

A lateralized avian hippocampus: preferential role of the left hippocampal formation in homing pigeon sun compass-based spatial learning

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Abstract

The hippocampal formation (HF) plays a crucial role in amniote spatial cognition. There are also indications of functional lateralization in the contribution of the left and right HF in processes that enable birds to navigate space. The experiments described in this study were designed to examine left and right HF differences in a task of sun compass-based spatial learning in homing pigeons (*Columba livia*). Control, left (HFL) and right (HFR) HF lesioned pigeons were trained in an outdoor arena to locate a food reward using their sun compass in the presence or absence of alternative feature cues. Subsequent to training, the pigeons were subjected to test sessions to determine if they learned to represent the goal location with their sun compass and the relative importance of the sun compass vs. feature cues. Under all test conditions, the control pigeons demonstrated preferential use of the sun compass in locating the goal. By contrast, the HFL pigeons demonstrated no ability to locate the goal by the sun compass but an ability to use the feature cues. The behaviour of the HFR pigeons demonstrated that an intact left HF is sufficient to support sun compass-based learning, but in conflict situations and in contrast to controls, they often relied on feature cues. In conclusion, only the left HF is capable of supporting sun compass-based learning. However, preferential use of the sun compass for learning requires an intact right HF. The data support the hypothesis that the left and right HF make different but complementary contributions toward avian spatial cognition.

Introduction

Functional lateralization is not a uniquely human or even mammalian feature of brain organization (Vallortigara *et al.*, 1999; Vallortigara & Rogers, 2005). In birds, numerous eye occlusion experiments (Güntürkün, 1997) investigating spatial (Rashid & Andrew, 1989; Andrew, 1991; Clayton, 1993; Ulrich *et al.*, 1999; Gagliardo *et al.*, 2001; Prior & Güntürkün, 2001; Tommasi & Vallortigara, 2001; Wiltschko *et al.*, 2002; Prior *et al.*, 2004) and nonspatial (Horn, 1986; Vallortigara & Andrew, 1994; Sui & Rose, 1997; Rogers *et al.*, 2004) behaviour have demonstrated hemispheric asymmetry in the control of behaviour.

The avian hippocampal formation (HF) has been extensively studied for its participation in a variety of navigational processes that support homing (Bingman & Able, 2002; Bingman *et al.*, 2005). After displacement to a distant, unfamiliar location, the homing pigeon navigational map enables pigeons to determine their location relative to the home loft (Papi, 1991; Wallraff, 2004). When learning a navigational map within an enclosed outdoor aviary (Ioalè *et al.*, 2000), lesions to HF of the left hemisphere impair acquisition while lesions to the right HF do not (Gagliardo *et al.*, 2001). In addition to the navigational map and navigation by familiar landmarks, the homing behaviour of pigeons is dependent on their sense of direction

determined by the sun compass and earth's magnetic field. The sun compass not only enables birds to orientate in space during navigation, but likely provides an essential directional framework in support of spatial learning (Bingman & Jones, 1994; Gagliardo *et al.*, 1996). Therefore, the more important role of the left HF in navigational map learning promotes the hypothesis that the left HF is similarly more important in enabling the sun compass to serve as a directional reference for spatial learning.

The aim of the present study is to better characterize the functional lateralization of the avian hippocampus by testing whether the preferential role of the left HF in navigational map learning is paralleled by a similar preferential role of the left HF in sun compass-based spatial learning.

Materials and methods

Subjects and surgery

Sixty adult homing pigeons (*Columba livia*), with homing experience from different sites up to 20 km from home, were used in the experiments, which took place at the Arnino Field Station (Pisa, Italy). The birds were divided into three experimental groups of 20 subjects each that underwent surgery at least one month before any behavioural training. HFL-birds were subjected to the ablation of the left hippocampal formation; HFR-birds were subjected to the ablation of the right hippocampal formation; C birds were sham operated controls.

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For the HFL and HFR surgery, pigeons were deeply anaesthetised with an intramuscular injection (2 mL/kg) of a 20% solution of chloral hydrate in saline and then placed in a stereotaxic apparatus. Lesions were made by aspiration under a surgical microscope following the procedure of (Bingman *et al.*, 1984). The hippocampal formation was stereotaxically identified according to the coordinates from Karten & Hodos (1967). The sham operated controls (C) were deeply anaesthetised, subjected to scalp incision and then sutured. The surgical procedure was approved by the Ethical Committee of the University of Pisa (CASA).

Throughout the experiments the birds had free access to water and grit; food was provided in the experimental apparatus during the training sessions. Training and test sessions occurred when the sun was visible. On days when the birds could not be trained they received approximately 20 g of mixed grain each.

Experimental apparatus

The experimental apparatus consisted of an octagonal arena (2.6 m in diameter and 0.75 m high; Chappell & Guilford, 1995; Gagliardo *et al.*, 1996). The arena, placed in the middle of an open field, had opaque walls and a roof made of a thin net. From inside the arena, the birds had no visual access to landscape features, but had an unobstructed view of the sky overhead. In the centre of the arena there was a remotely operated holding cage (30 × 30 × 30 cm), which was used to release a pigeon at the beginning of a trial. A goal box (eight total) was placed on the outer perimeter of each wall. Goal boxes were accessible by a 25 × 25 cm hole in each wall. A wooden barrier within each goal box prevented pigeons from seeing any food reward before entering the box. Depending on experimental procedure (see below), distinct feature cues may have been present above each goal entrance. When present, visual feature cues consisted of a square wood panel (25 × 25 cm) coloured yellow, green, blue, red, black, grey, blue and green stripes, and white and orange stripes.

General behavioural training procedures

To familiarize the birds with the test arena, a pre-training procedure was employed. On the first day of pre-training, each goal box was rewarded with food visible from the centre of the arena. On the second day of pre-training, each goal box was rewarded, but food was hidden behind the wooden barrier in each goal box. A pigeon was removed from the arena when all the food was eaten or after 30 min. For the third day and last session of pre-training, food was now placed in only one goal box (target goal) arbitrarily assigned to each subject. For each bird the compass direction of the goal, and when available the associated feature cues (see below), remained the same throughout the session and subsequent experimental training. On each trial of the last pre-training session ($n = 10$), a subject was removed from the arena only after having found the goal and eaten the food; in other words the subject was given the possibility to make mistakes until finding the reward (a correction procedure).

Following the last pre-training session, experimental training began. On each trial (ten trials per session), a pigeon was allowed to enter one goal box before being removed from the arena (i.e. no correction procedure was employed), and the trial scored correct if the target box (same as the last day of pre-training) was entered. Birds that were being tested at the same time were alternately given trials. Therefore, the within session, inter-trial interval for any individual ranged from approximately 15 to 40 min. Subsequent test-probe sessions consisted of five trials during which no food reward

was made available. All training and test sessions took place during the first half of sunny days.

Four experimental procedures were used.

Experiment 1: training in the absence of feature cues and test after clock-shift

C ($n = 9$), HFR ($n = 9$) and HFL ($n = 9$) experimentally naïve pigeons were trained and tested in the arena in the absence of any feature cues. The birds were trained to a pre-established points criteria. If a bird chose the rewarded box on a trial it was awarded one point. Because birds will occasionally move away from the centre of the arena before making a choice, a directional response reliant on the sun could lead a pigeon to orientate correctly but end up at a box adjacent to the goal location. Therefore, choices to the two boxes immediately adjacent to the goal box were awarded a half point in recognition that a subject orientated in the appropriate goal direction. Criteria were set at a minimum of 24 points over three consecutive sessions, with at least eight points awarded on the last session. One HFL pigeon that failed to reach criteria had its training terminated after 20 sessions.

After reaching criteria, a bird was housed for six days in a light-proof room where the artificial day started and ended 6 h before (fast clock-shift) the natural light-phase. During the first three days of the clock-shift treatment the birds were fed *ad libitum*. The food bowl was removed on the fourth day. Over the next three days birds were food deprived, having access only to the food left on the floor. Pigeons were then tested after completion of the clock-shift treatment to determine if they had learned the arena goal location with their sun compass. The test session consisted of five un-reinforced trials.

Experiment 2: training in the presence of feature cues and testing in the presence of feature cues after clock-shift

C ($n = 6$), HFR ($n = 6$) and HFL ($n = 6$) experimentally naïve pigeons were trained and tested in the arena provided with feature cues, and therefore could solve the task either by their sun compass or by relying on the distinctive feature cue over the target goal box. A trial was scored correct when a pigeon entered in the correct target box (goal). They were trained for six training sessions in the absence of any explicit learning criterion. Previous work, e.g. (Gagliardo *et al.*, 1996), has shown that the presence of sun compass and feature cue information enables both control and bilateral HF lesioned pigeons to generally learn this task in fewer than six sessions. The additional presence of feature cues, which stabilizes the orientation of the pigeons, also obviates the need of a points-system criteria as used in Experiment 1. Once the training phase ended, each bird was subjected to a 6 h fast clock-shift manipulation for at least 6 days (see Experiment 1 for details), following which the test session occurred. During the test session, which consisted of five un-reinforced trials, the sun compass and feature cues were set in conflict with each other.

Experiment 3: training in the arena provided with feature cues and test after rotation of the arena (feature cue shift)

C ($n = 8$), HFR ($n = 8$) and HFL ($n = 8$) experimentally naïve pigeons were trained and tested in the arena provided with feature cues as in Experiment 2. The birds were trained for six training sessions in the absence of any explicit learning criterion (see Experiment 2 for rationale). A trial was scored correct when a pigeon entered the correct target goal. Once training ended, the birds were given one test session with the arena and associated feature cues rotated of

90° counter-clockwise. Again, during the test session, which consisted of five un-reinforced trials, the sun compass and feature cues were set in conflict with each other.

Experiment 4: training in the arena provided with feature cues and test in the arena in the absence of feature cues after clock-shift

Six birds from each experimental group of Experiment 2 and four birds from each group of Experiment 3 participated in this experiment (C, $n = 10$; HFR, $n = 10$ and HFL, $n = 10$). After the test session of a bird's previous experiment, it was re-trained with feature cues present for 2 or 3 days (20–30 trials, the pigeons of Experiment 2 were first allowed to re-adapt to the natural photoperiod for at least one week) until they made no more than three errors in a ten trial session. A trial was scored correct when a pigeon entered in the correct target goal. Once re-training was completed, all pigeons were subjected to a 6 h fast clock-shift manipulation for at least six days. The pigeons were then tested in the arena in the absence of the feature cues to determine if they could demonstrate sun compass learning when the feature cues were not present. This test session consisted of five un-reinforced trials.

Histology and lesion reconstruction

As a first assessment of lesion damage, 12 HFR and 12 HFL pigeons of Experiments 1 and 2 were killed for lesion reconstruction. The birds were deeply anaesthetized with an overdose of a 20% solution of chloral hydrate and perfused intracardially with 10% formalin. Once extracted, the brains were cut coronally, in 50- μ m sections, with a freezing sliding microtome. The sections were stained with Cresyl violet, and with the aid of a macroprojector, the lesions were reconstructed on standard coronal sections derived from the atlas of Karten & Hodos (1967). The range of lesion damage for each group was visualized as a summary figure (see Fig. 5).

As a second assessment, eight HFR and seven HFL pigeons were similarly treated, and HF lesion damage volume as a per cent of total HF volume (for one hemisphere) was calculated for each bird. A Pearson rank correlation was then carried out to determine if there was any relationship between lesion damage volume and behavioural performance during the test sessions of Experiment 3 (an analysis with a smaller sample of the same birds was also carried out for Experiment 4). The analysis was carried out for Experiment 3 because the behaviour the HFR group birds was characterized by two distinct categories suitable for this kind of correlation analysis.

Data analysis and statistics

To test for group differences in learning, an analysis of variance was applied to the number of sessions taken by the pigeons of the three experimental groups to reach the criterion of Experiment 1. Additionally, a two-way analysis of variance for repeated measures was applied to the scores awarded to the birds across the first ten sessions of training. For birds that had met criteria before ten sessions, we simulated eight points per session out to ten sessions. For Experiments 2 and 3, a two-way analysis of variance for repeated measures was applied to the number of correct responses recorded across training. Because Experiment 4 was a composite of birds with previous experience, no learning analysis took place.

The directional choices of each pigeon over the first five trials of the last training session and the five trials of the test session were used to

calculate an individual mean vector for training and test, respectively (Batschelet, 1981). To normalize the data across subjects, we arbitrarily assigned a direction of 360° to the midpoint of the target goal during training. The individual mean vectors were used to calculate the group mean vectors. The Moore's test was used to test whether the directions of the individual mean vectors for each treatment group deviated from uniform; the Mardia two-sample test was used to test for between-group differences in directional choices. The Hotelling test for paired data was applied to the individual mean vectors to test for differences in the comparison between training and test orientation (Batschelet, 1981).

Results

Experiment 1: training in the absence of feature cues and test after clock-shift

Training

All but one pigeon (pigeon R of the HFL group) reached the training criteria in 20 sessions or less. Although the two lesion groups were somewhat slower in reaching criteria (C, mean 10.9, SE 1.59; HFL, mean 12.8, SE 1.68; HFR, mean 13.3, SE 1.53), there was no between group difference (one-way ANOVA, $F_{2,24} = 0.64$, $P > 0.5$). The two-way ANOVA for repeated measures applied to the points awarded through the first ten sessions (see Materials and methods for details) revealed no between group difference in learning rate ($F_{2,24} = 0.99$, $P > 0.3$). The performance of the three groups significantly improved across sessions ($F_{2,9} = 6.04$, $P < 0.0001$) at the same pace (interaction between treatment and sessions $F_{2,18} = 0.74$, $P > 0.5$).

Summarized in Fig. 1A are the individual directional choices and mean vectors of each pigeon for the first five trials of the last training session. It is clear that by the end of training the pigeons were accurately locating the food reward in the experimental arena (including bird R). For the first five trials of the last training session all groups were orientated in the training direction (one-sample Moore test, $P < 0.002$ for all groups, C mean vector angle 360°, mean vector length, 0.89; HFL 352°, 0.95; HFR 355°, 0.92), and no difference in the group mean vectors were observed between C and HFR, C and HFL, and HFR and HFL pigeons (two-sample Mardia test, $P > 0.5$ for all comparisons).

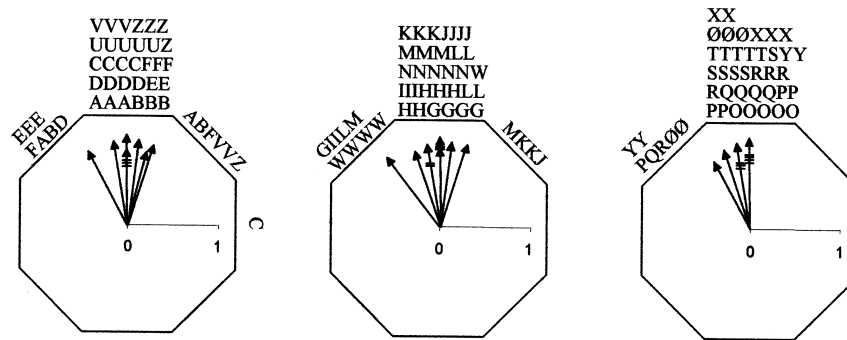
Phase-shift probe trial session

The training data demonstrate that the pigeons in all groups learned the directional location of the food reward. To determine if the directional learning was mediated by the sun compass, the pigeons were subjected to a phase-shift of the light-dark cycle. If reliant on the sun compass to directionally locate the goal during training, a pigeon should show an approximately 90° counter-clockwise shift of its directional choices during the subsequent phase-shift probe session.

Summarized in Fig. 1B are the individual directional choices and mean vectors of each pigeon for the five trials of the probe session. The Hotelling test for paired data revealed that all three groups of pigeons displayed test distributions significantly different from their respective training distribution ($P < 0.0005$ for both C and HFR pigeons; $P < 0.05$ for the HFL birds). However, only the C pigeons and the HFR pigeons displayed appropriate shifts in their orientated directional choices reflective of sun compass use (C, mean vector direction, 285°, mean vector length, 0.67; HFR, 279°, 0.62; one-sample Moore test, $P < 0.002$ and $P < 0.01$, respectively), and no between group difference was found (two-sample Mardia test, $P > 0.5$).

Experiment 1

(A) Training without Feature Cues



(B) Test after Phase-shift without Feature Cues

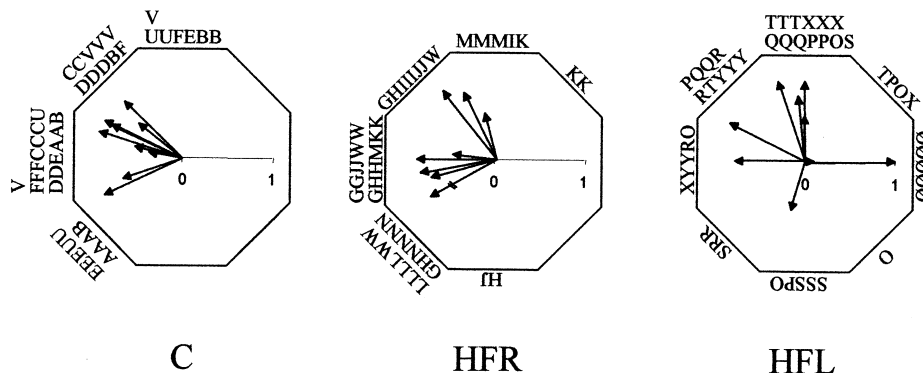


FIG. 1. Directional choices of the control (C), right HF-lesioned (HFR) and left HF-lesioned (HFL) pigeons during the last training session (A) and test session (B) of Experiment 1. Letters on the outside of each octagon represent the trial choices of each individual (each letter represents one subject). Arrows inside each octagon represent the mean vector of one pigeon, the length of which can be read from the scale. Arrows with lines cutting through them represent additional birds with the same mean vector. The top of the octagon identifies the normalized training direction for all pigeons.

By contrast, as a group, the directional choices of the HFL pigeons were not orientated (mean vector direction 327° , mean vector length 0.23, one-sample Moore test $P > 0.2$), and differed significantly from both the C and HFR pigeons (two-sample Mardia test, $P < 0.05$ in both comparisons). The data support the hypothesis that the left HF plays a more substantial role in sun compass-based spatial learning than the right HF.

Experiment 2: training in the presence of feature cues and test in the arena provided with feature cues after clock-shift

Training

All groups learned the task, displaying an increase in the number of correct responses across training (two-factor ANOVA within groups, $F_{5,107} = 3.676$, $P < 0.005$). The learning performance of the three experimental groups was not statistically different (two-factor ANOVA among groups, $F_{2,107} = 2.509$, $P > 0.05$). Furthermore, the three groups learned at the same rate (two-factor ANOVA interaction ($F_{10,107} = 0.869$, $P > 0.5$)).

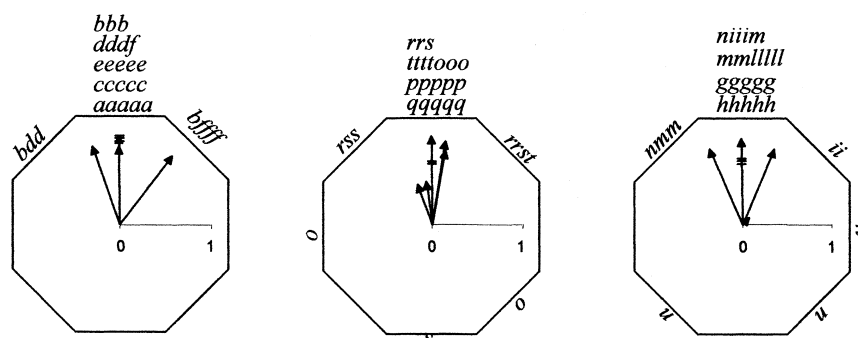
Summarized in Fig. 2A are the individual directional choices and mean vectors of each pigeon for the first five trials of the last training session. It is clear that by the end of training all pigeons were accurately locating the food reward in the experimental arena, except for the subject 'n' belonging to the HFL group. Overall, each group displayed a significantly orientated distribution of choices in the training direction (one-sample Moore test, $P < 0.001$ for all groups, C, mean vector angle 003° , mean vector length, 0.92; HFL, 001° , 0.80; HFR, 001° , 0.78), and no difference in the group mean vectors were observed between C and HFR (two-sample Mardia test, $P > 0.5$), HFL and C ($P > 0.1$) and HFR and HFL ($P > 0.5$) pigeons.

Phase-shift probe trial session

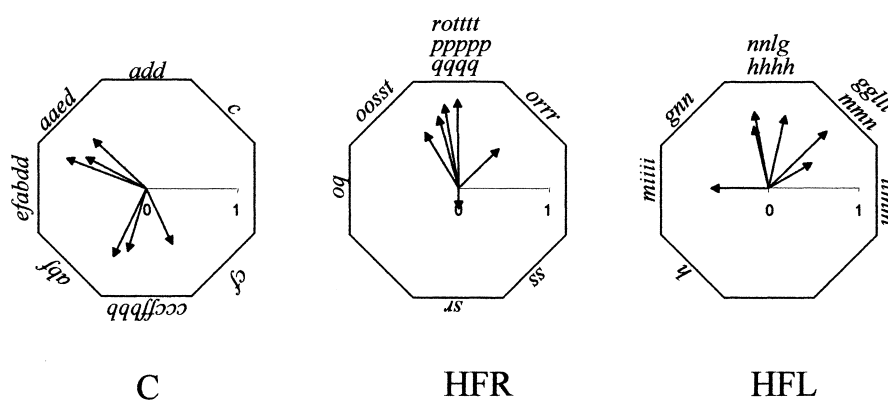
The training data demonstrate that the pigeons from all groups learned the location of the food reward. However, in principle, the birds might have relied on the sun compass, on the feature cues or both. The birds were then tested after a clock-shift treatment, which put in conflict directional information given by the sun compass and the visual information characterising the arena (the colour feature cues identifying each sector).

Experiment 2

(A) Training with Feature Cues



(B) Test after Phase-shift with Feature Cues



C

HFR

HFL

FIG. 2. Directional choices of the control (C), right HF-lesioned (HFR) and left HF-lesioned (HFL) pigeons during the last training session (A) and test session (B) of Experiment 2. See Fig. 1 for further explanation.

Summarized in Fig. 2B are the individual directional choices and mean vectors of each pigeon for the five trials of the probe session. The Hotelling test for paired data revealed that the control birds distribution differed between the training and test session ($P < 0.025$). By contrast, both lesioned groups displayed similar distributions before and after the clock-shift manipulation (Hotelling test for paired data, HFR, $P > 0.1$; HFL, $P > 0.25$). All the C pigeons displayed an orientated shift in their directional choices reflective of sun compass use, but because of the apparent bimodality in the extent of the shift, the vector distribution failed to reach significance (C, mean vector direction, 251° , mean vector length, 0.40; one-sample Moore test $P > 0.05$). By contrast, both right and left lesioned pigeons tended, in general, to orientate in the training direction (HFR, 356° , 0.60; HFL 007° , 0.53; one-sample Moore test, $P < 0.025$ and $P < 0.05$, respectively). However, because of the considerable scatter in the choices of the HFL pigeons, particularly the anomalous behaviour of pigeon 'i', a significant difference was found only in the comparison between the C and the HFR distributions (two-sample Mardia test, C vs. HFR $P < 0.05$; C vs. HFL $P > 0.1$; HFR vs. HFL $P > 0.5$).

In general, both unilateral lesion groups seemed to rely preferentially on the feature cues when set in conflict with the sun compass. By contrast, the intact control pigeons clearly shifted their directional choices in response to the clock-shift manipulation indicating preferential use of their sun compass.

Experiment 3: training in the presence of feature cues and test after the rotation of the arena (feature cue shift)

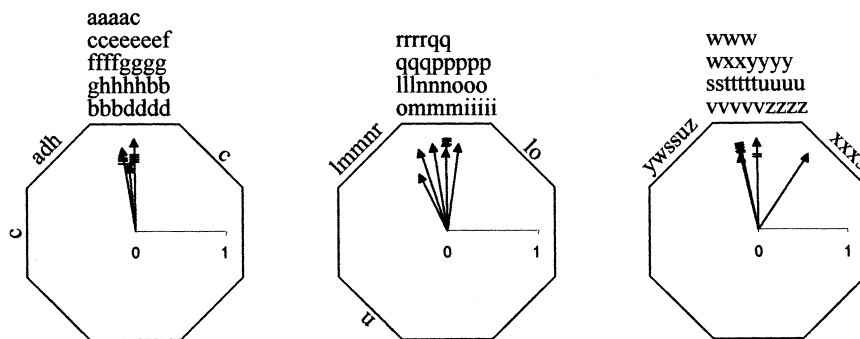
Training

Pigeons from all groups were performing well above chance starting with Session 1 (median number of correct responses, C 6, HFR 6, HFL 4) and improved their performance during training (two-factor ANOVA within groups, $F_{5,107} = 6.190$, $P < 0.0001$). However, the learning performance of the three experimental groups was statistically different (two-factor ANOVA among groups, $F_{2,107} = 4.279$, $P < 0.02$). *Posthoc* analysis (Student–Newman Keuls method) revealed that the HFR pigeons were worse than controls in learning the task ($P < 0.05$). Nevertheless the three groups improved their performance at the same rate, as there was no statistically significant interaction between treatment and the session (two-factor ANOVA interaction $F_{10,107} = 0.784$, $p > 0.5$).

Summarized in Fig. 3A are the individual directional choices and mean vectors of each pigeon for the first five trials of the last training session. Despite the higher number of errors made by the HFR birds during training, by the end of training all three experimental groups were accurately locating the food reward in the experimental arena. For the first five trials of the last training session, each group displayed a significantly orientated distribution of choices in the training direction (one-sample Moore test, $P < 0.001$ for all groups, C, mean vector angle 356° , mean vector length, 0.95; HFL, 358° , 0.93; HFR,

Experiment 3

(A) Training with Feature Cues



(B) Test after Rotation of the Arena/Feature Cues

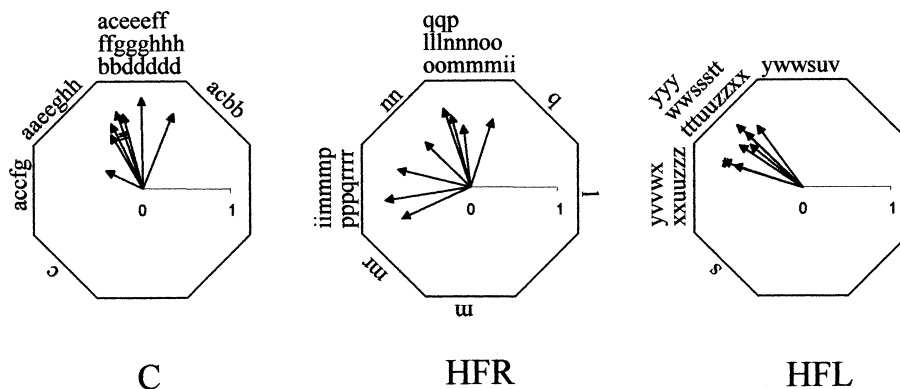


FIG. 3. Directional choices of the control (C), right HF-lesioned (HFR) and left HF-lesioned (HFL) pigeons during the last training session (A) and test session (B) of Experiment 3. See Fig. 1 for further explanation.

355°, 0.91), and no difference in the group mean vectors were observed between C and HFR, C and HFL and HFR and HFL pigeons (two-sample Mardia test, $P > 0.5$ for all comparison).

Feature cue-shift probe trial session

The training data demonstrate that the pigeons from all groups learned the location of the food reward, but similar to Experiment 2, the birds might have relied on the sun compass, the feature cues or both. Rotating the arena and associated feature cues again put into conflict directional information from the sun compass and the visual information of the arena.

Summarized in Fig. 3B are the individual mean vectors and directional choices of each pigeon for the five trials of the probe session. All the C pigeons tended to prefer their training direction regardless of the new position of the arena and feature cues, as previously reported (Gagliardo *et al.*, 1996). They continued to essentially choose the compass direction learned during training phase (C, mean vector direction, 346°, mean vector length, 0.77; one-sample Moore test $P < 0.001$; Hotelling test for paired data, training vs. test $P > 0.05$). By contrast, the left HF lesioned pigeons had their directional choices clearly influenced by the new position of the feature cues (HFL 304°, 0.84; one-sample Moore test, $P < 0.001$; Hotelling test for paired data, training vs. test, $P < 0.001$). The behaviour of the HFR pigeons was characterized by considerable

inter-individual variability that resulted in an overall shift in the group's orientation compared to training (HFR, 315°, 0.58 one-sample Moore test $P < 0.05$). However, the extent of the shift was not so marked as to produce significantly different orientation with respect to training (Hotelling test for paired data $P > 0.1$).

A two-sample Mardia test revealed a significant difference only in the comparison between the C and the HFL distributions (two-sample Mardia test, C vs. HFL $P < 0.005$; C vs. HFR and HFR vs. HFL both $P > 0.5$). From Fig. 3B, it is apparent that the behaviour of the HFL birds was more homogeneous than the HFR pigeons. In fact, all the HFL birds seemed to be similarly affected by the new position of the feature cues. By contrast, the HFR pigeons seemed to break down into a group that maintained the training compass direction and a group that followed the feature cues (see lesion damage correlational analysis below).

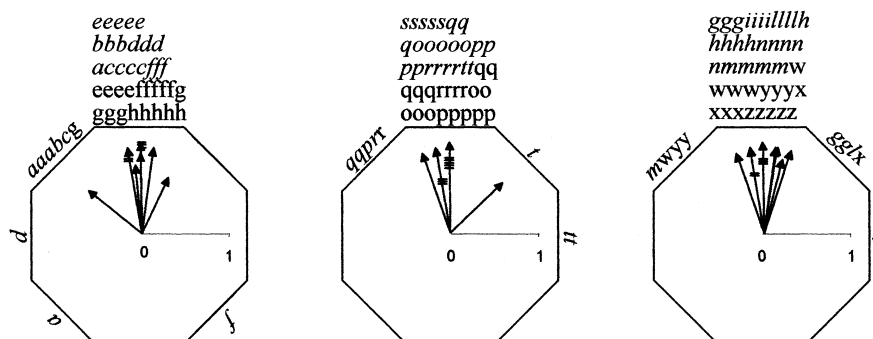
Experiment 4: training in the presence of feature cues and test in the absence of feature cues after clock-shift

Training

Summarized in Fig. 4A are the individual directional choices and mean vectors of each pigeon for the first five trials of the last training session (recall that the pigeons that participated in this experiment had previously participated in either Experiment 2 or Experiment 3). On

Experiment 4

(A) Training with Feature Cues



(B) Test after Phase-shift without Feature Cues

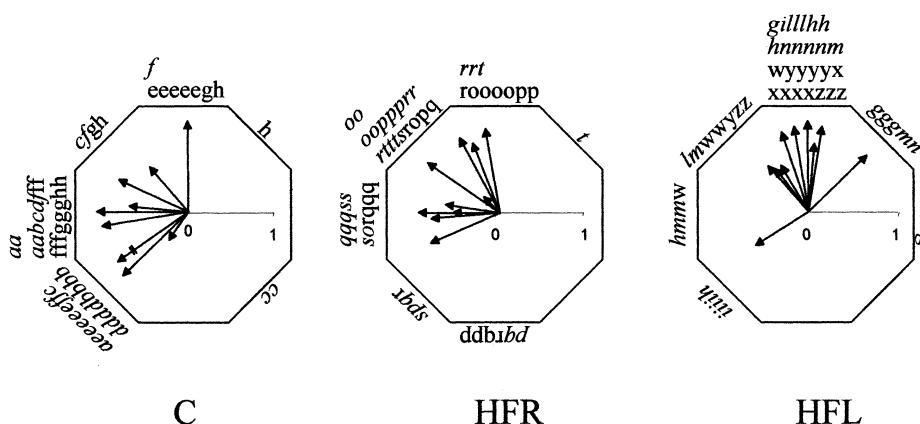


FIG. 4. Directional choices of the control (C), right HF-lesioned (HFR) and left HF-lesioned (HFL) pigeons during the last training session (A) and test session (B) of Experiment 4. See Fig. 1 for further explanation.

the last training day, each group displayed a significantly orientated distribution of choices in the training direction (one-sample Moore test, $P < 0.001$ for all groups, C, mean vector angle 356° , mean vector length, 0.85; HFL, 001° , 0.93; HFR, 356° , 0.86), and no difference in the group mean vectors were observed (two-sample Mardia test, $P > 0.5$ in all comparisons).

Phase-shift probe trial session

The training data demonstrate that the pigeons in all groups learned the location of the food reward, which could be identified as a compass direction and by the feature cues. In the present experiment we were particularly interested in determining if either of the lesion groups could demonstrate sun compass-based learning when feature cues were not present at test.

Summarized in Fig. 4B are the individual mean vectors and directional choices of each pigeon for the five trials of the probe session. The vast majority of C pigeons tended to shift their directional choices in a counter-clockwise direction as expected on the basis of directional learning mediated by the sun compass. (C, mean vector direction, 266° , mean vector length, 0.62; one-sample Moore test $P < 0.025$). In general, the HFR pigeons were similarly affected by the clock-shift, although some individuals tended to prefer the training sector (HFR, 299° , 0.59; one-sample Moore test, $P < 0.005$). Compared to the HFR group, the HFL pigeons were again more

homogeneous in their behaviour (but see pigeon 'i'), seemingly unaffected by the clock-shift manipulation (HFL, 359° , 0.88, one-sample Moore test $P < 0.001$). Because of the inter-individual variability of the HFR pigeons, a two-sample Mardia test revealed a significant difference only in the comparison between the C and the HFL pigeons (two-sample Mardia test, C vs. HFL $P < 0.025$; C vs. HFR and HFR vs. HFL $P > 0.5$ for both). However, while the orientation of the HFL pigeons was not affected by the clock-shift manipulation (Hotelling test for paired data, training vs. test, $P > 0.05$), both the C and the HFR pigeons displayed significantly different distributions during test compared to training (Hotelling test for paired data, training vs. test, C $P < 0.001$, HFR $P < 0.0025$).

Histology and lesion-performance correlation analysis

As a first assessment, 12 pigeons from each of the HFR and HFL groups of Experiments 1 and 2 were killed for lesion reconstruction. A summary of the reconstructions can be found in Fig. 5. As in our previous unilateral ablation work (e.g. Gagliardo *et al.*, 2002), all birds sustained damage to the targeted hemisphere, with generally substantial damage to the hippocampus proper and more variable damage to the parahippocampus. A few pigeons sustained some peripheral damage to the neighbouring hyperpallium apicale (HA), mesopallium (M) and nidopallium (N).

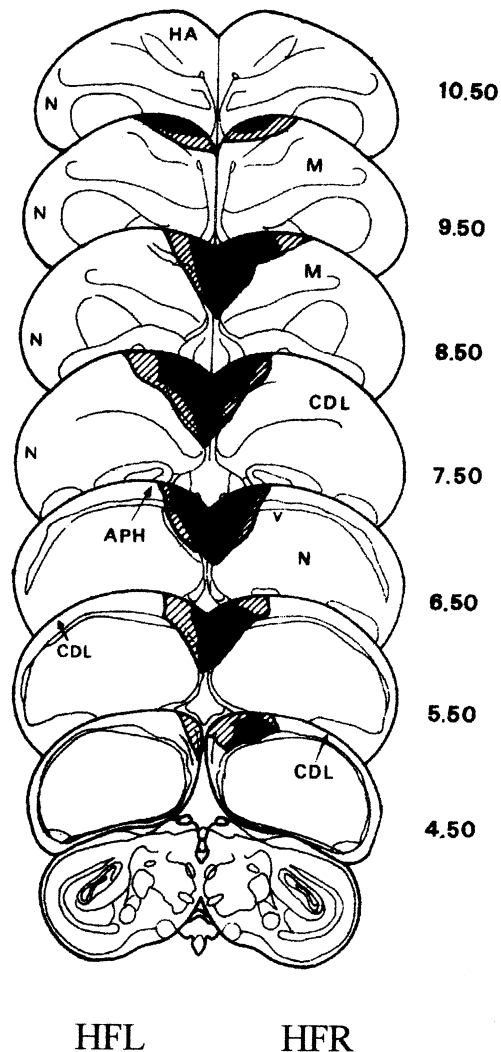


FIG. 5. Summary lesion reconstruction of 12 sampled HFL pigeons (left) and 12 sampled HFR pigeons (right). Areas covered in black identify damage common to at least nine of 12 pigeons. Striped areas identify damage common to at least four of 12 pigeons. Templates of coronal sections adapted from the atlas of Karten & Hodos (1967); numbers refer to anterior-posterior coordinates. Abbreviations (from Reiner *et al.*, 2004): APH, parahippocampus; CDL, area corticoidae dorsolateralis; HA, hyperpallium apicale; M, mesopallium; N, nidopallium; v, ventricle.

The test data of the lesioned birds was often characterized by considerable inter-individual variability, which was most apparent for the HFR birds of Experiment 3. This naturally raises the question of whether the extent of lesion damage could explain the variability in behavioural performance. To examine this issue, eight HFR pigeons and seven HFL pigeons of Experiment 3 were perfused, lesion reconstructed and HF damage quantified as a per-cent of overall HF volume (unilateral). A similar profile of lesion damage, including somewhat larger right HF lesions than left HF lesions (Fig. 5), was also found in this sample. A Spearman-rank correlation analysis was then carried out examining the relationship between HF lesion damage and the difference in an individual's directional choices between training and test session. For the eight HFR birds, which showed considerable inter-individual differences in behaviour during test (Fig. 3B), the per cent HF damage (limited to the right side only) ranged from 51% to 90%. Nonetheless, the per-cent lesion damage could not explain the behavioural variability ($r = 0.19$, $P > 0.50$). For

the seven HFL birds, which showed little inter-individual differences in behaviour, the per-cent HF damage (limited to the left side only) ranged from 8% to 88%. Despite the lower behavioural variability, the correlation was higher but not significant ($r = 0.54$, $P > 0.2$; noteworthy, the bird with 8% damage deviated the least, and therefore, behaved most like the controls). Four birds in each group also participated in Experiment 4, and for both the HFR and HFL pigeons there was no indication that per cent HF damage predicted behaviour. The origins of the behavioural variability found during test in the HFR and HFL pigeons are of interest, but it does not appear that once HF damage goes beyond an unspecified minimum that it can explain much of the behavioural variability.

Discussion

This investigation began with the question of whether the left and right HF of homing pigeons contributed differently to the sun compass representation of a goal location in an experimental arena and the influence supplemental feature cues might have on any lateralized HF lesion effect. Because of the large amount of data presented, we offer first a summary of our interpretation of the representational strategies employed by the different experimental groups.

Controls

Regardless of task parameters, the control birds displayed a preferential sun compass-based, goal location representational strategy as reflected in their appropriately shifted directional choices following clock-shift (see Fig. 6, Experiments 1, 2 and 4) and their failure to change their directional choices in parallel with the rotated feature cues of Experiment 3 (see Fig. 6). Only in Experiment 2 did the control pigeons not behave uniformly as a group, suggesting some effect of the misalignment of the sun compass and feature cue information on their behaviour. However, even in Experiment 2 there was no indication that anything but the sun compass guided their behaviour. As such, the data are consistent with previous studies (Chappell & Guilford, 1995; Gagliardo *et al.*, 1996) demonstrating the primacy of the sun compass over local feature cues in controlling the spatial behaviour of homing pigeons in tasks of this type.

Left hippocampal lesioned (HFL) pigeons

The homing pigeons with only an intact right HF also behaved consistently across the various tasks. Under clock-shift test conditions (Fig. 6, Experiments 1, 2 and 4), there was no indication of an appropriate shift in orientation indicative of sun compass use. In the feature cue-rotation test of Experiment 3 (see Fig. 6), the directional choices of the HFL pigeons shifted with the feature cues, but not perfectly. Their choices on this test (Fig. 3B) were perhaps better characterized as being substantially controlled by the feature cues, but with a bias towards the correct training direction. Finally, it is noteworthy that in addition to feature cues the HFL pigeons also could rely on some other unspecified source of information to guide their directional choices. The HFL pigeons of Experiment 1 learned the task in the absence of any feature cues. During the test of Experiment 4, when no feature cues were present, they continued to orientate in the training direction. Possible sources of directional information include markings in the test arena, stable sources of nonvisual environmental information (e.g. sounds originating from the nearby sea) or learning a fixed-angle response to the sun (all training and test sessions took place during the morning portion of the day). In

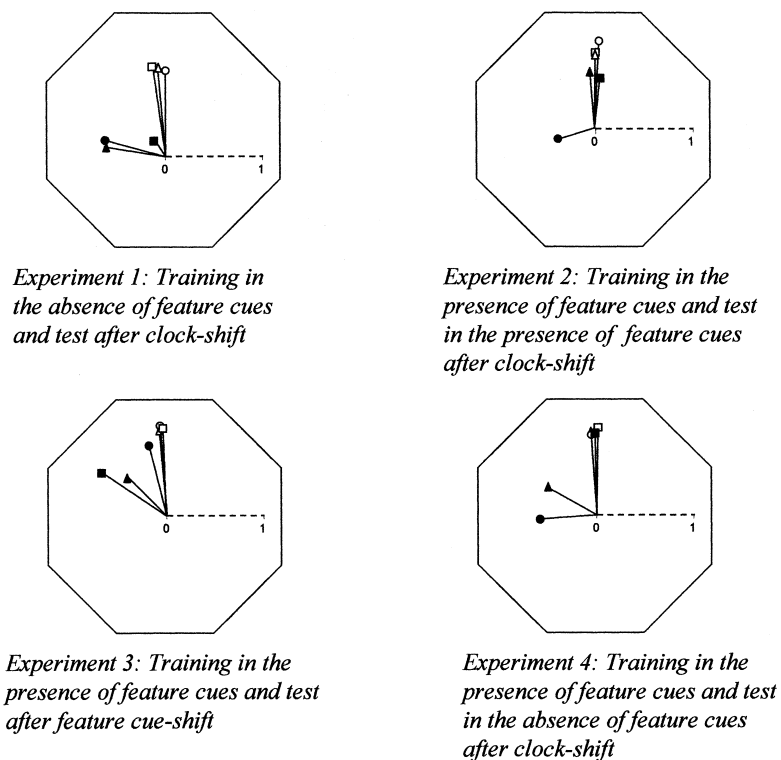


FIG. 6. Summary of the directional choices of the three groups of pigeons (C, open and filled circles for training and test, respectively), right HF-lesioned (HFR, open and filled triangles for training and test, respectively) and left HF-lesioned (HFL, open and filled squares for training and test, respectively). Group mean vectors, derived from the individual mean vectors, are represented.

summary, while successfully learning to use the feature cues to locate the goal location (Experiment 3), there was no indication that the HFL pigeons could use their sun compass. As such, left HF lesions produce a sun compass learning deficit similar to bilateral HF lesions (Bingman & Jones, 1994; Gagliardo *et al.*, 1996).

Right hippocampal lesioned (HFR) pigeons

Certainly the most interesting group were the pigeons with an intact left HF. During the test of Experiment 1 (see Fig. 6) they displayed appropriately shifted directional choices demonstrative of a sun compass-representational strategy. A similar result was found in the test of Experiment 4 (training with feature cues, clock-shift test in the absence of feature cues, see also Fig. 6). A left HF, in contrast to the right HF, is sufficient to support sun compass-based spatial learning. However, compared to controls, the behaviour of the HFR pigeons became curious in the presence of feature cues during test. The clock-shifted directional choices of HFR pigeons when tested without feature cues disappeared when training and testing occurred in the presence of feature cues (Experiment 2). When tested with rotated feature cues (Experiment 3), as a group the HFR pigeons showed a mixed preference between sun compass- and feature cue-information (a mixed preference that could not be explained by the extent of HF damage). In our view, the best interpretation of these findings is that a left and only a left HF is capable of supporting sun compass-based spatial learning. However, in the absence of a companion right HF, the preferential use of sun compass over feature cue information, so apparent in the control pigeons, changed to preferential (Experiment 2) or at least more inclusive (Experiment 3) use of feature cue information. In summary, overall the data support the crucial

conclusion that the left HF plays a substantially more important role in sun compass-based spatial learning than the right HF.

General discussion

The demonstration of left HF participation in a brain system that controls sun compass-based learning is the most recent of a growing list of findings identifying the left HF as preferentially involved in a range of spatial behaviour in homing pigeons and other species of birds. Only the left HF has been found to be necessary for navigational map learning (Gagliardo *et al.*, 2001), and there are suggestive data indicating that the left HF is more important for landmark goal navigation in a laboratory environment (Kahn & Bingman, 2004). If one assumes that conclusions drawn from eye occlusion studies on the spatial-functional characteristics of the left hemisphere reflect in part HF lateralization, then there is an even larger body of evidence indicating a left HF sensitivity to goal navigation (e.g. Rashid & Andrew, 1989; Ulrich *et al.*, 1999; Prior & Güntürkün, 2001; Tommasi & Vallortigara, 2001; Prior *et al.*, 2002; Prior *et al.*, 2004). Also, it is only in the left HF that one encounters neurons that display spatial response properties sensitive to the routes or paths a pigeon takes to reach a goal location (Hough & Bingman, 2004; Siegel *et al.*, 2005).

But what about the right HF? The results of the current experiments did not identify any direct participation of the right HF in learning a sun compass-based goal representation. It is important to note that Tommasi *et al.* (2003) suggested that the right HF in chicks participates in representing the geometric properties of an enclosed laboratory environment (see also Vallortigara *et al.*, 2004), and a similar result has been reported in homing pigeons (Kahn & Bingman,

2004). These findings hint that the right HF may participate more in representing goal locations within some geometric framework rather than the computation of routes among goals. Therefore, the existing data are consistent with the idea of at least a partial segregation of the spatial processing elements involved in sun compass-based navigational learning (left HF) and recognizing a goal location within some geometrical framework (right HF). Interestingly, evidence for a different pattern of lateralization in the presence of conflicting cues (described as local and global) has been reported recently in chick working memory tasks (Regolin *et al.*, 2004). However, our suggested model of HF functional lateralization is clearly an oversimplification in light of the interesting finding that right HF lesions can impact the hierarchical relationship between a left HF-dependent sun compass and a left HF-independent feature cue representation of the goal location. In contrast to controls, which preferentially relied on the sun compass under all of the test conditions, the HFR pigeons, while capable of learning to represent the goal location with the sun compass (Experiments 1 and 4), displayed a preference to orientate by the feature cues when available (Experiment 2 and to some extent Experiment 3).

Bilateral HF lesions do not impact the ability of homing pigeons to locate a goal in an outdoor arena based on visual feature cues (Gagliardo *et al.*, 1996). Therefore, we can assume that when using feature cues the HFR pigeons relied on an extra-hippocampal neural circuit to identify the goal sector. In the HFR pigeons, identifying the goal sector using this extra-hippocampal circuit could supercede the left HF-dependent sun compass-representational mechanism in controlling behaviour. These findings support the hypothesis that the right HF plays a difficult to describe but important role in promoting the primacy of a sun compass-representational strategy as seen in the control pigeons. These considerations highlight an important point. There appear to be at least two partially dissociable anatomical neural circuits that can be used to represent a goal, only one of which necessarily recruits participation of HF. Similar dissociable memory circuits have been proposed in mammals (Mizumori *et al.*, 2004).

At this point it is important to recall that although the temptation in lateralization studies is to view the two hemispheres as independent operating systems, they are nonetheless integrated. The left and right HF are connected by a large hippocampal commissure (Casini *et al.*, 1986) reflecting a lateralized but integrated cognitive system that may explain, in part, the spatial ability of birds. Despite the difficulty in characterizing the role of the right HF in spatial behaviour, it clearly interfaces with the left HF in supporting spatial cognition. Indeed, homing pigeons with either left or right HF lesions are impaired in learning the challenging task of navigating home by piloting to landscape features (Gagliardo *et al.*, 2002) in a manner similar to bilateral lesioned pigeons (Gagliardo *et al.*, 1999). Monocular occlusion of the left or right eye seems to have similar effects on navigating by familiar landmarks in a laboratory environment (Prior *et al.*, 2002). We predict that experimental designs that challenge the spatial representational abilities of birds, including some kind of geometrical processing of goal location, are most likely to reveal the importance of the right HF and the nature of the interaction between left and right HF in support of avian spatial behaviour.

In summary, the results of the present study reinforce the notion of a lateralized hippocampal formation in birds by demonstrating a necessary role of the left but not right HF in sun compass-based learning. As a comparison, it is curious that functional lateralization is not readily observable in the hippocampus of laboratory rodents (but see Kawakami *et al.*, 2003). (Lateralization has been demonstrated for

other regions of the rat brain involved in solving spatial tasks, e.g. Adelstein & Crowne, 1991; King & Corwin, 1992) By contrast, hippocampal lateralization in humans is well known (Smith & Milner, 1989), but it is not necessarily different aspects of spatial cognition that lateralize. In humans, the right hippocampus has been reported to be more heavily recruited in tasks of navigation, the left more heavily recruited in the encoding of episodic memories (Burgess *et al.*, 2002). Like birds, however, assigning a discrete functional dichotomy to the left and right hippocampus of humans is almost certainly an oversimplification (Grön *et al.*, 2000). Nonetheless, we are tempted by the speculation that, in birds, the occurrence of hippocampal lateralization may represent an important evolutionary adaptation determining the properties of avian spatial cognition.

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Abbreviations

C, sham operated controls; HF, hippocampal formation; HFL, left hippocampal formation lesioned; HFR, right hippocampal formation lesioned.

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