

RESEARCH ARTICLE

Perception of echo delay is disrupted by small temporal misalignment of echo harmonics in bat sonar

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SUMMARY

Echolocating big brown bats emit ultrasonic frequency-modulated (FM) biosonar sounds containing two prominent downward-sweeping harmonics (FM1 and FM2) and perceive target distance from echo delay. In naturally occurring echoes, FM1 and FM2 are delayed by the same amount. Even though echoes from targets located off-axis or far away are lowpass filtered, which weakens FM2 relative to FM1, their delays remain the same. We show here that misalignment of FM2 with FM1 by only 2.6 μs is sufficient to significantly disrupt acuity, which then persists for larger misalignments up to 300 μs. However, when FM2 is eliminated entirely rather than just misaligned, acuity is effectively restored. For naturally occurring, lowpass-filtered echoes, neuronal responses to weakened FM2 are retarded relative to FM1 because of amplitude-latency trading, which misaligns the harmonics in the bat's internal auditory representations. Electronically delaying FM2 relative to FM1 mimics the retarded neuronal responses for FM2 relative to FM1 caused by amplitude-latency trading. Echoes with either electronically or physiologically misaligned harmonics are not perceived as having a clearly defined delay. This virtual collapse of delay acuity may suppress interference from off-axis or distant clutter through degradation of delay images for clutter in contrast to sharp images for nearer, frontal targets.

Key words: echolocating bat, biosonar, echo delay, clutter interference, FM harmonics, echo spectrum, lowpass filtering.

INTRODUCTION

The biosonar sounds of big brown bats, *Eptesicus fuscus* (Chiroptera: Vespertilionidae), are frequency modulated (FM) and contain several harmonics, the most prominent being the first (FM1; sweeping down from ~55 to 22 kHz) and the second (FM2; sweeping down from ~105 to 45 kHz) (Saillant et al., 2007; Surlykke and Moss, 2000). The bat's signals contain these harmonics when target classification or echo discrimination takes place and during interception, when accurate localization is necessary for tracking (Ghose and Moss, 2003; Ghose and Moss, 2006; Simmons et al., 1995). Furthermore, recordings of sonar signals actually impinging on a suspended target confirm that the sounds contain these harmonics throughout aerial interception maneuvers (Saillant et al., 2007). The presence of harmonics widens the effective bandwidth of the transmitted signals (Simmons and Stein, 1980), which increases the sharpness of biosonar images (Simmons et al., 1998; Simmons et al., 2004) and enhances the bat's ability to perceive objects close to background clutter (Siemers and Schnitzler, 2004).

There is new evidence that the harmonic structure of biosonar sounds may play a more specific role in echo processing than that which is evident from bandwidth considerations alone. Across the 22 to 105 kHz band encompassing both FM1 and FM2, sound velocity is constant, so frequencies in both harmonics arrive from a target at the same delay. That is, harmonics in naturally occurring echoes are coherent, with an exact 2:1 ratio of frequencies (FM1:FM2) from moment to moment throughout the FM sweep. When trained to discriminate electronically generated 'normal' echoes in two-choice tests, big brown bats exhibit a delay acuity of

approximately 50 μs (Moss and Schnitzler, 1995; Simmons et al., 1995). However, in new two-choice experiments with split-harmonic echoes, the bat's echo delay acuity is hugely disrupted – it deteriorates to approximately 800 μs – if the principal harmonics, FM1 and FM2, are deliberately misaligned in time by 300 μs or altered in relative strength so that neuronal responses to the harmonics are misaligned as a result of amplitude-latency trading (Bates and Simmons, 2010; Stamper et al., 2009).

In the present paper we measure the size of the smallest temporal misalignment of FM2 relative to FM1 that causes the big brown bat's delay acuity to undergo the severe deterioration that is already known to occur for a relatively large 300 μs harmonic misalignment (Stamper et al., 2009). Using electronically generated split-harmonic stimuli, the time separation between FM2 and FM1 was decreased from 300 to 0 μs to determine the threshold for disruption of echo-delay perception. There are two possibilities for how the disruption of acuity will be manifested. First, if the disruptive effect builds up gradually as the time interval between harmonics is increased, and even then only occurs for large harmonic misalignments (e.g. 300 μs), it may not be particularly significant under ordinary conditions of echolocation because acoustic propagation in air cannot dissociate harmonics even by much smaller amounts. (The velocity of sound across the bat's echolocation frequencies is constant.) Second, if the disruption of delay acuity occurs abruptly for temporal misalignments much smaller than 300 μs, it may prove to be an important aspect of echo processing, even under natural conditions. Although harmonic misalignment cannot occur acoustically, it could occur internal to the bat's auditory system.

Physiological mechanisms that retard neuronal response latencies (amplitude-latency trading) will create a wide range of different-sized temporal misalignments of responses evoked by FM2 relative to FM1 because FM2 is normally attenuated more than FM1 in many echoes.

Many bats regularly fly and forage amidst clutter, such as dense vegetation, in conditions where the ability to perceive the space immediately to the front is crucial not only to locate targets but also to be sure no obstacles occupy this space (Jensen et al., 2001; Moss et al., 2006; Schnitzler et al., 2003; Siemers and Schnitzler, 2000; Siemers and Schnitzler, 2004; Simmons, 2005; Simmons et al., 2001). Echoes from surrounding clutter typically arrive at the same time as echoes from potential targets or obstacles located in the frontal zone and have to be classified as clutter. In an experiment investigating what is perhaps the worst case of dense, range-extended clutter, big brown bats were flown in an obstacle array made of large numbers of plastic chains hanging in rows from the ceiling of a flight room (Hiryu et al., 2010). The chain array was dense enough to require the bat to emit sounds at short intervals to update its images, and deep enough that echoes of each broadcast continued to arrive after the next broadcast was sent out. When all of the echoes from the first broadcast had not yet returned but the second sound was emitted anyway, the bat encountered potential pulse-echo ambiguity, defined as difficulty in determining which echoes belong to which emissions. Big brown bats solve this problem – that is, disambiguate the echoes to associate them with the corresponding emissions – by making slight changes of several kilohertz in the ending FM1 frequency of successive broadcasts. This subtle difference between successive broadcasts (only a few percent of the total 80-kHz frequency band of the sounds) causes proportionately much larger changes in the time-frequency spectrograms of their echoes, which the bats evidently used to group the echoes with the correct broadcasts. From this result, the hypothesis for the experiments reported here is that big brown bats will be sensitive to disparities introduced into the time-frequency pattern of echo spectrograms. The simplest way to test whether bats are sensitive to small disparities in the spectrograms of echoes is to deliberately misalign the harmonics (separating FM1 and FM2 by controlled time intervals) and then to measure the size of the smallest misalignment that the bat can just perceive. The particular behavioral effect is, however, not whether bats can detect different degrees of misalignment, but the effect of misalignment on the acuity of echo-delay discrimination.

MATERIALS AND METHODS

Subjects

Four adult big brown bats (*Eptesicus fuscus* Palisot de Beauvois 1796; three males and one female) were wild-caught in Rhode Island with a permit from the state's Department of Environmental Management. They were housed in individual cages at 22–23°C and 50–60% relative humidity with free access to vitamin-enriched water. The day–night cycle of the room was reversed to 12h:12h dark:light so experiments could be conducted during the daytime. Bats' weights were maintained between 14.5 and 15.5 g by adjusting the number of mealworms (*Tenebrio* larvae) that they were fed daily. Experiments complied with NIH Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985). Experimental procedures were approved by the Brown University Animal Care and Use Committee.

Procedure

The split-harmonic echo-delay discrimination experiment was run in a 4.0×3.0×2.5 m room with panels of sound-absorbent foam

(SONEX[®], Pinta Acoustic, Minneapolis, MN, USA) lining the floor and walls. Light levels were kept dim (~10 lux) during experimental trials to avoid disturbing the bats. The equipment for producing the electronic echo stimuli was located in an adjacent room. All trials were run double-blind; there were no visual cues available to the bats or to the trainers. The bats' task was to discriminate between electronically generated echoes that differed in arrival time while their harmonic structure was manipulated.

Each bat was trained to sit on an elevated Y-platform (Fig. 1) and broadcast its echolocation sounds toward two ultrasonic microphones (Brüel and Kjør model 4138 1/8-inch condenser microphones, Nærum, Denmark), one located on each end of the platform. The signals picked up at these microphones were used to generate the echoes that served as stimuli for the experiments. Microphones were mounted 20 cm away and separated by 40 deg. Echolocation sounds emitted by the bat were picked up by the microphones, filtered and delayed electronically and then delivered back to the bat from small electrostatic loudspeakers 15 mm in diameter (RCA, model 112343, Hauppauge, NY, USA). These speakers were mounted next to the microphones at the ends of both arms of the platform, 20 cm away from the bat and 50 deg apart. The stimuli delivered to the bat were delayed by 1160 μs through the combined acoustic paths from bat to microphone and from the loudspeaker to the bat, plus electronic delays that varied from 2000 to 2800 μs according to conditions.

The bat was rewarded with a piece of mealworm for walking down the arm of the Y-platform corresponding to the loudspeaker that delivered the positive stimulus (S+; see below), which was presented on either the left or right side in a pseudorandomized sequence (Gellerman, 1933). If the bat made an incorrect response, i.e. to the negative stimulus (S–; see below), a broadband sound was made to signal to the bat that it made an error, and a 5-s pause in the experiment ensued. All trials were run using a double-blind procedure. Two experimenters were present while each bat was run: a trainer who handled the bat and was blind to the position of the correct choice, and a recorder who controlled which loudspeakers generated the stimuli and recorded the bat's response. The recorder observed the bat using a black and white CCD video camera (Supercircuits, Inc., Type 166 15-CB22-1, Austin, TX, USA) mounted on the ceiling above the Y-platform. Illumination for the camera was provided by two infrared LED panels (Supercircuits, Inc.) located on either side of the video camera. The recorder was able to monitor the bat's performance on a Sony digital 8-mm video Walkman[®] recorder (New York, NY, USA) located behind the trainer and the Y-platform.

Sets of 50 experimental trials were conducted each day, with a total of 3 days for every condition. Each bat thus completed 150 trials over 3 days for each stimulus condition, for which the percentage of correct responses was recorded. All data were analyzed using binomial probability tests, which are the appropriate way to process data from independent-trial, two-alternative forced-choice experiments such as those used here (SPSS v. 16.0, Chicago, IL, USA).

Electronic stimuli

The two-channel electronic target-simulator system has been described fully elsewhere (Bates and Simmons, 2010) [see fig. 4 in Stamper et al. (Stamper et al., 2009)]; no changes were made in the acoustic or electronic components for these new experiments. The stimuli were electronically generated echoes of the bat's own biosonar broadcasts. They differed in delay, which was regulated by digital electronic delay lines, and in harmonic structure, which

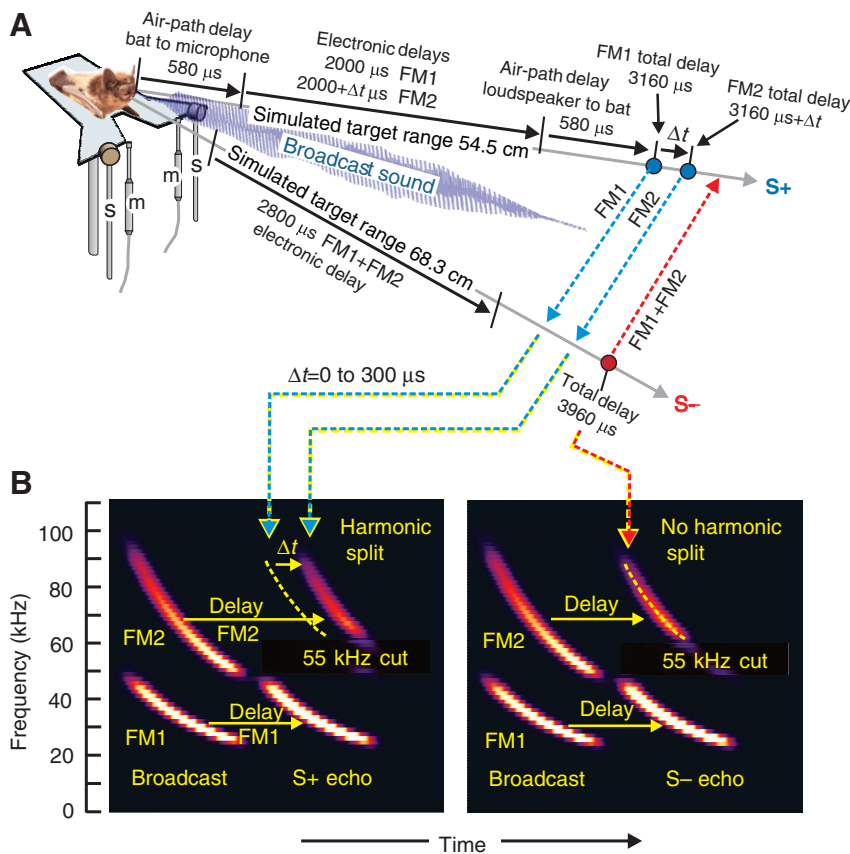


Fig. 1. Experimental method for assessing bats' perception of delay for split-harmonic echoes. (A) Diagram of a two-choice procedure, with the bat on a Y-shaped platform. An electronic target simulator picks up bat signals at the microphones (m), filters them to segregate the two harmonics and returns them as delayed echoes from loudspeakers (s). (B) Representative spectrograms show the bat's broadcast containing harmonics FM1 and FM2 with echo for S+ (left) and S- (right). Harmonics are split (FM1 by lowpass filters at 44 kHz and FM2 by highpass filters at 66 kHz), leaving a narrow band around 55 kHz cut out of both FM1 and FM2. For S+, the simulator delivers FM1 and FM2 at different delays, with small steps of added delay (Δt) from 0 to 300 μ s for FM2 (dashed yellow curve on S+ echo). For S-, the simulator delivers FM1 and FM2 together at the same delay even though the harmonic split is present. The relatively large 800 μ s delay difference between S+ and S- is the stimulus feature to be discriminated; the experiment measures the effects of different-sized harmonic misalignments (Δt) on performance.

was regulated by electronic filters in series with the delay lines (see below). The basic procedure was to present bats with both S+ and S- that were designed to incorporate split-harmonic echoes into an ordinary two-choice echo-delay discrimination protocol. For all conditions, S- consisted of a single-glint echo at a specific delay. (A single-glint echo is a single reflected replica of the broadcast at a specific delay. A two-glint echo contains two reflected replicas at slightly different delays.) S+ for the experimental condition consisted of a split-harmonic single-glint echo with varying amounts of time separation between the harmonics. S+ for the control condition was a two-glint echo with the first glint at the same delay as FM1 in the split-harmonic condition and the second glint at the same delay as FM2. Thus, experimental data were collected using time separations between FM1 and FM2 that could be compared with data collected using the same time separations between two glints. Although two-glint echoes commonly occur (Simmons and Chen, 1989), split-harmonic echoes would not occur in nature because the velocity of sound is the same at all bat frequencies. Nevertheless, electronic manipulation of harmonic delays is used here as a tool to examine the bats' sensitivity to disruption of echo spectrograms on the time-frequency plane. Bats perceive the delay for each glint in a two-glint echo for separations larger than 2 μ s (Simmons et al., 1998), but the errors bats make in this task are confined to a narrow span of delays close to the glint delays themselves. In split-harmonic experiments (Bates and Simmons, 2010; Stamper et al., 2009), bats make errors over a wide span of delays extending hundreds of microseconds away from each harmonic delay. For this reason, in the present study two-glint stimuli were used as control stimuli so that all the effects of electronic delays, filtering and simulation of echoes from loudspeakers could be combined to create data with which to assess the added effects of the harmonic misalignment.

The stimuli are best described in terms of changes imposed on the signals between their being picked up by the microphones and their being broadcast back to the bat from the loudspeakers. After being picked up by the left and right microphones (Fig. 1), the bats' signals passing through the left and right simulator channels were highpass (HP) filtered at 20 kHz (Rockland model 442 variable electronic filters; 36 dB octave⁻¹; San Diego, CA, USA) and lowpass (LP) filtered at 90 kHz (Stewart model vbf-8 variable electronic filter; 48 dB octave⁻¹; Beckenham, Kent, UK) to set the overall boundaries of the echo band delivered to the bat and to remove low-frequency background noise. In each simulator channel, these filtered signals were then delayed by specially built digital delay lines (1.3- μ s delay steps, 10-bit digitizing accuracy from analog input to digital delay and back to analog output). There were two parallel electronic delay lines in each simulator channel (left and right). The output of one was HP filtered and the output of the other was LP filtered (Rockland model 753A variable electronic filters; 112 dB octave⁻¹). The delayed HP and LP filtered signals in each simulator channel were then summed by an analog mixer, again LP filtered at 90 kHz (Rockland model 442 variable electronic filters; 36 dB octave⁻¹), and finally delivered to the left and right loudspeakers (RCA type 112343), which returned them to the bat as acoustic echoes.

The stimuli in the experimental and control conditions differed in the delay settings of the two delay lines in each simulator channel and in the HP and LP filter frequencies. In the experimental condition (split-harmonic S+; Fig. 1B), the LP filter in each simulator channel was set to 44 kHz to select only FM1 from the signals whereas the HP filter was set to 66 kHz to select only FM2. A narrow frequency band around 55 kHz was removed (cut) entirely from both S+ and S- to eliminate frequencies at which FM1 and FM2 might overlap. For experimental S+, the overall delay of the filter-selected FM1

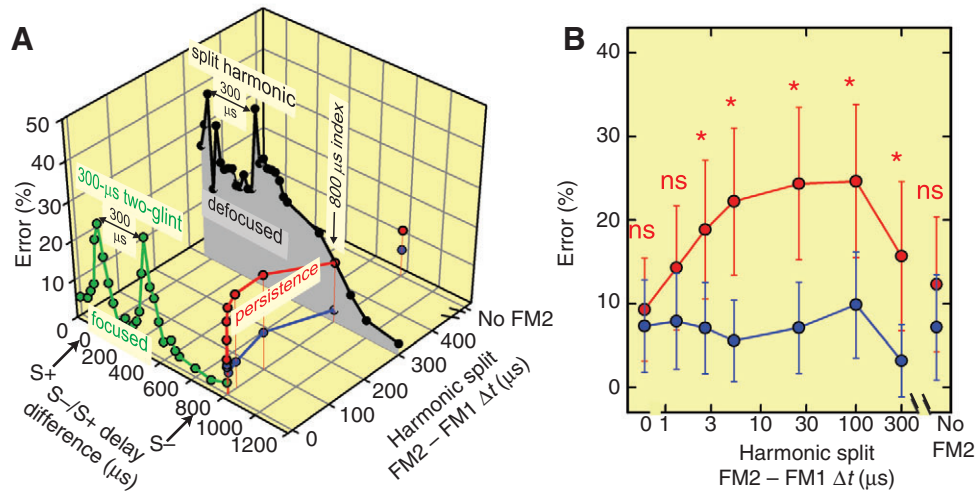


Fig. 2. Image defocusing caused by harmonic misalignment. (A) Explanation of defocusing. Green 300 μ s two-glint curve shows percent errors in delay-discrimination tests with S+ as a two-glint echo with 300 μ s glint separation and S- as a one-glint echo at different delays relative to both glints in S+. Discrete error peaks at 0 and 300 μ s show that bats separately perceive the delay of each reflection in the two-glint echo. The gray shaded area shows percent errors with S+ as a split-harmonic echo having FM2 at a 300 μ s longer delay than FM1. Again, S- is a one-glint echo presented at different delays to probe for perceived delays in S+. Error peaks at 0 and 300 μ s show that the bats separately perceive the delay of each harmonic (FM1 at 0 μ s, FM2 at 300 μ s), but these peaks appear as modulations on a broad pedestal of errors (gray area) stretching from 0 to >800 μ s. The pedestal of errors illustrates a blurred or defocused delay image, whereas the well-defined peaks in the two-glint curve (green) illustrate a focused image that resolves the two glints. In the present experiments, the bats' performance at a normally easily delay-discriminated difference of 800 μ s between S+ and S- is used as the index of defocusing while the harmonic split (Δt) is decreased stepwise from 300 μ s to 0 μ s (red). Significant defocusing of the bats' delay image begins for harmonic offsets as small as 2.6 μ s and persists for offsets up to 300 μ s. If FM2 is not delayed but removed entirely, performance returns to normal for focused images. (B) Mean (± 1 s.d.) performance of four bats on an 800 μ s delay-discrimination task in a split-harmonic experiment (red) and in two-glint control (blue). ns, $P > 0.007$; *, statistically significant differences ($P < 0.007$) between the two-glint control and split-harmonic experimental conditions based on binomial tests.

was set to 3160 μ s (2000 μ s electronic delay + 1160 μ s sound-path delay, corresponding to a target distance of 54.5 cm) whereas the delay of the filter-selected FM2 was increased above 3160 μ s by varying amounts of electronic delay between 2300 and 2000 μ s. The split-harmonic delay difference between FM1 and FM2 (Δt) thus varied over steps of 300, 100, 25, 5.2, 2.6, 1.3 or 0.0 μ s. For experimental S-, the overall delays of the filter-selected FM1 and FM2 were set to the same value of 3960 μ s (2800 μ s electronic delay + 1160 μ s sound-path delay, corresponding to a target distance of 68.3 cm). Under ordinary conditions, this 800 μ s delay difference is easily discriminated by big brown bats (Moss and Schnitzler, 1995; Simmons et al., 1995). The crucial feature of the experimental condition is that both S+ and S- undergo the same electronic filtering; only the electronic delays of FM1 and FM2 differed, by varying amounts of Δt for S+ and by 0 μ s for S-.

In the control condition (two-glint S+), the LP filter in each simulator channel was set to 90 kHz whereas the HP filter was set to 15 kHz, to allow both FM1 and FM2 to pass unobstructed into the echoes returned to the bat. For control S+, the overall delay of the first glint was set to 3160 μ s (2000 μ s electronic delay + 1160 μ s sound-path delay) whereas the delay of the second glint was increased to >3160 μ s by varying amounts of electronic delay between 2300 and 2000 μ s. The two-glint delay difference (Δt) thus varied over steps of 300, 100, 25, 5.2, 2.6, 1.3 or 0.0 μ s. For control S-, the overall delay of the two glints was set to the same value of 3960 μ s (2800 μ s electronic delay + 1160 μ s sound-path delay). The crucial feature of the control condition is that both S+ and S- again undergo the same electronic filtering; only the electronic delays of the two glints differed, by varying amounts of Δt for S+ and by 0 μ s for S-.

The time differences between FM2 and FM1 (Δt) for the harmonic split were the same as those used for the two-glint separation, so that comparison of the bats' performance in split-harmonic and two-glint conditions would reveal the effects of the harmonic delay separation. Besides the varying time differences for FM2 relative to FM1, or between the first and second glints, the only difference between the experimental and control conditions was the presence of the harmonic-split filter settings (i.e. the narrow filtered region around 55 kHz removed from the split-harmonic stimuli; see Fig. 1B). The effects of this 55 kHz cut can be evaluated by comparing the performance of the bats under experimental and control conditions at $\Delta t = 0$ μ s. If their performance is the same under both conditions, then the split-harmonic filters had no effect of their own beyond the delay differences between FM2 and FM1.

From bat sound at the microphone to its echo at the bat's location on the platform, the total gain of the target simulator system was approximately -35 dB for S+ and -40 dB for S-. The big brown bat's sensitivity for echo detection is not a fixed quantity; thresholds for echo detection are high immediately following the broadcast and decline by approximately 11 dB per doubling of delay thereafter for at least 6-10 ms (Kick and Simmons, 1984). The 5 dB attenuation of S- relative to S+ in the present study compensates for the 5 dB increased sensitivity of the bat for echoes at the longer delay of 3960 μ s compared with the shorter delay of 3160 μ s.

RESULTS

Fig. 2A plots the results of the present experimental and control conditions relative to data previously collected with respect to echo-delay acuity in big brown bats. These curves all show mean percentage errors achieved by multiple bats. First, plotted in green, is a complete

two-glint performance curve that shows percent errors made on an echo-delay discrimination task where S+ is a two-glint echo at a delay of 3.2 ms (300- μ s glint separation) and S- is a single glint echo at various delays from 3.1 to 3.9 ms (mean performance on 60 trials per bat, $N=2$ bats). [These data are from previous experiments (Simmons et al., 1990b); that they were not plotted in the original paper is solely due to limitations of space.] In that experiment, the delay of S- was moved along the horizontal S-/S+ delay difference axis (Fig. 2A) to probe for locations (delays of S-) where bats would perceive S- and either glint of S+ as having the same delay. The discrete error peaks at 0 and 300 μ s (green curve) show that the bats separately perceive the delay of each reflection in the two-glint S+.

Results from split-harmonic electronic echoes using an S+ with a 300 μ s offset of FM2 relative to FM1 are plotted in black (Fig. 2A) [150 trials per bat, $N=4$ bats; re-plotted from (Stamper et al., 2009)]. For the S+ in that experiment, FM1 delay was 3160 μ s and FM2 delay was 3460 μ s. Again, S- is a one-glint echo presented at different delays to probe for perceived delays associated with each of the harmonics in S+. This black error curve has prominent error peaks at delays of S- that correspond to the delay of either FM1 or FM2 in S+ (at 0 and 300 μ s), indicating that the bats perceived the delay of each harmonic separately. However, instead of error peaks sharply localized to the immediate region of the delay for FM1 or FM2, as occurs for the two-glint curve (green), there is a broad pedestal of errors (gray shaded area) surrounding these peaks and extending continuously from the arrival time of FM1 to longer delays past 800 μ s. This pedestal of errors extends more than 800 μ s past the arrival time of FM2, which itself is only 300 μ s after FM1. The pedestal is referred to here as a blurred or defocused delay image because the bats' performance reveals a lack of sharply perceived delay for the 300 μ s split-harmonic stimuli. By contrast, the well-defined peaks in the two-glint (green) curve illustrate a focused image that resolves the two glints as having separate delays.

The present experiment with a variable offset of FM2 relative to FM1 addresses the sensitivity of the perceptual mechanism underlying the defocusing effect illustrated by the gray area in Fig. 2A: how small a misalignment of FM2 from FM1 is sufficient to initiate the pedestal of errors? A stimulus delay difference of 800 μ s is used as an index of blurring because it is remote from any of the ordinary effects of glints separated by 300 μ s (green curve).

In Fig. 2A,B, the red and blue curves (split-harmonic persistence and two-glint control) are the mean split-harmonic experimental (red) and two-glint control (blue) data (mean \pm s.d. percent errors from 150 trials per bat, $N=4$ bats) from the present experiment. The mean results are plotted on Fig. 2A on the linear scale of the time separation (Δt) of FM2 from FM1 from 300 to 0 μ s. An additional data point is shown for the condition of no FM2 (i.e. only FM1) (Bates and Simmons, 2010). These same data are replotted in Fig. 2B on a logarithmic scale for the separation (Δt) of FM2 from FM1 to spread out the abrupt onset of the loss in performance, or defocusing effect, when FM2 is delayed by only a few microseconds relative to FM1. A Bonferroni correction resulted in a significant alpha level of 0.007. There was a significant decrement in performance (increase in percentage errors) for the experimental echoes compared with the control echoes beginning with the 2.6 μ s harmonic separation. That is, a significant defocusing effect already is evident at the 2.6 μ s harmonic separation, and it increases further as harmonic separation increases to 25 μ s and greater. This defocusing effect then persists as Δt increases to 300 μ s. It was only at the 0 and 1.3 μ s harmonic separation that the mean performance of the bats was statistically indistinguishable between conditions (Fig. 2B). Comparison of experimental and control performance at the stimulus condition of

$\Delta t=0$ μ s tests for the effects of the split-harmonic filters themselves (55 kHz cut; Fig. 1B); the statistical similarity of performance indicates that the experimental and control results are not influenced by a bias associated with these filter settings.

When FM2 was removed entirely from S+ echoes, instead of being delayed relative to FM1, mean performance then returned to being statistically indistinguishable between control and experimental conditions (Fig. 2B). The delay acuity of the bats did not seem to be compromised when only FM1 was present in returning echoes.

To summarize, when the temporal relationship between FM1 and FM2 is gradually disrupted through shifting the arrival time of the two harmonics by Δt , the bats abruptly lose acuity for performing the nominally easy 800 μ s delay-discrimination task through a process that amounts to defocusing of the image (Fig. 2A). Misalignments of FM2 with FM1 as small as 2.6 μ s cause the occurrence of significant defocusing (Fig. 2B).

DISCUSSION

The results described above show that big brown bats perceive echoes with misaligned harmonics – even echoes misaligned by as little as 2.6 μ s – as having a poorly defined delay. This effect is described here as defocusing of the bat's delay image. Use of the term defocusing is justified by the wide spread of poor performance, extending out to 800 μ s longer than the objective arrival time of the echoes. By contrast, two-glint echoes with glint separations (Δt) from 0 to 300 μ s (equal to the harmonic separations in Fig. 2) are perceived with very few errors in the 800 μ s discrimination task because 800 μ s is so remote from the delay of either the first or the second glint (green curve; Fig. 2A). Most importantly, two-glint echoes with Δt from 10 to 300 μ s are perceived as having two glints at their corresponding delays (Simmons et al., 1990a; Simmons et al., 1995; Simmons et al., 1998). The green two-glint control curve in Fig. 2A illustrates how the two-glint image is in focus; i.e. the glints are registered separately at their correct delays, with a narrow spread of errors (width of green error peaks) over approximately ± 50 μ s. The intervening space does not contain many errors and keeps the peaks separate.

Several studies involving a different approach than using electronically generated echoes have reported decrements in delay-discrimination performance when the time-frequency structure of echoes is altered in ways other than temporal misalignment of harmonics (Masters and Jacobs, 1989; Masters and Raver, 2000; Surlykke, 1992). These experiments used an electronically generated model echo – a single transmitted waveform selected from a series of each bat's sounds and stored digitally to replace actual echoes of individual broadcasts. This model echo is triggered by the bat's sounds to arrive at a fixed delay for a delay-discrimination task (two-choice or go/no-go) (see Moss and Schnitzler, 1995). In various model echo tests, the parameters of echo duration, amplitude of FM2 relative to FM1, signal frequency and the curvature of FM sweeps (adjusted by changing the decay constant of an exponential curve used as the modulation function) were changed to assess the effect on delay-discrimination acuity. Besides the dramatic loss in acuity caused when echoes were reversed in time so that they swept upward in frequency instead of downward (Masters and Jacobs, 1989; Surlykke, 1992), only changes in the curvature of the FM sweeps yielded changes in performance that could be detected with the discrimination procedure (Masters and Raver, 2000). In the context of the present results, this is the only manipulation that led to differences in the spectrograms of echoes relative to broadcasts

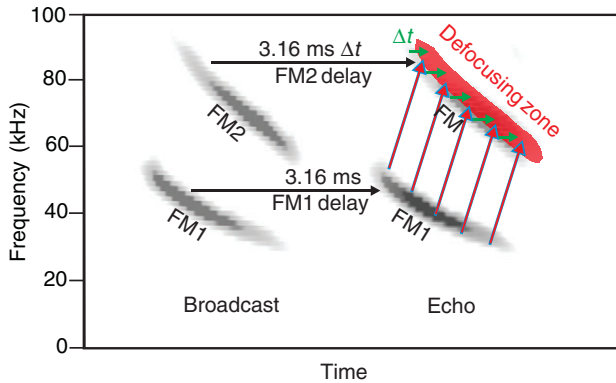


Fig. 3. Region of harmonic misalignment for image defocusing. Spectrograms of broadcast and echo showing harmonic sweeps, FM1 and FM2, and the bats' representation of echo delay from the time that elapses between broadcast and echo spectrograms (horizontal black arrows). When FM2 is offset in time (Δt ; horizontal green arrows) relative to FM1, the bats' delay acuity deteriorates, but recovers if FM2 is removed entirely. Neural responses evoked by FM2 that fall in a time window after the 'correct' time aligned with FM1 (red arrows pointing to red 'defocusing zone') interact with responses triggered by FM1 to blur the bats' delay image. Onset of defocusing is abrupt; it reaches significant strength only for the $2.6\ \mu\text{s}$ harmonic offset and it persists for at least $300\ \mu\text{s}$.

comparable to the differences produced in split-harmonic echoes (Fig. 1B).

The diagram in Fig. 3 addresses implications of the defocusing effect caused by misaligning harmonics in echoes. A target represented by split-harmonic echoes would be perceived as being badly smeared along its echo delay axis, or as having a defocused distance and shape. By contrast, a target represented by echoes with correctly aligned harmonics, or echoes that contain FM1 alone, would be perceived as having a well-defined distance and shape. That is, its image would be in focus. The bat's sharp threshold sensitivity ($2.6\ \mu\text{s}$), along with the persistence of defocusing for all values of harmonic separation from 2.6 to $300\ \mu\text{s}$, implies that some auditory mechanism detects harmonic misalignment of any size and then imposes strong defocusing on the resulting images. The virtual disappearance of defocusing when FM2 is removed completely, in contrast to the persistence of defocusing for various harmonic misalignments, argues for a zone of time immediately following the nominal, or correct, time-of-occurrence of the FM2 sweep relative to the FM1 sweep, within which the anomalous presence of the FM2 sweep initiates the defocusing effect. That is, the correct alignment of FM2 with FM1 is not being detected; otherwise, removal of FM2 would cause defocusing to occur. Instead, the misalignment of FM2 over a range from $2.6\ \mu\text{s}$ to at least $300\ \mu\text{s}$ is being detected. The crucial zone of time for misalignment begins almost immediately (i.e. $2.6\ \mu\text{s}$) following the nominal position of FM2 and extends to at least $300\ \mu\text{s}$ later without serious deterioration of the defocusing effect. This pattern of results suggests that, when responses to FM2 are allowed to shift to a later time than normal, they activate neuronal inhibition that initiates a cascade of responses designed to register the shape of targets from echo interference spectra (see Sanderson and Simmons, 2000; Sanderson and Simmons, 2002).

What is the significance under natural conditions of defocusing caused by temporal misalignment of echo harmonics if the atmosphere is a nondispersive medium for propagation of sound, especially over the short distances of up to 10 m that are relevant for echolocating bats? Biosonar in air is dominated acoustically not

by differences in the velocity of sound across frequencies but by LP effects related to propagation and directional beaming. First, atmospheric absorption attenuates higher frequencies relative to lower frequencies in proportion to target distance. The degree of LP filtering present in the outward-propagating sound increases with distance, and then it doubles over the echo's return path (Lawrence and Simmons, 1982). For a target at a distance of 3 m, for example, frequencies of 50–100 kHz in FM2 would undergo excess attenuation from atmospheric absorption by 9 to 18 dB whereas frequencies of 25–50 kHz in FM1 would be attenuated by <4 to 9 dB. Consequently, FM2 would be attenuated by approximately 5 to 9 dB more than FM1. Second, the bat's broadcasts are projected in a beam towards the front, with sharper directionality at high frequencies than at low frequencies (Ghose and Moss, 2003; Hartley and Suthers, 1989). The greater the eccentricity, or off-axis position, of the target, the more the incident sound undergoes LP filtering before it impinges on the target. The degree of LP filtering in echoes thus increases with both distance and off-axis position. In relation to harmonic structure, echoes from targets located straight ahead and at short range reach the bat's ears with FM1 and FM2 largely at the same strength, but as target range or off-axis location increases, FM2 is disproportionately attenuated in echoes relative to FM1. The opposite effect – HP filtering to remove FM1 so that echoes contain primarily FM2 – does not occur under natural conditions because the dimensions of flying insects and virtually all other objects, such as leaves, branches or the ground, are larger in proportion to the incident wavelengths than the Raleigh region for scattering (Fenton et al., 1998; Houston et al., 2004; Moss and Zageski, 1994; Simmons and Chen, 1989). That is, natural targets create echoes in an acoustic regime where the full bandwidth of incident sounds returns at an amplitude related to the target's cross-sectional area (the target's acoustic 'size').

Insect-sized targets affect echo spectra, but not as global LP filtering. The target's contribution instead consists of local notches in the spectrum due to reinforcement and cancellation caused by interference between overlapping reflections from multiple parts of the target, or glints (the target's acoustic 'shape') (Kober and Schnitzler, 1990; Moss and Zagaeski, 1994; Simmons and Chen, 1989). Behavioral, neurophysiological and computational studies have identified a process, called spectrogram correlation and transformation (SCAT), that has been hypothesized to explain how big brown bats locate the frequencies of these interference notches and reconstruct the corresponding delay differences between different parts of the target (Matsuo et al., 2004; Neretti et al., 2003; Peremans and Hallam, 1998; Saillant et al., 1993; Sanderson and Simmons 2000; Sanderson and Simmons, 2002; Sanderson and Simmons, 2005; Simmons et al., 1995; Simmons et al., 1998). Using a combination of overall echo delay and the echo interference spectrum as cues, these bats recreate for each echo an image depicting the object as a small number of glints on a perceptual axis of distance.

In contrast to specific shape-related spectral signatures caused by interference between glints, the presence of LP filtering can reliably be exploited to distinguish all echoes reflected by off-axis or distant clutter from echoes reflected by targets of interest located to the immediate front of the bat. How is this distinction represented in the bat's auditory system? Neurons at successive stages (cochlear nucleus to auditory cortex) in the big brown bat's auditory system are tuned to different frequencies across the echolocation band of roughly 20–100 kHz (Dear et al., 1993a; Dear et al., 1993b; Ferragamo et al., 1998; Haplea et al., 1994; Jen et al., 1989; Ma and Suga, 2008; Pollak et al., 1977; Sanderson and Simmons, 2000; Sanderson and Simmons, 2002). Results of these experiments

document a basic echo-delay processing scheme that uses dispersed response latencies as delay lines in the inferior colliculus followed by coincidence-detecting neurons in the auditory cortex. Delay-tuned neurons created at the auditory cortex by this processing cascade respond at a wide range of latencies. The only extraneous influence on the accuracy of echo-delay coding is amplitude-latency trading, a consequence of increased response latencies for echoes that are reduced in amplitude (Bodenhamer and Pollak, 1981) [in big brown bats, the trading ratio is approximately $-16\mu\text{s dB}^{-1}$ (Burkhard and Moss, 1994; Ma and Suga, 2008; Simmons et al., 1990a; Simmons et al., 1990b)]. The majority of delay-tuned neurons in the cortex are selective not only for a particular echo delay but also for the presence of an interference notch at their tuned frequencies. That is, although inferior colliculus neurons fail to respond if an interference notch is present at their tuned frequency (Sanderson and Simmons, 2000; Sanderson and Simmons, 2005), cortical neurons only respond if there is a notch at their tuned frequency, an inversion of the neuronal representation that depends on the evocation of inhibition by the ‘missing’ responses in the inferior colliculus (Sanderson and Simmons, 2002). Individual delay-line cells in the inferior colliculus produce an average of only one spike for each broadcast or echo (Sanderson and Simmons, 2000; Sanderson and Simmons, 2005), so if a particular frequency is reduced in strength in an echo due to interference, the single spikes evoked in neurons tuned to that frequency are retarded in latency by amplitude-latency trading (Burkhard and Moss, 1994; Ma and Suga, 2008; Simmons et al., 1990b). When some frequencies in echoes are reduced in amplitude by interference, the spikes representing these frequencies thus shift to longer latencies up to several hundred microseconds following the latencies at which they would have responded if no reduction in amplitude had occurred. The resulting temporal misalignment of responses at specific frequencies in the inferior colliculus may initiate the inhibitory process that inverts the notch representation and displays the glints in the cortex (Sanderson and Simmons, 2002). The size of the time window containing neuronal responses that have retarded latencies due to interference notches thus coincides with the time window (Fig. 3) into which the shift of FM2 causes defocusing of the delay image.

We speculate that the time-sensitive inhibitory process the big brown bat uses for registering individual interference notches is also activated by intrusion of responses evoked by electronically delayed FM2. However, although interference notches are distributed systematically across several discrete frequencies in echoes according to the time separation of the glint reflections (Moss and Schnitzler, 1995; Simmons et al., 1995), we hypothesize that the shift of the entire range of frequencies in FM2 into the inhibitory time window causes many different notch-selective neurons to be activated simultaneously, in effect ‘turning on’ a large number of perceived glints and defocusing the image. Delay separation of FM2 from FM1 does not occur acoustically in natural echoes. However, the decline in amplitude of FM2 relative to FM1 due to off-axis location or long target range leads to greater amplitude-latency trading of responses evoked by FM2. In effect, the auditory representation of FM2 is split from the auditory representation of FM1. For example, the LP effect described above for echoes arriving from a target at a range of 3 m results in attenuation of frequencies in FM2 by 5 to 9 dB relative to frequencies in FM1. The corresponding retardation of responses to FM2 due to amplitude-latency trading (at $16\mu\text{s dB}^{-1}$ attenuation) is 80 to 140 μs , which is solidly within the time window for defocusing of the bat’s delay image (Fig. 2B).

The bat’s delay images for targets located straight ahead and at short range will have high delay accuracy and resolution because the only spectral effect is interference due to the glint structure of targets, not a global LP effect. By contrast, images of background objects, or clutter, located farther away or off to the side will be rendered with very low delay acuity due to defocusing caused by LP filtering, which retards the auditory representation of FM2 relative to FM1. The imaging process in biosonar thus may be analogous to high-resolution foveal vision and low-resolution peripheral vision, except that objects are rendered in depth, not in direction.

LIST OF ABBREVIATIONS AND SYMBOLS

FM	frequency modulated
FM1	first harmonic
FM2	second harmonic
HP	highpass
LP	lowpass
S+	positive stimulus
S-	negative stimulus

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