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Wild rufous hummingbirds use local landmarks to return to rewarded locations**David J. Pritchard^{1*}, Renee D. Scott², Susan D. Healy¹ and T. Andrew Hurly²**¹School of Biology, University of St Andrews, Fife, UK²Department of Biological Sciences, University of Lethbridge, Alberta, Canada* Corresponding author: djp4@st-andrews.ac.uk

Highlights

- We trained wild hummingbirds to feed from a location surrounded by three landmarks.
- When we expanded the landmark array, the hummingbirds shifted where they searched.
- We suggest that hummingbirds use nearby landmarks to remember reward locations.

Abstract

Animals may remember an important location with reference to one or more visual landmarks. In the laboratory, birds and mammals often preferentially use landmarks near a goal (“local landmarks”) to return to that location at a later date. Although we know very little about how animals in the wild use landmarks to remember locations, mammals in the wild appear to prefer to use distant landmarks to return to rewarded locations. To examine what cues wild birds use when returning to a goal, we trained free-living hummingbirds to search for a reward at a location that was specified by three nearby visual landmarks. Following training we expanded the landmark array to test the extent that the birds relied on the local landmarks to return to the reward. During the test the hummingbirds’ search was best explained by the birds having used the experimental landmarks to remember the reward location. How the birds used the landmarks was not clear and seemed to change over the course of each test. These wild hummingbirds, then, can learn locations in reference to nearby visual landmarks.

keywords: spatial cognition, navigation, landmarks, spatial learning, hummingbirds

Introduction

Many species rely on visual information in the environment to return to important locations (Collett et al., 2013; Gould et al., 2010). They may use this information by matching their entire current view with a previously experienced visual panorama (Zeil, 2012) or by extracting prominent visual features and encoding the position of a location relative to such “landmarks” (Wiener et al., 2011). In theory, any visual feature at any distance could act as a landmark, however, animals tested in the laboratory generally show a preference for landmarks closer to the goal, rather than for alternatives further away (Bennett, 1993; Chamizo et al., 2006; Gould-Beierle and Kamil, 1996; Spetch, 1995).

Despite decades of work elucidating how vertebrates, primarily birds and rodents, use landmarks to return to locations in the laboratory, it is unclear how relevant those results are for such species living in the wild. In laboratory-like tests of spatial memory, wild, free-living birds appear to perform similarly to laboratory species (e.g. Healy & Hurly, 1995; Hurly, Fox, Zwueste, & Healy, 2014; Hurly & Healy, 2002; Hurly, 1996). With the significant exception of homing pigeons (e.g. Biro, Guilford, & Dawkins, 2003; Guilford & Biro, 2014; Schiffner & Wiltschko, 2013), however, there are few data concerning the cues that vertebrates in the wild use to remember locations. This stands in stark contrast to the wealth of knowledge about small-scale navigation, especially landmark use, by birds in the laboratory (Cheng et al., 2006; Gould et al., 2010).

Thus far, in fact, there is very little evidence that birds in the wild even use local landmarks to remember locations. Most of the experiments examining small-scale navigation in the wild have been focussed on wild rodents, such as ground squirrels *Spermophilus columbianus* and fox squirrels *Sciurus niger*, which do not preferentially use landmarks nearby the goal, and in some cases actively prefer more distant cues (Lavenex et al., 1998; Vlasak, 2006). The best evidence for local landmark use in wild birds comes from field experiments with hummingbirds. Wild hummingbirds can be readily worked with in the wild (Healy & Hurly, 2013) and primarily remember flowers in their territory in terms of their spatial location (Hurly & Healy, 1996, 2002). There is some evidence that this spatial memory is based on visual landmarks close to the flower’s location as hummingbirds will choose which of an array of four flowers to visit based on the presence of a landmark close to that flower (Hurly et al., 2014) and these birds can learn to search for a flower at the correct distance and in the correct direction from a pair of landmarks (Pritchard, Hurly, & Healy, 2015). What is still unknown, however, is whether wild hummingbirds will spontaneously acquire and/or weight distance and direction information from closer landmarks over other sources of spatial information, without being trained to use local landmarks. Rather than moving landmarks between flowers, as was done by Hurly et al. (2014), we trained rufous hummingbirds *Selasphorus rufus* in the wild to visit one of three locations on a board in which many different wells had been drilled. The rewarded locations were

presented within an array of three nearby landmarks (all on the board). To test the degree to which the birds relied on these landmarks that were in very close proximity to the goal, we presented the trained birds with a test in which we expanded the landmark array (Figure 1 & 2a). If the birds used the local landmarks to remember the rewarded location, they should shift the location in which they searched for the reward accordingly.

In addition to investigating whether the birds used local landmarks at all, we also examined whether the birds weighted the local landmarks by proximity to the rewarded location or whether they attended to the shape of the entire array of landmarks. To do this we investigated how the birds responded to the expansion of the landmark array when trained to each of the different rewarded locations. The three rewarded locations differed in the proximity of the three landmarks to the goal: (A) the rewarded location was closest to one landmark and equally far from the other two; (B) each of the three landmarks was at different distances to the goal; and (C) two landmarks were equally close to the reward while the third was further away. If the hummingbirds weighted local landmarks by their proximity to the goal, they should specifically shift their search in the direction of the closest landmark or landmarks.

Methods

Subjects and Experimental Site

The subjects of this experiment were six male rufous hummingbirds and the work was carried out in meadows along a valley in the Rocky Mountains, southwest of Beaver Mines, Alberta, Canada. Here, male rufous hummingbirds establish and defend feeding territories from mid-May to mid-July. Trials were conducted between 0730 and 1930h Mountain Standard Time.

The work was conducted with approval by the University of Lethbridge Animal Welfare Committee and under permits from Alberta Environmental Protection and the Canadian Wildlife Service.

Initial Training

Artificial feeders containing 14% sucrose solution were hung at several meadow sites along the valley in early-May. By mid- to late-May, males had established territories around the artificial feeders. Once it was evident that a male was successfully defending a feeder, it was marked with a coloured, non-toxic ink patch on its pale chest feathers to allow for identification. The ink was applied via a spray attached to a perch on the feeder, which meant that we did not have to catch or handle birds in order to mark them. All males resumed feeding within 15 minutes and we saw no diminution in territorial

holding ability as a result of the mark, which remained visible for the duration of the field season (about six weeks).

Once marked, we trained birds to feed from a dull grey Plexiglas[®] board (500mm x 500mm x 19mm), which was placed on a tripod at a height of 60cm from the ground and at an angle of 45° (Figure 1a). The 95 wells on the board were arranged in a hexagonal pattern with all nearest neighbours a distance of 52.4mm apart. Although each well could contain as much as 180 µL we filled only one with sucrose reward (20% sucrose). This well was marked by red tape around its edges. To train the bird to use the red tape to identify the rewarded well, after each visit we moved the rewarded location (with its red tape) to a new position on the board.

Experimental Trials

For experimental trials, we placed three landmarks on the board, 104.8mm from one another, in the shape of a half hexagon. The landmarks were bottle caps, 30mm in diameter, which were painted either a single colour or a combination of two different colours (pink, blue, green, white, purple, orange, light blue, red, light green). No two landmarks were the same, we changed the landmarks between trials and birds never experienced the same landmark twice.

At any one time we filled only one well with reward in one of three positions on the board relative to the landmarks, designated A, B, or C (Figure 2a): in treatment A, the birds were trained to find the reward in a well that was halfway between Landmarks 1 and 3; in treatment B, the birds were trained to find the reward in a well that was east of Landmark 1, and south of Landmark 2; and in treatment C, the birds were trained to find the reward in a well that was halfway between Landmarks 2 and 3 (Figure 2a). In all cases the bird could have learned the location of the reward with regard to the shape of the entire landmark array (e.g. Figure 3a) or in terms of the distance and direction of the closest landmark/s (Landmark 2 in A, Landmark 1 in B e.g. Figure 3b, Landmarks 2 and 3 in C).

The rewarded well contained 120 µL of 20% sucrose, an amount that the bird did not normally consume entirely. Previous experiments indicated that birds detected reward only by probing a well with its bill and tongue and could not detect reward through visual or olfactory cues (Healy and Hurly 1995; Hurly 1996).

Each bird completed three blocks of trials, each of which contained three trials, one of each treatment (reward at A, B, or C), randomised in order, for a total of nine trials overall. Each trial consisted of two parts: a learning phase and a test phase. In the learning phase, we selected the position of Landmark 1 randomly from one of 21 possible positions. We then placed the other two landmarks in their relative positions to create a half hexagon shape, and baited the rewarded well according to which of the three possible locations (A, B, or C) was to be rewarded. Between trials, we

rotated the board using one of four randomly selected rotations (0° , 90° , 180° , 270°). The board was cleaned as needed with a wet cloth to remove any smudges or other obvious marks the bird could use as a landmark cue.

For each trial we initially marked the rewarded well with red tape, which we removed after the bird had visited the rewarded location three times. If the bird was unable to locate the reward at his first unmarked visit, we replaced the red tape. After the bird had visited the rewarded well twice more, we removed the red tape again. If the bird was still unable to locate the reward, the red tape was replaced and gradually made smaller with each visit until the bird could no longer rely on it as a cue to locate the rewarded well. Once we had removed the tape for the final time, the bird had to make three consecutive visits to the rewarded well, and only the rewarded well, before we considered that the learning phase was completed. We emptied and refilled the well with $120\mu\text{L}$ of sucrose after each visit.

Once the bird had reached the three-visit criterion, we expanded the landmark array by moving each landmark two well sites (104.8mm) outward (Figure 2a). Although the movement of the landmarks during the expansion was the same in A, B, and C, as the rewarded location in each treatment was in a different position relative to the landmarks, the predicted locations in the expanded array differed among the treatments. In addition, in the expanded array, different landmark cues, such as the closest landmark/s or the overall shape of the array, indicated different locations on the board: in A, the predicted location based on shape fell halfway between Landmarks 1 and 3 and 104.8mm southeast of Landmark 2, whereas the predicted location based on the distance and direction from the closest landmark (Landmark 2) fell 52.4mm southeast of Landmark 2; in B, the predicted location based on shape fell due south of Landmark 2 and 104.8mm east of Landmark 1, while the predicted location based on the closest landmarks (Landmark 1) 52.4mm east of Landmark 1 (Figure 3c); finally, in C, the predicted location lay halfway between Landmarks 2 and 3, 104.8mm from each, whereas the predicted locations based on the closest landmarks (Landmarks 2 and 3 were equally close) were either 52.4mm east of Landmark 2 (referred to as Cl-1 in Figure 4C) or 52.4mm west of Landmark 3 (referred to as Cl-2 in Figure 4C). The expansion of the array marked the beginning of the test phase. No well contained reward in the test phase. On the bird's return to the board, we videotaped the wells he visited. A choice was defined as the bird stopping in flight and inserting his bill and tongue into a well. Once the bird flew away from the unrewarded board, we considered the test phase to be finished and the board was reconfigured for another trial.

Data analysis

On completion of the experiment we transcribed the videotape data and recorded the positions of each hummingbird's first three choices for each trial. We then calculated average x- and y-coordinates for the first, second, and third choices for each bird within each treatment (e.g. mean location of first choices for each bird when the reward was at A). Half of the trials of B and C were bilateral reflections about the central landmark and were integrated into a single location for analysis (Figures 2b & c). Using the calculations in Batschelet (1981), we used these mean search locations to construct 95% confidence ellipses to show the area in which the birds searched for the reward following the expansion, and to test our hypotheses by determining if important locations such as the original rewarded well, or the predicted locations, fell within the confidence ellipses or not. In order to analyse the direction in which the birds shifted their search and the extent to which they followed the landmarks we calculated the vector between the average locations of the first, second, or third stops and the previously rewarded well for each treatment and transformed those data to polar coordinates, which represent magnitude in millimetres and direction in degrees. To analyse whether birds shifted their search in a particular direction during the test, we used Raleigh tests to test whether the directions that the birds searched from the previous rewarded well were significantly different to a uniform distribution in which all directions are equally represented (Batschelet, 1981). We also used Raleigh tests to determine whether the directions that the birds searched were significantly similar to key predicted directions: West, the direction in which we moved Landmark 1 during the test; Northwest, the direction in which we moved Landmark 2 during the test; Northeast, the direction in which we moved Landmark 3 during the test; and for C, North, the direction of the predicted location based on shape, relative to the previous reward location. Finally, we analysed the distances that individuals shifted their mean search location from that of the previous reward using GLM with individual identity ("Bird") as a random factor.

Results

Training

Following the removal of the red tape which signalled the location of the rewarded well, birds took between 3 to 49 trials (mean \pm SE = 15.42 ± 1.42), with each trial recorded as a visit to the board, before reaching the criterion of visiting the rewarded well before any others for three trials in a row. Despite this variation, individual birds did not differ in the number of trials taken to reach criterion (GLM of log trials to criterion, with Treatment and Bird as a random factor: Bird: $F_{6,11.90} = 1.37$, $P = 0.304$), and there was no differences between the number of trials taken in treatments A, B, and C (Treatment: $F_{2,9.63} = 0.35$, $P = 0.72$; Treatment x Bird: $F_{9,32} = 1.10$, $P = 0.39$).

Did the hummingbirds use the landmarks?

Following the expansion of the landmark array, the majority of birds did not continue to search at the previously rewarded well (“o” on Figure 4), suggesting that they responded to the movement of the landmarks by changing where they searched. This shift in their mean search location could be the result of either: 1) the birds following the landmarks following the expansion, providing some evidence that the hummingbirds use local landmarks to remember rewarded locations; 2) a general decrease in search accuracy following the changing of the board.

We predicted that if the birds were following the landmarks we would see the mean search locations by the birds shift in a consistent direction, for example following one of the landmarks, resulting in the location of the previous reward falling outside of the confidence ellipse. If the birds were simply less accurate following the expansion of the landmark array, we predicted that the mean search locations by the birds would scatter evenly around the previously rewarded well, showing no overall change in direction. For the first choices in both A, where the reward fell between Landmarks 1 and 3, and C, where the reward fell between Landmarks 2 and 3, the previously rewarded well fell outside the 95% confidence ellipses (Figure 4), as the birds shifted their search in a consistent north to north-west direction away from the previous location (Table 1). When the birds were trained to B, in which the reward was south of Landmark 2 and east of Landmark 3, the previous rewarded well lay inside the confidence ellipse of the mean first choices but was at the very edge of the ellipse (Figure 4), and the birds still shifted their search in a similar consistent north-westerly direction to that seen in A and C (Table 1, Figure 4). In A, B and C, the ellipses of the mean second and third choices by the birds were also all shifted away from the original reward in a consistent direction, north to north-west for A and C, and increasingly westwards for B, but only in C did the original reward fall outside of the ellipse. These data suggest that following the expansion of the landmark array, the landmarks were guiding where the birds searched for the reward.

How did the hummingbirds use the landmarks?

As the hummingbirds searched away from the previously trained location when we expanded the array of landmarks, apparently following the landmarks, we next asked how the birds used the landmarks to remember the location of the reward, for example whether or not the birds followed the landmark that was closest to the reward during training.

To see if the birds followed the closest landmark, we first looked at the confidence ellipses we fitted earlier. If the well the same distance and direction from the closest landmark during training (“Cl” in Figure 4A and B, “Cl-1” or “Cl-2” in “C”) fell outside of the ellipse, this would suggest that the birds were not searching at that predicted location, and were, not following the closest landmark.

Similarly, if the well in the correct relative position with regard to the overall shape of the landmark array (“Sh” in Figure 4) fell outside the ellipse, this would suggest that the birds did not use the overall shape of the array. The results from the confidence ellipses are, however, less than clear. When birds had been trained to A, their search area in the test encompassed the locations predicted by following the closest landmark and the shape of the array (Figure 4A), but the birds’ mean search locations were not focussed on either of those locations, rather they were scattered between the previous rewarded well and landmark 2. When birds had been trained to B, the predicted location next to the closest landmark fell outside of the confidence ellipses for all three choices by the birds, while the predicted location relative to the overall shape of the array fell outside the ellipse for the first two choices (Figure 4B). Similarly, when birds had been trained to C, the predicted location relative to the overall shape of the array was only within the confidence ellipse for the first choice by the birds, while the predicted locations next to each of the equally closest landmarks both fell outwith the ellipses during all three choices (Figure 4C). Based on the data from the confidence ellipses, we were not able to determine how the birds might have been using the landmarks.

Although the hummingbirds did not obviously search at either the well that was the same distance and direction from the closest landmark or at the well in the same position relative to the shape of the landmark array, the birds did tend to shift their search in the direction of the predicted locations during the tests (Table 1, Figure 5). For example, when they were trained to A or C, the birds consistently searched north-northwest of the previously rewarded well, in the direction of landmark 2 (Figure 5). One exception is how the birds responded to the expansion of the landmark array having been trained to visit B. Similar to when the birds were trained to A and C, the birds shifted their search to the north of the previous reward, despite the closest landmark, landmark 1, and the predicted location relative to the shape of the array lying to the west of the previously rewarded well (Figure 5). Unlike in A and C, however, the birds did not continue to search north of the previous reward. By the third choice, the birds had shifted anti-clockwise, such that the birds were predominantly searching to the west, in the direction of the closest landmark and the predicted location relative to the shape of the three landmarks.

Although individual birds all shifted their search in the same direction, which suggests that the birds responded to the expansion of the landmark array, individuals could have differed in the extent to which they moved away from the original rewarded location. To determine whether individuals differed in the distance that they shifted their search, we carried out a GLM on the distance of the mean search location for each bird from the originally rewarded well, with treatments and choice as factors, and birds as a random factor. Although the distance of the mean search location from the original reward location for each bird did not differ across individual birds (Bird: $F_{6,11.04} = 1.612$, $P =$

0.233), the treatments (GLM of distance: treatment: $F_{2,11.04} = 1.37$, $P = 0.292$) or the birds' first, second or third choices (Choice: $F_{2,12.29} = 0.94$, $P = 0.417$), individual birds did differ in the distance they shifted their search across the different treatments (Bird*Treatment: $F_{11,20} = 3.96$, $P = 0.004$; all other interactions $P > 0.05$). In A, the distance of the mean search location from the previously rewarded well ranged from 0mm to 136.16mm (mean \pm SE = 69.69 ± 7.63 mm), in B from 0mm to 107.18mm (mean \pm SE = 49.14 ± 6.62 mm), and in C from 12.91mm to 107.18mm (mean \pm SE = 61.81 ± 6.01 mm).

Discussion

Following the expansion of a landmark array, rufous hummingbirds in the wild did not search around the location at which they had been trained to locate a reward. Rather, they responded to the expansion by shifting their searches in a consistent direction, although individual birds differed in the distance that they shifted their search in each treatment. Irrespective of the location of the reward relative to the landmarks during training, the mean location of the first wells chosen by the birds in the tests was in the direction that landmark 2 moved during the expansion. Landmark 2 was one of the closest landmarks to the reward when the birds had been trained to visit A and C, but not when the birds were trained to visit B, and in a similar direction to the predicted location relative to the overall shape of the landmark array for A and C, but not B. The birds' subsequent choices in the test also depended on whether they had been trained to A, B or C: only when they had been trained to visit B did the mean locations of the birds' second and third choices change. They shifted their search increasingly towards the landmark that had been closest to the reward during training and the predicted location based on the shape of the landmark array.

There are two classes of questions about landmark use that we wanted to address with this experiment: did the wild hummingbirds use local landmarks? And how did the hummingbirds use those cues? Despite the variability of our data, we believe that we can answer the first of those questions. Firstly, and importantly, the hummingbirds all used the local landmarks to relocate the reward. This was shown by all of the hummingbirds responding to the expansion of the landmarks array by changing where they searched in the test. Secondly, the birds' search patterns were not scattered in all directions, but shifted search in a consistent direction, which again suggests that they used the landmarks in their search. It is also the case, however, that the distance the birds shifted their search was sometimes less than we would have expected if the birds used only the landmarks we provided. This suggests that they may also have used the edges or shape of the board, or cues outwith the experimental apparatus. Individual variation in the distance that the birds shifted their search might, therefore, be the result of birds attending to both the local landmarks and these board or extra-board cues, but individuals differing in how they weight these different sources of spatial information.

Birds relying more on the local landmarks would shift their search closer to the distance moved by the landmarks, while, birds relying more on the board or extra-board cues would shift their search less and continue to search closer to the previous location. To determine whether these individual differences are indeed due to differences in the weighting that the birds gave the landmarks rather than, for example, differences in how the birds used the landmarks, requires further experiments in which cues other than the landmarks are made unreliable. Despite this variation between individuals, the fact that all the birds relied to some degree on the local board landmarks contrasts with the reliance by free-ranging rodents on distant cues rather than landmarks 25cm or 65cm from the goal (Lavenex et al., 1998; Vlasak, 2006), and is more reminiscent of the preference for near landmarks by rats (Chamizo et al., 2006) and by birds tested in the laboratory (Gould-Beierle and Kamil, 1999; Spetch, 1995) and the field (Hurly et al., 2014).

There was too much variation in our data for us to answer definitively our second question as to *how* the hummingbirds used the landmarks. The directions in which the birds searched do, however, suggest some features of hummingbird landmark use for future detailed investigation. First, the mean location of the first well probed by the birds following the expansion of the landmark array was always in the same direction regardless of whether the birds had been trained to find reward at A, B, or C. This consistency across the different treatments suggests that the birds were influenced less by the closest landmark/s or the relative shape of the whole array, the predicted locations for which differed across treatments, than by the distances and directions moved by of all the landmarks together, which was consistent across treatments. Birds in the lab can use learned vectors from multiple landmarks to relocate a goal, weighting each vector and searching at the weighted average location (Cheng, 1994, 1988; Gibson and McGowan, 2014) and our data suggest that birds in the wild may use landmarks in a similar way. Further experiments are required, however, before we can make any conclusions regarding the similarities or not of landmark use by birds in the wild to that of birds in the laboratory.

Second, when the birds had been trained to locate the reward at B, they moved where they searched over the course of the test. They did not do this when they had been trained to A or C. When the birds had been trained to B, however, the birds increasingly searched in the direction of the closest landmark and the correct location relative to the shape of the whole array. If the hummingbirds did this by averaging vectors from all the landmarks, this change in B would suggest that the weightings of the vectors changed across the test, from equal weighting to favouring the closest landmark. This may also have been the case when the birds had been trained to A and C, but as this would have lead to birds searching in the same location as they did initially, we cannot be sure. This shift in landmark hierarchy is consistent with the data from birds in the lab using multiple sets of cues to remember a location (Brodbeck, 1994; Shettleworth and Sutton, 2005; Thiele and Winter, 2005). Although our data

are too variable to determine why the hummingbirds changed their strategy, this result suggests attention should be paid to investigating where animals search within a test, in addition to determining the location of their first choice or the average location across all choices. The variability of the field environment, where the availability of different cues may change over time, may lead animals in the field to use more than one strategy to return to a goal, just as do homing pigeons (Wallraff, 2001; Wiltschko and Wiltschko, 2003).

The data collected here add to the very small pool of experimental data on landmark use by vertebrates in the wild (e.g. Lavenex, Shiflett, Lee, & Jacobs, 1998; Vlasak, 2006). Such data are difficult to obtain, as wild animals typically have to be trained to interact with test apparatus, which they can choose to avoid at any time (thus reducing sample sizes, within and across individuals), and by their nature field experiments allow less control than for experiments performed in the laboratory. As a result field data are more variable than is typically possible in the laboratory. Despite this, we believe by virtue of being collected in the wild, these data have particular value because although over the past several decades there has been increasing interest in the role that cognition plays in animals' lives outside of the laboratory (e.g. Healy & Braithwaite, 2000; Kamil, 1987), the difficulties of testing cognitive abilities in the wild have actually limited most of this work to the laboratory. Furthermore, variability itself is becoming an increasingly attractive component of animal behaviour for investigation. To fully understand the use to which animal cognition, here specifically landmark use, is put, we need to acknowledge the variation as well as the average signal. Doing this, we suggest, will allow the testing of the hypotheses raised here with greater accuracy and make good on the promise of bringing the laboratory out in to the wild (Healy and Hurly, 2004, 2003).

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Figure Legends

Figure 1. (a) A diagram of the experimental apparatus, showing the position of the landmarks during training, and the relative size of the hummingbirds. The birds were trained to a Plexiglas™ board, with wells 52.4mm apart drilled in a hexagonal pattern, attached to a tripod 60cm above the ground at an angle of 45°. The location of a rewarded well, containing 120 µL of 20% sucrose, was in a constant position relative to three individually coloured bottle tops acting as landmarks.

Figure 2 a). The location of the landmarks and rewarded well in each treatment. The closed, black circles show the locations of the three landmarks before the expansion, the open, white circles show the locations of the landmarks following the expansion. The landmarks are numbered as they are referred to in the text. In treatment A, the birds were trained to a well halfway between Landmarks 1 and 3, shown by a red circle. In treatment B, the birds were trained to a well east of Landmark 1, and south of Landmark 2, shown by a blue circle. In treatment C, the birds were trained to a well halfway between landmarks 2 and 3, shown by a green circle.

b). The locations of B during the experiment. B was at one of two locations that were bilateral reflections of each other. For analysis, the data for B were all integrated into a single location for analysis (in bold and underlined).

c). The two locations of C, bilateral reflections of one another, that were integrated into a single location (in bold and underlined) for analysis.

Figure 3. The locations of the reward for training treatment B predicted by the closest landmark or the shape of the whole array. The landmarks could be used to remember the reward location by: a). using the shape of the array of landmarks and the reward, or b). by remembering the distance and direction from a single landmark, in this case the closest landmark. c). Following the expansion of the landmark array, these cues indicate different locations from one another and from the original reward location (iii).

Figure 4. The mean locations of the first (Δ), second (+), and third choices (X) in the three treatments A, B, and C which differed in the location of the reward during training (expanded array locations are black circles, original landmark locations are open circles). The ellipses represent 95% confidence intervals in two dimensions around the mean visit locations, combined across all of the birds (first choices: closed line; second choices: dashed line, third choices: dotted line). The coloured symbols denote the predicted locations, Cl for the location predicted by following the closest landmark (Cl-1 and Cl-2 for the two possible locations in C), Sh for the location predicted by using the shape of the

landmark array, and O for the original rewarded location. In C, the embedded compass denotes the frame of reference used in the analysis and discussion of the data. As the board was rotated between different treatments, “North” does not correspond with the true direction of north during testing, but instead allows results from different treatments to be compared.

Figure 5. The directions of the locations the birds visited across their first three choices (1,2,3) in each treatment (A,B,C) relative to the locations rewarded in training. The central arrow shows the mean direction of the choices made by the birds, with the length of the arrow indicating the variance around this mean (non-uniformity in Table 1), the longer the arrow, the smaller the variance. The dashed lines on each circular plot indicate the directions of predicted locations, following the individual landmarks or the configuration of all the landmarks, with the asterisks representing the results of the Rayleigh tests from Table 1, which tested how significantly the choice directions were oriented in a particular predicted direction (* <0.05, ** <0.01, *** <0.001, **** < 0.0001). The direction that the birds were predicted to shift their search if following the closest landmark is indicated by “CI” or CI-1 and CI-2 in C in which two landmarks were equally close to the reward, while the direction that the birds would shift their search if using the configuration of all three landmarks is indicated with an “Sh”. The first choices by the birds were all in the direction that Landmark 2, and for A and B, that Landmark 3 were moved. For A and C, but not for B, this direction corresponded with both the closest landmark and the location predicted by the shape of the landmark array. When birds were trained to A or C, the direction that the birds searched relative to the original reward locations remained consistent over the following two choices, however, when the birds were trained to B, they searched increasingly in the direction predicted by following the closest landmark or using the shape of the array during the second and third visit.

Figure 1



Figure 2

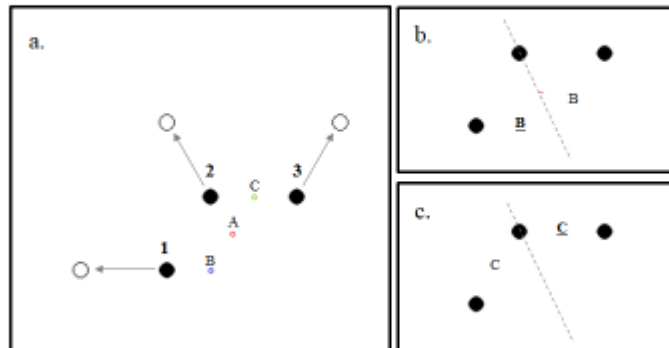


Figure 3

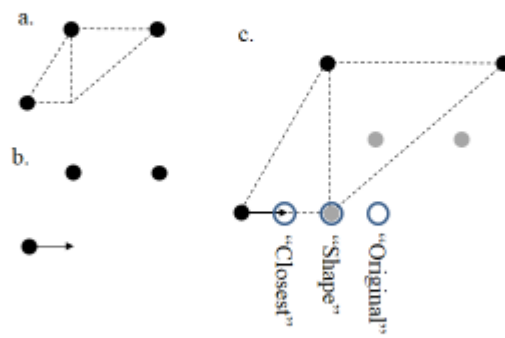


Figure.4

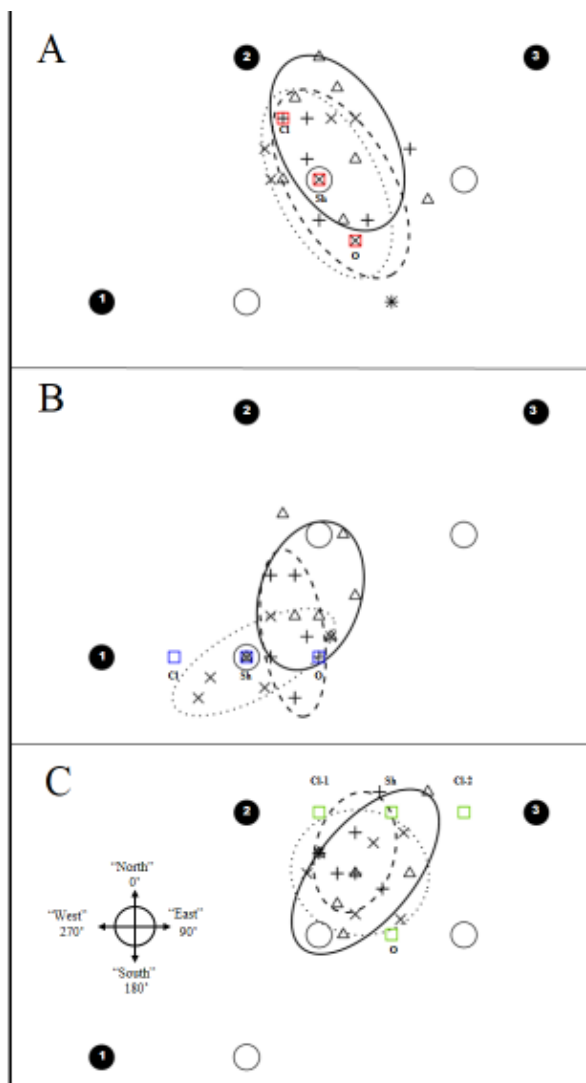


Figure.5

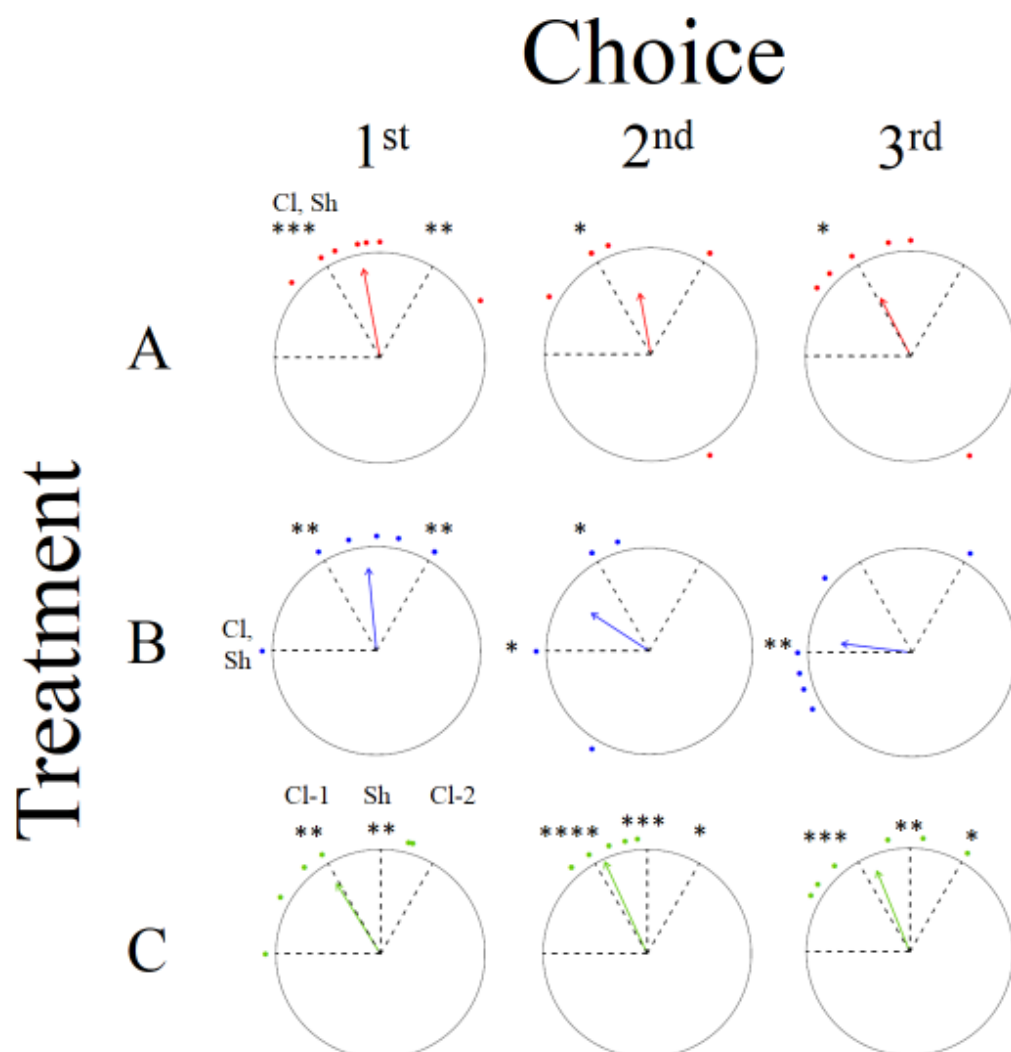


Table Legends

Table 1: The mean distances and directions of the locations the birds visited in the test trials relative to the locations rewarded in training. Non-uniformity shows the degree to which the directions that the birds shifted their search differed from a uniform circular distribution, i.e. whether the birds searched in a significantly consistent direction. Non-uniformity was tested using Rayleigh tests in R (R Development Core Team, 2012). The final columns show the degree to which the birds shifted their search in the direction that the individual landmarks moved: west, northwest or northeast. For the birds trained to C we also tested whether they were directed in the mean direction of the two closest landmarks, north. Directionality was tested using Rayleigh tests with one of the predicted directions (West, Northwest, Northeast, North) used as a reference, allowing us to determine whether the choices by the birds were significantly oriented in that direction. The closer the directional component (V) is to 1, the more oriented the choices were to the reference direction. Among treatments and across visits the birds did not differ in the distance at which they searched from the well rewarded in training. However, while the search direction when birds were trained to A and C remained consistent over the three visits, when the birds were trained to B they searched increasingly west of the original reward during the second and third visit.

Previous reward location	Visit	Mean Distance (mm ± SE)	Mean Direction (degrees)	Non-uniformity	Directional Components (V)			
					Directed West (270°)	Directed Northwest (330°)	Directed Northeast (30°)	Directed North (0°)
A	1	80.95	349.48	0.86**	0.16	0.81***	0.65**	
	2	63.49	349.71	0.58	0.1	0.55*	0.44	
	3	64.62	332.62	0.62	0.28	0.62*	0.33	
B	1	54.77	354.38	0.79*	0.08	0.72**	0.64**	
	2	36.23	303.01	0.67	0.56*	0.59*	0.03	
	3	55.48	277.16	0.68	0.68*	0.41	-0.26	
C	1	60.76	328.09	0.79**	0.42	0.79**	0.37	0.67**
	2	67.88	335.21	0.96***	0.41	0.96****	0.55*	0.88***
	3	56.78	337.76	0.84**	0.31	0.83***	0.51*	0.78**

* <0.05, ** <0.01, *** <0.001, **** < 0.0001.