

# Biosonar interpulse intervals and pulse-echo ambiguity in four species of echolocating bats

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**Summary statement:** Four species of echolocating bats emit signals with different interpulse-interval strategies for managing pulse-echo ambiguity in a complex test scene where multiple echo streams arrive together to be processed simultaneously.

## ABSTRACT

In complex biosonar scenes, the delay of echoes represents the spatial distribution of objects in depth. To avoid overlap of echo streams from successive broadcasts, individual echolocation sounds should only be emitted after all echoes of previous sounds have returned. However, close proximity of obstacles demands rapid pulse updates for steering to avoid collisions, which often means emitting a new sound before all of the previous echoes have returned. When two echo streams overlap, there is ambiguity about assigning echoes to the corresponding broadcasts. In laboratory tests of flight in dense, cluttered scenes, four species of echolocating bats exhibited different patterns of pulse emissions to accommodate potential pulse-echo ambiguity. *Miniopterus fuliginosus* emitted individual FM pulses only after all echoes of previous pulses had returned, with no alternating between long and short intervals. *Pipistrellus abramus* and *Eptesicus fuscus* alternated between emitting long FM pulse intervals to receive all echoes before the next pulse, and short intervals to update the rapidly changing scene while accepting partial overlap of successive echo streams. *Rhinolophus ferrumequinum* transmitted CF/FM pulses in alternating short and long intervals, usually two to four closely spaced sounds that produced overlapping echo streams, followed by a longer interval that separated echo streams. *Rhinolophus* is a statistical outlier from the three FM species, which are more similar to each other. The repeated overlap of CF/FM echo streams suggests that CF components have a distinct role in rejection of clutter and mitigation of ambiguity.

**Key Words:** biosonar, guiding flight, echo delay, interpulse intervals, plus-echo ambiguity, phantom images

## INTRODUCTION

Echolocating bats emit trains of intense biosonar pulses to perceive objects in the surrounding scene from echoes that return to their ears (Fenton, et al., 2016; Griffin, 1958; Neuweiler, 2000; Surlykke, et al., 2014). Each biosonar broadcast is a probe into the scene that yields echoes at different delays according to distances to objects ( $\sim 6$  ms/meter of target range; Chiu, et al., 2009; Surlykke and Moss, 2010; Wohlgemuth, et al., 2016). In practice, small, insect-sized targets are detectable out to no more than 5-10 m, for echo delays of 30-60 ms, while larger background objects would be detectable out to about 20-40 m (Stilz and Schnitzler, 2012). The depth of realistic scenes thus potentially encompasses several tens of meters, for echo delays up to at least 100-200 ms. The most elemental problem for the bat to solve is when to transmit individual echolocation sounds (*i.e.*, at what interpulse intervals, or IPIs), particularly in relation to the returning streams of echoes (Kothari, et al., 2014).

The experiments reported here examined the biosonar sound emission patterns of four species of bats flying in a complex sonar scene—a room containing rows of vertically-hanging plastic chains with a meter-wide corridor for the bat to fly along (Fig. 1A; Barchi, et al., 2015; Petrites, et al., 2009; Wheeler, et al., 2017). As each of the bat's emitted pulses propagates along the room (Fig. 1B), it reflects off successive rows of the chains (Fig. 1C). The echoes returning to the bat during the epoch of time immediately following the broadcast represents the distances to the different objects. In the simplest case, in Fig. 1D, the bat waits until all of the echoes from one broadcast have returned before emitting the next broadcast (IPI  $>$  maximum delay, T). This keeps echoes in the epoch of one broadcast from mingling with echoes in the epoch of the next broadcast. However, bats frequently fly in acoustically distinctive and often highly cluttered surroundings, such as vegetation (Grunwald, et al., 2004; Ming, et al., 2017; Müller, 2003; Müller and Kuc, 2000; Yovel, et al., 2008, 2009), where some of the objects are in close proximity while others extend farther away. Prompt

responses are necessary to avoid collisions, which requires emitting broadcasts at short intervals to keep up with rapidly-evolving conditions. Nevertheless, other objects still are present at longer ranges, and their echoes necessarily reach the bat at longer delays. If a new broadcast is emitted while echoes of the previous broadcast are still traveling through the air, the echo-stream epochs of the two broadcasts will overlap ( $IPI < T$ ; Fig. 1E). The long-delayed echoes of the first broadcast are liable to be judged as having shorter delays relative to the most immediate broadcast, not the correct, longer delays relative to the earlier broadcast. To the bat, phantom objects might suddenly appear at close range and will have to be avoided as hazards to flight even though no such objects actually are present. Misattribution of echoes to the most recent broadcast represents “pulse-echo” or “range” ambiguity, a major problem for radar or sonar systems operating in surroundings where objects are distributed in depth (Skolnik, 1980; Stimson, 1998).

In principle, there are three ways to mitigate pulse-echo ambiguity: The first solution is to emit sounds at IPIs long enough to accommodate all of the returning echoes before the next broadcast ( $IPI > T$ ; see Fig. 1D). This entails accepting the risk that nearby obstacles will have to be avoided using information acquired at the slower update rate necessitated by waiting until all echoes have returned. The second solution is to alternate between long and short IPIs. Each long IPI probes the whole scene out to its maximum depth ( $IPI > T$ ), while each short IPI probes for nearby objects that need rapid action for guidance ( $IPI < T$ ; Fig. 1E). In this solution, ambiguous echoes do arrive after the short IPIs, but, by keeping track of apparent echo delays that occur after both long and short IPIs, the spurious delays that follow the short IPIs can be identified and disregarded (Skolnik, 1980). The third solution is to transmit pulses that alternate in some identifiable acoustic feature, usually frequency or phase. So called “frequency hopping” (Markley and Antheil, 1942) is a method for coping with ambiguity and is a staple for communications security. Each broadcast “tags” its echoes

as distinct from the other broadcast's echoes. Echoes are assigned only to the broadcast having the corresponding frequency (Skolnik, 1980). By any of these three solutions, effective segregation of echo streams is achievable, but the underlying receiver mechanisms will be different.

Biosonar broadcasts have been examined specifically with regard to the pattern of IPIs related to overlap of pulse-echo epochs and emergence of ambiguous echoes (Melcón et al., 2011; Petrites et al., 2009; Barchi et al., 2013; Falk et al., 2014; Kothari et al., 2014; Sändig et al., 2014; Knowles et al., 2015; Wheeler et al., 2016; Accomando, et al., 2018). In addition, the strategy of frequency hopping from one pulse to the next is used by some bat species in situations where ambiguity likely occurs (Mora, et al., 2004; Guillén-Servent & Ibáñez, 2007; Hiryu, et al., 2010). These studies developed tools for analyzing IPIs to address the problem of pulse-echo ambiguity. Here, those tools are used to compare four different species of echolocating bats performing the same task.

## **MATERIALS AND METHODS**

**Animals:** Individuals of four species of adult, wild-caught bats were tested in flights through arrays of vertically-hanging plastic chains that served as a complex biosonar scene for assessing guidance by echolocation. The task challenged their ability to follow a straight corridor from a release point to the far end of the array while receiving echoes from chains at all points in the array (Fig. 1A; Petrites, et al., 2009). The challenge to the bats comes from both the presence of multiple near obstacles to be avoided and the long extent of the scene, which delivers echoes over a wide span of delays from as little as 3 ms to as much as 30-35 ms. Big brown bats (*Eptesicus fuscus*, Palisot de Beauvais) were flown in tests conducted at Brown University in Providence Rhode Island, USA. They were captured from house colonies in Rhode Island under a state scientific collection permit. Japanese house bats

(*Pipistrellus abramus*, Temminck), bent-winged bats (*Miniopterus fuliginosus*, Hodgson), and greater horseshoe bats (*Rhinolophis ferrumequinum Nippon*, Schreber) were flown in tests conducted at Doshisha University in Kyotanabe, Japan. The *Pipistrellus* were captured in Kyoto Prefecture; *Miniopterus*, and *Rhinolophus* were captured in Hyogo Prefecture. The bats were maintained in individual cages or a larger colony room in temperature- and humidity-controlled colony rooms (22-24° C. and 40-60% relative humidity) that were kept on a reverse light/dark cycle with 12 h dark/12 h light. Experiments were conducted during the bats' subjective night. All bats were fed live mealworms (larval *Tenebrio molitor*), with daily rations adjusted to keep individual body mass in a healthy range between 5 g and 30 g depending on the species. All of the animals had free access to vitamin-enriched water. For *Eptesicus*, husbandry and experimental procedures complied with Principles of Animal Care, publication #86-23 (1985) of the U.S. National Institutes of Health, and were approved by the Brown University Institutional Animal Care and Use Committee. For the other three species, procedures complied with the same NIH document, and current Japanese laws, approved by the Animal Experiment Committee at Doshisha University.

**Experimental procedures:** Experiments took place in two custom-built flight rooms. The facility at Brown University was 8.5 m long, 3.3 m wide, and 2.4 m high; insulated acoustically and electrically from external noise, including commercial FM radio signals. To accommodate recording equipment, a net across the width of the room restricted the effective flight space from the release point to the far wall to be 6 m long. The walls and ceiling of the room were entirely covered in fireproof and anechoic acoustic foam (SONEX<sup>®</sup>, Pinta Acoustic, Minneapolis, MN, USA) that dampened any residual wall reflections by 20-25 dB, and the floor was carpeted to minimize reverberation. During flights, the only illumination was from low-level red LEDs distributed around the room's walls (Barchi, et al., 2013). The facility at Doshisha University was an experimental chamber 8 m long, 3 m wide, and 2 m

high. Considering the release point for each flight, the useful flight space was about 6 m long. This chamber was constructed of steel plates bolted together to minimize interference from external electromagnetic noise, particularly commercial FM radio stations. During experiments, long-wavelength lighting that filtered out wavelengths below 650 nm was used to prevent the bat from using visual information.

Each flight room contained multiple rows of closely-spaced plastic chains suspended vertically from ceiling to floor for 1.8-2.0 m. They were arranged to leave a 1-m wide corridor along which the bats could fly (Fig. 1B). This basic chain configuration, but with either straight or curved corridors of varying widths, has been used previously to examine echolocation during flight (Petrites et al., 2009; Knowles et al., 2015; Wheeler et al., 2016). Because the task is a useful laboratory model for guidance in clutter, it also has been used to assess the effects of intense sound exposure on bats (Hom, et al., 2016; Simmons, et al., 2018). In the Brown University flight room, individual links in the chains measured 4.0 cm wide, 7.5 cm long, and 1.0 cm thick. At Doshisha University, the chain links were 4.0 cm wide, 7.0 cm long, and 0.8 cm thick. The vertically-extended chains are strong reflectors of the bat's incident emissions, returning echoes that decline only slightly with increasing distance due to their extended shape. Tests with artificial bat sounds to measure echoes from chains at distances from 30 cm to 5 m yielded echoes 11 to 16 dB weaker over that span of distances (Knowles, et al., 2015). Longer distances would eventually encounter large losses from atmospheric absorption, but within the confines of the flight space, all of the rows of chains in the array returned easily-detected echoes for individual bat sounds, thus posing a problem of pulse-echo ambiguity. Fig. 1A,B illustrates the series of echoes recorded from a series of broadcasts emitted during a representative flight by one species (*Miniopterus*). The indicated broadcast was followed by a long train of echoes that extended over 25 ms, culminating in an echo from the wall at 30 ms delay. The chains were spaced 30 cm apart

left to right in each row, and successive rows were separated by 40 cm (Fig. 1B). This configuration creates a complex acoustic echo scene combining proximity, density, and spatial extent. It challenges the bat's biosonar capabilities for flight guidance (Barchi et al., 2013). The 1-m corridor width was chosen for these experiments because it is enough more cluttered than an open room to evoke changes in broadcast patterns by *Eptesicus* (Knowles, et al., 2015; Petrites, et al., 2009; Wheeler et al., 2016).

### **Sound recording and analysis**

During each flight, the trains of pulses emitted by each bat were recorded with several different ultrasonic microphones positioned in the scene to acquire the bat's emitted pulses (Telemike on bat or Knowles microphone on wall in Fig. 1A; FG-3325 or SMG-0291 Knowles Electronics, Itasca, IL, USA) or the echoes from the chains (Anabat mike in Fig. 1A; Titley Scientific, Brendale, QLD, Australia). The microphones were positioned so that they could record all of the bat's pulses from the time of release until the time of landing on the back wall of the flight room. The microphone on the wall was used to register the broadcasts for calculating IPI values during all flights. The Telemike was used for recording the CF-FM sounds emitted by *Rhinolophus* because the presence of CF components interspersed between FM signals was a special concern for understanding how the FM components were separated in time. The large Anabat microphone was positioned behind the bat as it flew into the array of chains to document the occurrence of a long sequence of echoes reflected back to the bat after each broadcast. The recorded sounds were digitized at 192 kHz (Model 702T, Sound Devices recorder, Reedsburg, WI, USA) and saved as stereo .wav files. Each recording was manually started by one experimenter before the release of the bat from the other experimenter's hand, and then manually stopped once the bat had landed on the wall. Off-line analyses of the recorded sounds were performed using custom-written MATLAB procedures (R2014a; MathWorks, Cambridge, MA, USA). For each



flight, the recordings were first digitally high-pass filtered at 15 kHz to remove ambient noise. “Audio trials” representing the duration of each flight down the corridor towards the wall were selected in the following manner: First, the stereo sound file containing the recording from the end point of the flight was defined by the onset of a burst of rapidly-emitted pulses characteristic of the landing maneuver (“landing buzz,” Petrites et al., 2009; Wheeler, et al., 2016). Moving backward in time from the first pulse in the landing buzz, a time interval of 1-2 s was selected for analysis. This interval covers the time for a bat to fly down to the end of the corridor after being released, but does not include any pulses emitted by the bat prior to being released, or the landing buzz itself. In each audio trial, the time of each individual pulse was marked as the point at which its envelope reached its maximum amplitude. In some cases, the bat flew several times along the corridor, turning back at the far wall, returning, and then again flying along the corridor towards the far wall. The flight segments when the bat flew away from the Anabat mike and towards the far wall were identified from the echoes that the Anabat mike recorded from the chains, which only appeared during the bat’s movement away from the Anabat mike.

IPIs were calculated as the time intervals between the amplitude maxima of successive pulses. This yielded a series of IPIs for a given flight. Then, for each individual pulse, IPIs were labeled as “pre-IPI” (the IPI before the pulse) and “post-IPI” (the IPI after that pulse). This pair of intervals was determined for all but the first (no pre-IPI) and last (no post-IPI) pulses in the entire audio trial. The patterning of IPIs in each audio trial was analyzed using three metrics—(1) the distribution of IPIs for all flights of a given species (plotted as a histogram), (2) the contingency distribution of pre-IPI and post-IPI values for each pulse (plotted as a two-dimensional dot distribution), and (3) the distribution of post-IPI/pre-IPI values for each sound (Wheeler et al., 2016). This ratio metric treats successive inter-pulse intervals as proportions so that if the bat were to change the absolute size of the

IPIs, this metric would still capture their proportional relation. It allows analysis of how intervals before and after each pulse are related to one another and does not rely on a particular definition of a “sonar sound group” or “strobe group” (Moss et al., 2006; Petrites et al., 2009; Kothari et al., 2014), which are groups of pulses with short, stable IPIs surrounded by longer IPIs.

## RESULTS

Bats of all four species successfully made multiple flights along the corridor through the array of chains. Data were analyzed for a minimum of 2 *Miniopterus*, 2 *Pipistrellus*, 2 *Eptesicus*, and 2 *Rhinolophus*. To facilitate direct comparison of the four species, IPIs extracted from the recordings are presented in the same format—first, representative spectrograms from one flight, then a series of plots that summarize IPIs from all of the flights.

***Miniopterus*:** Fig. 2A shows spectrograms for a series of FM broadcasts emitted by *Miniopterus* during one flight along the corridor through the chain array to land on the far wall. These sounds were recorded with the Anabat mike (Fig. 1A), which emphasizes the sequence of echoes reflected back to the bat. Each sound in A appears blurred because it is followed by a stream of echoes from all of the chains situated in front of the bat. The flight terminates in a landing buzz of multiple, closely-spaced sounds that shift down to half of the frequencies contained in the FM sounds emitted along the flight path leading up to the landing. Fig. 2B zooms in two successive broadcasts (FM pulses #1 and #2, delineated by two vertical dashed lines in Fig. 2A), each followed by a long stream of echoes from the chains. Fig. 2A indicates the two IPI metrics extracted from the recordings—the interval before each sound (pre-IPI) and the interval after each sound (post-IPI). In Fig. 2B, the IPI between pulse #1 and pulse #2 is 34 ms, while from pulse #2 to the next pulse is 27 ms. Fig.

3A shows the IPIs from a series of broadcasts recorded during a 1.5-s segment of one flight by *Miniopterus*. The trace shifts up and down slowly across time, marking the relative consistency of IPIs between successive broadcasts. Fig. 3B shows a histogram of IPIs from all flights. Mean IPI is about 50.0 ms with only slight skew to shorter IPIs. The smoothness of the IPI sequences in the example in Fig. 3A is fully captured in Fig. 3C, a contingency plot of post-IPIs relative to pre-IPIs for each sound in the data-set. The diagonal line in Fig. 3C traces where post IPIs equal pre-IPIs. The data-points cluster along the diagonal line, indicating that the IPI after each sound is about the same length as the IPI preceding that sound. The distribution of the ratio of post-IPIs to pre-IPIs in Fig. 3D is narrow and symmetric. The mean ratio is about 1.

***Pipistrellus*:** Fig. 4A shows spectrograms for a series of FM broadcasts emitted by *Pipistrellus* during one flight along the corridor through the chain array, which culminates in a turn back into the corridor. They also were recorded with the Anabat mike (Fig. 1A) to emphasize the sequence of echoes reflected back to the bat. Here, the bat does not land, and the sounds sequence does not end with a landing buzz. Fig. 4B zooms in on seven successive broadcasts (segment delineated by two vertical dashed lines in Fig. 4A). Each pulse is followed by a long stream of echoes from the chains. The rhythm of IPIs used by *Pipistrellus* is irregular, with long and short IPIs intermingled. The IPI after pulse #1 is 62 ms, while the IPI after pulse #2 is only 24 ms, and after pulse #3 it is 47 ms. In this instance, the interval between pulse #2 and pulse #3 is shorter than the epoch of echo arrivals, as explained in Fig. 1E, indicating that some pulse-echo ambiguity occurs. Fig. 5A shows alternation between long and short IPIs in a series of pulses recorded during a 1.5-s segment of one flight. These alternations reveal sound (strobe) group doublets, with a few triplets marked by adjacent short IPIs. In Fig. 5B, the histogram of IPIs from all flights has a mean of about 40 ms, but the distribution is distinctly bimodal, with a narrow peak at 25 ms and broad peak at 30-60

ms. The contingency plot of post-IPIs relative to pre-IPIs in Fig. 5C reflects the bimodal distribution for IPIs less than 60 ms. This part of the plot has two clusters for alternating intervals—short pre-IPIs followed by long post-IPIs and long pre-IPIs followed by short post-IPIs. In Fig. 5C, for pre-IPIs longer than 60 ms, however, post-IPIs are similar. The histogram of post-IPI/pre-IPI ratios in Fig. 5D is strongly bimodal, with one peak at 0.7 and a second peak at 1.2. A lower, broader region of ratios also extends to 2.0.

***Eptesicus*:** Fig. 6A shows spectrograms for a series of FM broadcasts emitted by *Eptesicus* during one flight prior to landing on the end wall—again, recorded with the Anabat mike. Fig. 6B zooms in on five successive broadcasts (segment delineated by two vertical dashed lines in Fig. 8A). In this example, the pulses were recorded with the electret microphone on the wall (Fig. 1A) to register broadcasts without their echoes. Like *Pipistrellus* in Fig. 4A, the rhythm of IPIs used by *Eptesicus* is staggered, with long and short IPIs intermingled. Fig. 6B shows a doublet sound (strobe) group followed by a triplet. Fig. 7A shows a series of IPIs used by *Eptesicus* during one flight segment with strongly alternating long and short IPIs. Some sound (strobe) groups are doublets, some are triplets. In Fig. 7B, the distribution of all IPIs is notably trimodal, with a peak around 30 ms for IPIs within triplets, a peak around 60 ms for IPIs within doublets, and a peak around 100 ms for the longer intervals between sound groups. Intervals shorter than about 30 ms expose the bat to pulse-echo ambiguity at the beginning of flights when the bat is presented with full depth of the chain array. The contingency plot of post-IPIs to pre-IPIs in Fig. 7C reveals a bifurcated distribution where short IPIs are followed by long IPIs, reflecting the alternation from within to between sound groups. Sometimes the shortest IPIs are followed by similarly short IPIs, which indicates the triplets. The histogram of post- to pre-IPI ratios in Fig. 7D has two peaks—one at about 0.7, the other at about 1.8, which is dominated by IPIs within *versus* between sound groups.

***Rhinolophus***: Fig. 8A shows spectrograms for a series of CF/FM broadcasts emitted by *Rhinolophus* during one flight prior to turning away from the end wall. These sounds were recorded with the Anabat mike located behind the flying bat to emphasize the stream of echoes after each pulse. Each sound in A appears blurred because it is followed by a stream of echoes from all of the chains situated in front of the bat. Fig. 8B zooms in on seven successive broadcasts—a quadruplet followed by a triplet (segment delineated by two vertical dashed lines in A). Each pulse is followed by a long stream of echoes from the chains and then from the far wall. Like *Pipistrellus* in Fig. 4A and *Eptesicus* in Fig. 6A, the rhythm of IPIs used by *Rhinolophus* in Fig. 8A is irregular, with several short-IPI pulses grouped together and the groups separated by a longer IPI. Fig. 8C shows spectrograms of the same sounds recorded by the Telemike carried on the bat to pick up broadcasts without echoes from the chains as in Fig. 8A. The grouping of several broadcasts is more evident with the echoes removed. Fig. 8D shows the same seven broadcasts as in Fig. 8B but recorded by the Telemike without the echoes. The closely-spaced pulses in Fig. 8D are shown as intermingled with echoes in Fig. 8B. Overlap of echoes streams occurs for the closely-spaced pulses comprising the quadruplet and triplet, so pulse-echo ambiguity occurs often. Staggering of several short IPIs followed by a long IPI separates the overlapping echo streams, but only intermittently. The sample sequence of IPIs in Fig. 9A shows regular alternation between several short IPIs and one long IPI, creating groups of sounds that make up the whole sequence. In Fig. 9B, the distribution of IPIs is strongly skewed to short intervals, with a sharp peak around 25 ms between members of sound (strobe) groups. For inter-group intervals, the distribution spreads to 40-60 ms. From Fig. 8, IPIs shorter than about 30 ms regularly expose the bat to overlapping echo streams and consequent pulse-echo ambiguity. The dot pattern for the contingent distribution of post- and pre-IPIs in Fig. 9C has two widely-separated clusters lying above and below the diagonal line, signifying the

alternation from long IPIs between sound groups to short IPIs within groups. Short IPIs often repeat, signifying the presence of triplets and quadruplets in the sound groups. In Fig. 9D, the histogram for the distribution of post- to pre-IPI ratios is markedly trimodal, with peaks at 0.5, 1.1, and 2.5.

## DISCUSSION

Four species of echolocating bats were tested in flight experiments that exposed each species to the same task (Fig. 1), with presumably substantially the same degree of difficulty. All four species successfully flew along the corridor past multiple rows of vertically-suspended chains, in a corridor 1 m wide between the closest chains on the bat's left and right. In previous flight tests with *Eptesicus*, the corridor width of 1 m is narrow enough to begin imposing enough difficulty on orientation that bats shift from using relatively long IPIs to pulse timing patterns that intersperse short and long intervals (Knowles, et al., 2015; Petrites, et al., 2009; Wheeler, et al., 2017; Accomando, et al., 2018). In the tests described here, *Eptesicus* again conformed to alternating short and long IPIs.

The broadcast patterns of the four species varied from the simplest IPI distribution to more complex patterns (Figs. 3, 5, 7, 9). These consist of (1) a succession of relatively stable time intervals (*Miniopterus*), (2) increasingly complex sequences that contain groups of sounds (strobe groups) with two or three sounds each (*Pipistrellus*, *Eptesicus*), and (3) three or four closely-spaced sounds in each group (*Rhinolophus*). When viewed in the light of the extended echo streams from multiple rows of chains, especially near the start of each flight pass when the entire depth of the chain array faced the bat (Fig. 1A), these differences can be considered as distinct strategies for coping with the potential interference caused by overlap of echo streams. This particular kind of interference is pulse-echo ambiguity (Skolnik, 1980). *Miniopterus* largely avoids pulse-echo ambiguity by keeping intervals between sounds longer

than the echo streams that follow individual sounds. How *Miniopterus* deals with demands on rapid maneuvering is an open question, but the behavior of *Eptesicus*, a similar-sized bat, suggests that the task is sufficiently difficult that ambiguity and maneuvering capability both pose challenges on the bats. The alternating short and long IPIs used by *Pipistrellus* and *Eptesicus* seem designed to probe into the entire scene, out to the longest delays in the room, while next looking closely to nearby objects to facilitate rapid guidance reactions.

*Miniopterus* uses FM sounds similar to those recorded in flights here to hunt for flying insects (Wei and Zhang, 2011), and they change the terminal frequency of their FM sweeps when flying in each other's company to avoid mutual jamming (Maitani, et al., 2018). For *Rhinolophus*, whose reliance on the long CF components for Doppler-shift perception, there is a different factor to consider; the FM components may be constrained to be far enough apart to accommodate the minimum adequate duration for the intervening CF component. This species uses an exaggerated timing pattern of several closely-spaced FM signals to form triplets or quadruplets, followed by long intervals between these grouped sounds. The intervening time intervals still are filled by the CF components, though. It seems likely that the added information supplied by the CF echoes, presumably from Doppler shifts in the echoes returned by the nearest chains in each row, is important for guidance—a feature of the system used by *Rhinolophus* that is not shared by the other bat species.

The ordering of species from *Miniopterus* to *Pipistrellus* to *Eptesicus* to *Rhinolophus* that seems reasonable from visual inspection of the IPI histograms and the IPI ratio histograms in Figs. 3B D, 5B D, 7B D, and 9B D is supported statistically by the probabilities assigned to the pairwise comparisons of these two distributions using the Kolmogorov-Smirnov tests in Table 1. *Miniopterus* uses a timing pattern at one end of a continuum and *Rhinolophus* at the other end, with statistical similarity among the three FM species and statistical difference for *Rhinolophus*.

Pulse-echo ambiguity is one of the largest problems for sonar and radar, and several technical solutions are typically employed to cope with it. The diversity of interpulse interval patterns shows that bats have evolved implementations of two known solutions that involve intervals. (The third, frequency hopping, is known in bats that emit CF search signals, and in FM bats that are flown in the densest clutter.) We observe that *Pipistrellus* and *Eptesicus* use one of the classical solutions of alternating long and short intervals to “look” far and then near. *Rhinolophus* also alternates intervals, but the pattern is more complex, possibly due to the presence of CF components that have a minimum duration of about 5 ms. The presence of these CF components fills the time between successive FM components and may be a factor in the horseshoe bat’s use of groups of three or four closely-spaced FM signals with CF components filling much of the intervening times. The duty-cycle is very high—roughly 90% compared to the roughly 5% used by any of the FM bats. We have added these details in the discussion, plus two new references (Müller and Schnitzler, 1999,2000) regarding the greater complexity of potential flow-field cues available to CF-FM bats for flying in complex scenes such as the array of hanging chains. In relation to flow-field perception, we do not know what the use of fixed intervals by *Miniopterus* means, but the cues described in the new references may point to a fourth pulse-echo ambiguity strategy involving the entire flow-field instead of alternating long and short “looks.”



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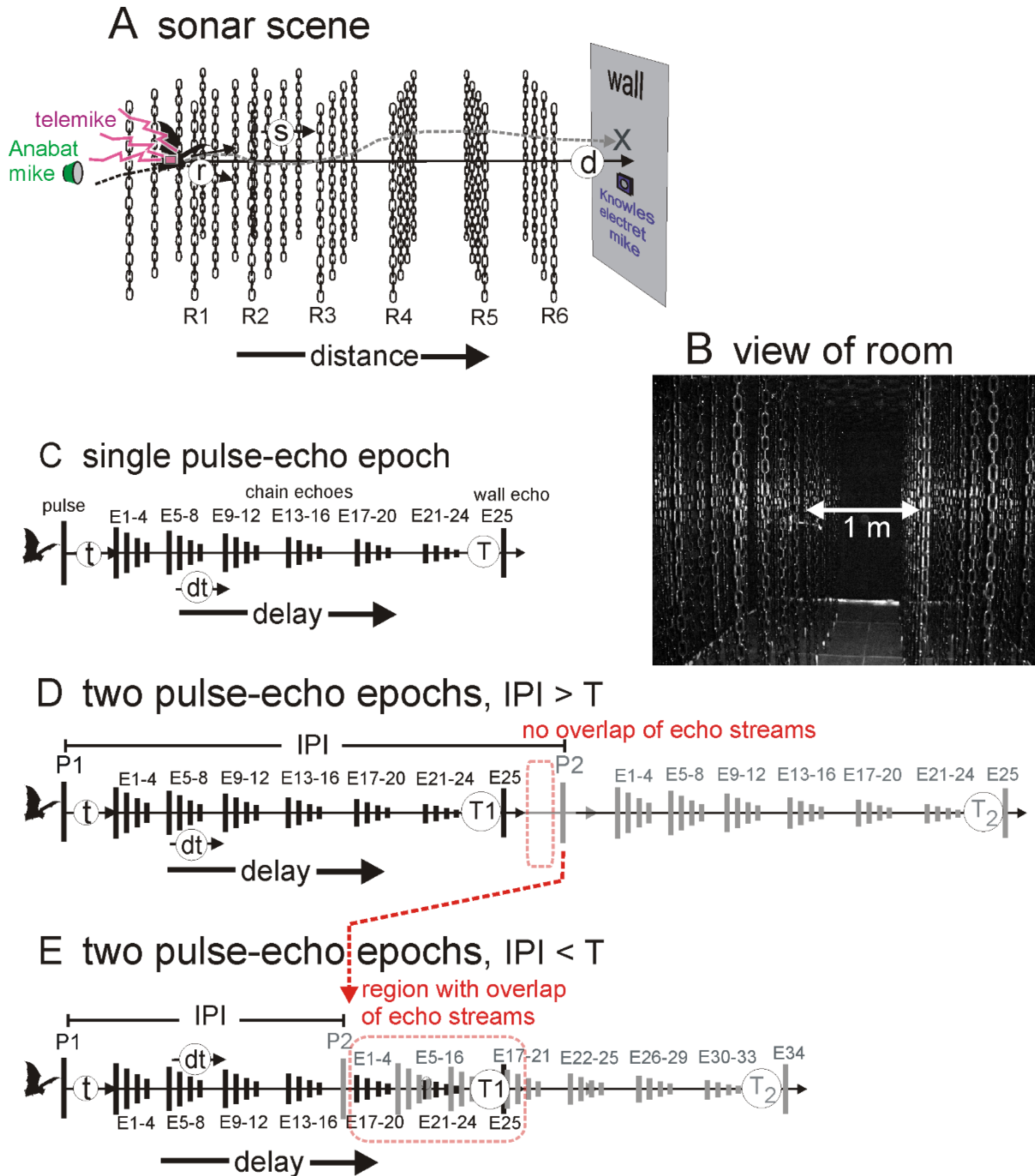
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**Table 1.** White-filled cells at top show probabilities for chance occurrence of comparisons among four species *IPI distributions* (Kolmogorov-Smirnov Test probabilities for pairwise differences between distributions from Figs. 4B, 6B, 8B, 10B). Dark-gray-filled cells at bottom show probabilities for chance occurrence of comparisons among four species *IPI ratio distributions* (Kolmogorov-Smirnov Test probabilities for pairwise differences between distributions from Figs. 4D, 6D, 8D, 10D). Diagonal line of light-gray cells show probabilities for chance occurrence of comparisons of each species with itself on either the IPI or the IPI ratio distributions, so all probabilities are 1.0. (\*  $p < 0.01$ , with Bonferroni correction)

Species	<i>Miniopterus</i>	<i>Pipistrellus</i>	<i>Eptesicus</i>	<i>Rhinolophus</i>
<i>Miniopterus</i>	1.0	0.107	0.037	<0.001 *
<i>Pipistrellus</i>	0.029	1.0	0.011	<0.001 *
<i>Eptesicus</i>	0.449	0.494	1.0	0.107
<i>Rhinolophus</i>	<0.001 *	0.029	<0.001 *	1.0

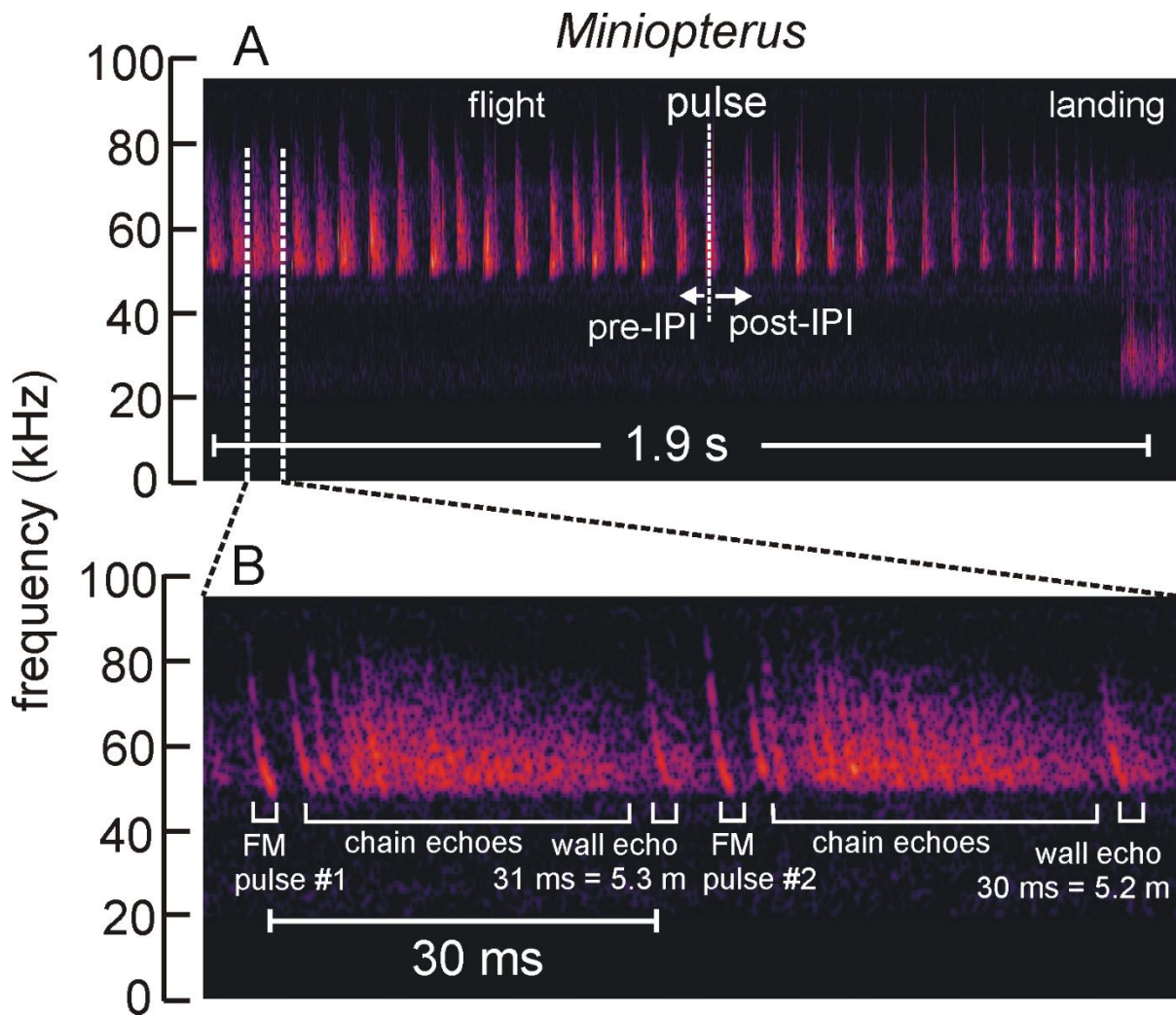
## Figures





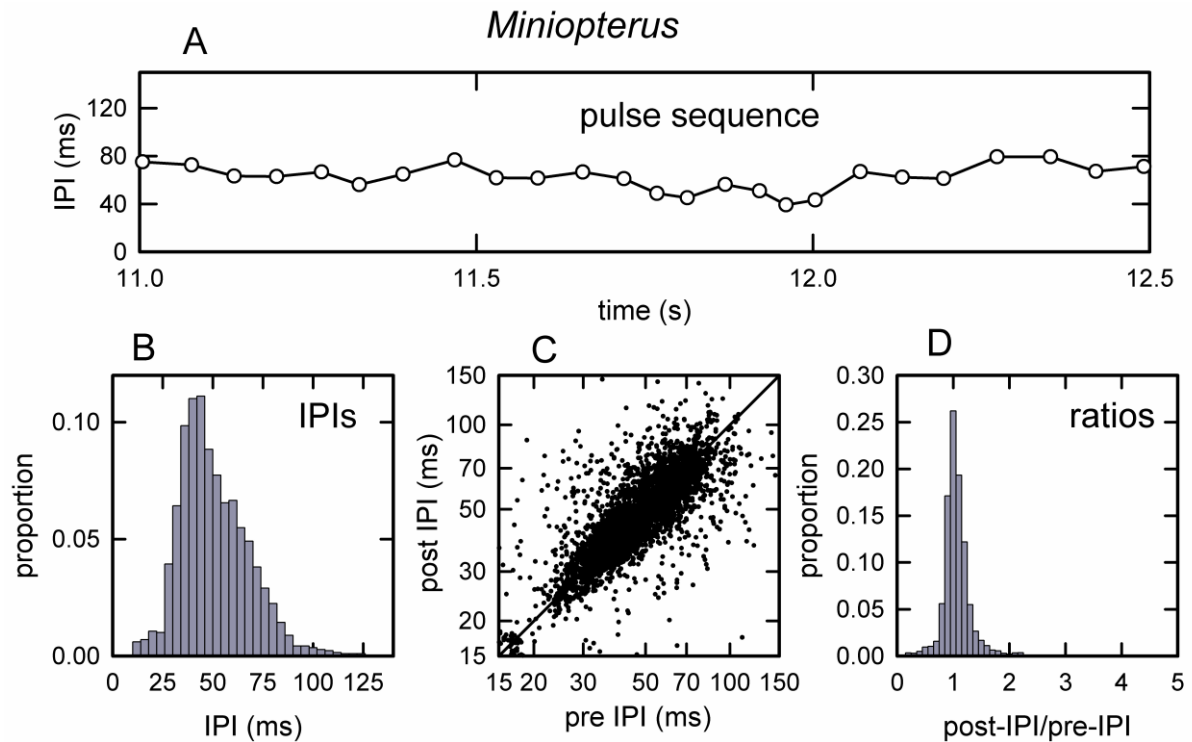
**Fig. 1.** Experimental set-up for flight tests in complex sonar scene. **(A)** Diagram showing bat flying along corridor past seven (six upcoming) rows of chains (R1-R6) to land on the far wall. Distances to nearest chains ( $r$ ) and spacing of successive rows ( $s$ ) are much shorter than the total distance to the wall ( $d$ ). Bat's biosonar pulses are recorded by three means—a radiomicrophone (telemike, magenta) carried by the bat to pick up broadcasts (Hiryu, et al., 2010), a large Anabat condenser microphone, green) located behind the bat to pick up backscattered echoes from the chains, or a Knowles electret microphone (blue) mounted on the landing wall to pick up direct broadcasts. **(B)** View of flight room (Brown Univ.) showing rows of chains and 1-m corridor for bat to negotiate. **(C)** Translation of spatial scene from Fig. 1A into acoustic echo stream received by the bat for the time epoch following a single transmitted pulse. Individual rows of chains yield clusters of echoes at progressively longer delays (*e.g.*, E1-4, E5-8) that extend in time out to the delay ( $T$ ) of the final echo from the wall (E25). Delays of echoes from nearest chains ( $t$ ) and delay separations of successive echo clusters ( $dt$ ) are much shorter than delay of wall echo ( $T$ ), which is the farthest reach of the scene. **(D)** Sequence of echoes received during two time epochs that follow each of two successive pulses (P1, P2) emitted far enough apart that the IPI is longer than the delay of echoes from the farthest point in the scene ( $T$ ). Each pulse yields a stream of echoes (*e.g.*, E1-4, E5-8) that evolves over time until the last echo (E25) returns. The IPI is long enough to accommodate all echoes for the first pulse. No echoes from P2 are received until all echoes of P1 have been returned, and no pulse-echo ambiguity occurs. **(E)** Sequence of echoes received during the two epochs that follow each of two successive pulses (P1, P2) emitted so close together that the IPI is shorter than the delay of echoes from the farthest point in the scene ( $T$ ). Each pulse yields a stream of echoes (*e.g.*,

E1-4, E5-8) that evolve over time until the last echo (E25) returns. However, now, some long-delay echoes of the second pulse, P2, arrive overlapping with short-delay echoes of P1. (Zone of overlap traced by red dashed box.) These echoes of P1 mingle with echoes of P2 to create a new echo stream for P2 (*e.g.*, E1-4, E5-16, E7-21) that registers both real chains in relation to P2 as well as multiple “phantom” chains located at short distances. The epoch following P2 continues to evolve until all remaining echoes have been received, but the early mixing of echoes of P1 and P2 misrepresents the spatial scene, creating ambiguity about which echoes are related to which pulses.

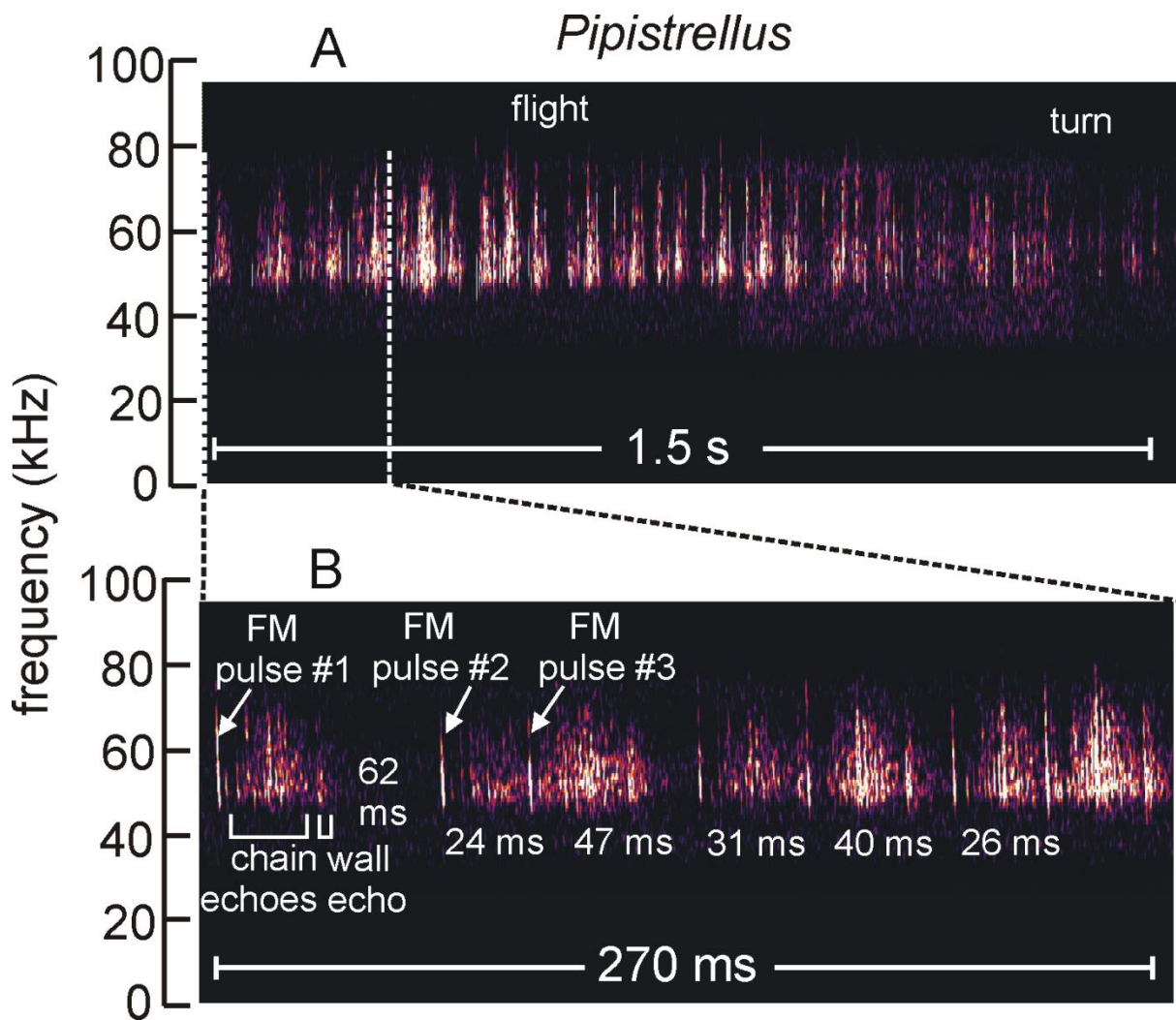


**Fig. 2.** (A) Spectrograms for a series of FM broadcasts emitted by *Miniopterus* during one flight along the corridor through the chain array to land on the far wall. These sounds were recorded with the Anabat mike (Fig. 1A) to emphasize the sequence of echoes reflected back to the bat. The flight terminates in a landing “buzz” of multiple, closely-spaced sounds that shift down to half of the frequencies contained in the FM sounds emitted along the flight path leading up to the landing. Each sound in A appears blurred because it is followed by a stream of echoes from all of the chains situated in front of the bat. (B) Illustration zooming in on two successive broadcasts (FM pulses #1 and #2, delineated by two vertical dashed lines in A), each followed by a long stream of echoes from the chains. This is a real example of the

conditions illustrated in Fig. 1D. A shows the two IPI metrics extracted from the recordings—the interval before each sound (pre-IPI) and the interval after each sound (post-IPI). The IPI between pulse #1 and pulse #2 is 34 ms, longer than the echo-returning epoch of 27 ms.

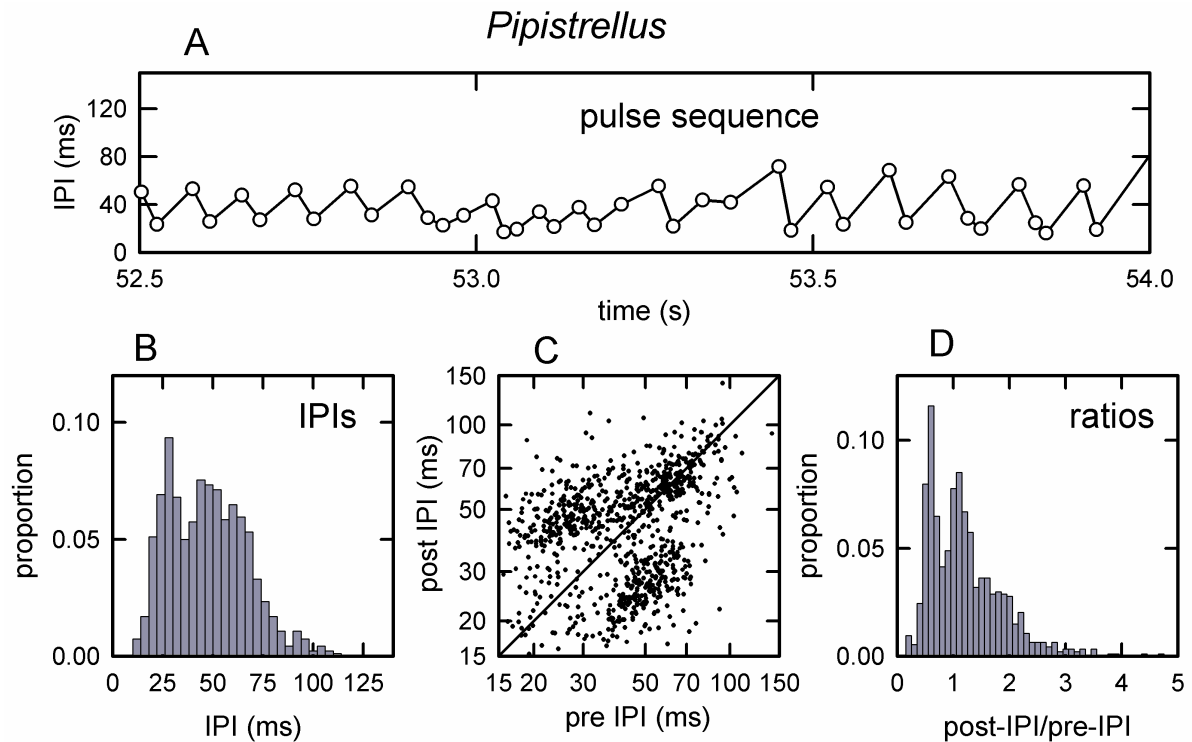


**Fig. 3.** Interpulse intervals used by *Miniopterus*. (A), Series of IPIs recorded during a 1.5-s segment of one flight by *Miniopterus*. (B) Histogram of IPIs from all flights. Mean IPI is about 50.0 ms with only slight skew to longer IPIs. (C) Contingency plot of post-IPIs relative to pre-IPIs for each sound in the data-set. The diagonal line indicates where post IPIs equal pre-IPIs. Data-points cluster along diagonal line, indicating that the IPI after each sound follows the IPI preceding that sound. Alternation between long and short IPIs is not present. The smooth trace in A reflects the continuous nature of successive IPIs. (D) Histogram of post-IPI/pre-IPI ratios. Distribution is narrow and symmetric; mean ratio is about 1.



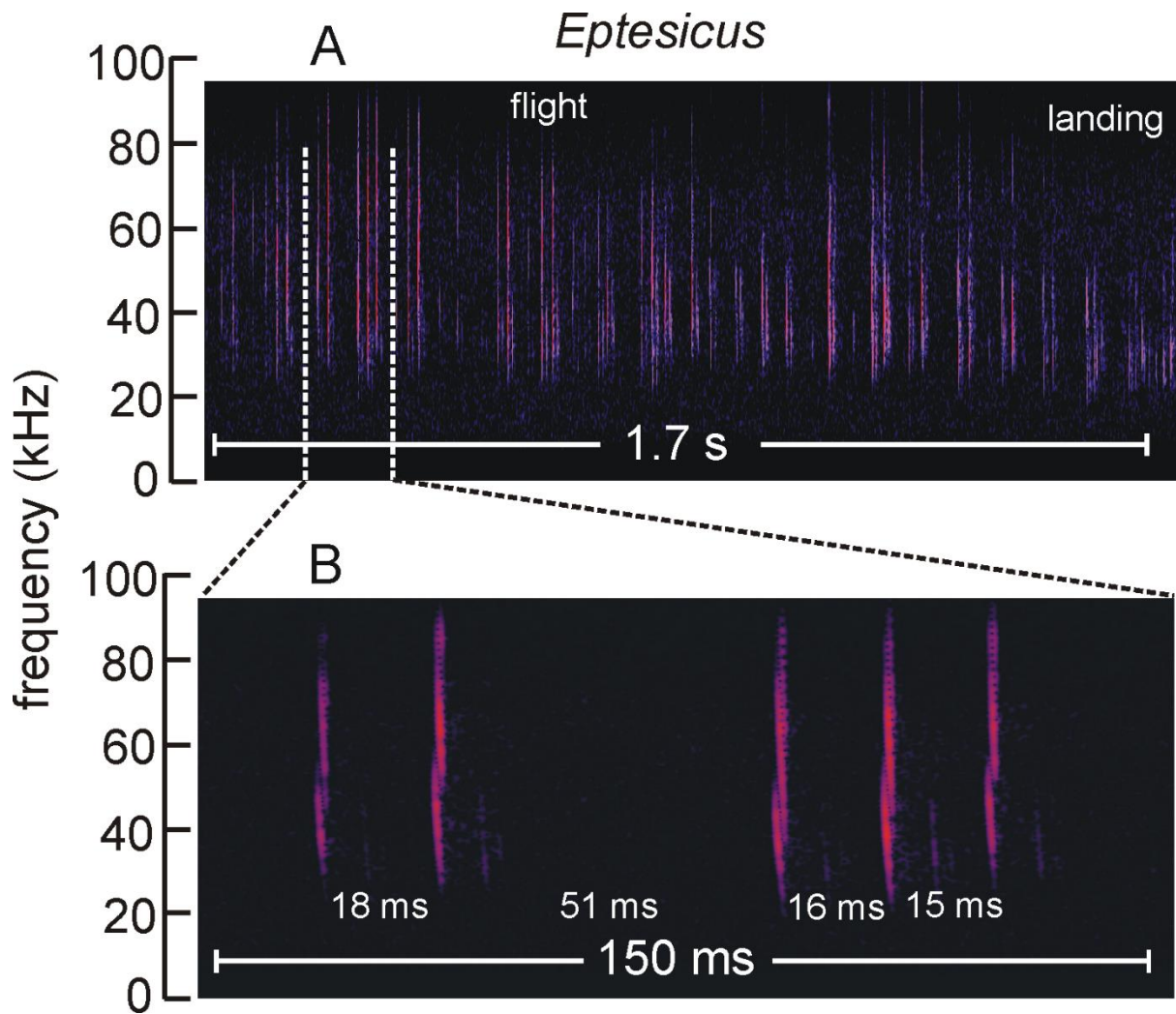
**Fig. 4.** (A) Spectrograms for a series of FM broadcasts emitted by *Pipistrellus* during one flight prior to turning at the end wall. These sounds also were recorded with the Anabat mike (Fig. 1A) to emphasize the sequence of echoes reflected back to the bat. In this flight, the bat does not land, and the sounds sequence does not end with a “landing buzz.” Each sound in A appears blurred because it is followed by a stream of echoes from all of the chains situated in front of the bat. (B) Illustration zooming in on seven successive broadcasts (segment delineated by two vertical dashed lines in A). Each pulse is followed by a long stream of echoes from the chains. Unlike *Miniopterus* in Fig.2A, the rhythm of IPIs used by *Pipistrellus* is irregular, with long and short IPIs intermingled. The IPI after pulse #1 is 62

ms, while the IPI after pulse #2 is only 24 ms, and after pulse #3 it is 47 ms. The interval between pulse #2 and pulse #3 is shorter than the epoch of echo arrivals, as illustrated in Fig. 1E, indicating that some pulse-echo ambiguity occurs.

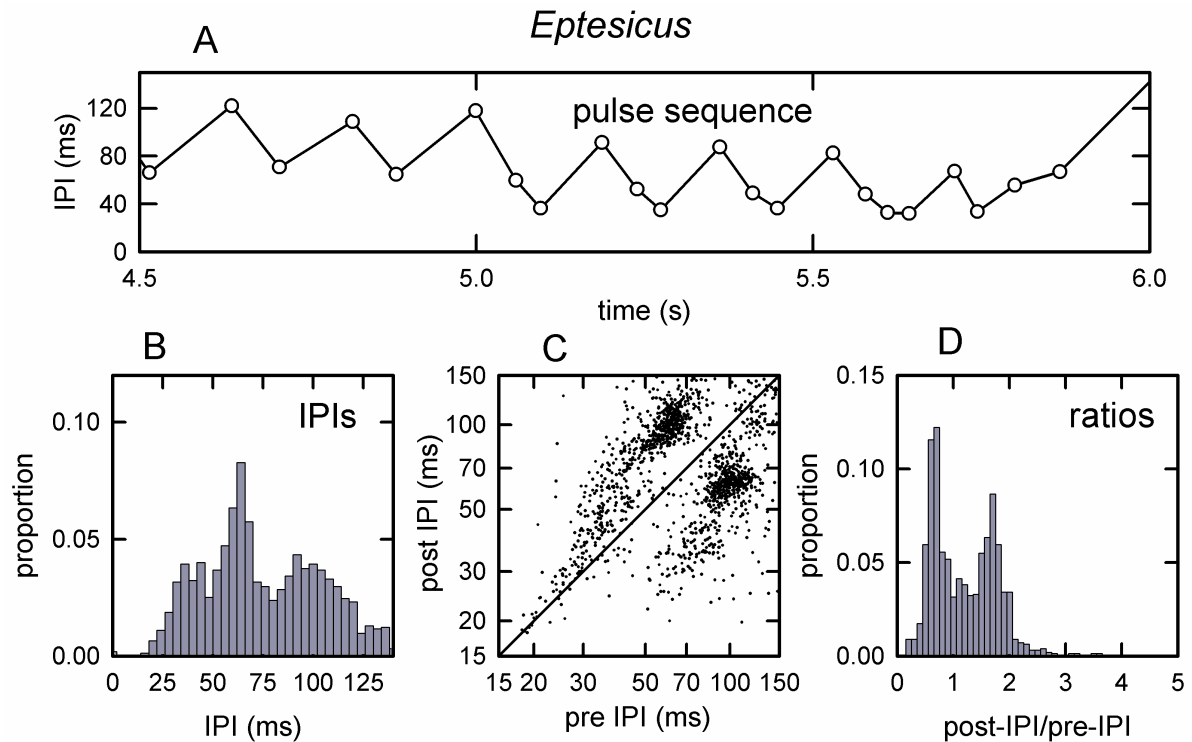


**Fig. 5.** Interpulse intervals used by *Pipistrellus*. **(A)**, Series of IPIs recorded during a 1.5-s segment of one flight. IPI values alternate between long and short intervals, indicating sound (strobe) group doublets, with a few triplets revealed by two adjacent short IPIs. **(B)** Histogram of IPIs from all flights. Distribution has mean of about 50 ms but is bimodal, with a narrow peak at 25 ms and broad peak at 30-60 ms. **(C)** Contingency plot of post-IPIs relative to pre-IPIs for each sound in the data-set. The diagonal line indicates where post IPIs equal pre-IPIs. For IPIs less than 60 ms, the distribution has two clusters reflecting alternating intervals—short pre-IPIs followed by long post-IPIs and long pre-IPIs followed by short post-IPIs. For pre-IPIs longer than 60 ms, post-IPIs are similar, indicating that longer IPIs after each sound follow longer IPIs preceding that sound. **(D)** Histogram of post-IPI/pre-IPI ratios. The distribution is strongly bimodal, with one peak at 0.7 and a second peak at 1.2. A lower, broader region of ratios extends to 2.0.

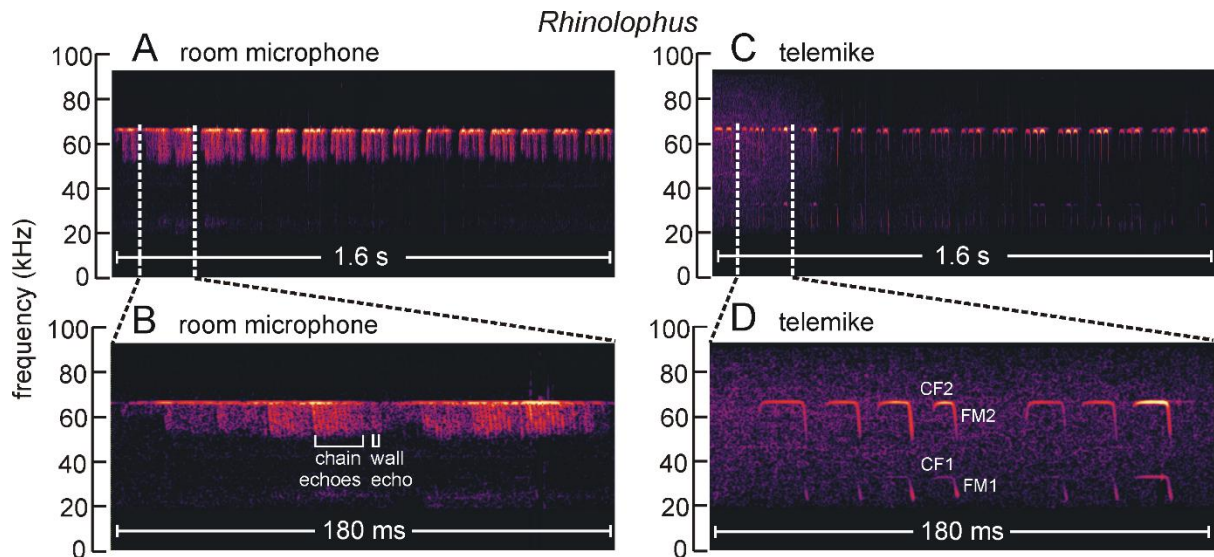




**Fig. 6.** (A) Spectrograms for a series of FM broadcasts emitted by *Eptesicus* during one flight prior to landing on the end wall. These sounds were recorded with the Anabat mike (Fig. 1A) to emphasize the sequence of echoes reflected back to the bat. Each sound in A appears blurred because it is followed by a stream of echoes from all of the chains situated in front of the bat. (B) Illustration zooming in on five successive broadcasts (segment delineated by two vertical dashed lines in A). These pulses were recorded with the electret microphone on the wall (Fig. 1A) to register broadcasts without their echoes. Like *Pipistrellus* in Fig.4A, the rhythm of IPIs used by *Eptesicus* in A is staggered, with long and short IPIs intermingled.

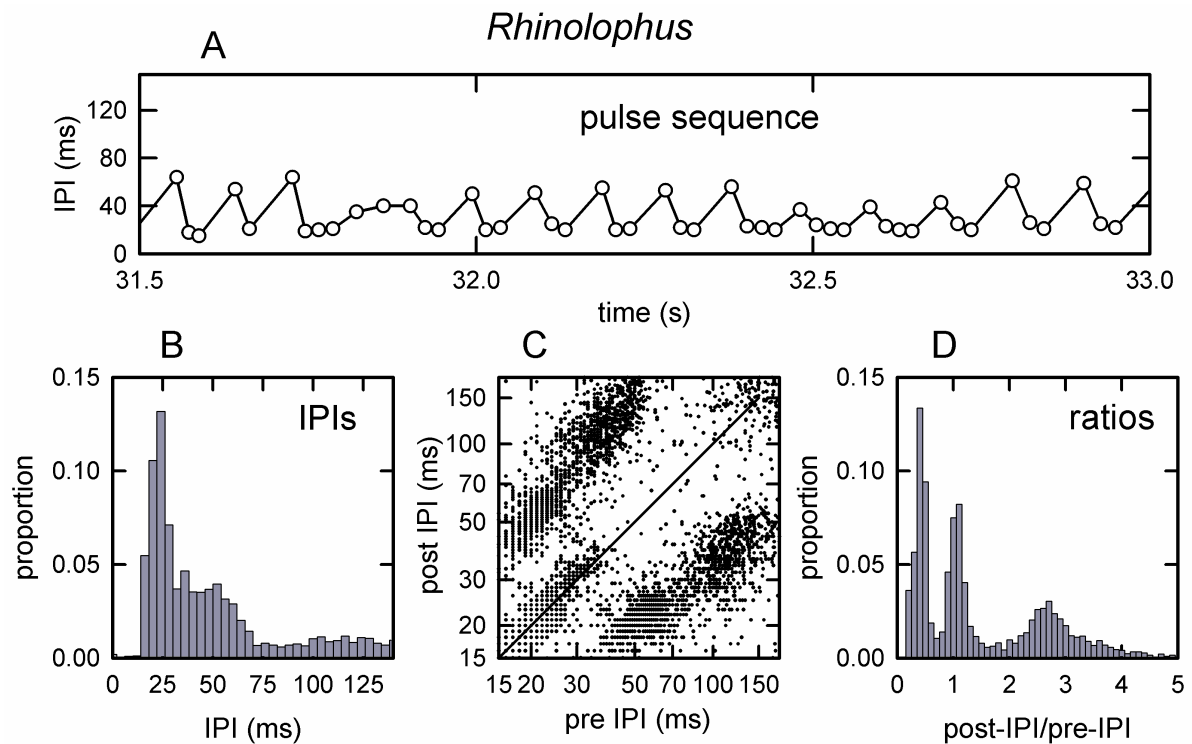


**Fig. 7.** IPIs used by *Eptesicus* during flights along 100-cm wide corridor through the array of chains. **(A)** Short (1.5 s) segment illustrates alternating long and short IPIs. Some sound (strobe) groups are doublets, some are triplets. **(B)** Distribution of IPIs is notably trimodal, with a peak around 30 ms for IPIs within triplets, a peak around 60 ms for IPIs within doublets, and a peak around 100 ms for the longer intervals between sound groups. Intervals shorter than about 30 ms expose the bat to pulse-echo ambiguity. **(C)** The dot pattern plotting post-IPIs to pre-IPIs reveals a bifurcated distribution where short IPIs are followed by long IPIs, reflecting alternation from within to between sound groups, and the shortest IPIs are followed by similarly short IPIs, reflecting within the triplets. **(D)** Histogram showing the distribution of post- to pre-IPI ratios has two peaks—one at about 0.7, the other at about 1.8, which is dominated by IPIs within *versus* between sound groups.



**Fig. 8.** (A) Spectrograms for a series of FM broadcasts emitted by *Rhinolophus* during one flight prior to turning away from the end wall. These sounds were recorded with the Anabat mike (Fig. 1A) to emphasize the sequence of echoes reflected back to the bat. Each sound in A appears blurred because it is followed by a stream of echoes from all of the chains situated in front of the bat. (B) Illustration zooming in on seven successive broadcasts (segment delineated by two vertical dashed lines in A). Each pulse is followed by a long stream of echoes from the chains and then from the far wall. The complex structure of the *Rhinolophus* CF/FM broadcasts only adds to the difficulty of separating the closely-spaced echoes. Like *Pipistrellus* in Fig.4A, the rhythm of IPIs used by *Rhinolophus* in A is irregular, with several short-IPI pulses grouped together and separated by a longer IPI. (C) Spectrograms of the same sounds recorded by the Telemike carried by the bat to pick up broadcasts without reverberation from the chains as in A. The grouping of several broadcasts and the longer IPIs between groups are more evident with the echoes removed. (D) The same seven broadcasts as in B but recorded without the echoes. The closely-spaced pulses in D are intermingled with echoes in B, showing not only that pulse-echo ambiguity occurs but that the bat seems not to alternate IPIs or shift its broadcast frequencies in the presence of the ambiguity. The

frequency-shift strategy to avoid ambiguity is complicated by the use of shifting CF frequencies to compensate for echo Doppler shifts, a unique feature of CF/FM echolocation by *Rhinolophus* and other CF/FM species.



**Fig. 9.** IPIs used by *Rhinolophus* during flights. **(A)** The sample sequence shows regular alternation between several short IPIs and one intervening long IPI. This same pattern appears in Fig. 8. **(B)** The distribution of IPIs is strongly but not smoothly skewed, with a sharp peak around 25 ms for the shorter IPIs between members of sound (strobe) groups, and a broader spread to 40-60 ms for the longer IPIs between groups. Intervals shorter than about 30 ms regularly expose the bat to pulse-echo ambiguity. **(C)** The dot pattern for the distribution of post- and pre-IPIs has two widely-separated clusters lying above and below the diagonal line, signifying the alternation to long IPIs between sound groups, plus three zones at Short IPIs representing the repetition of these IPIs within sound groups. **(D)** Histogram for the distribution of post- to pre-IPI ratios is markedly trimodal, with peaks at 0.5, 1.1, and 2.5.