

## RESEARCH ARTICLE

# Echolocating bats inspect and discriminate landmark features to guide navigation

Chao Yu, Jinhong Luo\*, Melville Wohlgenuth and Cynthia F. Moss<sup>‡</sup>

## ABSTRACT

Landmark-guided navigation is a common behavioral strategy for way-finding, yet prior studies have not examined how animals collect sensory information to discriminate landmark features. We investigated this question in animals that rely on active sensing to guide navigation. Four echolocating bats (*Eptesicus fuscus*) were trained to use an acoustic landmark to find and navigate through a net opening for a food reward. In experimental trials, an object serving as a landmark was placed adjacent to a net opening and an object serving as a distractor was placed next to a barrier (covered opening). The location of the opening, barrier and objects were moved between trials, but the spatial relationships between the landmark and opening, and between the distractor and barrier were maintained. In probe trials, the landmark was placed next to a barrier, while the distractor was placed next to the opening, to test whether the bats relied on the landmark to guide navigation. Vocal and flight behaviors were recorded with an array of ultrasound microphones and high-speed infrared motion-capture cameras. All bats successfully learned to use the landmark to guide navigation through the net opening. Probe trials yielded an increase in both the time to complete the task and the number of net crashes, confirming that the bats relied largely on the landmark to find the net opening. Further, landmark acoustic distinctiveness influenced performance in probe trials and sonar inspection behaviors. Analyses of the animals' vocal behaviors also revealed differences between call features of bats inspecting landmarks compared with distractors, suggesting increased sonar attention to objects used to guide navigation.

**KEY WORDS:** Acoustic orientation, Active sensing, Way-finding, Biosonar, Spatial perception

## INTRODUCTION

Landmark-guided navigation, a strategy by which animals find their way through the environment, has been studied in a wide range of species (Chamizo et al., 2012; Cheng, 1986; Collett et al., 1986; Foo et al., 2007; Jensen et al., 2005; Milgram et al., 1999; Nemmi et al., 2013; Rodrigo et al., 2014; Zhao and Warren, 2015). Past work has demonstrated that the spatial location (Biegler and Morris, 1993) and physical features (Chan et al., 2012; Nothegger et al., 2004; Sorrows and Hirtle, 1999) of landmarks can influence navigation

behaviors, but the active inspection of landmarks by animals engaged in navigation tasks has not previously been investigated.

Biegler and Morris (1993) reported that the constant spatial location of a landmark is required for an animal to show landmark learning. They trained rats to locate food that was hidden at a fixed distance and direction to a landmark. When the landmark remained at a constant location, the rats learned to search around the marked location to find food, even when the food was absent. However, when the landmark was moved from trial to trial, animals did not search at locations designated by the landmark, suggesting that the animal failed to learn to use the object as a landmark when its location was variable.

Other research findings challenge the conclusions of Biegler and Morris (1993) by demonstrating landmark-enhanced learning in animals tested in a dynamic environment (Roberts and Pearce, 1998). Specifically, rats trained with a moving landmark and platform in the Morris water maze task showed higher success and faster response times in reaching a submerged platform compared with rats trained with a static landmark–platform configuration, suggesting accelerated learning in animals exposed to changing spatial locations of the landmark. Roberts and Pearce (1998) attribute the discrepancy between their findings and those reported by Biegler and Morris (1993) to the richness of the animal's environment. They reasoned that their test environment contained many distal cues, which could provide the animals with directional information and thus facilitate goal localization.

The conflicting findings from past research on landmark-guided navigation in rodents raise the question of whether environmental test conditions and/or stimulus parameters influence landmark-guided navigation in different species. Past research on bats, for example, indicates that landmark navigation differs across species and tasks. Nectarivorous and frugivorous bats rely preferentially on spatial location cues over stimulus cues when feeder locations remain constant on a wall during training (Carter et al., 2010; Thiele and Winter, 2005). However, in conditions under which feeding locations were not constant across trials during training, insectivorous bats instead learned to use object features to find a goal (Hulgard and Ratcliffe, 2014; Siemers, 2001). Additionally, insectivorous big brown bats can learn to rely on the location of a moving landmark to guide navigation (Jensen et al., 2005).

Although the spatial location of a landmark can provide an important cue for navigation, the features of a landmark may sometimes provide more useful information than its location. For example, humans may learn to make a left turn at the Starbucks in the city center, without storing information about the specific location of the building. In other situations, humans can use a sign above a doorway to find an exit from a building. Here, the physical features of the sign, rather than its location in the building, serve as the primary cue to select a navigation path.

The physical features of a landmark (shape, color, size, etc.) have been shown to contribute differentially in guiding navigation

Department of Psychological and Brain Sciences, Johns Hopkins University, 3400 N Charles Street, Baltimore, MD 21218, USA.

\*Present address: School of Life Sciences, Central China Normal University, Wuhan, Hubei 430079, China.

<sup>‡</sup>Author for correspondence (cynthia.moss@jhu.edu)

 C.F.M., 0000-0001-6916-0000

Received 7 September 2018; Accepted 26 March 2019

(Chan et al., 2012). Specifically, past work has demonstrated that the more salient or distinct an object, the more likely such an object will be used as a landmark (Nothegger et al., 2004; Sorrows and Hirtle, 1999; Stankiewicz and Kalia, 2007), and by extension it may also invoke salience-dependent inspection behaviors. However, these previous studies only examined the influence of physical characteristics on an animal's use of a landmark for navigation by either directly changing the landmark features, or by correlating a change in an animal's behaviors with different landmark features. A direct measure of an animal's active inspection of physical features of landmarks during navigation has not been previously explored.

Animals that use active sensing to localize objects in the environment are well suited for laboratory studies of landmark inspection during spatial navigation. For example, *Eptesicus fuscus*, an insectivorous North American bat, adapts the time–frequency structure and directionality of echolocation calls to actively gather information from the environment to discriminate and localize objects (Falk et al., 2011; Moss et al., 2006, 2011; Simmons et al., 1979). The bat's adaptive control over sonar call features therefore provides explicit indicators of inspection behaviors in the context of spatial navigation.

The present study quantified the sonar inspection behaviors of bats engaged in a spatial navigation task. Bats were trained to discriminate between two objects (a landmark and a distractor object), which were similar in size but differed in physical characteristics, and to use the landmark to guide navigation through an opening in a net to access a food reward. We monitored the bats' sonar inspection of the objects by recording and analyzing the directional aim and temporal features of their echolocation calls as they performed the landmark-discrimination task. We hypothesized that bats show increased sonar-guided attention to landmarks than to distractors.

## MATERIALS AND METHODS

### Animals

Four wild-caught bats [*Eptesicus fuscus* (Palisot de Beauvois 1796); two males and two females] served as subjects in this study. The bats were collected in the State of Maryland under permit number 55440. The animals were housed in animal facilities at the Johns Hopkins University under a reversed light:dark cycle (12 h:12 h dark:light). The Institutional Animal Care and Use Committees at the Johns Hopkins University approved all procedures.

### Apparatus and data acquisition

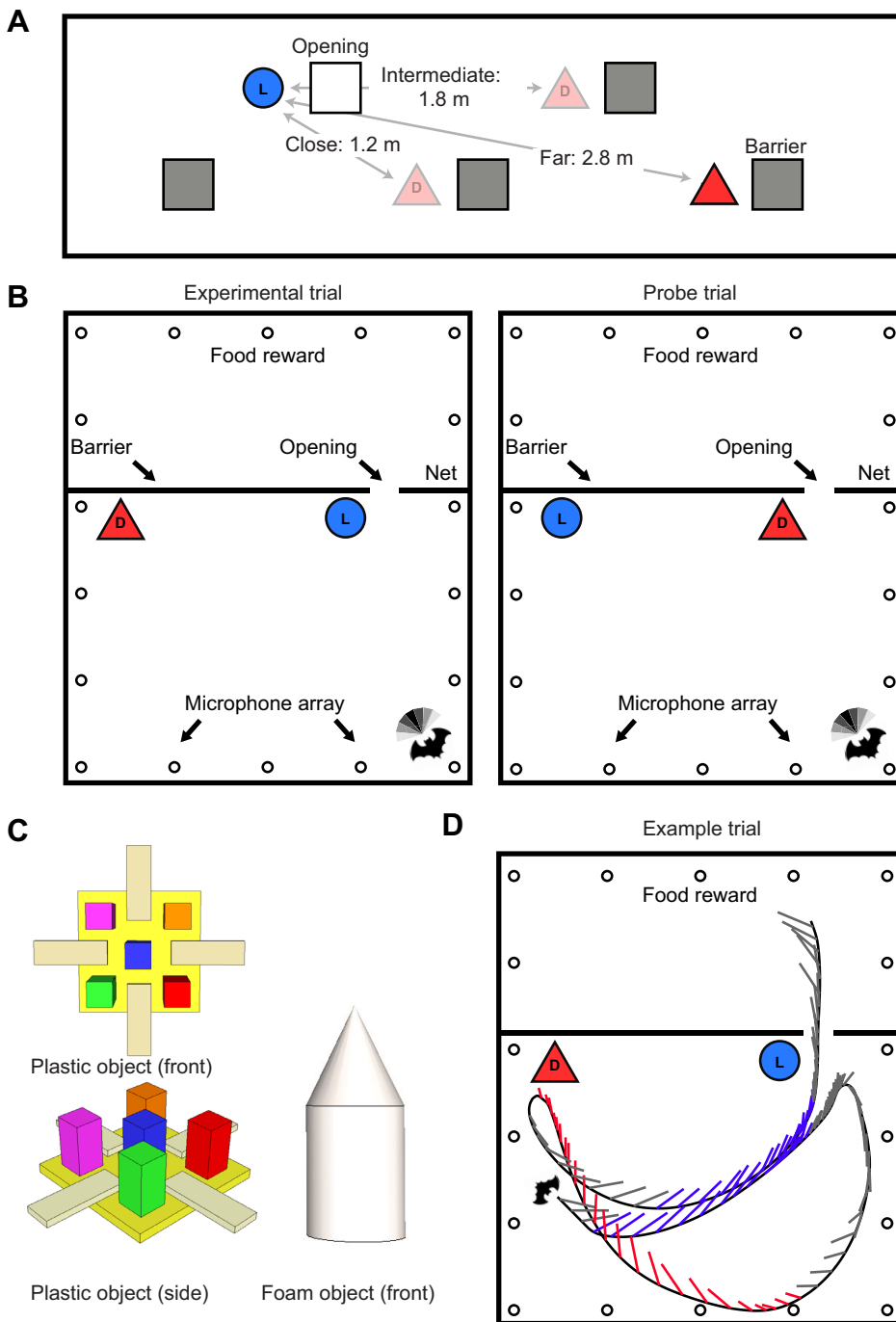
The experiment was performed in a large flight room, 7×6×2.5 m, with walls and ceiling lined with acoustic foam to reduce acoustic reverberation. The room was partitioned into two compartments with deer blocking net. The bat was always released on one side and trained to fly through a 30×30 cm opening in the deer blocking net to the other side of the room to receive a food reward, a mealworm. Schematics of the experimental setup are presented in Fig. 1. The net opening positions varied across trials. There were five possible locations of the opening in the net, differing in elevation and azimuth (Fig. 1A). In each trial, four of the five locations were covered with deer blocking net pieces so that only one opening was available for the bat to fly through to obtain its food reward on the other side of the room (Fig. 1B). The landmarks used for the current experiment were two similarly sized (approximately 20 cm in length) objects: a plastic multi-faceted object and a foam cylinder with a conical top (Fig. 1C). The plastic object consisted of a square base plate, four arms coming off the center of each side of the square plate, and five spines extending orthogonally. The foam cylinder

consisted of a foam column at the bottom and a cone shape on the top. In each experimental trial, one of the objects, which the bats learned to use as a landmark, was positioned 15 cm to the left of an opening in the net. The other object, here referred to as a distractor object, was placed 15 cm to the left of a different barrier (one of the covered openings, Fig. 1A). Both objects were hung from the ceiling with fishing line at pre-determined locations adjacent to the net. We identified three possible spatial separations of interest, defined as: close (1.2 m), intermediate (1.8 m) and far (2.8 m) between the two objects (Fig. 1A). We used a MATLAB program to generate a pseudo-random sequence of the opening and barrier positions along the net. The positions were selected so that neither the opening nor the barrier repeated its position on two consecutive trials. Bats were trained to use different objects as the landmark to guide navigation (see below).

The room was illuminated by infrared LEDs outside the visible range of *E. fuscus* (Hope and Bhatnagar, 1979). We used a night shot video camera (SONY HDR-PJ790V, Japan) to record entire experimental sessions and measure trial completion times. Eighteen motion-capture cameras (T40 and T40s Vicon Motion Systems Ltd, UK), operating at a sampling rate of 100 Hz, were mounted to the ceiling of the room, to record and later reconstruct the bats' 3D flight trajectories. These cameras tracked the positions of infrared reflective markers placed on the bat. In addition, an 18-channel microphone array (D500X external microphone, Petterson Elektronik, Uppsala, Sweden) was used to record the bat's echolocation calls and reconstruct the beam axis, i.e. where the bat directed the center of its sonar beam (Ghose and Moss, 2003). Signals from each microphone channel in the array were filtered between 10 and 100 kHz (USBPB-S1, Alligator Technology, CA, USA) and sampled at 250 kHz (NI PXI-6143, National Instruments, Austin, TX, USA). The microphones were fixed on the wall, approximately 1.2 m above the floor, and distributed evenly along the walls. Each microphone was tagged with two reflective markers that aligned with the direction it was pointing, to obtain its position and vector in the video data. One example trial showing the flight trajectory and sonar beam direction of a bat is presented in Fig. 1D. We used an end-trigger to capture 8 s of video and audio data, just before the bat successfully flew through the net or crashed into the net on each trial. The video equipment and microphones were synchronized with a common transistor–transistor logic (TTL) trigger signal.

### Echo recording and measurement

We recorded and analyzed echoes from the two objects by broadcasting a 1 ms synthetic bat echolocation signal, comprising a two harmonic FM down sweep (50–20 kHz and 100–40 kHz) over 20 repetitions. With the 1 ms synthetic chirp duration, there was no overlap between the broadcast signal and the echo return. The outgoing sonar signals were amplified (model 7500, Krone-Hite) and broadcast through a custom electrostatic loudspeaker at 1 m distance from the objects. We designed a compensatory impulse response of the loudspeaker frequency response using the maximum length sequence method to generate a broadcast signal with equal energy in the range of 20–100 kHz ( $\pm 1$  dB) (Luo et al., 2015). Echoes were recorded with a ¼ inch microphone with the grid on (model 7016, ACO Pacific), calibrated using a Brüel & Kjær type 4231 calibrator. The signals were amplified and recorded at 250 kHz, and then digitized (PXIe 8135, with two data acquisition cards PXIe 6358, National Instruments). The loudspeaker and microphone were both directed at the center of the front surface of the objects (Fig. 1C). The microphone was placed at a distance of



**Fig. 1. Experimental setup and example trial.**

(A) Front view of the net partition. Five possible net opening positions are shown in square shapes. One net opening was randomly chosen during each experimental trial, shown in white. Other positions were covered during the experiment, shown in gray. The landmark (L) is shown in blue, and the distractor (D) is shown in red. Three spatial separations between two objects are shown: close (1.2 m), intermediate (1.8 m) and far (2.8 m). (B) Schematics of experimental procedures. A net wall (shown in A) partitioned the flight room into two parts. An 18-channel microphone array was mounted and distributed along the walls of the flight room. The left panel shows the schematic of an experimental trial: the landmark was placed next to the opening, and the distractor was placed next to a covered opening; the right panel shows the schematic of a probe trial: the landmark was placed next to a covered opening, and the distractor was placed next to the opening. (C) Schematics of the two objects used in the experiment. Two bats (plastic group) were trained to use the plastic object (left) as the landmark and the foam object (right) as the distractor; the other two bats (foam group) were trained to use the foam object as the landmark (right) and the plastic object (left) as the distractor. The plastic object is presented at two view angles to show the asymmetric structure. (D) Example experimental trial. The bat inspected both objects before it flew through the opening. The flight path is shown in black, and the aim and duration of each vocalization is represented by a vector along the flight path. The vector direction indicates the sonar beam aim, and the vector length indicates the inner window ( $0.5 \times \text{call duration} \times \text{speed of sound}$ ) of the vocalization. The calls that are directed toward the landmark are in blue, and the calls that are directed toward the distractor are in red.

50 cm from the object to increase the signal-to-noise ratio. The broadcast was 75 dB SPL at the object location.

For the sound recordings, we manually marked the onset and offset of each recorded echo returned from the objects. Then we computed the root mean square (RMS) SPL of the echo for each repetition and calculated the mean of all 20 repetitions. We used the RMS echo SPL to calculate target strength, i.e. the amplitude difference between the sound impinging on the object and the echo, at a reference distance of 1 m.

### Experimental procedures

Four bats were first trained to fly through a net opening, marked by a learned landmark, to receive a mealworm reward on the other side of the room. Two of the four bats (one male and one female) used the

plastic object as the landmark and the foam object as the distractor; we refer to this group of bats as the plastic group. The other two bats (one male and one female) used the foam object as the landmark and the plastic object as the distractor; we refer to this group of bats as the foam group. In experimental trials, the landmark was always placed 15 cm to the left of the net opening, whose location changed from trial to trial. Therefore, the landmark provided a reliable spatial cue to the bat for the net opening location. The distractor object was placed to the left of a barrier in each trial, except in probe trials, where we positioned the distractor object next to the opening and the landmark adjacent to a barrier. We used probe trials to test whether the bat had learned to use the features of the landmark to find its way through the net rather than searching for the net opening directly. The probe trials occurred in 10% of the total trials and were

interleaved in between the experimental trials. We used a block design with a pre-determined number of experimental trials and probe trials in each block before each day's experimental session. We included one probe trial and, on average, nine experimental trials in each block. After all the bats learned to fly through the net openings to receive a food reward, the experimental sessions started.

We prevented the bat from exploring the experimental setup prior to each trial by keeping it in a cage covered with felt while we changed the positions of the two objects and the net opening location. The bat was always released from the same corner of the flight room (Fig. 1B) and was captured with a butterfly net after it successfully completed or failed a trial. We calculated the time from the bat's release to its flight through the opening, and the number of crashes adjacent to the trained landmark for each trial (within 20 cm of the opening/barrier position) from the video recordings of the night shot camera. The latencies and the number of crashes were compared between experimental and probe trials to determine whether the bat was using the landmark to find the opening. During the experiment, if the bat took more than 60 s to fly through the net, we ended the trial and the bat was captured, and the latency was recorded as 60 s. Such trials were considered failures to use the landmark to guide navigation. After the bat was captured, it remained in the cage until the next trial, or the experiment ended for the day.

### Video analysis

The 3D flight trajectories of the bats were reconstructed in Nexus (Vicon Motion Systems Ltd) using data collected with the Vicon motion capture cameras running at 100 frames  $s^{-1}$ . Each marker (those on the bat and on the objects) was manually labeled in Nexus and then exported to MATLAB. We adhered two markers to the bat, one on the head and the other on the upper body. Typically, we used the head marker to track the bat's position. However, in cases where the head marker was not visible in the cameras, we used the upper body marker. The two markers were placed approximately 5 cm apart. We adhered reflective tape to the four edges of the plastic object, and we put reflective tape on the top and bottom edge of the foam object. We averaged the marker positions on each object to obtain each object's center position, which was approximately 15 cm left to the opening or the barrier. We used the front marker (the marker closest to the microphone tip) to mark each microphone's position. The microphone vector was calculated by subtracting the front marker's position from the back marker's position (the marker closer to the wall).

### Audio analysis

The audio data were analyzed using custom MATLAB programs. We used the microphone channel in the array that yielded the best signal-to-noise ratio to manually label the call times and call durations. Pulse interval (PI) was calculated as the time interval between the onsets of two consecutive calls, after compensating for sound travel time at the bat's instantaneous distance from the microphone for each call. PI is a metric used to quantify the sonar vocal rate of the bat. We identified sonar sound groups (SSGs), clusters of calls with shorter PIs, flanked by longer intervals, in all the audio files, following the methods described in Kothari et al. (2014) and Moss et al. (2006). Briefly, the SSGs were identified as clusters of two or more vocalizations where the PIs of the flanking calls are at least 1.2 times longer than the intra-group PIs. Additionally, if there are three or more calls, the PIs within the SSG must have a relatively stable PI (less than 5% error with respect to the mean PI). We then calculated the percentage of the calls that

belonged to an SSG for both calls directed at the landmark or the distractor object. The beam axis analysis was computed following the methods described in earlier publications (Ghose and Moss, 2003; Lee et al., 2017). Briefly, for each call, we first compensated the energy spectral density (ESD) measured at each microphone with microphone sensitivity, directionality and transmission attenuation. We then estimated the direction vectors from the bat to each of the microphones based on the instantaneous slope of the bat's flight trajectory. Finally, we computed the sonar beam axis as the center of the best-fitting Gaussian between the ESD and bat-to-microphone angles at 35 kHz.

Using the beam axis of each call produced by the bat before it flew through the net opening, we calculated the tracking angles to the landmark and distractor objects. The tracking angle was defined as the angle between the beam axis and direction to one object. For example, a tracking angle of 0 deg to the landmark indicates that the bat was pointing its sonar beam axis directly at the object. We then labeled these calls as either inspecting the landmark or distractor object for all tracking angles less than 30 deg. The 30 deg criterion was chosen because past literature reported that the 6 dB sonar beam width of *E. fuscus* is approximately  $\pm 45$  deg at around 35 kHz, the spectral region of greatest energy of this species' call (Hartley and Suthers, 1989), and importantly, that the big brown bat aims its sonar beam with an accuracy of approximately  $\pm 3$  deg at a target it prepares to intercept (Ghose and Moss, 2003). To be conservative, we used  $\pm 30$  deg as our criteria to consider the bat's reception of off-axis echoes. In situations where the tracking angles to both objects were similar (difference less than 20 deg), we excluded labeling calls, because that the bat could be inspecting both objects at the same time. In addition, we calculated the minimum physical distance between the bat and a target for which there was no overlap between the outgoing call and incoming echoes, and refer to this measure as the 'inner window' (Kalko and Schnitzler, 1993). The window is calculated based on call duration:  $0.5 \times \text{call duration} \times \text{speed of sound}$ . FM bats typically avoid call-echo overlap of objects they are attending, and call duration (inner window) therefore provides an indicator of the bat's acoustic gaze along the range axis (Surlykke et al., 2009). When the bat's call duration creates an overlap between emissions and object/net echoes, this implies that the bat has shifted its attention to more distant objects.

The trials with either object located at the left-most location (Fig. 1A) in the room were excluded from all acoustic analysis because the object was very close the left wall (approximate 30 cm) and a microphone that was positioned next to the object. Consequently, we cannot differentiate calls that were directed at the object from those that were directed at the microphone. In addition, past research has suggested that clutter around a target can influence a bat's vocal behaviors (Moss et al., 2006).

The distributions of tracking angles were obtained by transforming the data into histograms with 10-deg bins. Then, we computed the percentage of calls that occurred in each 10-deg tracking angle bin (see Fig. 4). We compared the tracking angles to the landmark and to the distractor for bats in the plastic landmark group and bats in the foam landmark group. To compare the difference between tracking angle distributions to the landmark and the distractor, we calculated the mean squared error (MSE) for each pair of distributions (e.g. plastic landmark-foam distractor and foam landmark-plastic distractor). Thus, a larger MSE value indicates that the two distributions are more different from each other. In addition to MSE, we calculated the half-width (HW) of each distribution of tracking angles. A small HW value indicates that the



bat restricted its sonar inspection within a smaller window of interest, i.e. towards the landmark or distractor object. To statistically quantify the differences between the distributions, we used permutation tests (Good, 2004). Specifically, we pooled the data across conditions (e.g. plastic landmark, foam distractor) and then randomly assigned data points to two new distributions of equal sizes as the original (plastic group:  $N=23,636$ ; foam group:  $N=11,489$ ). For comparisons between tracking angles to landmarks and distractors, we randomized the label of each pair of tracking angles (each vocalization has a tracking angle to the landmark and a tracking angle to the distractor) for every given pair. For comparisons between plastic and foam landmark groups, we randomized the labels of the object serving as the landmark. After this, we obtained the MSE and HW values from the new distributions with the same methods described above. The permutation test procedures were repeated 10,000 times.

When analyzing the sonar beam directing behaviors of the bats as they inspected the objects, we only included calls that were directed at either the landmark or the distractor, categorized with the  $\pm 30$  deg tracking angle criteria (total number of calls: 35,125; number of calls included here: 19,692). Prior work showed that distance to a landmark strongly influences the sonar behaviors of the bat (Jensen et al., 2005), and the bat does not take identical flight paths across trials. To control for the effects of bat–object distance, we binned the response variables (PIs and call durations) into 25 cm distance intervals with respect to the landmark and the distractor, so that each distance was included an equal number of times in the analyses. We then fit all three of these response variables to a general linear model (GLM) using a backward stepwise procedure. The bat's distance to the landmark and its distance to the distractor were set as continuous predictor variables. The object that the bat was inspecting (landmark or distractor), the object that the bat used as a landmark (plastic or foam) and the spatial separation of the two objects (close, intermediate and far; Fig. 1A) were categorized as nominal predictors in the GLMs. In addition, we also assessed interactions between the object that the bat was inspecting (landmark versus distractor) and the object that served as the landmark (plastic versus foam group).

## RESULTS

In this experiment, we trained echolocating big brown bats to discriminate between an acoustic landmark and a distractor, and to use the landmark to find an adjacent opening in the net to access the food reward (Fig. 1).

### Echo measurements from the objects

We first measured spectral and amplitude differences in the echoes reflected from each object used in the study (Fig. 2). The amplitudes of the echoes returned by both objects were similar. Specifically, the target strength of the foam and plastic objects at 1 m were  $-24$  and  $-25.7$  dB, respectively. The echoes returning from the foam object had a similar power spectral density (PSD) distribution to that of the artificial broadcast sonar signal (Fig. 2A). However, the echoes returning from the plastic object revealed spectral notches that arose from interference between echoes returning from closely spaced surfaces of the object. This can be seen in the PSD distribution, which shows prominent notches at several different frequencies when compared with the foam object (Fig. 2A). These spectral notches are more apparent when subtracting the power of the original digitized playback signal from the power of echo recordings of each object (Fig. 2B). Additionally, owing to the complex shape of the plastic object, the echo pattern would be expected to vary

when the bat approached it from different angles, providing additional shape cues to the bat.

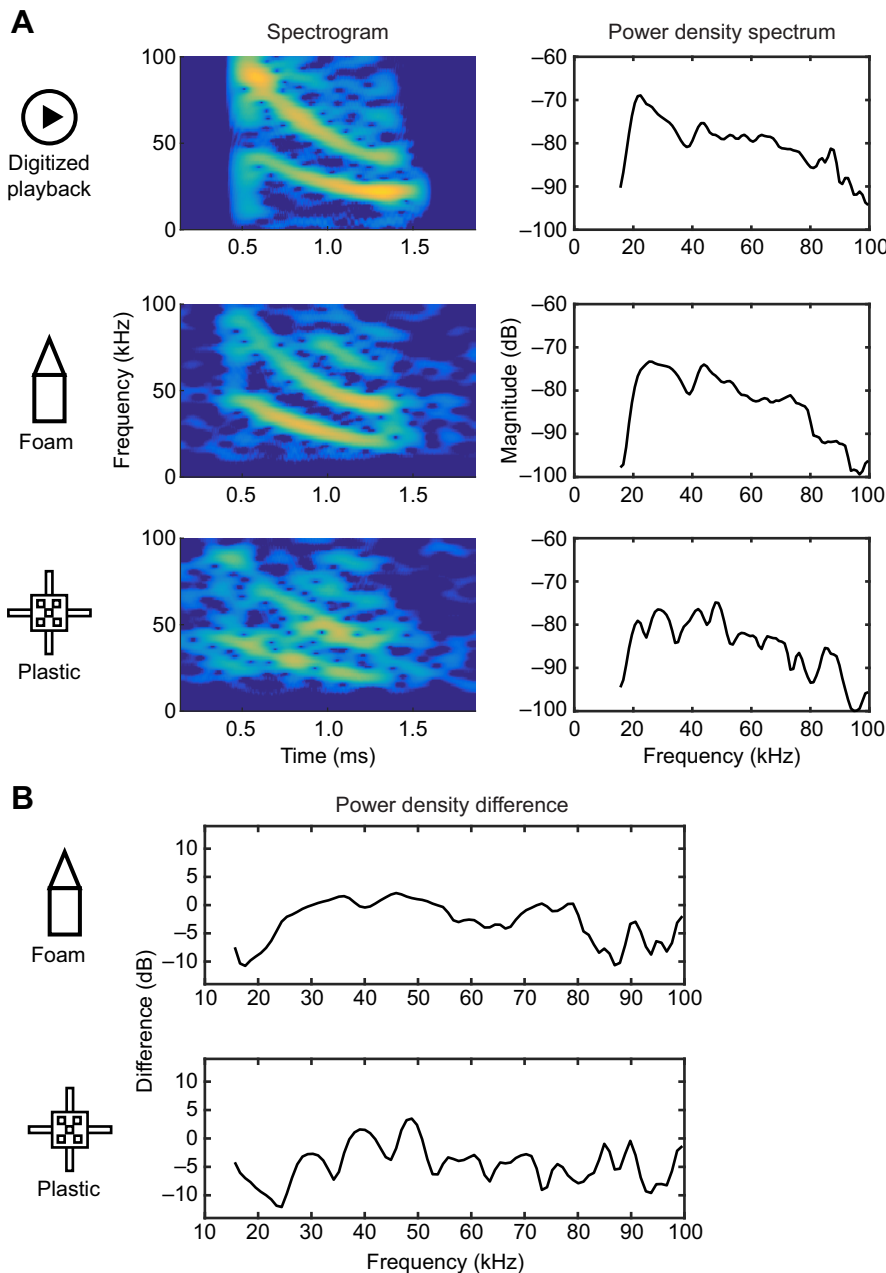
### Behavioral performance

We hypothesized that bat's adaptive echolocation behavior contributes to object discrimination, and thus enables the bat to perform landmark-feature-guided navigation. We used probe trials, where the landmark was placed at a location adjacent to a covered net opening, to determine whether the bat was using the landmark or features of the net to guide navigation. We averaged the latency of the bat to fly through the net and net crashes for experimental trials in each block and compared these measures with the results of the probe trials (distractor object placed adjacent to net opening) to test whether the bat had learned to discriminate between the two objects and relied largely on the landmark to guide navigation. The behavioral performance is shown in Fig. 3. On average, the bats took a longer time (Fig. 3A) and showed increased frequency of net crashes (Fig. 3B) in the probe trials than the experimental trials, suggesting that placing the landmark away from the opening in probe trials interfered with the bats' ability to find the net opening (two-way ANOVA, time:  $F=191.1$ ,  $P<0.001$ ; net crashes:  $F=39.0$ ,  $P<0.001$ ). There was an interaction between the landmarks (the object that was used as the landmark) and experimental conditions (experimental versus probe trials;  $F=45.2$ ,  $P<0.001$ ). Specifically, the bats in the plastic group took a significantly longer time to find the opening and made more net crashes in probe trials compared with the bats in the foam group (time:  $t=7.2$ ,  $P<0.001$ ; net crashes:  $t=5.1$ ,  $P<0.001$ ; Fig. 3A,B).

We used 60 s as a cut-off criterion for determining whether the bat was successful on a given trial (Fig. 3C). The success rate, calculated as  $N_{\text{success}}/N_{\text{total}}$ , was significantly higher in experimental trials than in probe trials in both groups of bats that were trained to use either the foam object or the plastic object as the landmark ( $\chi^2=129.89$ ,  $P<0.001$ ). In experimental trials, the success rates were not different between the bats in the foam group (92.5%) and the bats in the plastic group (92%) ( $\chi^2=0.15$ ,  $P=0.698$ ). In the probe trials, the success rates significantly dropped in both groups, and chi-squared tests revealed a significant difference between success rates in probe trials between the two groups (foam group: 67%; plastic group: 10%;  $\chi^2=26.2$ ,  $P<0.001$ ). The bats in the plastic group showed a lower success rate in probe trials, suggesting that these bats relied heavily on this landmark to find the net opening.

### Echolocation behavior

We compared the distributions of all sonar tracking angles to either the landmark or the distractor in bats using the plastic object and the foam object as the landmark (referred to as the plastic and foam groups) (Fig. 4). The average median tracking angles of the four distributions were 13 deg (plastic group: 17.8 deg for landmark, 4.1 deg for distractor; foam group: 14.5 deg for landmark, 15.2 deg for distractor). Positive tracking angles indicate that the bat was directing its beam to the right of the object, in the direction of the net opening or barrier (Fig. 1A). In both groups, the HWs of the tracking angle distributions to the landmark were smaller than the HW tracking angle distributions to the distractor (permutation test, plastic group: difference in HW= $-139.49$ ,  $P<0.001$ ; foam group: difference in HW= $-22.79$ ,  $P<0.001$ ). Additionally, the MSEs were significantly different between tracking angles to the landmark and to the distractor in both groups (permutation test, plastic group: MSE=6.89,  $P<0.001$ ; foam group: MSE=2.24,  $P<0.001$ ). These results suggest that when inspecting the landmark, the bat restricted its beam aim over a smaller region of interest compared with the



**Fig. 2. Echo recordings and spectral properties for the playback, foam object and plastic object.**

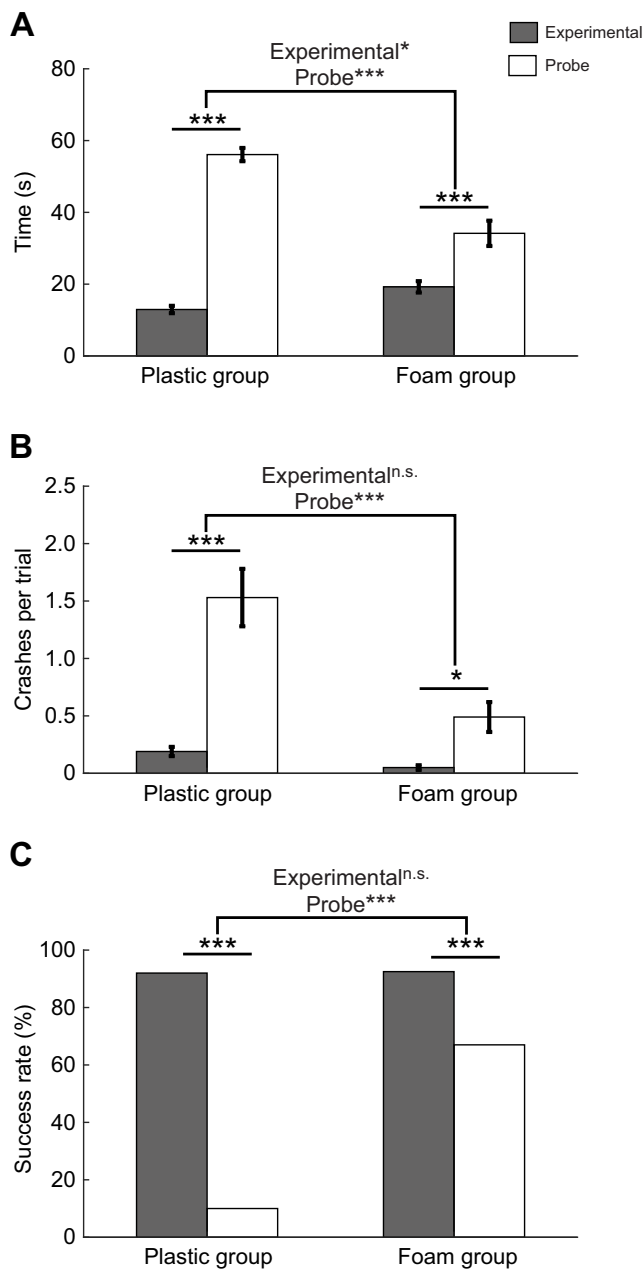
(A) The left panel, top to bottom, displays the spectrogram of the original digitized playback signal (top), an echo returned by the foam object (middle) and an echo returned by the plastic object (bottom). Sonar broadcasts were directed at the center of each of these objects, oriented towards the front of each object at a distance of 1 m as shown in Fig. 1. The right panel shows the power density spectrum (PDS). The echo returned by the foam object showed similar PDS to the playback; the echo returned by the plastic object contains spectral notches at several different frequencies. (B) The power density spectral differences between the digitized signal and the echo returned by the foam object (top), and by the plastic object (bottom).

distractor object. A comparison between distributions showed that the HW was larger for bats in the plastic group for both tracking angles to the landmark and tracking angles to the distractor (permutation test, landmark:  $P < 0.001$ ; distractor:  $P < 0.001$ ). The MSEs were also significantly different (landmark:  $P < 0.001$ ; distractor:  $P < 0.001$ ).

We then analyzed the bat's adaptive echolocation behaviors when it approached both the landmark and distractor objects to investigate sonar inspection strategies in the experimental trials of the task. An example trial is shown in Fig. 1D. We limited our acoustic analysis to the calls ( $n = 19,692$ ) that were directed at either the landmark or the distractor object to quantify the bat's vocal adjustments when inspecting the objects. We calculated the 'inner window' based on sonar call duration (see Materials and Methods), which is defined as the minimum distance between the bat and an object producing no call–echo overlap. Here, we used the inner window calculation to determine whether the bat shifted its acoustic gaze to more distant

objects behind the net while performing this task (see Surlykke et al., 2009). We found that a negligible number of calls ( $n = 29$ , or 0.15% of the sonar calls directed at either the landmark or the distractor) overlapped echoes from the landmark or distractor, suggesting that the bat's acoustic gaze along the range axis in most trials was directed to the landmark and distractor objects, and not to objects beyond the net hole.

The results of adaptive echolocation behaviors as the bats inspected the landmark or distractor are summarized in Table 1. All four bats, when inspecting either the landmark or the distractor, reduced PIs as they approached the objects ( $F = 376.4$ ,  $P < 0.001$ ). The object being inspected was characterized using the sonar beam axis data, i.e. the object to which the bat directed its sonar beam with a tracking angle of  $\pm 30$  deg (see Materials and Methods). Similarly, the bats reduced call duration as they approached each object ( $F = 339.9$ ,  $P < 0.001$ ). We found that the bats in the foam group produced significantly shorter PIs ( $F = 74.7$ ,  $P < 0.001$ ; Fig. 5A) and



**Fig. 3. Behavioral differences between experimental and probe trials.** The bats in the plastic group are shown on the left; the bats in the foam group are shown on the right. Error bars indicate  $\pm$ s.e.m. Experimental trials are shown in grey ( $N=77$ ), and the probe trials are shown in white ( $N=77$ ). (A) Average duration to fly through the net opening in experimental and probe trials. Bats in both the plastic and foam groups took longer to fly through the opening in probe trials than in experimental trials (two-way ANOVA,  $F=182.1$ ,  $P<0.001$ ). The data for bats that used the plastic and foam objects show differences: trial duration was shorter in experimental trials ( $t=-2.1$ ,  $P<0.02$ ) and longer in probe trials ( $t=7.2$ ,  $P<0.001$ ) for bats in the plastic group compared with the foam group. (B) Average number of crashes per trial in experimental and probe trials. Bats in both plastic and foam landmark groups showed more crashes in the net in probe trials compared with the experimental trials (two-way ANOVA,  $F=39.0$ ,  $P<0.001$ ); however, bats in the plastic group showed significantly more crashes in probe trials compared with bats in the foam group ( $t=5.1$ ,  $P<0.001$ ). (C) Success rate of bats in both the plastic and foam groups showed differences between experimental and probe trials. The success rate of all bats dropped during probe trials compared with experimental trials ( $\chi^2=129.89$ ,  $P<0.001$ ). The success rate was significantly lower in probe trials for bats in the plastic group than the foam group ( $\chi^2=26.2$ ,  $P<0.001$ ). n.s., not significant; \* $P<0.05$ ; \*\*\* $P<0.001$ .

**Table 1. General linear model results for three acoustic parameters (pulse interval, call duration and sonar sound groups)**

	Pulse interval	Call duration	Sonar sound groups
Distance to landmark	376.4 (7.1)***	339.9 (0.3)***	–
Distance to distractor	21.8 (1.8)***	131.7 (0.2)***	–
Plastic versus foam	74.7 (3.3)***	1.1 (0.01)	138.1 (9.1)***
Landmark versus distractor	14.9 (1.5)***	10.4 (0.06)**	100.7 (7.7)***
Landmark separation	5.07 (1.3)*	2.7 (0.04)	10.1 (4.2)**
Plastic versus foam $\times$ landmark versus distractor	19.3 (1.6)***	5.0 (0.04)*	59.8 (6.0)***

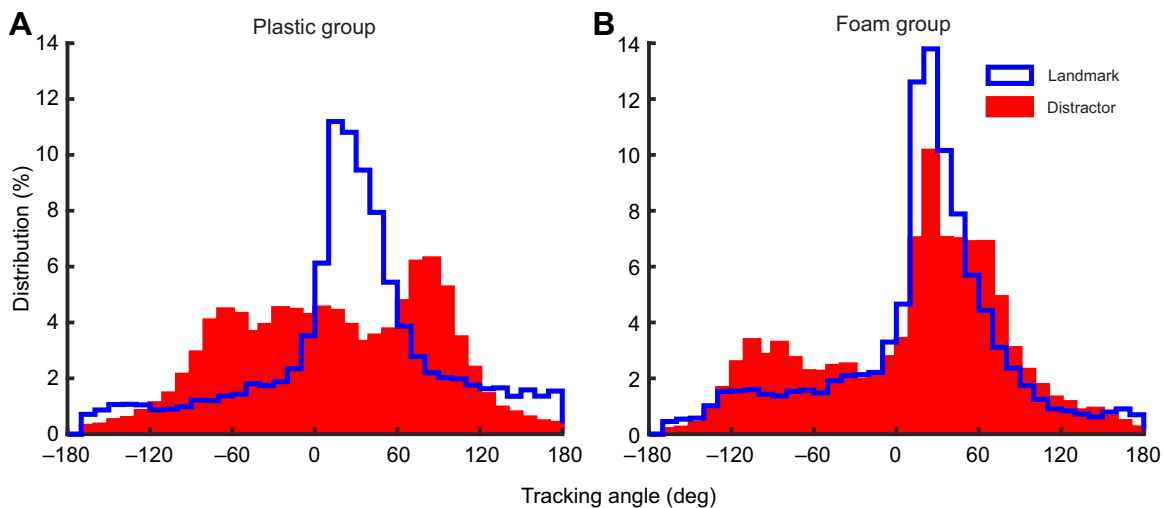
$F$ -values are shown for all factors included in each model. The numbers in parentheses show the estimated coefficients for each factor in the GLMs. The asterisks indicate the level of significance of each factor (\* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ ).

more SSGs ( $F=138.1$ ,  $P<0.001$ ; Fig. 5E) compared with bats in the plastic group. Additionally, for all three acoustic parameters, we found significant interactions between the object serving as the landmark (i.e. plastic versus foam group) and the object that the bat was inspecting (i.e. landmark versus distractor) (PIs:  $F=19.3$ ,  $P<0.001$ ; call durations:  $F=5.0$ ,  $P=0.0248$ ; SSGs:  $F=59.8$ ,  $P<0.001$ ). *Post hoc* Student's  $t$ -tests (with Bonferroni corrections) revealed that the bats in the plastic group produced shorter PIs ( $t=-6.8$ ,  $P<0.001$ ; Fig. 5A) and call durations ( $t=-4.4$ ,  $P<0.001$ ; Fig. 5C), and used more SSGs ( $t=15.1$ ,  $P<0.001$ ; Fig. 5E) when directing the sonar beam at the landmark than the distractor object. However, these differences in sonar call parameters were not significant in bats in the foam group (PIs:  $t=-0.3$ ,  $P>0.05$ ; call durations:  $t=-0.6$ ,  $P>0.05$ ; SSGs:  $t=1.4$ ,  $P>0.05$ ). In addition, when inspecting the landmark, the bats in the plastic group produced longer PIs ( $t=3.4$ ,  $P<0.001$ ) and fewer SSGs ( $t=-2.8$ ,  $P=0.005$ ) compared with bats in the foam group. Similarly, when inspecting the distractor, the bats in the plastic group produced longer PIs ( $t=8.7$ ,  $P<0.001$ ) and call durations ( $t=2.2$ ,  $P<0.027$ ), and more SSGs ( $t=-13.8$ ,  $P<0.001$ ) compared with bats in the foam group.

We also analyzed the bat's echolocation behavior to determine whether the spatial separation of the landmark and distractor objects influenced its control over sonar call parameters. We separately measured the PIs, call durations and number of SSGs when the spatial separation between the landmark and the distractor object was (1) close, 1.2 m apart; (2) intermediate, 1.8 m apart; or (3) far, 2.8 m apart (Fig. 1A). We found that the bats produced shorter PIs when the objects were placed closer together ( $F=5.1$ ,  $P=0.024$ ; Fig. 5B). Bats also produced a higher percentage of SSGs when the landmark and distractor objects were closer together ( $F=10.1$ ,  $P<0.01$ ; Fig. 5F). However, the spatial separation between the landmark and distractor object did not influence call duration ( $F=2.7$ ,  $P=0.098$ ; Fig. 5D).

## DISCUSSION

The goal of this study was to quantify natural inspection behaviors and performance of bats engaged in a landmark navigation task. We trained four echolocating big brown bats, *E. fuscus*, to discriminate between two objects, and to use one of the objects as a landmark to guide navigation. Our behavioral data show that bats successfully discriminated between the two objects in the task, as evidenced by behavior in probe trials, in which bats crashed more frequently and found the net opening at a longer latency when the distractor object was placed adjacent to the net opening. The bats' sonar inspection behaviors also differed between the landmarks and distractors: when



**Fig. 4. Distributions of tracking angles of echolocation calls.** (A) Distributions of tracking angles with respect to the landmark (blue) and to the distractor (red) of bats in the plastic group ( $N=23,636$ ). The mean squared error between tracking angles to the landmark and the tracking angles to the distractor were significantly different from zero (permutation test,  $P<0.001$ ). The half-width (HW) of the tracking angle distribution to the landmark was smaller than the HW tracking angle distribution to the distractor (permutation test,  $P<0.001$ ). (B) Distributions of tracking angles with respect to the landmark (blue) and to the distractor (red) of bats in the foam group ( $N=11,489$ ). The mean squared error (MSE) between tracking angle distribution to the landmark and the tracking angle distribution to the distractor were significantly different from zero (permutation test,  $P<0.001$ ). The HW of tracking angle distribution to the landmark was smaller than the HW tracking angle distribution to the distractor (permutation test,  $P<0.001$ ). The bats in the foam group showed a smaller HW in tracking angle distribution both to the landmark and to the distractors, compared with bats in the plastic group (permutation test,  $P<0.001$ ).

inspecting the landmark, the bats directed their sonar beam axis over a smaller region of interest compared with the distractor object.

For successful landmark discrimination and recognition, past findings suggest that a critical feature of a landmark is its saliency or distinctiveness (Chan et al., 2012; Stankiewicz and Kalia, 2007). In the present study, echoes returning from the two objects used as sonar landmarks showed very different acoustic properties (Fig. 2), with the plastic object returning echoes with distinct spectral notches and the foam object returning echoes with more uniform spectral distribution. If the spectrally complex plastic object echoes provided a strong acoustic cue to the bat, this object would serve as a more distinct landmark, leading to the prediction that bats using the plastic object as a landmark (plastic group) would outperform the bats using the foam object as a landmark (foam group) in experimental trials. Indeed, the bats in the plastic group were able to find the opening significantly faster than bats in the foam group; in probe trials, where the plastic landmark was moved away from the opening, bats in this group found the opening only 10% of the time, compared with 67% for the bats in the foam group, suggesting that the bats' navigation behavior was strongly influenced by the distinctiveness of the landmark. Specifically, bats trained to use the plastic object as a landmark had difficulty modifying their navigation behavior in probe trials when this acoustically distinct object provided invalid information about the net opening.

One of our primary goals in this study was to investigate the echolocating bat's active sensing behaviors in a landmark-feature-guided navigation task. Past studies report that the bat's PI (the reciprocal of call rate) and production of SSGs are related to the difficulty of an echolocation task (Kothari et al., 2014, 2018a,b; Moss et al., 2006). In the present experiment, we found that the bats employed different sonar inspection strategies with the two different objects: bats in the plastic group produced calls at significantly longer intervals, with fewer SSGs, compared with bats in the foam group. Additionally, bats showed significant interactions in sonar behaviors between the objects serving as the landmark (i.e. plastic

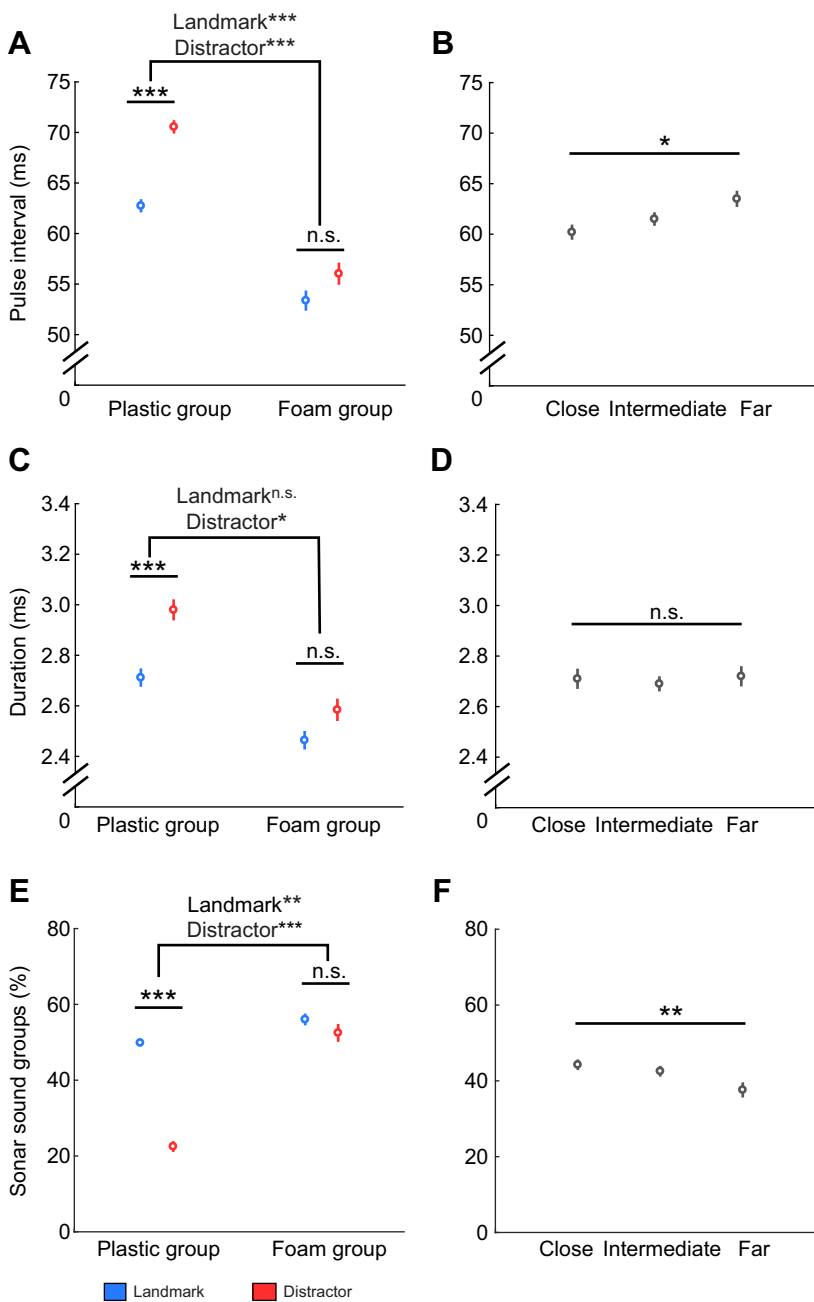
versus foam group) and the object the bat was inspecting (i.e. landmark versus distractor).

Why did the bats in plastic group produce calls with longer PIs and fewer SSGs when inspecting objects? Prior work on bat echolocation has reported that complex spectral profiles of echoes can facilitate discrimination between different objects (Müller and Kuc, 2000; Falk et al., 2011). In the present study, the bats trained to use the more complex, and therefore acoustically distinct, plastic object as a landmark may have obtained adequate echo information to guide their navigation with longer PIs (lower call rates) and fewer SSGs. On the flip side, bats in the foam group may have decreased PIs (increased call rates) and produced more SSGs to gather more information about the landmark to perform the task. We interpret these findings to suggest that the foam landmark required increased sonar-guided attention compared with the acoustically distinct plastic landmark.

Additionally, we observed differences between bats in the plastic landmark group when they inspected the landmark compared with the distractor. The bats trained to use the plastic object as a landmark directed a larger proportion of calls at the landmark, as revealed in the tracking angle distribution, which shows a clear peak around the landmark (Fig. 4A). By contrast, the distribution of the tracking angles towards the foam distractor in the plastic landmark group shows no peak, but instead a broad range of tracking angles around the distractor. However, the tracking angle distributions of bats trained to use the foam object as the landmark suggest that the bats in this group often directly inspected both the landmark and the distractor. When bats in the plastic group inspected the landmark, they reduced PIs and produced more SSGs than when they inspected the distractor. Bats in the foam group did not show these differences in sonar call parameters between inspection of the landmark and the distractor.

Ensonification of the two objects showed similar target strengths at 1 m, with a difference of 1.7 dB. However, echoes returned by the plastic object showed spectral notches at multiple frequencies, compared with the more uniformly distributed power spectral





**Fig. 5. Bats adapt vocal behavior to inspect objects.** Left column (A,C,E) shows the adaptive vocal behaviors of the bat as it inspects the landmark (blue) and the distractor (red). Error bars indicate  $\pm$ s.e.m. Bats in the plastic group reduced pulse interval (A;  $t=-6.8$ ,  $P<0.001$ ) and call duration (C;  $t=-4.4$ ,  $P<0.001$ ) and used more sonar sound groups (E;  $t=15.1$ ,  $P<0.001$ ) when inspecting the landmark compared with the distractor. These differences were not statistically reliable in bats in the foam group. Right column shows the average pulse interval, call duration and percent of sonar sound groups across trials when the spatial separation between the landmark and distractor objects was close, intermediate and far. Bats used shorter pulse intervals (B;  $F=2.25$ ,  $P=0.024$ ) and more sonar sound groups (F;  $F=10.1$ ,  $P<0.01$ ) when the objects were closer together. However, the call durations were not affected by the object separation (D). Asterisks indicate the overall significance of each factor included in the GLMs (n.s., not significant; \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$ ).

density of the echoes returned by the foam object. These data suggest that the plastic object returned a distinct spectral pattern. These measurements, together with echolocation call analyses, demonstrate that landmark distinctiveness influences the sonar inspection behaviors of big brown bats. Specifically, when bats were trained to use an acoustically distinct plastic landmark to guide navigation, they showed longer PIs, fewer SSGs and more variable beam-directing behavior around the distractor object compared with the landmark. By contrast, when bats were trained to use the less acoustically distinct foam landmark, they showed more directed sonar inspection of both the landmark and the acoustically distinct plastic distractor, as they performed the navigation task.

When echolocating bats navigate in cluttered environments, they adapt vocal behaviors with respect to the distance and spatial configuration of obstacles, reducing PIs and increasing SSGs compared with open environments (Falk et al., 2014; Moss et al.,

2006). Likewise, bats tracking a moving target from a stationary position decrease PIs and increase SSG production when clutter objects are positioned close to a moving target (Mao et al., 2016). We found a similar pattern in the bats' adaptive echolocation behavior (e.g. an increase in SSG production) when the distractor object was placed close to the landmark. One interpretation of these data is that the distractor object created clutter echoes when it was positioned close to the trained landmark, making the task more challenging for the bats.

Our study aimed to understand the navigation and acoustic behaviors of the bat in the presence of a landmark. However, there are other potential cues that the bat could have used to perform the task. One of the possible cues is the echoes returned by the edges of the net openings. However, the increased crash rate in the probe trials provides evidence that the bat learned to use the landmark to guide its navigation. Thus, even though we cannot rule out the

possibility that the net opening served as a cue for the bat's navigation, data suggest that the landmark and/or the distractor cues carried more weight. Additionally, the bat could have potentially used echoes returning from the barrier or a covered opening (Fig. 1A) to guide navigation. In our experiment, four of the five openings were covered by a small piece of net in every trial, so that these four locations would provide similar cues to the bat. Therefore, the only distinct acoustic cues available to the bats for navigation were the objects themselves (landmark or distractor).

Previous studies of landmark-guided navigation in rodents have reported conflicting results with respect to landmark stability (Biegler and Morris, 1993; Roberts and Pearce, 1998). Roberts and Pearce (1998) argued that rats can learn to use a moving landmark in an enriched environment by combining information acquired from both landmark and distal cues in the environment. Our study, performed in a relatively complex environment with a landmark whose position changed from trial to trial, showed results similar to Roberts and Pearce's (1998) experiment. Specifically, regardless of trial-to-trial changes in the location of a landmark and distractor, bats could discriminate the two objects and reference the position of the landmark to navigate successfully. Directional information may have been enhanced through cues from the global environment, where we mounted microphones and video cameras along the walls and ceiling, or through spatial memory of the boundaries of the flight room.

Although environmental cues can be used to guide spatial navigation, the use of landmark features may depend on the natural behaviors of a given species. For example, some bat species feed on flowers or fruits that are typically static in the environment. Studies of nectarivorous and frugivorous bats (*Glossophaga soricina* and *Carollia perspicillata*) showed that these animals relied preferentially on spatial cues over features of objects for navigation (Carter et al., 2010; Thiele and Winter, 2005). Bats were trained to take food at one of the feeders with a stable location (spatial cues), and a distinct scent and shape (feature cues). When tested in cue-conflicting conditions with no reward, both nectarivorous and frugivorous bat species preferred to visit the feeder consistent with the spatial cues (Carter et al., 2010; Thiele and Winter, 2005). By contrast, studies of insectivorous species (*Myotis nattereri*), which normally chase moving prey, showed that bats can rely on feature cues to locate food (Hulgard and Ratcliffe, 2014; Siemers, 2001). Siemers (2001) trained gleaning bats (*Myotis nattereri*) to feed in a round-shaped feeder. When tested in a two-choice task with a novel feeder of a different shape, these bats flew repeatedly to the trained round-shaped feeder, even though it was empty. Furthermore, Hulgard and Ratcliffe (2014) reported that object feature learning can inhibit later spatial location learning in *M. nattereri*. In our study, the insectivorous big brown bat (*E. fuscus*) learned to use the features of a landmark to guide its navigation, despite the changing landmark position across trials. Our experiment adds further support to the hypothesis proposed by Stich and Winter (2006) that an animal's natural foraging behaviors can influence the strategy it employs to navigate through the environment: the nectarivorous and frugivorous bats rely heavily on spatial cues because most flowers and fruits remain available at a constant location for extended periods, whereas insectivorous bats may need to monitor dynamic environmental features.

In summary, animals navigating in the natural environment may sometimes rely more on the features of landmarks than on their spatial locations. Feature-guided navigation necessarily invokes inspection behaviors, which are difficult to monitor in visually guided animals. This study directly monitored and measured the

echolocation behavior of bats engaged in a spatial navigation task, and our data suggest that active sensory inspection plays a central role in landmark-feature-guided navigation. Further, distinct landmarks may facilitate the bat's navigation by returning spectrally rich echo patterns. Future research in other animals can serve to advance a broader understanding of the general and species-specific contributions of active sensing behaviors in guiding landmark navigation.

#### Acknowledgements

We would like to thank Dr Kirsten Bohn for advice on statistics. Dr Benjamin Falk helped measure microphone sensitivity and directionality; Dr Angeles Salles and our two reviewers each provided valuable comments and helped us to improve the manuscript.

#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: C.Y., J.L., M.W., C.F.M.; Methodology: C.Y., C.F.M.; Formal analysis: C.Y.; Investigation: C.Y., J.L.; Resources: C.F.M.; Data curation: C.Y., M.W., C.F.M.; Writing - original draft: C.Y.; Writing - review & editing: C.Y., J.L., M.W., C.F.M.; Supervision: C.F.M.; Project administration: C.F.M.; Funding acquisition: C.F.M.

#### Funding

This research was supported by the National Science Foundation (1734744), the Office of Naval Research (N00014-12-1-0339; N00014-17-1-2736) and the Air Force Office of Scientific Research (FA9550-14-1-0398). J.L. was funded by a long-term postdoctoral fellowship from the Human Frontier Science Program (LT000279/2016-L). C.F. M. gratefully acknowledges a sabbatical award from the James McKeen Cattell Fund, which supported work on the revision of this manuscript.

#### References

- Biegler, R. and Morris, R. G. M. (1993). Landmark stability is a prerequisite for spatial but not discrimination learning. *Nature* **361**, 631-633. doi:10.1038/361631a0
- Carter, G. G., Ratcliffe, J. M. and Galef, B. G. (2010). Flower bats (*Glossophaga soricina*) and fruit bats (*Carollia perspicillata*) rely on spatial cues over shapes and scents when relocating food. *PLoS ONE* **5**, e10808. doi:10.1371/journal.pone.0010808
- Chamizo, V. D., Rodríguez, C. A., Espinet, A. and Mackintosh, N. J. (2012). Generalization decrement and not overshadowing by associative competition among pairs of landmarks in a navigation task. *J. Exp. Psychol. Anim. Behav. Process.* **38**, 255-265. doi:10.1037/a0029015
- Chan, E., Baumann, O., Bellgrove, M. A. and Mattingley, J. B. (2012). From objects to landmarks: the function of visual location information in spatial navigation. *Front. Psychol.* **3**, 1-11. doi:10.3389/fpsyg.2012.00304
- Cheng, K. (1986). A purely geometric module in the rat's spatial representation. *Cognition* **23**, 149-178. doi:10.1016/0010-0277(86)90041-7
- Collett, T. S., Cartwright, B. A. and Smith, B. A. (1986). Landmark learning and visuo-spatial memories in gerbils. *J. Comp. Psychol.* **158**, 835-851. doi:10.1007/BF01324825
- Falk, B., Williams, T., Aytakin, M. and Moss, C. F. (2011). Adaptive behavior for texture discrimination by the free-flying big brown bat, *Eptesicus fuscus*. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **197**, 491-503. doi:10.1007/s00359-010-0621-6
- Falk, B., Jakobsen, L., Surlykke, A. and Moss, C. F. (2014). Bats coordinate sonar and flight behavior as they forage in open and cluttered environments. *J. Exp. Biol.* **217**, 4356-4364. doi:10.1242/jeb.114132
- Foo, P., Duchon, A., Warren, W. H. and Tarr, M. J. (2007). Humans do not switch between path knowledge and landmarks when learning a new environment. *Psychol. Res.* **71**, 240-251. doi:10.1007/s00426-006-0080-4
- Ghose, K. and Moss, C. F. (2003). The sonar beam pattern of a flying bat as it tracks tethered insects. *J. Acoust. Soc. Am.* **114**, 1120-1131. doi:10.1121/1.1589754
- Good, P. I. (2004). *Permutation, Parametric, and Bootstrap Tests of Hypotheses*. New York: Springer Science+Business Media Inc.
- Hartley, D. J. and Suthers, R. A. (1989). The sound emission pattern of the echolocating bat, *Eptesicus fuscus*. *J. Acoust. Soc. Am.* **85**, 1348-1351. doi:10.1121/1.397466
- Hope, G. M. and Bhatnagar, K. P. (1979). Electrical response of bat retina to spectral stimulation: comparison of four microchiropteran species. *Experientia* **35**, 1189-1191. doi:10.1007/bf01963279

- Hulgard, K. and Ratcliffe, J. M.** (2014). Niche-specific cognitive strategies: object memory interferes with spatial memory in the predatory bat *Myotis nattereri*. *J. Exp. Biol.* **217**, 3293-3300. doi:10.1242/jeb.103549
- Jensen, M. E., Moss, C. F. and Surlykke, A.** (2005). Echolocating bats can use acoustic landmarks for spatial orientation. *J. Exp. Biol.* **208**, 4399-4410. doi:10.1242/jeb.01901
- Kalko, E. K. V. and Schnitzler, H.-U.** (1993). Plasticity in echolocation signals of European pipistrelle bats in search flight: implications for habitat use and prey detection. *Behav. Ecol. Sociobiol.* **33**, 415-428. doi:10.1007/BF00170257
- Kothari, N. B., Wohlgemuth, M. J., Hulgard, K., Surlykke, A. and Moss, C. F.** (2014). Timing matters: sonar call groups facilitate target localization in bats. *Front. Physiol.* **5**, 168. doi:10.3389/fphys.2014.00168
- Kothari, N. B., Wohlgemuth, M. J. and Moss, C. F.** (2018a). Dynamic representation of 3D auditory space in the midbrain of the free-flying echolocating bat. *eLife* **7**, 1-29. doi:10.7554/eLife.29053
- Kothari, N. B., Wohlgemuth, M. J. and Moss, C. F.** (2018b). Adaptive sonar call timing supports target tracking in echolocating bats. *J. Exp. Biol.* **221**, jeb.176537.
- Lee, W. J., Falk, B., Chiu, C., Krishnan, A., Arbour, J. H. and Moss, C. F.** (2017). Tongue-driven sonar beam steering by a lingual-echolocating fruit bat. *PLoS Biol.* **15**, 1-15. doi:10.1371/journal.pbio.2003148
- Luo, J., Goerlitz, H. R., Brumm, H. and Wiegrebe, L.** (2015). Linking the sender to the receiver: vocal adjustments by bats to maintain signal detection in noise. *Sci. Rep.* **5**, 1-11. doi:10.1038/srep18556
- Mao, B., Aytikin, M., Wilkinson, G. S. and Moss, C. F.** (2016). Big brown bats (*Eptesicus fuscus*) reveal diverse strategies for sonar target tracking in clutter. *J. Acoust. Soc. Am.* **140**, 1839-1849. doi:10.1121/1.4962496
- Milgram, N. W., Adams, B., Callahan, H., Head, E., Mackay, B., Thirlwell, C. and Cotman, C. W.** (1999). Landmark discrimination learning in the dog. *Learn. Mem.* **6**, 54-61.
- Moss, C. F., Chiu, C. and Surlykke, A.** (2011). Adaptive vocal behavior drives perception by echolocation in bats. *Curr. Opin. Neurobiol.* **21**, 645-652. doi:10.1016/j.conb.2011.05.028
- Moss, C. F., Bohn, K., Gilkenson, H. and Surlykke, A.** (2006). Active listening for spatial orientation in a complex auditory scene. *PLoS Biol.* **4**, 615-626. doi:10.1371/journal.pbio.0040079
- Müller, R. and Kuc, R.** (2000). Foliage echoes: a probe into the ecological acoustics of bat echolocation. *J. Acoust. Soc. Am.* **108**, 836-845. doi:10.1121/1.429617
- Nemmi, F., Piras, F., Péran, P., Incoccia, C., Sabatini, U. and Guariglia, C.** (2013). Landmark sequencing and route knowledge: an fMRI study. *Cortex* **49**, 507-519. doi:10.1016/j.cortex.2011.11.016
- Nothegger, C., Winter, S. and Raubal, M.** (2004). Selection of salient features for route directions. *Spat. Cogn. Comput.* **4**, 113-136. doi:10.1207/s15427633scc0402\_1
- Roberts, A. D. L. and Pearce, J. M.** (1998). Control of spatial behavior by an unstable landmark. *J. Exp. Psychol. Anim. Behav. Process.* **24**, 172-184. doi:10.1037/0097-7403.24.2.172
- Rodrigo, T., Gimeno, E., Ayguasanosa, M. and Chamizo, V. D.** (2014). Navigation with two landmarks in rats (*Rattus norvegicus*): the role of landmark salience. *J. Comp. Psychol.* **128**, 378-386. doi:10.1037/a0036544
- Siemers, B. M.** (2001). Finding prey by associative learning in gleaning bats: experiments with a Natterer's bat *Myotis nattereri*. *Acta Chiropt.* **3**, 211-215.
- Simmons, J., Fenton, M. and O'Farrell, M.** (1979). Echolocation and pursuit of prey by bats. *Science* **203**, 16-21. doi:10.1126/science.758674
- Sorrows, M. E. and Hirtle, S. C.** (1999). The nature of landmarks for real and electronic spaces. In *Spatial Information Theory. Cognitive and Computational Foundations of Geographic Information Science* (ed. C. Freksa and D. M. Mark), pp. 37-50. Springer Berlin Heidelberg.
- Stankiewicz, B. J. and Kalia, A. A.** (2007). Acquisition of structural versus object landmark knowledge. *J. Exp. Psychol. Hum. Percept. Perform.* **33**, 378-390. doi:10.1037/0096-1523.33.2.378
- Stich, K. P. and Winter, Y.** (2006). Lack of generalization of object discrimination between spatial contexts by a bat. *J. Exp. Biol.* **209**, 4802-4808. doi:10.1242/jeb.02574
- Surlykke, A., Ghose, K. and Moss, C. F.** (2009). Acoustic scanning of natural scenes by echolocation in the big brown bat, *Eptesicus fuscus*. *J. Exp. Biol.* **212**, 1011-1020. doi:10.1242/jeb.024620
- Thiele, J. and Winter, Y.** (2005). Hierarchical strategy for relocating food targets in flower bats: spatial memory versus cue-directed search. *Anim. Behav.* **69**, 315-327. doi:10.1016/j.anbehav.2004.05.012
- Zhao, M. and Warren, W. H.** (2015). How you get there from here: interaction of visual landmarks and path integration in human navigation. *Psychol. Sci.* **26**, 915-924. doi:10.1177/0956797615574952