

RESEARCH ARTICLE

Adaptive sonar call timing supports target tracking in echolocating bats

Ninad B. Kothari^{1,*}, Melville J. Wohlgeuth¹ and Cynthia F. Moss^{1,2,3,4}

ABSTRACT

Echolocating bats dynamically adapt the features of their sonar calls as they approach obstacles and track targets. As insectivorous bats forage, they increase sonar call rate with decreasing prey distance, and often embedded in bat insect approach sequences are clusters of sonar sounds, termed sonar sound groups (SSGs). The bat's production of SSGs has been observed in both field and laboratory conditions, and is hypothesized to sharpen spatiotemporal sonar resolution. When insectivorous bats hunt, they may encounter erratically moving prey, which increases the demands on the bat's sonar imaging system. Here, we studied the bat's adaptive vocal behavior in an experimentally controlled insect-tracking task, allowing us to manipulate the predictability of target trajectories and measure the prevalence of SSGs. With this system, we trained bats to remain stationary on a platform and track a moving prey item, whose trajectory was programmed either to approach the bat, or to move back and forth, before arriving at the bat. We manipulated target motion predictability by varying the order in which different target trajectories were presented to the bats. During all trials, we recorded the bat's sonar calls and later analysed the incidence of SSG production during the different target tracking conditions. Our results demonstrate that bats increase the production of SSGs when target unpredictability increases, and decrease the production of SSGs when target motion predictability increases. Furthermore, bats produce the same number of sonar vocalizations irrespective of the target motion predictability, indicating that the animal's temporal clustering of sonar call sequences to produce SSGs is purposeful, and therefore involves sensorimotor planning.

KEY WORDS: *Eptesicus fuscus*, Sonar sound groups, Auditory localization, Sonar ranging

INTRODUCTION

Echolocating bats navigate and capture prey by producing ultrasonic signals and listening to echo returns from objects in the environment (Griffin, 1958). Fundamental to echolocation is the bat's dynamic modification of sonar call parameters, such as pulse duration, pulse intensity, pulse interval and spectral content, in response to information carried by returning echoes (Griffin et al., 1960;

Simmons, 1979; Moss and Surlykke, 2010). In their natural habitats, bats often pursue evasive and unpredictably moving insects in cluttered conditions, further complicating the task of localizing and intercepting prey (Kalko and Schnitzler, 1989; Schnitzler and Kalko, 2001). We hypothesize that the bat's adaptive temporal patterning of sonar sounds sharpens its localization accuracy and allows it to integrate information across sonar sequences to track unpredictably moving targets (Moss and Surlykke, 2010; Lewicki et al., 2014).

Insectivorous bats reduce the interval between sonar calls as the distance to prey decreases (Simmons et al., 1979; Kalko and Schnitzler, 1989; Moss and Surlykke, 2001), and embedded in foraging call sequences are sonar sound groups (SSGs), which are defined as clusters of echolocation signals at comparatively short and stable pulse intervals, flanked by signals at longer intervals (Moss and Surlykke, 2001; Moss et al., 2006; Kothari et al., 2014). The production of SSGs is an adaptive sonar behavior that has been reported in both laboratory and field studies of bat echolocation, in free-flying animals (Surlykke and Moss, 2000; Moss and Surlykke, 2001; Moss et al., 2006; Petrites et al., 2009; Kothari et al., 2014; Sändig et al., 2014; Falk et al., 2015; Wheeler et al., 2016) and those tracking moving prey from a stationary position (Aytekin et al., 2010; Kothari et al., 2014). Past studies have shown that bats temporally cluster echolocation calls to produce SSGs when they are engaged in tasks that require higher spatiotemporal resolution. For example, in a laboratory study, Moss et al. (2006) reported that big brown bats increased the production of SSGs when they captured insects in the vicinity of clutter; and similarly, Falk et al. (2014) found that this species produced more SSGs as they foraged in an artificial forest, compared with an open room. Petrites et al. (2009) also reported that bats increased the production of SSGs when navigating in highly cluttered environments. A related finding, reported by Sändig et al. (2014), showed that bats performing a wire-avoidance task increased the production of SSGs with increasing task difficulty. In a more recent study, Wheeler et al. (2016) reported that big brown bats not only increased the number of SSGs, but also the number of sonar vocalizations contained in each SSG, as they encountered greater clutter along their flight path. These observations support the hypothesis that the bat's production of SSGs serves to improve its spatiotemporal resolution of objects (targets or obstacles) in the environment.

In the natural environment, bats frequently intercept free-flying, erratically moving prey (Roeder, 1962, 1967), a task that requires high spatiotemporal sonar resolution and the integration of echoes over time to predict future target location. Unpredictable insect flight trajectories create uncertainty about prey location, and it has been shown that bats tracking free-flying insect prey produce SSGs (Triblehorn et al., 2008; Ghose et al., 2009), but past studies have not directly studied the bat's sonar behavior under conditions where target motion predictability is systematically manipulated.

To rigorously investigate if SSGs are used by bats to localize unpredictably moving targets, we designed an experiment that

¹Department of Psychological & Brain Sciences, Krieger School of Arts and Sciences, Johns Hopkins University, Baltimore, MD 21218, USA. ²The Solomon H. Snyder Department of Neuroscience, School of Medicine, Johns Hopkins University, Baltimore, MD 21205, USA. ³Department of Mechanical Engineering, Whiting School of Engineering, Johns Hopkins University, Baltimore, MD 21218, USA. ⁴Behavioral Biology Program Chair, Johns Hopkins University, Baltimore, MD 21218, USA.

*Author for correspondence (ninadbkothari@jhu.edu)

 N.B.K., 0000-0001-5543-6459; M.J.W., 0000-0003-4779-4154; C.F.M., 0000-0001-6916-0000

engages an animal in the insect-tracking task, while also permitting precise control over the relative bat-target position and systematic manipulation of the predictability in target trajectory over successive trials. We hypothesize that bats increase the production of SSGs with increasing unpredictability of the target's motion.

MATERIALS AND METHODS

Experimental set-up and animal training

Four big brown bats, *Eptesicus fuscus* (Palisot de Beauvois 1796), were trained to rest on a platform and track a moving prey item (mealworm). The experimental set-up is described in detail in Kothari et al. (2014) and is presented briefly here. A tethered insect food reward was suspended from a rectangular loop of fishing line, and its motion was controlled using a rotary servomotor (Aerotech BMS60 brushless, slotless rotary servomotor attached to an Ensemble MP10 motor controller; Pittsburgh, PA, USA), interfaced with custom MATLAB software (2012a) that controlled the velocity, acceleration, deceleration and distance the target traveled (Fig. 1A). Experiments were carried out under low-level, long-wavelength

illumination that precluded the bat's use of vision (Hope and Bhatnagar, 1979). This method engaged the bat in naturalistic sonar tracking behavior, and it also allowed the experimenter precise control and repeatability over the relative motion between the bat and target, which is not possible in free-flight experiments.

Bat sonar vocalizations were recorded using two Ultra Sound Advice UM3 microphones (London, UK; see 'Pulse microphone' and 'Echo microphone' in Fig. 1A) and were digitized using a National Instruments (NI) A/D PCIe card interfaced with MATLAB (2012a). The triggering of the Aerotech servomotors and the start of the microphone recording were synchronized using a single TTL pulse generated via the MATLAB–NI interface. Details regarding the initial training paradigm are also described in Kothari et al. (2014).

Briefly, individual bats were trained on single-target motion tracking, wherein the target started at a distance of 2.5 m, accelerated at a rate of 7 m s^{-2} , traveled a distance of 2 m with constant velocity of 4 m s^{-1} , mimicking the approximate flight velocity of a bat during the approach phase of insect capture (Hayward and Davis, 1964), and then decelerated at a rate of

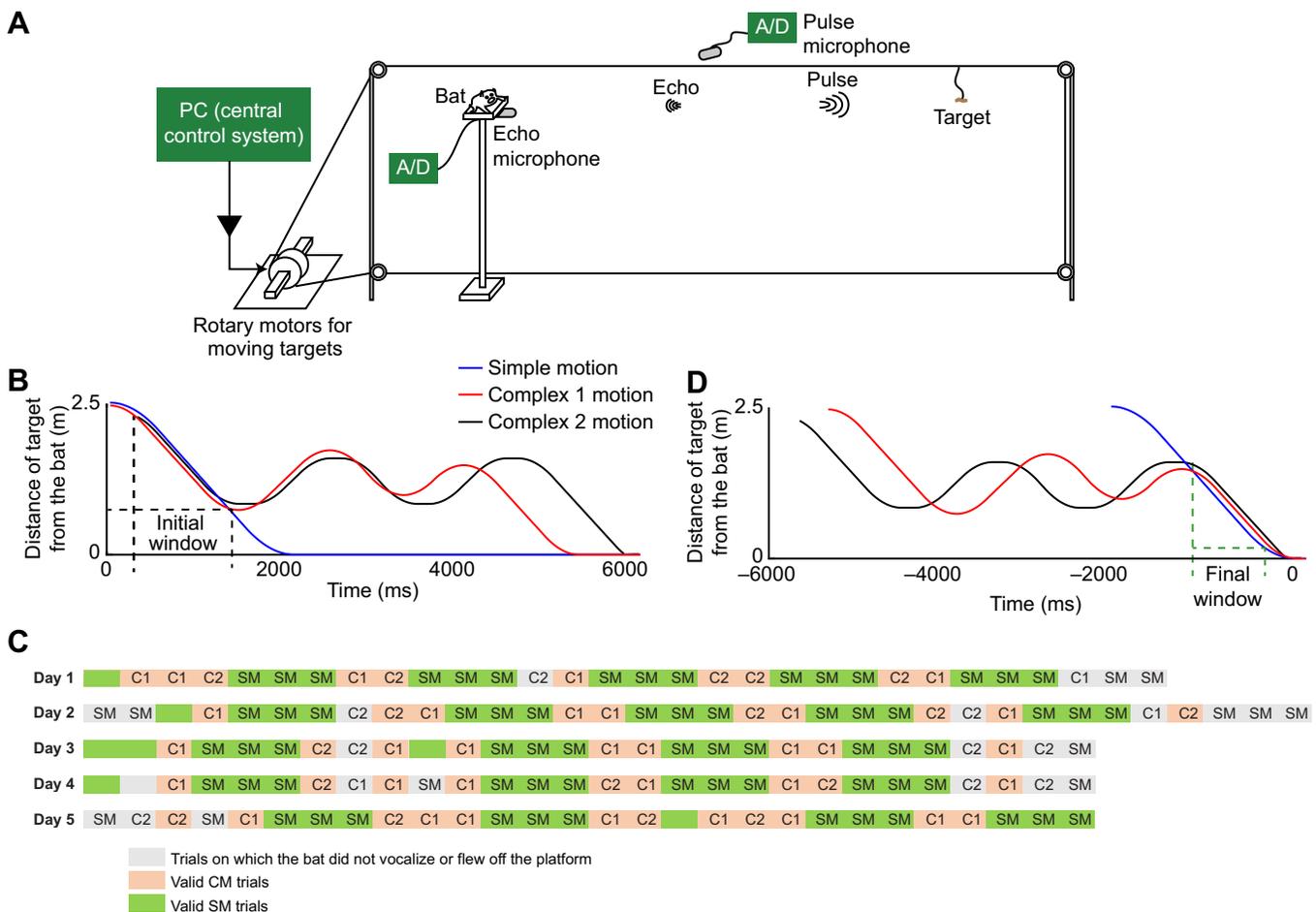


Fig. 1. Experimental design. (A) Bats (*Eptesicus fuscus*) were trained to rest on a platform and track an approaching target (shown at the far end) suspended from a loop of monofilament wire. The wire was connected to a motor via a pulley system. Microphones connected to A/D converters (shown in green) were used to collect sonar calls of the bat. (B) Displacement of the target, with the x-axis denoting the time of each trial where zero indicates the start of the trial. The y-axis is the distance of the target from the bat, so when the target starts it is 2.5 m from the bat. The simple motion (SM) and complex motion (CM1 and CM2) target motions are shown in blue, red and black, respectively. Each target motion has been slightly displaced to clearly display the overlap of the motions in the initial phase. (C) All trial sequences from all recorded sessions of Bat C. Green and red indicate valid SM and CM trials, respectively. Gray indicates trials when the bat did not vocalize or flew off the platform. (D) To demonstrate that the final approach motions of the targets for each of the SM, CM1 and CM2 are identical, the motion sequences have been redrawn according to when the target reached the bat. Here, the x-axis is the trial time, with zero indicating the time of arrival of the target at the bat. The y-axis is the target distance from the bat. Here again, the target motions have been displaced to clearly indicate the overlap of the final approach phase (indicated by the two green lines) of the target.

5 m s^{-2} . The displacement and velocity with respect to the stationary bat are shown in Fig. 1B (blue trace). We refer to this motion as simple motion (SM). Additionally, we introduced catch trials (on about 10% of trials) during the training to the bat. In these catch trials, the target was suspended at the same initial position, but on another tether that was not connected to the motor system. When we initiated a catch trial, the motor was active, but the target did not move. These catch trials were added to decouple the sounds of the motors from the motion of the target. On such trials, bats either stop echolocating, or did not produce any pulse interval changes in their calls, presumably because they received feedback that the target was not moving. When data collection began, bats encountered new target motion trajectories, as detailed below.

Experimental design

Once the bat reliably tracked the tethered insect following the SM trajectory, two novel types of target motion trajectories were introduced to the bat on the day of the experiment. We refer to these target motion trajectories as complex motion 1 and 2 (CM1 and CM2, respectively). In the novel complex motion trajectories the target first moved towards the bat, after which it oscillated back and forth, before finally reaching the bat. The target displacement and velocities relative to the stationary bat are shown in Fig. 1B (CM1: red; CM2: black). The simple and two complex motion trajectories were designed with the following criteria.

Initial target motion phase (initial time window)

The initial phase of target motion was comparable across motion trajectory conditions. This phase is marked by the two dashed black lines in Fig. 1B. After the initial phase, the target motion paths diverged and followed pre-determined trajectories, with the target approaching the bats directly in the SM trials, while oscillating back and forth before arriving at the bat in the CM trials. By presenting the same initial trajectories across target motion conditions, the bat's echolocation behavior can be directly compared at the start of each trial for SM and CM trajectories.

Trials were presented to the bat in a random order. We manipulated predictability of target motion by repeating consecutively three SM trials at randomly chosen points during each session. Bats were not cued when the sequence of SM trials would start or end. We hypothesized that during the random presentation of trials, changes in target trajectory would evoke an increased production of SSGs. Furthermore, consecutive repetition of the SM trials, target motions on which bats were already familiar, would evoke fewer SSGs as the target trajectories would be more predictable. Figure 1C shows 5 days of trial sequences that were presented to 'Bat C'.

Trials were presented in a series of 20–30 per day (see Fig. 1C for trial sequences presented to Bat C). Each consecutive trial was generally started as soon as the bat intercepted and consumed the mealworm reward. It should be noted that bats were given no cue regarding which trial would be presented (SM, CM1 or CM2). Furthermore, as the initial target trajectory was similar for each of the motion paths, bats would only be able to identify the trial type after the target traveled towards the bat for approximately 1400 ms, referred to as the initial window (see Fig. 1B).

Final target motion phase (final time window)

Figure 1D shows the motion trajectories aligned with respect to when the target reached the bat. As illustrated in Fig. 1D, the final motion phase for each of the target trajectories was also designed to be comparable across trials. This 'target arrival window' is marked

by the two dashed green lines in Fig. 1D. By examining the target arrival window, we could analyse how the bat's sonar behavior changed as its familiarity with the complex trajectories (CM1 and CM2) increased over a period of days.

Analysis techniques

The digitized sonar vocalizations were analysed and identified using custom-written MATLAB (2012a) routines. Once the sonar calls were identified using the automatic routine, they were manually verified and corrected for errors. Call parameters such as pulse interval (PI), pulse duration (PD) and pulse onset (PO) were computed from the identified and corrected calls. Measures of start and end call frequency, sweep rate and bandwidth were not computed, as the frequency response of microphones was not calibrated.

SSGs were identified according to the following criteria: two or more vocalizations flanked by calls with larger PI at the ends (a minimum of 1.2 times larger PI). When three or more calls occurred within a SSG, a PI stability criterion was also applied: PI stability with 5% tolerance with respect to the mean PI of the SSG (Moss et al., 2006; Kothari et al., 2014). Figure 2 shows an example trial sequence CM–SM1–SM2–SM3–CM–CM, with dashed black lines highlighting the initial time window (identical motion phase); the trace in blue is the raw audio trace of the bat's vocalizations, and SSGs are identified with red horizontal lines.

RESULTS

Four big brown bats (*E. fuscus*) were trained to rest on a platform and track an insect reward tethered from a fishing line and delivered via a pulley system to the bat, as shown in Fig. 1A. Once the bats reliably tracked the food reward in the simple-motion target trajectory condition, they were introduced to the two novel complex motion (CM1 and CM2) target trajectories on the day of the experiment. Data were collected from Bats A and C, for 5 days, and from Bats B and D, for 6 days.

Bats increased the production of sonar sound groups when the presentation of target trajectories changed across trials, but they produced the same average number of calls. Figure 2 shows an example trial sequence when a bat was presented with a sequence of CM1–SM1–SM2–SM3–CM1–CM2 target trajectories. By counting the number of SSGs during the initial window of the SM1, SM2 and SM3 trial sequence, we can determine the effect of increasing target motion predictability upon the rate of SSG production. Figure 2 illustrates that the bat reduced the production of SSGs (within the initial target motion phase, indicated by two dashed black lines, see 'Materials and Methods') as the SM trajectory was presented repeatedly to the bat over consecutive trials. The data show that as the SM trajectory was repeated, and the predictability of the target's trajectory over trials increased, the bat reduced the number of SSGs (shown in red). Furthermore, the bat showed reduced SSG production for the CM2 trial that followed a sequence of three SM trials, followed by an increase in SSGs on the subsequent CM1 trial.

To quantify changes in the production of SSGs across trials following a CM–SM1–SM2–SM3 sequence, Fig. 3 shows a comparison of the mean number of SSGs produced in each of these trials. Mean and s.e.m. values for all bats are summarized in Table 1. A one-way repeated measures ANOVA (MATLAB procedure 'anova1') compared number of SSGs, within the initial time window (indicated by the dashed black lines in Fig. 2), across all CM–SM1–SM2–SM3 trial sequences. All four bats showed a main effect of trajectory condition on the number of SSGs. As the

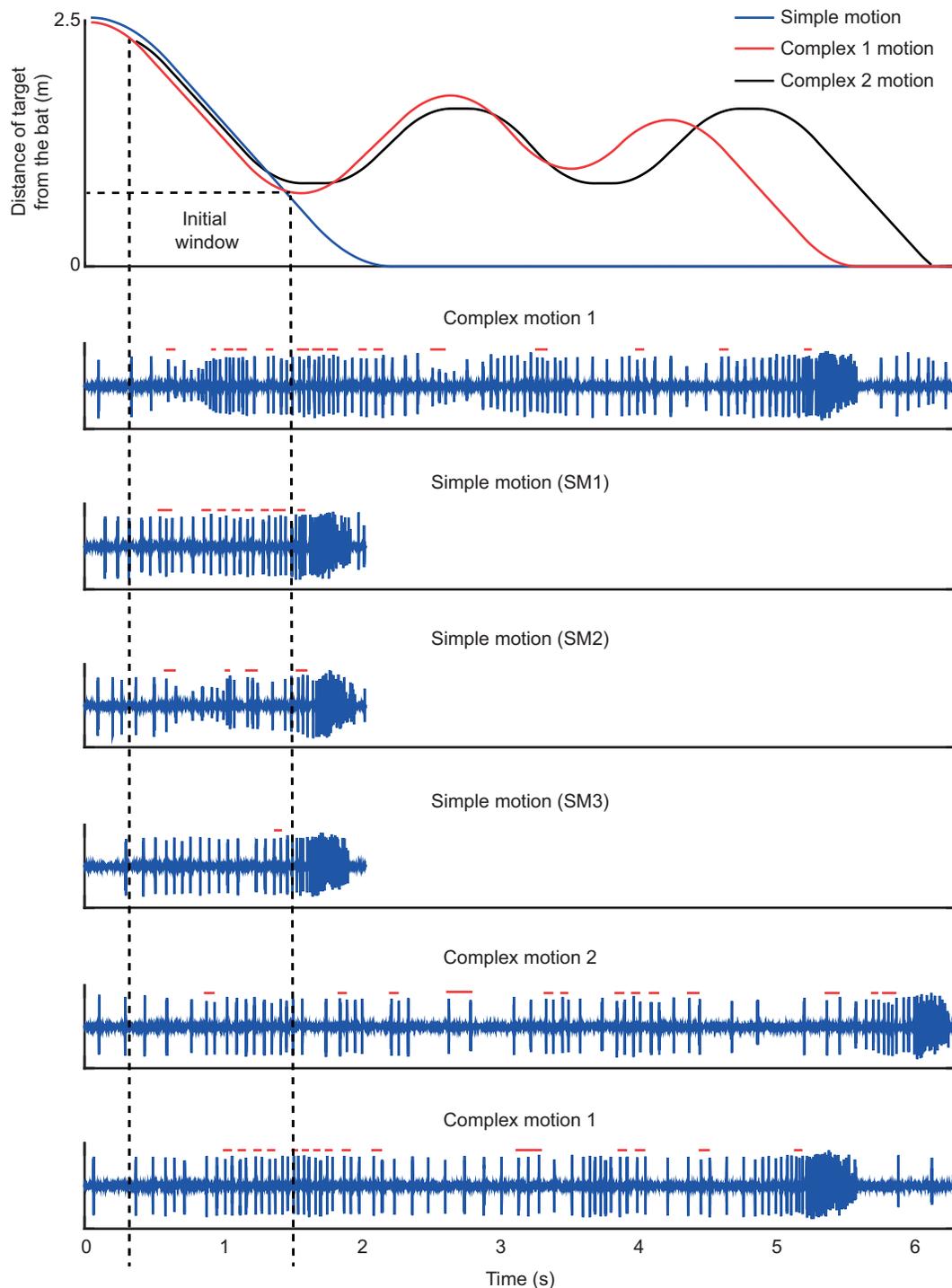


Fig. 2. Example trial sequence. The top panel shows the target displacement for each motion (simple motion, SM; complex motion, CM1 and CM2) presented to the bat. In the SM target motion trajectory, the target starts at 2.5 m from the bat and travels with uniform velocity to the bat. In the CM trajectories, the target first moved towards the bat, after which it oscillated back and forth, before finally reaching the bat. The x-axis shows time, and the y-axis shows the distance of the target from the bat. The black dashed lines extending throughout the figure indicate the initial time window of analysis where the target motions for each motion trajectory were nearly identical. The panels below show an example trial sequence. The raw audio recording at the microphone is shown in blue, with sonar sound groups (SSGs) indicated in red. The trial sequence in this example is CM1–SM1–SM2–SM3–CM2–CM1. It can be seen that as the predictability in target motion decreased (down and across the repeated SM motions), bats reduced the production of SSGs (red horizontal lines) within the initial time window. The number of SSGs in the following CM2 trial was also reduced, after which, in the following CM1 motion, the bat produced more SSGs due to increased target motion unpredictability.

target trajectory repeated over sequential presentation of SM trials, the mean number of SSGs decreased ($P < 10^{-4}$), indicating that bats reduce the number of SSGs as the predictability in target trajectory

increased from SM1 to SM3. *Post hoc* comparisons were carried out between CM–SM1, CM–SM3 and SM1–SM3 using the Holm–Bonferroni method, confirming that the bats significantly reduced

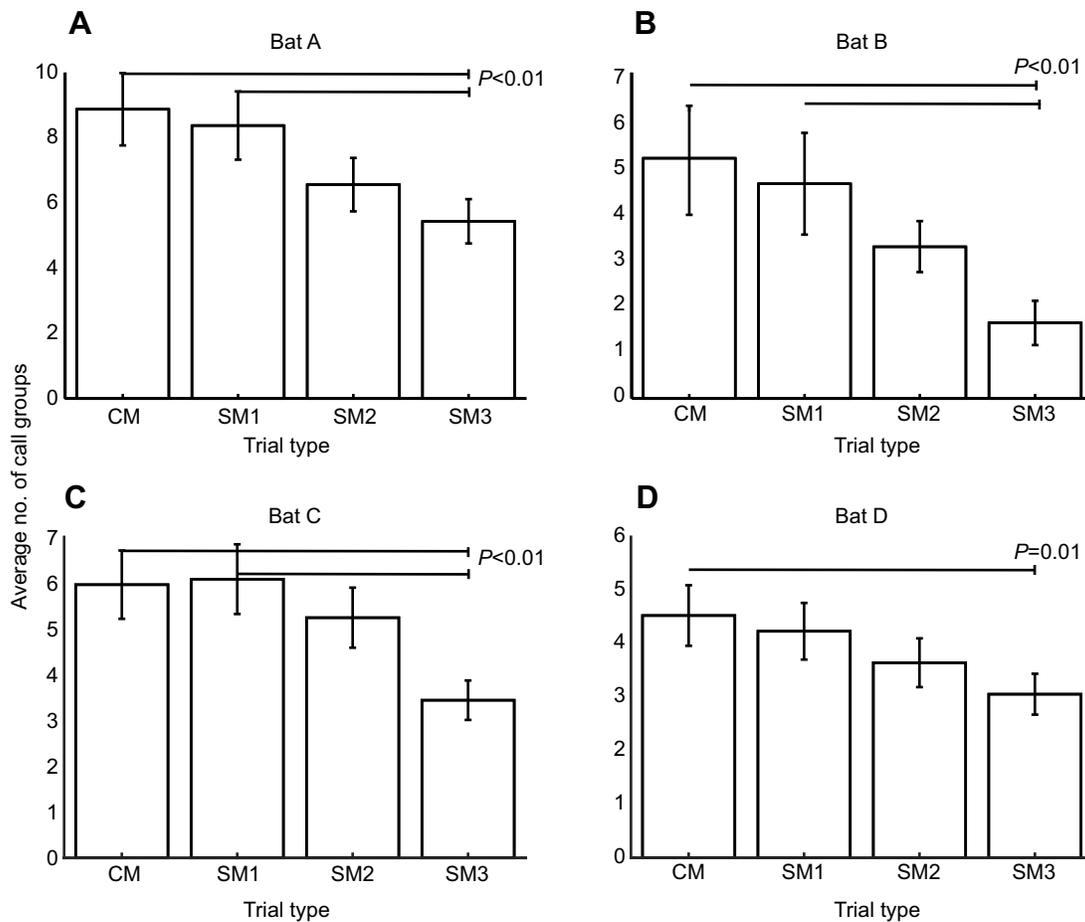


Fig. 3. Bats reduce the production of sonar sound groups as target trajectory becomes more predictable. (A–D) Average number of SSGs produced by all four bats when presented with the unpredictable complex target motion (CM1 or CM2) followed by more predictable simple target motion (SM1, SM2 and SM3). The error bars indicate s.e.m.

the number of SSGs between CM–SM3 and SM1–SM3 ($P < 0.001$ for Bats A, B and C and $P < 0.05$ for Bat D).

To investigate if changes in the bat's SSG production depended on repeating any target trajectory (SM or CM), we also analysed the times when CM trials were repeated consecutively. Here CM could be either CM1 or CM2. We observed no statistical differences in SSGs produced by bats presented with the trial sequence SM–CM–CM–CM (see Fig. 4A). Here, data have been combined across bats by normalizing the number of SSGs produced in each sequence of SM–CM–CM–CM (to the first SM trial). We also analysed the number of SSGs produced by the bat in the CM trial immediately following the sequential presentation of three SM trials (as shown in Fig. 2). In other words, we analysed SSG production in the trial sequence CM–SM–SM–SM–CM (here again, CM could be CM1 or CM2). This analysis revealed that for the trial after the sequential SM presentations, the bat produced fewer SSGs in the initial

window of the CM trial (Fig. 4B). Here, data have been combined across bats by normalizing the number of SSGs produced in each sequence of CM–SM–SM–SM–CM (to the first CM trial, and thus the first column has the value 1).

We also investigated if the bats produced more echolocation calls when target motion trajectory changed (i.e. reduced predictability, indicated by the dashed black lines in Fig. 2). Figure 5 shows the average number of sonar calls produced by all four bats (error bars are s.e.m.) in the CM–SM1–SM2–SM3 trial sequence during the 'initial window' of the target trajectory (indicated by dashed black lines in Fig. 2). The average number of sonar calls produced during the initial time window was not statistically different across the sequentially presented trials (one-way repeated measures ANOVA, $P > 0.1$). Thus, bats increased the production of SSGs when target trajectory changed from trial to trial, but they did not change the average number of sonar calls produced.

Table 1. Reduction in sonar sound groups as predictability in target trajectory increases sequentially from CM–SM1–SM2–SM3

	CM	SM1	SM2	SM3
Bat A	8.93±1.32	8.41±1.38	6.39±0.94	5.51±0.75
Bat B	5.44±1.15	4.78±1.03	2.78±0.97	1.778±0.59
Bat C	5.97±0.87	6.07±0.91	5.24±0.71	3.32±0.41
Bat D	4.59±0.68	4.27±0.72	3.61±0.48	3.02±0.39

Data are the mean (±s.e.m.) number of sonar sound groups produced.

Bats reduce production of sonar sound groups as target trajectory repeats over multiple days

Figure 1D shows the distance of the target from the bat on the y-axis, and the SM and CM target trajectories are aligned in the final approach of the food reward, with time 0 ms corresponding to when the target reached the bat. This figure illustrates that the end trajectories of the complex motions (CM1 and CM2) are comparable to the SM, and this time window is marked by two

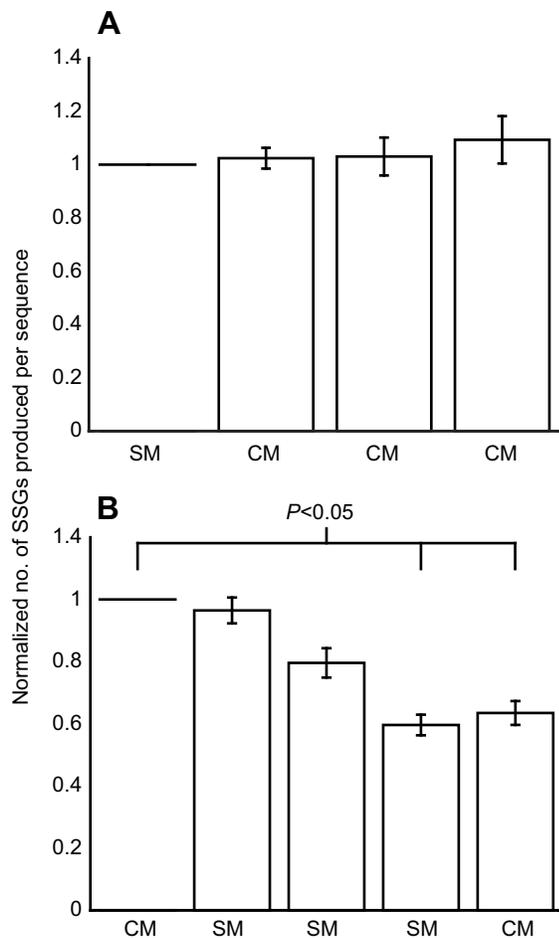


Fig. 4. Number of SSGs produced for different trial sequences.

(A) Number of SSGs produced by the bats when three consecutive CM (here CM could be CM1 or CM2, randomly chosen) trials were presented to the bats. For comparing number of SSGs produced per sequence across bats, number of SSGs were normalized to the number of SSGs produced in the SM trial preceding the three CM trials. (B) Number of SSGs produced by the bats when a CM–SM–SM–SM–CM sequence occurred. Here, CM could be either CM1 or CM2, and randomly chosen. For comparing number of SSGs produced per sequence across bats, number of SSGs produced in any trial of the sequence was normalized to the number of SSGs produced in the first CM trial of the sequence. For both panels, data were combined from all bats and only the initial window was analysed. The error bars indicate s.e.m.

dashed green vertical lines. We refer to this as the final time window. In Fig. 6, the SSG data analysis was restricted to the final time window, when the target trajectories of the CM trials were identical. Figure 6 shows box plots of the SSGs produced by the bats in the final time window for the CM1 and CM2 target trajectories, for the first day (when the two complex target trajectories were first introduced to the bats), an intermediate day (day 3) and the last day of data collection (for Bats A and C, last day was day 5, while for Bats B and D, last day was day 6). All bats showed a reduction in the production of SSGs across days, as their familiarity with the complex target trajectories increased.

We also investigated if the bats produced fewer sonar calls during the ‘final window’ (Fig. 1D, green lines) as the bats tracked targets in repeated complex target motion trials, over a period of days. We calculated the total number of calls produced by bats during the final window and compared these over a period of days (day 1, day 3 and last day) and found no significant change in the total number of calls

(one-way repeated measures ANOVA, $P > 0.2$) although the bats did show a trend of reducing the production of SSGs over days (see Table 2).

Comparison of pulse duration and interval of sonar sound groups across test days

To investigate if bats adapted PD and/or PI of calls over repeated presentations of complex target trajectories, we compared the PD and PI of calls within SSGs for complex motion (CM1 and CM2) trajectories across days. Figure 7 shows the mean and s.e.m. changes in PD and PI, respectively, with respect to target distance and across days (day 1, day 3 and last day are plotted in red, blue and green, respectively). Day 1 was the first day when the bats were introduced to complex motion and last day was the final day of data collection. For Bats A and C, the last day was day 5 while for Bats B and D, the last day was day 6. To investigate if bats adapted the PD and PI of SSGs across days and target distance, we performed a two-way repeated measures ANOVA. ‘Days’ and ‘target distance’ were included as the two main variables of the ANOVA. There was a significant change in both PD and PI for target distance ($P < 0.001$) but no significant change was observed in these call parameters (PD and PI) across days ($P > 0.2$). In addition, no significant interaction effect was observed ($P > 0.4$).

DISCUSSION

When insectivorous bats pursue moving prey, they must precisely track changing target position by processing information carried in sonar echoes. Accurate target localization is crucial for the bat’s planning of subsequent motor behaviors, such as timing the production of sonar calls and the trajectory of flight. Bats dynamically adapt sonar call parameters to extract task-relevant echo information from the environment, and the temporal patterning of sonar calls (sonar sound groups, SSGs) is a component of the bat’s adaptive vocal behavior. The production of SSGs has been recorded in echolocating bats in the field and in the laboratory (Moss and Surlykke, 2001, 2010; Moss et al., 2006; Petrites et al., 2009; Surlykke et al., 2009; Aytekin et al., 2010; Hiryu et al., 2010; Falk et al., 2014; Kothari et al., 2014; Sändig et al., 2014; Wheeler et al., 2016). Insectivorous bats typically track moving prey, often with erratic trajectories, and in this study we show that bats increase the production of SSGs when target motion is unpredictable. Our results are consistent with the hypothesis that task demands for spatial localization accuracy evoke temporal clustering of echolocation calls into SSGs.

Temporal patterning of sonar sounds may sharpen localization of unpredictably moving targets

Many insects have evolved erratic flight trajectories to evade predation by echolocating bats. Insects that hear ultrasound, for instance, exhibit evasive flight maneuvers in response to echolocation signals (Roeder, 1962, 1967; Triblehorn and Yager, 2005). In the experiments presented here, we experimentally manipulated the predictability of target trajectories presented to bats tracking moving prey. Our results show that bats significantly increase the production of SSGs when the unpredictability of target motion increases (Fig. 3), consistent with the hypothesis that SSGs are used by echolocating bats to increase spatiotemporal resolution of target position when demands for sonar localization accuracy are high (Moss et al., 2006; Petrites et al., 2009; Kothari et al., 2014; Sändig et al., 2014; Kothari et al., 2018). These results raise the possibility that bats integrate echoes returning from SSGs to aid target trajectory prediction and sensorimotor planning.

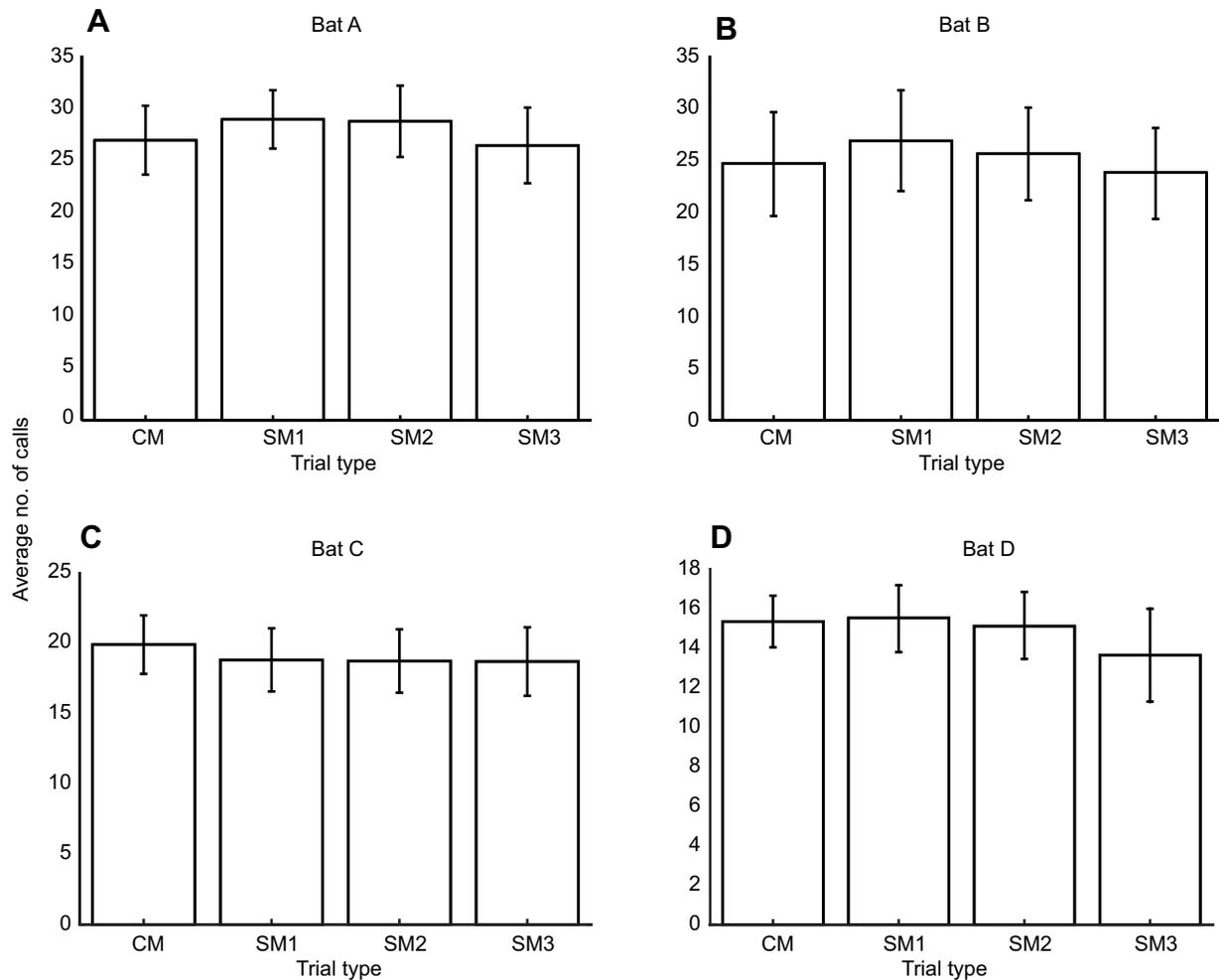


Fig. 5. Bats produce the same number of calls in the initial time window, irrespective of target motion unpredictability. (A–D) Average number of sonar calls produced by all four bats within the initial time window (see black lines in Fig. 2) when presented with unpredictable complex target motion (CM1 or CM2) followed by more predictable simple target motion (SM1, SM2 and SM3). The error bars indicate s.e.m.

Bats reduce production of sonar sound groups as target trajectory repeats

On the first day of data collection, the bats were familiar with the simple motion target trajectory, while complex motion trajectories (CM1 and CM2) were novel to the bat. The CM and SM motion trajectories were designed to have nearly identical motion paths during their final approach (final window indicated by two dashed green lines in Fig. 1D). Our results (Fig. 6) demonstrate that as bats are exposed to the complex motion trajectories (both CM1 and CM2), over a period of days, they reduce the production of SSGs during the more predictable and familiar trajectory in the final window, which is common across all motion conditions (CM1, CM2 and SM). This result demonstrates that the production of SSGs is modulated by target motion predictability.

Bats dynamically adjust PD to avoid the overlap between incoming echoes and the outgoing sonar call. In addition, bats can also adjust the PI of sonar calls to avoid ambiguity of echo assignment over successive calls (Moss and Surlykke, 2001; Schnitzler and Kalko, 2001; Wilson and Moss, 2004; Falk et al., 2015). In our study, only the predictability of target motion was manipulated across trials and days. Furthermore, we found no significant change in either PD or PI as the predictability in the target motion increased (Fig. 1C) across the SM1–SM2–SM3 target

trajectory presentations. Similarly, we did not find any significant change in PD or PI across days, as the bats became more familiar with the novel complex motions (Fig. 7), lending support to the hypothesis that bats specifically increase SSG production when tracking unpredictably moving targets.

SSGs: created through control of temporal patterning or addition of calls?

Our results demonstrate that bats actively increase the production of SSGs as the target unpredictability increases (Fig. 3). In addition, bats also decrease SSG production as they become more familiar with complex target trajectories over a period of days (Fig. 6). This raises the question of whether bats increase the number of sonar calls to produce SSGs (call addition), or temporally rearrange calls to produce SSGs while producing the same number of sonar calls (temporal patterning)? Both strategies are illustrated schematically in Fig. 8: (1) call addition: by adding extra calls to a sonar call stream, sonar sound groups could be produced; or (2) temporal rearrangement: here, the bat actively modifies the time of emission of the sonar calls to group them into clusters of vocalizations.

The call addition strategy asserts that the unpredictability of CM target trajectories would evoke more echolocation calls and, as a by-product, more SSGs. If this was the case, the total number of sonar

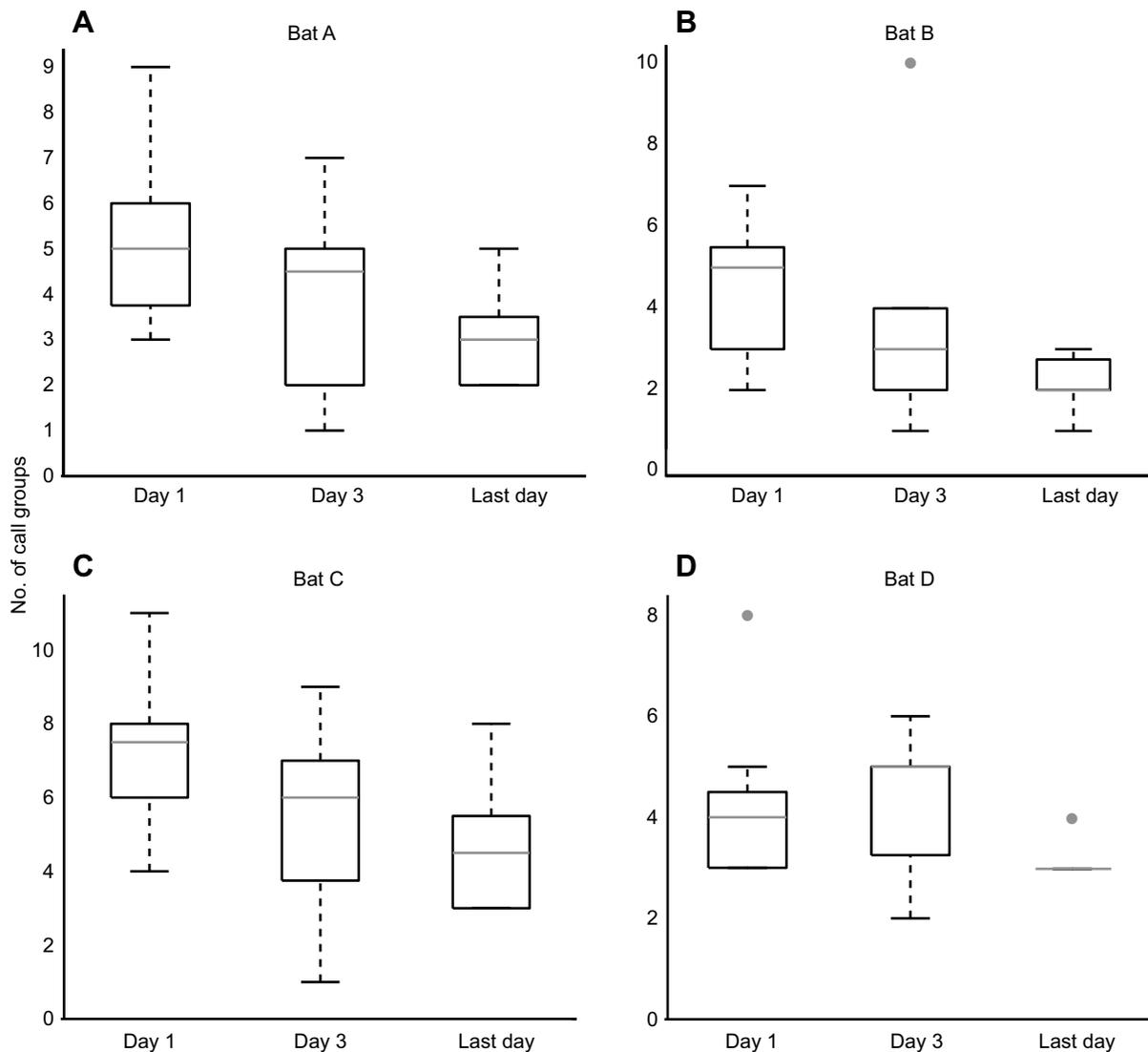


Fig. 6. Bats produce fewer sonar sound groups as familiarity with novel target trajectories increases. (A–D) Box plots of the number of SSGs produced by all four bats in the final time window (indicated by two green lines in Fig. 1D) as their familiarity with the novel complex motions (CM1 and CM2) increased over a period of days. On the last day (day 6), Bat D only produced three SSGs in each trial, which is why there is only the median (gray line) represented without a box.

calls produced by the bat would be greater in CM and SM1 trials, compared with SM3. Our data (Fig. 5) show that there is no statistically significant difference between the number of calls produced by the bats during the initial phase of CM, SM1, SM2 or SM3.

The temporal rearrangement strategy suggests that the bats do not increase the number of sonar calls to track an unpredictably moving target, but instead actively adjust the temporal patterning of calls to produce SSGs. All bats in this study produced the same mean number of calls (Fig. 5) in the initial time window (indicated by the two black lines in Fig. 2), consistent with the temporal

rearrangement strategy. In other words, the data show that bats actively cluster their sonar calls to produce SSGs. The temporal rearrangement strategy implies that SSGs have behavioral significance for the bat tracking a target with an unpredictable trajectory. Moreover, to perform this behavior would involve sensorimotor planning, as information from previous calls is used to adjust the temporal patterning of subsequent sonar calls to extract relevant information from the environment (Ulanovsky and Moss, 2008; Koblitz et al., 2010; Moss and Surlykke, 2010).

Possible relevance of temporal patterning of sonar calls for intercepting eared insects

In some insect species, the temporal pattern of ultrasonic vocalizations produced by attacking echolocating bats can provide acoustic cues to trigger evasive and unpredictable maneuvers, like diving and erratic trajectories (Roeder, 1962, 1967; Triplehorn and Yager, 2005; Corcoran et al., 2009; Ghose et al., 2009). Foraging bats gradually decrease pulse interval as the distance to the insect prey decreases (Simmons et al., 1979; Surlykke and Moss, 2000; Moss and Surlykke, 2001; Schnitzler and Kalko, 2001), and

Table 2. Mean (\pm s.e.m.) number of sonar calls produced in final window for CM trials over a period of days

	Day 1	Day 3	Last day
Bat A	35.22 \pm 3.78	37.86 \pm 3.81	36.97 \pm 4.15
Bat B	35.62 \pm 3.58	34.24 \pm 3.17	36.42 \pm 3.27
Bat C	31.00 \pm 2.11	33.33 \pm 2.34	35.32 \pm 4.92
Bat D	30.75 \pm 2.23	27.86 \pm 2.58	29.01 \pm 2.73

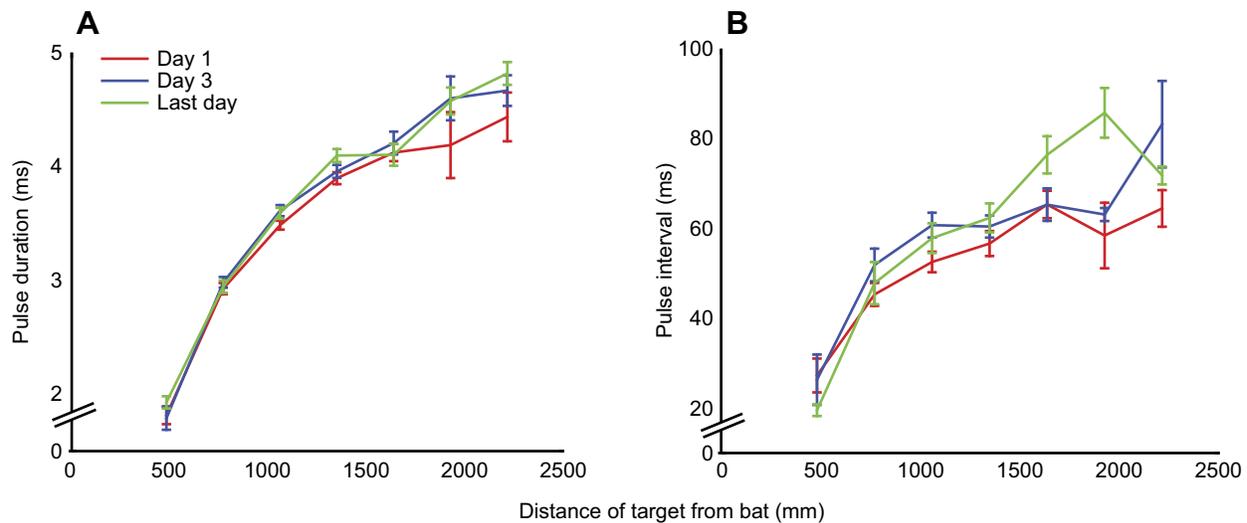


Fig. 7. Comparison of pulse duration and pulse interval of sonar sound groups as bats learned a novel target trajectory. Comparison of pulse duration (A) and pulse interval (B) over a period of days as their familiarity with the novel complex target motions (CM1 and CM2) increased.

Tribblehorn et al. (Tribblehorn and Yager, 2005; Tribblehorn et al., 2008) found that the gradual decrease in pulse interval provides insects with more time to trigger evasive responses and escape capture. However, in cases where bats temporally pattern their sonar calls to create rapid changes in pulse intervals (SSGs), hearing insects may delay the initiation of evasive maneuvers and escape rates would decrease. Ghose et al. (2009) also reported that bats produce SSGs while tracking erratically moving insects.

Does the production of SSGs benefit the echolocating bat as it forages for insect prey? Previous work suggests that the bat's temporal patterning of sonar calls influences the probability of successful insect capture (Tribblehorn and Yager, 2005; Tribblehorn et al., 2008; Ghose et al., 2009). Our data suggest that actively producing SSGs also aids the bat with the important task of tracking an unpredictably moving target.

Implications for range tuning of neurons in the auditory systems of echolocating bats

Bats estimate target distance by measuring the time delay between sonar sound production and the reception of echoes (Hartridge, 1945; Simmons, 1973). Neural representations of target range have been studied by presenting pairs of sounds (mimicking the bat's own call and the returning echo) to passively listening bats and

recording from auditory neurons, which show pulse-echo (P/E) delay facilitation and tuning for particular P/E delay pairs. Echo delay-tuned neurons have been characterized in the auditory cortex (Suga and O'Neill, 1979; O'Neill and Suga, 1982; Schuller et al., 1991; Hagemann et al., 2010), thalamus (Olsen and Suga, 1991; Yan and Suga, 1996) and midbrain (Dear and Suga, 1995; Ehrlich et al., 1997; Valentine and Moss, 1997; Portfors and Wenstrup, 1999) of echolocating bats.

Delay tuning of neurons has been shown to be modulated by the temporal patterning and repetition rate of P/E pairs (O'Neill and Suga, 1979; Hechavarria et al., 2013; Bartenstein et al., 2014). The delay tuning curves become sharper/narrower for shorter pulse intervals, thus implying that there may be perceptual sharpening of range estimation. Our findings are consistent with the previous proposals of Moss et al. (Moss and Surlykke, 2001; Moss et al., 2006) and Sändig et al. (2014) that SSGs may serve to sharpen sonar range perception in bats operating under challenging conditions. Preliminary data suggest the dependence of echo delay tuning in the bat auditory midbrain on the stability of pulse intervals of P/E pairs, which mimic sonar sound groups (Ulanovsky and Moss, 2008; see their fig. 2). We believe that a critical test of sonar image sharpening with SSG production would be the characterization of neural echo delay tuning in the auditory system of an actively echolocating bat engaged in target tracking. Kothari et al. (2018) reported that representation of target depth in auditory neurons in the superior colliculus of free-flying bats sharpened when bats produced SSGs compared with single sonar calls.

In summary, our results demonstrate that echolocating big brown bats actively increase the production of SSGs when target motion is unpredictable, and decrease the production of SSGs when the motion is more predictable, thus supporting the hypothesis that temporal patterning of sonar calls sharpens sonar resolution for tracking erratically moving targets. Furthermore, our finding that bats produce sonar sound groups without increasing the total number of calls suggests that this adaptive sonar behavior involves sensorimotor planning.

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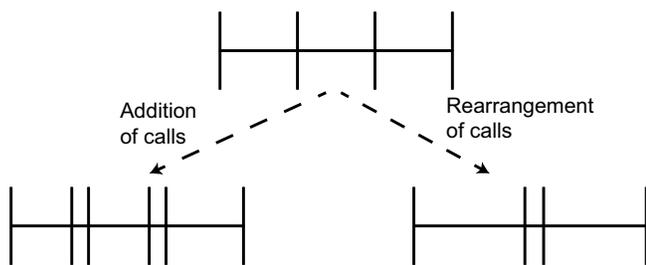


Fig. 8. The call addition versus temporal rearrangement strategies. This figure demonstrates two ways in which bats can produce SSGs in the same time interval (shown in the top panel). The top panel shows a sequence of four calls produced by a bat. By producing two extra calls, a bat could produce SSGs by adding calls (bottom left panel), while SSGs could also be produced using the same number of calls by temporally rearranging the calls (bottom right panel).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: N.B.K.; Methodology: N.B.K., C.F.M.; Software: N.B.K., M.J.W.; Validation: N.B.K.; Formal analysis: N.B.K.; Investigation: N.B.K., M.J.W.; Resources: C.F.M.; Data curation: N.B.K.; Writing - original draft: N.B.K.; Writing - review & editing: N.B.K., M.J.W., C.F.M.; Visualization: N.B.K.; Supervision: C.F.M.; Project administration: C.F.M.; Funding acquisition: C.F.M.

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