

RESEARCH ARTICLE

A magnetic pulse does not affect homing pigeon navigation: a GPS tracking experiment

Richard Holland^{1,*}, Caterina Filannino² and Anna Gagliardo²

¹School of Biological Sciences, Queen's University Belfast, 97 Lisburn Road, Belfast, BT9 7BL, UK and ²Department of Biology, University of Pisa, Via Volta 6, 56126 Pisa, Italy

*Author for correspondence (r.holland@qub.ac.uk)

SUMMARY

The cues by which homing pigeons are able to return to a home loft after displacement to unfamiliar release sites remain debated. A number of experiments in which migratory birds have been treated with a magnetic pulse have produced a disruption in their orientation, which argues that a ferrimagnetic sense is used for navigation in birds. One previous experiment has also indicated an effect of magnetic pulses on homing pigeon navigation, although with inconsistent results. Previous studies have shown that some magnetic-related information is transmitted by the trigeminal nerve to the brain in some bird species, including the homing pigeon. The function of this information is still unclear. It has been suggested that this information is important for navigation. Previous studies with trigeminal nerve lesioned homing pigeons have clearly shown that the lack of trigeminally mediated information, even if magnetic, is not crucial for homing performance. However, this result does not completely exclude the possibility that other ferrimagnetic receptors in the homing pigeon play a role in navigation. Additionally, recent studies on homing pigeons suggested the existence of a ferrimagnetic sense in a novel location presumably located in the inner ear (lagena). In the present study, we tested whether any ferrimagnetic magnetoreceptors, irrespective of their location in the bird's head, are involved in pigeons' homing. To do this, we treated homing pigeons with a strong magnetic pulse before release, tracked birds with GPS loggers and analyzed whether this treatment affected homing performance. In the single previous magnetic pulse experiment on homing pigeons, only initial orientation at a release site was considered and the results were inconsistent. We observed no effect of the magnetic pulse at any of the sites used on initial orientation, homing performance, tortuosity or track efficiency, which does not support a role for the ferrimagnetic sense in homing pigeon navigation, at least not in this geographic area, where magnetic field variations are in the region of 200 nT intensity and 0.8 deg inclination.

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INTRODUCTION

True navigation describes behaviour in which an animal is able to return to a known goal from an unfamiliar area without access to cues emanating from it or information from the displacement to the unfamiliar site (Able, 2001; Griffin, 1952; Keeton, 1974). The homing pigeon is a long-established model for the study of true navigation because of its motivation to return to a home loft rapidly after displacement to an unfamiliar place (Wallraff, 2005). However, despite more than 40 years of experimental investigation, the cues used by pigeons for true navigation remain debated (Phillips et al., 2006; Wallraff, 2005). According to the olfactory navigation hypothesis, pigeons perform true navigation by relying on the spatial distribution of environmental odours learned at the home loft area by associating the wind-borne odours with the wind directions (Papi et al., 1972; Wallraff, 1991). An alternative hypothesis argues that one or more magnetic field parameters can be used for finding the animal's position (Lohmann et al., 2007; Putman et al., 2011), although in some regions of the Earth it seems to be difficult to use a bi-coordinate magnetic map because different magnetic parameters are distributed at a relatively small or parallel angle to each other (Åkesson and Alerstam, 1998; Boström et al., 2012). A number of experiments in which the orientation performance of pigeons

appeared to be affected in various ways by releases in magnetic anomalies have been argued to support this hypothesis, although only with indirect evidence (Dennis et al., 2007; Mora and Walker, 2009; Walcott, 1991; Wiltschko et al., 2009a).

As it has been proposed that birds, including pigeons, sense the magnetic field through sensory cells that contain ferrimagnetic material such as magnetite (Kirschvink and Gould, 1981), another line of evidence in favour of a role for magnetic cues in pigeon navigation comes from experimental manipulation of the presumed sensory system. Brief, strong magnetic pulses that would re-magnetise magnetic material have been shown to disrupt the orientation of migratory birds in the laboratory (Beason et al., 1995; Munro et al., 1997; Wiltschko et al., 1994) and in the field (Holland and Helm, 2013; Holland, 2010). This sensory system appears to detect some aspect of the magnetic field, and magnetic information would be conveyed to the brain by the ophthalmic branch of the trigeminal nerve (hereafter V1) (Beason and Semm, 1996; Heyers et al., 2010; Mora et al., 2004; Semm and Beason, 1990a). The fact that magnetic pulses affect adult but not juvenile migrating birds (Munro et al., 1997) and the trigeminal nerve does not play a role in the magnetic compass response (Beason et al., 1997; Zapka et al., 2009) has been used

to argue that this sensory system is used for map-based navigation in migrating birds (Wiltschko and Wiltschko, 2006). A magnetic pulse has been shown to disrupt the orientation performance of homing pigeons, although not homing performance (Beason et al., 1997). Beason and colleagues argued that this effect was on a map rather than a compass as releases were under sunny conditions, where the sun compass has been shown to dominate over the magnetic compass in the cue hierarchy. Local anaesthetic applied to the beak area, a putative site of magnetoreception in pigeons (Fleissner et al., 2003), has also been shown to reduce the disrupting effect of being released in a magnetic anomaly (Wiltschko et al., 2010). It is argued that these results provide evidence that pigeons use a ferrimagnetic sense as part of a redundant navigation system. However, the results reported by Wiltschko et al. actually speak against crucial involvement of the trigeminal system in navigation, as the anaesthetised pigeons showed unimpaired navigational abilities. This is consistent with what was reported in a series of experiments in which the V1 of homing pigeons were lesioned before release at unfamiliar sites (Gagliardo et al., 2006; Gagliardo et al., 2008; Gagliardo et al., 2009). In no case did lesioning V1 result in impairment in homing performance, and strikingly, birds with an intact V1 were unable to home if the olfactory nerve was severed. This suggested that V1 was neither sufficient nor necessary for true navigation in these birds.

Initially, iron-containing cells found in the upper beak of homing pigeons and other birds (Beason and Nichols, 1984; Falkenberg et al., 2010; Fleissner et al., 2003; Fleissner et al., 2007; Williams and Wild, 2001) were suggested as magnetoreceptors innervated *via* the V1. However, a recent and thorough study made on homing pigeons (Treiber et al., 2012) strongly suggested that the majority, if not all, of Fe-positive cells both in the upper beak and other parts, such as skin, respiratory epithelium and feathers folliculi, are represented by macrophages. This finding questions the previous model of the upper beak organ (Fleissner et al., 2003; Fleissner et al., 2007) and clearly shows that the widely used method of Prussian Blue staining alone is not enough when it comes to identification of iron-containing magnetoreceptors. Nevertheless, the study by Treiber et al. (Treiber et al., 2012) does not completely rule out the possibility that trigeminal ferrimagnetoreception in birds exists. There is independent evidence suggesting that V1 in homing pigeons (Mora et al., 2004) and European robins (Heyers et al., 2010) does transmit magnetic-related information to the brain [although see Kishkinev et al. (Kishkinev et al., 2012) for failure to replicate Mora et al. (Mora et al., 2004) with European robins]. These data still support the notion that there are magnetoreceptors in the areas innervated *via* V1 (the upper beak, cere, skin at the frontal part of the bird's head or adjacent areas), though these magnetoreceptors have not yet been identified with certainty (Heyers et al., 2010; Mouritsen, 2012; Treiber et al., 2012). Even if V1 does transmit magnetic information in homing pigeons, previous studies with trigeminal nerve lesioned pigeons (Gagliardo et al., 2006; Gagliardo et al., 2008; Gagliardo et al., 2009) have clearly shown that V1-carried information is not crucial for homing performance in this species. However, this result does not completely exclude that non-trigeminally mediated ferrimagnetic receptors in the homing pigeon play role in navigation. Recent studies by Wu and Dickman (Wu and Dickman, 2011; Wu and Dickman, 2012) on homing pigeons suggested the existence of a ferrimagnetic sense in a novel location, presumably in the inner ear (lagena). Despite this neurological evidence, the effect of inner ear lesions on pigeon homing performance has provided mixed results, with one experiment failing

to find an effect on homing (Wallraff, 1972). Another study did record deficits in performance (Harada et al., 2001), but only tested birds in a familiar area, and the results suggest that the effect was due to non-specific effects of the surgery rather than an impairment of navigation by removal of a sensory system [see Wallraff (Wallraff, 2005) for discussion]. Taken together, there is a possibility that ferrimagnetoreceptors, irrespective of their location in the bird's head, are involved in pigeons' homing as a compass, map or both. To test this hypothesis, we treated homing pigeons with strong magnetic pulse before release, tracked birds with GPS loggers and analysed whether this treatment affects homing performance. In the single previous magnetic pulse experiment on homing pigeons, only initial orientation shortly after release, not the whole homing tracks, were considered, and the result was inconsistent, indicating a possible effect of the pulse treatments at some release sites but not others (Beason et al., 1997).

MATERIALS AND METHODS

General procedure

One hundred and twenty-five adult homing pigeons, *Columba livia* Gmelin 1789, of at least 6 months of age and hatched at the Arnino field station (latitude 43°39'26"N, longitude 10°18'14"E), Pisa, Italy, were used in the study, which took place in 2010 and 2011. The pigeons were raised as free flyers and were kept and manipulated according to Italian law on animal welfare. The birds had been subjected to training releases up to 5–7 km around the loft in different directions. Twenty days prior to the experimental releases all the birds were equipped with a PVC dummy weight, similar in dimension and weight to the GPS data logger they would be carrying, in order to accustom them to flying with a load. The dummy was attached to the pigeons' back by means of a Velcro strip glued on the feathers, which had been trimmed.

GPS data logger

Technosmart and iGotU (Mobile Action Technology, New Taipei City, Xindian District, Taiwan) GPS data loggers were used in 2010 and 2011, respectively. They recorded positional data of flying birds every second, and only in a few cases at a lower sampling rate (6–10 s). The positional information stored by a GPS data logger includes latitude, longitude and time of recording. The devices also provide information about altitude, but with insufficient precision to allow a reliable analysis. The tracks for each pigeon for each recorded release were visualised in Google Earth.

Test releases

Before displacement the birds were divided into two groups and subjected to two different treatments. Magnetically manipulated (MP) birds received a strong brief pulse from an SCR-fired capacitive discharge unit (SOTA magnetic pulser, Sota Instruments, Penticton, BC, Canada) modified by the addition of a double-wrapped, 10 cm diameter Lee Whittling coil (Kirschvink, 1992). The coil system produced a unidirectional magnetic pulse of ~0.1 ms duration, with peak amplitude slightly over 0.1 T, and a rise time of ~100 ns. The pulse was administered from a solenoid aligned perpendicular to the Earth's magnetic field, applied 'south anterior' as defined by Beason et al. (Beason et al., 1995) (see Fig. 1). A pulse of this strength is sufficiently large to overcome the coercivity of all known biogenic magnetite and thus re-magnetise it in the direction of the applied pulse (Dunlop, 1981; Kirschvink, 1983). After homing, the MP birds were excluded from the subsequent releases. Control (C) birds were placed in the pulser, but although the capacitor charged and fired in the same way as for the MP

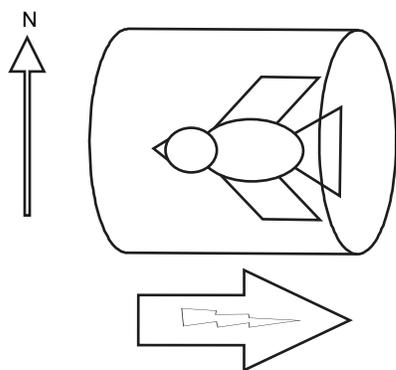


Fig. 1. Schematic of the orientation of the bird to the pulse when placed in the pulse coil. The horizontal arrow with the lightning bolt indicates the direction of the pulse. The vertical arrow indicates the orientation of the Earth's magnetic field (after Holland, 2010).

pigeons, the current was not delivered into the coil and so the magnetic pulse was not delivered. After homing, the C birds tested at one site could be released at another site either in the C or the MP group in a balanced number.

All the birds were released at unfamiliar locations (see Table 1 for details). Prior to release, the dummy on the back of each pigeon was replaced with a GPS data logger. Each pigeon was released singly, with at least 10 min between releases. All the experimental releases took place under sunny conditions, with no or light wind.

Quantitative analyses and statistical procedures

All tracks interrupted within 4 km distance from the release site were excluded from the analysis. If pigeons belonging to two different experimental groups joined after release they were excluded from the analysis. If two pigeons belonging to the same group joined, only the one released later was included in the analysis.

Homing success was recorded and the performances (homed *versus* lost) of the two groups were compared using the χ^2 -test. For each pigeon we analysed the following aspects of the flight path.

Distance from home

The distance from home observed every each hour after release was analysed for the first 10 h of tracking. The performances of the two groups were compared with a two-way repeated-measures (RM) ANOVA.

Stops

Stops were recorded as the percentage of time the birds were perching with respect to the time of recording.

Efficiency index

To compare the length of the homing journey we considered the efficiency index, which we calculated as the ratio between the track length and the beeline distance between the release site and home. When the tracks were not complete we added the linear distance from the end of the track to the loft. The efficiency indices of the experimental groups were compared with the Mann–Whitney *U*-test. Tracks shorter than the 50% of the beeline were excluded from the analysis.

Tortuosity per kilometre

To perform this analysis we drew concentric circles around the release site with a radii increasing by 1 km from 2 to 10 km, and we considered separately the portions of the tracks included in the ring delimited by two consecutive circles (for details, see Gagliardo et al., 2011). For each portion we considered the direction taken by the bird moving from one point to the next and from this we calculated the mean vector. We applied a two-way RM ANOVA on the mean vector lengths relative to portions of the tracks recorded at increasing distances in order to compare the tortuosity in the flight path of the two experimental groups.

Mean vector analysis

The procedure described above was applied to the section of each track within a range of 1–10 km from the release site. The resulting mean vector gives a good description of the initial orientation of the bird and the tortuosity of its flight path. For each group the mean vector distributions were tested for randomness using the one-sample Hotelling test. At each release site the mean vector distributions of the two groups were compared using the two-sample Hotelling test.

Virtual vanishing bearings

We recorded the direction of the birds at 2 km from the release site. The circular distributions were tested for randomness using both the Rayleigh test and the *V*-test, and the two groups were compared with the Watson *U*²-test (Batschelet, 1981).

RESULTS

The tracks used in the analysis are reported in Figs 2–5 for Chiesina Uzzanese, La Sterza, Montespertoli and Braccagni, respectively.

Table 1. Homing success of birds released at the four test sites

Release site	Treatment	<i>N</i>	<i>n</i>	Homed	Lost	χ^2
La Sterza	C	18	16	14	2	1.26 ^{ns}
299 deg, 39 km	MP	19	17	11	6	
29–30/07/2011						
Chiesina Uzzanese	C	12	10	8	2	0.20 ^{ns}
241 deg, 40.3 km	MP	12	11	9	2	
28/07/2011						
Montespertoli	C	17	17	16	16	0.47 ^{ns}
270 deg, 61 km	MP	17	16	15	18	
15/08/2010						
29–30/07/2011						
Braccagni	C	38	38	22	16	0.01 ^{ns}
326 deg, 106.3 km	MP	40	39	21	18	
7–10/08/2010						
1/08/2011						

C, control; MP, magnetic pulse. *N*, number of birds released; *n*, number of birds considered in the analysis.

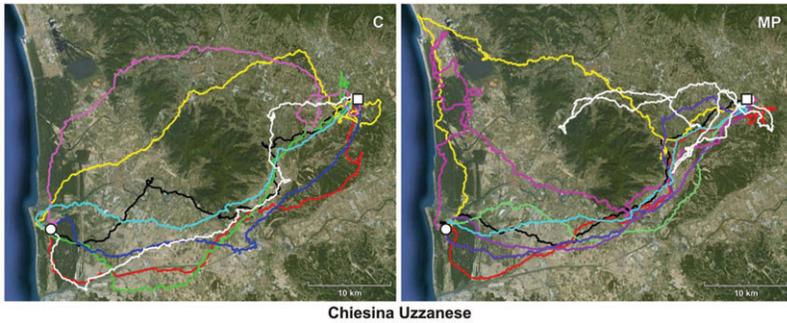


Fig. 2. Release from Chiesina Uzzanese. Tracks of control (C) and magnetic pulsed (MP) pigeons are reported in the left and right panels, respectively. Release and home sites are indicated by the white square and circle, respectively. Within the same panel, different tracks are indicated with different coloured lines. The tracks are plotted over a Google Earth map.

Homing performance

The homing success of C and MP pigeons were not statistically different according to the χ^2 test in each of the four release tests (Table 1). The two-way RM ANOVA applied to the distance from home recorded in the first 10 h after release did not reveal a significant difference between the C and MP pigeons at any site (factor treatment; $P > 0.1$ in all tests; Chiesina Uzzanese $F = 1.825$, La Sterza $F = 0.168$, Montespertoli $F = 2.131$, Braccagni $F = 0.770$; Fig. 6). The distance from home significantly decreased over time (factor hours from release; $P < 0.001$ in all tests; Chiesina Uzzanese $F = 19.347$, La Sterza $F = 22.055$, Montespertoli $F = 18.905$, Braccagni $F = 19.610$; see Fig. 2). The interaction between treatment and time turned out to be significant in one ($P < 0.001$; Chiesina Uzzanese $F = 4.718$) out of four test releases ($P > 0.1$; La Sterza $F = 1.468$, Montespertoli $F = 0.455$, Braccagni $F = 0.444$). In fact, at Chiesina Uzzanese the MP pigeons approached home significantly faster than the C birds in the first 3 h after release (Student–Newman–Keuls test, C vs MP, Hour 1 $q = 5.282$, $P < 0.001$; Hour 2 $q = 4.042$, $P < 0.01$; Hour 3 $q = 3.618$, $P < 0.05$).

Stops

The percentage of time the birds spent sitting during the track recording was significantly different between the two groups of pigeons only at Montespertoli (Mann–Whitney U -test $P < 0.05$, median C 57%, MP 79%). In the other three release tests the difference between C and MP pigeons never reached significance (Chiesina Uzzanese, C 68%, MP 61%; La Sterza, C 74%, MP 77%; Braccagni C 81%, MP 77%).

Tortuosity

The analysis of the tortuosity applied on sectors of the tracks at increasing distances from the release site did not show a significant difference between treatments at any site ($P > 0.1$, Chiesina Uzzanese $F = 0.009$; La Sterza $F = 0.346$; Montespertoli $F = 0.109$; Braccagni $F = 0.002$). The tortuosity was not significantly different at various

distances from the release site ($P > 0.05$, La Sterza $F = 1.677$; Montespertoli $F = 0.611$; Braccagni $F = 1.141$), except in the release from Chiesina Uzzanese, where the tortuosity was significantly higher ($P < 0.05$, $F = 2.471$) at a distance ranging between 2 and 3 km than at a distance ranging from 8 to 9 km from the release site ($P < 0.05$, Tukey's test). At no site was there a significant interaction between treatment and distance (La Sterza $F = 1.413$, Chiesina Uzzanese $F = 0.809$; Montespertoli $F = 1.127$; Braccagni $F = 0.745$).

Efficiency index

The efficiency index (EI) did not differ between groups according to the Mann–Whitney U -test in the four test releases (median EI: Chiesina Uzzanese, C $N = 8$, EI = 0.52, MP $N = 9$, EI = 0.64; La Sterza, C $N = 14$, EI = 0.56, MP $N = 11$, EI = 0.58; Montespertoli C $N = 11$, EI = 0.74, MP $N = 10$, EI = 0.67; Braccagni C $N = 12$, EI = 0.71, MP $N = 8$, EI = 0.72].

Virtual vanishing bearings

The two groups of pigeons were significantly oriented at 2 km from the release site and their mean vector direction was close to the home direction in all tests (Fig. 7, Table 2). The Watson U^2 -test did not reveal a significant difference between C and MP pigeons at any site ($P > 0.1$ in all tests).

Mean vector analysis

The initial orientation of the birds at a distance ranging between 1 and 10 km from the release site is summarised by the mean vector diagrams represented in Fig. 8 (see also Table 2 for the second-order mean vector values). The Hotelling test applied to the mean vector distributions revealed that both C and MP group mean vector distributions were different from random at all release sites (Chiesina Uzzanese C $P < 0.01$, MP $P < 0.001$; La Sterza C $P < 0.001$, MP $P < 0.01$, Montespertoli and Braccagni both C and MP $P < 0.001$). The two-sample Hotelling test did not reveal a difference between the C and MP distribution in any of the release tests ($P > 0.1$).



Fig. 3. Release from La Sterza. Other explanations as in Fig. 2.

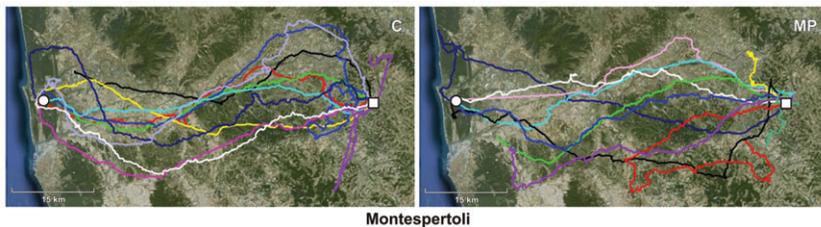


Fig. 4. Release from Montespertoli. Other explanations as in Fig. 2.

DISCUSSION

It has been argued that if animals possess a ferrimagnetic sense, then applying strong magnetic fields should disrupt or damage the sensory receptor, as shown by *in vitro* experimental tests demonstrating the disruption of a cluster of superparamagnetic particles when treated with a strong magnetic pulse (Davila et al., 2005). Therefore, such a treatment is expected to produce loss or change of magnetic-information-based navigation performance (Kirschvink and Gould, 1981). Magnetic pulse treatments have previously been shown to affect the orientation of migratory birds in laboratory- (Wiltshcko et al., 1994) and field-based tests (Holland and Helm, 2013; Holland, 2010). In studies using orientation cages, the cancelling of the effect when a local anaesthetic is applied to the trigeminal nerve (Beason and Semm, 1996) or the upper beak (Wiltshcko et al., 2009b) is argued to indicate that the effect is on a magnetosensory receptor involved in navigation, located in the beak and mediated by the trigeminal nerve. A magnetic pulse has also been shown to affect the orientation of homing pigeons in some circumstances (Beason et al., 1997). A significant difference was only observed at two out of 16 sites in the comparable south anterior treatment, and not consistently [fig. 2, table 1 in Beason et al. (Beason et al., 1997)]. Our results show no difference in orientation or homing performance between C and MP-treated birds at any distance, with a maximum (but not significant) difference in mean vectors of 23 deg at Braccagni, 106 km from the loft. Taken together with the results of Gagliardo et al. (Gagliardo et al., 2006; Gagliardo et al., 2008; Gagliardo et al., 2009), this result strongly suggests that putative ferrimagnetic receptors in the upper beak, lagena or elsewhere do not play a role in homing of pigeons in this study. Whether this result is because pigeons do not use magnetic cues for map navigation is a matter of discussion. The results of Gagliardo et al. (Gagliardo et al., 2006; Gagliardo et al., 2008; Gagliardo et al., 2009) indicate that an intact trigeminal nerve does not allow anosmic pigeons to home within 100 km of the home loft. This finding speaks against a multicue system for true navigation in homing pigeons at these distances. However, Wiltshcko et al. (Wiltshcko et al., 2010) argue that improved initial orientation performance at magnetic anomalies located within 90 km from home after the application of local anaesthesia on the beak was due to the removal of confusing magnetic information. The results of Treiber et al. (Treiber et al., 2012), which indicate that the putative site of magnetoreception in the upper beak appear to be macrophages, make it unclear as to whether this treatment would affect a magnetoreceptor [although see Wiltshcko and Wiltshcko (Wiltshcko and Wiltshcko, 2012) for other behavioural evidence consistent with magnetoreceptors in this region and discussion of this issue in the Introduction].

Possible experimental differences between this study and that of Beason et al. (Beason et al., 1997) include the pulse strength and the distance of release sites. The pulse strength varied between the present study and that of Beason et al., with a 0.1 T pulse used in this study and a 0.5 T pulse in Beason et al. However, previous experiments (Holland, 2010; Holland et al., 2008; Holland and Helm,

2013) using this pulse device have indicated significant effects on initial orientation in both bats and migratory birds. Furthermore, 0.1 T is greater than the coercivity of all known biogenic magnetite, so this seems an unlikely explanation for these results (Dunlop, 1981; Kirschvink, 1983).

Why is the distance of release argued to be important? One hypothesis suggests that difference in intensity of the magnetic field between the current position and the desired goal provides a cue to latitude (Phillips et al., 2006). Differences in intensity are argued to be too variable or too small at distances <75 km from the goal (Phillips, 1996). We acknowledge that Beason et al. (Beason et al., 1997) had several release sites more distant than our 106 km from the loft, although significant effects were seen at only two of those in the south anterior group. The sample sizes between papers were comparable, however, with no more than 12 bearings analysed at any site in the prior study. However, distance alone is not an explanation unless it corresponds with differences in the magnetic field parameters that the birds were exposed to in the two regions. Examining the magnetic field parameters of the four release sites used in our experiment with respect to home highlights the variability inherent in the magnetic field (supplementary material Table S1, values obtained from a global field model). While three of the sites (Braccagni, La Sterza and Chiesina Uzzanese) have field parameters that vary as expected in comparison to the home loft values (i.e. increasing inclination and total intensity from south to north), one site, Montespertoli, has a total intensity that is greater than the home site, despite being on the same latitude. The largest difference in intensity is between Braccagni and home (214 nT less at Braccagni than at the loft), as one would expect because it is approximately south of the loft and 106 km away, i.e. the furthest latitudinal displacement. Beason et al. (Beason et al., 1997) found a correlation between distance of release site from home and the size of deflection from controls. They also had three sites at distances further than our furthest release site (123, 128 and 169 km). However, this does not take into account intensity or inclination differences between the home loft and the release site, upon which the hypothesis of magnetic navigation is based [see supplementary material Table S1

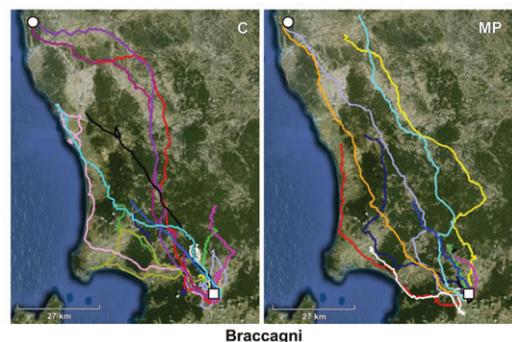


Fig. 5. Release from Braccagni. Other explanations as in Fig. 2.

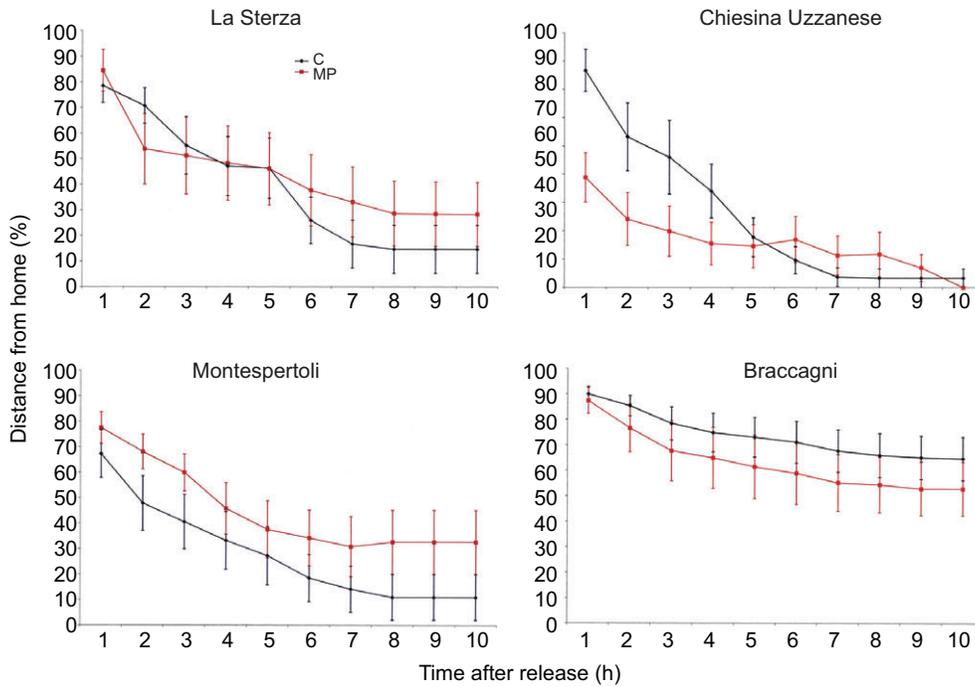


Fig. 6. Distance from home plotted as a function of time for experimental and control groups at the four release sites. Control birds (C) are plotted in red and experimental birds (MP) are plotted in blue in all graphs.

for values of Beason et al. (Beason et al., 1997)]. Closer examination of the intensity and inclination parameters at the sites used in both studies in relation to observed significance of the effect reveal a more complicated picture that is not easy to explain by a simple intensity/inclination/distance *versus* effect relationship. First, if significantly oriented groups are considered, Beason et al. (Beason et al., 1997) only had two sites out of 16 at which a significant effect of treatment was observed in the south anterior treatment (comparable to ours). Two of these releases were at a site 107.8 km from the loft, with distance and inclination values comparable to

our furthest site, but with a higher intensity (60 nT higher). The other site where the experimental group became disoriented, 169 km from the loft, had a lower intensity (40 nT lower) and inclination (0.39 deg lower). The other two sites at greater distance than our furthest site (123 and 128 km) did not show a significant effect in the comparable south anterior treatment, in fact showing smaller deflection from controls (12 and 14 deg) than at our furthest site (23 deg), despite in one case (123 km) having the highest intensity and inclination values that of any site used (294 nT). The fact that the intensity and inclination difference of our furthest site, 106 km from the loft, is

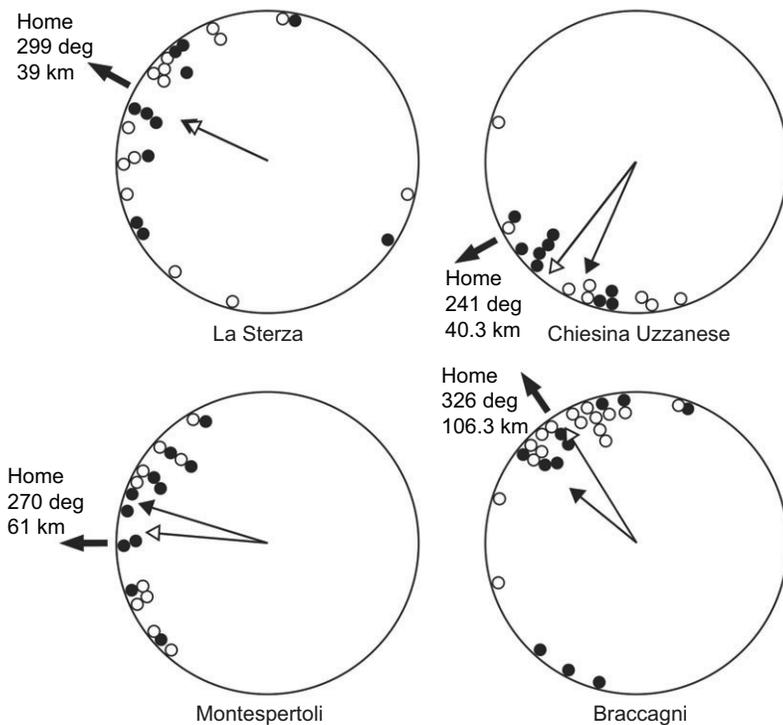


Fig. 7. Virtual vanishing bearings of C (open circles) and MP (filled circles) groups at each of the four release sites. Mean vectors are shown (C, open arrowhead; MP, filled arrowhead). Arrow outside the circle represents the home direction.

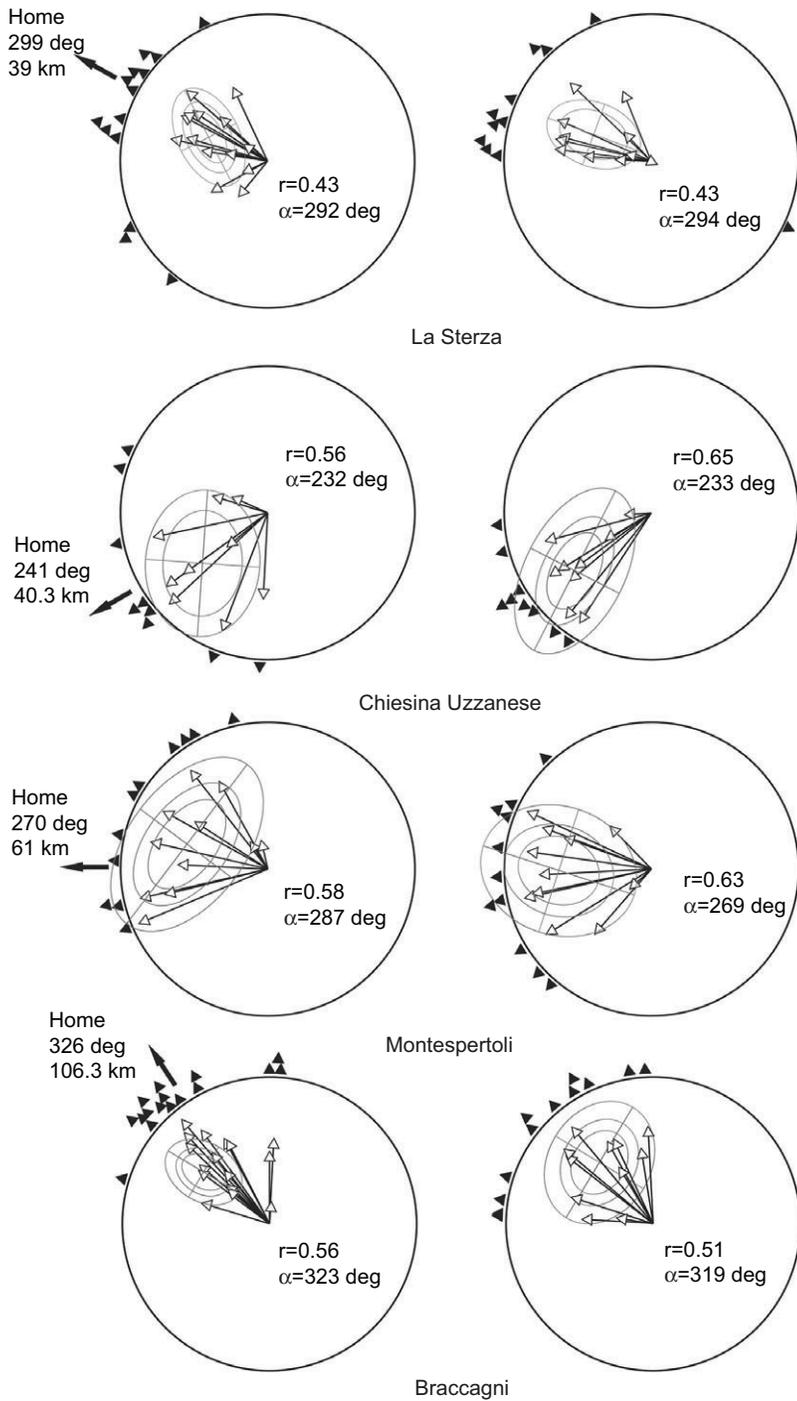


Fig. 8. Initial orientation analysis performed on the section of the tracks recorded from 1 to 10 km from the release site. Each mean vector has been calculated from the directions taken by a bird while moving from one fix to the next (see Materials and methods for details). Second-order mean vector length (r) and direction (α) are reported for each diagram. Triangles outside each diagram represent the direction of the mean vectors plotted inside each circle. Confidence ellipses of the distributions according to the one-sample Hotelling test are reported: significance levels of $P < 0.05$, $P < 0.01$ and $P < 0.001$ are represented by the inner, intermediate and outer ellipses, respectively.

greater than the difference in the site at 169 km in Beason et al. (Beason et al., 1997) also highlights that greater distances in the Beason et al. (Beason et al., 1997) study *per se* do not necessarily mean greater differences between magnetic parameters and are not necessarily a stronger predictor of effect. Thus, if the effects seen in the Beason et al. study are related to magnetic parameters, it is in a way that is not explained simply by greater differences in magnetic parameters and it does not easily explain the lack of an effect at our furthest site. It is not currently clear whether the magnetic sense of a homing pigeon is sensitive enough to detect the changes in intensity reported here. Changes in intensity of 3000 nT have been shown to affect the orientation behaviour of

migratory birds (Henshaw et al., 2010), and electrophysiology studies suggest sensitivity between 50 and 200 nT (Beason and Semm, 1987; Semm and Beason, 1990b) [but see Mouritsen and Hore (Mouritsen and Hore, 2012) for criticisms of early electrophysiology work]. No direct behavioural test indicates sensitivity as low as required for detecting 200 nT changes. On this basis, it would be important to test the effect of magnetic pulses, selecting sites where intensity differences are clearer indications of differences in latitude and approach potentially more realistic sensory thresholds.

A number of tracking studies that tested the navigation ability of *Procellariiformes* bearing strong magnets on their head failed to find

Table 2. Initial orientation at the four test release sites

Release site	Treatment	n	Virtual vanishing bearing			Mean vector 1–10 km		
			r	α (deg)	Homeward component	r	α (deg)	Homeward component
La Sterza	C	14	0.59**	295	+0.59***	0.43***	292	+0.43
	MP	11	0.64**	295	+0.64**	0.43**	294	+0.43
Chiesina Uzzanese	C	8	0.80**	204	+0.64**	0.56**	232	+0.63
	MP	9	0.94***	218	+0.87***	0.65***	233	+0.64
Montespertoli	C	10	0.80***	275	+0.80***	0.58***	287	+0.55
	MP	11	0.88***	287	+0.84***	0.63***	269	+0.63
Braccagni	C	16	0.89***	328	+0.89***	0.56***	323	+0.56
	MP	11	0.56*	305	+0.54**	0.51***	319	+0.51

C, control; MP, magnetic pulse; r, mean vector length; α , mean vector direction. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

an effect of the treatment on navigation (Benhamou et al., 2003; Bonadonna et al., 2005; Mouritsen et al., 2003), suggesting that they rely on other cues, possibly olfaction, for navigation (Nevitt and Bonadonna, 2005). The only previous tracking study on the effect of magnetic pulses on bird navigation was conducted on catbirds displaced during migration (Holland et al., 2009) and tracked by aerial survey from a light aircraft. This study also failed to find an effect on birds' orientation. However, in the latter case, the delay between treatment and departure meant that it was possible that the birds had recalibrated, as previous evidence suggests the pulse is temporary, lasting ~10 days (Holland and Helm, 2013; Wiltschko et al., 1998). In the present experiment, homing pigeons homed within hours of the treatment, so this cannot be an explanation for the lack of an effect. This strongly suggests that ferrimagnetic receptors do not play a role in homing of pigeons. This result, together with those from previous studies using trigeminal nerve lesioned pigeons (Gagliardo et al., 2006; Gagliardo et al., 2008; Gagliardo et al., 2009), questions the hypothesis of magnetic-based navigation in homing pigeons at least for intensity differences in the range of 200 nT. Whether (ferri)magnetoreceptors and the trigeminal nerve play a crucial role in long-distance navigation in birds remains to be demonstrated.

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AUTHOR CONTRIBUTIONS

R.H and A.G conceived the study. R.H, C.F. and A.G. carried out the experiments. R.H and A.G. wrote the paper.

COMPETING INTERESTS

No competing interests declared.

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