

1     **Conditioned Discrimination of Magnetic Inclination in a Spatial-Orientation**  
2             **Arena Task by Homing Pigeons (*Columba livia*)**

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10    Running Head: Pigeons detect magnetic inclination cues

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The Journal of Experimental Biology – ACCEPTED AUTHOR MANUSCRIPT

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### Summary

13 It has been well established that homing pigeons are able to use the Earth's magnetic field to obtain  
14 directional information when returning to their loft and that their magnetic compass is based, at least in  
15 part, on the perception of magnetic inclination. Magnetic inclination has also been hypothesized in  
16 pigeons and other long-distance navigators, such as sea turtles, to play a role providing positional  
17 information as part of a map. Here we developed a behavioural paradigm which allows us to condition  
18 homing pigeons to discriminate magnetic inclination cues in a spatial-orientation arena task. Six homing  
19 pigeons were required to discriminate in a circular arena between feeders located either in a zone with a  
20 close to 0° inclination cue or in a zone with a rapidly changing inclination cue (-3° to +85° when  
21 approaching the feeder and +85° to -3° when moving away from the feeder) to obtain a food reward. The  
22 pigeons consistently performed this task above chance level. Control experiments, during which the coils  
23 were turned off or the current was running anti-parallel through the double-wound coils system,  
24 confirmed that no alternative cues were used by the birds in the discrimination task. The results show that  
25 homing pigeons can be conditioned to discriminate differences in magnetic field inclination, enabling  
26 investigation into the peripheral and central neural processing of geomagnetic inclination under controlled  
27 laboratory conditions.

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29 Keywords: Homing pigeon – magnetic inclination – compass - conditioning.

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## Introduction

33 Homing pigeons (*Columba livia*), selectively bred for several thousand years for their ability to return to  
34 their loft from distant and unfamiliar sites, have been one of the main model species for studying the  
35 sensory mechanisms underpinning navigation behaviour. It is generally accepted that true navigation  
36 requires the use of a map and a compass, as originally suggested by Kramer (1953, 1961), to determine  
37 position relative to the goal and to set and maintain an appropriate direction of movement, respectively  
38 (for reviews see: e.g., Able, 2000; Wiltschko and Wiltschko, 2009; Wallraff, 2005). Over the last few  
39 decades, considerable evidence has accumulated that spatial information provided by the Earth's  
40 magnetic field plays an important role during pigeon navigation (for reviews see: e.g., Wiltschko and  
41 Wiltschko, 1995; Wiltschko and Wiltschko, 2009).

42 Firstly, homing pigeons possess a magnetic compass. Previous studies conducted with migratory  
43 birds had shown that the birds adjusted their preferred direction for orientation during migratory  
44 restlessness whenever the horizontal component of the magnetic field vector experienced by the birds  
45 inside Emlen funnels was rotated by magnetic coils surrounding the funnels (Wiltschko, 1968).  
46 Analogous to this, pigeons carrying battery-operated magnetic coils atop their heads flew in the opposite  
47 direction from home under overcast conditions (i.e., without the availability of their sun compass)  
48 whenever the vertical component of the local magnetic field vector was inverted (Walcott and Green,  
49 1974; Benvenuti *et al.*, 1982). Thus, the avian magnetic compass, unlike a human-made magnetic  
50 compass, is an “inclination” compass (for review see e.g., Wiltschko and Wiltschko, 2007; Wiltschko and  
51 Wiltschko, 2009), which does not respond to polarity, but instead utilizes the direction of the magnetic  
52 field vector to determine the North-South axis and the inclination angle to distinguish between  
53 “poleward” (downward inclination) and “equatorward” (upward inclination) direction.

54 Secondly, indirect evidence from field studies has suggested that pigeons may also, under some  
55 conditions, rely on spatial information provided by the Earth's magnetic field to determine their position  
56 with the vanishing directions of pigeons having been correlated to temporal and spatial variations in the  
57 local magnetic field under sunny conditions (Keeton *et al.*, 1974; Frei and Wagner, 1976; Wagner, 1976;  
58 Walcott, 1978; Frei, 1982). Furthermore, magnetic pulse treatments under sunny conditions shifted the  
59 initial orientation direction of homing pigeons thus providing further indication of the possible existence  
60 of a magnetic map (Beason *et al.*, 1997). It should be noted though that such a pulse effect was not  
61 replicated in a recent study with GPS-tracked homing pigeons (Holland *et al.*, 2013), which found no  
62 evidence of impairment of either initial orientation or navigation performance. Furthermore, there is also  
63 considerable evidence, which is not necessarily mutually exclusive to the possibility of the existence of a

64 magnetic map, that homing pigeons use odours in the atmosphere at least at some locations on Earth to  
65 determine their position (for reviews see Papi, 1992 and Wallraff, 2004; 2006).

66 Relevant for the current study, it is noteworthy that although discussion of a hypothetical  
67 geomagnetic map generally focuses on spatial variation in intensity (Walker, 1998; Walker, 1999; Dennis  
68 *et al.*, 2007; Mora and Walker, 2009; Wiltschko *et al.*, 2009; Postlethwaite and Walker, 2011; Mora and  
69 Walker, 2012; Postlethwaite *et al.*, 2012), magnetic inclination (the angle between the magnetic field  
70 vector and the Earth's surface) is also suitable for determining latitude (Gould, 1982) as it varies  
71 systematically, just like intensity, with latitude from the equator ( $0^\circ$ ) to the poles ( $+90^\circ$  and  $-90^\circ$  at the  
72 magnetic North and South poles respectively). For example, it been suggested that newts, which generally  
73 move over relatively short distances of only a few kilometers during homing (e.g., Fischer *et al.*, 2001;  
74 Phillips *et al.*, 2002), use magnetic inclination to determine latitude. More impressive, several studies  
75 have also indicated that inclination has an important role in position determination for marine long-  
76 distance migrators. Loggerhead sea turtles (*Caretta caretta*) can detect the magnetic inclination angle  
77 (Lohmann and Lohmann, 1994) and it has been even suggested that sea turtles possess a magnetic map  
78 consisting of a grid of magnetic intensity and inclination values (e.g., Lohmann and Lohmann, 1996;  
79 Lohmann *et al.*, 2007) While this hypothesis may not be globally applicable (lines of equal intensity and  
80 inclination intersect at sufficiently large angles only in small geographic areas) nor evolutionary stable  
81 due to gradual shifts in the Earth's magnetic poles over time (Courtilot *et al.*, 1997; Walker *et al.*, 2002),  
82 magnetic inclination remains an intriguing possibility that may allow animals such as sea turtles and  
83 birds, if they are sensitive to it, to locate their position at least on one axis of a bi-coordinate map.

84 Conditioning experiments in the laboratory provide one useful avenue to investigate sensory  
85 mechanisms as well as perception limits. Previous attempts to condition homing pigeons or other birds to  
86 magnetic stimuli have focused either on magnetic anomalies, which varied both in magnetic intensity and  
87 inclination in an uncontrolled way (Mora *et al.*, 2004; Thalau *et al.*, 2007; Freire *et al.*, 2012), or on  
88 changes in the horizontal component (Freire *et al.*, 2005; Voss *et al.*, 2007; Keary *et al.*, 2009; Wilzeck *et al.*,  
89 2010). To investigate whether homing pigeons could be trained to discriminate differences in  
90 magnetic field inclination only, we developed a behavioural conditioning paradigm that required pigeons  
91 to discriminate changes in magnetic inclination to obtain a food reward in a spatial-orientation arena task.  
92 In addition to demonstrating that our experimental pigeons can discriminate inclination differences, the  
93 results of our study open up the possibility for future investigation into the peripheral and central neural  
94 processing of magnetic inclination, potentially offering insight into the neural organization of the avian  
95 magnetic compass as well as provide a new approach to test the hypothesis of a geomagnetic "inclination  
96 map" component under controlled laboratory conditions.

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## Results

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### *Discrimination of Magnetic Inclination Zones*

99 All experiments were conducted in a circular arena situated centrally atop cinder blocks inside a 3-axis  
100 magnetic coil system (Fig. 1a) with pigeons being individually harnessed to a horizontal tracker arm (Fig.  
101 1b). Four automated feeder-response units were situated against the wall of the circular arena aligned with  
102 the four cardinal directions in the testing room (geographic North (N), South (S), East (E), and West (W);  
103 Fig. 1c; see also Methods and Materials and Supplementary Text). During the initial conditioning series,  
104 six pigeons were required to select one of two possible feeders associated with changing inclination out of  
105 a total of four feeders available (chance level 50%; Fig. 2). Responses to feeders in the “Zero Inclination”  
106 zones were not rewarded. Our results clearly show that homing pigeons are able to discriminate changes  
107 in magnetic inclination as they were able to distinguish the two feeders associated with a rapid change in  
108 inclination ( $-3^\circ$  to  $+85^\circ$ ) from the two feeders associated with a constant, low inclination value ( $-3^\circ$ ) (Fig.  
109 3a).).

110 Furthermore, we observed a statistically significant improvement in the pigeons’ performance  
111 over the course of the sessions (Linear Mixed Model ANOVA, type III test of fixed effects:  $F_{\text{Session}} =$   
112  $26.085$ ,  $p < 0.001$ ). However, it is important to note that reported data do not take the form of a traditional  
113 learning curve as most birds were already above chance level in the first session after having received  
114 prior discrimination training with the magnetic stimulus during the pre-training phase. Because pre-  
115 training was customized for each bird’s acquisition of the task (see Supplementary Text), discrimination  
116 performances during pre-training were not comparable for individual birds and therefore are not included  
117 in the graph. A systematic difference between subjects was detected (Linear Mixed Model ANOVA, type  
118 III test of fixed effects:  $F_{\text{Subject}} = 16.202$ ,  $p < 0.001$ ) due to birds 703 and 724 taking longer than the other  
119 birds to acquire a performance level that was consistently above chance (they did so toward the end of the  
120 initial conditioning series and performed well during the parallel/anti-parallel control series). After the  
121 initial eight sessions, the standard error for the mean performance across birds for each session no longer  
122 included the chance level (50%) for almost all sessions (for sessions 14 and 16 the standard error crossed  
123 the chance level, although it is not clear why the birds performed more poorly on these two days). We  
124 then calculated the mean performance over these last 17 sessions for each bird and then the mean  
125 discrimination performance across all birds ( $n=6$ , mean  $64.98\% \pm 3.31$  SE, 95% confidence interval  
126  $56.47\%$  to  $73.49\%$ ). This was significantly different from chance level (50%), both when comparing  
127 individual mean bird performances to chance level (un-paired T-test:  $T = 4.442$ ,  $p < 0.01$ ) as well as when  
128 looking at the mean performance of all birds being above chance level over the course of all sessions in  
129 this series (Wilcoxon Signed Ranks Test: T-Value = 0,  $p < 0.001$ ). In summary, the pigeons were

130 successfully able to perform the discrimination task and that performance was consistently above chance  
131 level over time.

### 132 *Coil On-Off Control Sessions*

133 White noise was used to mask any humming sounds emanating from the coils. Furthermore, the axis of  
134 correct feeder choices (North-South or East-West) was disassociated from any visual cues in the  
135 experimental room by selecting from a pseudorandom schedule which of the two feeder axes in the arena  
136 was associated with the changing inclination values for a given trial. To test whether any other alternative  
137 cues may have been used by the pigeons to identify a correct feeder, we conducted a coils On-Off series  
138 of control sessions with four of the original six pigeons. Four sets of two Coils-Off control sessions (no  
139 current sent to coils) were alternated with two consecutive standard sessions (same procedure as for the  
140 initial conditioning series) (Fig. 3b).

141 Mean discrimination performance averaged across all birds for all standard sessions of the coils  
142 On-Off control series was  $66.95\% \pm 2.16$  SE ( $n=4$ , 95% confidence interval 60.06% to 73.83%). This was  
143 significantly above chance level (50%; un-paired T-test:  $T = 7.550$ ,  $p < 0.001$ ) and slightly higher than the  
144 performance during the initial conditioning series. The birds' mean performance for each session was also  
145 consistently above 50% over time (Wilcoxon Signed Ranks Test: T-Value = 0,  $p < 0.001$ ). The  
146 performance in standard sessions was in contrast to the performance for the Coils-Off sessions ( $n=4$ ,  
147 mean  $50.30\% \pm 1.68$  SE, 95% confidence interval 44.94% to 55.65%), during which performance fell to  
148 around chance level (50%; un-paired T-test:  $T = 0.176$ ,  $p > 0.05$ ; Wilcoxon Signed Ranks Test: T-Value =  
149 14,  $p > 0.05$ ). Furthermore, each bird's individual performance was significantly different from its  
150 performance during Coils-Off sessions (paired T-test: T-value = 7.2289,  $p < 0.01$ ). Therefore, the pigeons  
151 were not able to perform the discrimination task when the coils were turned off.

### 152 *Coils Parallel/Anti-Parallel Control Sessions*

153 Next we conducted a parallel/anti-parallel control series using double-wrapped coils as suggested by  
154 Kirschvink *et al.* (2010) with seven pigeons (three from the initial conditioning series as well as four  
155 additional birds, which were pre-trained until a comparable average performance as in the initial  
156 conditioning series was achieved). The purpose of this was to eliminate the possibility of the white noise  
157 not having masked all sounds emanating from the experimental equipment and thus being used as  
158 alternative cues. This approach also eliminated the use of any other alternative cues (e.g., heat or  
159 vibration) potentially associated with the varying amounts of current passing through the coils during a  
160 trial.

161 Four sets of two consecutive sessions with the current running through the double-wound coils in  
162 the same direction (parallel sessions, i.e., same magnetic inclination cues as for the initial conditioning  
163 series) were alternated with three sets of two consecutive sessions with the current running in the opposite  
164 direction (anti-parallel sessions, i.e., background magnetic inclination cues) (Fig. 3c). Similar to the coils  
165 On-Off control experiment, for parallel coil sessions the mean discrimination performance averaged  
166 across all birds ( $n=7$ , mean  $69.08\% \pm 0.91$  SE, 95% confidence interval  $66.86\%$  to  $71.30\%$ ) was  
167 significantly above chance level (50%; un-paired T-test:  $T = 19.763$ ,  $P < 0.001$ ; Wilcoxon Signed Ranks  
168 Test:  $T\text{-Value} = 0$ ,  $p < 0.001$ ). This was significantly different (paired T-test:  $T\text{-value} = 9.4691$ ,  $p <$   
169  $0.001$ ) from the mean performance for each bird for the anti-parallel coils sessions ( $n=7$ , mean  $49.73\% \pm$   
170  $1.47$  SE, 95% confidence interval  $46.13\%$  to  $53.32\%$ ; chance level 50%; un-paired T-test:  $T = 0.186$ ,  $P >$   
171  $0.05$ ; Wilcoxon Signed Ranks Test:  $T\text{-Value} = 9$ ,  $p > 0.05$ ). This shows that the pigeons were not able to  
172 perform the discrimination task when the current ran anti-parallel through the coils. One curious finding  
173 was that the relatively small standard error associated with the discrimination performances during the  
174 earlier parallel sessions was not maintained during the last four parallel sessions. We do not know why  
175 this occurred, but complex conditioning paradigms inherently yield a certain amount of variability in the  
176 discrimination performances.

177 Mean discrimination performance during both Coils On-Off and the Parallel-Antiparallel series  
178 fell to around chance level with relatively little variance. The birds were still very motivated during Coils-  
179 Off trials as well as during anti-parallel sessions to move between feeders and peck the response keys  
180 when they were lit, i.e., they did not make their choices completely randomly nor did they just sit in front  
181 of a single feeder for the entire session pecking only that response key. Instead they sometimes adopted a  
182 combination of alternative choice behaviours, with the combination being unique to each bird.

183 We are confident for two reasons that such alternative strategies did not significantly contribute to  
184 the discrimination performance observed during standard sessions. Firstly, these strategies were not  
185 sufficient to raise performance above chance level during the two control series for which no magnetic  
186 discrimination cues were available to the birds. Secondly, an analysis of the discrimination performance  
187 for the last 10 standard sessions in Fig. 3a revealed that all six pigeons generally favoured choosing a  
188 different feeder on a subsequent trial irrespective of whether they were rewarded (Win-Shift: mean =  
189  $70.24\%$  of subsequent trials with choices to a different feeder  $\pm 1.75$  SE) or not (Loose-Shift: mean =  
190  $73.51\%$  of trials  $\pm 2.93$  SE). This is as would be expected, if the birds did not follow any other alternative  
191 behavioral strategy in making feeder choices. This is because when four feeders are available then an  
192 unbiased behavioral strategy would result in  $\frac{1}{4}$  of the time the bird choosing the same feeder as during the  
193 previous trial and  $\frac{3}{4}$  of the time choosing one of the other three feeders. We would like to point out here

194 that shifting to another feeder even though the stimulus presented could be the same as in the previous  
195 trial can still result in a correct response, because for each trial both feeders on the axis associated with  
196 the changing magnetic inclination stimulus were rewarded. Therefore, we observed no behavioral-  
197 strategy bias in their feeder choices across trials that could have influenced the pigeons' discrimination  
198 performance on inclination-meaningful test trials.

199 As described above, retro-fitting our coil system for the anti-parallel sessions resulted in a weak  
200 residual magnetic intensity gradient being produced by the coils instead of complete cancellation of the  
201 coils' fields, yet the pigeons' discrimination performance fell to chance level during anti-parallel sessions.  
202 This is not surprising as such a weak stimulus would be considerably more difficult to discriminate and  
203 thus the birds were highly likely to switch for the same level of motivation (85% free-feeding weight and  
204 10s feeder access per correct choice) to alternative behavioural strategies (see above), which still yielded  
205 a reward for 50% of the trials. This is especially true given that the birds were only exposed to this  
206 weaker stimulus for two sessions at a time and for a total of only eight sessions. Therefore, no  
207 conclusions can be drawn from this control experiment about whether or not pigeons are able to perceive  
208 such small changes in magnetic inclination. To test such a possibility, a carefully designed threshold  
209 study will need to be performed in the future.

210 In summary, because discrimination performance fell to chance level not only when current to the  
211 coils was disconnected, but also when current ran through the coil system in an anti-parallel fashion, the  
212 two control experiments demonstrated that neither the current itself nor any other alternate non-magnetic  
213 cues could have been used by the birds to discriminate the magnetic intensity cues in this experimental  
214 setup. This result is consistent with the fact that the coils felt barely warm to touch during sessions, the  
215 arena's support base rested on a concrete floor without contact to the coils, and auditory as well as visual  
216 cues were controlled for.

### 217 *Truncated Inclination Range Sessions*

218 To eliminate the possibility that the pigeons used differences in declination between North-South and  
219 East-West trials to solve the discrimination task, we conducted with five of the seven birds from the  
220 parallel/anti-parallel series 15 sessions for which the upper magnetic range was truncated (see methods  
221 above) (Fig. 3d).

222 Our results show that homing pigeons are able to discriminate magnetic inclination changes from  
223  $-3^\circ$  to  $+71^\circ$  from inclination values held constant at  $-3^\circ$  with the mean performance of birds ranging  
224 between 64% and 71%. We next calculated the mean performance over all sessions for each bird and then  
225 the mean discrimination performance across all birds ( $n=5$ , mean  $68.97\% \pm 1.26$  SE, 95% confidence



226 interval 65.72% to 72.22%). The latter was slightly higher than the mean performance during the initial  
227 conditioning series and the Coils-On sessions of the first control series, but comparable to the  
228 performance level during the parallel sessions of the second control series. Furthermore, the  
229 discrimination performance for the truncated inclination range was significantly different from chance  
230 level (50%). This was true when comparing individual mean bird performances to chance level (un-paired  
231 T-test:  $T = 14.432$ ,  $p < 0.001$ ) as well as when looking at the mean performance of all birds being above  
232 chance level over the course of all sessions in this series (Wilcoxon Signed Ranks Test:  $T\text{-Value} = 0$ ,  $p <$   
233  $0.001$ ). Therefore the pigeons were able to perform the discrimination task and that performance was  
234 consistently above chance level over time.

235 We did not observe a statistically significant change in the pigeons' performance over the course  
236 of the sessions of the truncated inclination range series (Linear Mixed Model ANOVA, type III test of  
237 fixed effects:  $F_{\text{Session}} = 2.520$ ,  $p = 0.117$ ). That is, there was no traditional acquisition curve for the  
238 conditioned response to this inclination stimulus, which was not surprising given the considerable  
239 experience the birds already had with the overall conditioning paradigm. A systematic difference between  
240 subjects was detected (Linear Mixed Model ANOVA, type III test of fixed effects:  $F_{\text{Subject}} = 4.556$ ,  $p =$   
241  $0.003$ ) due to bird 259's slightly poorer and more variable performance level.

242

243

## Discussion

244 The results presented here provide evidence that homing pigeons are able to discriminate differences in  
245 the properties of magnetic inclination during a conditioning task. During the initial conditioning series,  
246 homing pigeons were required to select within a circular arena one of two possible feeders associated with  
247 a rapid change in inclination value ( $-3^\circ$  to  $+85^\circ$ ) to obtain a food reward whilst the other two available  
248 feeders were associated with an unchanging inclination value of close to  $0^\circ$ . All pigeons performed  
249 consistently above chance level (50%).

250 During two series of control experiments, the possibility that alternative cues (e.g., sound,  
251 vibration, or pseudorandom order of cue presentation) were used by the pigeons to select correct feeders  
252 was tested with discrimination performances dropping to chance level whenever the magnetic coils were  
253 switched off or current through the coils was run in an anti-parallel fashion. During the latter series, the  
254 residual changes in magnetic intensity of up to 4,300nT, as experienced by the pigeons during anti-  
255 parallel conditions, could not be used by the pigeons to identify the correct feeders. These intensity  
256 changes were greater than the maximally 3,500nT changes occurring during parallel sessions, thus

257 indicating that the birds were not able to use intensity changes in this conditioning paradigm to locate  
258 rewarded feeders.

259 During the truncated inclination range series, the rewarded inclination stimulus was reduced in  
260 range ( $-3^\circ$  to  $+71^\circ$ ) to test the possibility of simultaneous residual changes in magnetic declination serving  
261 as a discrimination cue on their own. The pigeons continued to perform the discrimination task during the  
262 truncated series consistently above chance level whilst changes in both intensity and declination were  
263 below the levels that the birds had previously failed to discriminate during the anti-parallel control series.

264 This series therefore confirmed that the pigeons were able to detect and use magnetic inclination  
265 on its own as a discrimination cue to solve the discrimination task. Previous successful magnetic  
266 conditioning studies with pigeons had trained the animals to discriminate magnetic anomalies, which  
267 consisted of both changes in magnetic intensity and inclination, and were generated either by magnetic  
268 coils or a group of bar magnets (Mora *et al.*, 2004; Thalau *et al.*, 2007; Freire *et al.*, 2012). Studies with  
269 homing pigeons (Wilzeck *et al.*, 2010) as well as two other bird species, the domestic chicken (*Gallus*  
270 *gallus*; Freire *et al.*, 2005) and zebra finches (*Taeniopygia guttata*; Voss *et al.*, 2007; Keary *et al.*, 2009),  
271 conditioned the birds to a shift in the horizontal component of the magnetic field.

272 Overall the discrimination performance during the initial conditioning series was very similar to a  
273 previous conditioning study that required homing pigeons to discriminate the presence and absence of a  
274 magnetic anomaly varying in both intensity and inclination (mean discrimination performance of around  
275 65% with a chance level of 50%; Mora *et al.*, 2004). Mean discrimination performances presented here  
276 increased slightly over the course of the study to almost 70% during the parallel sessions of the second  
277 control series, most likely as the result of increased experience with the experimental task. A similar level  
278 of performance was also achieved during the truncated inclination range series. Another recent study,  
279 which required homing pigeons to discriminate magnetic intensity cues within a virtual magnetic map  
280 paradigm had achieved relatively better performance (45 to 55% with 25% chance level; Mora and  
281 Bingman, 2013), but this was a substantially different type of discrimination task, which makes  
282 comparisons in performance levels difficult.

283 It is well established in animal psychobiology literature that discrimination tasks, which require  
284 the animal to move between different manipulanda (feeder-response units), consequently cause a  
285 separation of the stimulus (magnetic inclination cues), response (key pecking) and reinforcement (food  
286 reward) in space and time (for a review see: Mora *et al.*, 2009). This results then in a considerably lower  
287 discrimination performance, as observed here, compared to a discrimination task in a traditional Skinner  
288 box, which requires a stationary pigeon to discriminate sensory cues (typically 90–100% performance in a  
289 simple visual discrimination task). Consequently, how high above chance level the discrimination

290 performance resides does not necessarily reflect the birds' sensitivity to magnetic inclination in the field  
291 in the behavioural context of free-flying navigation. To determine how sensitive pigeons are to inclination  
292 cues, a threshold study will have to be conducted, which progressively reduces the size of the stimulus to  
293 be discriminated until discrimination performance consistently resides around chance levels.

294 The pigeons in this study could have (1) used the actual inclination values at the individual  
295 feeders to solve the discrimination task, (2) compared the changing inclination with the steady value that  
296 differentiated the areas on either side of the two feeder types, or (3) utilized a combination of these two  
297 strategies. Further experiments are needed to differentiate these possibilities, but it appears likely that the  
298 pigeons are at least capable of the first option as birds inexperienced with the task spent a considerable  
299 amount of the sampling period walking in circles in the arena from feeder to feeder. In contrast to this,  
300 during the truncated inclination range sessions, birds typically either remained during the sampling period  
301 at the feeder chosen during the previous trial or moved clockwise (or counterclockwise) by 90 °to the  
302 neighbouring feeder before making their choice. That is, they either only minimally moved around the  
303 arena or not at all depending on whether they chose the same feeder as during the previous trial or one of  
304 the neighbouring feeders.

305 The question, however, also arises as to whether absolute inclination values and/or the switch  
306 from positive to negative inclination were detected. The latter refers to the fact that because the  
307 inclination changed from -3° at the unrewarded feeders to +85° (+71° for the truncated series) at the  
308 rewarded feeders, there was a moment where inclination switched from negative to positive. In all the  
309 species studied so far, the avian magnetic compass has been shown to be an inclination compass, i.e.,  
310 birds tend to disregard the polarity of the magnetic field vector, but only attend to the downward- versus  
311 upward-pointing aspect of the vector to discern pole-ward versus equator-ward directions along the  
312 North-South axis (for review see e.g., Wiltschko and Wiltschko, 2007; Wiltschko and Wiltschko, 2009).  
313 Therefore, the fact that the inclination with our magnetic coil setup decreased from +85° (later +71°) to -  
314 3°, and thus changed polarity, should not have been relevant to the discrimination task. This is because the  
315 birds would have experienced with their inclination compass only an absolute change from 85° (later 71°)  
316 to 0° and then a slight increase again to 3° when moving from a rewarded feeder toward an unrewarded  
317 feeder on either side of the rewarded one.

318 In terms of sensitivity, it had been previously shown that pigeons are able to discriminate the  
319 presence and absence of a magnetic anomaly varying mostly in intensity (peak intensity of 189  $\mu$ T  
320 compared to 44  $\mu$ T background intensity), but nevertheless varying also significantly in inclination (peak  
321 inclination of -80° compared to -64°; Mora *et al.*, 2004). The results presented here show that pigeons are  
322 able to detect differences in magnetic inclination independent of any meaningful variation in intensity. To

323 facilitate learning of the discrimination task, the size of the inclination change was initially set to be as  
324 close as possible to the maximum change of  $90^\circ$  whilst minimizing any simultaneous changes in magnetic  
325 inclination and declination. The truncated inclination range series indicates that the birds are at least able  
326 to discriminate a minimum inclination change of  $70^\circ$ . The tracker arm was, however, typically not exactly  
327 lined up with the feeder position as the pigeon was sitting in front of a feeder. Furthermore, inclination  
328 changed by  $1.6^\circ$  per  $1^\circ$  rotation of the tracker arm ( $70^\circ$  over  $45^\circ$  arena zone). Therefore, a tracker arm  
329 positioned  $15^\circ$  to either side of a feeder, which was frequently observed, would reduce the inclination  
330 change experienced by the bird to approximately  $50^\circ$ . Given that birds use inclination cues as part of their  
331 magnetic compass during homing, sensitivity to inclination cues considerably greater than the one  
332 indirectly inferred by our results is almost certain.

333 Irrespective of what property of the inclination stimulus the pigeons utilized, the fact that the  
334 pigeons were able to discriminate differences in magnetic inclination provides evidence that they must  
335 possess the ability to detect, differentiate, and process magnetic inclination information. Still very little is  
336 known about how magnetic inclination is perceived, or where and how such information is processed in  
337 the brain. Researchers have been looking for a candidate magnetoreceptor in homing pigeons and  
338 migratory birds for several decades. Some progress has been made in recent years in relation to a putative  
339 receptor system for the magnetic compass in the retina of migratory birds (recent reviews: Mouritsen,  
340 2012; Mouritsen and Hore, 2012). A candidate molecule (cryptochrome) is thought to alternate between a  
341 singlet and a triplet state in a light-mediated radical-pair process with the ratio of the two states depending  
342 on the orientation of the magnetic field vector axis (Ritz *et al.*, 2000). Such a system would, however, not  
343 be suitable to determine the vector's inclination angle. It is this angle that is thought to help distinguish  
344 poleward from equatorward directionality during the compass-step of navigation.

345 A second theoretical mechanism, based on behavioural, electrophysiological and anatomical work  
346 in rainbow trout (*Oncorhynchus mykiss*) (Walker *et al.*, 1997), proposes an iron-mineral-based receptor in  
347 the olfactory epithelium, which responds to magnetic field intensity changes and potentially also to  
348 changes in inclination angle (Walker *et al.*, 2002). However, it should be noted that the existence of a  
349 magnetite and maghemite-containing receptor structure on the underside of the pigeon's upper beak as  
350 proposed by Fleissner *et al.* (2007) has been recently called into question as macrophages may have been  
351 interpreted as iron-mineral particles (Treiber *et al.*, 2012). Most recently, the lagena otolith organ in the  
352 pigeon's inner ear has also been raised as a possible location for a third type of magnetoreceptor (Wu and  
353 Dickman, 2011). Future impairment studies with the conditioning paradigm presented here should  
354 provide the opportunity to distinguish between the various alternative mechanisms of inclination

355 perception described above as well to identify the regions in the brain where such information is  
356 processed.

357 In terms of the nerve carrying magnetic inclination information to the brain, the ophthalmic  
358 branch of the trigeminal nerve was identified early on as a likely candidate. Beason and Semm (1996)  
359 were first to demonstrate in a bird species, the bobolink (*Dolichonyx oryzivorus*), that the ophthalmic  
360 branch of the trigeminal nerve carries magnetic information, although it was not clear which component  
361 of the magnetic field (magnetic intensity, magnetic inclination or possibly both) was transmitted. Also,  
362 the ability to discriminate the presence and absence of a magnetic anomaly with changes in intensity and  
363 inclination was abolished following the sectioning of this nerve in homing pigeons (Mora *et al.*, 2004).  
364 Whilst a possible role of the trigeminal nerve during homing by pigeons in Italy at distances of up to 105  
365 km has been dismissed (Gagliardo *et al.*, 2006; 2009), several recent studies have investigated in  
366 migratory and non-migratory birds the role of the ophthalmic branch of the trigeminal nerve in  
367 transmitting magnetic information to the brain (European robins (*Erithacus rubecula*), Heyers *et al.*  
368 (2010); Pekin duck (*Anas platyrhynchos domestica*), Freire *et al.* (2012)) and its role in correcting for  
369 displacement during migration (reed warblers (*Acrocephalus scirpaceus*), Kishkinev *et al.*, 2013). Two  
370 recent studies by Wu and Dickman (2011, 2012) have also shown involvement of pigeon's trigeminal  
371 neurons in magnetoreception as well as recorded neuronal responses in the pigeon's brainstem in response  
372 to changes in magnetic field direction, intensity, and polarity.

## 373 **Methods and Materials**

### 374 *Magnetic Inclination Stimulus*

375 The 3-axis magnetic coil system (four 240 x 240 cm square coils per axis with a coil spacing of  
376 89/62/89 cm; coil winding ratio of 26:11:11:26; 14 AWG, PVC-insulated copper coil wire, aluminium  
377 frame, adapted from Merritt (1983); Fig. 1a) was powered by three power supplies (BK Precision, Model  
378 9123A, 0-30V/0-5A Single Output Programmable DC power supply with constant current output), one  
379 assigned to each axis (x, y, and z) of the coil system. This coil system was able to generate a sphere-  
380 shaped area in the center of the coils, approximately the size of the diameter of the experimental arena,  
381 within which the generated magnetic field was very uniform, albeit not perfectly uniform, as is typically  
382 the case with this type of coil system. That is, the magnetic field vector was very similar in terms of  
383 spatial orientation (inclination and declination) and length (intensity) for all spatial points inside this  
384 "bubble". By changing independently the current output to each of the three coil axes, we were able to  
385 either increase or decrease the magnetic field inclination *in real time* and relatively *uniformly* throughout  
386 the entire experimental arena. A white noise generator positioned next to the coil system masked any

387 potential humming noise emanating from the coil system. The power supplies and associated relays were  
388 fully automated and located in a control room adjacent to the room containing the coil system. The coil  
389 wiring remained cool to the touch throughout the conditioning sessions.

390 The amount of current supplied to each coil axis was fully automated via custom-written  
391 software. The arena was divided into four 90° zones with each zone extending 45° on either side of one of  
392 the four feeders (Fig. 2). As the pigeon rotated the horizontal tracker arm while walking around the  
393 periphery of the arena, the position of the tracker arm was determined by the angular decoder. Based on  
394 this position, the software simultaneously generated a magnetic field vector, whose inclination was  
395 adjusted to match that required for the tracker arm's position for one of two possible patterns as follows.  
396 For North-South trials (see magnetic conditioning procedure below), inclination was held constant at  
397 close to 0° in the zones with the East and West feeders at their center. Whenever the pigeon moved from  
398 the border of the East or West zone toward the North or South feeders, inclination rapidly increased from  
399 -3° at the border to +85° at the feeder and then decreased again from +85° at the North or South feeders to  
400 -3° at the border of the North and South zone with the East and West zones. For East-West trials, this  
401 pattern of inclination change was rotated by 90°. That is, inclination was held constant at close to 0°  
402 around the North and South feeders, but changed rapidly around the East and West feeders.

403 The background field and the magnetic field parameters generated by the coil system were  
404 characterized with a FVM handheld 3-axis vector fluxgate magnetometer (Meda Inc.) at the head-height  
405 of a walking pigeon and at a distance of 30 cm from the center of the arena. Due to structural steel and  
406 electrical circuits in the walls of the experimental room, the background inclination and declination varied  
407 between +61.9° and +68.2° (Mean +65.0° ±0.4 SE) and +4.8° and + 24.5° (Mean +17.6° ±1.2 SE),  
408 respectively (see top left panel of Supplementary Fig 2a-c in Mora and Bingman, 2013). Background  
409 magnetic intensity varied around the periphery of the arena along a SW to NE gradient (mean 47,300 nT  
410 ±330 SE with values ranging from 45,350 to 49,100 nT).

411 In contrast to the variations in the background field, the coil system itself generated a magnetic  
412 field vector whose inclination varied, as expected, strongly during the trial from -3° to +85° when the  
413 birds moved 360° around the periphery of the arena from feeder to feeder with the pattern of change being  
414 90° out of phase for the North-South and East-West trials (Supplementary Figure 1a). Magnetic intensity  
415 and declination changes experienced by the bird whilst walking from feeder to feeder around the  
416 periphery of the arena ranged for intensity from 45,500nT to 49,000nT (Supplementary Figure 1b) and for  
417 declination from +9° to +26° (Supplementary Figure 1c) for both North-South and East-West trials due to  
418 the variations in the background field described above. During control sessions with anti-parallel current  
419 (see below), there were very small changes in inclination (62° to 66°) as well as some changes in intensity



452 the pigeons using any visual cues to solve the spatial conditioning task. At the end of the 15 s sampling  
453 period, all four feeders' pecking lights were illuminated. The pigeons were trained to choose one of the  
454 two feeders associated with the rapidly increasing magnetic inclination. A correct choice was rewarded  
455 with a 10-s access to the food magazine, whereas incorrect choices resulted in a time penalty of 10 s being  
456 added to the 5-s inter-trial interval (ITI), during which the arena was dark and only the background  
457 magnetic field was present. The magnetic stimulus for the next trial depended on a pseudo-random  
458 sequence rather than the correctness of the choice made during the preceding trial. For further details see  
459 Supplementary Text.

#### 460 *Coil Control Procedures*

461 For the Coils On-Off controls series, Coils-On sessions were alternated with Coils-Off sessions. During a  
462 Coils-On session the procedures described above were followed. By contrast, during Coils-Off sessions  
463 the custom-written software did not supply any current output from the three power supplies to the 3-axis  
464 magnetic coil system. Therefore, the relays in the control room were still producing audible clicks as if  
465 the direction of current coming from one or more of the power supplies were switched from clockwise to  
466 counterclockwise for a coil axis, but no magnetic field was produced by the coil system.

467 For the Parallel-Antiparallel control series, the number of each coil's wire loops was halved and a  
468 switch added that allowed the current in both halves of the coil to run either parallel (in the same  
469 direction) or anti-parallel (in opposite directions). Whilst the outer coil's 26 loops were halved into two  
470 sets of 13 loops, for the 11 loops of the inner coils, we added an additional loop of wire that was only  
471 supplied with current during the anti-parallel setting so that current ran through 6 loops in one direction  
472 and through 5 + 1 loops in the other direction. When running parallel, the same magnetic field inclination  
473 was produced as for standard sessions, but when running anti-parallel the two coil halves cancelled each  
474 other effectively out (Supplementary Figure 1d-f). A mean residual magnetic field inclination change of  
475  $0.5^\circ \pm 0.08^\circ$  SE (mean residual intensity and declination were  $1,366\text{nT} \pm 98\text{nT}$  SE and  $1.0^\circ \pm 0.2^\circ$  SE  
476 respectively) remained that was probably due to the retro-fitting of the double coils system having not  
477 exactly halved the coils.

#### 478 *Statistical Analysis*

479 For each session performed by each bird, the percentage of correct choices out of 32 trials was calculated.  
480 We also calculated for each session the mean discrimination performance across all birds, which was  
481 graphed together with the individual birds' percentage of correct choices for each session (Fig. 3). For  
482 further details see Supplementary Text. All statistical tests see Zar (1999).



483

### Ethics Statement

484 This study was carried out in strict accordance with the recommendations in the Guide for the Care and  
485 Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the  
486 Institutional Animal Care and Use Committee of Bowling Green State University (Permit Numbers: 09-  
487 001 and 12-002).

488

### Acknowledgements

489 We sincerely thank Russell Mora for the development of the conditioning software, Andrew Wickiser for  
490 the construction of the experimental setup, as well as Tom Barnhardt and Tom van Handel for their  
491 generous supply of thorough-bred racing pigeons. We greatly appreciated the invaluable input by  
492 Shannon Thompson, Jean Adelphi-Long, and Susan Orosz during the development of the pigeon harness.  
493 Lindsey Cunningham was also involved with the pre-training of the pigeons.

494

### Funding

495 This work was supported by a grant from The National Science Foundation [IOS-0922508 to V.P.B. and  
496 C.V.M.] as well as a Research Incentive Grant provided by the Faculty Research Committee at Bowling  
497 Green State University to V.P.B..

498

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- 625

626

**Figure Captions**

627 Fig. 1: Experimental setup for magnetic inclination conditioning paradigm (not drawn to scale). a)  
 628 Circular orientation arena (diameter 110 cm) surrounded by three-axis coils system (red lines;  
 629 adapted from Merritt (1983)), which generated a spatially uniform magnetic field inclination cue  
 630 throughout the entire arena. This type of magnetic cue is in contrast to spatially variable magnetic  
 631 anomalies used by past conditioning studies (e.g., Mora *et al.*, 2004). Magnetic field intensity in  
 632 arena was controlled in real time via customized software based on the position of the horizontal  
 633 tracker arm over time. Note that the arena's four feeders-response units are not shown for clarity.  
 634 b) Pigeon walking in arena whilst attached via harness (red) to horizontal tracker arm (adapted  
 635 from previous sea turtle studies (e.g., Lohmann, 1991)), with tracker arm orientation in the arena  
 636 detected by angular decoder every 200 ms. Note that the arena's four feeders-response units are  
 637 not shown for clarity. c) Top view of arena showing pigeon attached to horizontal tracker arm as  
 638 well as position of four feeder-response units (grey rectangles), each with a pecking key above an  
 639 automated food reservoir, located around periphery of the circular arena in the four cardinal  
 640 directions (geographic North, South, East and West; dashed lines).

641 Fig. 2: Arrangement of reinforcement contingencies for North-South and East-West trials. During North-  
 642 South trials the 90° zones with either the North and South feeders at their center were associated  
 643 with magnetic inclination rapidly increasing (red arrow) from -3° to a maximum inclination value  
 644 of +85° (-3° to +71° for truncated magnetic inclination range series) when the pigeon approached  
 645 either of these two feeders. In contrast to this, the 90° zones with either the East or West feeders  
 646 at their center were associated with a constant value of -3° ("Zero Inclination" zones). During  
 647 these trials a pecking response at either the North or South feeder was rewarded with access to the  
 648 food reservoir for 10 seconds. During East-West trials the East and West feeders were associated  
 649 with the rapid inclination change and responses at these feeders rewarded with food access.  
 650 North-South and East-West trials were presented in pseudo-random order during each 32-trial  
 651 session. Feeders indicated by yellow boxes labeled with cardinal direction and rewarded axis  
 652 indicated by red line.

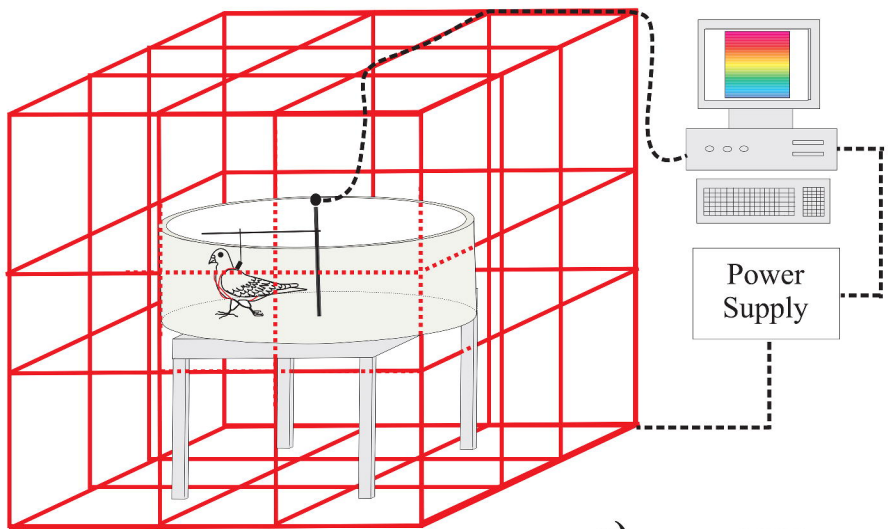
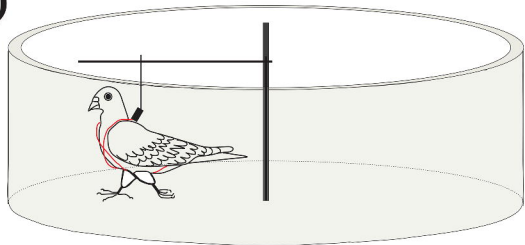
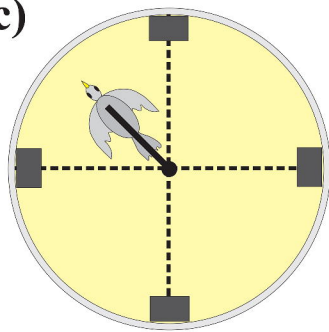
653 Fig. 3: Percentage of correct choices made by individual pigeons during each session as well as mean  
 654 performance across all birds for each session. a) Initial conditioning series (chance level 50%). b)  
 655 Coils On-Off control series consisting of standard sessions as well as control sessions during the  
 656 latter of which the coils system had no current input. c) Parallel-Antiparallel control series for  
 657 which sessions with current running parallel through a double-wound coil system were alternated

658 with sessions with current running anti-parallel through the coil system. d) Truncated magnetic  
659 inclination range series for which the range in magnetic inclination changes was reduced from  $-3^\circ$   
660 to  $+85^\circ$  to a narrower range of  $-3^\circ$  to  $+71^\circ$  to eliminated simultaneous changes in declination being  
661 a possible alternate discrimination cue.

662 Supplementary Fig. 1: Total magnetic field experienced by pigeon at 16 locations around periphery of  
663 circular orientation arena during parallel (standard) sessions. Measurements were  
664 taken with a FVM handheld 3-axis vector fluxgate magnetometer (Meda Inc.) at  
665 the head height of a walking pigeon and at a distance of 30 cm from the center of  
666 the arena. a) Magnetic inclination angle. b) Magnetic field intensity. c) Magnetic  
667 declination angle.

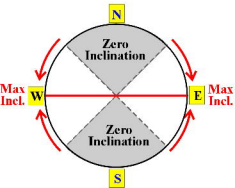
668 Supplementary Fig. 2: Total magnetic field experienced by pigeon at 16 locations around periphery of  
669 circular orientation arena during anti-parallel sessions. Measurements taken as  
670 described for Figure 1. a) Magnetic inclination angle. b) Magnetic field intensity.  
671 c) Magnetic declination angle.

672 Supplementary Fig. 3: Total magnetic field experienced by pigeon at 16 locations around periphery of  
673 circular orientation arena during parallel (standard) sessions with a truncated  
674 magnetic inclination range. Measurements taken as described for Figure 1. a)  
675 Magnetic inclination angle. b) Magnetic field intensity. c) Magnetic declination  
676 angle.

**a)****b)****c)**



**East-West Axis  
Rewarded**



**North-South Axis  
Rewarded**

