

## RESEARCH ARTICLE

# Spatial memory and stereotypy of flight paths by big brown bats in cluttered surroundings

Jonathan R. Barchi\*, Jeffrey M. Knowles and James A. Simmons

Department of Neuroscience, Brown University, Providence, RI 02912, USA

\*Author for correspondence (barchi@brown.edu)

### SUMMARY

The big brown bat, *Eptesicus fuscus*, uses echolocation for foraging and orientation. The limited operating range of biosonar implies that bats must rely upon spatial memory in familiar spaces with dimensions larger than a few meters. Prior experiments with bats flying in obstacle arrays have revealed differences in flight and acoustic emission patterns depending on the density and spatial extent of the obstacles. Using the same method, combined with acoustic microphone array tracking, we flew big brown bats in an obstacle array that varied in density and distribution in different locations in the flight room. In the initial experiment, six bats learned individually stereotyped flight patterns as they became familiar with the space. After the first day, the repetition rate of sonar broadcasts dropped to a stable level, consistent with low-density clutter. In a second experiment, after acquiring their stable paths, each bat was released from each of two unfamiliar locations in the room. Each bat still followed the same flight path it learned originally. In a third experiment, performed 1 month after the first two experiments, three of the bats were re-flown in the same configuration of obstacles; these three resumed flying in their accustomed path. The other three bats were flown in a mirror-image reconfiguration of the obstacles; these bats quickly found stable flight paths that differed from their originally learned paths. Overall, the flight patterns indicate that the bats perceive the cluttered space as a single scene through which they develop globally organized flight paths.

Key words: bat echolocation, biosonar scene, biosonar sounds, flight dynamics, spatial memory.

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### INTRODUCTION

*Eptesicus fuscus* (Palisot de Beauvois 1796), the big brown bat, is an insectivorous echolocating bat in the family Vespertilionidae (Kurta and Baker, 1990). Such bats have limited eyesight (Suthers, 1966) and rely primarily on biological sonar for orientation, obstacle avoidance, and detection and capture of prey (Neuweiler, 2000). Echolocation is an active sensory process that involves specializations in the auditory system (Kick and Simmons, 1984), in sound production mechanisms (Neuweiler, 2000) and in vocal-motor control (Moss and Surlykke, 2010). Flying by sonar also requires coupling between the bat's echolocation system and the neural control of flight (Moss et al., 2006). While flying at speeds up to  $3\text{ m s}^{-1}$ , bats segregate sources of returning echoes, construct an internal representation of the three dimensional (3D) auditory scene, and respond to salient stimuli with rapid but intricate locomotor behavior.

Echolocation broadcasts are brief, ultrasonic frequency-modulated (FM) chirps consisting of several harmonic frequency sweeps that collectively cover frequencies from about 20 to 100 kHz. The operating range of the big brown bat's echolocation system is limited, by spherical spreading and atmospheric attenuation of ultrasound, to an effective range of about 5 m for detecting insects (Kick and Simmons, 1984). The effective angular field-of-view is less limited by the transmit and receive beam patterns, which are very broad (140 deg at 25 kHz to 60 deg at 80 kHz,  $-6\text{ dB}$ ) (Aytekin et al., 2004; Ghose and Moss, 2003; Hartley and Suthers, 1989; Jen and Chen, 1988; Wotton et al., 1995), than by auditory computations that create a narrower (20–25 deg) frontal zone of protection from

clutter masking (Bates et al., 2011; Sümer et al., 2009). In sparsely cluttered environments, *E. fuscus* scans its surroundings by aiming its sonar beam at relevant objects (Surlykke et al., 2009). They also change their pulse repetition rate – and thus their primary sensory update rate – based on environmental conditions such as the proximity of targets and clutter density. In open spaces or only moderately dense clutter, the steady-state call rate is 5–20 Hz, but it rises to 40–50 Hz in dense clutter (Hiryu et al., 2010; Moss and Surlykke, 2010; Petrites et al., 2009; Saillant et al., 2007). These bats receive essentially stroboscopic updates of the world around them (Surlykke and Moss, 2000).

Despite these apparent sensory limitations, *E. fuscus* is able to fly and orient by sonar with great agility in three dimensions over a broad range of spatial scales, from feeding sites in corners of fields or clearings in woods with dimensions of a few meters, to home ranges from roosts to feeding sites that might be kilometers in size (Bernard and Fenton, 2003; Lewis, 1995). Moreover, they frequent the same feeding and drinking locations by flying from their roosts and back night after night (Williams and Williams, 1970). This suggests that echolocating bats place an especially high demand on spatial memory and self-motion information – such as proprioception and vestibular input – in order to stay oriented in the world over spaces much larger than their sonar can penetrate. There is good evidence that echolocating bats have a strong sense of space. Spear-nosed bats (*Phyllostomus discolor*) have been observed to fly routes of hundreds or thousands of meters between day roost and foraging sites, using consistent paths that vary between individuals (William and Williams, 1970). Bats of several species have also been

observed to home when captured in their day roost and released elsewhere, over a range of 10 miles (~16 km) or more, and to return to the same cave roost in multiple years after annual migrations of hundreds of miles (Holland et al., 2006; Mueller and Emlen, 1957; Tsoar et al., 2011). In the laboratory, big brown bats have been shown to use acoustic landmarks for spatial orientation on a centimeter scale (Jensen et al., 2005), but this study did not consider the bats' navigational precision outside of a very small test region in which they were required to fly through a hole in a mesh. An additional factor is that echolocating bats fly at velocities of several meters per second. With a small-target operating range of only about 5 m (Kick and Simmons, 1984), they typically cross the operating range of their primary sense in a few seconds. Flights over longer distances require operation over successive range segments that must be stitched together to maintain a sense of the space as a whole.

In the dark, a big brown bat must rely on echolocation to sense its environment, and it cannot pause mid-flight to re-orient, as *E. fuscus* cannot hover (Neuweiler, 2000; Petrites et al., 2009). In contrast, terrestrial mammals such as rodents that are generally used in studies of spatial learning and memory can stop at any time to examine their surroundings. Because of the short operating range of their biosonar, the bat's sensing of local space has to be intimately tied with memory, with an internal map of the space as a whole. Because flying bats move so rapidly through their perceptual range, this places a premium on the coupling of spatial memory to the sensing of location and to locomotion. (Ulanovsky and Moss, 2008; Ulanovsky and Moss, 2011). These circumstances present an opportunity to exploit the patterns of movement of bats flying within spaces with dimensions of a few meters, such as a laboratory flight room, as a tool for directly observing the internal representations of space that guide the animal's actions. Because bats actively adapt their echolocation calls depending on their perceptual needs and their surroundings, analysis of calling behavior provides a real-time indicator of bats' perceptions (Moss and Surlykke, 2010). Similarly, analyzing flight dynamics as bats navigate novel and familiar environments can provide insights into their internal representation of the space in which they are flying. Experimental analysis of their flight dynamics on a spatial scale of several meters – comparable to their operating range – may reveal aspects of spatial processing complementary to those observed in observations of bats flying over longer routes to reach feeding sites or their roost.

In this study, we recorded echolocation behavior and flight dynamics as big brown bats were introduced to a novel flight space, and observed how their behavior changed over the course of several days of exposure and in response to changes in the space or the flight conditions. As the bats became familiar with the space, they quickly developed individual stereotyped flight patterns – repetitive loops along a stable trajectory. Analysis of this echolocation and flight behavior provides insight into the interaction between sonar perception, spatial memory and flight control as bats learn to navigate in a new space.

## MATERIALS AND METHODS

### Animals

Six big brown bats, *E. fuscus*, were used for these experiments. These bats were wild-caught from homes in Rhode Island under a scientific collecting permit issued by the state Department of Environmental Management, and housed individually according to protocols approved by the Brown University IACUC. The bats were maintained in an environmentally controlled colony, at 22–24°C and 40–60% relative humidity. Animals were fed mealworms, larval *Tenebrio molitor*, and their food intake was adjusted individually

to maintain their mass in the range 16–20 g. They had free access to vitamin-supplemented water at all times. The light cycle of the bat colony was reversed, 12 h/12 h dark/light, so that experiments could be conducted during the daytime on alert bats.

### Flights and recording

Flight experiments were conducted in an acoustically and electrically isolated flight chamber, depicted in Fig. 1A,B (Hiryu et al., 2010; Petrites et al., 2009). The flight chamber was 9.0 m long, 3.3 m wide and 2.4 m high, and a pair of overlapping vertical nets restricted the bat's flight to a rectangular test volume 6.0 m long. Black plastic chains were hung from floor to ceiling in this volume, at positions shown in Fig. 1. These chains could easily be moved to reconfigure the test environment, and they provided strong acoustic reflections along their entire length from their repeated individual links (Petrites et al., 2009).

Two experimenters participated in the conduct of all trials presented here. One person, inside the flight chamber, released the bat at the beginning of each trial and retrieved it at the end. The second person, outside of the flight chamber, operated the recording system, logged flight parameters, and gave the first experimenter instructions about the experiment. The flight chamber was completely dark during experimental trials, so the animal had to rely solely on its biosonar and spatial memory for navigation and obstacle avoidance. The person in the flight chamber, deprived of vision, was told where to retrieve the bat by the outside person based on the thermal video feed.

For all experiments, a flight trial was defined as one release into the test volume. The bat was allowed to fly from release until it landed, and time in flight was logged for each flight where the bat made at least one full circuit of the volume; that is, from the release point, to the opposite end of the room, and back to the release end of the room again. A single flight session continued until the bat had accumulated at least 5 min of flight, or for 20 flight trials. The goal was to accumulate at least 5 min of flight time for each bat on each day; the 20-trial limit prevented sessions from growing arbitrarily long (in wall time) if flight performance was poor. In practice, bats often reached 5 min of flight time well before they had completed 20 trials; in a few cases a bat flew for 5 min in a single trial. After each sustained flight, the bat was rewarded with small pieces of mealworm, offered in forceps. For a given bat, flight sessions were always performed at roughly the same time of day; a day's block of flight sessions generally started at 13:00 h, and the block start time was constant across all sessions for any given animal.

To track the bats and record their sonar broadcasts, the flight chamber was instrumented with two thermal video cameras and an ultrasonic microphone array attached to a high sample rate recording system. The thermal infrared cameras (Merlin mid-range and/or Photon 320, FLIR Systems, Boston, MA, USA) were mounted at one end of the room, and aimed so that their fields of view overlap in most of the space occupied by the bat during flight. Their signals were multiplexed and simultaneously captured to computer disk using a commercial video digitization system (V4HD, MOTU, Cambridge, MA, USA) and displayed on a monitor at the recording workstation. Twenty-four ultrasonic microphone/preamplifier/filter boards of our own design were mounted throughout the room to transduce audio, which was digitized at 192 kHz per channel and saved to computer disk using a commercial audio capture system (PCI-424/2xHD192/AudioDesk 3.0, MOTU). The ultrasonic audio and video data streams were synchronized using SMPTE time code, encoded as an audio-rate digital signal called longitudinal time code (LTC). The video streams were used to monitor and locate the bat

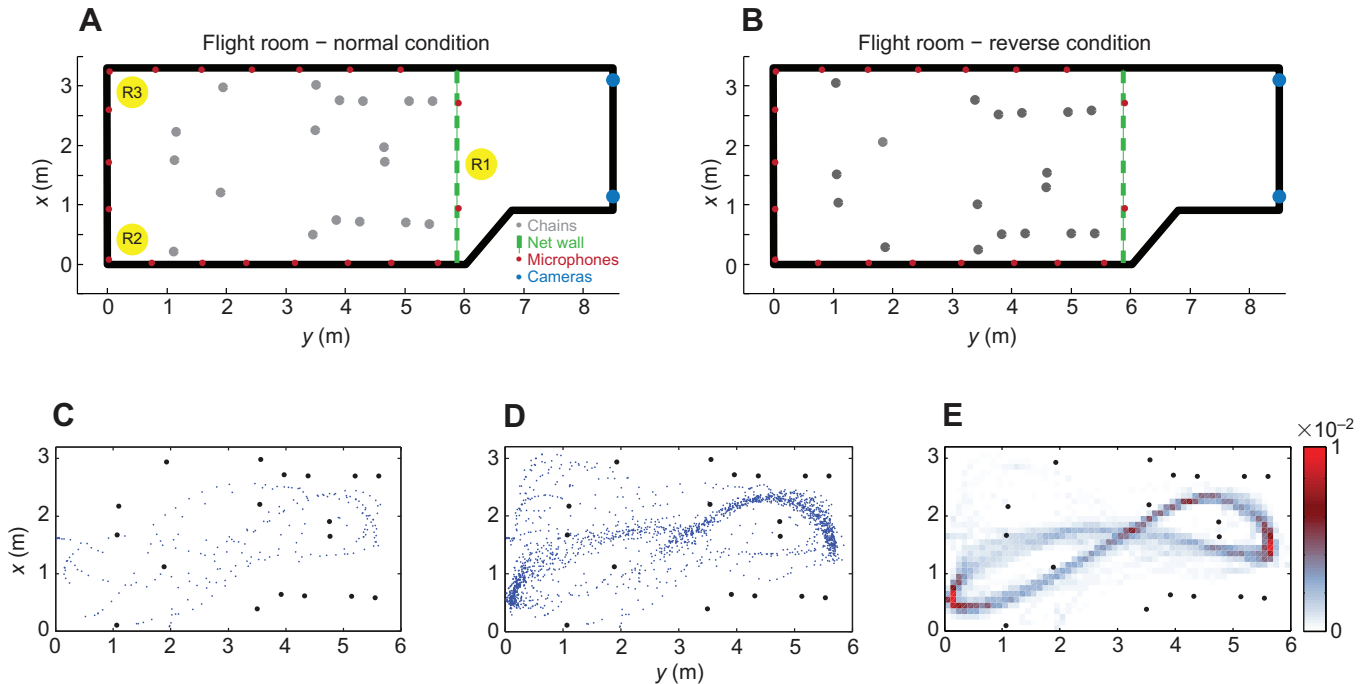


Fig. 1. Methods for conducting flight experiments and construction of flight occupancy histograms. (A,B) Plan projections of the flight room for normal (A) and reverse (B) configuration of obstacles, showing the net wall restricting the flight volume, vertical plastic chains around which the bat must navigate, positions of the array microphones on the walls, the two thermal cameras used to record bat flights, and release positions for multiple release point (MRP) experiments (A only; yellow circles labeled R1, R2, R3). The reverse condition shows the mirror-image chain configuration used in the second time course experiment (TC2) for bats in the reverse group. (C–E) Construction of flight occupancy histogram. (C) Reconstructed call positions (small blue dots) from one complete, looping flight of one bat. (D) All reconstructed call positions from all flights by the same bat on the same day as C. (E) The  $x$ - $y$  occupancy histogram computed from call positions in D.

during flight, and saved as a secondary record of the experiments. The audio data were further processed off-line using custom-written MATLAB (MathWorks, Natick, MA, USA) software developed in our lab, as described below.

#### Position reconstruction

The bat's position during flight was reconstructed off-line from ultrasonic audio records using a time difference of arrival (TDOA) method implemented in MATLAB code. Briefly, bat calls were detected on any recorded channel based on their energy, and a window of time surrounding each detected call was extracted from all channels. These per-channel records were then cross-correlated in time to obtain the relative time of arrival of the call on each channel, and thus at each microphone position. From these time differences of arrival, and precise knowledge of the location of each microphone, an estimate of the bat's position was obtained at each time when it made a call (Fujioka et al., 2011; Gillette and Silverman, 2008; Wei and Ye, 2008). Using the microphone array and TDOA algorithms for localization has proven to be more reliable than two-camera direct linear transform (DLT) methods, which reconstruct 3D information from sets of two-dimensional (2D) images captured by the thermal cameras. Because microphones are distributed around the perimeter of the room, at several heights, the array is much less sensitive than the camera pair to occlusion of the bat by obstacles, such as the chains. It also allows for accurate localization in all three dimensions, while the accuracy of two-camera DLT perpendicular to the inter-camera axis (i.e. along the length of the flight chamber) is limited by inter-camera spacing and by distance to the subject.

After a bat's successive echolocation call positions along its flight path were reconstructed from the acoustic data, the records were programmatically trimmed. We removed physically implausible outliers, points for which the associated instantaneous velocity or acceleration was inconsistent with biology, and long series of points when the animal was stopped, such as the beginning and end of flights, which would bias the occupancy analysis described below. Approximately 10% of total raw points were removed by this process, the majority of which were stopped segments prior to release and after landing. The trajectory was then fitted with a piecewise-cubic smoothing spline and resampled at regular temporal intervals. This allowed analysis of the bat's flight track through space independent of the repetition rate of its sounds, which defined the initial sampling points of the position. The spline was used solely to resample the data for regular temporal sampling, and did not change any position estimates enough to affect subsequent analysis – the mean change in position due to spline resampling was <10 cm across all points in the data analyzed here.

#### Analysis

After being reconstructed, tracked flight paths were used to create occupancy histograms as illustrated in Fig. 1C–E, using a custom-written MATLAB code. These histograms were created by dividing the flight volume into regular cubic spatial bins with edge length 10 cm, and counting the number of points in a set of flight paths that fall into each bin. These 3D histograms were normalized by dividing the number of points that fall into each bin by the total number of points in the set of flights to give each bin a probability density, which allows them to be combined for further analysis (e.g.

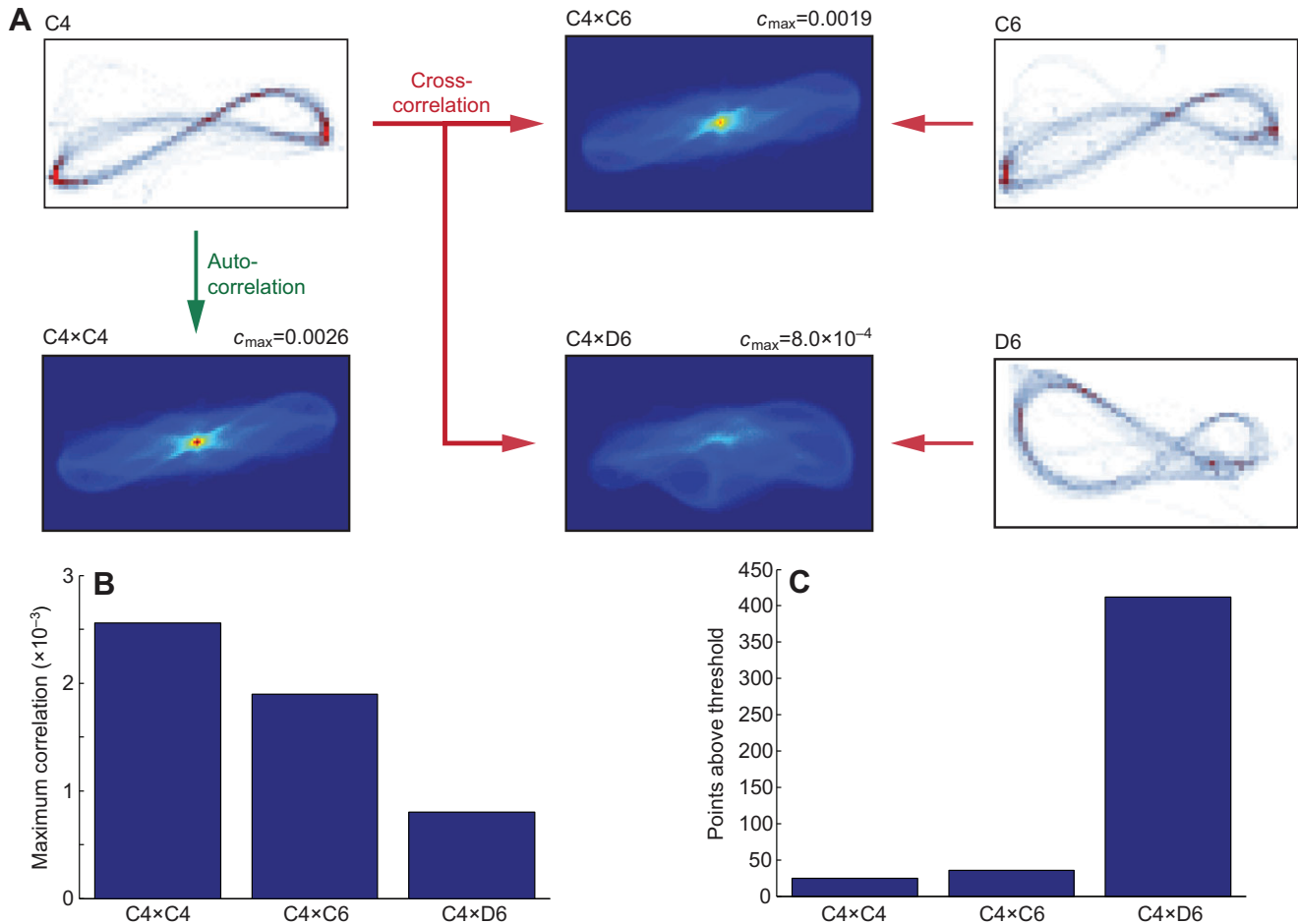


Fig. 2. Using two-dimensional (2-D) cross-correlation of flight occupancy histograms to quantify path sharpness and similarity. (A) Computation of representative occupancy histogram cross-correlations for two bats from the first time course experiment (TC1). Three plots show occupancy histograms for bat C on day 4 (C4, top left), bat C on day 6 (C6, top right) and bat D on day 6 (D6, bottom right). Three other plots show the auto-correlation of the path for bat C, day 4 (C4×C4, bottom left), cross-correlation of paths for bat C, days 4 and 6 (C4×C6, top center), and cross-correlation of paths for bat C, day 4 and bat D, day 6 (C4×D6, bottom center). Maximum correlation coefficient ( $c_{\max}$ ) is printed above the top-right corner of each plot. Note the gross similarity between flight patterns for the two bats. Both within-bat C central correlation peaks (C4×C4, C4×C6) are tightly concentrated with high peaks (red–orange points), while the cross-bats C and D correlation peak (C4×D6) is broadly spread and low (light blue spread) despite the qualitative similarity of the flight paths. (B) Quantification of the three correlations from A by the maximum correlation coefficient ( $c_{\max}$ ; height of central peak on 2D surface). (C) Quantification of the same correlations by the number of points on the 2D surface above threshold at 60% of peak value (points above threshold; spread of central peak).

across bats). The binned positions were collapsed along one dimension to create 2D occupancy histograms for presentation and further analysis. Further analysis focused on the plan ( $x,y$ ) projection of the flight volume, as illustrated in Fig. 1E, as the chain obstacles are distributed in this plane and the bats did not change their altitude much during flight.

Summary quantitative analysis of the flight path data was performed in MATLAB by using a 2D cross-correlation between different sets of occupancy histograms, as illustrated in Fig. 2A. The cross-correlation of two similar histograms, or of one histogram with itself (autocorrelation), yields a sharp central correlation peak and little energy off-center; this can be seen in the lower-left and upper-middle panels of Fig. 2A. In the autocorrelation, the width and maximum height of this central peak both reflect the sharpness of the underlying histogram. The cross-correlation of two dissimilar histograms has a lower and broader central peak, and much more off-center energy, as shown in the lower-middle panel of Fig. 2A. In order to obtain a numeric score for the similarity between two

distributions, and by proxy for the convergence and stability of flight patterns, we measured the maximum correlation value and the number of points in the distribution that were greater than 60% of this maximum value for each computed correlation. These metrics are plotted in Fig. 2B,C for each of the cross-correlations shown graphically in Fig. 2A, and are analogous to the central peak height and width, respectively. As the two correlated histograms become sharper and more alike, the correlation maximum increases and the peak width decreases, reducing the number of points above a given threshold. When computed, statistical tests were performed using functions from the MATLAB Statistics Toolbox (MathWorks).

The reconstructed flight paths were also used to guide a second round of call detection for analysis of echolocation behavior, implemented as a final step of the position reconstruction code. After reconstruction and spline resampling, the reconstructed position of the bat over time was used to steer the room microphone array as a sum-and-delay beam former to search for echolocation calls along the bat's flight path. This array focusing provided a higher signal-



to-noise ratio for the resultant record than that obtained on any single microphone channel, facilitating more effective detection of calls. Detected calls that fell within the analyzed flight segments were used to compute the average echolocation call rate during these intervals.

### Experimental design

We hypothesized that the bats would develop stable flight paths as they became familiar with the locations of obstacles in a test environment, and that these stable flight paths would persist over time and in the face of small perturbations of the obstacles. The first experiment (first time course; TC1) consisted of six flight sessions. For each trial, the bat was released into the test volume from the same point at the front of the room, release point 1 (R1; Fig. 1A). The bats had no prior experience with the flight chamber or the configuration of the obstacles within. The goal of this experiment was to observe the bat's behavior as it explored, and became familiar with, a new flight space. Specifically, we expected that the bats would develop stable, efficient paths through the flight chamber as they learned its configuration.

The second experiment (multiple release points; MRP) was performed in three additional flight sessions immediately following the six sessions of series TC1. For these sessions, the bat was released for each trial from one of three different points in the flight chamber (R1, R2 and R3; Fig. 1A), according to a random schedule. MRP was used in this experiment to assess whether the new initial conditions (i.e. starting position for flight) affected flight behavior in an otherwise familiar situation, with attention focused on the bats' ability to return to an established flight path and on the nature of the path itself. Specifically, when released from a new place, we expected that each bat would return to the same flight path it had previously learned.

After series TC1 and MRP, the bats were maintained in the colony without further exposure to the flight chamber for one full month. In the third experiment (second time course; TC2), the bats were brought back to the flight chamber for another six session series, analogous to TC1. Three of the six bats were presented with the room obstacles in the same configuration as in series TC1 ('same' condition, 'normal' panel, Fig. 1A), while the other three bats were presented with the obstacles mirrored left-to-right across the long axis of the room ('reverse' condition, Fig. 1B). This mirror-image transformation was chosen because it changed the overall configuration of obstacles in the room, and thus the most efficient paths through them, without major changes in the global or local density of chains (clutter) that might affect flight behavior just locally or for non-specific sensory reasons such as the total number of echo sources. This experiment had two main goals – to assess whether the bats would return to their previously learned flight paths after a month outside the test environment, and to see how they would respond to a change in the configuration of the obstacles within the flight chamber. We hypothesized first, that the bats would settle into stable flight patterns more quickly in series TC2 than in TC1, and second, that at least those bats exposed to the same configuration as in series TC1 would return to the flight paths they had previously learned. We expected that bats exposed to the 'reverse' condition would still retain information from their previous exposure to the flight chamber, and they would converge to flight paths that were distorted or altogether different from those learned in series TC1.

## RESULTS

The evolution of flight path occupancy during experiment TC1 is illustrated graphically for all six bats in Fig. 3. Each column

represents one flight session (1 day), and each row represents one bat. Two important results are illustrated here. First, five of the six bats (Fig. 3B–F) developed, within the first 3 days of TC1, a pattern of space occupancy that did not evenly sample the room, but was instead confined to sharp, tightly defined flight tracks. The sixth bat (bat A) converged toward a few path options, but it took longer (until day 6) and its occupancy of the space remained more scattered than any of the others. Second, the flight path through the room varied across individuals – each bat found an individual solution for flight through the obstacle set and exploration of the space. The bats thus developed individually stereotyped flight tracks as their experience in the flight chamber progressed. The fact that different bats learned different paths through the space further suggests a spatial learning process, and discounts the possibility that these results may reflect a globally optimal solution to which all bats were constrained.

Bat A eventually developed a weakly stable flight pattern, but took almost all of TC1 to do so, and flew inconsistently throughout all three experiments. This bat was an outlier in other ways too. For example, her average flight velocity was nearly 50% higher than that of the other bats – so much higher that she could be identified based on velocity by experimenters watching the video feed. For these reasons, data from bat A have been excluded from subsequent pooled numerical analyses.

Fig. 4 presents summary cross-correlation results from experiment TC1, showing the day-to-day trend of convergence to a stable flight path as measured by cross-correlation peak spread (method explained in Fig. 2). For each bat, cross-correlations were performed between the occupancy histogram for each day and the occupancy histogram for day 6 (the day 6 correlation is an autocorrelation), and quantified by the spread of the cross-correlation peak on the 2D surface as described in Materials and methods. Fig. 4 plots the daily means ( $\pm$ s.e.m.) of these values across the bats, with bat A excluded (Fig. 4A) and with bat A included (Fig. 4B). These graphs quantify the effect that is visually apparent in Fig. 3 – as the bats become familiar with flying in the normal flight chamber configuration (Fig. 1A), their flight paths become more stable, so that each day's occupancy histogram becomes more closely correlated with that from day 6.

The second experiment, MRP, varied the release point in the room. Fig. 5 shows occupancy histograms pooled by release condition (R1, R2, R3 in Fig. 1A), along with a reference plot of day 6 from the original TC1 series. As in Fig. 3, each row of plots is one animal and each column is one release condition (release point 1, 2 or 3, or TC1 day 6). Comparison of the histograms from each release point and initial data from the first experiment (TC1) shows visually that the bats returned to their established flight path within the test volume regardless of the initial release conditions; there was no distortion of the overall flight pattern, and no increase in off-track points or spreading of the track width beyond what was observed in experiment TC1. Fig. 6 shows quantification of each bat's retention of its flight path from the original release point (R1) for all three release points in experiment MRP (R1, R2, R3). For each bat, cross-correlations were computed between the occupancy histograms for each of the three release conditions (R1, R2, R3) and the histograms from days 1 and 6 of experiment TC1 (D1 and D2, respectively). Following initial experience in experiment TC1, when the bats were flown from different release points in MRP, the spread of the cross-correlation peak (at 60% of peak maximum) and the peak height are similar for correlations of R1, R2 and R3 with TC1 day 6. For the correlations of R1, R2, and R3 with TC1 day 1, the peak spread is wider and the peak height is lower. Testing the

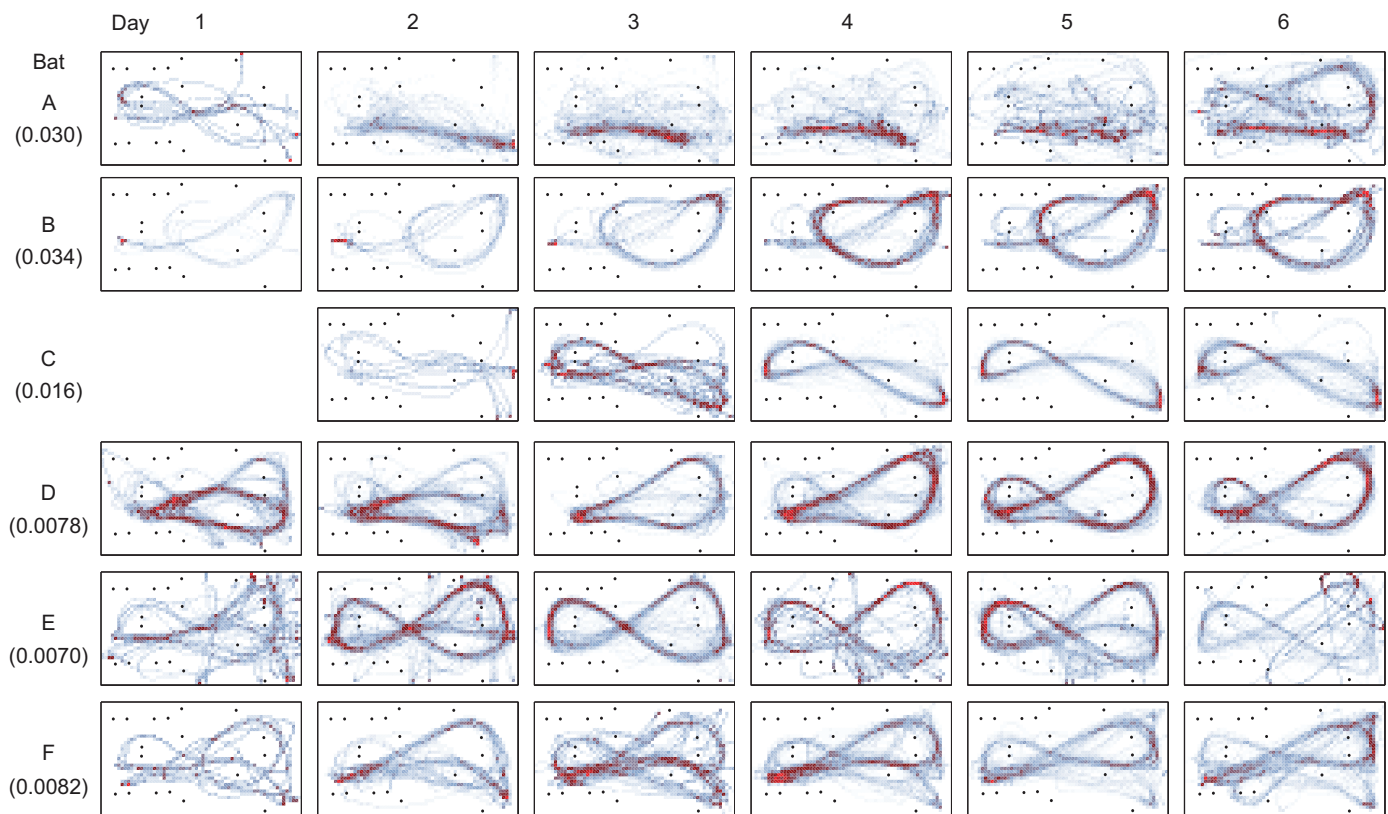


Fig. 3. Plot of individual per-day flight path occupancy histograms from experiment TC1 for all six bats. Each row of plots traces an individual bat across 6 days; each column of plots represents 1 day of the time course experiment for all six bats. Each plot collapses all flights from 1 day and one bat. Black dots on each plot indicate the positions of chain obstacles, and the number under each bat's identifier is the maximum observed occupancy probability – to which that bat's plots are normalized. Note the overall effect of progressive tightening for paths for all bats, particularly after the first day. (Bat C made too few flights on day 1 to analyze.)

contrast that correlations between release flights and TC1 day 6 are sharper and stronger than correlations between release flights and TC1 day 1, the null hypotheses for both Fig. 6A and 6B were rejected using the Kruskal–Wallis ANOVA ( $P < 0.01$  and  $P < 0.05$ , respectively). Thus, the rotations of the initial view of the scene achieved by shifting the release points did not prevent the bats from quickly resuming their individual accustomed flight paths.

Experiment TC2 brought the same six bats back into the flight room for further testing after a 1 month interval – three bats to the same configuration of the chains (normal, Fig. 1A) and three bats to the reversed configuration (Fig. 1B). Fig. 7 shows a representative overview of flight occupancy histograms from experiment TC2 – 3 days each for two bats in the ‘same’ group and two bats in the ‘reverse’ group, alongside reference plots of the same bats’

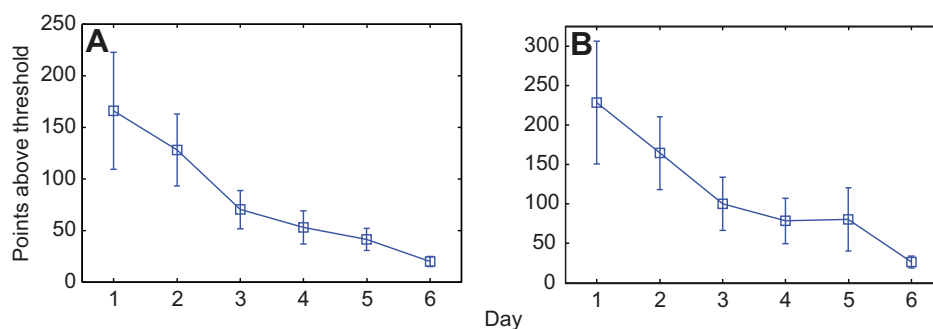


Fig. 4. Quantification of the results of experiment TC1 by the cross-correlation method illustrated in Fig. 2. The spread of the central cross-correlation peak across the 2D surface is the metric. Cross-correlations were computed between each day of experiment TC1 and day 6 of experiment TC1 (as shown in Fig. 2A, upper-center panel), and then their spread was quantified by the number of points above 60% of the maximum height of the central peak (points above threshold). A broad peak spread, indicated by many points above threshold, indicates poorer correlation. These values were averaged across bats for each day and plotted as means  $\pm$  s.e.m. (A) Values for 2D spread of the above-threshold auto-correlation functions for each day of experiment TC1, excluding bat A (see Fig. 3 and Results). (B) Values for 2D spread of the above-threshold auto-correlation functions for each day of experiment TC1, including all bats.

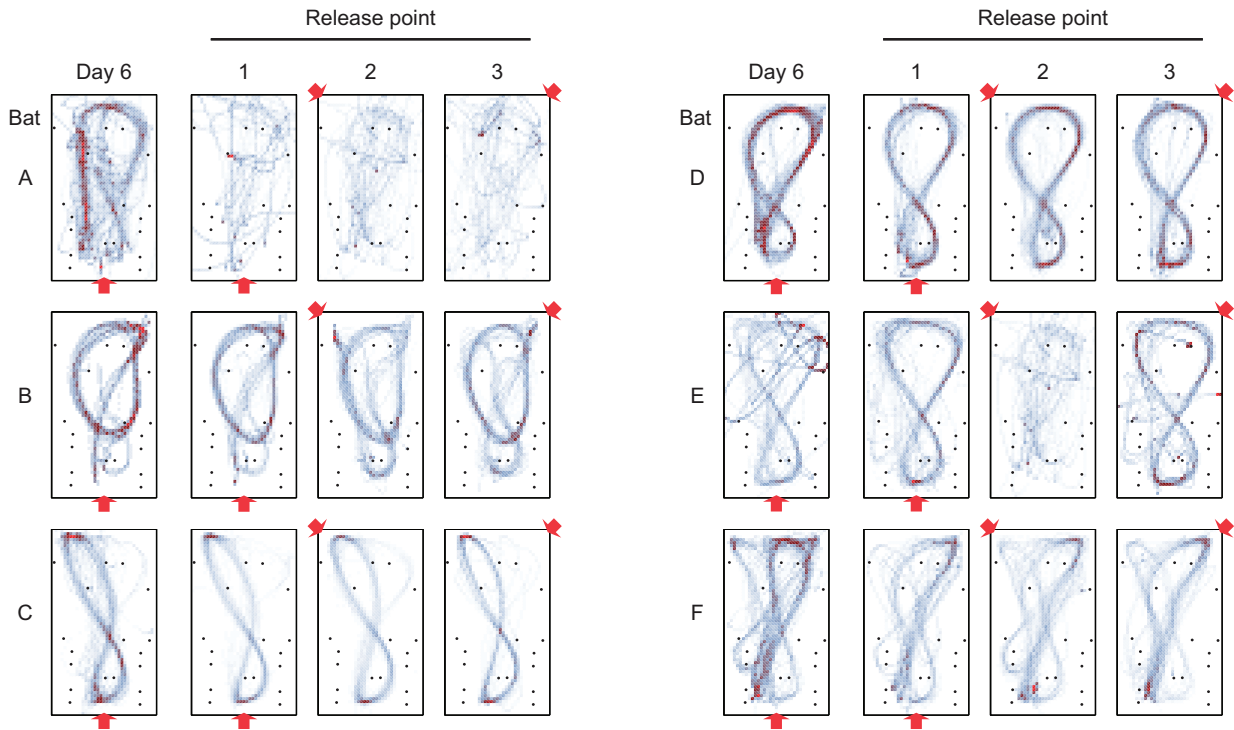


Fig. 5. Flight path occupancy histograms from multiple release points in experiment MRP. Data were pooled across 3 days of the experiment, and separated according to the (numbered) release point of the flights (red arrows; R1, R2, R3 in Fig. 1A). Each row represents one bat, and each column one release point. For comparison, the occupancy histograms for the final day of experiment TC1, day 6, are replotted from Fig. 3.

occupancy histograms from day 6 of experiment TC1. The same flight path occupancy histograms were computed as in TC1, and cross-correlations were computed between occupancy histograms for each of the 6 days of TC2 and day 6 of series TC2, for each bat. Fig. 8A shows the spread of the day-to-day cross-correlation functions (at 60% of peak height) for the five bats in both configurations of TC2. For comparison, the data from TC1 are replotted from Fig. 4A. As an index of the bats' 'echolocation effort', the sounds recorded with the microphone array and used to track the bats (Fig. 1) were processed to measure the mean broadcast call rate during the flights accumulated in the cross-correlations for both TC1 and TC2. Mean broadcast call rates are plotted in Fig. 8B. Both

the cross-correlation peak-spread area (Fig. 8A) and the call rate (Fig. 8B) started lower and stayed lower during series TC2 compared with series TC1; in both cases, all days of TC2 were close to the value only reached on day 6 of TC1. These effects were evaluated with the Kruskal-Wallis ANOVA, comparing pooled data from series TC1, all days, with pooled data from series TC2, all days. TC2 was significantly different from TC1 in both cases, with  $P < 0.01$  for the cross-correlation data in Fig. 8A and  $P < 0.05$  for the call rate data in Fig. 8B.

Comparisons were also performed within the data from experiment TC2, between the two bats in the 'same' group (excluding bat A) and the three bats in the 'reverse' group. Means

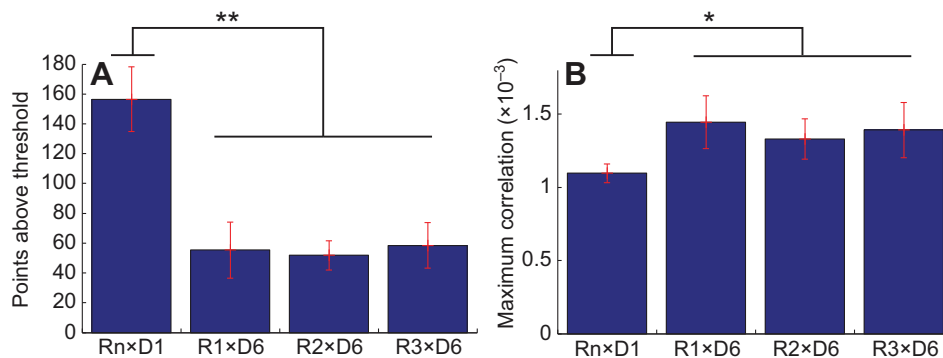


Fig. 6. Quantification of experiment MRP. Cross-correlations were computed, for each bat, between flights from release groups 1–3 (R1–R3), and days 1 and 6 from the original experiment TC1. Correlation results from Release (all) (Rn) × TC1 day 1 (D1) are quantified in the first column of each plot (mean ± s.e.m.), and results from each Release (day) (R1–R3) × TC1 day 6 (D6) condition are summarized in the following columns (mean ± s.e.m.). (A) The spread of the cross-correlation peak over the 2D surface above 60% of maximum peak height (points above threshold) is the metric (see Fig. 2). (B) Height of the 2D cross-correlation peak for the same comparisons. Once each bat converged on a stable path by day 6 in TC1, changing the release point in MRP did not disrupt path sharpness. \*Significant difference at  $P < 0.05$ ; \*\*significant difference at  $P < 0.01$ .

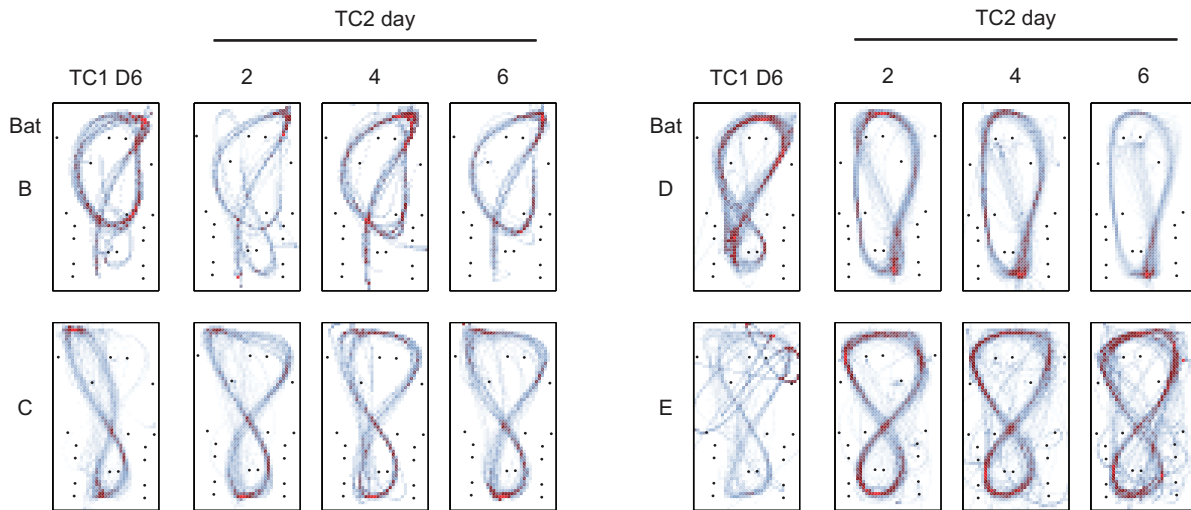


Fig. 7. Flight path occupancy histograms from representative days and animals in experiment TC2. Each row represents one bat, and each column one experimental day in TC2 (days 2, 4 and 6). For comparison, the occupancy histograms for the final day of experiment TC1, day 6, are replotted from Fig. 3.

are plotted for both cross-correlation peak-spread (with TC2, day 6; Fig. 9A) and echolocation call rate (Fig. 9B). In contrast to the same comparisons obtained for cross-correlation and call rate between TC1 and TC2 in Fig. 8, Fig. 9 shows a reversal of the effect between the two conditions within TC2. The cross-correlation peak spread (Fig. 9A) was higher across days for the reverse condition encountered by three bats than for the repeat of the normal condition encountered by the other three bats (two analyzed). However, the call rate (Fig. 9B) was lower across days for the reverse condition than for the repeat of the normal condition. For both measures, the difference between ‘reverse’ and ‘same’ pooled across days was significant (Kruskal–Wallis ANOVA) with  $P < 0.01$ . By either measure, the bats in the ‘reverse’ group never converged to the same level of performance as the bats in the ‘same’ group. The bats’ accumulated previous experience with the normal configuration of the obstacles in experiment TC1 was retained in the second exposure to the same configuration in experiment TC2 (Fig. 8), but it was detrimental to their ability to adapt to the reversed obstacle configuration in TC2 (Fig. 9).

A final comparison was performed between results from the ‘same’ and ‘reverse’ groups in order to quantify the similarity of the paths used by the bats in experiment TC2 to the paths used by the same bats in experiment TC1. For this comparison, histogram cross-correlations were computed between each day in experiment TC2 and the corresponding day in experiment TC1, for each bat.

The means ( $\pm$ s.e.m.) are plotted for cross-correlation peak spread, grouped by experimental condition, in Fig. 10. This cross-correlation, between corresponding days of experiment TC2 and TC1, is a measure of the similarity of the flight paths that the bats used in experiment TC2 to those that they used in experiment TC1. The two groups are similar for days 1 and 2 of experiment TC2, and diverge for days 3–6. For days 3–6 of experiment TC2, the bats in the ‘same’ group used flight paths very similar to the flight paths that they used in experiment TC1, as indicated by the low correlation peak spread. The correlation peak spread for the bats in the ‘reverse’ group is higher than that of the bats in the ‘same’ group, and never drops from the initially high value seen in both groups on days 1 and 2 of experiment TC2. The difference between the ‘same’ and ‘reverse’ groups is significant ( $P < 0.01$ , Kruskal–Wallis ANOVA). This indicates that the bats in the ‘reverse’ group, unlike those in the ‘same’ group, never returned to the same flight paths that they developed in experiment TC1.

**DISCUSSION**

The results from the three experiments described above demonstrate that big brown bats learn and use individual, stable flight paths as they become familiar with a cluttered space. They develop these flight tracks rapidly over several days (Figs 3, 4). The tracks remain stable over time (Fig. 7A) and in the face of perturbations of their initial conditions (Figs 5, 6). By the second day of experiment TC1,

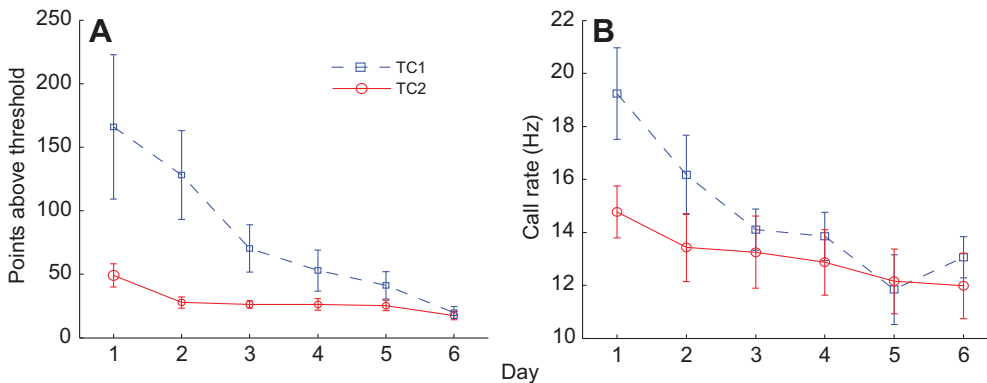


Fig. 8. Comparison of results from experiment TC1 and experiment TC2. (A) Plot of mean cross-correlation peak 2D spread values by day for all bats in experiments TC1 and TC2; cross-correlations were computed for both series as described in Fig. 4. (B) Graph of mean echolocation call rate by day for experiment TC1 and experiment TC2. Call rate is plotted as a per-day mean across all bats, with error bars indicating  $\pm$ s.e.m. The pooled data are significantly different between TC2 and TC1 in both cases ( $P < 0.01$  in A;  $P < 0.05$  in B).



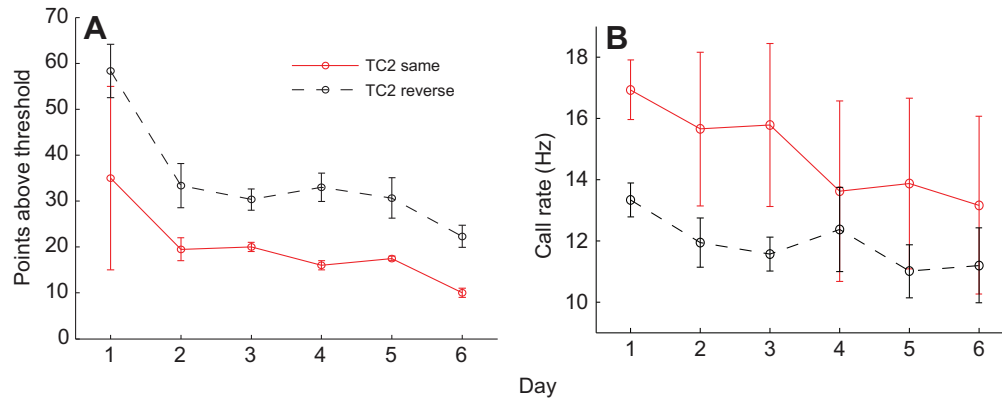


Fig. 9. Comparison of results from two conditions of experiment TC2. (A) Plot of mean cross-correlation peak 2D spread values by day for two bats in experiment TC2 exposed to the same configuration of chains and the three bats exposed to the reverse condition. (B) Plot of mean broadcast call rates by day for two bats in experiment TC2 exposed to same configuration of chains and the three bats exposed to the reverse condition. Data are plotted as means across all bats, with error bars indicating  $\pm$ s.e.m. The pooled data are significantly different between TC2 same and TC2 reverse ( $P < 0.01$ , both panels).

five of the six bats had converged on their own stable flight path. The sixth bat adopted one very simple flight path after the first day, and later developed a second, more looping flight path that more closely resembled the flight paths of the other bats. Each bat adhered to a unique path within the space; that is, each bat developed its own preferred path rather than all bats converging on a globally optimal route. The development of stable flight paths thus must depend on the sensory and motor experiences of each individual bat over the course of the experiment. These observations are consistent with each bat developing a stereotyped flight path through the space as a spatial memory closely tied to echolocation and to flight dynamics.

Over the first 3 days of exposure to the flight chamber, the bats' echolocation call rates declined from an average of about 20 Hz and approached a stable rate of about 12 Hz (Fig. 8B), consistent with previous observations made in cluttered environments (Petrites et al., 2009). This decrease in calling rate suggests that as bats become familiar with their flight space, they can tolerate a slower rate of sensory updates from successive echolocation

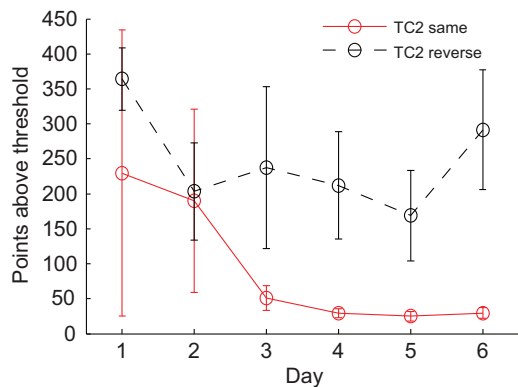


Fig. 10. Comparison of results from two conditions of experiment TC2 against original performance in experiment TC1. Cross-correlations were computed, for each bat, between each day of experiment TC2 and the corresponding day of experiment TC1, and their spread was quantified by the number of points above 60% of the maximum height of the central peak. These values were grouped and averaged according to experimental group: two bats exposed to the same configuration of chains and the three bats exposed to the reverse configuration of chains. The pooled data are significantly different between TC2 same and TC2 reverse ( $P < 0.01$ ).

broadcasts. We postulate that the development of stable flight paths allows bats to depend on their accumulated internal representation of a space as well as on moment-to-moment echolocation, and to navigate within the space as a whole. The bats' ability to quickly track into their familiar paths when released from different locations around the room confirms this suggestion. The qualitative impression given by previous studies of spatial memory in bats – that they are able to form stable memories relative to a point in space (Jensen et al., 2005) – is supported quantitatively by the results of our experiments.

Two manipulations were used to probe the stability of these flight tracks in the presence of different initial conditions (experiment MRP) or changed configuration of the obstacles (experiment TC2). In both cases, stable flight paths survived the transitions. The bats' ability to reacquire their previously established flight pattern when released in different places suggests that their image of the scene is anchored to the room rather than to the bat. In effect, rotation of the space with respect to the bat's release point did not cause the bat to treat it as a new space. These results show that the bats perceive the space globally, rather than as a series of independent local waypoints (i.e. as a succession of more or less independent obstacle-avoidance maneuvers). That is, they do not treat the individual obstacles just as something to be avoided at the last moment, but as elements of a structured environment through which they navigate based on knowledge of the whole scene. Previously published studies of bats flying in the chain array in this room show that all of the chains and all of the features of the room (wall, ceiling, floor, etc.) yield echoes that are audible to the bat from any point in its flight (Hiryu et al., 2010; Petrites et al., 2009). Besides revealing that the bats perceive the room globally in terms of stable flight paths under different modifications of the space and conditions, those previous studies documented changes in acoustic behavior related to the perception of multiple objects in the room at different distances. In other words, at least on the spatial scale of this room, the bats are not processing the scene incrementally in pieces but instead as a cohesive whole (Moss and Surlykke, 2010).

That the bats are treating the flight chamber as an integrated scene through which they must navigate is further supported by the results of experiment TC2. After learning the flight space, and their own individual flight solutions within it, the bats were rested for a month. They had no intervening exposure to the flight chamber, but when they returned to it for experiment TC2 they returned almost

immediately to a level of stable flight performance similar to that at the end of experiment TC1. Bats exposed to the same room configuration in experiments TC1 and TC2 resumed using the same flight paths they had previously learned, with the same precision they displayed at the end of experiment TC1. Bats confronted with a different configuration of obstacles in experiment TC2 from those they learned in experiment TC1 found new flight paths that were measurably different from the ones they had previously learned, and never quite returned to the same level of precision they had developed in the first experiment. In effect, the memory of the room configuration this group of bats developed during experiment TC1 inhibited, or at least delayed, their ability to learn and precisely execute flight trajectories through the slightly different configuration of obstacles they faced in experiment TC2.

All of these results support the hypothesis that big brown bats presented with a new cluttered space, like those spaces in which they naturally navigate and forage, quickly translate it into an internal map in which they develop precisely coordinated flight paths. From an ethological standpoint, this makes intuitive sense. When flying in a cluttered environment, a big brown bat covers the range of its primary sense every couple of seconds (Kick and Simmons, 1984). For flight like this to be anything more than a succession of obstacle-avoidance maneuvers, a detailed internal map is highly desirable; repeatedly dodging obstacles is not an efficient way to fly, and efficiency is important for an animal that must spend so much time flying (Neuweiler, 2000). Moreover, a flying bat is often a foraging bat, and foraging raises two more considerations. First, detection of prey requires an active sensory search, and navigating from an internal map might allow sensory attention to be devoted to prey detection rather than tracking and avoiding environmental objects. Second, capture of prey following detection requires quickly and efficiently navigating a path to the target, in a cluttered 3D environment, while tracking it as it moves. A detailed internal map, intimately tied to sensation and locomotion, would greatly facilitate this process.

The results of our experiments also suggest some interesting predictions about the neural substrates of spatial memory. Physiological experiments in rodents and in bats (*E. fuscus* and *Rousettus aegyptiacus*) have found cells in the hippocampus and the entorhinal cortex with great selectivity for locations in space. The firing of some hippocampal pyramidal neurons, called 'place' cells, is strongly modulated by an animal's position in a given environment (Ahmed and Mehta, 2009; Ulanovsky and Moss, 2007; Ulanovsky and Moss, 2011; Wilson and McNaughton, 1993). In the entorhinal cortex, the firing of so-called 'grid' cells varies in a regular grid-like fashion in any environment (Fyhn et al., 2004; Hafting et al., 2005; Yartsev et al., 2011). The firing rates of an ensemble of place or grid cells can be used to accurately reconstruct an animal's position (McNaughton et al., 2006), but recent work in rats suggests that the temporal relationship of spikes and rhythmic activity in the hippocampus also encodes spatial information (Ahmed and Mehta, 2009).

In both rats and bats, the size of commonly recorded place fields is of the order of tens of centimeters. This is based on recordings in crawling animals (rats and crawling bats), but it provides a starting estimate. Bats present an interesting system for the study of hippocampal dynamics because when flying at several meters per second, they spend very little time in each place field. Because the big brown bat cannot stop during flight, its dwell time on small segments of the path will be very short. In fact, even if we assume that place fields in a flying bat are 10 times larger than those observed in crawling animals, a flying bat would traverse each place field in

just a few hundred milliseconds. During their flights in the present experiments, the bats flew at horizontal speeds of  $\sim 2.5 \text{ m s}^{-1}$  (e.g.  $2.7 \pm 0.23 \text{ m s}^{-1}$  for experiment TC1) while emitting 10–20 calls  $\text{s}^{-1}$  on average. This means that the interval between broadcasts (i.e. the sensory update rate) is associated with movement across distances of several tens of centimeters in a few hundred milliseconds. The rapidity of bat flight suggests that only a few spikes are likely to occur when the animals occupy a particular neuron's place field; this is in contrast to spatial memory studies on rodents, which can stop to reorient themselves while learning a new task or a new space. These considerations suggest that temporal coding might play a particularly important and visible role in the bat hippocampus: as bats fly through a sequence of place fields, the corresponding cells are activated in a temporally precise manner, with a sparse neuronal code. The behavior described in the experiments above could serve as an important model for further research into the role of temporal coding in the hippocampus and associated spatially selective circuits.

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