push/pull principle, with inhibitory feedback lowering and excitatory feedback increasing call frequencies. While the behavioral significance of an active compensation for echo frequencies below RF remains unclear, these behavioral results are crucial for determining the neural implementation of audio-vocal feedback control in horseshoe bats and possibly in mammals in general.

Key words: horseshoe bat, *Rhinolophus ferrumequinum*, hearing, echolocation, audio-vocal feedback, Doppler-shift compensation behaviour.

Introduction

While the importance of auditory feedback for vocal learning, particularly in birds, is well documented (Griffin, 1958; Rübsamen and Schäfer, 1990; Janik and Slater, 1997; Okanoya and Yamaguchi, 1997; Doupe and Kuhl, 1999; Leonardo and Konishi, 1999), its role in adulthood is much less understood. In the avian song system, it has recently been demonstrated that auditory feedback can play a major role in the control of song throughout a bird's life (e.g. Okanoya and Yamaguchi, 1997; Woolley and Rubel, 1997; Leonardo and Konishi, 1999). Although the evidence is patchy, among adult mammals, only humans, bats and possibly cetaceans appear to require auditory feedback for the maintenance of basic parameters of species-specific vocalizations (Tyler, 1993; Janik and Slater, 1997; McCowan and Reiss, 1997). In adult humans, for example, modified formants in the playback of a test subject's voice affect the fundamental frequency of her/his vocal utterances (Houde and Jordan, 1998).

Although auditory feedback does not seem to affect vocalizations in various adult non-human primates and in adult cats (Janik and Slater, 1997; Jürgens, 1998), it is essential in bats (Griffin, 1958). Horseshoe bats, for instance, specialize in adjusting the frequency of their calls depending on the pitch of the echo signal. During flight, the dominant constant-frequency component of their distinctive calls is

shifted as a result of Doppler effects. The bats compensate for these shifts by adjusting the frequency of their subsequent calls (Schnitzler, 1968). This ensures that the echo of interest remains within a narrow frequency range stimulating a region of the cochlea innervated by a disproportionately large neuronal population with exceptionally sharp tuning properties, termed the 'auditory fovea' (Schuller and Pollak, 1979) (see Fig. 1). This so-called Doppler-shift compensation (DSC) behavior (Schnitzler, 1968) represents one of the most precise forms of sensory-motor integration known. It has been compared with visual fixation, in which eye movements keep an image of interest centered on the fovea, a region of the retina with densely packed receptors and neurons with small receptive fields (Schuller and Pollak, 1979). DSC behavior can even be elicited in stationary horseshoe bats by presenting echo mimics, i.e. electronically delayed and frequency-shifted playbacks of the bat's own calls (Schuller et al., 1974, 1975). DSC behavior is not limited to horseshoe bats. The Central and South American mustache bat *Pteronotus parnellii* produces echolocation calls that are very similar to those of horseshoe bats and also compensates for Doppler-shifted echoes (Henson et al., 1985; Keating et al., 1994).

For the past three decades, we have commonly believed that in both groups of bats only echo frequencies returning above the bat's resting frequency (RF; i.e. the frequency the bat emits and hears when not flying) affect DSC behavior, causing the bat to lower its vocalization frequency (horseshoe bats, e.g. Schnitzler, 1968, 1973; Schuller et al., 1974, 1975; Simmons, 1974; Metzner, 1989, 1993b, 1996; Tian and Schnitzler, 1997; Pillat and Schuller, 1998; Behrend and Schuller, 2000; mustache bats, e.g. Henson et al., 1985; Suga et al., 1987; Pollak and Casseday, 1989; Gaioni et al., 1990; Suga, 1990; Keating et al., 1994). Echo frequencies returning below the RF, where auditory thresholds are up to 30 dB higher (see Fig. 1), were believed to provide no auditory feedback and only allow the call frequency to return passively to the RF. This appeared plausible since under natural conditions, when horseshoe (or mustache) bats approach a background target, the bats experience only positive Doppler shifts. However, the literature also contains some, though mostly neglected, evidence that echo frequencies below the RF might also drive DSC behavior. Schnitzler himself in his original publication (Schnitzler, 1968) shows a horseshoe bat lowering and raising its call frequency below and above the RF (his Fig. 12), in response to a large ball swinging in front of the bat. Similarly, when Gaioni et al. (1990) tested DSC behavior in mustache bats by swinging the bats on a pendulum, two bats raised their call frequencies by 200–400 Hz above the RF during the backward swing (their Fig. 1). Nevertheless, they state that mustache bats 'did not show DSC on the backswing' (Gaioni et al., 1990). Finally, results from deafening experiments in horseshoe bats suggested that, to maintain RF in normal hearing bats, auditory feedback was required from frequencies not only above but also below the RF (Rübsamen and Schäfer, 1990). Therefore, it appears that the literature does indeed contain some conflicting reports on whether bats compensate for echo frequencies below the RF.

The present study was designed to re-assess the range of echo frequencies eliciting DSC behavior. This information is indispensable in evaluating the circuitry and neural mechanisms for auditory feedback control of DSC behavior.

Materials and methods

Twelve greater horseshoe bats, *Rhinolophus ferrumequinum* (Rhinolophidae, Chiroptera), from the People's Republic of China were trained to compensate for artificially frequency-shifted playbacks of their own echolocation calls. Procedures were in accordance with National Institutes of Health guidelines for experiments involving vertebrate animals and were approved by the local Animal Use and Care Committee. Animals were screened for optimum DSC behavior. Bats were only chosen for the experiments if they consistently vocalized spontaneously and compensated for at least 90 % of the maximum size of positive shift for sinusoidal frequency modulations (0.03 Hz modulation rate, 3 kHz maximum frequency shift). Six bats were used for the experiments following paradigm 1 (see Results) and three for paradigm 2 (which represents a much less natural stimulus condition and is therefore more difficult for the bats). The electronic arrangement for the generation of the frequency-shifted echo mimics followed a design described elsewhere (Schuller et al., 1974, 1975; Metzner, 1993b), modified with custom-built hardware and software devices (see below).

The following gives a brief theoretical outline of how double heterodyning and filtering yields frequency-shifted playback signals (for further details, see Schuller et al., 1974, 1975). In the first heterodyning step, the bat's call is recorded (say call frequency is at 80 kHz) and 'mixed' (electronic multiplication) with one pure-tone signal (say 60 kHz) resulting in two signals, one at 140 kHz (=80+60 kHz) and one at 20 kHz (=80–60 kHz). This output consisting of signals at 20 and 140 kHz is highpass-filtered at 99 kHz, resulting in cancellation of the 20 kHz component. In the subsequent second heterodyning step, the remaining 140 kHz component is then mixed with a second pure-tone signal (say 62 kHz). The outcome is a signal composed of components at 202 kHz and 82 kHz. Lowpass-filtering at 99 kHz cancels the high-frequency component (202 kHz) and transmits the signal at 82 kHz, which is the frequency of the playback signal delivered to the bat. Hence, it simulates an echo that is shifted 2 kHz above the bat's own call frequency. The difference between the first and second pure-tone signals used for heterodyning therefore determines the size of the frequency shift induced in the playback signal. Since each heterodyning step results in two components that are far more than one octave apart (20 *versus* 140 kHz, and 82 *versus* 202 kHz, respectively), they can be easily and reliably separated by filtering.

Call frequency, call amplitude and time course and the size of the induced frequency shift in the echo mimic were analyzed using commercially available signal-analysis statistics

software ('Signal' Engineering Design, Belmont, MA, USA; SigmaStat and SigmaPlot, Jandel Corp., San Rafael, CA, USA).

Experiments were performed in an anechoic chamber (28 °C, >50 % relative humidity) where echoes reflected from the walls were below the noise level of our recording system (i.e. <45 dB SPL). The bats' calls were recorded by a $\frac{1}{4}$-inch ultrasonic microphone and amplifier (Brüel & Kjær; Nærum, Denmark) positioned 15 cm in front of the bat's nostrils, electronically delayed by 4 ms (custom-built delay line), heterodyned (model DS335 function generators, accuracy greater than 0.01 Hz at 80 kHz; Stanford Research Systems, Sunnyvale, CA, USA), high- and subsequently lowpass-filtered (99 kHz each; digital two-channel filter, model SR650, roll-off 115 dB per octave; Stanford Research Systems, Sunnyvale, CA, USA) and then played back *via* a power amplifier (Krohn-Hite, model 7500, Avon, MA, USA) and a condenser-type ultrasonic loudspeaker (Panasonic Inc.; Secaucus, NJ, USA).

The loudspeaker was positioned at a distance of 15 cm from the bat's right or left pinna and at angles of approximately 30° lateral from (azimuth) and 15° below (elevation) the midline, roughly corresponding to the best direction of hearing in these bats (Grinnell and Schnitzler, 1977). Bats could move their head freely. The transfer function of the loudspeaker allowed the delivery of pure-tone pulses of up to 122 dB SPL measured at the position of the bats' pinnae and ±5 kHz around the bats' RFs, which ranged from 76.5 to 78.8 kHz. A spectrographic analysis revealed that the amplitude of harmonics for pure-tone signals in this frequency range was less than 60 dB SPL. Calibration of the playback system was performed with a $\frac{1}{4}$-inch ultrasonic microphone and power amplifier (Brüel & Kjær) using commercial signal-analysis software ('Signal', Engineering Design, Belmont, MA, USA). The frequency and amplitude of the bats' calls were extracted from a custom-built frequency-to-voltage and a.c./d.c. converter, respectively. The accuracy for determining call frequency and amplitude was ±24 Hz and ±3 dB, respectively. Call frequency, call amplitude and time course and the size of the induced frequency shift in the echo mimic were continuously monitored and recorded on video tape using a recording adapter (Vetter 3000A, Rebersburg, PA, USA; sample rate 40 kHz per channel).

Results

Two experimental approaches were chosen, both designed to quantify the effects of auditory feedback from frequencies above as well as below RF on the bats' call frequency. First, we electronically altered the frequency of playbacks of the bat's own vocalizations (=echo mimics) and slowly, sinusoidally modulated them around the bat's RF. Frequency shifts above (positive shifts) or below RF (negative shifts) were generated. In contrast to previous investigations (e.g. Schuller et al., 1974, 1975), however, the echo mimics were delivered at similar sensation levels, i.e. at similar intensities above hearing threshold for both conditions (Fig. 1). The bats'
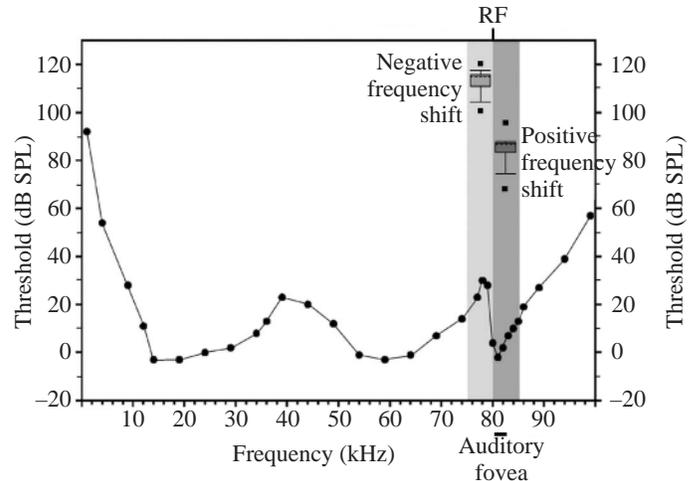

Fig. 1. Behavioral audiogram of the greater horseshoe bat *Rhinolophus ferrumequinum* (Long and Schnitzler, 1975). The resting frequency (RF) is normalized to 80 kHz. The frequency ranges over which Doppler-shift-compensation behavior was tested are indicated in dark (Doppler shifts above RF) and light (Doppler shifts below RF) gray shading. Within these ranges, two examples are given for the intensity ranges tested. The numbers of playback signals analysed for this graph were 512 for positive and 537 for negative Doppler shifts. The lower and upper ends of the boxes indicate the twenty-fifth and seventy-fifth percentile, respectively, with a broken horizontal line at the median. Error bars indicate the tenth and ninetieth percentiles and squares indicate outliers. The frequency range just above RF where thresholds reach very low levels is also referred to as the 'auditory fovea' (short horizontal bar beneath the abscissa) (Schuller and Pollak, 1979). Note that the difference between the medians of the intensity ranges tested for positive and negative Doppler shifts corresponds approximately to the difference in the hearing threshold for these frequency ranges.

compensation performance was then correlated with various modulation depths and rates in the artificial echo. Second, we analyzed the time courses of DSC behavior in response to stepwise shifts in the playback's frequency (see Fig. 3A for details). Although this paradigm represents a rather artificial situation not encountered naturally by the bats, it allows us to analyze better various aspects of DSC behavior, such as its temporal characteristics or the effects of varying echo frequency (Simmons, 1974; Schuller et al., 1975; Schuller and Suga, 1976a). We used this approach to determine how varying the intensity of the echo mimics affected the speed of compensation for positive or negative frequency shifts. If, as we had previously believed, auditory feedback from frequencies below RF has no effect on DSC behavior, bats should not compensate for sinusoidal negative frequency shifts in the first experimental paradigm and the time course of DSC behavior during the negative frequency step in the second set of experiments (see Fig. 3A) should be independent of the playback's intensity.

In the first series of experiments, the rates of sinusoidal change in the playback frequency were 0.1 or 0.03 Hz (depending on the bat's preference) and reached a maximum
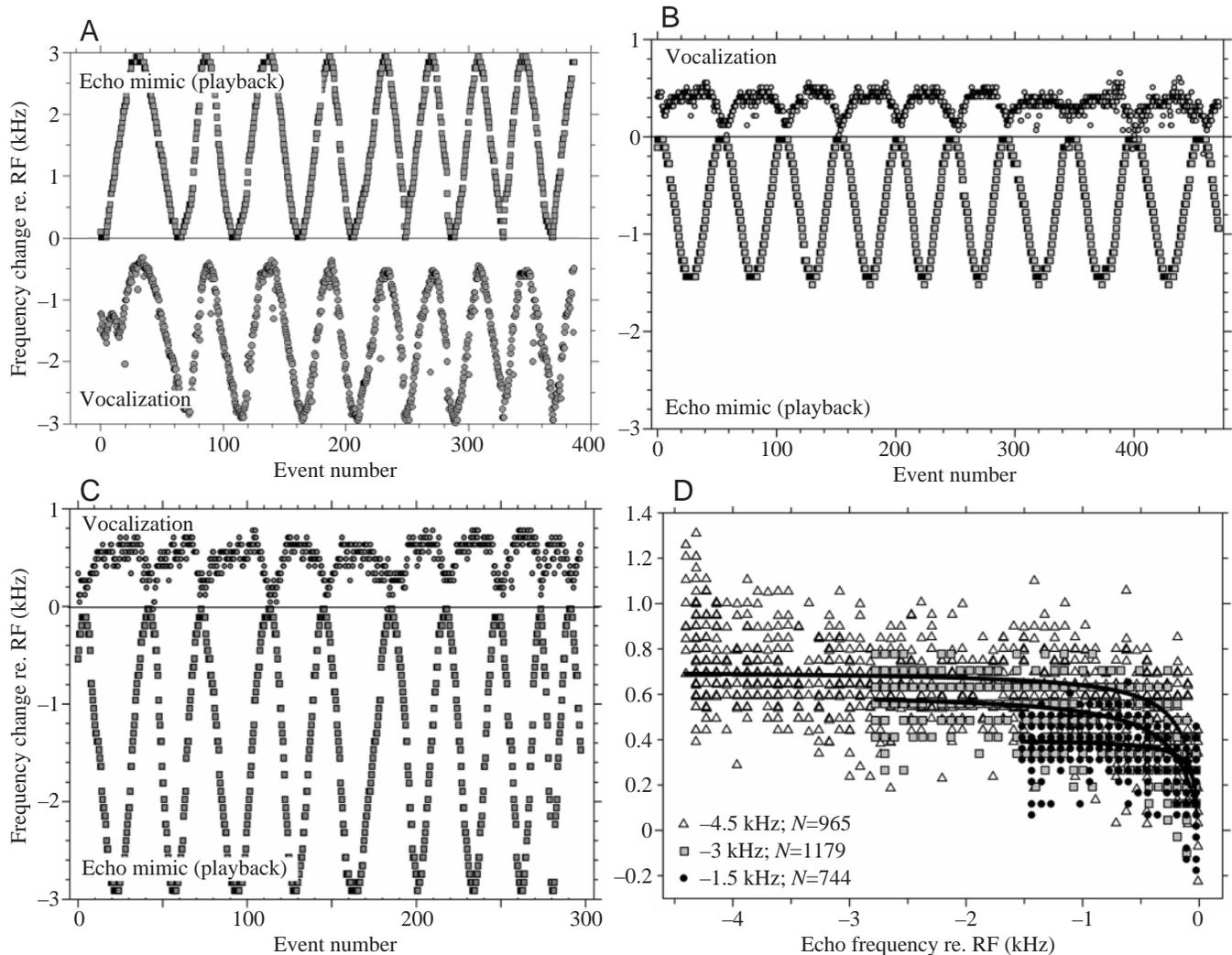
Fig. 2. Doppler-shift compensation behavior in response to sinusoidal negative (below the resting frequency, RF) shifts in the playback frequency. The frequency of the echo mimics was shifted sinusoidally above ('normal' DSC; A) or below the bat's resting frequency ('inverse' DSC; B–D) at intensity levels approximately 60–80 dB above threshold (see Fig. 1). Each parameter combination was tested at least 10 times with at least 10 modulation cycles per bat and experimental session. All data are representative examples obtained from one bat (RF7). (A) Example of 'normal' DSC behavior, i.e. lowering of call frequencies below RF (circles, bottom trace) in response to playback frequencies above RF (squares, top trace). For each vocalization (=event), the call's maximum frequency (circles) and the corresponding frequency shift introduced in the echo mimic (squares) were determined. Maximum frequency shift in the echo mimic, +3 kHz relative to RF; modulation rate, 0.1 Hz, 40 dB attenuation. (B) Example of 'inverse' DSC behavior, i.e. raising of call frequencies above RF (circles, top trace) in response to playback frequencies below RF (squares, bottom trace). Same conventions as in A. Maximum frequency shift in echo mimic, –1.5 kHz relative to RF; modulation rate, 0.1 Hz, 20 dB attenuation. (C) Maximum frequency shift in echo mimic, –3 kHz relative to RF; modulation rate, 0.1 Hz, 20 dB attenuation. (D) Maximum frequencies of calls relative to RF (ordinate) plotted against the corresponding playback frequencies relative to RF (abscissa) for three different maximum frequency shifts (squares, –1.5 kHz; circles, –3 kHz; triangles, –4.5 kHz; N, number of calls analyzed for three bats). Modulation rate, 0.1 Hz, 20 dB attenuation. The three curves are the result of a non-linear regression analysis and are significantly different (Kruskal–Wallis one-way analysis of variance on ranks; $P<0.001$).

shift of 1.5, 3 or 4.5 kHz above or below the bat's RF. The stimulus intensity varied between 0 and 30 dB attenuation relative to the intensity of the bat's call (corresponding to approximately 95 and 115 dB SPL) for frequency shifts below RF and 20 and 50 dB attenuation (65–95 dB SPL) for shifts above RF (see Fig. 1). As expected, all six bats tested in this paradigm compensated in the usual fashion for approximately 95 % of the maximum positive frequency shift (Fig. 2A; see

also Schuller et al., 1974, 1975). Surprisingly, however, these bats also compensated for negative shifts in the frequency of echo mimics (Fig. 2B–D). These changes in the call frequency did not occur randomly but, instead, followed the sinusoidal changes in stimulus frequency (Fig. 2B,C) with a correlation coefficient of greater than 0.65 for all three ranges tested (Pearson product moment correlation; $P\leqslant0.001$). However, compensation for negative shifts was slightly more erratic than

that for positive shifts. While it is difficult to quantify the variability of constantly changing call frequencies emitted in response to sinusoidally modulated playback frequencies, we chose to use the standard deviation relative to the mean call frequency emitted during DSC behavior as a first approximation. We found that, in the typical example for a response to positive shifts (Fig. 2A), it was 33 %; in the responses to negative shifts depicted in Fig. 2B,C, it increased to 53 % and 50 %, respectively.

Another more dramatic difference between compensation for positive and negative shifts was that the bats never fully compensated for negative shifts (Fig. 2B–D). The greatest increase in call frequency observed was +1.51 kHz in response to a –4.5 kHz shift in the artificial echo, and the mean maximum compensation performance was 22.0 % (*N*=500 cycles), 16.9 % (*N*=500 cycles) and 14.9 % (*N*=200 cycles) of the maximum frequency shifts of –1.5, –3 and –4.5 kHz, respectively. Nevertheless, the overall changes in call frequency in response to the three different echo frequency shifts tested were significantly different (Fig. 2D). For comparison, call frequencies emitted at rest by an individual bat show standard deviations of only approximately ±50 Hz, which is less than 0.1 % of RF (Schuller et al., 1974).

In this first experimental paradigm, horseshoe bats compensated only for up to 22 % of the frequency range covered by negative shifts (Fig. 2B–D), whereas they compensated for 95 % of positive shifts (Fig. 2A) (Schnitzler, 1968; Schuller et al., 1974; Tian and Schnitzler, 1997). This asymmetry was not based upon a lack of auditory input from echo frequencies below RF since different modulation depths below RF had significantly different effects on DSC behavior (Fig. 2B–D). Instead, this difference appears to be caused by limitations on the (pre)motor control side, since even electrical stimulation of the superior laryngeal nerve, which is the motor nerve innervating the larynx and controlling call frequency (Schuller and Rübsamen, 1981), was unable to raise call frequencies by more than 1.2 kHz for a stimulation near saturation of the firing rate of the nerve (Schuller and Suga, 1976b). The peculiar mechanics of sound production in the larynx of bats probably causes such a constraint (Suthers and Fattu, 1982): in bats, the precise timing between glottal activity and the activity of the cricothyroid muscle, which is particularly important for producing high-pitched vocalizations, limits the generation of increases in call frequencies.

The results presented in Fig. 2 therefore indicate (i) that, in addition to frequencies above RF (Fig. 2A), those below RF (Fig. 2B–D) also provide auditory feedback for the control of DSC behavior, and (ii) that horseshoe bats can systematically increase their vocalization frequency even above the RF (Fig. 2B–D).

To verify the former point, the time courses were measured for decreases and increases in vocalization frequency during stepwise positive (up to 4.5 kHz above RF) and negative (return to RF) shifts in echo frequency, respectively (Fig. 3A). This paradigm had previously been used to yield important insights into, for instance, the effects of varying step size on the time course of compensation and to compare the speed of compensation for positive with that for negative steps (Simmons, 1974; Schuller et al., 1975; Schuller and Suga, 1976a). These studies demonstrated that compensation became faster with increasing step size and that, for the same absolute intensity level, responses to positive steps were faster than those to negative steps. The results also showed that information about the size of the frequency shift in the last echo heard could be stored for several minutes, being significantly reset only when a new call had been emitted and the corresponding echo signal had been heard at a different frequency (the 'sample-and-hold' analogy of Schuller and Suga, 1976a).

What had been missing so far, however, was information on how varying intensity levels for positive and negative steps affect the speed of compensation. Hence, in our second series of experiments, we tested four different intensities ranging, in steps of 10 dB, from 0 to 30 dB attenuation relative to the bat's own call (corresponding to intensities of approximately 85–115 dB SPL). If, as indicated by the results from our first experimental paradigm (see Fig. 2), both frequency ranges provide auditory feedback, call frequencies during both positive and negative shifts in stimulus frequency should change more rapidly with increasing intensity. This was indeed the case in all three bats tested (Fig. 3C,D; a representative example is given in Fig. 3B). The median time constants for negative shifts shortened from 2.28 s at 30 dB attenuation to 0.89 s at 0 dB attenuation (Fig. 3B,C); for positive shifts, the median time constants shortened from 1.64 s at 30 dB to 0.75 s at 0 dB attenuation (Fig. 3D). While the time courses of responses to positive steps were slightly more variable (S.D. ranging from 1.37 to 0.22 s) than those for negative steps (S.D. between 0.38 and 0.17 s), the trend was nevertheless significant (all pairwise multiple comparison procedure, Dunn's method, *P*<0.05). The speed of DSC responses was directly correlated with the size of the initial change in call frequency: the first call during faster DSC responses to positive steps, for instance, was emitted at lower frequencies than during slower responses (data not shown; see also Schuller, 1986).

Discussion

These results raise two main questions: (i) what is the potential behavioral significance of compensating for both positive and negative frequency shifts and (ii) how does this affect our view of any underlying neural substrates and feedback mechanisms?

It is apparent that horseshoe bats approaching a background target should compensate for flight-induced increases in the echo frequency to maintain echoes within their auditory fovea. But what is the purpose of compensating for negative frequency shifts? Normally, only echoes returning from larger background targets and not those from small prey objects are loud enough to elicit DSC behavior (e.g. Schnitzler, 1968, 1973; Trappe and Schnitzler, 1982). Thus, it has commonly
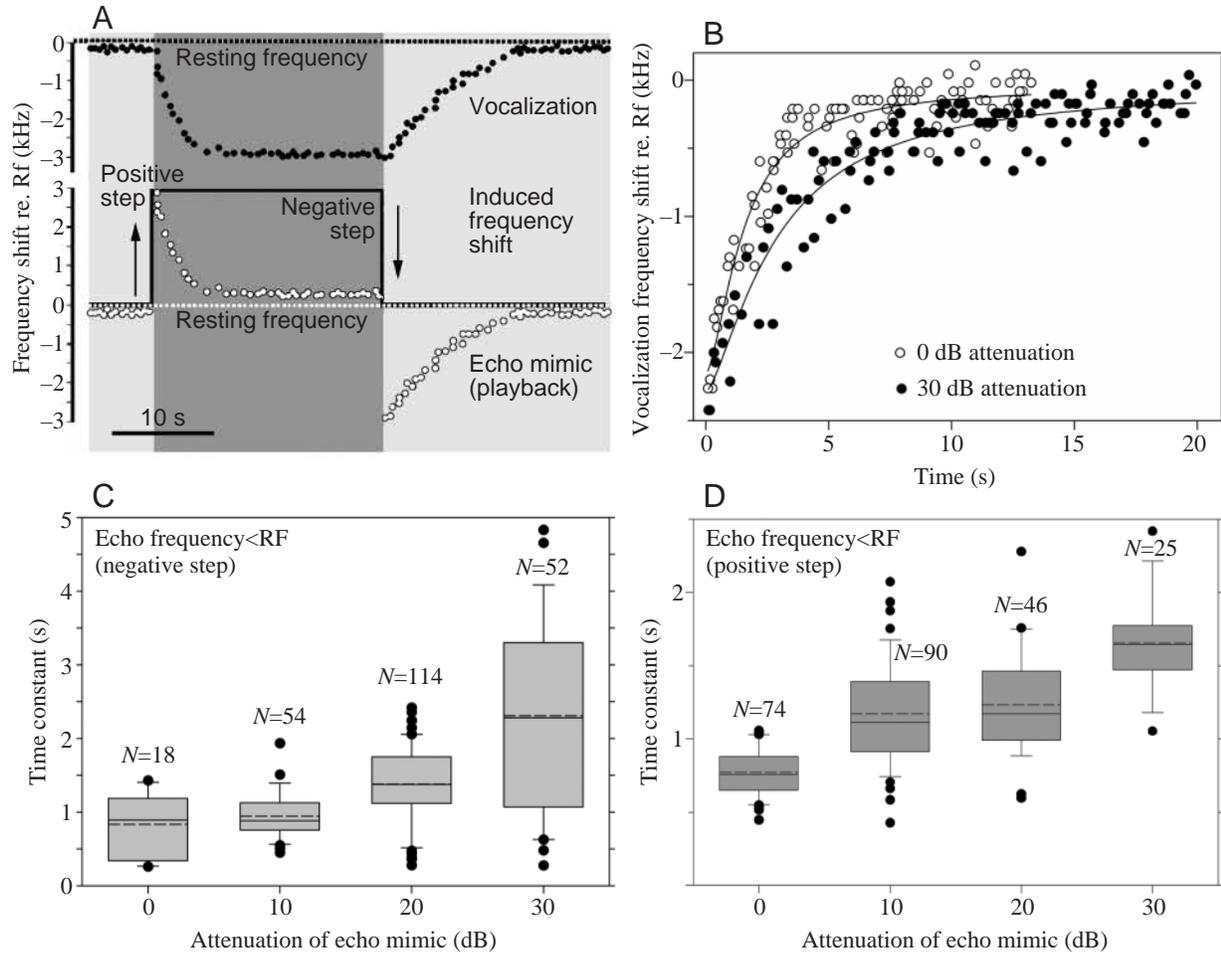
Fig. 3. Doppler-shift compensation behavior in response to stepwise changes in the frequency of echo mimics. (A) Time courses of the frequency shift in echo mimics (white circles, bottom trace) and corresponding call frequencies (black circles, top trace; only the highest frequency measured in each call is given). An initial positive shift in playback frequency causes playback frequencies to rise above the resting frequency (RF). Call frequencies are therefore lowered below RF. The subsequent negative step back to zero shift causes playbacks to return at frequencies below RF (since call frequencies are still below RF). Consequently, the bat increases its call frequencies. Each step was maintained for up to 30 s until the bat had reached its compensation frequency. At least 10 repetitions of each parameter combination were presented per bat and session. Time constants were determined by measuring the time until the call frequency had changed by 67 % in response to positive and negative shifts in stimulus frequency. (B) Time courses of call frequency increases (3 kHz above RF) in response to negative steps in playback frequency to zero shift for two different attenuations (bat dsb6). Each symbol represents the maximum frequency in one call. Louder playback signals (0 dB attenuation, open circles; N=226) cause call frequency to increase faster than weaker playback signals (30 dB attenuation, filled circles; N=103). The difference between the two conditions is significant (all pairwise multiple comparison procedure, Dunn's method, $P<0.05$). Frequency shifts of 1.5 kHz and 4.5 kHz above RF yielded similar results (not shown). (C) Mean time constants for call frequency increases analyzed in all three bats tested in response to negative steps in playback frequency. N is the number of time constants analyzed. The numbers of calls analyzed for each condition were 366 (0 dB), 354 (10 dB), 292 (20 dB) and 169 (30 dB). Significant differences exist for 0 dB *versus* 20 dB and 30 dB, for 10 dB *versus* 20 dB and 30 dB and for 20 dB *versus* 0 dB, 10 dB and 30 dB (all pairwise multiple comparison procedure, Dunn's method, $P<0.05$). For an explanation of the box and whisker plots, see Fig. 2. (D) Mean time constants for lowering of call frequency in response to positive steps in playback frequency. Same conventions as in C. The numbers of calls analyzed for each condition were 401 (0 dB), 278 (10 dB), 332 (20 dB) and 175 (30 dB). Significant differences exist for 0 dB *versus* 10 dB, 20 dB and 30 dB, for 10 dB *versus* 0 dB and 30 dB and for 20 dB *versus* 0 dB and 30 dB (all pairwise multiple comparison procedure, Dunn's method, $P<0.05$).

been believed that only frequencies above RF are encountered naturally (Schnitzler, 1968; Schuller et al., 1974, 1975; Schuller and Suga, 1976b; Schuller, 1986; Metzner, 1989, 1993b; Tian and Schnitzler, 1997; Pillat and Schuller, 1998). Although some of the data originally describing DSC behavior in horseshoe and mustache bats indeed showed that these bats

could also compensate for negative shifts (see Fig. 12 in Schnitzler, 1968; see Fig. 1 in Gaioni et al., 1990), this observation had soon been discounted. This failure to notice the importance of feedback from echo frequencies below RF did not change when results from deafening experiments demonstrated that the RF of deaf horseshoe bats changed

'unsystematically, and some even nearly maintain the presurgical values' (Rübsamen and Schäfer, 1990). The authors suggested that, to maintain RF, auditory feedback was required not only from frequencies above but also from those below RF since the absence of negative feedback only from frequencies above RF should have caused deafened bats to produce call frequencies that were different from the pre-deafened value.

However, there are some circumstances during normal echolocation behavior when echo frequencies could return below the RF and elicit compensation behavior. For instance, during final target approach, such as before landing on a cave wall, flight speed is gradually reduced, which causes echo frequencies to fall below RF as a result of 'overcompensation' by the bat. Hence, bats start to increase their call frequencies. During these final approach stages, calls are still emitted at very high levels of approximately 120 dB SPL, and thus echo intensities also remain high (Tian and Schnitzler, 1997). Even when adding a transmission loss of up to 20 dB for the corresponding target distances (Lawrence and Simmons, 1982), these calls generate echoes returning at least 70 dB above the auditory threshold for these frequencies (Fig. 1, light gray area). This corresponds to intensities that also elicit the lowering of call frequencies in response to positive Doppler shifts (Schuller et al., 1974) (Fig. 1, dark gray area). Active compensation for these negative frequency shifts during final target approach would enable the bat to increase its call frequency faster, and thus more efficiently, than with a purely passive mechanism (Schuller, 1986).

Another instance when horseshoe bats might experience echo frequencies shifting below RF is during somersault landings, which they quite frequently perform (W. M. and S. Z., personal observations). During such flight maneuvers, the position of the bat's head and ears changes rapidly relative to a stationary background, such as a cave wall, and this might be sufficient to induce small negative Doppler shifts. However, in the absence of any documented echo signals recorded during natural flight maneuvers in Doppler-compensating bats, these scenarios have to be considered speculative.

What are the consequences for the neural substrates and sensory feedback mechanisms involved in controlling DSC behavior? The observation that horseshoe bats actively compensate for both positive and negative shifts in echo frequency suggests that DSC behavior is not controlled by a unidirectional audio-vocal feedback mechanism, as has been assumed over the past three decades (Schnitzler, 1968, 1973, 1986; Schuller et al., 1974, 1975; Simmons, 1974; Schuller and Suga, 1976b; Metzner, 1989, 1993b; Tian and Schnitzler, 1997; Pillat and Schuller, 1998). Since echo frequencies below RF also elicit DSC behavior, one can no longer assume that only populations of neurons tuned to frequencies above RF are potential candidates for audio-vocal interfaces (Metzner, 1989, 1993b, 1996; Pillat and Schuller, 1998; Behrend and Schuller, 2000). Neurons tuned to frequencies below RF obviously play a role as well.

More importantly, however, the findings described here revise our current understanding of the audio-vocal feedback mechanism that controls DSC behavior. Previously, a single inhibitory (Metzner, 1989, 1993b, 1996; Pillat and Schuller, 1998; Behrend and Schuller, 2000) or excitatory feedback mechanism was considered to be sufficient to account for the lowering of call frequencies in response to positive Doppler shifts. However, the active response to both positive and negative Doppler shifts (Figs 2, 3) suggests that a single inhibitory or a single excitatory feedback mechanism is insufficient. This is illustrated in Fig. 4. The motor command for generating different call frequencies appears to be the same in all mammals studied so far, including humans (Fig. 4A). As indicated by a white arrow, lower vocalization frequencies (VF_1 in Fig. 4A) are caused by a lower level of activity of the motor output, e.g. the superior laryngeal nerve (see the corresponding motor activity level MA_1 in Fig. 4A) (Schuller and Suga, 1976b; Schuller and Rübsamen, 1981; Yajima and Hayashi, 1983; Larson et al., 1987). Conversely, higher pre-motor activity (MA_2) generates higher call frequencies (VF_2), as shown by a black arrow. Any sensory feedback mechanism must ultimately conform to this relationship, i.e. sensory information about different echo frequencies must converge at the level of the sensory-motor interface in such a way as to allow the motor pattern described above to be generated in response.

The three simplest scenarios for such an integration of echo frequencies and the resulting auditory feedback control of call frequencies during DSC behavior are depicted in Fig. 4B–D. Generally, during DSC behavior, echo frequencies above RF (such as EF_1 in Fig. 4B–D; white arrows) generate lower vocalization frequencies (VF_1 in Fig. 4A), as is seen in any 'normal' DSC behavior (see Fig. 2A). However, auditory feedback from frequencies below RF (EF_2 in Fig. 4B–D; black arrows) produces call frequencies above RF (VF_2 in Fig. 4A), as we have shown in Fig. 2B–D ('inverse' DSC behavior).

Let us now consider how these different echo frequencies above and below RF (EF_1 and EF_2) yield call frequencies below and above RF, respectively, assuming that a purely inhibitory feedback mechanism is at work at the level of the sensory-motor interface (Fig. 4B). We had originally suggested this scenario largely on the basis of neurophysiological data (Metzner, 1989, 1993b). First, let us look at echo frequencies above RF, such as EF_1 in Fig. 4B. We know that they lower call frequencies (such as VF_1 in Fig. 4A) and we also know that a lowering of call frequency requires a decrease in motor activity (Fig. 4A, white arrow). Assuming purely inhibitory feedback, reduced motor activity can be caused only by inhibition that is stronger than at rest. The corresponding echo frequencies above RF can create such stronger inhibition only when sensory activity levels increase with increasing echo frequencies (white arrow in Fig. 4B). Conversely, lower echo frequencies (black arrow in Fig. 4B) exhibit a lower level of sensory activity, leading to less inhibition of the motor side and thus causing call frequencies to rise (black arrow in Fig. 4A).

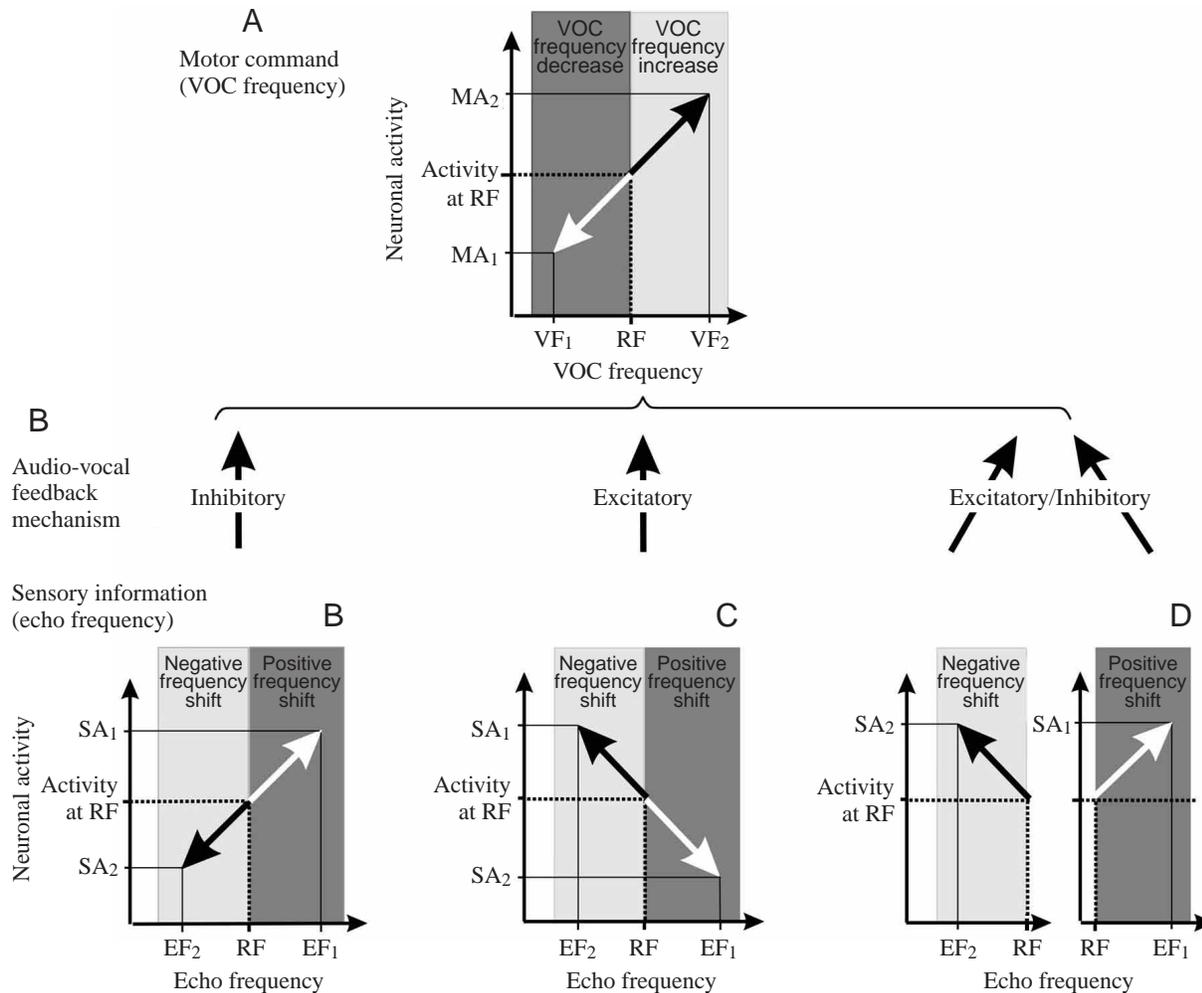If we assume instead an all-excitatory feedback mechanism

Fig. 4. Effects of different audio-vocal feedback mechanisms on Doppler-shift compensation (DSC) behavior. Sensory information about different echo frequencies (B–D) is translated into motor activity that generates the corresponding call frequencies (A) using a purely inhibitory (B), all-excitatory (C) or combined excitatory/inhibitory (D) audio-vocal feedback mechanism. Note that the discussed non-linearity in the motor control system limiting call frequency increases (see Fig. 2; see also Schuller and Suga, 1976b; Suthers and Fattu, 1982) has been omitted for clarity. (A) In the motor nerve controlling the frequency of sound production by the larynx, vocalization (VOC) frequencies below resting frequency (RF) (VF_1; white arrow), such as during 'normal' DSC behavior (see Fig. 2A), are caused by a level of motor activity (MA_1) that is lower than that generated at RF. Conversely, call frequencies above RF (VF_2; black arrow), such as during 'inverse' DSC behavior (see Fig. 2B–D), require a level of motor activity (MA_2) that is above the resting value. (B–D) Illustrations of the three basic scenarios for how different sensory activity levels (SA_1 and SA_2) that are caused by echo frequencies above RF (EF_1; white arrows) or below RF (EF_2; black arrows) have to be integrated in a sensory-motor interface to yield the appropriate motor commands that ultimately lower and raise call frequencies (as shown in A).

(Fig. 4C), the relationship between varying echo frequencies and the resulting changes in sensory activity levels simply have to be reversed to yield the appropriate motor commands (Fig. 4A).

Audio-vocal feedback control during DSC behavior inevitably requires convergence and pooling of frequency information from all frequency channels involved. As a corollary, firing rates in neurons integrating this frequency information become ambiguous: their level of activity is determined by the size of the frequency shift (as deduced above) but also, much as in individual auditory neurons, by the intensity of the echo. Hence, during auditory feedback control, frequency information is at least to a certain degree traded for

intensity information and *vice versa*. This is essential when analyzing the effects we observed while varying intensity levels during stepwise changes in echo frequency (see Fig. 3) in the light of an all-inhibitory (Fig. 4B) or a purely excitatory (Fig. 4C) feedback mechanism. These experiments demonstrated that after both positive and negative steps higher echo intensities caused call frequencies between successive calls to change faster, resulting in shorter time constants of the DSC responses (Fig. 3B,C). However, neither a purely inhibitory nor a purely excitatory scenario is consistent with these results, as outlined below.

If auditory feedback control were purely inhibitory, as assumed in Fig. 4B, louder echoes at any frequency (above or

below RF), by pushing the sensory activity to higher levels, would result in stronger inhibition of the motor side over the entire frequency range (below and above RF, respectively). An overall stronger inhibitory effect on the motor side, however, had opposite consequences on the raising and lowering of call frequencies: louder echoes at frequencies above RF would cause call frequencies to drop faster because of stronger inhibition (in Fig. 4A, the new motor activity level would fall below MA$_1$). Louder echoes below RF, however, which also exert more inhibition on the motor side, would result in a slower rise in call frequency (see Fig. 4A: the new motor activity level would fall below MA$_2$ as well). This, however, is contradicted by our experimental results (Fig. 3B,C). Our results are also inconsistent with a purely excitatory feedback mechanism (Fig. 4C), which predicts that louder echoes below RF should accelerate the DSC response whereas echoes above RF should slow it down.

Only an antagonistically acting control mechanism, combining excitatory and inhibitory feedback as depicted in Fig. 4C, is fully consistent with our experimental results. In such a 'push/pull' operational mode, inhibitory feedback would originate from a neuronal population encoding for positive Doppler shifts (Fig. 4C, right portion) and excitatory feedback from another neuronal population encoding for negative Doppler shifts (Fig. 4C, left portion). Hence, call frequencies would decrease and increase, respectively, by modulating the motor activity around an intermediate level corresponding to the RF (Fig. 4A).

Indeed, our recent results from pharmacological studies indicate that a small brainstem area controls DSC behavior *via* such an antagonistically acting mechanism utilizing inhibitory feedback from frequencies above RF mediated by γ-aminobutyric acid (GABA$_A$) and excitatory feedback from frequencies below RF mediated by glutamate (AMPA) (Smotherman and Metzner, 2000).

'Push/pull' operational modes appear to control a variety of behaviors, such as compensatory eye movements in both vertebrates and invertebrates (Moschovakis et al., 1996), antagonistically acting motor outputs during various forms of locomotion (Stein et al., 1997; Shaw and Kristan, 1999) and an electromotor behavior related to orientation and object detection in weakly electric fish, the 'jamming avoidance response' in *Eigenmannia* (Metzner, 1993a). DSC behavior might therefore reflect general principles of sensory-motor control of motor outputs. It may even share basic aspects with audio-vocal feedback controlling the pitch of vocal utterances in other mammals (Janik and Slater, 1997; McCowan and Reiss, 1997), including the involuntary response to 'pitch-shifted feedback' in humans (Burnett et al., 1998; Houde and Jordan, 1998; Jones and Munhall, 2000). However small a potential adaptive advantage of compensating for negative frequency shifts might have been, it is evolutionarily probably more appropriate to consider that, as long as maintaining a presumably universal neural basis did not place the system at any disadvantage, there was also no selective pressure acting to eliminate it.

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