Echolocating bats can use acoustic landmarks for spatial orientation

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Summary

We investigated the echolocating bat's use of an acoustic landmark for orientation in a complex environment with no visual information. Three bats of the species *Eptesicus fuscus* were trained to fly through a hole in a mist net to receive a food reward on the other side. In all experiments, the vocal behavior of the bats was recorded simultaneously using a high-speed video recording system, allowing for a 3D reconstruction of the flight path. We ran three types of experiments, with different spatial relations between the landmark and net hole. In the first experiment, the bat's behavior was studied in test trials with the landmark placed 10 cm to the left of the net opening; between test trials, the positions of the net opening and landmark were moved, but the spatial relationship between the two remained fixed. With the landmark adjacent to the net opening, the bats quickly found the hole. In the second experiment, bats were tested in control trials in which the landmark was moved independently of the hole, breaking the established spatial relationship between the two. In control trials the bats repeatedly crashed into the net next to the landmark, and inspected the area around it. In the final experiment, the landmark was removed altogether from the set-up. Here the bats spent more time per trial searching for the net opening with an increased number of inspections as well as crashes into the net. However, over the course of a test day without the landmark, bats reduced the time spent per trial and focused inspections and crashes around the hole. The behavioral data show for the first time that the echolocating bat can learn to rely on an acoustic landmark to guide spatial orientation.

Key words: bat, *Eptesicus fuscus*, echolocation, acoustic landmark, orientation.

Introduction

Animals depend heavily on spatial information from the environment to find food, mates, a roost or a nest. Local visual cues or landmarks are widely used by a variety of animals when they operate in familiar surroundings. For example, pigeons (Braithwaite and Guilford, 1991; Burt et al., 1997), arthropods (Tinbergen and Kruyt, 1938; Wehner et al., 1996), corvids (Kamil and Jones, 1997), electric fish (Cain and Malwal, 2002), rodents (Collet et al., 1986; Save et al., 1998) and other mammals (Cheng and Spetch, 1998) all use local visual landmarks for orientation (Vander Wall, 1990; Devenport et al., 2000; Shettleworth, 2000).

Surprisingly, the exclusive use of acoustic landmarks has never before been reported for any species. Even in rats, animals that have well developed hearing, there are only negative findings for the use of acoustic landmarks alone for spatial orienting. For example, Rossier et al. (2000) demonstrated that the addition of acoustic cues improved the performance of a rat using a visual landmark in a water maze, but a sound stimulus alone was not sufficient for the rat to orient properly. Indeed, there is no evidence for exclusive use of acoustic landmarks for spatial orientation, even among auditory specialists like echolocating bats. This raises the question of whether distal sensing of sound can serve as a reliable reference for spatial orientation.

Microchiropteran bats use echolocation for spatial orientation, along with the detection and tracking of prey (Griffin, 1958). These animals probe the surroundings with high frequency sound pulses and listen for information about objects carried by their reflected echoes. They can detect echoes from small objects at a distance up to 5 m (Kick, 1982). For object localization, bats compute the direction using interaural differences in the returning echoes and the distance from the time delay between sound emission and returning echo. The spatial resolution of the bat's directional hearing is comparable to many mammals, approximately 1° in the horizontal plane (Masters et al., 1995; Simmons et al., 1983) and 3° in the vertical plane (Lawrence and Simmons, 1982). By contrast, the bat's range resolution is highly specialized (reviewed in Moss and Schnitzler, 1995). The bat processes information about the environment from the direction and distance information extracted from sonar echoes, computing a three-dimensional (3-D) acoustic representation which, in turn, can be used to establish acoustic spatial landmarks for orientation.

Although bats have a high resolution echolocation system that allows them to operate in complete darkness, their vision is adequate to provide spatial information about landmarks in the environment (Suthers, 1970; Neuweiler, 1999). The bat retina responds best at low light levels and saturates at medium light levels (Hope and Bhatnagar, 1979). Visual acuity of bats is poor compared with diurnal mammals, but is comparable with many other nocturnal mammals (Pettigrew et al., 1988). Visual acuity of the big brown bat is about 1° (Bell and Fenton, 1986), similar to that of rodents.

Almost 30 years ago, Williams et al. (1966) reported that phyllostomid bats can indeed use vision to reference landmarks as they navigate in familiar territory over several kilometers. This was demonstrated in homing experiments, in which bats were wearing either opaque masks that eliminated spatial vision or identical transparent masks, and were able to home only with the transparent masks that permitted the use of vision.

Field observations also suggest that bats may use spatial memory in favor of echolocation. For example, bats living in large numbers in caves appear to rely on spatial memory when exiting at dusk. Under these conditions, bats produce echolocation pulses, but if a barricade is placed at the opening of a cave they crash into it (Griffin, 1958), which indicates that they may rely on spatial memory, not echoes to orient in familiar environments.

That bats establish spatial memory of the environment is also suggested by the results of laboratory studies. For example, Griffin (1958) required a bat to perform an S-shaped flight path between a roost site and a feeding site in a room. Once the bat had become familiar with the room, one of the obstacles, a large masonite plate, was moved so that the opening was now at the opposite site. The bat consistently produced around 20 echolocation pulses per second when flying and yet, when the plate was moved the bat crashed into it at the previous position of the opening. In another study, bats of the species *Megaderma lyra* were trained to fly through a mesh with 70 squares, each 14 cm×14 cm, to access a feeding station. Both vision and echolocation were available to the bat in this experiment, and the results showed that each bat picked a preferred opening to fly through, and remembered the position of this opening with an accuracy of 2 cm (Neuweiler and Möhres, 1967). Also under conditions of illumination that would permit the bat's use of vision, studies have also demonstrated egocentric navigation in *Phyllostomus discolor* (Höller, 1995; Höller and Schmidt, 1996), and allocentric navigation in *Eptesicus fuscus* (Mueller and Mueller, 1979).

More recent field experiments (Helversen and Helversen, 2003), and laboratory experiments in a naturalistic environment (Winter and Stich, 2005), demonstrated that phyllostomid bats have a large capacity for spatial memory. Also, vespertilionid bats like *Eptesicus nilssonii* visit the same feeding patches and return to the same roost, not only after a night's hunt, but also year after year (Rydell, 1990). These studies do not exclude the bat's use of vision either, but they show that bats establish a memory of the area in which they live and forage, and such spatial representations can persist for a long time.

Although it has been demonstrated conclusively that echolocating bats can use hearing to represent space with high resolution (Moss and Schnitzler, 1995) and that they can rely on 3-D acoustic information to orient in complex environments (Moss and Surlykke, 2001), the bat's exclusive use of echolocation to establish spatial landmarks has not been previously demonstrated. A more complete study of the bat's use of sensory information to build representations of space is important, not only for understanding the orientation behavior of this animal in particular, but for learning more about spatial memory systems in general.

To determine whether an animal can use acoustic landmarks for spatial orientation, we conducted a series of experiments with echolocating bats under conditions that precluded their use of vision. We examined details of dynamic changes in sonar signal production and flight behavior when bats used echolocation alone to navigate and seize prey in a complicated environment, where it might improve its success if it made use of acoustic landmarks. *Eptesicus fuscus* was chosen as the study animal because its adaptive echolocation behavior is well described both in the field and the laboratory. We used the bat's adaptive response in sonar pulse design as an indicator of how it perceives its immediate surroundings, to reveal how it exploits landmarks and/or spatial memory while navigating in a complex, but familiar, environment and, importantly, in the absence of visual cues. Our results show that echolocating bats can make use of acoustic landmarks to guide 3-D flight paths.

Materials and methods
Animals

We initiated training of nine big brown bats *Eptesicus fuscus* Beauvois, of which three completed the tasks. These three bats were trained to perform in an obstacle avoidance and prey capture task in a large laboratory flight room. Two bats were collected from the attics of private homes in Maryland, USA, whereas the third bat was collected from a winter colony in Ontario, Canada. One male (M1) and one female (F1) were run in the summer and fall of 2002. The female (F1) and a different male (M2) were run in the spring 2003. M1 unfortunately died over the winter. The bats were housed in a vivarium at the University of Maryland under constant temperature (25°C) and humidity (50%). The light:dark cycle of the vivarium was 12 h:12 h with lights switched off at 08:00 h and turned on at 20:00 h. This reversed light cycle enabled the experiments to be conducted during daytime when the bats would otherwise be inactive. The bats were fed on mealworms, which they caught during experiments. Animals had access to freshwater in their cages at all times, and vitamins were provided in the drinking water every second day. Bats were weighed every day before the start of an experiment, and their mass was maintained between 12 and 15 g.
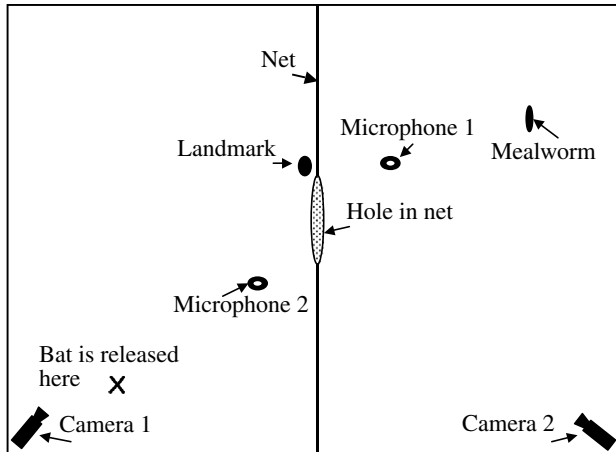
Fig. 1. A schematic drawing of the experimental setup (not to scale). The net divided a large flight room in two, and the net position could be adjusted to change the location of the opening in the horizontal axis. The center of the hole was approximately 1.5 m above the floor. Two high speed cameras were placed in corners of the room and recorded the bat's flight path. Two microphones placed 30 cm above the floor, one on each side of the net (both 50 cm from the net), recorded the bat's echolocation pulses. A landmark (photo tripod) was placed on the left side of the hole, and a tethered mealworm was hung from the ceiling at various positions on the other side of the net. The bat had to find its way through the net opening in order to get the mealworm reward.

Experimental set-up

During experiments the flight room was illuminated with low level (less than 0.05 lux) long-wavelength light (>650 nm), precluding the bats from using vision to perform the task (Hope and Bhatnagar, 1979). Experiments were conducted in a large flight room (6.5 m×7 m×3 m) lined with acoustic foam. The room was divided by a mist net (Avinet, Dryden, NY, USA; Fig. 1) made of 0.1 mm diameter threads, and the space between adjacent threads was 25 mm. The knots that tied the threads together in a diamond pattern were 0.4 mm thick. A hole with a diameter of 35 cm was cut in the net. The bats were trained to fly through this hole to gain access to a food reward on the other side (Fig. 1). The position of the net opening was adjacent to a landmark in some experiments (see below). For practical reasons the net opening could only be moved in the horizontal plane, and no vertical movement was possible. The center of the hole was approximately 1.5 m above the floor. The behavior of the bats was recorded on two gen-locked, high-speed video cameras (Kodak Motion Corder; 240 frames s^{-1}) placed in two corners of the room. Using a calibration frame and commercial software (Motus 3.2, Centennial, CO, USA) this setup allowed us to reconstruct the 3-D flight path of the bats. Simultaneous with the video recordings, echolocation calls were picked up by two ultrasound microphones (Ultrasound Advice; 3.5 cm in diameter) on the floor one on each side of the net, as shown in Fig. 1. The echolocation pulses were amplified (40 dB), band pass filtered (10–100 kHz, ±3 dB, Stanford Research Systems,

Sunnyvale, CA, USA), and recorded onto two channels on a Wavebook (IoTech, Cleveland, OH, USA) with sampling rate of 250 kHz for each channel. A manual end-trigger system was connected to both the video and sound recording systems. Upon triggering, the preceding 8 s from a buffer of the audio and video recordings were stored. This set-up allowed us to correlate the bat's acoustic behavior with its flight behavior in each trial.

Sound analysis

The echolocation sounds were analyzed using a custom MatLab program (BatGadget, written by Aaron Shurger and modified by Amaya Perez). Sound parameters analyzed were signal duration, signal interval (measured from start of one signal to the start of the next), start- and end-frequency of the first harmonic, and the bandwidth between these two measurements. Time parameters were measured from the oscillogram, whereas the frequency parameters were measured from the spectrogram. Spectrograms were made of 256 points Fast Fourier Transform (FFT), using a Hanning window and 45% overlap between consecutive FFTs. Bats using frequency modulated (FM) signals reduce signal duration as they approach an object, thus continually avoiding an overlap between outgoing cry and returning echo (Cahlander et al., 1964; Kalko and Schnitzler, 1989; Hartley, 1992). The time, or space, in which such an overlap would occur, has been referred to as the pulse–echo overlap zone (Kalko and Schnitzler, 1993) or the 'inner window' (Wilson and Moss, 2004). It is assumed that this zone or inner window is an indicator of the shortest range at which a bat is searching for prey. We used the bats' pulse duration to calculate the size of the inner window as ($\frac{1}{2}$) × signal duration × speed of sound (Kalko and Schnitzler, 1993), i.e. the minimum target distance in front of the bat where there is just no overlap between outgoing pulse and returning target echo.

Video analysis

For each video frame, the positions of the bat, the landmark, each microphone, the edge of the net hole (using eight evenly spaced markings) and the mealworm were digitized. Each object was marked with a different color to identify it and match up the spatial coordinates of recordings in the two cameras. The coordinates were then exported to a database and combined with the sound recordings to generate a 3-D animation of the bat's flight path in relation to the hole, the landmark and the worm using a MatLab program (written by Aaron Schurger and modified by Amaya Perez).

Echo measurements

We measured the echoes from the net, the worm and the landmark. Sounds were generated using a Tucker-Davis-Technologies System 2 (Alachua, FL, USA; hardware and software), amplified, filtered (Stanford Research Systems) and broadcast through a speaker [Tweeter LT800; frequency response flat (±3 dB up to 100 kHz)] powered with Krone-Hite DC amplifier. Echoes were picked up with a GRAS $\frac{1}{4}''$

microphone (40BF; Holte, Denmark) amplified 40 dB by a Larsen and Davis amplifier/power supply, filtered by a WaveTech filter (Karachi, Pakistan; band pass filtered 10–100 kHz) and digitized on-line with a Wavebook (Iotech), using a sampling rate of 500 kHz. The emitted signal was 1 ms in duration and consisted of a downward linear frequency modulated (FM) sweep, from 90 kHz to 20 kHz, shaped by a Hanning window function. The source level from the speaker was 77 dB re SPL at 10 cm. The signal was repeated every 0.05 s. The speaker and the microphone pointed towards the same position on the target, but the microphone was placed 10 cm closer to the target than the speaker to maximize the signal-to-noise ratio (SNR). The speaker was placed 50 cm from the target. With a signal duration of 1 ms there was no overlap between outgoing sound and incoming echo. We measured the target strength of the net at two angles, 90° and 45°, respectively, between the net and the sound beam. Target strengths of the landmark and the mealworm were only measured from one angle. All target strength calculations are referenced to 10 cm such that the target strength was measured in dB as the echo level 10 cm from the target relative to the incoming sound level at the target. The target strength of the net measured at an angle of 90° was –26 dB, whereas the target strength measured at an angle of 45° was –22 dB. The target strength of the tethered worm was –16 dB, and that of the landmark was measured to –1 dB. All sound levels are given in dB SPL re 0.0002 Pa rms. The microphones were calibrated using a Sound Level Calibrator (Brüel & Kjær 4231; Nærum, Denmark).

Behavioral experiments

Since the only source of light came from low level (<0.05 lux), long wavelength (>650 nm) illumination, the bats could only obtain information about the room through echolocation (Hope and Bhatnagar, 1979). The bats were trained to fly through the hole in a mist net (see details above) and catch a tethered mealworm on the other side. After the initial training a landmark, a photo tripod, was introduced and placed 10 cm to the left of the hole, adjacent to it with the top of the tripod at level with the center of the hole. The bats were released from the same area (within 1 m^3) in all trials. In the first 5–6 sessions, the setup was moved every fourth trial. The landmark was always at that same position relative to the hole, whereas the mealworm was moved to a new position between each trial. We did this to show the bats that the landmark provided them with reliable information about the hole's position. We introduced control trials, in which only the landmark was moved to a new position, whereas the hole remained in the same position. Only one control trial was run in each session. After 5–6 sessions with the setup being moved every fourth trial, we started moving the setup between every trial. Again one control trial was conducted in each session. At the end of each trial the bat was caught in a butterfly net and held in that while the setup was moved and the video and sound recordings were downloaded for off-line analyses. This break between trials lasted for approximately 5 min. In control trials

when only the landmark was moved, we caught the bat and made the same sounds as when we moved the net, and the break lasted as long as between test trials. Bats were tested 5–6 days a week. Each test day is referred to as a session, with 5–11 trials run for each bat in each session. We defined a trial as the time from the release of the bat until it flew through the hole or the experimenter aborted the trial. An aborted trial occurred when the bat stopped attempts to fly through the hole, hung on the wall producing few or no echolocation pulses, or repeatedly crashed into the net in the same position. In control trials the experimenter terminated a trial after the bat had crashed into the net three times. From the time the bat was released until it passed through the net opening it was free to fly around on the release side, exploring the net, the landmark and the hole. The bat's behaviors were noted on data sheets, and the duration of each trial was determined. We also noted how often and where the bat crashed into the net, and how often it inspected the net, and the hole. In control trials it was further noted if the bats inspected the landmark. In test trials it was not possible to separate inspections of the hole from inspections of the landmark, since these were too closely spaced. The bat's behavior was classified as an inspection when it flew up close to the net (or the hole and the landmark) and then away, or flew close (within 50 cm of the net) and parallel to the net using an increased repetition rate of echolocation pulses relative to the repetition rate used before closing in on the net. The start of the inspection was noted as the point where the bat came within 50 cm of the net.

Finally, we conducted a series of experiments in which the landmark was removed and the bats were required to find the opening in the net using echolocation and/or spatial memory of the setup. The opening in the net was moved to a new position at the start of the no-landmark sessions and remained in that same position throughout the session. In this way we could test if the bats developed a memory of such a setup within a short time of exposure (six trials per session) as some earlier anecdotal reports have suggested. The bats were still caught between trials and remained in the capture net while video and audio data were downloaded.

Results

Database

There was no significant difference in the behavior of the bats between sessions in which the setup was moved every trial and sessions in which the setup was moved every fourth trial (as indicated by the number of successful flights through the hole, number of crashes into the net, number of inspections, and time spent per trial; $P<0.05$). Thus, we pooled the data for these two experiments. There were, however, some differences in the details of the three bats' behavior, and therefore the data from the three bats were analyzed separately. Table 1 summarizes the number of sessions and trials analyzed for the different setups for each bat. Behavioral data were noted for every trial and in some trials the high quality video recordings were analysed in detail, allowing for correlation between the

Table 1. *Number of sessions and trials analyzed*

	Behavioral data					Video and sound analysis	
	Landmark present			No landmark		Number of trials	
	Sessions	Trials					
Bat	Total	LM at hole	LM moved	Sessions	Trials	LM present	No LM
F1	38	235	23	7	40	44	13
M2	17	114	12	9	52	35	15
M1	32	214	31	–	–	30	–

Bat M1 died before the experiment with no landmark (LM) was conducted.

acoustic behavior and flight behavior (Table 1). In the course of one trial the bat may show several behaviors, i.e. inspections, crashes into the net, and flying through the hole. Thus, in some trials the sound and video recordings were divided into sections and analyzed accordingly, with each section containing a particular category of behavior.

General behavior

The three bats had individual approaches to solving the task. When the female F1 was released, she flew directly to the same spot on the wall from which she produced echolocation pulses of long duration (around 6–8 ms). The time she spent on the wall was highly variable, lasting between a few seconds and

several minutes. When taking off, she reduced pulse duration to ca. 3 ms and either flew one or two rounds before going back to the wall, or made an attempt to go through the hole. If the attempt resulted in a crash into the net she returned to the same spot on the wall. The male M1 never landed on the wall, but flew continually through an entire trial. Upon release he used echolocation pulses with durations around 3 ms. The signal duration was reduced as he approached the net. Most often M1 went straight from the release site to the hole and attempted to go through it. If he crashed into the net, he flew one or two rounds in the room and then made a new attempt to go through the hole. The second male M2 also flew throughout an entire trial and used the same range of pulse durations as M1.
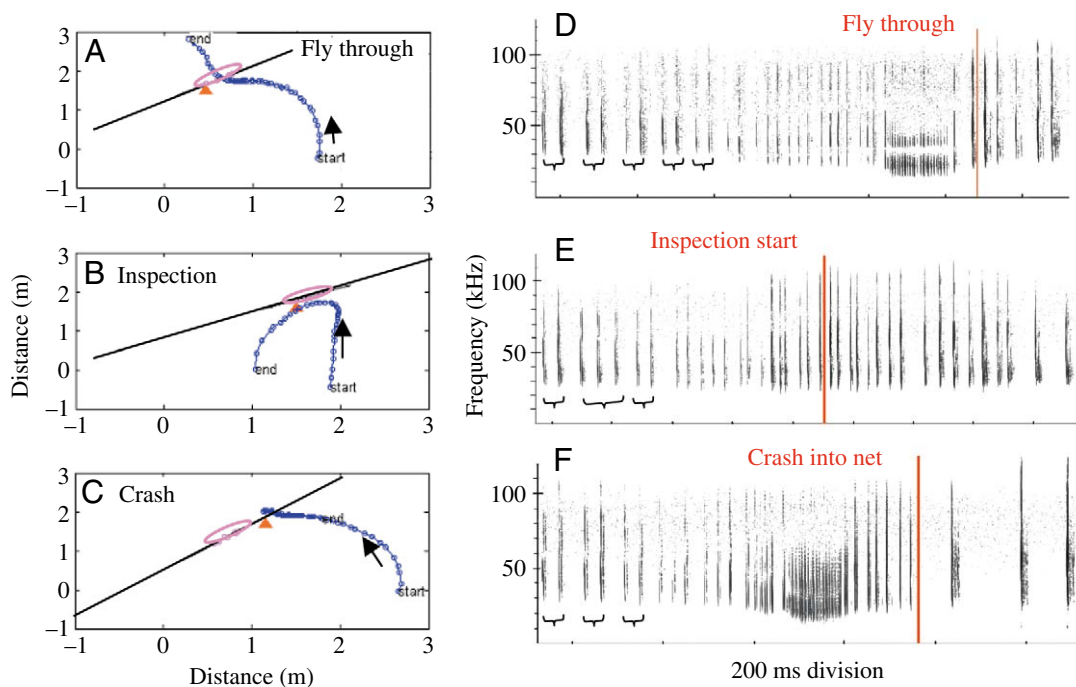

Fig. 2. Flight patterns for three different behaviors (A–C), along with the corresponding spectrograms of the sounds produced by the bats (D–F). (A,D) A bat flew through the hole. (B,E) A bat inspected the hole. (C,F) A bat crashed into the net next to the landmark, at the usual position of the hole relative to the landmark. (A–C) The bats' flight paths are displayed as viewed from above. The net is indicated by a diagonal line and the opening is marked in pink. The pink triangle represents the landmark's position along the net in the specific trial. Blue circles denote the bat's position at the time of vocalization and black arrows indicate flight direction. (D–F) In the spectrograms, some of the pulses are clustered together in sound groups with two or three pulses (bracketed). A vertical red line in the spectrogram shows the time the bat flew through the hole, crashed into the net or started an inspection.

However, M2 flew a couple of rounds after release and inspected the hole and/or landmark more frequently than the other bats before he attempted to go through the hole. Thus, the individual behaviors of the three bats were different, but as the results show, the relative changes in behavior and performance with changes in the setup were similar for the three animals.

Behavioral data

Three types of behaviors were possible in each trial: (1) inspection of the net, (2) crashing into the net and (3) flying through the hole. A bat might show all three in one trial, i.e. first crash into the net, then make an inspection of the net, and finally fly through the opening. We would then record a count of one for finding the hole, but also counts for each crash and inspection. Examples of these behaviors are shown in Fig. 2.

With the landmark next to the hole, the bats found the opening in 99–100% of all these trials (Fig. 3). Since they could only fly through the hole once per trial, an average score of 1 in Fig. 3 means that the bat eventually found the hole in all trials. Before finding the opening in the net, the bats occasionally flew into parts of the net. Such crashes into the net occurred on average between 0.3 and 0.4 times per trial when the landmark was adjacent to the hole (Fig. 3A). The vast majority of those few crashes into the net happened just around the hole, with the remaining crashes occurring at the position where the hole had been in the previous trial (4–7%, Table 2).

In control trials, the landmark was moved to a position away from the hole. In these trials one bat (M1) never found the hole (thus a value of 0 in Fig. 3B), whereas the two other bats (M2 and F1) found it in 20% of the trials (Fig. 3B). All bats crashed repeatedly into the net next to the landmark where the hole normally was found relative to the landmark. The average number of crashes when the landmark was moved away from

Table 2. *Percentage of all crashes into the net that occurred at the previous position of the hole and the percentage of crashes that occurred right around the hole*

	Crashes with landmark		Crashes without landmark	
Bat	Previous	Hole	Previous	Hole
F1	4.5 (1)	95.5 (21)	44.0 (32)	42.0 (30)
M2	5.0 (1)	95.0 (20)	55.0 (76)	29.0 (40)
M1	7.5 (3)	80.0 (32)	–	–

Numbers in parentheses refer to the actual number of crashes at the specific position. With the landmark next to the hole (test trials) the bats rarely crashed at the previous position of the hole. With no landmark, the bats crashed into the net at a position different both from the previous position and from the opening in 14% (F1) and 16% (M2) of all crashes. No data were obtained for M1 with the landmark removed.

the hole was between 1.8 and 3 per trial for the three bats (Fig. 3B), which is significantly more crashes than when the landmark was next to the opening (within bats $P<0.05$, Student's t-test). For example, M1 flew directly into the net next to the landmark where the net hole was previously found relative to the landmark. He crashed in this position without hesitation three times in a row. Also F1 crashed repeatedly next to the landmark in these control trials. However, the other male bat, M2, did not crash as consistently as the two others. This bat sometimes stalled in front of the net and flew away, as if detecting the faint echoes from the net. Yet, this did not prevent M2 from crashing into the net at this position in the next attempt.

In the experiments with no landmark the two bats (M2 and F1) crashed as often as 1.4 and 2.2 times on average per trial, but eventually found their way through the hole in 82–85% of

Fig. 3. (A–C) Average number of crashes and flights through the hole per trial, and (D) the average duration of trials. Values are means +1 S.D. Data are shown for each bat (M1, M2, F1) separately. (A) The landmark is placed next to the hole. In the fly through data F1 failed to find the hole in one trial, which explains the error bar for that bat. (B) The landmark is moved to a position away from the hole. (C) Here there was no landmark available. (D) The average duration of a trial, when the landmark was next to the hole, moved away from the hole, or no landmark available.
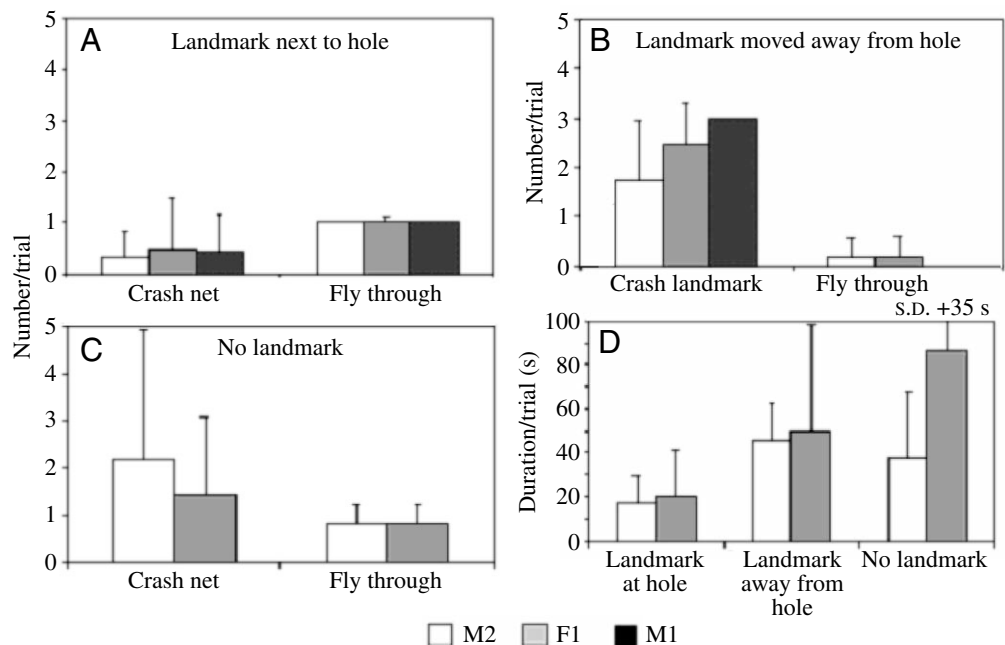
the trials (Fig. 3C). The bats did not crash at random into the net. In fact, the majority of crashes were at the position where the opening had been on the previous session, and a considerable number of crashes occurred just around the hole (within 15 cm from the rim; numbers are listed in Table 2). Bat M1 died before it could be tested in the no-landmark setup.

On average the bats took less than 20 s per trial when the landmark was placed adjacent to the hole, whereas they spent on average 40 s or more when the landmark was moved away from the hole or was absent (Fig. 3D). This is consistent with the increased number of inspections and crashes when the landmark was moved or absent (Figs 3 and 4).

We classified flight behavior as inspections if either the bat flew close to the net (within 50 cm of the net), parallel to the net, or flew up to the net or the hole and stalled or made loops in front of the net. All bats inspected the net more frequently in control trials and when there was no landmark, compared to trials in which the landmark was adjacent to the hole (Fig. 4). With the landmark placed adjacent to the opening (test trials), the bats inspected the net between 0.1 and 1.4 times per trial on average (Fig. 4A). This was also the case in control trials, but in addition to inspecting the net including the hole, the bats also
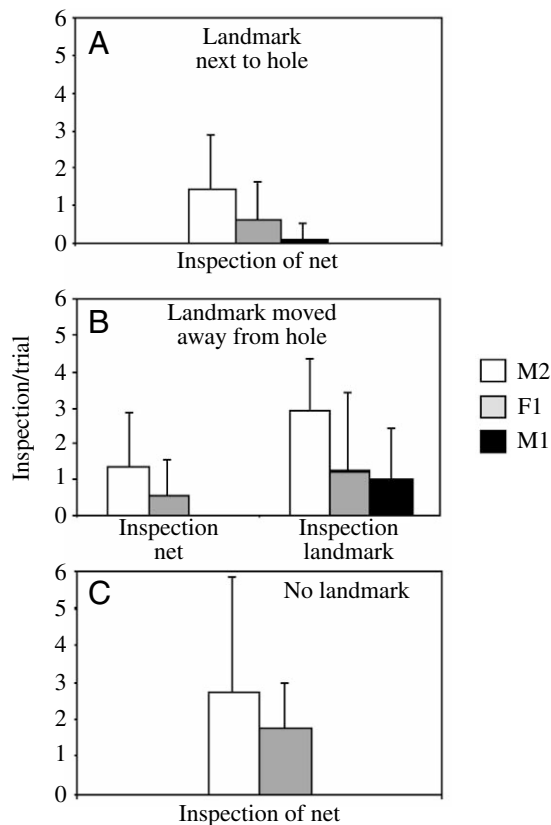

Fig. 4. Average number of inspections per trial when (A) the landmark was next to the hole, (B) the landmark was moved to another position and (C) there was no landmark available. A and C show all inspections recorded, whereas in B inspections are separated into inspections of landmark and inspections of the rest of the net including the hole. Values are means +1 s.d.

inspected the landmark, which was now between 1 and 3 m from the landmark position adjacent to the hole in test trials (Fig. 4B). When the landmark was removed the bats inspected the net between 1.5 and 2.5 times per trial on average (Fig. 4C). The average number of inspections differed between the three bats, with the male M2 making by far the most inspections in all situations [*P*<0.05, ANOVA and Tukey's HSD test (multiple comparison, honest significant difference test)].

In the no landmark experiments, the hole remained in the same position throughout the session, which made it possible to investigate whether the bats improved their performance over the course of a session. The average performance improved from trial to trial through the sessions and the bats spent less time seeking the net opening in the later trials compared to the first ones across sessions (Fig. 5A,B). This trend is most clear for the bat F1 (Fig. 5B). The total number of crashes and inspections per trial remain relatively unchanged in the course of a session, but after parsing the crashes into 'crash into net' and 'crash into net near hole', a different pattern appears (Fig. 5C,D). From trial to trial the number of crashes at the previous position of the hole decreased and by trial 5 or 6, both bats rarely crashed into the old hole position. After 5–6 trials F1 flew through the new hole without much difficulty, whereas M2 also aimed at the new hole, but had some trouble determining the exact position, and hence crashed now and then.
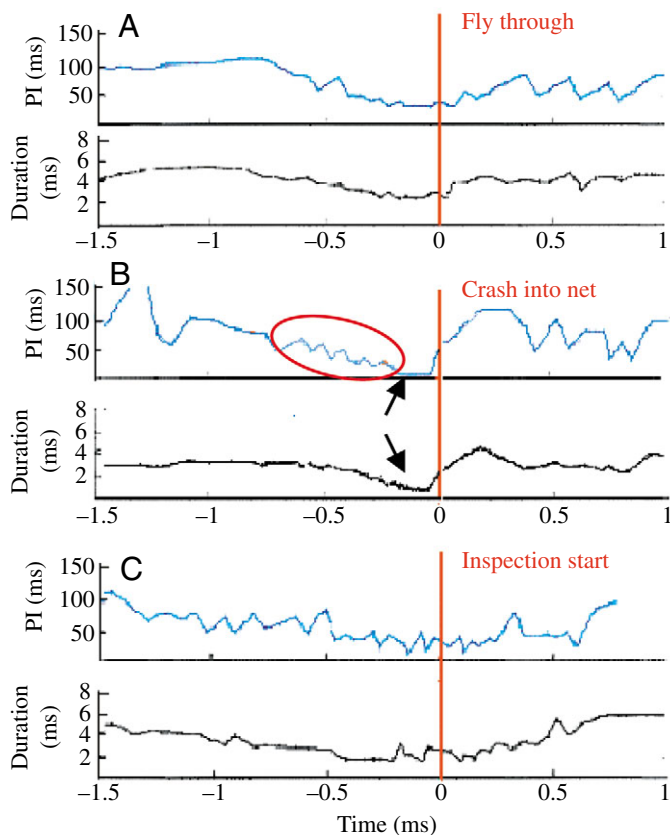
Acoustic behavior

All three bats showed similar acoustic behavior once they were flying and approaching the net. They all reduced the signal duration and pulse interval (PI) as they approached the net, regardless of whether they flew through the hole, crashed into the net, or flew up to inspect the net (Figs 2 and 6). The reduction of the pulse duration was a relatively smooth transition, whereas the PI pattern was more irregular, because often the pulses were produced in sound groups, typically consisting of 2–5 signals (Figs 2 and 6B). As the bat approached the net, the PI within a sound group was reduced from sound group to sound group. Also, the interval between sound groups was reduced as the bat approached the net. This was clearly seen for M1 and M2 whereas F1 rarely produced these sound groups and thus had a smoother reduction in the PI as it approached the net (Fig. 6A). A distinct acoustic behavior was observed on several occasions, either when the bat flew up to the net, or before it flew through the hole or crashed into the net. It consisted of a group of 5–25 echolocation pulses in which the signal duration and PI were reduced to less than 1 ms and 10 ms, respectively (Fig. 6B). It shows some resemblance to a feeding buzz (the terminal phase of an insect pursuit), but also some distinct differences. In order to prevent confusion, we refer to this sound group as a 'high rate sound group'. High rate sound groups occurred in 42% of the trials analyzed for M2, both before it flew through the hole or crashed into the net. Thus, it was not possible to use the bats' acoustic behavior to predict the bats' success in flying through the hole.

Fig. 5. Average performance of the bats as a function of trial number over a session in the experiment with no landmark at the hole. (A) The average success of flying through the hole. (B) The average time spent on the trials. (C) The average number of crashes into the net far away from the hole and (D) the average number of crashes into the net around the hole. Values are means +1 S.D.
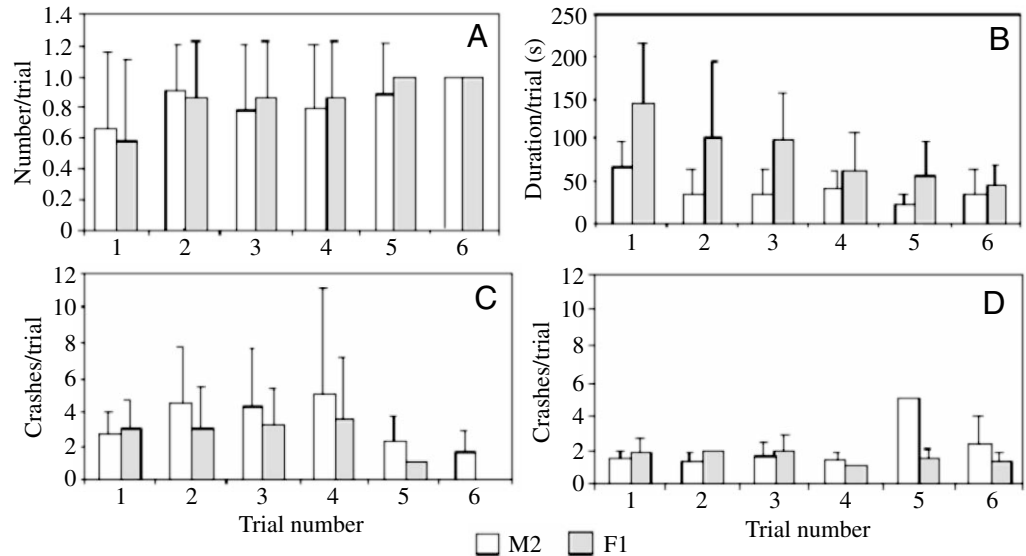
Inner window

The inner window is defined as the range from the bats mouth to the minimum target distance in front of the bat, where there is just no overlap between outgoing pulse and returning target echo. The exact size of the inner window is solely determined by the sonar signal duration used by the bat (see Materials and methods for further details). As described above, the bats reduced sonar signal duration as they approached the net. Presumably, this reduction of signal duration reflects their efforts to avoid overlap between their outgoing sonar pulse and the echoes from the net or the landmark. However, before the bats actually flew through the hole or crashed into the net they increased the signal duration to between 3–5 ms (Fig. 6), corresponding to an inner window range between 52 and 86 cm. This increase in signal duration resulted in an overlap between outgoing pulse and incoming net echo between 50 and 330 ms before the bat flew through the hole (examples in Fig. 7A–D). The overlap between the bat's sonar pulse and the net echo suggest that its acoustic gaze was shifted to a location beyond the net; however, it was not possible to use changes in signal duration to make inferences about which objects in the room the bat have been attending. Pulse–echo overlap did not occur during inspections, since the bat only increased signal duration after it turned to fly away from the net. In some trials M2 first produced a high rate sound group and then increased the signal duration 50–150 ms before flying through the hole or crashing into the net. The increase in signal duration caused an overlap between pulses and net echoes (Fig. 7B,C). In trials with no high rate sound groups M2 increased the pulse duration earlier, thus experiencing pulse–net echo overlap earlier, i.e. between 125 and 315 ms before flying through the hole or crashing into the net (Fig. 7A,D). The four examples of overlap between the inner window and the net echo in Fig. 7 illustrate that there is no systematic correlation between the time where the overlap first occurs and the outcome of the net approach (fly through vs crash into net). Neither is there a direct relation between the

Fig. 6. Typical trials showing signal duration (black line) and pulse interval (PI; blue line) when the bat (A) flew through the hole (F1), (B) crashed into the net (M2), or (C) inspected the net (M2). In C the zero indicates the start of the inspection behavior (see Materials and methods and Fig. 2B). Note in B how the pulse interval shifted up and down as the pulses were produced in little sound groups (in red circle). Further, note the high rate sound group (indicated by arrows) just before the bat crashed, showing very short signal durations and intervals.
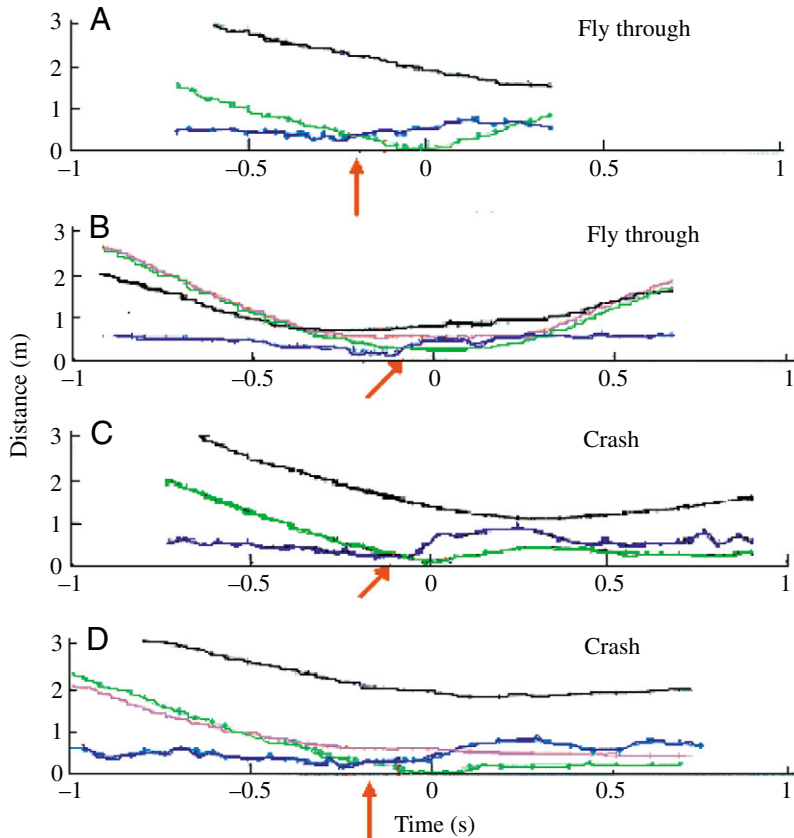
time of overlap and the presence/absence of the landmark (Fig. 7). F1 never produced high rate sound groups before it flew through the hole or crashed into the net. F1 tolerated pulse–net echo overlap between 100 and 330 ms prior to these interactions. This bat never used signal durations shorter than 2 ms when flying through the hole, crashing into the net, or in most inspections. Only in two inspections containing a high rate sound group did the bat's signal duration become shorter than 2 ms. In contrast to the acoustic behavior in response to the net, bats consistently adjusted the duration of their signals to avoid overlap between pulses and landmark echoes

The acoustic behavior indicates that the bats crashed into the net because they did not detect it. There were no obvious differences between the sonar behavior of a bat that crashed into the net and one that flew successfully through the hole. In fact, it was not possible to infer from its echolocation pattern if the bat had a successful fly-through or crashed into the net.

Discussion

The results of this study offer the first demonstration that an animal can rely exclusively on acoustic landmarks to guide spatial orientation. Past research, including studies on animals with well-developed hearing, such as rodents and echolocating bats, have permitted the use of visual cues, sometimes in combination with acoustic cues, for spatial landmark orientation (Hahn, 1908; Neuweiler and Möhres, 1967; Höller, 1995; Rossier et al., 2000).

In the present study, an echolocating bat searched for an opening in a mist net, which allowed it access to a food reward. The bat performed this task under conditions that excluded its use of vision, and therefore only echoes from its sonar vocalizations provided spatial information about the environment. An echo-reflecting landmark provided the bat with a spatial reference to find the opening in the net. After the bat had learned that the landmark provided spatial information about the opening in the mist net, it successfully found its way to the food reward. In experimental trials, the relative position of the landmark and net opening was held constant, though they were both moved inbetween trials. In control trials, the landmark and net opening were moved independently. Although the sonar returns from the mist net can be detected by the echolocating bat, the animals favored use of the landmark over the net echoes to guide orientation behavior. The bat's reliance on the acoustic landmark was indicated by repeated crashes into the net in control trials, in which the spatial cue was invalid.

The bats produced echolocation calls continuously during all trials, but since the animals crashed into the net adjacent to the landmark in control trials, they either ignored or failed to perceive the echoes from the net. The echoes from the landmark were 21–25 dB more intense than the net echoes. Listening for the intense echo from the landmark is undoubtedly an easier task than searching for opening of the net that produced echoes with a target strength around -22 dB and -26 dB at 10 cm, which is less than target strengths

measured from some of the smallest moths that bats feed on (around –17 dB at 10 cm, at 30 kHz; Surlykke et al., 1999).

When the landmark was removed altogether from the setup, the bats spent more time searching for the hole, and they crashed into the net more frequently than when the landmark was present and positioned adjacent to the net opening. Over the course of a test day when the landmark was absent, the bats spent less time searching for the net opening and found the net opening more consistently. Furthermore, in the first trials of a session the bats crashed most frequently into the net at the position where the hole had been on the previous session, and in the later trials, when bats crashed into the net, they did so in regions adjacent to the opening. We therefore conjecture that the bats had adequate acoustic information to detect the net, but relied more on the strong echoes obtained from the landmark when it was available to reference the position of the net opening.

The bats' crashes around the net opening in the final trials of a session without the adjacent landmark suggest that they learned the approximate position of the opening, likely referenced to other fixed objects in the room, such as the microphones on the floor, but that the resolution of the spatial reference was too low for complete clearance through the opening in each attempt. Other studies of spatial memory in different bat species report higher memory resolution than we found in the present study of *E. fuscus* (Höller, 1995; Neuweiler and Möhres, 1967). In fact, the bat, *M. lyra,* remembered the position of an opening with an accuracy of 2 cm (Neuweiler and Möhres, 1967). One might therefore infer that *M. lyra* shows much better resolution in spatial memory than the bats in our study. However, in the study on *M. lyra* the bats had several days (the exact number of days is not provided by the authors) to learn the position of a preferred opening in the grid, whereas the bats in our study only had one session (consisting of 6 trials) to learn the position of the net opening. Furthermore, the lights were on during the experiment with *M. lyra,* and the authors argue that bats require visual cues to obtain spatial memory of an area (Neuweiler and Möhres, 1967). This argument is based on the observation that sighted bats found their roost with a higher success rate than blinded bats (Barbour et al., 1966; Williams et al., 1966).

It is indeed noteworthy that all prior studies of spatial memory in bats have been conducted with illumination permitting the use of vision (Hahn, 1908; Neuweiler and Möhres, 1967; Höller, 1995). The reduced performance accuracy we find in the present study, compared to the study on *M. lyra*, can be explained by a combination of species differences, lack of visual cues, or the difference in time allowed for the bats to develop a high resolution spatial representation of the environment, or perhaps all three possibilities. It is likely that a combination of visual and auditory cues can improve the bats' performance, as has been shown for rats (Rossier et al., 2000), and this could possibly be true for other animals as well. Importantly, the data reported here demonstrate that bats can develop spatial references through the auditory system alone.

In the present study, the bats produced echolocation sounds throughout a trial and thus had the possibility of using sonar returns to find the opening in the net, rather than using spatial memory. The echo measurements of the net revealed that the sonar returns were weak, but also that their strength depended on the angle of ensonification, such that a bat approaching the net at a perpendicular angle received a weaker echo than a bat approaching it at a smaller angle. During inspections, the bats often flew nearly parallel to the net, thus increasing the net echo strength. This may have helped them to detect the part of the net with the opening, even though the apparent size of the opening is reduced when the angle of approach diminished. Regardless of the angle of approach, the bats had difficulties finding the opening, suggesting that the contrast between the net and the hole was poor, due to the faint echoes from the net. Therefore, the use of spatial landmarks would offer the animal an advantage in finding its way through the net opening.

Acoustic behavior

There were no obvious differences between the patterns of vocal production from a bat flying successfully through the net opening and those from a bat flying into the net. In both situations, the bats decreased signal duration and interval as they approached the net, responding in both cases as if they were approaching an object. This suggests a dissociation between processing and responding to echoes that would inform the bats of an imminent crash.

When the bats inspected the net, their acoustic behavior was not entirely predictable. The signal duration and interval decreased with approach to the net, but the reduction was not as pronounced as when they flew through the opening or crashed into the net. All bats occasionally produced high rate sound groups during an inspection of the net, and this was always accompanied by sharp turns and/or loops in the flight path. The high rate sound groups have been described in a number of situations, for example a bat landing at a roost site or inspecting an object (Faure and Barclay, 1994). As mentioned above, these high rate sound groups resemble the feeding buzz, but the pulse duration and pulse interval never become as short as in a buzz that precedes insect capture. In the feeding buzz of *E. fuscus,* pulse duration and interval can become as short as 0.5 ms and 5.5 ms, respectively. A feeding buzz in *E. fuscus* can also be divided into a buzz I and a buzz II phase (Surlykke and Moss, 2000; Griffin et al., 1960). In the high rate sound group the pulse duration drops below 1 ms, but the pulse interval never falls below 10 ms, and this vocal behavior pattern never develops into the buzz II phase, which is characteristically produced before feeding. The high rate sound groups provide the bat with a higher rate of acoustic sampling, and it may be that approach to a roosting perch, or in this case passage through an obstacle, requires increased accuracy in sonar localization.

The reduced sonar signal duration and interval is an expected response from a bat approaching an object. However, at some point before flying through the hole, or crashing into the net, the bats abruptly increased sonar signal duration, which

resulted in an overlap between the outgoing cry and the net echo. Since bats that use frequency modulated sonar vocalizations actively avoid pulse–echo overlap under most conditions (Cahlander et al., 1964; Kalko and Schnitzler, 1989; Hartley, 1992), the abrupt increase in cry duration indicates that the bat shifted its acoustic gaze to a position further away, i.e. to the other side of the net. Exactly, how far out along the range axis the bat shifted its gaze is not possible to determine from the signal duration, which only indicates the shortest object range to which the bat is responding. Indeed, the bat may be attending to echoes from objects at a distance that extend beyond the inner window constrained by sonar signal duration. During inspections, the bats adjusted their sonar signal duration consistently to avoid pulse–echo overlap, even in situations when no high rate sound groups were produced. Therefore, during inspections, the bats' acoustic gaze was presumably at the net and the landmark, but not the other side of the net.

In this study, we found that bats can rely on landmarks perceived through auditory cues alone. This, of course, does not rule out the bat's use of visual cues in the development of a spatial representation of the natural environment. Bats often fly out at dusk, and during the commute between the roost and the hunting area, there will often be some level of light available, especially in regions with long dusks and light nights. In some parts of the world, dusk lasts for more than an hour, and it may never get completely dark overnight, leaving the bat with reliable visual cues to orient. In areas where the nights can get very dark, moonlight may provide the bats with sufficient illumination for using vision, along with echolocation to successfully establish and reference spatial landmarks.

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