

RESEARCH ARTICLE

Bats coordinate sonar and flight behavior as they forage in open and cluttered environments

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ABSTRACT

Echolocating bats use active sensing as they emit sounds and listen to the returning echoes to probe their environment for navigation, obstacle avoidance and pursuit of prey. The sensing behavior of bats includes the planning of 3D spatial trajectory paths, which are guided by echo information. In this study, we examined the relationship between active sonar sampling and flight motor output as bats changed environments from open space to an artificial forest in a laboratory flight room. Using high-speed video and audio recordings, we reconstructed and analyzed 3D flight trajectories, sonar beam aim and acoustic sonar emission patterns as the bats captured prey. We found that big brown bats adjusted their sonar call structure, temporal patterning and flight speed in response to environmental change. The sonar beam aim of the bats predicted the flight turn rate in both the open room and the forest. However, the relationship between sonar beam aim and turn rate changed in the forest during the final stage of prey pursuit, during which the bat made shallower turns. We found flight stereotypy developed over multiple days in the forest, but did not find evidence for a reduction in active sonar sampling with experience. The temporal patterning of sonar sound groups was related to path planning around obstacles in the forest. Together, these results contribute to our understanding of how bats coordinate echolocation and flight behavior to represent and navigate their environment.

KEY WORDS: Echolocation, Flight behavior, Spatial memory, Path planning

INTRODUCTION

Echolocating bats actively probe the environment with ultrasonic signals to build a spatial representation of a sonar scene from information carried by returning echoes (Griffin, 1958). Each scene is dynamic: the animal and its prey are moving through space, which produces changes in the features of echo returns. The task is further complicated in a cluttered environment where each sonar emission results in a cascade of echoes arriving from different locations, which the bat must organize into a coherent representation (Moss et al., 2006; Moss et al., 2011; Simmons et al., 1988). Acoustic cues, such as interaural time, intensity and spectral differences, provide information about the direction of a sonar object (Shimozawa et al., 1974; Simmons et al., 1983), whereas echo arrival time provides

information about its distance (Ewer, 1945; Simmons, 1973). The bat's own actions, coupled with information carried by dynamic echo streams, may be key to understanding how the bat can operate in a complex environment.

There are over 1100 species of bats (Simmons, 2005), of which ~70% echolocate using the larynx (Jones and Teeling, 2006). These species have evolved varied biosonar signal designs to enable successful foraging in their natural habitats (Schnitzler and Kalko, 2001). Detecting prey, parsing the acoustic scene and localizing objects require sonar signals tailored to each task. Bats that emit short-frequency-modulated calls of low duty cycle to capture insects on the wing reduce the duration of calls in cluttered environments to minimize pulse–echo overlap (Cahlander et al., 1964; Kalko and Schnitzler, 1989; Schnitzler et al., 1987) and widen call bandwidth to increase information about object location (Faure and Barclay, 1994; Hartley, 1992; Jensen and Miller, 1999; Kalko and Schnitzler, 1993; Surlykke et al., 1993). These bats also adjust the interval between successive calls to receive echoes from relevant objects before producing the next call (Moss and Surlykke, 2001; Surlykke and Moss, 2000). This serves to avoid ambiguity about the echo arrival time from each sonar emission. In highly cluttered environments where pulse intervals (PIs) cannot be adequately adapted to avoid ambiguity in call–echo assignment, bats make further adjustment in spectral structure of closely spaced calls in a group (Hiryu et al., 2010).

Although bats often decrease the interval of sounds in a continuous manner as they near obstacles, the temporal structure of sonar call sequences can be more complex. Occasionally, sound groups, clusters of several signals that contain a shorter PI than surrounding calls, occur embedded within a sequence of calls (Moss and Surlykke, 2001; Moss et al., 2006). Sound groups have been implicated in resolving spatial information in more detail (Kothari et al., 2014) and have been found to be more prevalent in complex acoustic scenes (Moss et al., 2006; Petrites et al., 2009; Sändig et al., 2014). These findings suggest that bats are actively controlling the features and timing of the sonar sounds they produce in order to build a representation of a complex environment; however, the echo scene, and thus the perception of the environment, is also dependent on the bat's own movement through space and time.

A more complete understanding of the echolocating bat's dynamic sonar scene must take into account the animal's active control of sonar signals in relation to its flight. Bat sonar sounds are directional (Hartley and Suthers, 1989; Jakobsen et al., 2013; Simmons, 1969), which restricts the animal's view of 3D space. Bats must therefore control the aim of the sonar beam to spatially sample the environment and objects of interest (Falk et al., 2011; Ghose and Moss, 2003; Seibert et al., 2013; Surlykke et al., 2009). The sonar beam aim has been shown to predict flight motor output (Ghose and Moss, 2006). The time delay, or lag, between the sonar gaze angle and turn rate was found to be greater than zero for all phases of echolocation (Ghose and Moss, 2006). The gain, or slope, in the

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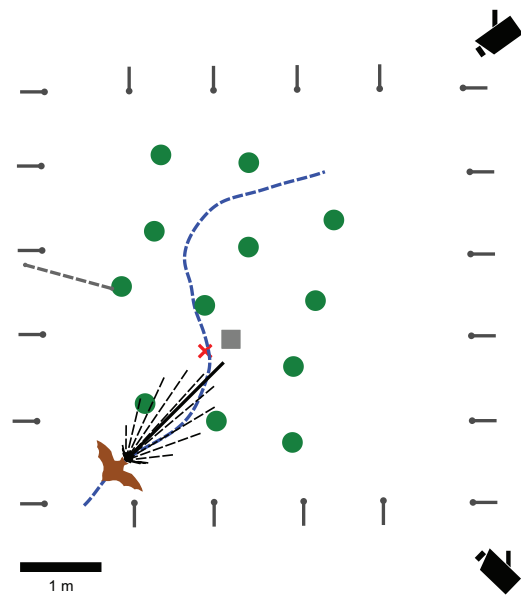


Fig. 1. Top view schematic of 'forest' flight room with artificial trees. The bat is represented in diagram, and the bat's flight path through the artificial trees shown as a dashed blue line. The tethered mealworm is represented as a red cross. The artificial trees are shown as green circles and, with respect to the room, plotted to scale. The left wall net is shown as a gray dashed line. The microphones from the planar array bordering the room are represented as gray circles. They are offset from the walls by 0.3 m sticks, represented in gray. An example vocalization is depicted as the corrected intensity recorded at each of the microphones in the array (dashed black lines). The estimated beam direction is shown as a thick black line. The wideband microphone is represented by a gray square, and was placed 0.3 m above the floor. High-speed cameras are represented in diagram, and were positioned in the top corners of the room. The open room had an identical setup without artificial trees or side wall net.

linear model predicting turn rate from gaze angle increased as the bats transitioned through the echolocation phases of insect capture. This adaptive linear relationship between acoustic gaze and flight behavior connects the bat's attentional control and motor planning behaviors. Together, these results indicate that the bat is guided by spatial sampling of its environment, which is an interaction between the actively controlled aim of the sonar beam, the temporal patterning of its sonar signals and its flight trajectory.

Here, we analyze the coordinated adjustments in echolocation and flight behavior of the insectivorous big brown bat *Eptesicus fuscus* Palisot de Beauvois 1796 (Vespertilionidae), which forages in open spaces as well as in and among vegetation (Simmons et al., 2001). We hypothesize that changes to the environment will result in adjustments to sonar signal design, sonar temporal patterning, sonar directional aim, and flight steering and flight speed. Characterization of these adjustments can reveal adaptive behaviors for navigating clutter, the dynamics of echo information flow and the representation of a bat's sonar scene.

RESULTS

Performance

Nine wild, big brown bats were trained to catch tethered mealworms in an open laboratory room. Once bats reached proficiency at foraging in the open room, they were tested in an artificial forest (Fig. 1). While foraging, the bats were able to successfully maneuver without hitting trees ($79 \pm 6\%$ of trials with no crashes averaged across 9 bats, mean \pm s.e.m.). Although individual differences in the approach and flight trajectories existed, each bat was successful in the task.

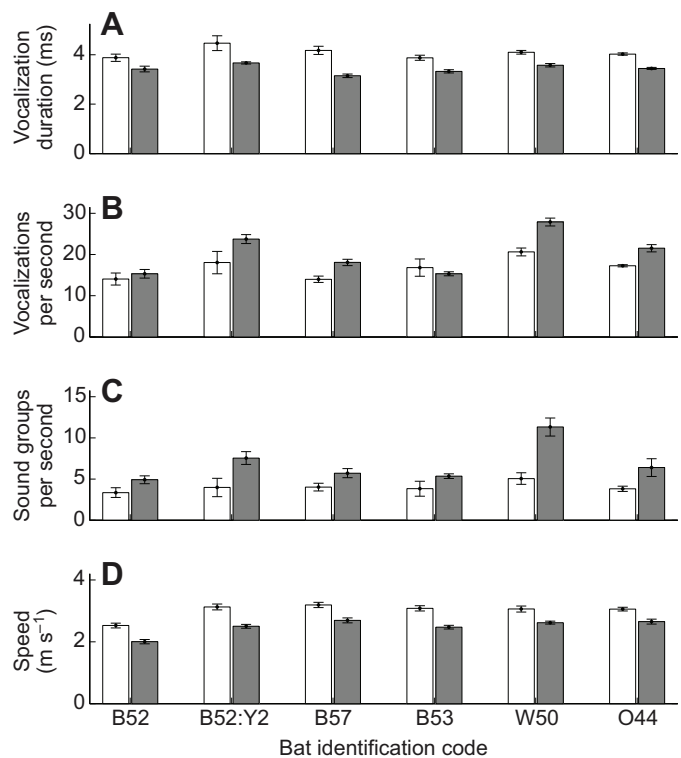


Fig. 2. Behavioral changes in big brown bats (*Eptesicus fuscus*) between the open and forest flight rooms. Individual bat averages across trials for (A) vocalization duration excluding buzz calls, (B) vocalizations per second excluding buzz calls, (C) vocalizations in sound groups per second and (D) flight speed for each bat. Results are means \pm s.e.m. White bars, open room recordings; gray bars, forest recordings.

Adaptive sonar and flight behavior

When foraging in the forest, the bats adapted the structure and temporal patterning of their sonar calls as well as their flight kinematics. We characterized these changes between the open room and the forest (Fig. 2, paired two-tailed *t*-test for each comparison). We found that bats in the forest emitted shorter duration vocalizations, mean 3.43 ms compared with 4.09 ms ($t_5=7.46$, $P<0.001$), indicating that the bats avoided pulse-echo overlap when flying near obstacles. The bats vocalized at a higher repetition rate in the forest, mean of 20.31 compared with 16.79 vocalizations per second ($t_5=-2.73$, $P=0.041$), which is indicative of sonar approach sequences but was also observed when bats were near obstacles. An increased repetition rate can increase the localizing resolution of sonar by providing more echoes from the surroundings. The bats not only increased the rate of calling, but also changed the temporal patterning of their vocalizations when in the forest. The bats emitted a higher rate of sound-group vocalizations – clusters of closely spaced emitted calls – in the forest, with a mean of 6.87 compared with 4.00 sound groups per second in the open room ($t_5=-3.80$, $P=0.013$). Bats flew at slower speeds in the forest than in the open room with a mean of 2.49 m s^{-1} compared with 3.01 m s^{-1} ($t_5=14.22$, $P<0.001$), which is consistent with bats flying between obstacles. A decrease in flight speed allowed bats additional time to react to obstacles and maneuver through the forest.

The decrease in flight speed in the forest also served to increase the pulse density, or the number of vocalizations emitted per meter traveled, with a mean of 8.25 vocalizations per meter in the forest compared with 5.77 in the open room ($t_5=-4.80$, $P=0.005$). The

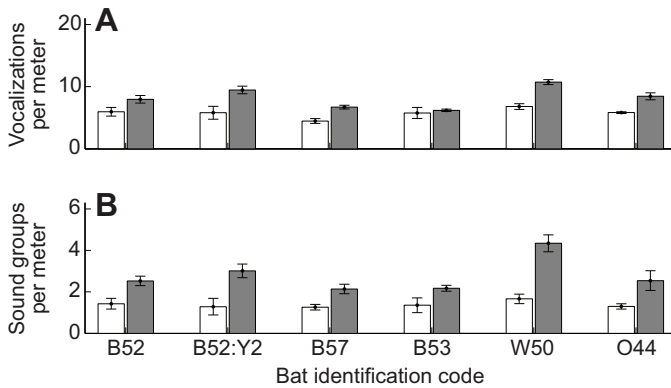


Fig. 3. Sonar behavior in relation to distance travelled in big brown bats. Individual bat averages across trials for (A) pulse density, or number of vocalizations per meter traveled, and (B) sound-group density, or number of sound-group vocalizations per meter traveled. Results are means \pm s.e.m. White bars, open room recordings; gray bars, forest recordings.

sound-group density also increased in the forest, with a mean of 2.79 sound group vocalizations emitted per meter traveled compared with 1.38 in the open room ($t_5 = -4.91$, $P = 0.004$; Fig. 3). An increase in pulse density and sound-group density increased the information flow by increasing the number of echoes returning from obstacles as the bats navigated the artificial forest.

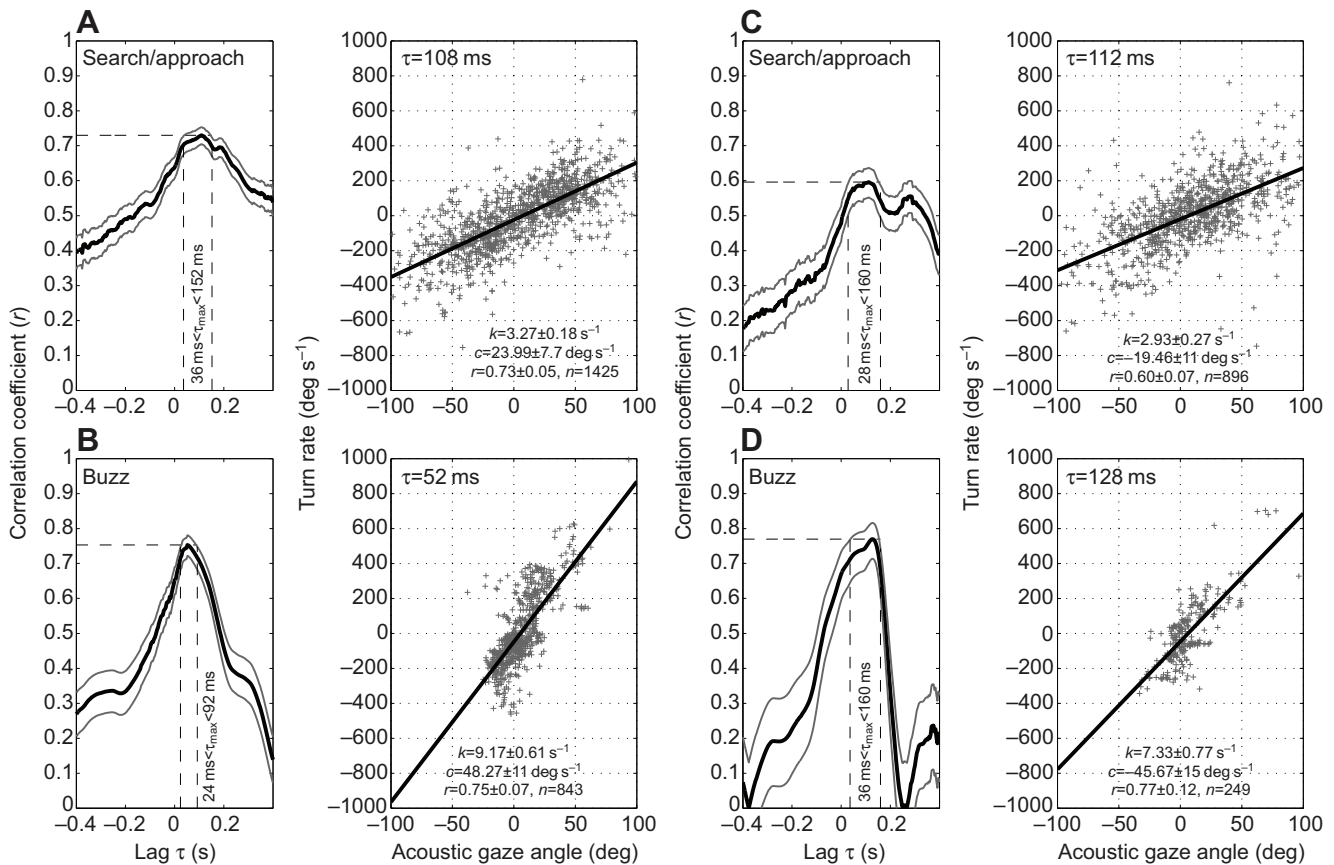


Fig. 4. Sonar beam direction and turn rate correlation analysis between open room and forest during search/approach and buzz phases of echolocation. Open room (A,B) and forest (C,D) and search/approach (A,C) and buzz (B,D). Left panels show calculated correlation, r (black line, CI in gray), between acoustic gaze angle and turn rate for time lags, -0.4 to 0.4 s. The peak correlation, found at τ_{\max} , is indicated with a dashed horizontal line. CI for τ_{\max} is indicated with dashed vertical lines. Right panels show the linear correlation at τ_{\max} . The gaze angle and corresponding turn rate for each vocalization are plotted (gray crosshairs). The fitted linear relationship is plotted in black. k refers to the slope of the fitted line, c refers to the intercept, r is the correlation and n is the number of vocalizations.

Steering by hearing in the forest

A bat's sonar gaze angle (beam aim relative to flight direction) has been found to be linked to its flight motor output through a delayed linear model (Ghose and Moss, 2006). The general control law was defined as the linear relationship:

$$\dot{\theta}_{\text{flight}}(t + \tau) = k\theta_{\text{gaze}}(t), \quad (1)$$

where θ_{gaze} is the gaze angle (the angle between the beam axis and flight vector), $\dot{\theta}_{\text{flight}}(t + \tau)$ is the rate at which the bat turns, k is a state-dependent gain factor, and τ is the constant time by which the flight lags the gaze direction. In the original study (Ghose and Moss, 2006), this linear relationship varied depending on the phase of the sonar sequence (search, approach, or buzz or terminal phase). We examined whether a change in environment could alter the link between the sonar acoustic gaze and turn rate.

A linear relationship between acoustic gaze and flight turn rate was determined for search/approach ($PI > 20$ ms) and buzz ($PI < 10$ ms) phases of the sonar sequence (Fig. 4). The correlation r was found for different time delays τ , relative to each vocalization. The maximum correlation τ_{\max} was compared between open room and forest for the search/approach and buzz echolocation phases (Table 1). Late-approach phase vocalizations (PI between 10 and 20 ms) were not well represented in the present dataset (6% of vocalizations in the open room and 4% in the forest) and were excluded from this analysis. No statistical differences between the values of τ_{\max} were found; however, all were significantly above zero (Table 1).

Table 1. Relationship between acoustic gaze angle and turn rate in the big brown bat *Eptesicus fuscus*

	Room	τ_{\max}	CI	r	k	n
Search/approach	Open	108	36–152	0.73±0.05	3.27±0.18 s ⁻¹	1425
	Forest	112	28–160	0.60±0.07	2.93±0.27 s ⁻¹	896
Buzz	Open	52	24–92	0.75±0.07	9.17±0.61 s ⁻¹	843
	Forest	128	36–160	0.77±0.12	7.33±0.77 s ⁻¹	249

Peak correlation (r), the delay at the peak (τ_{\max}), gain (k) at τ_{\max} and the number of vocalizations (n) for open room and forest in search/approach and buzz echolocation behavior. Values for r and k are means ± 95% confidence intervals.

The linear model at τ_{\max} was examined for open room and forest and across the phases of echolocation sequence (ANCOVA). Pairwise comparisons of the gain, or slope, of the linear relationship showed a larger gain in the buzz phase than in the search/approach phase (Table 1). This result is consistent with the finding in Ghose and Moss (Ghose and Moss, 2006) in the open room alone. The gain was larger in the open room than in the forest during the sonar buzz phase (Bonferroni correction, Table 1). The larger gain in the open room during the buzz phase coincided with an overall decrease in turn rate in the forest during buzz phase (two-sample t -test, $t_{904}=3.57$, $P<0.001$) with no change gaze angle ($t_{904}=0.42$, $P=0.67$). These findings indicate that the environment shapes the relationship between gaze angle and flight motor output.

Spatial memory versus active sensing

Stereotyped flight paths which develop over time indicate that bats are relying on their spatial memory, and they have been found to coincide with a reduction in active sensing in non-foraging bats navigating obstacles (Barchi et al., 2013). We tested foraging bats in the artificial forest for these effects over several days (see Table 2 for number of trials collected). We calculated 2D occupancy histograms from bat flight trajectories (Fig. 5) and cross correlated the occupancy histograms of the last day in the forest with each previous day for each bat. We calculated the peak (maximum) and spread (number of points above 60% of the maximum) of each cross correlation for each bat to measure the development of flight stereotypy over time (Fig. 6). A repeated-measures ANOVA for both the peak and the spread of the cross correlations resulted in a statistically significant change in peak ($F_9=11.12$, $P=0.002$) but not spread ($F_9=2.12$, $P=0.17$). A pairwise comparison on the peak found an increase between the first and last cross correlation (difference 3.13×10^{-4} , $P=0.02$, Holm–Bonferroni adjustment), indicating that flight patterns became more similar between the first and last day in the forest.

Table 2. Trials collected for each bat and day

Bat	Open	F1	F2	F3	F4	F5
B52	11	9	7	0	0	0
B52:Y2	4	10	6 (10)	10	10	10
B57	6	10	5	7	8	0
B57:Y2	5	5 (9)	0 (10)	10	10	10
B53	11	11	8	0 (9)	9	0
W50	14	16	0	0	0	0
O44	16	10	0	0	0	0
O40:Y2	0	7 (8)	7	0 (8)	6	6
B59:Y2	0	6	6	0 (6)	7	5 (7)

Open and F1–F5 indicate the condition, either open room or forest day 1 to day 5. Trial numbers are for both audio and trajectory analysis unless number in parentheses present. Trial numbers in parentheses indicate the number of flight trajectory trials if different from the number of audio trials. These differences arose from problems in the wideband audio data collection.

We then investigated whether bats altered their active sensing or flight kinematics over time in the forest by measuring vocalization duration, number of emitted vocalizations per second, sound-group vocalizations emitted per second and flight speed. We found no evidence for changes over successive days in the forest (Fig. 7A–D). Similarly, we found no changes in the crash rate over time in the forest (Fig. 7E). These findings indicate that the bats did not rely on spatial memory in place of active sensing.

Path planning and sonar temporal patterning

Bats use a complex sampling strategy when foraging in the forest; the spatial locations and temporal patterning of the emitted sonar calls are both varied and systematic. Bats emitted sonar sound groups while navigating the forest, often at high rates (see Fig. 2). Sound groups may provide additional spatial resolution for localization of nearby objects. The temporal patterning of the emitted sonar calls and their relation to objects in the room along with each bat's movement through space was further investigated.

We examined the relationship between the temporal patterning of sonar calls and the resulting flight path navigation. We calculated the distance from the bat to the nearest tree and time-aligned the resulting distance to the sonar sound groups or single vocal emissions. In a period of 100–300 ms after the onset of vocalization, bats were closer to the nearest tree for sound-group emissions than single-call emissions (Fig. 8, two-tailed two-sample t -test, mean of 0.51 m for strobe calls compared with 0.58 m, $t_{16}=-2.35$, $P=0.032$). The average duration of a complete sound group (onset of first call to onset of last call) for bats in this study was 106 ms for doublets (82% of sound groups) and 136 ms for triplets (15% of sound groups). These results indicate that bats navigating the forest use sonar sound groups before approaching the trees.

DISCUSSION

Bats foraging in different environments face a variety of challenges, from target detection to figure–ground segregation. Here, we find that this bat species adapts sonar and flight behavior in concert when shifting from open to cluttered environments.

Adaptive behavior to obstacles

We found that big brown bats shortened their sonar signal duration and increased their repetition rate in the artificial forest compared with the open room (Fig. 2A,B). Shortening the signal duration decreases pulse–echo overlap (Kalko and Schnitzler, 1989; Kalko and Schnitzler, 1993), which allows a bat to more easily process echoes from closely spaced objects. A higher signal-repetition rate increases the rate of echo sampling of the environment. Both of these echolocation adaptations may have served to increase localization accuracy for the bats as they navigated the artificial forest in this study.

Our data point to a potential link between sound groups and flight-path planning. The bats in the present study increased the rate of emitted sound groups in the forest (Fig. 2C). An increase in

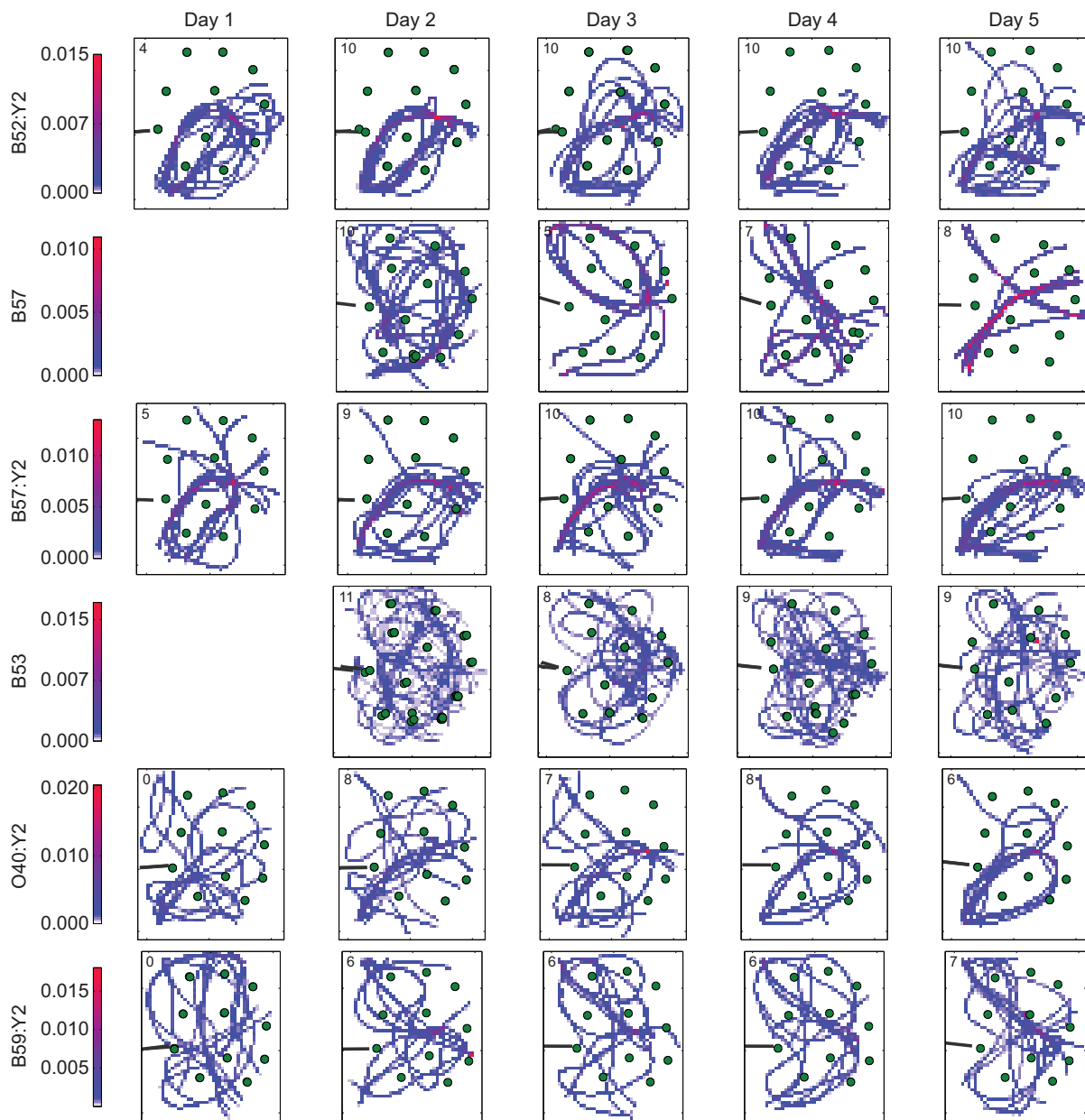


Fig. 5. Trajectory–occupancy histograms for each bat and each day represented as heat map in top view. Tree positions for each trial are overlaid represented as green circles. Occupancy as a percentage of time is shown by change in color from white to blue to red (indicated in scale on left), in order of least likely to most likely to occur. Number of trials per day is shown in top left corner of each panel.

sound-group production has been reported when bats encounter clutter or nearby obstacles (Moss and Surlykke, 2001; Moss et al., 2006; Petrites et al., 2009; Sändig et al., 2014). We found that when bats emitted sonar sound groups in the forest, they were closer to trees between 100 and 300 ms after the vocalization emission time, compared with when they produced single calls (Fig. 8), indicating that the bats organized calls into groups when they were preparing to approach artificial trees. On average, each sound group was completed (including echo arrival time) before the bats reached the closest distance to a tree. Sound groups often occur at a different phase of the wing beat than single calls (Moss et al., 2006), and the timing of the sound group, in relation to wing beat, is probably planned prior to the sound-group initiation (Koblitz et al., 2010). The delay between the emitted sound group and the resulting trajectory distance to a tree allows for sonar ranging before nearing

obstacles and for path planning. Therefore, production of sound groups seems to be involved in path planning around obstacles.

We observed that bats flew slower in the forest (Fig. 2D). Studies on other species of bats and birds have found that slow flight incurs significant energetic costs (Norberg, 1990; Thomas, 1975). Wing morphology measures of aspect ratio for *E. fuscus* (Farney and Fleharty, 1969) indicate that this species is not well adapted for slow flight and that energetic costs would be higher when flying at slow speeds. However, by flying slower, the bats can more easily maneuver around obstacles without crashing. Slower flight, together with the changes in sonar behavior in the forest, resulted in an increase in pulse density and sound-group density, which provided additional echo information for localizing obstacles. Slow flight also provided time for processing echoes and path planning. Slow flight in the forest served multiple

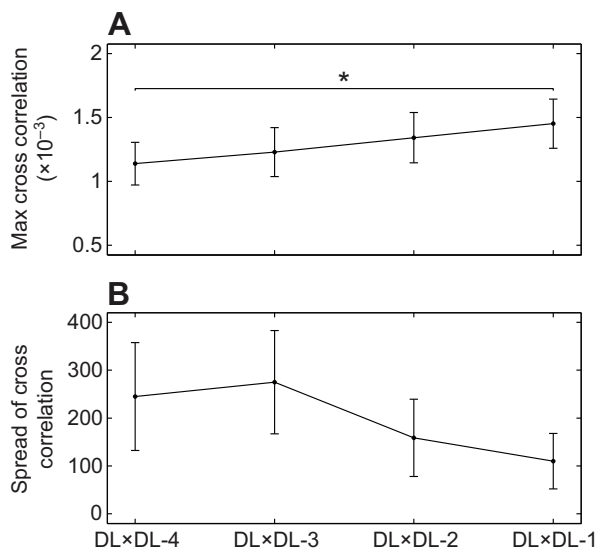


Fig. 6. Measures of cross correlation of occupancy histogram of last day in forest with each previous day in forest. Along x-axis, DL indicates last day in the forest for each bat. The cross correlation day is relative to the last day in the forest, between 1 and 4 days before the last day (DL-1 to DL-4). (A) Maximum (peak) of each cross correlation. (B) Spread (number of points above 60% of the maximum) of each cross correlation. These values were averaged across bats and plotted as the means \pm s.e.m. The only significant change ($P=0.002$) is shown with an asterisk between the cross correlation with DL-4 and DL-1 for the maximum cross correlation.

functions, affecting both flight maneuverability and biosonar performance.

Linking between acoustic gaze and flight motor output

Ghose and Moss (Ghose and Moss, 2006), studying bats foraging in an open room, reported a linear relationship between gaze angle and a positively delayed turn rate. In our experiment, we compared this relationship in both the open room and the forest (Fig. 4). A cluttered environment might alter this relationship because the bats need to respond to nearby obstacles quickly, the flight motor output may rely more on spatial memory instead of sonar, reducing the time lag or even making it negative. However, we found that the lag time that produced the maximum correlation was positive in the forest as well as the open room, confirming that the bats' acoustic gaze leads flight motor output, even in a cluttered environment.

Ghose and Moss (Ghose and Moss, 2006) also found that the gain, or slope, in the linear correlation between gaze angle and turn rate, was higher in the sonar buzz phase than the search/approach phase. We found a similar increase in gain between the buzz phase and search/approach phase, regardless of environmental condition. This result suggests that the behavioral state of the animal has a strong influence on the link between gaze angle and turn rate. In this analysis, we compared the gain between the open room and the forest and found a decrease in the sonar buzz phase in the forest, as well as an overall decrease in turn rate in the forest during the buzz phase. The shallower turn rate during the buzz phase in the forest was not observed during the search/approach phase. These findings highlight the important interaction between flight kinematics and sonar behavior. The gain of the relationship between gaze angle and turn rate depends on both the flight and sonar gaze, which are modified by the behavioral state of the animal, as well as the complexity of the environment.

The slope relating gaze angle and turn rate in the open room in our experiment was found to be higher than reported in Ghose and Moss for the buzz phase. In addition, the percentage of late approach (tracking) phase vocalizations was found to be lower in our experiment than that reported previously (Ghose and Moss, 2006). The Ghose and Moss study was conducted in an open room but the mealworm was dropped from a trap door towards the end of the trial instead of remaining stationary tethered from the ceiling. The certainty in the mealworm position in our experiment may have decreased late-approach-phase vocalizations and also decreased the acoustic gaze angle. A lower gaze angle could lead to the higher gain reported in our study.

Spatial memory versus active sensing

We did not find any changes in the sonar behavior as the bats gained experience in the forest, indicating that the bats in our study relied more on active sensing than on spatial memory (Fig. 7). However, we did find an increase in flight path stereotypy over the days (Fig. 6). Barchi et al. (Barchi et al., 2013) examined spatial memory with bats flying through a chain array and found a strong effect of stereotypy as well as a decrease in vocal repetition rate over time. However, in the previous study (Barchi et al., 2013), the bats were not foraging. We suspect that foraging places demands on bats that limit their use of spatial memory, because flight paths in the present study were more variable than those reported for bats navigating obstacles in the absence of prey (Barchi et al., 2013). Foraging requires accurate localization of obstacles and prey, and requires precise planning of complex flight motor behaviors in order to capture the prey item. These requirements probably force bats into a continuous active sensing mode and reduce their reliance on spatial memory.

A bat's representation of its environment is built upon its sonar system that processes echo information over time. In order for bats to successfully fly at high speeds, within a complex, cluttered environment, bats must continuously update their perception of space. Bats can rely on spatial memory for navigation, sometimes in place of active sensing (Barchi et al., 2013; Jensen et al., 2005), but in the present study, we find that requirements in path planning, object localization and foraging maneuvers require continuous active sensing. Bats in the present study were actively foraging and the results point to the importance of continuous sonar updates, in contrast to situations of 'transport' flight, where memory may take over in well-known surroundings.

Active perception is guided by action in a task-driven manner. However, motor behaviors subsequently propagate backwards to dictate scene sampling. Here, we sought to understand the motor actions driving the acquisition of information for scene analysis. This study links the animal's acoustic input and motor output to uncover adaptive active sensing behaviors, which depend on both the behavioral state of the animal and the environment.

MATERIALS AND METHODS

Experimental setup and data collection

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Maryland, College Park. Experiments were conducted in a large flight room (flight area $6.0 \times 5.7 \times 2.5$ m) lined with acoustic sound-absorbing foam (Sonex One, Acoustical Solutions, Inc.) in low, long wavelength light (>650 nm, incandescent light through infrared filters, Plexiglas G #2711, Atofina Chemicals). Animals used were nine adult, wild-caught *Eptesicus fuscus*, trained to catch insects (*Tenebrio molitor* larvae) tethered and hanging from the ceiling by monofilament fishing line (Berkley Trilene, 0.9 kg test, 0.13 mm diameter).

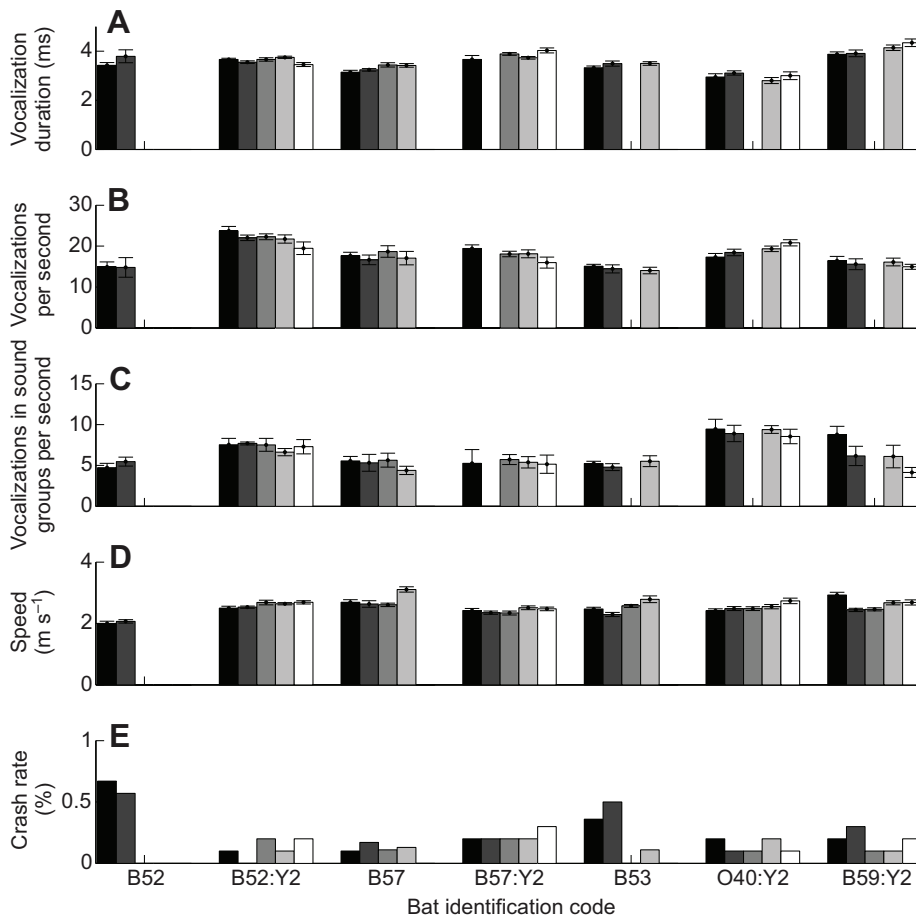


Fig. 7. Sonar and flight behavior over time in forest. Data for forest day 1 to forest day 5 are indicated by gradation in color from black to white (left to right) for each bat. Trial averages for (A) vocalization duration, (B) vocalizations per second, (C) vocalizations in sound groups per second and (D) flight speed. Values shown are means \pm s.e.m. (E) Crash rate as a percentage of flights per day. Gaps between data points in sonar data for bats B57:Y2, B53, O40:Y2 and B59:Y2 in A–C are due to absent microphone data. Bats had up to 5 days of flight in the forest but some had fewer (see Table 2).

Bats were trained in the open room. Once trained, a set of either 11 or 12 artificial trees as well as a net along one side of the room was introduced. The trees remained in the same position for subsequent trials for each bat; however, small changes to the tree positions occurred if the bats hit the trees during flight. Artificial trees were constructed using mist net (Avinet 38 mm mesh, 75 denier/2-ply) wrapped around two metallic rings (diameter 0.254 m) connected vertically with strings (length 2.1 m) for support, creating a cylinder that was hung from the ceiling. The netting, stretched by the weight of the bottom ring, became narrower in the middle. The artificial trees were spaced \sim 1 m apart from their centers. A net connected the side wall of the flight room created a barrier to encourage the bats to explore within the forest and not to circle around the trees. Within the artificial forest, the mealworm was hung in the space between trees. See Fig. 1 for schematic of experimental setup.

The number of trials for each bat is listed in Table 2. Note that two bats, O40:Y2 and B59:Y2, did not have open room data collected. Two other bats, B52 and B57 were tested for a second year, so they were not naive to the

forest. However, these two bats experienced a different orientation of the trees in year 2.

Experimental recording setup

Two high-speed cameras (Photron FASTCAM PCI R2) filmed the bats at 250 frames per second. We reconstructed the 3D flight paths using the direct linear transform algorithm with in-house software programmed in MATLAB and the KineMat toolbox (Reinschmidt and van den Bogert, 1997). A horizontally mounted microphone array (20 microphones) recorded the sound emission intensities of the bat vocalizations (see Ghose and Moss, 2003). This allowed reconstruction of the horizontal beam pattern and calculation of the sonar beam axis. An ultrasound-sensitive microphone (UltraSound Advice, SM2 microphone with SP2 amplifier) was used to record the wideband sonar emissions (bandpassed between the frequencies of 10 and 100 kHz, Wavetek-Rockland Dual Hi/Lo Filter) and recorded digitally (IoTech 512 Wavebook and computer). Data collection synchronization was achieved using a trigger switch connected to a TTL

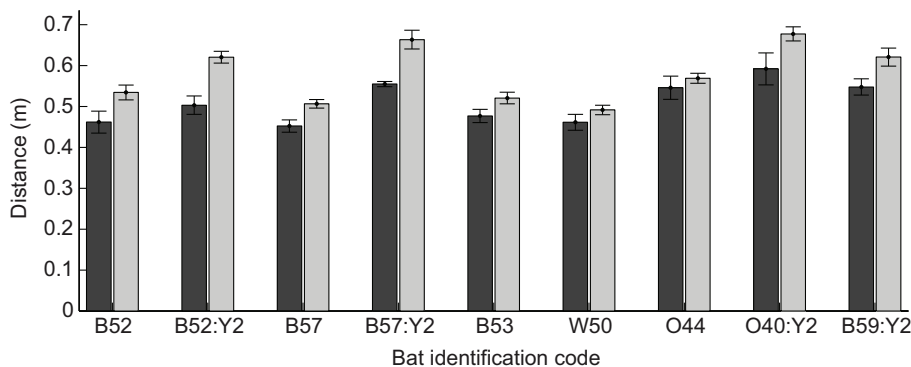


Fig. 8. Time-aligned distance to nearest tree for sound-group vocalizations compared with single-sound vocalizations. Bat averages across trials for distance to tree aligned to vocalization onset is shown (0.1–0.3 s after vocalization). Sound-group distances are in black; single-sound distances are gray. Error bars are s.e.m.

generator circuit that broadcast to each system. Each system was configured with an 8 second rolling buffer aligned to the onset of the TTL pulse. After each trial, data were downloaded.

Sound analysis

Sonar vocalizations on the wide-band recordings were processed using custom software written in MATLAB. Vocalization peak intensities were identified using the MATLAB findpeaks algorithm after squaring and smoothing the time-waveform and using a threshold based on the noise of the recordings. Echoes were automatically ignored when the ratio between peaks differed by a magnitude of five or higher and the interval between pulse and echo was below 15 ms. Vocalizations were manually checked for echoes and skipped vocalizations. The call onsets and offsets were identified by using a recursive search for changes in energy. The sonar calls were either high-passed or low-passed (Butterworth filter, frequency cutoff at 30 kHz) for the onset and offset markings and were manually checked. Vocalizations with a PI below 10 ms (buzz phase) or with a signal-to-noise ratio that was too low did not have onsets or offsets marked and were excluded from duration analysis. Vocalizations with a PI above 300 ms were not included in analyses (determined to be either silent or missed prior vocalizations).

Sound groups were identified when their PI was less than 1.2-times the PI of surrounding vocalizations. For vocalizations occurring in groups of three or more, the PI differences between the vocalizations needed to be below 5%. Vocalizations outside the range of 10 to 100 ms were excluded.

Trajectory analysis

Flight speed was calculated over a smoothed flight trajectory to remove artifacts of 2D digitization (cubic spline interpolation over a sampled subset of the 2D camera data). The average turn rate was calculated as a three-point moving average of the turn angle between each smoothed 3D position. Turn rates above 500 deg s⁻¹ (positioning errors) and turn rates of 0 deg s⁻¹ (stationary) were not included.

Occupancy histograms were created by collapsing the 3D trajectory data to 2D plan projection (x,y). The number of points across a set of flight paths were counted that fell inside 10 cm² bins. These points were converted to probabilities by dividing each bin count by the total number of points across each set of flights. After normalization, the occupancy histograms could be compared across days in the forest. Bats with two or fewer days in the forest were excluded from trajectory comparisons over time (B52, W50 and O44).

Aim of sonar beam in relation to flight turn rate

We examined the correlation at time offsets -0.4 to 0.4 s. To obtain 95% confidence intervals for the correlation coefficient r , we performed a Fisher transformation and calculated:

$$\pm z_{\alpha/2} \frac{1}{\sqrt{n-3}}, \quad (2)$$

where $z_{\alpha/2}$ is 1.96 for 95% confidence intervals and n is the sample size. The confidence interval for τ_{\max} was determined by considering the range of obtained r values not significantly different from the peak r value ($\pm 95\%$ confidence interval). The data from three bats were combined for this analysis (B52, B57 and B53).

Temporal relationship between sonar vocalizations and flight trajectories

In this analysis, the distance to the nearest tree, independent of direction, was calculated for each call. Call emission times were set to the onset time (corrected for time of flight to the microphone). For sound groups, the onset was the first vocalization of the sound group. All single sounds (non-sound-group calls) were included except for calls with a PI below 10 ms. The distance between the bat and nearest tree was calculated along the trajectory between 100 and 300 ms after each vocalization. This range of time was used because it represented a portion of the trajectory that could be influenced by sonar echoes returning and because of the difference observed in distance to the tree along this interval. For each bat, an average across trials of the distance to the nearest tree was calculated for both sound-group and single-sound emissions.

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Competing interests

The authors declare no competing financial interests.

Author contributions

L.J., C.F.M., A.S. conceived and designed the experiment. L.J. performed the experiments. B.F. analyzed the data. B.F. wrote the paper with assistance from C.F.M., L.J. and A.S.

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