

SI Guide

Supplementary methods:

Description of release procedures, experimental manipulation and statistical analysis of the data.

Supplementary figure 1:

Description of the different predicted outcomes of a shift in the magnetic field at sunset depending on the method of calibration.

Supplementary figure 1 legend:

Legend for figure S1.

Supplementary Table 1.

Details of release dates, wind speed and direction, track directions and headings at 5km.

Supplementary Methods

Experimental Subjects

Big brown bats *Eptesicus fuscus*, were captured at their roost in the barn at Princeton University field station. When a bat was caught it was fed meal worms and water and kept over night. The bat was fed meal worms and water again the next night before release. If weather conditions did not allow a release the second night the bat was released back into the barn.

Release procedure

Bats were displaced to a site at the edge of Neshanic Valley golf course, New Jersey, 20km north of their home by car and were fitted with a 0.5g radio-transmitter attached to shaved skin between the shoulders with super glue, just prior to release. The bats were then tracked from a small airplane (Cessna 152 or 170) until they returned to the home roost or stopped moving for more than one hour. Using a two antenna setup on the aircraft bats were located ($\pm 100\text{m}$) every several minutes and the flight path later reconstructed from the GPS waypoints recorded at these locations. A receiver at the home roost allowed us to record when a bat returned if it had to be abandoned because of lack of movement during the tracking period.

Magnetic field manipulation

To manipulate the magnetic field direction bats were captured as above and on the second evening they were put in a clear Perspex cage inside a Helmholtz coil next to the home roost. The bat was placed in the coil 45 minutes before to 45 minutes after sunset and had a clear view of the sun and horizon from inside the coil. The field was shifted either clockwise (CW) or counter clockwise (CCW) by 90° with respect to magnetic north by placing the coil at a 45° angle to the direction of shift and increasing the power to the coil until a professional compass placed in the centre of

the coil read 90°. For all groups, control, CW and CCW only one bat was released per night, except on the final release where a CW bat and a Control bat were released consecutively with a 15 minute interval between the two bats. Control releases were otherwise performed prior to the Helmholtz releases to determine whether un-manipulated bats showed a predictable homing response that would provide a baseline for further investigation. CW and CCW shifts were determined by a pseudorandom order with no more than 3 shifts consecutively in the same direction. CW and CCW bats were transported to the release site in the Perspex cage inside the Helmholtz coil. Control bats were transported to the release site in an opaque Perspex box.

Statistical analysis

Tracks (Fig. 1 B, C) were constructed from GPS waypoints. The bearings to the bats when they were 5km from the release site were selected as their track directions. Testing orientation at other distances between 3 and 8 km was not found to significantly alter the track directions. Wind speed was often a significant percentage of bat flight speed, thus track directions and headings often did not coincide. The experimental design meant that we could not control for wind speed and direction by releasing all bats under comparable conditions. There are no data on whether bats are able to correct for wind drift but some night migrating birds appear not to⁹. It is unlikely that bats can maintain the sensory contact with the ground that would be necessary to allow them to correct for wind drift given that their echolocation system has a maximum range of 25m¹⁰.

We therefore analysed the headings of the bats to get directional information about the effect of the magnetic field. Headings were calculated as the direction of the vector that was equal to the track vector (release to 5 km) minus the wind vector¹¹ (Table S1). Wind direction and speed were obtained from NOAA.gov. Mean headings

were analysed for significant orientation in the predicted direction by the V test and for a difference between groups by the Watson-Williams test¹².

Approval

These experiments were approved by Princeton University Institutional Animal Care and Use committee.

Supplementary references

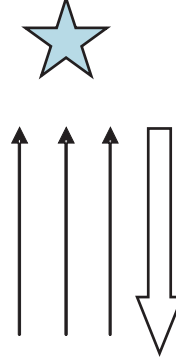
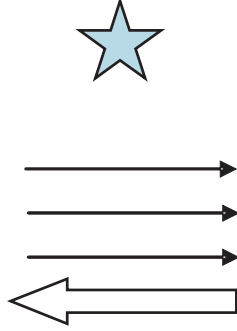
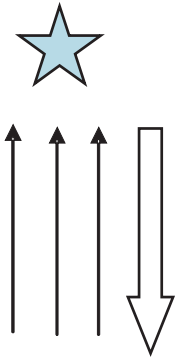
9. Cochran, W.W. & Kjos, C.G. *Illinois Natural History Survey Bulletin* **33**, 297-330 (1985).
10. Holderied, M. W. et al. *Journal of Experimental Biology* **208**, 1321-1327 (2005).
11. Green, M. & Alerstam, T. *Journal of Theoretical Biology* **218**, 485-496 (2002).
12. Batschelet, E. *Circular Statistics in Biology* (Academic Press, New York, 1981).

Natural condition

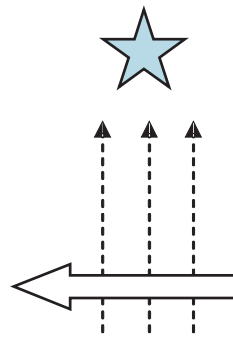
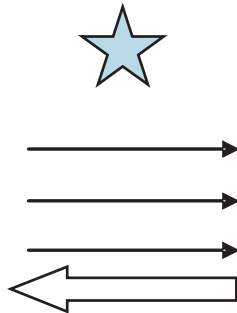
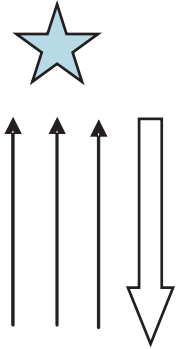
During treatment

At release site

A



B



C

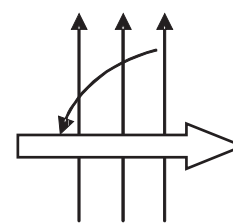
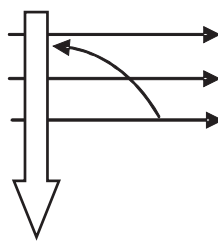
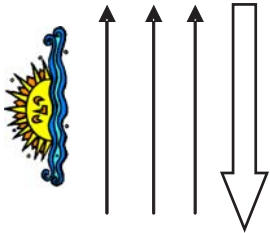


Figure S1. Schematic diagram to illustrate the predictions for direction of orientation after exposure to a magnetic field shifted clockwise (CW) through sunset depending on the interaction between magnetic and celestial cues. The 3 lined arrows in each diagram represent the magnetic field direction and the solid arrow represents the perceived direction of the home site for our bats. A: if there is simple dominance of the magnetic compass or a star compass, with no calibration then when released in a natural magnetic field at the release site the bats should fly south regardless of the direction of the field during treatment. B: if the magnetic field calibrates a star compass then during treatment the field is shifted CW, making the bat perceive north as east and therefore south as west. When released, a bat trying to fly south to reach home would thus fly west using its magnetic calibrated star compass. C: if sunset cues calibrate a magnetic compass then when the field is shifted east at sunset the bat will perceive that geographical south is 90° CW to the magnetic field direction. When released in a normal magnetic field, flying 90° CW to it will result in the bat flying east.

Table 1. Headings, wind strength and direction

date	Exp	5km_TD	5km_T	W_dir	W_kmh	H_day	H	R
08-Apr	control	164	00:20:39	338	12.880	1	202	0.751
09-Apr	control	159	00:15:59	338	11.270	1	161	1.999
11-Apr	control	165	00:19:32	180	16.100	1	173	10.154
15-Apr	control	233	00:17:09	225	24.150	2	228	11.875
29-May	control	122	00:22:32	180	8.050	1	143	7.082
17-May	CW	37	00:16:07	225	12.880	2	20	1.646
22-May	CW	104	00:13:12	293	12.880		93	2.245
27-May	CW	141	00:25:54	23	11.270	1	83	5.082
28-May	CW	128	00:13:42	90	8.050	1	118	6.547
29-May	CW	67	00:18:54	180	8.050		97	4.639
18-May	CCW	236	00:13:14		0.000		236	5.000
19-May	CCW	43	00:25:03	225	11.270		14	0.340
20-May	CCW	149	00:13:59	315	27.370	1	277	1.948
23-May	CCW	180	00:13:15	270	16.100	1	215	6.135
25-May	CCW	267	00:15:14		0.000		267	5.000

Exp= experimental condition, 5km_TD= 5km track direction, 5km_T=time to reach 5 km, W_dir=wind direction, W_kmh =wind speed, H_day =day on which the bat homed, left blank if the bat did not return, H= heading (after subtracting the wind vector from the bearing) and R=the length of the heading vector.