Science AAAS

Supplementary Materials for

Bats perceptually weight prey cues across sensory systems when hunting in noise

D. G. E. Gomes, R. A. Page, I. Geipel, R. C. Taylor, M. J. Ryan, W. Halfwerk

.

correspondence to: w.h.halfwerk@vu.nl

This PDF file includes:

Materials and Methods

Study system

Fringe-lipped bats (*Trachops cirrhosus*; N = 12) were captured in Soberanía National Park, Panamá, between January and May of 2015 via targeted mist-netting or hand-netting from of known roosts. Bats were given one night to acclimatize in a large outdoor flight cage (5x5x2.5 m) in Gamboa, Panamá. Bats were then trained for one night to attack our frog models (using mating sound of the frog only) and all bats readily attacked the models after two nights in captivity. We rewarded both models on every third trial with baited fish (rewarded did not differ in any response variable compared to unrewarded trials; see also [*21*] for more details and training and reward scheme). Each bat was injected with a subcutaneous passive integrative transponder (Trovan, Ltd) prior to release at the capture site to prevent retesting of individuals. All necessary permits were obtained from the Government of Panamá, and all research complied with the Institutional Animal Care and Use Committee (IACUC) protocols from the Smithsonian Tropical Research Institute (STRI).

Stimulus playback

We gave individuals a choice to attack one of two frog models that either emitted a multimodal cue (mating sound plus a dynamically inflating and deflating vocal sac) or a control cue (mating sound plus static vocal sac) to the bat. Each frog model was placed on the centre of a smooth-surfaced Plexiglas circular platform (diameter = 33 cm) that echo-acoustically mimics a water surface (Fig. S1 (*31*)). Speakers (Tymphany Peerless, 6 cm; powered by a Pyle PCA2 Stereo Power Amplifier 2 X 40W) were placed directly below each platform to playback a synthetic túngara frog (Physalaemus [=*Engystomops] pustulosus)* call (whine plus one chuck set to 76 dB at 1 m, C weighting, set to Max and Fast; Radioshack SPL meter). Holes were drilled through the Plexiglas to allow sound transfer. An inflatable silicon balloon was placed in front of the frog models (mimicking a vocal sac) and an air tube ran under the Plexiglas and out the backside of the platform. The tube from one model (hereafter referred to as the multimodal model) was connected to a gas-relay station in an adjacent room where the experimenters sat. The gas-relay station forced air into the tube, thereby inflating the silicon balloon in front of the robofrog model. The control model balloon was not inflated.

We broadcast both multimodal and control stimuli simultaneously at a rate of 0.5 calls per second using a laptop (Dell Latitude E4300) connected to a multi-channel setup (Edirol FA-101). We used three channels, two for the speakers and one for the gas relay station that was driven by a 19 kHz pure tone. The silicon vocal sac inflated from 12 mm (standing diameter) to 25 mm (maximum inflation) with call onset and deflated with call offset 400 ms later.

In previous experiments we used surgical catheters that produced a broad-band sound in the range of 10 - 70 kHz during inflation (see also (*21*)). To avoid these unwanted acoustic by-products created by the inflation of the catheters, we developed a new vocal sac using silicon for our experiments. We made recordings of our new dynamically inflating and deflating vocal sac in a sound attenuating chamber at a distance of 20 cm with an ultrasonic microphone (microphone capsule CM16, CMPA preamplifier unit, Avisoft Bioacoustics, Berlin, Germany) to confirm that no sound was produced in the detectable range (or at least not above the 18 dB SPL noise floor of our microphone).

Experimental design

Bats were given a choice to attack either multimodal or control platform in the presence and absence of different types of acoustic noise as well as clutter treatments. Experimental trials started with bats on their perch in a 60 cm x 60 cm roost made out of black cloth placed in a corner of the flight cage. Our two platforms were located 2.4 m, 3.2 m, or 4 m from this roost (Fig. S1) and were always placed 80 cm from each other (on centre). There were 9 locations for each platform, offering a possibility of 6-paired positions for the choice test (i.e. 1 and 2, 2 and 3…). The multimodal platform was pseudo-randomly placed on the right or left side position to control for side-biases.

We tested bat attack behavior during three acoustic noise treatments: 'masking noise' consisting of band-passed white noise in the range of 0.1 - 4.0 kHz set to 73 dB SPL (C- weighted, set to Max and Fast, at 1 m), 'non-masking noise' in the range of 4.0-8.0 kHz set to 73 dB and a control condition (ambient noise in our outdoor flight cage ranging from 55-60 dB SPL (C) with most energy in the 4.0 -10 kHz frequency range). White noise was generated and filtered in the program Audacity to create both band-passed noise treatments (using a 48 dB roll-off per octave). Noise playback was generated from a desktop computer (Dell Optiplex 790), amplified (Pyle PCA2 Stereo Power Amplifier, 2 X 40W), and produced through a speaker (Tymphany Peerless, 6 cm) that was attached to the ceiling (3 m above the platform) and faced downward over the centre of the platform positions. The experimental noise level was measured as 69 dB in the middle of the platform array (at the platform level) and as 66 dB at the perch.

Additionally we tested bat attack behavior in response to a clutter treatment. Objects surrounding a target also return echoes and this so-called background clutter is known to interfere with detection and/or processing of echo-acoustic target cues. We used dried leaves surrounding our target frog models to create additional background clutter (here after referred to as 'clutter treatment'). In half of the trials (clutter treatment) leaves were attached with double-sided tape to a Plexiglas ring (inside diameter 13.5 cm, outside diameter 33 cm) and in the other half (no clutter treatment) no leaves were attached to the Plexiglas (Fig. S1). Bats were tested on the full-factorial combination of acoustic noise and clutter treatment, resulting in six trials with different treatment combinations. Each trial type was repeated six times per individual and order of treatments was randomized and balanced across blocks of 6 trials. Every third trial was rewarded with small pieces of baitfish on top of both models to ensure foraging motivation. Noise playback started five minutes prior to stimulus playback to avoid startle responses by bats. Playback of control and multimodal cues lasted 1 minute, or was switched off directly after a bat made an attack.

Behavioral recordings

Attack sequences were recorded onto a desktop pc with a 4-camera home security system (Conrad) and three infrared sensitive cameras (2 CMOS 6 mm cameras fixed to the flight cage walls, 1 Sony CCD pinhole, 2.8 mm camera fixed to the ceiling providing top view over the platform array). An observer (blind to the hypotheses) scored attacks from the videos. We defined an attack preference for control or multimodal model as either retrieval of baitfish (rewarded trials), bite of the frog model (unrewarded trials), landing on or hovering over the platform. Bats almost always landed on a platform or attempted to bite a model frog during unrewarded trials and always attacked the side over which they hovered during these trials. Trials in which no clear attack preference could be scored (e.g. when bats circled around the collection of platforms or flew in figure eights around both platforms) were left out the analysis and repeated (only 14 trials out of 446). In addition to attack preference we scored flight time and attack latencies from the videos. Attack latency was defined as the time taken for a bat to leave the perch after the onset of stimulus playback and flight time was defined as the time taken between the onset of flight and making an attack attempt.

We recorded the echolocation behavior of six bats during the experiment with a condenser microphone (microphone capsule CM16, CMPA preamplifier unit, Avisoft Bioacoustics, Berlin, Germany) and digitized using a real time ultrasound acquisition board (UltraSoundGate 116, Avisoft Bioacoustics, Germany; 500 kHz sampling rate, 16 bit resolution). The microphone was always placed in between the two platforms facing the perch to record calls emitted by the bat in the direction of the target. We aligned these recordings with videos using the frog stimulus and the sound of bats hitting the target as time reference points. We identified the time point (with a resolution of ~ 0.1 s) of a bat taking flight for each recording and selected 1 s of recording prior to this time point. From this section we counted all the echolocation calls and randomly selected up to three calls per experimental trial for further analyzes. We analyzed these calls in the program Saslab Pro (version 5.2, Avisoft Bioacoustics, Glienicke, Germany). Call peak frequency (frequency of the loudest peak, in kHz) was measured using powerspectrum plots in Avisoft. Call duration (in ms) was obtained from the amplitude envelope, using an automatic threshold of -15 dB.

<u>Data analyses</u>

We assessed the effect of acoustic noise and clutter treatment as well as their interaction on bat attack behavior and preference for the multimodal signal in the program R v.2.15.1 (*32*). We constructed linear mixed models using the package *lme4.* We modelled random variation in the effect of explanatory parameters per individual bat by fitting random slopes as well as intercepts (*33*). We created models containing covariates, such as distance between perch and platforms and trial number as fixed effects and selected the optimal null model using Aikaike's Information Criterion corrected for small sample sizes (AICc) (*33*).

The optimal null models of echolocation call parameters all included distance as fixed effect, whereas models on latency and flight duration only include bat identity as random effect. We used likelihood ratio tests (using ML) between null models and models containing noise treatment, clutter treatment and their interaction as fixed effects (*33*). We followed up on significant treatment effects using post-hoc independent contrasts. The number of attacks on the multimodal model was compared with the number of attacks on the control model using the binding function of generalized linear mixed models, with a binomial error structure and a probit-link function and bat ID as random intercept. We compared this attack preference null model with models containing noise treatment, clutter treatment and their interaction as fixed effect. We followed up on significant fixed effects by testing for a significant positive intercept per treatment group.

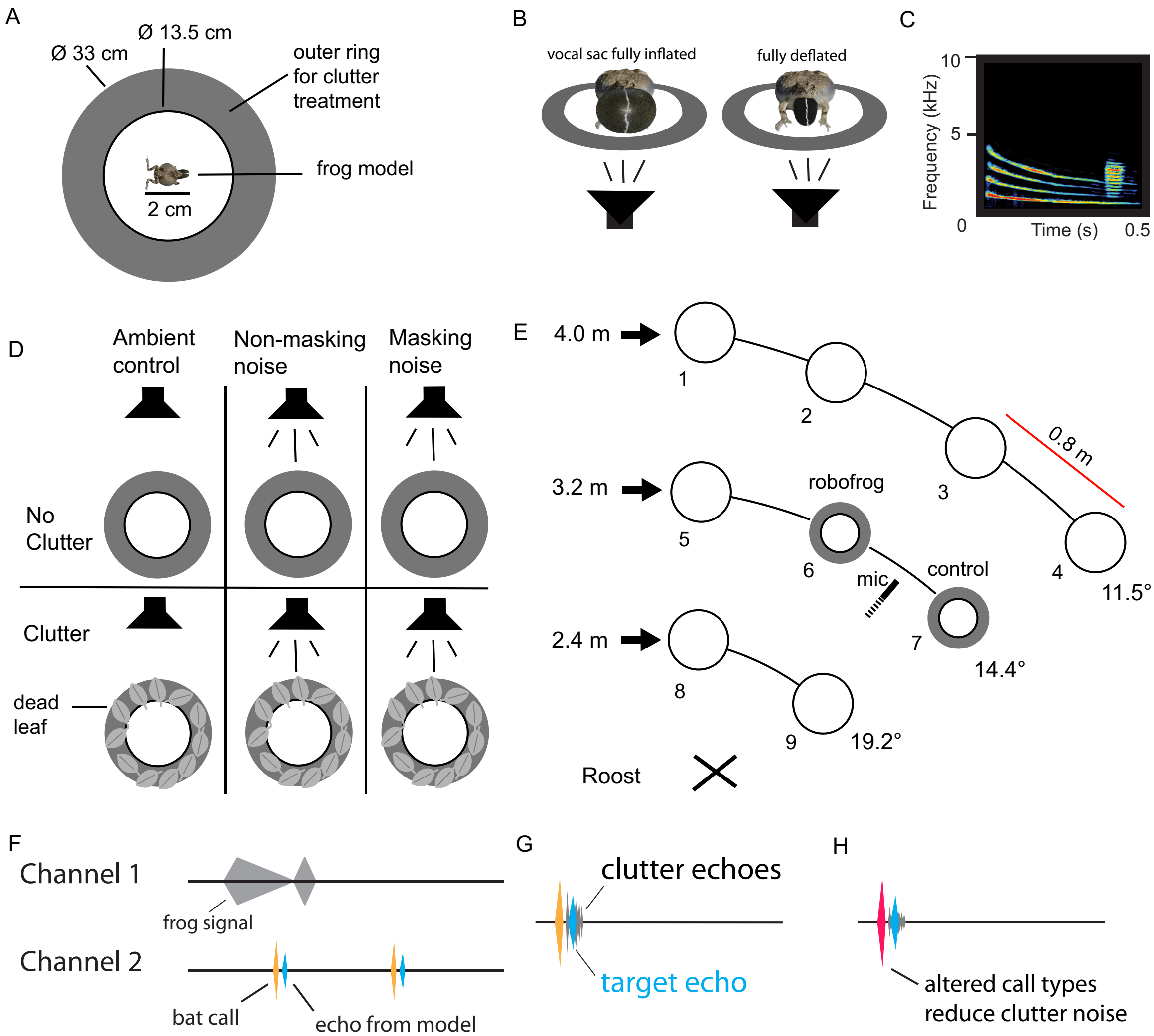

Fig. S1. Experimental setup and clutter treatment expectations. (**A**) Frog models were placed on an elevated platform with speakers playing the frog sound placed underneath the models. The platform was covered with a Plexiglas sheet. (**B**) Front view showing speaker placed underneath elevated platforms to broadcast frog sound at a rate of 0.5 calls/sec. The vocal sac of one frog model was actively inflated and deflated in sync with sound playback. (**C**) Spectrogram showing the main frequency range of the frog's mating sound (**D**) Full-factorial experimental design in which bats were tested on three different noise treatments and two different clutter treatments. For the clutter treatment we glued leaves collected from the forest floor onto an additional Plexiglas ring surrounding the frog models. (**E**) We placed the platforms on two of nine possible locations. The two platforms were always placed adjacent to each other at the same distance from the bat's perch. Locations were defined by three different distance categories (in relation to a reference point underneath the perch on the floor). We kept the distance between platforms constant (80 cm), which consequently led to differences in viewing angles from the perch per distance category. A microphone was placed between platforms to record echolocation calls made on the perch. (**F-H**) Expected bat responses to clutter treatment. (**F**) Bats can either use the sound of the frog as a cue (passive listening, channel 1) or the echo returning from the frog's moving vocal sac (active listening, channel 2) to locate their prey. (**G**) Leaves surrounding the frog model create additional echoes (clutter) that need to be processed by the bats echolocation system. Bats may either ignore echolocation cues and instead attend more to the frog's sound, or (**H**) alter their call design.

Dependent variable	fixed effects	parameter estimate	s.e.	χ^2	df	p-value
Attack latency	Intercept	1.87	0.52			
(5.5 s ± 8.5)	Noise treatment			22.09	2	< 0.001
	masking	2.87	0.60			
	non-masking	0.93	0.11			
	Clutter treatment	0.98	0.07	0.094	1	0.76
	distance	1.05	0.06			
Flight duration	Intercept	2.58	0.28			
(4.1 s ± 3.0)	Noise treatment			0.24	2	0.89
	masking	2.68	0.09			
	non-masking	2.68	0.09			
	Clutter treatment	4.29	0.43	17.04	1	< 0.001
	distance	2.75	0.06			
Attack preference	Intercept	0.48	0.04			
(for multimodal)	Noise treatment			7.63	2	0.022
	masking	0.6	0.04			
	non-masking	0.54	0.05			
	Clutter treatment	0.52	0.03	0.60	1	0.43
Attack latency	Intercept	2.25	0.41			
(post-hoc test)	masking	3.86	0.99			
	non-masking	-0.87	0.13			
	MM	-0.97	0.13			
	Noise | MM			11.82	2	0.003
	masking | MM	-0.64	0.12			
	non-masking | MM	1.14	0.22			

Table S1. Statistical details for the effect of noise and clutter treatment on bat attack behavior. Parameter estimates were calculated from full models, always including noise and clutter treatment as fixed effects. The distance between the perch and the platform containing both frog models had a significant effect on attack latency and flight duration and was therefore included in initial null models. The post-hoc analysis on the effect of robofrog choice on attack latencies only included noise treatment, robofrog choice and the interaction as fixed effects. MM refers to attack on the multimodal frog model. See supplementary methods for more details.