

## 1 **Introduction**

2 Anatomical and functional hemispheric asymmetries are a ubiquitous property of vertebrate  
3 brains (Vallortigara & Rogers, 2005; Ocklenburg & Güntürkün, 2012). Because of their strongly  
4 lateralized visual systems (Manns & Ströckens, 2014), research on birds has made a substantial  
5 contribution to our understanding of the evolution, function and neural basis of the lateralized  
6 control of visually guided behaviour. Although the pattern of asymmetry may vary in the two most  
7 commonly studied avian species, young chickens (*Gallus gallus domesticus*) and pigeons (*Columba*  
8 *livia*), there is evidence that the two brain hemispheres of all birds asymmetrically support visually  
9 guided spatial behaviour (Tommasi & Vallortigara, 2001; Nardi & Bingman, 2007).

10 Pigeon homing is supported by a range of navigation and orientation mechanisms including  
11 the so-called navigational map, used from unfamiliar locations, familiar landscape/landmark  
12 navigation, sun compass and geomagnetic compass (Wallraff, 2005). There is considerable  
13 evidence that the two brain hemispheres make different contributions supporting navigational  
14 mechanisms in pigeons. Navigation from unfamiliar sites, necessarily involving the navigational  
15 map and associated compass mechanisms, is principally controlled by the left brain hemisphere  
16 with respect to visual inputs (Prior, Wiltschko, Stapput, Güntürkün & Wiltschko, 2004),  
17 hippocampal control (Gagliardo, Ioalè, Odetti, Bingman, Siegel & Vallortigara, 2001; Gagliardo,  
18 Vallortigara, Nardi & Bingman, 2005) and control by the olfactory cortex (Gagliardo, Odetti, Ioalè,  
19 Pecchia & Vallortigara, 2005). The left hippocampal bias correlates with the recently discovered  
20 broader neural network organization of the left hippocampus compared to the right (Jonckers,  
21 Güntürkün, De Groof, Van der Linden & Bingman, 2015). By contrast, navigation by familiar  
22 landscape/landmarks appears more complicated and likely involves visual processing in both brain  
23 hemispheres (Diekamp, Prior, Ioalè, Odetti, Güntürkün & Gagliardo, 2002) as well as both the left  
24 and right hippocampus (Gagliardo, Odetti, Ioalè, Bingman, Tuttle & Vallortigara, 2002). However,  
25 interpretation of the familiar landscape/landmark lateralization data is confounded by the existence  
26 of two navigational strategies that can exploit topographic information in the control of homing

27 behaviour: site-specific compass orientation and pilotage (Gagliardo, Ioalè & Bingman, 1999;  
28 Guilford & Biro, 2014; Filannino, Armstrong, Guilford & Gagliardo, 2014). Using the first strategy,  
29 birds rely on memorised release site features learned in association with the homeward compass  
30 direction. By contrast, relying on the spatial relationship among familiar visual features would  
31 enable pigeons to pilot home without being dependent on compass information, as such, resembling  
32 more the “cognitive map” conceptualized by O’Keefe and Nadel (O’Keefe & Nadel, 1978). These  
33 two strategies can be experimentally dissociated by releasing pigeons within a familiar area after  
34 having manipulated their internal clock, which induces a deflection in orientation only when the sun  
35 compass is being used because of an error in the estimation of time of day. The relative importance  
36 of familiar topographical cues for the re-orientation of a pigeon can be evaluated by comparing the  
37 degree of deflection and/or the degree of overlap between tracks recorded before and after clock-  
38 shift. Using this protocol on pigeons housed in coastal Tuscany, the important influence of the sea  
39 as a major guiding feature in enabling re-orientation after clock-shift has often been discussed  
40 (Bonadonna, Holland, Dall’Antonia, Guilford & Benvenuti, 2000; Gagliardo, Odetti & Ioalè, 2005;  
41 Filannino, Armstrong, Guilford & Gagliardo, 2014). In fact, clock-shifted pigeons released from  
42 familiar locations outside visual contact with the coast consistently display a greater deviation from  
43 the home direction compared to pigeons released near the coastline, and this is true regardless of  
44 whether the phase-shift induced deflection led the birds to orient towards the sea or inland.  
45 Interestingly, hippocampal ablation impairs the ability to re-orient at familiar locations where the  
46 coast can be used as a topographic cue (Gagliardo, Ioalè, Savini, Dell’Omo & Bingman, 2009).

47         The possible differential involvement of left and right hemisphere for the visual processing  
48 of familiar landmarks for homing pigeon navigation remains uncertain, partly because until recently  
49 all experiments relied on vanishing bearing and homing speed data. In the current study, we applied  
50 GPS-tracking technology to record the entire flight tracks of the experimental birds in order to  
51 assess possible asymmetries in the contribution of left and right hemispheres in the visual  
52 processing supporting navigation by familiar landmarks or landscape features. Pigeons were first

53 trained under monocular conditions (only the left or right eye had access to landmark/landscape  
54 information) from two training sites located near the coast north and south of home, and then tested  
55 under binocular conditions from the same sites, but now after a phase-shift manipulation of the  
56 light-dark cycle. The phase-shift manipulation was implemented to lead the birds to fly off initially  
57 in a wrong direction (induced navigational error; Wallraff, 2005). Using this two-step protocol, we  
58 were able to study the development of flight path fidelity under monocular viewing, and whether  
59 the left or the right-eye visual system is advantaged in the learning of familiar topographical  
60 features during training that would enable a better landmark-based retracing of the acquired training  
61 route home after clock-shift. We expected to observe a higher level of route fidelity during training  
62 and/or after clock-shift in the pigeons trained with the hemisphere preferentially used for  
63 memorising and recalling visual landmarks and landscape features associated with route fidelity.  
64 Alternatively, in the absence of any brain asymmetry in landmark-based route fidelity, one would  
65 expect better re-orientation in the monocular group advantaged with accessibility to visual  
66 information from the coastline.

## 67 **Materials and methods**

### 68 **General procedure**

69 Sixty-four, first-year, naive pigeons (*Columba livia*) **of both sex**, hatched and kept at the  
70 Arnino field station (43°39'26"N, 10°18'14"E; Pisa, Italy), were used. After fledging the birds were  
71 allowed to perform spontaneous flights around the loft. All releases took place under sunny  
72 conditions, with no or light winds over two summers (2013 and 2014). The birds had access to  
73 food, grit and water ad libitum for the duration of the experiment. The project was approved by the  
74 Scientific Ethics Committee of the University of Pisa (permit N. 24300) and was carried out in  
75 accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific  
76 purposes. Two weeks before the beginning of the experiment, the birds were equipped with PVC  
77 dummies in order to accustom them to carrying a load. A dummy was dorsally attached to a pigeon

78 by means of a Velcro<sup>®</sup> strip glued to the feathers, previously trimmed. During the experimental  
79 releases, the dummy was replaced with a GPS data logger (Mobile Action IgotU, 20g), that stored  
80 every second latitude, longitude, speed and time of recording. The tracks recorded were visualized  
81 with QGIS (<http://www.qgis.org/>). All original track data are available at the data archive  
82 [www.movebank.org](http://www.movebank.org) (DOIs provided at publication).

83 Pigeons were randomly assigned to three groups. LH ( $N = 23$ ) birds were trained with the  
84 left eye covered with an eye cap (see below). Therefore, because of the strongly, lateralized,  
85 contralateral visual inputs to the telencephalon, these birds were prevalingly limited to left  
86 hemisphere visual processing (3). RH ( $N = 25$ ) birds were trained with the right eye covered, and  
87 therefore, right hemisphere visual processing. C ( $N = 16$ ) birds were trained with both eyes  
88 uncovered. The three groups of birds were subjected to the following release program from each  
89 of the two release sites chosen for the experiment (see below): 7 group training releases; 1  
90 individual release under non-clock-shifted conditions, 1 individual release following clock-shift.  
91 Both LH and RH birds wore the eye-cap during the 7 group releases and the individual release  
92 under non-clock-shift conditions, but were released in the binocular condition following clock-  
93 shift.

94 Three days before the beginning of the training releases a Velcro ring was glued with water-  
95 soluble, non-toxic glue to trimmed feathers around the left and right eye of the LH and RH birds,  
96 respectively. The complementary piece of Velcro ring was glued to a conical cap made of a double  
97 layer of translucent paper (diameter 30 mm), which allowed diffuse light to stimulate the eye, and  
98 therefore, eliminate possible effects on the internal clock. The birds were monitored daily in the  
99 loft to observe any possible effect of the monocular occlusion on their behaviour. After monocular  
100 occlusion the birds were able to feed and drink normally and seemed to get used to wear the eye-  
101 cap in a few hours. The LH and RH birds were released as a group with eye caps attached,  
102 together with the C birds, near (500 m) their loft in order to accustom them to flying with one eye  
103 covered. The three groups of pigeons together were then subjected to 7 group training releases

104 from each of the two release sites (Livorno, home direction and distance 341°, 12 km; La Sterpaia  
105 194°, 9.5 km), followed by an additional individual release from each training site. Both release  
106 sites were 4-5 km from the coast roughly North (La Sterpaia) and South (Livorno) with respect to  
107 home. These release sites were chosen such that the sea, constituting a salient topographical  
108 feature for re-orientation (Gagliardo, Ialò, Savini, Dell'Omo & Bingman, 2009; Filannino,  
109 Armstrong, Guilford & Gagliardo, 2014), was on the right side of a pigeon when heading home  
110 from La Sterpaia, and on the left side of a pigeon when heading home from Livorno. A  
111 consequence of the geographical position of the release sites is that after a fast clock-shift (see  
112 below) the expected anticlockwise deflection would lead the birds towards the sea when released  
113 from Livorno, and inland when released from La Sterpaia.

114 The GPS loggers were applied to the pigeons before displacement. The pigeons were  
115 transported in a crate with a removable wall. Once at the release site, the crate was placed on the  
116 ground and opened allowing the birds out as a group. During the single releases the birds were  
117 tossed singly at an interval of at least 10 minutes between individuals. The LH and RH pigeons  
118 wore the eye-caps continually for the duration of the training portion of the experiment (until the  
119 clock-shift manipulation, see below).

120 In 2013 (LH  $N = 15$ ; RH  $N = 17$ ; C  $N = 12$ ) not every bird was always provided with a GPS  
121 logger during the group training phase (see supplementary material for details). By contrast, in 2014  
122 (LH  $N = 8$ ; RH  $N = 8$ ; C  $N = 4$ ) all birds carried GPS loggers for every training and test release.  
123 However, during both years, all the birds were equipped with GPS loggers for the individual test  
124 releases before and after the clock-shift treatment (see below). After the last training release from  
125 each site (the individual releases), the pigeons that completed the entire training phase (LH  $N = 11$ ;  
126 RH  $N = 10$ , C  $N = 14$ ) were 6 hours fast shifted by placing them, with the eye caps now removed, in  
127 a light tight room ventilated by an aspirator and provided with perches and *ad libitum* food and  
128 water. The light-dark cycle in the room was shifted 6-hours fast with respect to the natural cycle.  
129 After at least 6 days of clock-shift treatment, each bird was released singly from each release site

130 under binocular conditions. After the first release under clock-shift, the birds, once they had homed,  
131 were immediately placed back in the clock-shift room in order to keep them phase-shifted until the  
132 second clock-shift release. **Birds moult out glued velcro strip after experiment. After homing**  
133 **from their second clock-shift release the birds were returned to live and breed at the lofts,**  
134 **allowing access to free flight daily.**

135

### 136 **Quantitative analyses and statistical procedures**

137 All the statistical analyses were limited to fixes recorded during active flight, which we defined as a  
138 GPS recorded speed greater than 5 km/h. Details on the tracks obtained and the analyses performed  
139 are reported in Table 1.

#### 140 *Tracks recorded during the training phase.*

141 For the tracks recorded during the training releases, we analysed the following parameters:

#### 142 *Social behaviour of monocularly occluded birds.*

143 For each group release (releases 1-7), we computed the pairing index (PI): the percentage of the  
144 fixes of each bird's track that were 80 m or closer to another pigeon at the same time. A Two Way  
145 RM ANOVA was applied to the mean individual PIs for each individual across the 7 training-group  
146 releases from each site in order to test for differences in gregariousness during homing among the  
147 experimental groups (factors: treatment and release site). Multiple, post-hoc comparisons were  
148 performed with the Bonferroni t-test. The PI analysis was conducted only on the 2014 data set,  
149 when all the pigeons carried GPS loggers during each group training release.

150

151 *Local navigation approaching the home loft:* To quantify the efficiency in localising the home loft  
152 during the last phase of the homing flight (approaching the home loft), we recorded the median  
153 length of the training routes starting with a first fix located beyond a line crossing the home site

154 perpendicularly to the beeline between the release site and the home site for each individual until it  
155 reached home (a pigeon that did not cross that line on a given training release was given a score of 0  
156 km). Between group differences in median kilometres flown beyond the home area were assessed  
157 with a Kruskal-Wallis test for each release site. The Mann-Whitney U-test was used to compare the  
158 performance of monocular birds released under their most favourable (home loft should have fallen  
159 within the visual field of the open eye as a pigeon approached the home loft; RH from Livorno  
160 versus LH from La Sterpaia) and most unfavourable (RH from La Sterpaia versus LH from  
161 Livorno) condition for visually locating the home loft during the final part of their homing journey.  
162 Only the birds with at least 6 out of 8 tracks available for a release site were included in the  
163 analysis.

164

165 *Spatial occupancy index.* Using QGIS, for each fix of each individual's track we arbitrarily  
166 considered the radius of 400 m around a fix to identify approximately the space that was visually  
167 accessible (what we term the "space buffer") for each training release excluding the first (when the  
168 pigeons were still unfamiliar with the route home). For each pigeon, we merged all the buffers  
169 separately for each release site and computed the obtained area in square kilometres. Based on this  
170 analysis, smaller areas would indicate both an efficient homing route (close to the "beeline" from  
171 release site to home loft) and/or a higher tendency to retrace previous routes or parts of them  
172 (fidelity). The spatial occupancy index was defined as the ratio obtained by dividing each  
173 individual's area by the minimal, theoretically possible area for each release site (the area obtained  
174 merging the buffers of the beeline between the release site and home: Livorno 9.6 km<sup>2</sup>, La Sterpaia  
175 7.6 km<sup>2</sup>). We then applied a Kruskal-Wallis (Livorno: C  $N = 7$ , RH  $N = 8$ ; LH  $N = 10$ ; La Sterpaia:  
176 C  $N = 8$ , RH  $N = 8$ ; LH  $N = 10$ ) test to compare spatial occupancy indices across groups for each  
177 release site. This was done to test the effect of different visual access to landscape features  
178 (seacoast, inland or both) on the spatial occupancy index displayed by the three experimental  
179 groups. The Wilcoxon test was used for within-group comparisons between the two release sites (C

180  $N = 7$ , RH  $N = 8$ ; LH  $N = 10$ ). Only the birds for which at least 6 out of 7 training tracks for each  
181 release site were available were included in the analysis.

182 ***Tracks recorded under clock-shift.***

183 Pigeons that flew together during the clock-shift test releases were excluded from the following  
184 analyses.

185 *Retracing the last training tracks after clock-shift.* We examined the percentage of fixes from La  
186 Sterpaia and Livorno flown by each bird during the last, individual training flight that were over-  
187 flown again (within 400 m distance) during homing flights performed after clock-shift. Fixes within  
188 1 km of the release site and home loft were excluded from this computation. The analysis was  
189 performed only on complete tracks from the release site to home. A Two-Way RM ANOVA was  
190 applied to the percentage of “over-flown fixes” in order to assess between group differences and  
191 any release-site effect with respect to the tendency to fly, after clock-shift, near landmarks  
192 experienced during the last, individual training flight. Post hoc comparisons were performed with  
193 the Bonferroni t test.

194

195 *Kilometres flown outside the total familiar area.* For each pigeon we computed the number of  
196 kilometres flown outside the previously visited areas (pooled training occupancy maps from both  
197 release sites including the first training release) when homing under the clock-shift condition. For  
198 the second track recorded after clock-shift, we amended the familiar area buffer map generated  
199 during the training flights (see above) to include the buffer areas recorded during the first homing  
200 flight under clock-shift. This analysis was conducted on the birds for which at least 12 out of 16  
201 training tracks were available (LH,  $N = 8$  and  $N = 9$ ; RH,  $N = 8$  and  $N = 8$ ; C,  $N = 3$  and  $N = 7$ , from  
202 Livorno and La Sterpaia, respectively). A One Way ANOVA or Kruskal-Wallis was used to test  
203 for between group differences. Multiple comparisons were performed with the Fisher method.

204 *Initial orientation before and under clock-shift.* For both the flight path recorded under clock-shift  
205 and the last training release before the shift treatment, we considered the section of each track from  
206 the release site out to a 10 km radius and we computed for each bird for each releases its mean  
207 vector by averaging the direction taken by the bird moving from one fix to the next. The mean  
208 vector distributions of the three groups were tested for randomness with the Hotelling test. A two  
209 sample Hotelling test was used to compare mean vector distributions. Virtual vanishing bearing data  
210 distributions (orientation of the birds at 2 km from the release site) are reported in Table 2.

211 *Deviation from the last training track.* This analysis was performed on the birds for which both the  
212 last training track and the track recorded under clock-shift were available. We computed the  
213 deviation of each individual's track recorded under clock-shift compared to its last training track  
214 and groups were compared with the Kruskal Wallis test.

215

## 216 **Results**

### 217 *Tracks recorded during the training phase*

#### 218 *Social behaviour of monocularly occluded birds.*

219 The analysis performed on the 2014 pigeons' pairing indices (PI), used to assess gregarious  
220 behaviour during the group training releases, revealed a significant difference between the  
221 treatments (C,  $N = 4$ ,  $PI = 0.88 \pm 0.13$ ; LH,  $N = 6$ ,  $PI = 0.63 \pm 0.13$ ; RH,  $N = 6$ ,  $PI = 0.40 \pm 0.05$ ; Two  
222 Way RM ANOVA  $F_{2,13} = 22.995$ ,  $P < 0.001$ ; Fig. A1). Being monocular turned out to impair the  
223 ability of a bird to keep close to a companion while homing (Bonferroni t-test, C versus RH,  $P <$   
224  $0.001$ ; C versus LH,  $P < 0.05$ ). However, the RH birds displayed a significantly smaller pairing  
225 index than LH pigeons (RH versus LH,  $P < 0.01$ ). This result confirmed what had been observed in  
226 2013, although for that year the missing tracking data for some pigeons did not allow us to measure  
227 with certainty the group-forming behaviour of the birds. The degree of gregariousness co-varied  
228 with release site ( $F_{1,13} = 29.86$ ,  $P < 0.001$ ; Livorno,  $PI = 0.52 \pm 0.27$ ; La Sterpaia  $PI = 0.69 \pm 0.18$ ),

229 with birds homing from La Sterpaia displaying higher PIs. This may be explained in part by the  
230 observation that from La Sterpaia many birds often landed and accumulated on the roof of a nearby  
231 building before starting their homing flight. This probably favoured their tendency to fly with a  
232 companion. A significant interaction between treatment and release site was also found ( $F_{2,13} =$   
233  $7.646$ ,  $P < 0.01$ ). In particular, from Livorno each group displayed a significantly different PI in  
234 comparison to the other two groups (C,  $PI = 0.82 \pm 0.19$ ; LH,  $PI = 0.60 \pm 0.15$ ; RH,  $PI = 0.24 \pm 0.07$ ; C  
235 versus LH,  $P < 0.05$ ; C versus RH and RH versus LH,  $P < 0.001$ ). By contrast, when flying from La  
236 Sterpaia the difference between LH and RH did not reach significance (C,  $PI = 0.93 \pm 0.09$ ; LH,  $PI =$   
237  $0.66 \pm 0.15$ ; RH,  $PI = 0.56 \pm 0.07$ ; C versus LH  $P = 0.01$ ; C versus RH  $P < 0.001$ ; LH versus RH  $P >$   
238  $0.1$ ). In addition, the RH pigeons displayed a PI significantly greater when homing from La Sterpaia  
239 compared to when homing from Livorno ( $P < 0.001$ ), while the C and LH pigeons displayed similar  
240 PIs from both release sites ( $P > 0.05$ ).

241

#### 242 *Local navigation approaching the home loft*

243 Visual inspection of the birds' tracks (Fig. A2) as they approached the home loft revealed that  
244 the LH birds tended to fly more inland from Livorno and nearer the coast from La Sterpaia; as  
245 expected, their movement drifted to the side of their open eye. The complementary pattern was  
246 displayed by the RH birds, who were more likely to fly near the coast from Livorno and inland from  
247 La Sterpaia. The consequence of this behaviour is that when approaching the home area, the LH  
248 birds homing from Livorno and the RH birds homing from la Sterpaia were more likely to have the  
249 home loft and immediate surroundings on the side of their occluded eye. In many cases these birds  
250 did not head directly to their home loft despite being close to it, but rather, continued to fly beyond  
251 the home area sometimes for several kilometres before turning and eventually reaching home (Fig.  
252 A3).

253 From Livorno the three groups displayed a significant difference in the number of kms flown  
254 to reach home after having crossed the line perpendicular to the beeline between home and the

255 release site (Fig. 1; Kruskal-Wallis test,  $P = 0.011$ ; median number of km: LH,  $N = 9$ , 2.3 km; RH,  
256  $N = 8$ , 0 km; C  $N = 10$ , 0.21 km). In fact, the LH birds were significantly less efficient in localising  
257 the loft during approach compared to the RH pigeons (Dunn's test, LH versus RH,  $P < 0.02$ ; both  
258 LH versus C and C versus RH,  $P > 0.05$ ). Interestingly, the groups' approach navigation was not  
259 significantly different from La Sterpaia. ( $P > 0.1$ ; LH,  $N = 9$ , 0 km; RH,  $N = 8$ , 0.55 km; C,  $N = 8$ , 0  
260 km), where the RH pigeons might have been expected to fly longer path.

261 A direct comparison between the monocular groups released from their respective less  
262 favourable release site (RH from La Sterpaia versus LH from Livorno) uncovered that the LH birds  
263 were more impaired than the RH pigeons in locating the loft (one tailed Mann-Whitney U test,  $P <$   
264  $0.05$ ). When released from their "favourable" release site (RH from Livorno and LH from La  
265 Sterpaia), the two monocular groups were equally efficient in locating the home loft (Mann-  
266 Whitney U test,  $P > 0.1$ ).

267  
268 *Spatial occupancy index.* From Livorno the RH birds displayed a smaller spatial occupancy index  
269 compared to the LH birds (Fig. 2a and Fig. A4; Kruskal-Wallis test,  $P < 0.01$ ; LH,  $N = 10$ , median  
270 ratio 5.49; RH,  $N = 8$ , 3.36; C,  $N = 7$ , 4.43; Dunn's test, LH versus RH  $P < 0.01$ ; both C versus LH  
271 and C versus RH  $P > 0.1$ ). By contrast, no between group difference emerged from La Sterpaia  
272 (Kruskal-Wallis test,  $P > 0.1$ ; LH,  $N = 10$ , 5.50; RH,  $N = 8$ , 5.50; C,  $N = 8$ , 7.31). The Wilcoxon  
273 signed rank test revealed that while LH birds displayed similar spatial occupancy indices when  
274 released from Livorno and La Sterpaia ( $P > 0.2$ ), both the C and RH pigeons displayed higher  
275 spatial occupancy indices from La Sterpaia compared to Livorno ( $P < 0.05$  and  $P < 0.02$ ,  
276 respectively).

277 This analysis indicates that the RH birds when flying with the leading line of the coast  
278 accessible to their open eye (from Livorno) were better at following the same route home compared  
279 to the LH pigeons (from La Sterpaia). In fact, the spatial occupancy indices of the RH birds from

280 Livorno were significantly lower than the same indices of the LH birds from La Sterpaia (Mann-  
281 Whitney U test,  $P < 0.001$ ).

### 282 ***Tracks recorded under clock-shift.***

283 *Retracing the last training tracks after clock-shift.* The analysis of the percentage of the positional  
284 fixes recorded from the last training (unshifted) flights that were re-approached (nearer than 400 m)  
285 during the homing flight under clock-shift significantly differed among the three groups (Two Way  
286 RM ANOVA,  $F_{2,25} = 6.168$ ,  $P < 0.01$ ; C,  $N = 7$  and  $N = 9$  from Livorno and La Sterpaia  
287 respectively; LH,  $N = 9$ , and RH,  $N = 8$ , from both release sites; Fig. 3). In particular, the RH birds  
288 displayed significantly more fixes within 400 m of the un-shifted flight path in comparison to both  
289 the LH and C groups (Bonferroni t test, RH versus LH,  $P < 0.01$ ; RH versus C,  $P < 0.05$ ; C versus  
290 LH,  $P > 0.5$ ). A difference between the release sites emerged, as the clock-shift tracks from  
291 Livorno, irrespective of group, were more likely to overlap the last training (un-shifted) flight paths  
292 ( $F_{1,19} = 4.44$ ,  $P < 0.05$ ). No significant interaction between treatment and release site was found  
293 ( $F_{2,19} = 1.814$ ,  $P > 0.1$ ).

294

295 *Kilometres flown outside the total familiar area.* The LH birds binocularly released under clock-  
296 shift tended to fly for longer distances outside the previously visited area compared to both RH and  
297 C pigeons (Fig. 2b and Fig. 4). However, this difference turned out to be statistically significant  
298 only for the clock-shift test from Livorno (One Way ANOVA, Livorno:  $F_{(2,16)} = 4.15$ ,  $P < 0.05$ ; LH,  
299  $N = 8$ , mean  $22.5 \pm 9.8$  km; RH,  $N = 8$ ,  $12.7 \pm 8.4$  km; C,  $N = 3$ ,  $6.7 \pm 8.8$ ; Fisher method LH versus  
300 RH and LH versus C  $P < 0.05$ , RH versus C  $P > 0.1$ ; La Sterpaia:  $P > 0.1$ , LH,  $N = 9$ , median 25.4  
301 km; RH,  $N = 8$ , 18.1 km; C,  $N = 7$ , 18.4 km).

302

303 *Initial orientation before and after clock-shift.* Over the 10 km radius centred on the release site,  
304 the three groups of birds on the last training (single) release displayed significantly oriented

305 distributions both from Livorno and La Sterpaia (Hotelling test  $P < 0.001$  in all cases). As expected,  
306 the initial orientation of the monocular birds reflected a bias towards the side of the open eye  
307 (Livorno, LH,  $N = 9$ ,  $r = 0.60$ ,  $\alpha = 347^\circ$ ; RH,  $N = 8$ ,  $r = 0.51$ ,  $\alpha = 303^\circ$ ; C,  $N = 7$ ,  $r = 0.71$ ,  $\alpha = 330^\circ$ ;  
308 La Sterpaia, LH,  $N = 9$ ,  $r = 0.54$ ,  $\alpha = 222^\circ$ ; RH,  $N = 8$ ,  $r = 0.60$ ,  $\alpha = 181^\circ$ ; C,  $N = 9$ ,  $r = 0.51$ ,  $\alpha =$   
309  $207^\circ$ ), such that at both La Sterpaia and Livorno the LH and RH pigeons displayed significantly  
310 different mean vector distributions (two sample Hotelling test, LH versus RH  $P < 0.01$  and  $P <$   
311  $0.001$  from La Sterpaia and Livorno, respectively). The monocularly occluded birds oriented  
312 differently from the binocular pigeons, with the exception of the LH pigeons at La Sterpaia (Two  
313 sample Hotelling test, C versus RH  $P < 0.05$  at both sites; C versus LH  $P < 0.05$  and  $P > 0.05$  at  
314 Livorno and La Sterpaia, respectively).

315 After clock shift the three groups of birds displayed significantly oriented mean vector  
316 distributions from both Livorno and La Sterpaia (Hotelling test  $P < 0.01$ ), with the exception of the  
317 RH pigeons released at La Sterpaia ( $P > 0.05$ ): Livorno, LH,  $N = 9$ ,  $r = 0.21$ ,  $\alpha = 282^\circ$ ; RH,  $N = 8$ ,  $r$   
318  $= 0.17$ ,  $\alpha = 262^\circ$ ; C,  $N = 8$ ,  $r = 0.21$ ,  $\alpha = 271^\circ$ ; La Sterpaia, LH,  $N = 9$ ,  $r = 0.34$ ,  $\alpha = 153^\circ$ ; RH,  $N =$   
319  $10$ ,  $r = 0.22$ ,  $\alpha = 143^\circ$ ; C,  $N = 11$ ,  $r = 0.41$ ,  $\alpha = 173^\circ$ . As expected after a fast-phase-shift treatment,  
320 the second order mean vector directions indicate a general anticlockwise deflection with respect to  
321 the mean orientation observed during the last training release. No significant difference between the  
322 distributions of the three clock-shifted groups at either release site was detected (Two Sample  
323 Hotelling test  $P > 0.05$  in all comparisons).

324

325 *Deviation from the last training track.* Figure 5 reports the individual initial (within 10 km radius  
326 from the release site) deviation of the birds following clock-shift from their own track recorded in  
327 the un-shifted condition (see also Fig. A5 for further details). The Kruskal Wallis test did not reveal  
328 any statistical difference between the initial deviation of the three groups ( $P > 0.05$ ) at both Livorno  
329 and La Sterpaia; median deviation: Livorno, LH  $-22^\circ$ , RH  $+2^\circ$ , C  $-20^\circ$ ; La Sterpaia LH  $-76^\circ$ , RH  $-$   
330  $42^\circ$ , C  $-34^\circ$ ).

332 **Discussion**

333 Pigeons learn to follow familiar routes home across the landscape as they gain experience  
334 from multiple releases from the same site, and the evidence suggests that this involves increasing  
335 reliance on piloting via familiar landmark/landscape information and a lessening of reliance on  
336 compass-based orientation. For the first time we provide cumulative evidence supporting the  
337 hypothesis that this learning is lateralized in pigeons, with the right hemisphere preferentially used  
338 in the process. Although training under monocular conditions may have resulted in an improvement  
339 in the re-tracing of the increasingly faithful route home using the non-preferred(left) hemisphere, a  
340 difference between the left and right monocularly occluded birds was evident. Birds trained with the  
341 left eye open, and hence with the right hemisphere the principal recipient of visual information  
342 (RH), are more faithful to a familiar flight corridor acquired during training compared to pigeons  
343 relying on their right eye/left brain hemisphere (LH). The RH pigeons remain more faithful even  
344 after having their time-compensated sun compass shifted. These results are partially consistent with  
345 a recent report by Martinho and colleagues (Martinho, Biro, Guilford, Gagliardo & Kacelnik, 2015).  
346 Although they found no advantage of the right hemisphere during the acquisition of route fidelity,  
347 monocular pigeons that acquired route stereotypy with the left eye/right hemisphere system  
348 displayed stronger route fidelity when subsequently tested with the contralateral system (the  
349 implication being more information transfer from the right to the left hemisphere). We explain their  
350 lack of asymmetry in favour of the right hemisphere during route learning as probably being due to  
351 the relatively easy task employed, with pigeons being released from only one site located a short  
352 distance from the loft. The results by Martinho and colleagues (Martinho, Biro, Guilford, Gagliardo  
353 & Kacelnik, 2015) offer potentially interesting implications for the interpretation of the results of  
354 the present experiment. In fact, it is possible that the more efficient landmark-based route retracing  
355 shown by the RH birds, particularly when tested under binocular conditions after clock-shift, might

356 be partly due to a greater transfer of information to the left hemisphere of the familiar route learned  
357 by the right hemisphere (Letzner, Patzke, Verhaal & Manns, 2014).

358           Generally, and consistent with previous reports (Diekamp, Prior, Ioalè, Odetti, Güntürkün &  
359 Gagliardo, 2002), monocular occlusion, irrespective of side, was associated during training with a  
360 route bias in the direction towards the side of the open eye (Fig. A3). As a consequence, the RH  
361 birds generally flew closer to the coast from Livorno than from La Sterpaia, while the opposite  
362 occurred for the LH pigeons. Therefore, during training the LH birds homing from Livorno and the  
363 RH pigeons homing from La Sterpaia were more likely to be eastward of home once reaching the  
364 home area, i.e., with the home loft on the side of the occluded eye. This likely resulted in locating  
365 the home loft being more difficult for the RH birds homing from La Sterpaia and the LH birds  
366 homing from Livorno. However, the RH and the LH pigeons were not equally affected by this  
367 potentially unfavourable situation. Specifically, the RH birds, when “blind” to the home loft from  
368 La Sterpaia, flew fewer kilometres beyond the home area, i.e., were more efficient, compared to the  
369 LH pigeons when “blind” to the home loft from Livorno (Fig. 1).

370           The poorer efficiency of the LH pigeons, compared to the RH birds, to localise the home loft  
371 in the last phase of their homing journey contributed to their larger spatial occupancy area during  
372 training flights from Livorno (Fig. 2a and Fig. A4). One explanation for this difference is that the  
373 greater efficiency of the RH birds in localising the loft resulted from better access to navigationally  
374 relevant landmarks and landscape features. Additionally, one could argue that the better  
375 performance of the RH birds from Livorno might have also resulted, in part, from the physical  
376 constraint of the coastline limiting how far they could drift westward (open-eye-ward) to the coast.  
377 This interpretation is consistent with the fact that RH birds displayed a significantly lower spatial  
378 occupancy index when homing from Livorno compared to La Sterpaia. However, if the only thing  
379 that mattered was the physical constraint of the sea, one would expect the LH pigeons from La  
380 Sterpaia to have displayed fewer “over flown” kms and lower spatial occupancy index compared to  
381 the RH pigeons. Actually, the LH birds demonstrated no advantage with respect to over-flown kms

382 and spatial occupancy when released from La Sterpaia compared to Livorno. Overall, we interpret  
383 the difference in the behaviour of the LH and RH pigeons during training as a reflection of a greater  
384 tendency on the part of the RH pigeons to fly faithfully over familiar space. It should be noted here  
385 as well that even when the RH birds had diminished visual access to the loft (from La Sterpaia),  
386 they did not display as large a tendency to fly beyond the home area compared to the LH pigeons  
387 from Livorno. Therefore, the RH pigeons also appeared superior in using landmark/landscape  
388 information to locate the loft when direct visual access to the loft was rendered less likely.

389 Consistent with the theme that the RH pigeons displayed greater fidelity in retracing the  
390 same routes home across releases was the notable similarity in the tracks they flew during their last  
391 training release (birds released singly) and their tracks following clock-shift (when they were  
392 returned to being binocular). The clock-shift test was performed to test specifically the ability of the  
393 pigeons to use familiar landmarks for re-orientation without use of the sun compass. The RH  
394 pigeons had a significantly higher percentage of fixes over the corridor flown on their last training  
395 release retraced under clock-shift compared to both the LH and Control pigeons. From a different  
396 perspective, it is also noteworthy that under clock-shift the LH pigeons, significantly from Livorno  
397 and trending from La Sterpaia (Fig. 2b and Fig. 4), flew over more unfamiliar space than did the  
398 RH or control pigeons.

399 Previous research has pointed out that visual access to the coastline enhances familiar  
400 landmark-based re-orientation in misdirected clock-shifted pigeons, perhaps because it acts as a  
401 major guiding feature (Bonadonna, Holland, Dall'Antonia, Guilford & Benvenuti, 2000; Gagliardo,  
402 Odetti & Ioalè, 2005; Gagliardo, Ioalè, Savini, Dell'Omo & Bingman, 2009; Filannino, Armstrong,  
403 Guilford & Gagliardo, 2014). For this reason, a lack of brain asymmetry in the acquisition and  
404 maintenance of route fidelity would have resulted in more robust landmark-based navigation in the  
405 RH birds compared to the LH birds when homing from Livorno (left eye exposed to the sea), and in  
406 the LH birds compared to the RH pigeons when homing from La Sterpaia (right eye exposed to the  
407 sea). In fact, the LH birds did not display significantly greater route fidelity than the RH pigeons

408 when the view of the sea was expected to enhance any acquired fidelity. This observation supports  
409 the hypothesis of a brain asymmetry in favour of the right hemisphere in the use of topographical  
410 cues in the context of route fidelity.

411 Taken together, the training and clock-shift data all point to a left eye/right brain hemisphere  
412 advantage in pigeons staying close to familiar space and previously flown routes, and perhaps under  
413 some conditions, being better at staying in the visual-catchment zone of the home loft (they tended  
414 to be less likely to fly beyond the home area). The attachment to familiar space displayed by the RH  
415 pigeons is likely to be supported by recognition of topographical features. One could argue that the  
416 heightened sensitivity of the RH birds to attend to visual landmarks might be a consequence of their  
417 tendency to fly alone rather than with others. It has been claimed, based on EEG recordings taken  
418 from flying pigeons (Vyssotski et al., 2009), that binocular pigeons flying in a flock attend less to  
419 visual landmarks than do birds flying alone. Therefore, one might expect that the control pigeons of  
420 the current study, having a higher tendency to fly in groups, would be less attentive to landscape  
421 features, and therefore, show less familiar space fidelity. However, the behaviour of the control  
422 pigeons was ambiguous, at times showing poorer fidelity with familiar space compared to the RH  
423 pigeons, and other times performing like the RH pigeons. There may also be a physiological factor  
424 that can explain the behaviour of the RH pigeons. In starlings (*Sturnus vulgaris*) and blue tits  
425 (*Cyanistes caeruleus*) the left eye is characterized by a larger number of single cones (Hart,  
426 Partridge & Cuthill, 2000; Hart, Partridge, Cuthill & Bennett, 2000), which may enable better  
427 colour vision (Maier & Bowmaker, 1993) and therefore perhaps landmark discrimination. If  
428 pigeons are like starlings and blue tits, then the RH advantage reported here may be in part due to  
429 better visual discrimination of landmarks. However, differences between the left and right eyes in  
430 colour discrimination have not been observed in laboratory studies, and in general, a left  
431 hemispheric dominance in discrimination tasks has been reported (Manns & Ströckens, 2014).  
432 Nevertheless, it is possible that the left-hemispheric dominance in visual discrimination tasks

433 typically found is not caused by differences in sensory, but by cognitive and/ or motor control  
434 processes.

435         The advantage of the left eye/right hemisphere system for familiar space fidelity and  
436 possibly visual landmark memory that can aid homing may partially depend on functional  
437 asymmetry of the hippocampal formation. Earlier homing experiments based on the recording of  
438 vanishing bearings generally revealed that unilateral hippocampal lesions (Gagliardo, Odetti, Ioalè,  
439 Bingman, Tuttle & Vallortigara, 2002) produced a similar orientation performance compared to  
440 bilateral lesions when birds are released from familiar locations after clock-shift (Gagliardo, Ioalè,  
441 Savini, Dell'Omo & Bingman, 2009). However, there is some evidence that the right hippocampus  
442 plays a more important role in local navigation near the home loft (Gagliardo, Ioalè, Odetti,  
443 Bingman, Siegel & Vallortigara, 2001).

444         Studies conducted on chicks in an indoor arena suggested that encoding the geometric  
445 features of an enclosure lateralizes to the right hippocampus (Tommasi, Gagliardo, Andrew &  
446 Vallortigara, 2003). Consistent with this observation, arena experiments with monocular occluded  
447 chicks (Tommasi & Vallortigara, 2004) and pigeons (Prior, Lingenauber, Nitschke & Güntürkün,  
448 2002) suggested an asymmetrical involvement of the right and left hemisphere in the processing of  
449 global and local spatial information, respectively. By contrast, Wilzeck, and colleagues (Wilzeck,  
450 Prior & Kelly, 2009) reported an equal contribution of both hemispheres in encoding geometric-  
451 global information. However, comparison between experimental arena and outdoor, large-scale  
452 navigation experiments is complicated by the fact that, in arenas, landmarks (local cues) are likely  
453 more easily represented as beacons rather than cues embedded in some spatial-relational map  
454 (Sherry & Vaccarino, 1989; **Vallortigara, Pagni & Sovrano, 2004**). However, the likely reliance  
455 on both landscape cues, such as the coastline, and the spatial relationship among familiar landmarks  
456 to support the observed route fidelity, especially in the RH pigeons, likely involves the processing  
457 of “global cues”, and as such, render our results consistent with what has been reported at least in  
458 some arena experiments (e.g., Tommasi & Vallortigara, 2004; **Rashid & Andrew, 1989**). In

459 addition, the apparent superiority of the right hemisphere in memorising topographical features in a  
460 natural, environmental setting (our data) is consistent with the better performance of the right  
461 hemisphere in learning complex stimuli in operant conditioning tasks (Yamazaki, Aust, Huber,  
462 Hausmann & Güntürkün, 2007). Finally, a systematic GPS study investigating possible differences  
463 in the role of the left and right hippocampus during homing is lacking. However, a left hippocampal  
464 advantage for navigating by geometry in an indoor arena has been reported in pigeons (Nardi &  
465 Bingman, 2007), while no functional asymmetry in the hippocampal formation has been reported to  
466 date in landmark-based navigation (Gagliardo, Odetti, Ioalè, Bingman, Tuttle & Vallortigara, 2002).

467 In the current experiment we reported an analysis of the relative tendency of pigeons to fly  
468 together in three treatment groups. Although the statistical analysis was conducted on a sub-sample  
469 of pigeons (only pigeons released in the 2014 when every track was recorded; see Material and  
470 methods for details), the general behavioural differences were apparent in the entire data set.  
471 Clearly further investigation is needed to better understand laterality as it relates to gregariousness,  
472 but we think it worth discussing the lateralized differences in gregariousness suggested by our  
473 analysis.

474 Although both the LH and RH pigeons were less likely than the control pigeons to fly with  
475 another bird during the training flights, the LH birds were nonetheless more gregarious than the RH  
476 birds. Recalling the retinal differences described above, the difference between the LH and RH  
477 pigeons might be at least partly due to an asymmetry in the number of double cones in the retina of  
478 the left and right eye if pigeons resemble starlings and blue tits (Hart, Partridge & Cuthill, 2000;  
479 Hart, Partridge, Cuthill & Bennett, 2000). In these two species it has been shown that double cones,  
480 specialised for movement detection (Campenhausen & Kirschfeld, 1998), are more abundant in the  
481 right eye. By contrast, single cones, as noted above, are more abundant in the left retina (Hart,  
482 Partridge & Cuthill, 2000; Hart, Partridge, Cuthill & Bennett, 2000). As such, a greater sensitivity  
483 of the right eye to motion may have facilitated detection and joining of other pigeons in the LH  
484 compared to the RH birds. However, the advantage of left hemisphere in processing and/or

485 integrating perceived motion reported in the literature (see Manns & Ströckens, 2014) might explain  
486 the more gregarious behaviour of the LH pigeons without implying a role of social signals in flock  
487 cohesion. That is because, according to previous investigations, social signals and individual  
488 recognition seem to be more controlled by the right hemisphere (Daisley, Mascalconi, Rosa-Salva,  
489 Rugani & Regolin, 2009). Nagy and colleagues (Nagy, Ákos, Biro & Vicsek, 2010) observed that  
490 pigeons following a familiar leader are more likely to keep it on their left side, presumably using the  
491 left eye/right hemisphere. However, Nagy and colleagues (Nagy, Ákos, Biro & Vicsek, 2010)  
492 recorded a difference in the reaction speed to turns of a following companion when the being  
493 followed is viewed by the left or right eye, and not an actual difference in attraction or pairing with  
494 a companion. Following a leader might reflect a process of individual recognition, which as stated  
495 above, is more strongly associated with the right hemisphere (see Manns & Ströckens, 2014). The  
496 suggestive stronger role of the left hemisphere in flock cohesion observed in the present experiment  
497 does not necessarily imply an involvement of right eye/left hemisphere visual system in individual  
498 recognition. **In fact, both hemispheres were found to be involved in avian social responses, but**  
499 **with different strategies: while the left hemisphere is engaged in categorising an object as**  
500 **social partner or not, the right hemisphere is specialised for individual recognition within the**  
501 **social partner category (Vallortigara & Andrew, 1991; Vallortigara & Andrew, 1994).**

502 In summary, our results suggest the intriguing hypothesis that a lateralized brain might have  
503 an evolutionary advantage in that the processing and memorizing visual landmarks in the context of  
504 route fidelity is mainly a property of the left eye/right hemisphere, while the right eye/left  
505 hemisphere is engaged in parsing flying objects, like companions or potential predators. Indeed, the  
506 partial segregation of such functions has been proposed to provide a selective advantage promoting  
507 the evolution of brain asymmetries in birds and other species groups (Vallortigara & Rogers, 2005;  
508 Rogers, Vallortigara & Andrew, 2013).

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615

616 Table 1. Number of tracks recorded for each bird and analyses conducted.

Treat	Birds	Year	Group releases		Individual releases		Clock-shift tests		Analyses	
			Livorno	Sterpaia	Livorno	Sterpaia	Livorno	Sterpaia	Livorno	Sterpaia
C	360	2013	6	7	1	1	inc	1	<i>b, c</i>	<i>b, c, d, e</i>
C	393	2013	6	7	1	1	0	inc	<i>b, c</i>	<i>b, c, e</i>
C	475	2013	2	3	1	1	1	inc	<i>d</i>	
C	479	2013	3	2	1	1	1	1	<i>d</i>	<i>d</i>
C	480	2013	4	4	1	1	1		<i>d</i>	<i>d</i>
C	488	2013	6	7	1	1	1	1	<i>b, c, d</i>	<i>c, d, e</i>
C	497	2013	5	7	1	1	1	1	<i>b, c, d</i>	<i>b, c, d, e</i>
C	509	2013	5	4	1	inc	1	1	<i>b</i>	
C	528	2013	5	3	1	1	1	1	<i>b, d</i>	<i>d</i>
C	530	2013	4	3	1	1	inc	1		<i>d</i>
C	369	2014	7	7	0	0	0	0	<i>a, b, c</i>	<i>a, b, c</i>
C	674	2014	7	7	1	1	1	1	<i>a, b, c, d, e</i>	<i>a, b, c, d, e</i>
C	869	2014	7	7	0	1	1	1	<i>a, b, c, e</i>	<i>a, b, c, e</i>
C	873	2014	7	7	1	1	1	1	<i>a, b, c, e</i>	<i>a, b, c, d, e</i>
LH	471	2013	6	7	1	1	inc	1	<i>b, c</i>	<i>b, c, d, e</i>
LH	483	2013	6	6	1	1	0	inc	<i>c</i>	<i>c</i>
LH	520	2013	7	7	1	1	1	1	<i>b, c, d, e</i>	<i>b, c, d, e</i>
LH	523	2013	1	2	1	1	1	1	<i>d</i>	<i>d</i>
LH	533	2013	6	7	1	1	1	1	<i>b, c, d, e</i>	<i>b, c, d, e</i>
LH	364	2014	7	7	1	1	1	1	<i>a, b, c, d, e</i>	<i>a, b, c, d, e</i>
LH	665	2014	7		1	1	1	inc	<i>a, b, c, d, e</i>	<i>a, b, c, e</i>
LH	868	2014	7	7	1	1	1	1	<i>a, b, c, d, e</i>	<i>a, b, c, d, e</i>
LH	882	2014	7	7	1	1	1	1	<i>a, b, c, d, e</i>	<i>a, b, c, d, e</i>
LH	886	2014	7	7	1	1	1	1	<i>a, b, c, d, e</i>	<i>a, b, c, d, e</i>
LH	892	2014	7	7	1	1	1	1	<i>a, b, c, d, e</i>	<i>a, b, c, d, e</i>
RH	411	2013	4	5	1	1	1	1	<i>d, e</i>	<i>d, e</i>
RH	481	2013	6	7	1	1	1	1	<i>b, c, d, e</i>	<i>b, c, d, e</i>
RH	501	2013	7	7	1	1	1	1	<i>b, c, d, e</i>	<i>b, c, d, e</i>
RH	511	2013	5	3	1	1	1	1	<i>d, e</i>	<i>d</i>
RH	372	2014	7	7	1	1	1	1	<i>a, b, c, d, e</i>	<i>a, b, c, d, e</i>
RH	375	2014	7	7	1	1	inc	inc	<i>a, b, c</i>	<i>a, b, c, e</i>
RH	393	2014	7	7	1	1	1	1	<i>a, b, c, d, e</i>	<i>a, b, c, d, e</i>

RH	662	2014	7	7	1	1	1	1	<i>a, b, c, d, e</i>	<i>a, b, c, d, e</i>
RH	863	2014	7	7	1	1	paired	inc	<i>a, b, c</i>	<i>a, b, c</i>
RH	887	2014	7	7	1	1	1	1	<i>a, b, c, d, e</i>	<i>a, b, c, d, e</i>

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617 Treat: experimental treatments (see Materials and methods for details); Birds: individual codes;  
618 Year: year of the experiment; Group releases: number of tracks obtained during the group training  
619 releases from Livorno and La Sterpaia; Individual training releases and Clock-shift tests: track  
620 obtained (1), track not obtained (0), incomplete (inc), bird joined to another bird (paired); Analyses,  
621 the codes indicate the analysis performed on the data relative to each subject as follows: *a* Pairing  
622 index; *b* Kilometres flown beyond the latitude of the home loft; *c* Spatial occupancy index; *d*  
623 Percentage of fixes of the last training tracks over-flown under clock-shift; *e* Kilometres flown  
624 outside the total familiar area. See materials and methods for further explanations.  
625

626

627 Table 2. Virtual vanishing bearing distributions

Release site	Treat	Last training individual release				Clock-shift test			
		<i>N</i>	$\alpha$	r	hc	<i>N</i>	$\alpha$	r	hc
La Sterpaia	C	14	213	0.86***	+0.81***	13	150	0.83***	+0.60***
	LH	11	227	0.83***	+0.70***	11	144	0.81**	+0.52**
	RH	10	182	0.95***	+0.93***	10	100	0.68**	-0.05
Livorno	C	12	327	0.98***	+0.95***	11	259	0.77***	+0.10
	LH	11	354	0.94***	+0.92***	11	265	0.64	+0.06
	RH	10	301	0.82***	+0.62**	10	238	0.88***	-0.20

628 *N*, number of virtual vanishing bearing considered in the analysis;  $\alpha$ , mean vector direction; r, mean  
 629 vector length; hc, homeward component  $hc = r\cos(\alpha-\beta)$ , where  $\beta$  is the home direction; asterisks in  
 630 the r and hc columns indicate the results of the Rayleigh and V-test respectively; \* $P \leq 0.05$ , \*\* $P \leq$   
 631 0.01, \*\*\* $P \leq 0.001$ . The V test has been performed considering the home direction as expected  
 632 direction  
 633

634 Figure Legends

635 Figure 1. Box plots represent individual, median kilometres flown beyond a line crossing the home  
636 site perpendicularly to the beeline between the release site and the home site (see Materials and  
637 methods) until eventually reaching the home loft, during training flights. Boxes represent the  
638 interquartile range (IQR) between first and third quartiles and the line inside represents the median.

639

640 Figure 2. Box plots representation of a) spatial occupancy index: ratios between the familiar area  
641 and the minimal theoretical area that can be over-flown homing from each of the two release sites  
642 during training; b) kilometres flown outside the familiar area in clock-shifted binocular condition.  
643 Boxes represent the interquartile range (IQR) between first and third quartiles and the line inside  
644 represents the median. Whiskers denote the lowest and highest values within  $1.5 \times \text{IQR}$  from the  
645 first and third quartiles, respectively. Circles represent outliers beyond the whiskers.

646

647 Figure 3 Each symbol represents the individual percentage of fixes of the training corridor (defined  
648 by the points distant less than 400 m from each fix of the last training tracks from Livorno and La  
649 Sterpaia) that were over-flown during the tests under clock-shift in binocular condition.

650

651 Figure 4 Clock-shifted birds' tracks included in the analysis plotted over their respective familiar  
652 area (barred area). Black and grey lines represent the tracks recorded under clock-shift in the first  
653 and second release, respectively. Livorno and La Sterpaia release sites are indicated with a triangle  
654 and circle, respectively; home is indicated with a pentagon. Individual pigeon codes are reported.

655

656 Figure 5 Individual initial deviations of clock-shifted track from the last training track. See  
657 materials and methods for other explanations.

658

659 Figure A1 Mean Pairing Index of binocular control birds (C), birds processing visual inputs with  
660 the left (LH) or the right (RH) hemisphere, at each release site. The total value computed averaging  
661 the individual PI relative to both release sites is also given. Error bars indicate the standard  
662 deviation.

663

664 Figure A2 Example of tracks showing the bias towards the open eye from both release sites. RH  
665 birds, black line; LH, broken line.

666

667 Figure A3 Representative examples of tracks in the last part of the homing journey of RH pigeons  
668 from La Sterpaia (upper panels) and LH pigeons from Livorno (lower panels). The red continuous  
669 line indicates the perpendicular to the beeline between the release site and home, which is marked  
670 with a red broken line.

671

672 Figure A4 Spatial occupancy area. Merged corridors over-flown from La Sterpaia (light grey) and  
673 Livorno (dark grey) during the training flights subsequent to the first one from each site. See text  
674 for further explanation. Livorno and La Sterpaia release sites are indicated with a triangle and  
675 circle, respectively; home is indicated with a pentagon. Individual pigeon codes are reported.

676

677 Figure A5 Observed mean and median deviation from the home direction of birds released under  
678 clock-shift at the two release sites every 500 meters from the release site. The mean deviation from  
679 home of the same birds released in the last training release and the expected deviation are also  
680 reported. Error bars represent standard errors. Negative and positive values of the ordinate axis  
681 represent counter-clockwise and clockwise deflections, respectively.

