Manipulating Environmental Clutter Reveals Dynamic Active Sensing Strategies in Big Brown Bats

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Abstract – Vocalizing animals confront acoustically challenging conditions in which background noise (clutter) can mask or shift attention away from biologically relevant signals. Echolocating big brown bats (*Eptesicus fuscus*) are excellent comparative models for studying how animals differentiate between multiple sound sources in complex acoustic scenes. We trained four big brown bats to fly down an asymmetrical corridor producing distinct clutter echoes from the two sides. While in flight, they were presented with playbacks of exemplars of an echolocation call, a social communication call, or waterfall noise, from one or both sides of this corridor; a silence condition served as a control. We predicted that bats would perceive the playbacks, as indexed by modifications of their vocalizations and shifts in their head aim. Bats completed flights at a high rate of success in all conditions. Although bats produced calls in similar sized sonar sound groups in playback and silent trials, they emitted more echolocation calls and shortened the time intervals between calls in response to playbacks. These comparisons suggest the playbacks increased the perceptual difficulty of the task to some extent. Bats aimed their heads towards the left side of the corridor where clutter echoes were acoustically stronger but also sparser. Changes in head aim in response to playbacks were small. Our data suggest that big brown bats flying through clutter detect differences in the information content of surrounding acoustic scenes and alter their echolocation behavior accordingly.

Keywords – Acoustic clutter, Active sensing, Big brown bat, Communication calls, Echolocation, *Eptesicus fuscus*, Noise

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Vocalizing animals communicate and navigate within environmental conditions that are acoustically challenging. Background noise sources, both biotic and abiotic, can mask or interfere with perception, recognition, and localization of biologically relevant signals. Nonetheless, animals are able to form acoustic scenes of their environment and can detect, discriminate, and shift attention between sound sources of immediate interest against background noise or clutter. Identification of the perceptual and cognitive strategies by which animals perform these tasks can elucidate mechanisms by which species adapt to and gain information from changing environments (Hulse, 2002).

Echolocating big brown bats (*Eptesicus fuscus*; Kurta & Baker, 1990) are excellent models for understanding how animals perceive and navigate through complex acoustic scenes. These nocturnal insectivores emit short duration, frequency-modulated (FM) ultrasonic echolocation calls with two harmonics, with the first harmonic (FM1) sweeping down from approximately 55 to 20 kHz and the second harmonic (FM2) sweeping down from 100 to 50 kHz. They receive and decode returning echoes to identify the distance, size, and shape of ensonified targets (such as insect prey or navigational

obstacles; Griffin, 1958). Big brown bats emit their echolocation calls in trains, at intercall (interpulse) intervals that they actively modify depending on the acoustic scene. Calling rate varies with distance to targets, in the presence of echoes from clutter (non-target objects) that might interfere with target detection, and by how challenging the bats perceive the task to be (Kothari et al., 2014; Moss et al., 2006; Petrites et al., 2009; Tuninetti et al., 2021). Moreover, like other species of FM bats, big brown bats dynamically and rapidly adjust the amplitude, duration, and spectral content of their echolocation calls as well as the directionality of their sonar beam when confronted with acoustic scenes of varying complexity (Accomando et al., 2018; Adams et al., 2019; Beetz et al., 2019; Fujioka et al., 2014; Hiryu et al., 2010; Hulgard & Ratcliffe, 2016; Knowles et al., 2015; Melcón et al., 2011; Ming et al., 2020; Sändig et al., 2014; Surlykke et al., 2009; Tuninetti et al., 2021; Wheeler et al., 2016). These changes in call production and beam aim serve as indices of the bat's perception of its surroundings and of its shifts in attention to specific cues (echoes) within the entire acoustic scene.

In their natural environments, bats are subjected to environmental noise from biotic (e.g., conspecific or heterospecific bats, insects) and abiotic (e.g., water, wind farms, traffic) sources. These noise sources impact acoustically guided foraging by echolocation (Allen et al., 2021; Bunkley et al., 2015; Gomes & Goerlitz, 2020; Luo et al., 2015; Schaub et al., 2008; Siemers & Schaub, 2011). In a study of five bat species foraging near natural gas compression stations emitting broadband noise below 24 kHz, Bunkley et al. (2015) found that the activity levels of one of these species, whose echolocation calls contain spectral energy within this range, were affected by the compressor noise; activity of other species broadcasting higher frequency echolocation calls was unaffected. Luo et al. (2015) showed that foraging activity of Daubenton's bats (*Myotis daubentonii*) was impaired in the presence of traffic noise, even though the spectrum of that noise did not overlap with their echolocation calls. Schaub et al. (2008) reported that foraging by gleaning greater mouse-eared bats (*Myotis myotis*) was affected more by continuous broadband noise than by transient traffic or vegetation noise. Besides echolocation calls, bats emit distinct calls for social communication with conspecifics, both in the roost and during flight (Barlow & Jones, 1997; Bastion & Schmidt, 2008; Gadziola et al., 2012; Götze et al., 2020; Montoya et al., 2022; Springall et al., 2019). These calls can function in either affiliative or agonistic interactions. Little is known about how environmental noise affects perception of social calls (Song et al., 2019). A full understanding of the impacts of noise on perception requires understanding how noise source and content affects both echolocation and communication calls.

The mechanisms driving the impact of environmental noise on behavior remain unclear. One possibility is that noise decreases the bat's hearing sensitivity. It has been shown, however, that big brown bats are not susceptible to temporary increases in hearing thresholds due to intense noise exposure (Hom et al., 2016; Simmons et al., 2017, 2018). Bats may find noise to be distracting, diverting attention away from foraging, even if hearing sensitivity is not affected (Allen et al., 2021). Finally, noise may produce a stress or an aversive (avoidance) response (Luo et al., 2015; Schaub et al., 2008). On the other hand, noise may be beneficial, providing information necessary for guiding complex behaviors (Giepel et al., 2019). This presumed informational role of noise assumes that bats can perceive and make judgments on the importance of particular noise sources for echolocation or communication.

In this experiment, we asked if bats performing a laboratory navigation task could differentiate between background noise sources of different acoustic complexity and presumed informational content. Bats flew down an unobstructed corridor surrounded on both sides by physical objects producing acoustic clutter of different strengths and spatial extent. We hypothesized that bats would bias their head aim towards the side of the corridor with the most informative clutter, expected to be the side leading to an open flight path. To increase the complexity of the surrounding acoustic scene, we broadcasted playbacks of intense sounds (species-specific and abiotic) from one or both sides of the corridor. We expected that the bats would perceive these playbacks and alter the timing of their own echolocation calls and their head aim as a result. We expected that these changes in calling and head aim would be modulated by the presumed informational content of the playbacks and their source. Our results address the question of how the addition of novel, intense acoustic stimuli in an already complex soundscape affects active sensing behaviors.

Methods

Ethics Statement

Capture of wild bats participating in these experiments was approved by collector permits (2015-37, 2016-32, 2019-48-W) from the state of Rhode Island. The experimental protocol and all laboratory animal care were approved (protocol #20-11-0002) by the Brown University Institutional Animal Care and Use Committee.

Animals

Four wild-caught adult big brown bats (two males, Jorge and Thor; two females, Freyja and Octavia) participated in the experiment. Because bats were wild-caught, their ages are unknown. Only four bats were available for this experiment, due to the limits for captures imposed by the state collector permit. Bats were vaccinated against rabies upon entering laboratory care and were individually identified by microchips (Trovan ID-100A RFID transponder, Trovan LID-573 microchip reader) implanted subcutaneously under the skin on their backs. They were housed in social groups in a temperature- and humidity-regulated laboratory space (22-24ºC and 40-60% relative humidity) on a reverse light cycle (12 hours dark, 12 hours light). All bats had free access to water and were fed live mealworms (*Tenebrio* larvae), either as rewards for successful flights during the experiment or in their home enclosures on non-experimental days. Food rations were regulated for each bat to maintain their weights in a healthy range between 15-20 grams. Both the colony room and the experimental flight room are classified as Biohazard Level 2 spaces, and all personnel had up-to-date rabies vaccination.
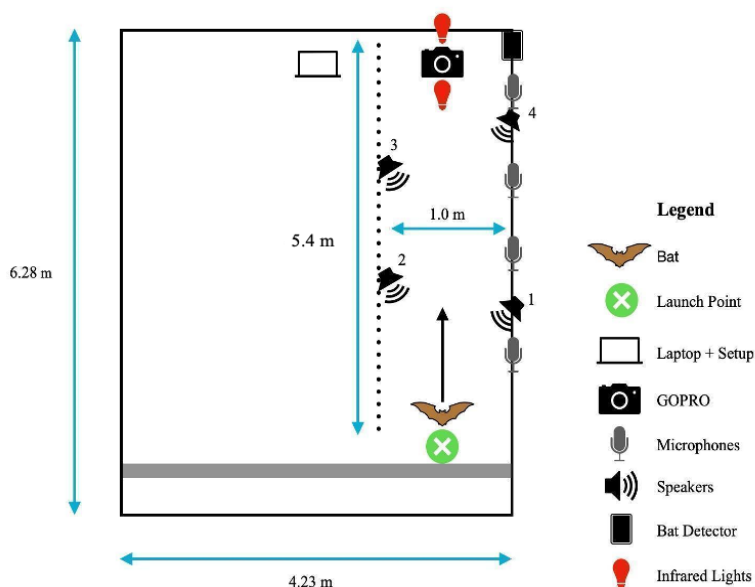
Flight Corridor

Experiments took place inside a dedicated room for bat experimentation (6.28 m X 4.23 m X 2.70 m). The four walls and the ceiling of the room were lined with acoustic foam (SONEX®, Pinta Acoustics) and padded panels covered with artificial turf were installed on the floor to dampen acoustic reflections and reduce ambient noise. The flight space was a straight, unobstructed flight corridor (5.4 m long, 1.0 m wide) physically and acoustically different (asymmetrical) on its two sides (Figure 1). On the left side, we hung black plastic chains (link size 4.0 cm wide, 7.5 cm long, 1.0 cm thick) at intervals of 20 cm, extending from the launch point to the far (end) wall of the corridor. The chains were suspended from PVC crossbars fixed to the ceiling and were weighted at the floor end to prevent swaying. Echoes produced by these chains mimic the extended sequences of echoes produced by foliage (Petrites et al., 2009). An open flight path existed behind these chains. The foam-covered wall of the room, on which were mounted four 1.5 cm diameter PVC frames and clamps, formed the right side of the corridor. The flight corridor was illuminated with infrared light panels, powered by an external 12-volt regulated power supply (EXTECH DC 382213), attached to the ceiling.

The bat's flight down the corridor was video recorded at 120 frames/s using a GoPRO camera (Hero 2 v312, customized to remove the camera's infrared filter) mounted atop a tripod at a height of 1.12 m centered at the far end of the corridor and facing the launch point. A bat flying down the midpoint of the corridor would be flying directly toward the camera and centered within its view. Four analog ultrasonic microphones (Dodotronic Momimic; frequency response +/- 4 dB from 20-100 kHz) were mounted at the same height 122 cm apart on the right wall. Four ultrasonic loudspeakers (Kenwood KFC-XTI5le; frequency response +/- 3 dB from 0.35-75 kHz; -12 dB at 80 kHz) were mounted along the right and left sides of the corridor (two on each side), angled at 45° so that acoustic stimuli would be broadcast towards the launch point. Heights of the loudspeakers (140-224 cm from the floor; Figure 1) were chosen based on prior experience of the heights at which different bats tend to fly.

Figure 1

Bird's Eye View of the Flight Room, with the Flight Corridor Along the Right Side

Note. Dimensions are shown by the blue lines and arrows. The left side of the corridor was bounded by a row of hanging plastic chains (dotted line, leading to an open flight path). Two loudspeakers (speakers 2 and 3) were mounted along the chains (at 156 and 216 cm from the floor, respectively). The right side of the corridor was bounded by a solid wall covered by acoustically absorbing foam panels. Clamps for two loudspeakers (speakers 1 and 4, mounted 140 and 224 cm from the floor) and for four ultrasonic microphones were mounted on this wall. The bat was released at the launch point (green X) and flew down the corridor (black line and arrow) to land on the far (end) wall. The GoPro video camera on the end wall recorded the bat's position during flights. LED infrared panels with no visible component illuminated the corridor for video recording.

Playback Stimuli

Three exemplar sounds, with distinct acoustic features and presumed information content, were used for playbacks (Figure 2). These were an Echolocation call exemplar, a communication call exemplar, and continuous Waterfall noise as an example of an abiotic, broadband source. Echolocation and communication calls were recorded from big brown bats in their home enclosures with a Wildlife Acoustics SM4 recorder (Wildlife Acoustics, Inc.; sampling rate of 192 kHz). The resulting audio files were cleaned in Adobe Audition 2022 ('silence frequencies brush') to eliminate background ventilation noise. From these recordings, we selected examples of calls with the highest signal-to-noise ratios to use as playback stimuli. For the Echolocation call exemplar, we selected two calls from one bat and, using Adobe Audition, repeated them in pairs separated by 36 ms with an inter-pair interval of 65 ms, for a total duration of 2 s. As an exemplar of a communication call, we used frequency-modulated bouts (FMB calls) recorded from a cage housing two male bats. FMB calls are distinguished acoustically from echolocation calls by their sweep shape, longer duration, and lower terminal frequency. These calls may communicate the presence of food, particularly in an agonistic context (Wright et al., 2013, 2014). We constructed the FMB exemplar by repeating two FMB calls at an interval of 18 ms, separated from another pair by an interval of 170 ms, for a total duration of 2 s. Due to the acoustic roll-offs of the Kenwood loudspeakers, energy in the Echolocation and FMB exemplars recorded in the flight corridor begins to attenuate above 60 kHz. Continuous Waterfall noise was procured from an online library of sounds (Mixkit.co). The noise was high-pass filtered at 6 kHz to remove frequencies outside the bat's range of hearing and cut to a duration of 2 s. No ventilation noise was present in the Waterfall stimulus file and so it was not filtered further. All audio files were normalized to the same nominal 0 dB RMS

(root mean square) value using Adobe Audition's 'Normalize' option. We chose to employ only one exemplar of each of the three playback sounds, because we were not interested in the bat's ability to generalize across different examples of sounds within these categories.

Figure 2

Sound Spectrograms of Playback Stimuli (Top Three Rows) Recorded in the Flight Corridor
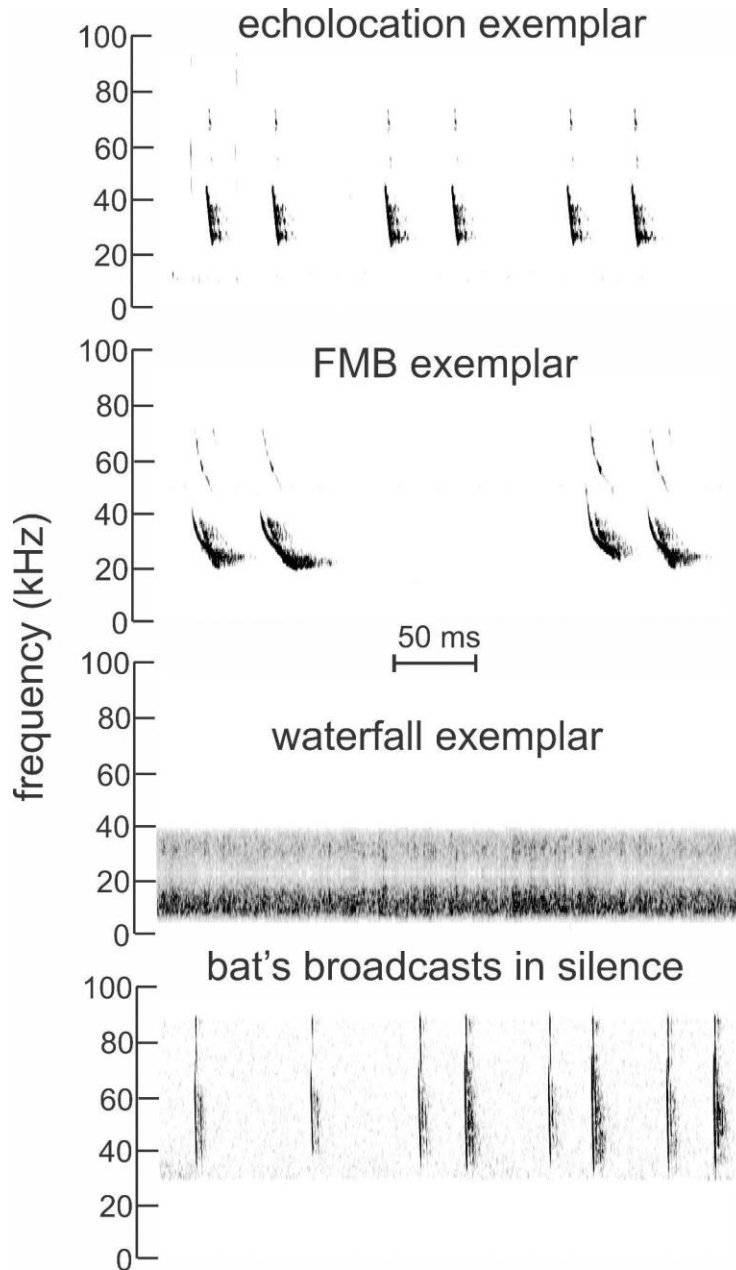
Note. Spectrograms for the Echolocation and FMB exemplars were computed in Adobe Audition at 192 kHz sampling rate, 512 resolution. The acoustic characteristics of the loudspeakers resulted in reduced energy in these signals above 60 kHz. The spectrogram for the Waterfall exemplar was computed at a sampling rate of 44.1 kHz, 512 resolution. All three playback sounds were normalized to 0 dB RMS for playback. The bottom row shows a spectrogram of the echolocation call of one bat flying down the corridor in the Silence condition. Because the bat's own call was not broadcast through loudspeakers, it contains more high frequency energy than the Echolocation or FMB exemplars. Time scale in all spectrograms is 50 ms.

Exemplars were stored as uncompressed .wav files on a Dell Latitude laptop running Windows 10 connected to a National Instruments Data Acquisition (DAQ) device (NI-USB-6361) and controlled with a custom-written MATLAB script. Analog output of the DAQ device was sent to a power amplifier (Harman Kardon PM645; frequency response range 0.5-150 kHz), connected to a series of analog switches used to route the sound signal to the loudspeakers (both sides, left side only, right side only) for playbacks.

To measure stimulus levels from the playbacks and from the sides of the corridor, we placed a Brüel & Kjaer Model 4135 (1/4 inch) condenser microphone in the center of the corridor, 75 cm from the launch point and at a height of loudspeaker #1. We measured the peak-to-peak voltage of the microphone signal on an oscilloscope (RIGOL DS1054Z digital 4 channel 50MHz) and converted these measures to decibel sound pressure level (dB SPL re 20 µPa). At 0 dB RMS, the pulsed Echolocation and FMB exemplars broadcast from the loudspeakers (both sides, left side, right side) range in level from 104-114 dB SPL, while levels of the continuous Waterfall noise range from 89-97 dB SPL. Playbacks at these levels do not induce temporary threshold shifts in big brown bats (Hom et al., 2016; Simmons et al., 2016, 2017, 2018).

Each of the hanging chains reflects an intense echo at a sound pressure of approximately 90 dB SPL when ensonified by bat-like FM pulses at 120 dB SPL. The whole row of chains reflects a series of echoes spaced 1.1 ms apart in delay. Each chain's links contribute separate, closely spaced reflections, so a single chain's echoes last for about 5 ms. As the chains recede in the distance from 1 to 4 m, their echoes decrease in amplitude only slightly, by about 4.4 dB. Over the same distance, the right side returns weaker, widely extended reflections, 10 dB lower (approximately 80 dB SPL) than the reflections from the chains. The PVC frames and clamps return echoes 4 dB stronger than those produced by the wall itself, so that the highest intensity of echoes from the right side of the corridor is 84 dB SPL, 6 dB less than those from the left.

Procedure

Experiments took place during the bats' subjective night. Three experimenters participated in data collection. The first experimenter released the bat at the launch point and retrieved it when it completed the flight. The second experimenter-initiated presentation of the playback stimuli, and the third experimenter controlled the video recording. Playback stimuli were presented, and video recording commenced, as soon as the bat was released.

Each bat flew at least 10 flights on each day of the experiment. Approximately 1% of trials were eliminated due to experimenter error in initiating or terminating stimulus presentations, due to the bats reaching their daily mealworm allotment before 10 flights could be completed, or due to a bat's unwillingness to launch from the experimenter's hand. On experimental days 1-8, the flying bat was presented with the Echolocation call exemplar, the FMB call exemplar, or Silence (no stimulus). Loudspeaker system noise was present during Silence trials. Waterfall noise was included as a separate condition on experimental days 9-15. These exemplars were presented in groups of three (based on loudspeaker side: both, left, right) in a random order. The first flight of the day was always in Silence. An example trial sequence is Trial 1, Silence; Trials 2-4, Echolocation exemplar, loudspeakers both, left, right; Trials 5-7: FMB exemplar, loudspeakers right, left, both; Trials 8-10: Waterfall exemplar, loudspeakers left, both, right; Trials 10-12: Silence.

Audio Acquisition and Analysis

The four wall-mounted microphones were used to record, identify, and localize each of the bat's own vocalizations (any echolocation or communication calls) during flights. Audio outputs from the microphones were routed to two stereo microphone preamplifiers (FMR Audio, RNP8380), filtered (Thor Laboratories 20kHz high pass filter), and recorded onto the first four channels of an 8-channel digital recorder (ZOOM F8, 192 kHz sampling rate). One additional audio channel recorded the playback stimuli

directly from the Dell laptop to mark stimulus onset time in relation to recordings of the bat's own calls. The output of an ultrasonic bat detector (Petterssen 240X) mounted on the ceiling at the end of the corridor was recorded on an additional audio channel.

Digital output files from the four microphone channels of the Zoom recorder were filtered in MATLAB (version 2021b) using a minimum order high pass FIR filter with a high frequency cutoff at 20 kHz and a stopband attenuation of 60 dB to remove low frequency noise. We developed a custom MATLAB code to separate out the bat's own calls from the recordings of the playback stimuli picked up by the microphones. Because the Echolocation and FMB exemplars when broadcast through the loudspeakers had minimal energy above 80 kHz, while the bat's own calls contained energy up to 90 kHz (Figure 2), we high-pass filtered the audio files from those trials at 80 kHz and identified the bat's own calls by the presence of high frequency energy above this cutoff. Audio files in the Waterfall condition were high-pass filtered at 40 kHz, and the bat's own calls were identified by energy above this cutoff. We used the *findpeaks* function in MATLAB to calculate the time of each of the bat's own calls, using spline interpolation over local maxima separated by at least 2 ms. The maximum distance between peaks was set to 10 ms, and the maximum peak width was set to 7 ms.

The bat's position in the corridor was estimated in MATLAB using the timing of its own calls as received by two (one pair) of the four microphones, using time-difference-of-arrival (Barchi et al., 2013; Sathyan et al., 2006; Smith & Abel, 1987). When calls were detected in more than two microphones, position was calculated as the average of the bat's distance from each microphone pair. The MATLAB script calculated the time of call emission and inter-pulse intervals (IPI) between these calls by comparing the timing across the four microphones. Any IPIs less than 7 ms were discarded to avoid mis identifying chain reverberations as emitted calls (Tuninetti et al., 2021; Wheeler et al., 2016). Position estimation using two microphones was not feasible in the Waterfall condition, because the bat's own calls were obscured by noise on three of the four microphone channels, making only one channel reliable for call identification. We categorized the bat's own echolocation calls into sonar sound groups (SSGs; Kothari et al., 2014), based on the relative time intervals between and within groups of pulses as used previously (Tuninetti et al., 2021). All statistical analyses were done in R Studio (v4.0.2).

Quantification of Head Aim

The aim of the bat's head during successful flights was quantified using a deep learning, marker less tracking network (DeepLabCut, Nath et al., 2019). To train the network, 20 frames from 20 videos were hand-labeled to track five points: the bat's left wingtip, left pinna tip, nose, right wingtip, and right pinna tip. The network identified and tracked the same five points through all frames in all videos. The result was a .csv file showing the (x,y) position of each tracked point and a video labeled with those points. The network also provided a confidence value for identification of each tracked point in each video frame. For statistical analysis, we included only those tracked points from the nose, right pinna tip, and left pinna tip that were each identified with a confidence greater than 90%.

A custom MATLAB script was used to calculate the direction in which the bat's head faced from the tracked position of its pinnae and nose. We calculated the offset in the bat's head aim as the ratio of the nose's horizontal offset from the middle of the two pinnae to the total distance between the two pinnae. As a bat flies down the corridor towards the camera on the end wall, the distance between the pinna tips increases. If the bat's nose is centered directly between the two pinnae, then the head aim offset is 0%, at all distances from the camera. As the bat turns its head away from the midline of the corridor, the position of its nose moves in the horizontal axis closer to one pinna tip and further from the other pinna tip. Therefore, we calculated head aim offset as the ratio of the nose's position between the pinnae to the overall distance between the pinnae.

Results

Performance

Total number of flight trials and performance (percent of successful flights, in which the bat reached the end wall) in all conditions are shown in Table 1. Any flights in which a bat landed in the corridor or turned back towards the launch point before reaching the end wall were labeled as unsuccessful. Performance data were averaged across the three loudspeaker sides (both, left, right). We compared performance in Silence to that in each playback condition using two-tailed exact binomial tests with a Bonferroni-corrected p-value of .017. Only one pairwise comparison was statistically significant: Bats performed significantly worse in Waterfall flights (91.3%) than in Silence flights (97.8%; $p = .005$). This result is likely driven by the poor performance of one bat (Freyja), where performance in that condition was only 67% successful.

Table 1

Number of Flight Trials (Flights) and Performance (% Successful Flights) for each Bat in Each Condition (Echolocation Exemplar, FMB Exemplar, Waterfall Exemplar, Silence)

Bat	Echolocation Exemplar		FMB Exemplar		Waterfall Exemplar		Silence	
	Flights	% Success	Flights	% Success	Flights	% Success	Flights	% Success
Freyja	42	83	42	88	18	67	23	96
Jorge	42	98	44	95	15	100	20	95
Octavia	46	100	46	100	18	100	24	100
Thor	45	98	45	98	18	100	27	100

Note. Performance is averaged over all three loudspeaker sides.

Modifications of Calling Behavior

Bats never emitted FMB or any other social calls during successful flights, even in the FMB condition. Numbers of echolocation calls emitted by the flying bat and IPIs between these calls in successful flights are shown in Table 2. Data were compiled from all three loudspeaker sides. Vocalizations during unsuccessful flights were not analyzed.

We developed two linear mixed models (LMM) to test the prediction that the number of emitted calls and IPIs between these calls would vary between conditions. In both models, condition was included as a fixed effect and bat as a random effect; loudspeaker side was not included as an effect. The models were built in RStudio using the *lme4* package, and significance of effects was determined using the *lmerTest* package, which performs F tests using Satterthwaite's degrees of freedom method (Brown, 2020; Kuznetsova et al., 2017).

Results of the two LMMs (number of calls, IPIs) are shown in Table 3. Bats emitted significantly more calls in the Echolocation and FMB conditions compared to Silence (estimates = +30.9 for Echolocation, $p < .001$; +23.3 for FMB, $p < .001$). There was no significant difference in number of calls between the Waterfall and Silence conditions ($p = .14$). Results of pairwise comparisons using the adjusted Tukey test show that number of emitted calls was higher in both the Echolocation (estimate = +24.5, $p < .001$) and the FMB (+16.8, $p = .0001$) conditions compared to the Waterfall condition, but there were no significant differences between the Echolocation and FMB conditions. Bats emitted shorter IPIs in playback conditions relative to flights in Silence (estimates = -9.5 ms in the Echolocation

condition, $p < .001$; -8.9 ms in the FMB condition, $p < .001$; and -5.9 ms in the Waterfall condition, $p < .001$). IPIs were significantly shorter in the Echolocation (-3.6, $p < .001$) and FMB (-2.9, $p < .001$) conditions compared to the Waterfall condition. IPIs were also shorter in the Echolocation condition compared to the FMB condition (-0.6, $p = .0107$).

Table 2

Mean Numbers of Echolocation Calls (Mean n Calls) and IPIs (Mean IPI ms) for Each Bat (Bat) in Each Condition (Condition)

Bat	Condition	Mean n Calls	Mean IPI (ms)	IPI (sd)
Freyja	Echolocation	77.3	31.0	23.0
	FMB	90.3	20.5	12.5
	Waterfall	61.7	26.0	17.6
	Silence	60.2	34.2	26.5
Jorge	Echolocation	79.6	21.4	13.0
	FMB	78.3	22.8	14.5
	Waterfall	60.8	30.0	28.7
	Silence	59.0	30.3	17.8
Octavia	Echolocation	91.6	22.9	17.2
	FMB	77.7	25.0	19.5
	Waterfall	84.4	21.6	20.0
	Silence	54.0	36.3	35.3
Thor	Echolocation	102.2	18.3	8.8
	FMB	72.7	24.0	17.1
	Waterfall	98.2	33.4	24.5
	Silence	54.4	33.7	24.4

Note. The standard deviation (sd) of IPI is also shown (IPI sd). Data are averaged over all loudspeaker sides.

Table 3

*Results of LMMs Predicting Number of Emitted Calls and IPI*

Predictors of Call n	Estimates	CI	p
(Intercept)	54.2	48.6 - 59.9	< .001
Stim:Echolocation	+30.9	+23.7 - +38.1	**< .001**
Stim:FMB	+23.3	+16.3 - +30.2	**< .001**
Stim:Waterfall	+6.4	-2.2 - +15.0	.14
Random effects (Call n)			
σ^2		641.3	
n Bat		4	
Observations		414	
Marginal R^2		0.2	
Predictors of IPI	Estimates	CI	p
(Intercept)	31.6	30.4 - 32.8	< .001
Stim:Echolocation	-9.5	-9.9 - -8.9	**< .001**
Stim:FMB	-8.9	-9.4 - -8.4	**< .001**
Stim:Waterfall	-5.9	-6.5 - -5.2	**< .001**
Random effects (IPI)			
σ^2		208.5	
n Bat		4	
Observations		30861	
Marginal R^2		.05	

Note. Results of the two LMMs testing changes in number of emitted calls (top) and in IPI (bottom) for each playback exemplar (Stim) compared to Silence. CI = 95% confidence intervals around the predicted estimate. Positive estimates indicate increases in call number or IPI and negative estimates indicate decreases in call number or IPI. The *p* values are calculated via F tests using Satterthwaite's degrees of freedom method. Statistically significant p values are in bold.

We examined any changes in the timing of the bat's own echolocation calls between conditions by calculating sonar sound groups (SSGs). In all conditions and for all bats, the most common SSG (mean proportion of total calls from all bats = .55) was a doublet (SSG = 2; Figure 3); single calls (SSG = 1) were the second most common pattern. The proportion of SSGs of sizes of 3 and greater were small in all conditions. We performed pairwise McNemar's repeated-measures chi-squared tests comparing the proportions of calls in Silence emitted as singles and as doublets to the same proportions emitted in playback conditions. Comparisons were run separately for individual bats, as individual bats can display different amounts of sonar sound grouping. Three pairwise comparisons were done per bat (Silence vs. Echolocation, Silence vs. FMB, and Silence vs. Waterfall), and a Bonferroni-corrected *p*-value of 0.017 was used to assess significance. All pairwise comparisons were significant ($p < .001$, χ^2 values ranging between 26 - 1191). Three bats emitted more single calls and fewer doublets in playback conditions than in Silence; the exception (Thor) emitted fewer single calls and more doublets in playback conditions than in Silence.

Figure 3

Sonar Sound Groups (SSGs, Group Sizes 1, 2, ≥ 3) Produced by Each Bat in Each Condition (Top Row) as a Proportion of Total Calls (Y Axis) for that Condition
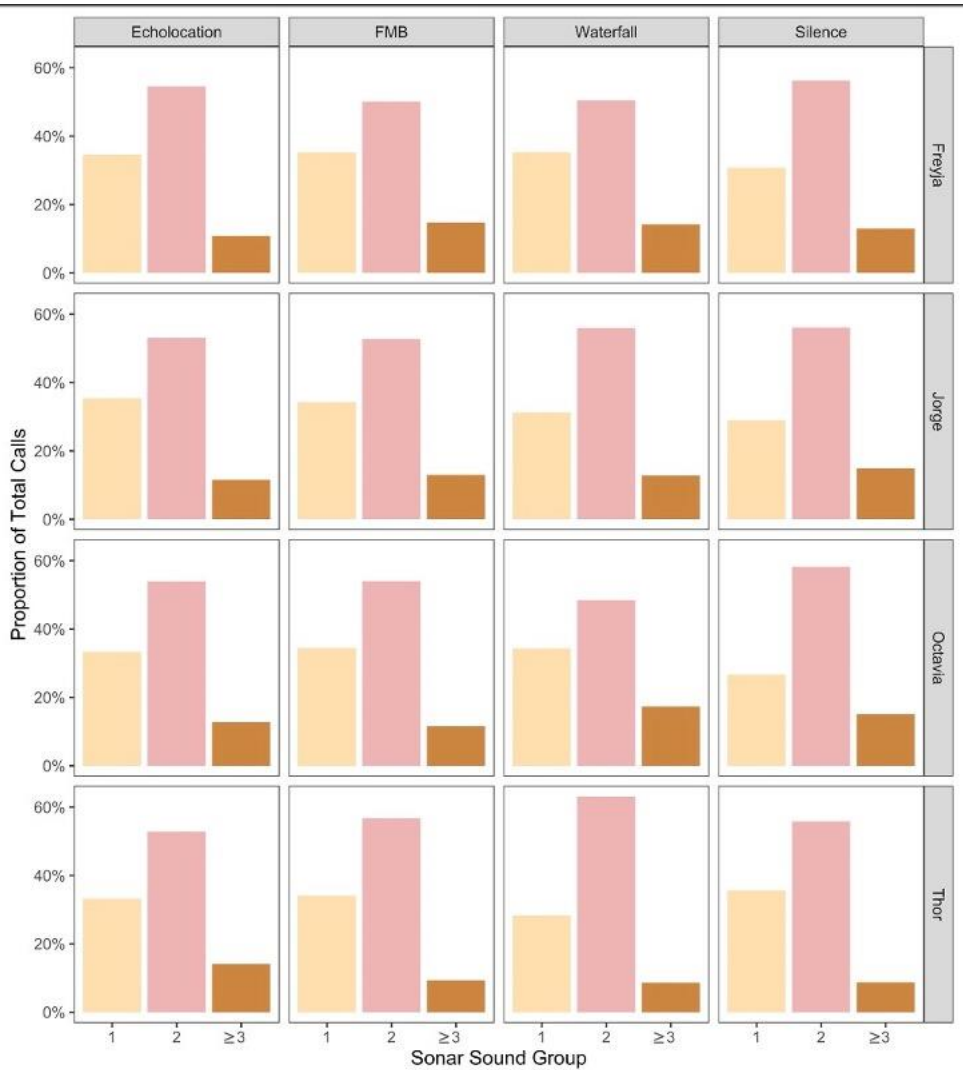
Note. Doublets (SSG = 2) followed by single calls (SSG = 1) were the most predominant SSG in all conditions. SSG groups of 3 and larger occurred rarely.

Shifts in Head Aim

We hypothesized that head aim would be biased towards the side of the corridor with the more informative but sparser clutter, i.e. the left side where hanging chains led to an open flight path (Figure 1; Tuninetti et al., 2021). We expected that the magnitude of this bias would be affected by the playbacks. Specifically, if playbacks (particularly the FMB exemplar; Wright et al., 2013) were aversive to the bat, we expected head aim to veer away from the source (loudspeaker side) of these playbacks. If bats were not attentive to the asymmetry in clutter from the corridor or to the playbacks, then we expected head aim to be centered around the midline in all conditions, regardless of the side of the active loudspeakers.

Figure 4 shows the best-fitting smoothed polynomial regression and 95% confidence intervals of estimates of head aim offset (i.e., the deviation in symmetry between the nose and the left and right pinna

tips, as a percentage) for all bats in all conditions. There were considerable individual differences in head aim offset, but some general trends emerge. At the beginning of flight, bats tended to aim their heads close to the midline (calculated head aim offset of 0%) or slightly towards the left (positive offset). As the bats progressed down the corridor, the left side bias in head aim offset tended to increase (up to 4-6%), then returned closer to the midline as bats reached the end wall. The exception to this trend is Thor, whose head aim offset remained biased towards the left throughout the entire flight.

Figure 4

Estimated Head Aim Offset in Percentage (Left Y-Axis) for each Individual Bat (Columns) in each Condition (Right Y-Axis), as a Function of Distance Between the Ears/Distance from Launch Point (X Axis)
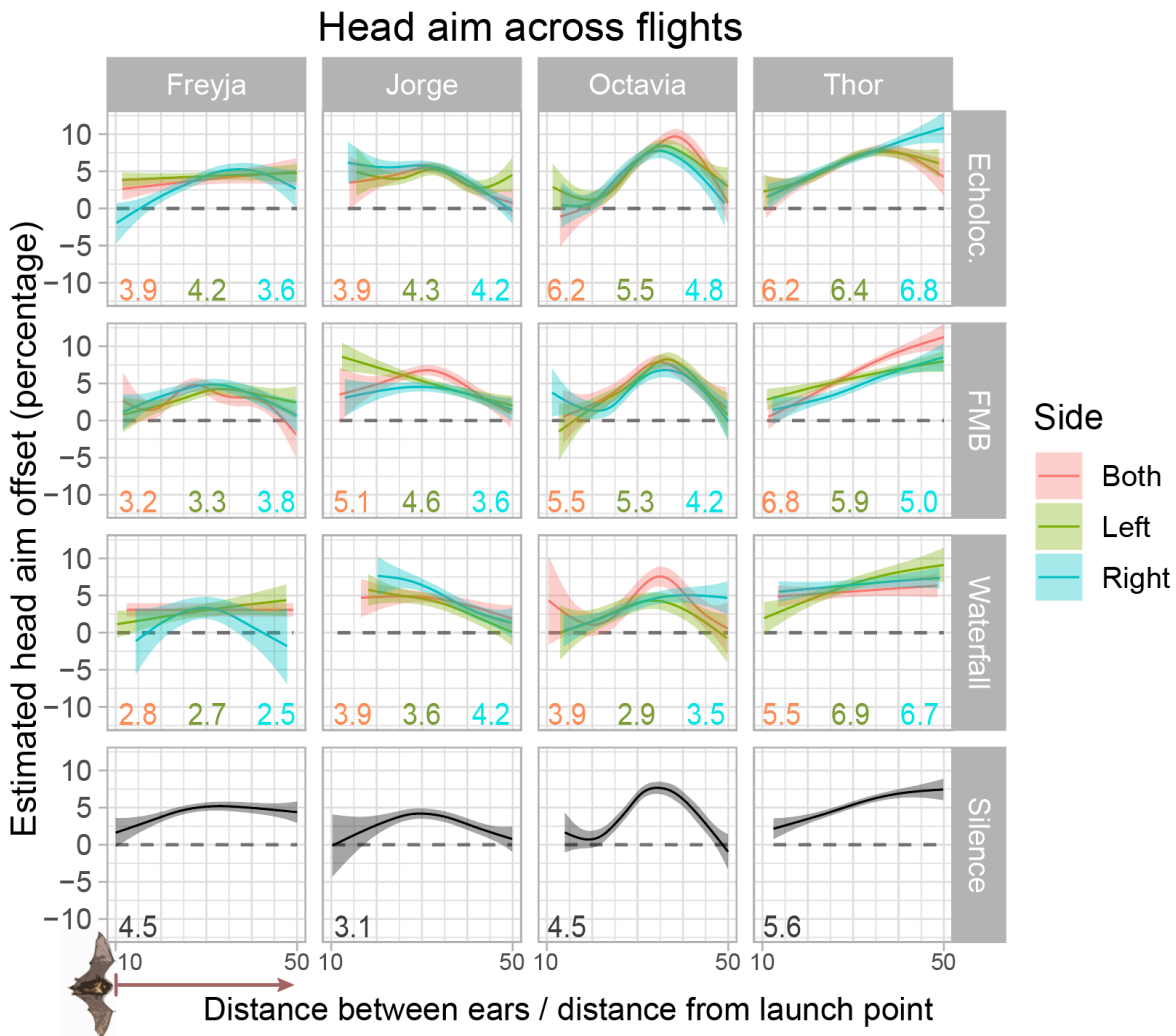
Note. Distance between the ears is calculated by number of pixels, where a pixel distance of 10 indicates that the bat is near the launch point and a pixel distance of 50 indicates that the bat is near the end wall. Head aim offset (percentage) is calculated from the ratio of distance of the bat's nose relative to each pinna tip. Offset calculations include only those estimates of the position of the nose and of the two pinna tips where DeepLabCut provided a confidence estimate of 90% or greater. Solid lines in each plot show the smoothed polynomial regression through the data, with shaded areas indicating 95% confidence intervals. Positive offsets indicate aiming toward the left (chains) side of the corridor, and negative offsets indicate aiming toward the right (wall) side of the corridor. Loudspeaker side is indicated by colors (right insert: both, left, right). Mean head aim offset (calculated from the raw data) for each loudspeaker side is shown along the bottom of each plot, in corresponding colors.

Using LMM, we tested if head aim offset was affected by the fixed effects of playback condition and loudspeaker side. The bat's progress down the corridor (i.e., how close the bat was to the video camera, as indicated by the absolute distance between its ears) was also included as a fixed effect. Bat was included as a random effect. To assist with model convergence, the ear distance measurement was scaled and centered around 0 using the base R scale function. Scaling and centering the ear distance variable does not change its effect or relationship with other variables, nor does it affect the interpretation of the effects of ear distance within the LMM. The only effect is to normalize the ear distance measurements and constrain the range of its values, thus making it possible for the LMM convergence process to calculate the large number of regression estimates required.

Table 4 shows the results of this model. There was an overall bias in head aim offset towards the left (chains) side of the corridor (positive intercept = +5.7, $p < .001$). Distance between the ears did not significantly affect these estimates (estimate = +1.1, $p = .3$), as expected, given the use of a ratio to calculate head aim offset. The left side bias was not affected by playbacks of the Echolocation exemplar, regardless of the loudspeaker side ($p > .1$ for all estimates). Playbacks of the FMB exemplar reduced, but did not eliminate, the left side bias by inducing a slight shift closer towards the right (negative estimates: both sides = -.5, $p = .005$; left side = -1.1, $p < .001$; right side = -.7, $p < .001$). This effect can be seen in plots from three of the four bats at both the beginning and the end of flights (Figure 4). Playbacks of the Waterfall exemplar also reduced but did not eliminate the left side bias (negative estimates: both sides = -.9, $p < .001$; left side = -0.8, $p < .001$; right side = -.9, $p < .001$). Results of running the model without the variable of loudspeaker side showed a significant effect of playback condition (estimate = +.3, CI = +.1 - +0.6, $p = .005$).

Table 4

Results of the LMM Testing Changes in Head Aim Offset Relative to Silence

Results of LMM predicting head aim offset			
Predictors	*Estimates*	*CI*	*p*
(Intercept)	+5.7	+4.2 - +7.2	**<0.001**
Ear distance (scaled)	+1.1	-1.0 - +3.1	0.3
Stim:Echolocation × Side:both	-0.1	-0.4 - +0.3	0.7
Stim:Echolocation × Side:left	-0.2	-0.5 - +0.2	0.4
Stim:Echolocation × Side:right	0.0	-0.3 - +0.3	0.9
Stim:FMB × Side:both	-0.5	-0.8 - -0.1	**0.005**
Stim:FMB × Side:left	-1.1	-1.4 - -0.7	**<0.001**
Stim:FMB × Side:right	-0.7	-1.0 - -0.4	**<0.001**
Stim:Waterfall × Side:both	-0.9	-1.4 - -0.4	**<0.001**
Stim:Waterfall × Side:left	-0.8	-1.3 - -0.4	**<0.001**
Stim:Waterfall × Side:right	-0.9	-1.3 - -0.4	**<0.001**
Random effects			
σ^2	36.2		
n_{Bat}	4		
Observations	20376		
Marginal R^2	0.01		

Note. CI = 95% confidence intervals around the predicted estimate. Positive estimates indicate bias towards the left side of the corridor, and negative estimates indicate bias towards the right side of the corridor, compared to results obtained in Silence. A value of 0 indicates no bias; that is, bats aimed their heads towards the midline. Statistically significant *p* values are bolded.

Discussion

For survival, animals must extract and act upon relevant information from a dynamic and complex sensory world. This can proceed by active, sequential sampling of the environment, by head or eye movements in visually dominant animals (Hayhoe & Ballard, 2005), or by adjusting timing of call emissions and sonar beam aim in echolocating bats and toothed whales (Surlykke et al., 2009; Tuninetti et al., 2021; Wisniewska et al., 2016). Research in active acoustic sensing has focused on understanding how animals separate signals of interest from background noise or clutter. But to do this, clutter must first be detected and then categorized as background (Bates et al., 2011). And, even background clutter may provide information needed to adjust behavior appropriately to the surrounding environment (Giepel et al., 2019).

In this experiment, we tasked bats to fly down a narrow, straight corridor surrounded by strong acoustic clutter that was 6-10 dB more intense on one side than the other. We attempted to increase the attentional demands of the task by challenging the flying bats with superimposed playbacks with different acoustic and presumed informational content (exemplars of two conspecific vocalizations and an abiotic sound). We expected that the bats would increase the numbers of calls they emitted and decrease the IPIs between their calls in response to playbacks of these exemplars as compared to the Silence condition. We expected bats to show a bias towards the side of the corridor with the most informative clutter, that is, the side leading to an open flight path (Tuninetti et al., 2021), but that this bias would vary in magnitude depending on the content of the exemplars and the side of the corridor from which they were broadcast.

Bats Dynamically Adjust Sonar Emissions When Flying in Tasks Demanding Attention

Big brown bats increase the numbers of echolocation calls they emit and decrease the IPIs between these calls in conditions of increasing task difficulty (Hiryu et al., 2010; Knowles et al., 2015; Kothari et al., 2014; Moss et al., 2006; Petrites et al., 2009; Sändig et al., 2014; Surlykke et al., 2009; Tuninetti et al., 2021; Wheeler et al., 2016). Bats foraging outdoors in clear, uncluttered surroundings emit single calls in a train, with IPIs gradually decreasing as they approach and then capture an insect target (the pursuit sequence; Simmons et al., 2001). In empty flight rooms or in surroundings with minimal acoustic clutter, IPIs are shorter, but calls are still likely to be emitted as ungrouped singles (Kothari et al., 2014; Moss et al., 2006). In contrast, when navigating through cluttered environments containing multiple ensonified objects, bats shorten IPIs still further, and also adopt a strategy of sonar sound grouping. In this strategy, individual calls are emitted in SSG groups of 2 to 6, with short IPIs between calls within a group and longer IPIs between these groups. This sonar sound grouping has been hypothesized to facilitate the task of isolating echoes from target objects against clutter, i.e., echoes from non-target, extraneous objects (Kothari et al., 2014; Petrites et al., 2009; Tuninetti et al., 2021; Wheeler et al., 2016).

The results of our experiment are consistent with this previous work in showing that, when confronted with playbacks of sounds broadcast against an already cluttered background, the bats significantly increased the numbers of calls they emitted and significantly decreased the IPIs between these calls, compared to their behavior in the Silence condition. Bats emitted more calls in response to broadcasts of the Echolocation and FMB exemplars than to the Waterfall exemplar, suggesting that they perceived the differences between these exemplars. All playbacks produced decreases in the IPIs between the bat's own calls, with the Echolocation exemplar producing shorter IPIs than the FMB exemplar, and the FMB producing shorter IPIs than the Waterfall exemplar. These data suggest that the bats differentiated between the playbacks, either in their acoustic content or in their informational content (i.e., signaling a biotic or an abiotic source; sound used for orientation/prey capture vs one used for communication).

It is possible that the bats' vocal responses were affected by the sound pressure levels of the playbacks. Even though all playbacks were broadcast at 0 dB RMS, the pulsed Echolocation and FMB exemplars had higher sound pressure levels than the continuous Waterfall exemplar. We emphasize,

however, that IPI differed significantly between Echolocation and FMB exemplars, which did not differ in sound pressure level. It is important to note that our stimuli were restricted to only one exemplar of each playback type. To ascertain more clearly whether bats can differentiate between Echolocation, FMB, and Waterfall sounds while in flight, or if acoustic compared to informational content is more important in driving their behavior, additional exemplar sounds within each category need to be tested.

We expected that bats would emit more calls as SSGs in playback conditions than they did in Silence. This expectation was not borne out in our data; instead, the most common SSG in all playback and Silence conditions was a doublet (SSG = 2). There were few instances of larger SSG groups, regardless of condition. This high proportion of doublets suggests that the creation of SSGs in our experiment was driven by the structure of the corridor and its surrounding clutter, rather than by the presence or absence of external acoustic stimuli. A high proportion of doublets was also observed in earlier experiments in which bats flew down corridors bordered by chains, both when the chain clutter on either flanking side was symmetric (Accomando et al., 2018; Tuninetti et al., 2021; Wheeler et al., 2016) and when it was asymmetric (Warnecke et al., 2016, 2018). It is possible that the tasks given to the bats in these experiments were not sufficiently difficult to induce the bat into producing more SSGs. Challenging bats with the more complex foraging tasks used in previous studies (e.g., Allen et al., 2021), combined with a more complex navigation task, might lead them to produce more complex SSGs. Still, we suggest that understanding the adjustments bats make in simpler navigation tasks is crucial for interpreting and modeling the plasticity of their behavior in more complex tasks.

Bats Shift Attention Towards More Informative Clutter

Big brown bats flying down corridors with symmetrical clutter on both sides, in the absence of superimposed playbacks, maintain a straight flight trajectory, with no significant side biases in head aim (Accomando et al., 2018; Knowles et al., 2015; Wheeler et al., 2016). In contrast, when flying down asymmetrical corridors, these bats alter their flight trajectories to steer away from the side with the denser clutter and towards the side with the sparser clutter (Warnecke et al., 2016, 2018). Those results suggest that bats perceive differences in the density of surrounding clutter (i.e., the number and spacing of echoes arriving from the left compared to the right) and use these differences to guide their attention.

Our results are consistent with those of Warnecke et al. (2016, 2018) in showing that big brown bats alter their head aim and flight trajectory to favor one side when flying down corridors with asymmetrical clutter. In all conditions in our experiment, including Silence, bats exhibited a bias in head aim towards the left (chains) side of the corridor. This left side bias decreased in response to playbacks of the FMB and Waterfall exemplars compared to the Echolocation exemplar, consistent with the hypothesis that bats differentiated the playbacks from the background clutter. Loudspeaker side did not significantly influence head aim bias, suggesting that none of the playbacks were aversive. This result also suggests that the background clutter was more salient to the bat than were the source of the playbacks.

Echoes from the left side chains were discrete and sparsely spaced at nominal 1.2 ms intervals (for their 20-cm spacing), even though each individually was 6-10 dB more intense than the fully continuous clutter that extended along the entire wall on the right. We suggest that the bats biased their head aim towards the sparser clutter from the chains, because this clutter was spatially interspersed with open intervals that signaled the presence of an open flight path beyond the chains. The bats potentially could escape from the corridor through the left side, by flying between the chains. The right   wall offered no such escape path. Aiming their heads to the left allows the bats to gather information about the open area lying beyond the chains. This interpretation is consistent with the data of Tuninetti et al. (2021), who showed that corridor flights requiring sharp turns are performed at a higher rate of success when the turn leads to an open flight path as opposed to a closed wall. We observed that, upon reaching the end of corridor, bats sometimes flew out towards the left instead of landing on the wall; they never flew towards the right. One limitation of our work is that we were unable to vary the nature of the corridor side leading to the open flight path. Future work in which corridor structure can be manipulated from flight-to-flight

will be important in determining how bats can differentiate asymmetric clutter to guide their flight trajectory.

Overall, our data suggest that bats perceive playbacks of intense sounds superimposed upon a cluttered background and use the information in both the background and the playbacks to adjust their audio-motor behaviors without impairing flight performance. Moreover, we suggest that the spectral and informational content of surrounding sound sources are important variables for understanding the impacts of noise on animal behavior.

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