

# Differential effects of magnetic pulses on the orientation of naturally migrating birds

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In migratory passerine birds, strong magnetic pulses are thought to be diagnostic of the remagnetization of iron minerals in a putative sensory system contained in the beak. Previous evidence suggests that while such a magnetic pulse affects the orientation of migratory birds in orientation cages, no effect was present when pulse-treated birds were tested in natural migration. Here we show that two migrating passerine birds treated with a strong magnetic pulse, designed to alter the magnetic sense, migrated in a direction that differed significantly from that of controls when tested in natural conditions. The orientation of treated birds was different depending on the alignment of the pulse with respect to the magnetic field. These results can aid in advancing understanding of how the putative iron-mineral-based receptors found in birds' beaks may be used to detect and signal the intensity and/or direction of the Earth's magnetic field.

**Keywords:** magnetite; polarity; migration; bird navigation; magnetic field

## 1. INTRODUCTION

A significant body of behavioural evidence indicates that birds use the magnetic field for orientation and navigation (Wiltschko & Wiltschko 1995), both to indicate direction (Wiltschko & Wiltschko 1972; Cochran *et al.* 2004) and also possibly as part of a position location system or 'map' (Freake *et al.* 2006). Despite this, the way in which the magnetic field is detected and used by animals for orientation and navigation remains somewhat controversial (Mouritsen & Ritz 2005; Kirschvink *et al.* 2010). Three distinct sensory mechanisms have been proposed for the detection of the magnetic field. It was initially proposed that a magnetic sense could be a by-product of electroreception in animals possessing ampullary canals (Kalmijn 1981). This sense could not function in a terrestrial environment, however, and so other mechanisms are necessary to explain in particular how birds are able to sense the magnetic field. Two hypotheses currently exist: a radical-pair mechanism and an iron-mineral-based mechanism. A growing body of evidence supports both mechanisms playing a role in the navigation system of birds (see table 1 for a summary of the role of the two mechanisms in navigation based on the magnetic field).

The radical-pair mechanism proposes that the Earth's magnetic field alters the unpaired electron spin state of photoreceptive chemicals in the eye, causing them to oscillate between singlet and triplet unpaired states (Schulten *et al.* 1978; Schulten &

Windemuth 1986; Ritz *et al.* 2000; Wang & Ritz 2006; Rogers & Hore 2009). It follows from this that the detection of the magnetic field is thus a by-product of the visual system (Heyers *et al.* 2007; Zapka *et al.* 2009). It appears that the region of the bird's brain responsible for nocturnal vision 'cluster N' is necessary for magnetic compass orientation (Mouritsen *et al.* 2005; Zapka *et al.* 2009). The most likely photoreceptive chemical is the blue light receptor protein cryptochrome (Ritz *et al.* 2000; Liedvogel *et al.* 2007; Solov'yov *et al.* 2007; Solov'yov & Schulten 2009). Evidence for a radical-pair magnetic compass based on cryptochromes comes from the fact that migratory birds tested in orientation cages are disoriented under certain wavelengths and intensities of light (Muheim *et al.* 2002; Wiltschko & Wiltschko 2002; Wiltschko *et al.* 2008). It is also predicted that oscillating magnetic fields should disrupt the radical-pair system (Canfield *et al.* 1994, 1995). Experiments in which migrating birds are subjected to oscillating magnetic fields do indeed disrupt their ability to use magnetic compass orientation (Ritz *et al.* 2004, 2009).

A second mechanism proposes that the magnetic field is detected by iron minerals, with magnetic remanence as part of a sensory system that signals the movement of these minerals in relation to the Earth's magnetic field. This movement could potentially be used to detect the intensity, inclination or polarity of the magnetic field (Kirschvink & Gould 1981; Kirschvink *et al.* 2001, 2010). Initial hypotheses were based on the fact that magnetotactic bacterial cells contain magnetite chains (Blakemore 1975; Kalmijn & Blakemore 1978; Blakemore *et al.* 1980) and that such cells might form

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Table 1. Proposed mechanisms of magnetoreception in birds.

mechanism	location	function	innervation	brain region	diagnostic
radical-pair chemical photoreceptor sensory cells	eye	inclination (compass)	not known	cluster N <sup>a,b</sup>	MHz oscillating magnetic fields <sup>c</sup>
containing iron minerals	beak <sup>d</sup>	intensity (for map?) <sup>e</sup>	trigeminal nerve (ramus ophthalmicus medius, ROM) <sup>d</sup>	not known	magnetic pulse, <sup>f</sup> conditioning to intensity anomaly and lesions of ROM <sup>e</sup>
sensory cells containing iron minerals	beak	polarity (compass?)	trigeminal nerve	not known	field reversal combined with local anaesthesia of beak <sup>g</sup>

<sup>a</sup>Mouritsen *et al.* (2005).<sup>b</sup>Zapka *et al.* (2009).<sup>c</sup>Ritz *et al.* (2004).<sup>d</sup>Fleissner *et al.* (2003).<sup>e</sup>Mora *et al.* (2004).<sup>f</sup>Wiltschko *et al.* (1994).<sup>g</sup>Wiltschko *et al.* (2005).

the basis of a ‘compass organelle’ (Kirschvink & Gould 1981). Evidence indicates that such iron-based minerals have been found in the tissue of a number of animal taxa (Wiltschko & Wiltschko 1995). Where the magnetic material has been identified within the tissue, it appears to be associated with the trigeminal nerve (Walker *et al.* 1997; Diebel *et al.* 2000; Fleissner *et al.* 2003, 2007). Two experimental techniques have been proposed to be diagnostic of a magnetic sense based on iron minerals. First, if the trigeminal nerve is the primary pathway of innervation of the iron based mineral magnetic sense, lesions or anaesthesia of the nerve should disrupt magnetoreception. Second, brief magnetic pulses strong enough to overcome the coercivity of the iron minerals should remagnetize them in the direction of the applied pulse and thus change the orientation of the bird. Both these techniques have been used and argue that an iron-mineral-based magnetic sense exists in birds. Trigeminal nerve section has been shown to stop pigeons from being able to detect a magnetic intensity anomaly (Mora *et al.* 2004), although it does not appear necessary for magnetic compass orientation in juvenile migrant robins (Zapka *et al.* 2009). Magnetic pulses have been shown to alter the orientation of birds (Wiltschko *et al.* 1994, 1998; Beason *et al.* 1995, 1997) as well as homing bats (Holland *et al.* 2008), mole rats (Marhold *et al.* 1997) and sea turtles (Irwin & Lohmann 2005). Magnetic pulses only appear to affect adult migratory birds, not juveniles, which indicates that iron-mineral-based magnetoreception plays a role in an experience-based mechanism, which is presumed to be the map, not the compass system of birds (Munro *et al.* 1997*a,b*; Wiltschko *et al.* 2006).

A recent discovery suggests that the iron-mineral-based sensory system of birds is also able to detect the polarity of the magnetic field as part of a ‘fixed direction response’ displayed by migrating birds orienting in darkness or monochromatic light (Wiltschko *et al.* 2005, 2008; Stapput *et al.* 2008). Whether this polarity-based response plays any role in naturally migrating birds during navigation in the wild is unclear

as it results in a season-independent orientation that does not match with normal orientation in the tested species.

Most magnetic pulse experiments have applied the pulse at a 90° angle to the magnetic field, which would have unknown effects on the iron minerals in the sensory cells. It has been argued that the correct application of a magnetic pulse could be diagnostic of the structure of the sensory system (Kirschvink *et al.* 1985). This is based on the observation that the swimming direction of magnetotactic bacteria is reversed by the application of a pulse antiparallel to the magnetic biasing field owing to remagnetization, whereas a pulse parallel to it has no effect (Blakemore 1975; Blakemore *et al.* 1980). There is partial support for this in a bat (*Eptesicus fuscus*), but the response of the crucial antiparallel group was too noisy to clearly indicate that the resulting change in orientation was purely due to a reversal of polarity of the magnetic material in the sensory cell (Holland *et al.* 2008). In the only test of this kind on birds in the Australian silvereye (*Zosterops l. lateralis*), neither the parallel-treated nor antiparallel-treated group responded in a way that was suggestive of a simple ‘magnetosome-like’ sensory organelle (Wiltschko *et al.* 2002). Indeed, recent evidence of the structure of the iron-mineral-based sense in birds indicates that this system is far more complex than the magnetosome model and is based on two magnetic materials, magnetite bullets and maghemite platelets, in sensory dendrites arranged in a three-dimensional architecture and innervated by the trigeminal nerve (Fleissner *et al.* 2003, 2007; although see Winklhofer (2009) for a discussion of the diagnostic power of the X-ray technique used). Models have shown that the maghemite platelets can amplify the magnetic field such that the magnetite bullets will pull on the nerve membrane and thus transduce a signal of the intensity of the magnetic field (Solov'yov & Greiner 2009*b*). This structure can also, in theory, detect the polarity and inclination of the magnetic field (Solov'yov & Greiner 2009*a*). These structures have been shown to be present in the beaks of four bird species that have different

lifestyles with regard to their movement ecology, including the European robin, which is one of the species in this study and so may be a common feature of birds (Falkenberg *et al.* 2010). While a magnetic pulse would be expected to affect this structure, there is no clear prediction as to how a pulse aligned either parallel/antiparallel to the magnetic field or perpendicular to it would be expected to alter the orientation of the treated animals. In fact, the pulse treatment remains something of a 'black box' as there have been no direct measurements of the effect of a pulse on the iron minerals in the bird magnetic sensory system, either *in vivo* or *in vitro* and so it is currently unknown precisely which aspect of the iron-mineral-based magnetoreception system the pulse affects (Fleissner *et al.* 2007).

A further confounding element in the iron-mineral-based magnetoreception system is that although laboratory-based tests have indicated the presence of this mechanism in birds, so far, there is less support for its use by birds in a natural setting. While pigeons have been demonstrated to be affected by a magnetic pulse (Beason *et al.* 1997), neither a pulse treatment on migrating birds (Holland *et al.* 2009) nor trigeminal nerve lesions in homing pigeons (Gagliardo *et al.* 2006, 2008, 2009) had an effect on the birds' orientation in the field. In the case of Holland *et al.* (2009), however, a significant delay between treatment and departure could have allowed the animals to recalibrate their sensory systems, as is the case in the laboratory (Wiltschko *et al.* 1998). The present paper thus aims to further investigate the role of the iron-mineral-based magnetoreceptor system in migratory birds using magnetic pulses. By using different alignments of the pulse to the magnetic field (perpendicular, parallel and antiparallel), the study investigates whether there are differential responses that may provide information about the structure and function of the iron-mineral-based magnetic sense in naturally migrating birds.

## 2. MATERIAL AND METHODS

### 2.1. Subjects

European robins (*Erithacus rubecula*) and reed warblers (*Acrocephalus scirpaceus*) were caught by mist netting at the Metnau monitoring station, Radolfzell, Germany between 10 April 2009 and 21 May 2009. Birds were weighed, and scored for migratory fattening from 1 to 5 using the Kaiser scale (Kaiser 1993). A total of 19 robins were caught between 10 April 2009 and 1 May 2009 and 57 reed warblers were caught between 20 April 2009 and 21 May 2009.

### 2.2. Experimental treatment

After processing at the ringing station, birds were subjected to a magnetic pulse. An SCR-fired capacitive discharge unit (an SOTA magnetic pulser) was 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 approximately 0.1 ms duration, with a peak amplitude slightly over 0.1 T, and a rise time of

approximately 100 ns. A pair of fine wire Helmholtz coils produced a 320  $\mu$ T biasing field that could be aligned parallel or antiparallel to the pulse direction.

*2.2.1. Perpendicular pulse.* Robins and reed warblers both received a treatment in which a magnetic pulse aligned perpendicular to the magnetic field was applied by placing the birds into the pulse coil for a duration of one pulse. The pulse coil was aligned with the direction of the pulse west to east and the birds were placed in the coil with their heads facing the direction of the pulse, 'south-anterior' (figure 1), as defined by Beason *et al.* (1995). Controls received the same treatment but the current in the double-wrapped coils was aligned in the opposite direction, so that no pulse was administered even though the capacitor charged and fired in the same way as in the experimental group. Six robins and 13 reed warblers were treated with the perpendicular magnetic pulse, and 13 robins and 13 reed warblers received the 'sham' control treatment.

*2.2.2. Parallel/antiparallel pulse.* Not enough robins were caught to also use the parallel/antiparallel pulse on this species, but 30 reed warblers were caught and treated with a pulse either parallel (13 birds) or antiparallel (17 birds) to the alignment of the magnetic field. The pulse was administered by aligning the solenoid west-east (direction of the pulse) and the biasing field was activated to be either parallel to the pulse (pointing east) or antiparallel to the pulse (pointing west). Birds were placed into the pulse coil south-anterior as in the perpendicular pulse experiment and received one pulse before being removed. This experiment overlapped with the perpendicular experiment and so no further controls were used (the last control departed on 17 May 2009 and the first experimental birds departed on 14 May 2009).

### 2.3. Data collection

After receiving the pulse treatment birds were fitted with a 0.4 g LB2N radio transmitter (Holohil Systems Ltd). The transmitter was attached by sewing it to a square of cloth and then attaching the cloth to the bird's back after removing the feathers. Latex based eyelash glue was used to attach the cloth, which would ensure that the transmitter would not fall off. This method has been extensively tested and the transmitter was found to stay attached to birds for up to 24 days (Raim 1978), which is longer than the predicted life of the transmitter (21 days). Once the glue was dry, the bird was released back on the Metnau near where it was caught. Birds were monitored for their direction of departure from a 30 m high observation tower at the Metnau Peninsula (47.729° N, 9.002° E) by two methods. The first was to use a hand-held receiver and a three-element yagi antenna. The transmitter frequencies of the birds which could be detected at the release point were scanned. When a 'take-off' event occurred, this was usually characterized by an increase in signal strength with more variability, followed by a decrease in signal strength, which could be measured as vanishing in a discrete direction.

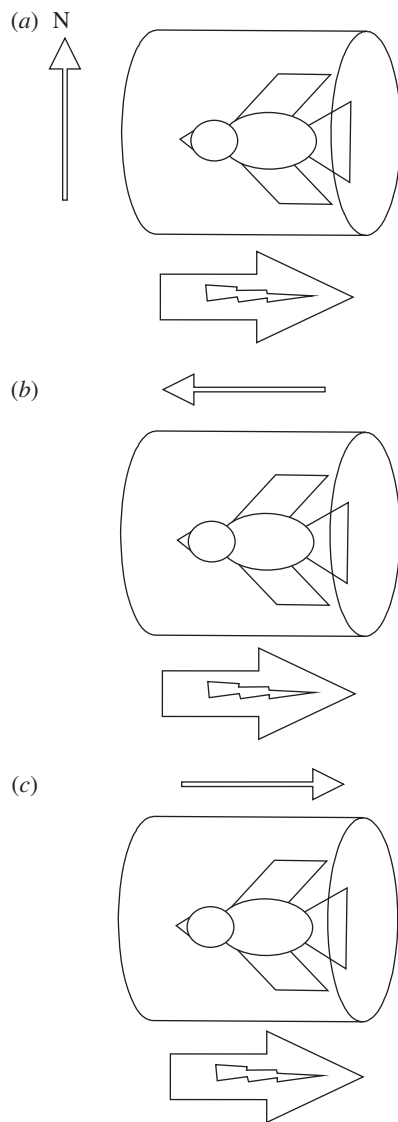


Figure 1. A schematic of the alignment of the pulse relative to the bird and the magnetic field or biasing field. (a) Perpendicular pulse. The bird is placed in the pulse coil with its head facing the direction of the pulse and no artificial biasing field. As the pulse coil is aligned west to east, the pulse is perpendicular to the only biasing field present, that of the Earth's magnetic field. This treatment shifts the orientation of migratory birds by  $90^\circ$  in orientation cages (e.g. Wiltschko *et al.* 1994). (b) Antiparallel pulse. The bird is placed in the pulse coil with its head facing the direction of the pulse. An artificially produced biasing field is activated in the opposite direction to the pulse. This treatment reverses the swimming direction of magnetotactic bacteria (Blakemore *et al.* 1980), but results in a bi-modal east–west orientation in migratory birds in an orientation cage (Wiltschko *et al.* 2002). (c) Parallel pulse. The same as in (b), except the artificial biasing field is aligned in the same direction as the Earth's magnetic field. This treatment does not change the swimming direction of magnetotactic bacteria, but results in a bi-modal east–west orientation in birds in an orientation cage (Wiltschko *et al.* 2002).

The bearing of the last point at which the signal was detected was scored as the departure direction. A transmitter placed at the tower on the Metnau was detectable from the top of a hill 5.5 km north of the release point and so this would be a likely detection

range for the departure bearing. During data collection, only the identifying frequencies of the birds were available to the observer (consisting of six-digit numbers), and not the treatment group of an individual bird. In the second method, an automatic receiver unit (ARU) recorded signal strength of transmitters from four four-element yagi antennae pointed north, south, east and west. The automated receiver scanned through the frequencies programmed into the memory, scanning through each antenna for a single frequency before moving to the next frequency. Departure directions were estimated from the signal strengths of the two antennae receiving the strongest signal at the last point at which signal strength was judged to be above a baseline level, indicating a signal was no longer detected (Cochran & Lord 1963; Crofoot *et al.* 2008). This level was estimated from observing a graphical representation of the signal trace before, during and after a departure. This was necessary because although the background noise level was recorded by the ARU, the antennae traces never precisely dropped to this level. As with hand-held bearings, only the identifying frequency number was available during analysis. The mean vector of the two direction vectors was used (Batschelet 1981), one from each antennae, with the direction of the respective antenna and length equal to the relative signal strength (signal strength–background noise) of the respective antennae. Only the signal strengths identified as being the departure point were analysed in the bearing calculator. When the observer was present, the bearing taken by hand was given priority, but comparison, where possible, between bearings taken by hand and those taken by the automatic receiver revealed no difference in the methods (all reed warblers, Watson–Williams test all cases: control, hand,  $n = 7$ , ARU,  $n = 6$ ,  $F_{1,11} = 0.34$ ; perpendicular pulse, hand,  $N = 5$ , ARU,  $n = 6$ ,  $F_{1,9} = 0.74$ ,  $p = 0.41$ ; parallel pulse, hand,  $n = 6$ , ARU,  $n = 5$ ,  $F_{1,9} = 0.27$ ,  $p = 0.62$ ; antiparallel pulse, hand,  $n = 6$ , ARU,  $n = 7$ ,  $F_{1,11} = 1.91$ ,  $p = 0.2$ ), and so the ARU and hand-held bearings were pooled for analysis (only one bearing in the perpendicular pulsed group was taken by the ARU, so no comparison was possible for robins). In the robin group, controls were tagged before experimental birds as it was first necessary to establish that a measurable baseline-oriented response was possible. In the reed warbler group, where daily numbers caught allowed, the treatment was balanced between controls and experimental groups. However, the date of departure of each bird was the crucial factor in whether the experimental and control groups had been exposed to different conditions that would affect their orientation, as even light winds ( $4\text{--}5\text{ m s}^{-1}$ , 10 m above ground level) have been shown to influence departure direction (Mouritsen 1998). Figure 2 shows the departure dates of each bird. No precise wind information was available for the exact departure of each bird, but wind data were obtained from [www.wetteronline.de](http://www.wetteronline.de) for Konstanz airport (11.5 km from the Metnau). Analysis was performed on the mean daily wind speed and mean daily wind direction measured at 10 m above ground level. Given that birds take off and rise to a height where they will

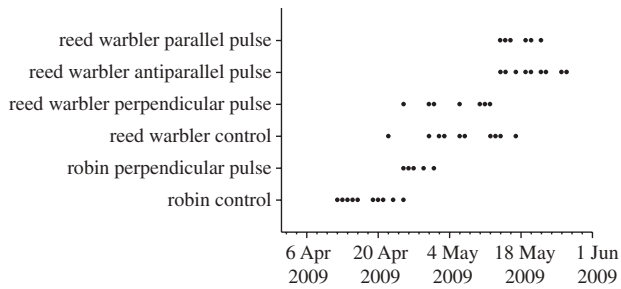


Figure 2. Departure dates of birds from each experimental group.

be exposed to different wind strengths (Mouritsen & Larsen 1998; Bowlin *et al.* 2005), these data should be viewed with caution. Analysis of wind speed and direction indicates that with four exceptions all birds departed in winds of  $0\text{--}0.2\text{ m s}^{-1}$  (1 on the Beaufort scale) or  $0.2\text{--}0.5\text{ m s}^{-1}$  (2 on the Beaufort scale). The four exceptions took off on days when the mean daily wind speed was  $0.5\text{--}1\text{ m s}^{-1}$  (3 on the Beaufort scale). Analysis of wind directions on departure dates for each bird did not indicate any differential assortment of mean daily wind directions between control and experimental groups in terms of wind exposure assigned as north, south, east or west ( $\chi^2$ -test, Yates correction: robins,  $\chi^2 = 1.47$ ,  $p = 0.69$ ; reed warblers, perpendicular pulse versus control,  $\chi^2 = 1.41$ ,  $p = 0.70$ , parallel versus antiparallel pulsed,  $\chi^2 = 0.58$ ,  $p = 0.47$ ).

### 3. RESULTS

#### 3.1. Perpendicular pulse

All groups displayed orientation significantly different from random (Rayleigh test: robins: control,  $r = 0.84$ ,  $p < 0.0001$ , experimental,  $r = 0.88$ ,  $p = 0.004$ ; reed warblers: control,  $r = 0.89$ ,  $p < 0.0001$ , experimental,  $r = 0.76$ ,  $p < 0.0001$ ). There was a significant angular difference between the orientation of the control and experimental groups in both robins (figure 3; Watson–Williams test,  $F = 28.689$ ,  $p < 0.0001$ ) and reed warblers (figure 4; Watson–Williams test,  $F = 12.182$ ,  $p = 0.002$ ) with a deflection of the experimental group east of controls in both cases. There was no significant difference in the time between tagging and departure of experimental birds and controls in either species (figure 5; ANOVA: robins:  $F_{1,15} = 0.025$ ,  $p = 0.88$ ; reed warblers:  $F_{1,23} = 0.0001$ ,  $p = 0.99$ ). Figure 2 shows the departure dates of birds in each group. There is an overlap in the departure dates of the reed warbler groups but the robin controls took off before the experimental birds.

#### 3.2. Parallel/antiparallel pulse

Both parallel- and antiparallel-treated reed warbler groups displayed orientation significantly different from random, although the antiparallel group is axially oriented (figure 6, Rayleigh test: parallel:  $r = 0.52$ ,  $p = 0.046$ ; antiparallel: unimodal,  $r = 0.27$ ,  $p = 0.39$ , bi-modal,  $r = 0.53$ ,  $p = 0.02$ ). There was a significant difference between the antiparallel group and the parallel group (Mardia–Watson–Wheeler test:  $W = 12.09$ ,

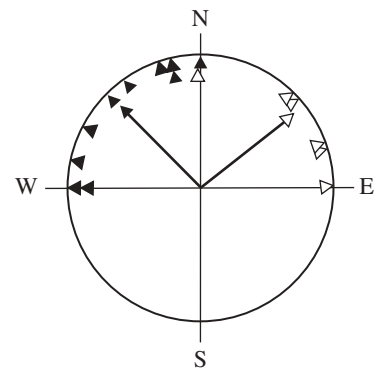


Figure 3. Circular diagram of departure bearings of control (black triangles,  $n = 10$ ) and experimental (open triangles,  $n = 6$ ) robins treated with a perpendicular magnetic pulse. The arrows represent the mean bearings and vector lengths of each group.

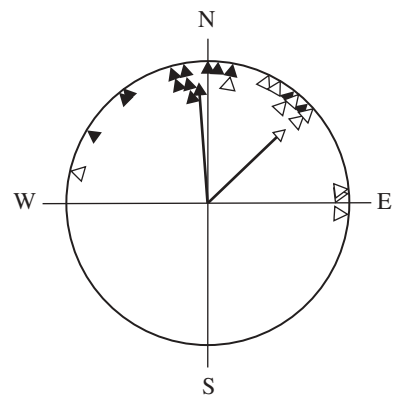


Figure 4. Circular diagram of departure bearings of control (black triangles,  $n = 13$ ) and experimental (open triangles,  $n = 11$ ) reed warblers treated with a perpendicular magnetic pulse. The arrows represent the mean bearings and vector lengths of each group.

$p = 0.002$ ) and between antiparallel birds and control birds (Mardia–Watson–Wheeler test:  $W = 18.88$ ,  $p < 0.0001$ ), but not between the parallel and control groups (Mardia–Watson–Wheeler test:  $W = 3.44$ ,  $p = 0.179$ ). There was a significant difference between the control and the parallel and antiparallel groups in the time between tagging and departure, with controls taking longer to depart, but not between the parallel and antiparallel groups (figure 5; ANOVA:  $F_{2,36} = 10.989$ ,  $p < 0.0001$ ; *post hoc* Bonferroni test: parallel versus control,  $p < 0.001$ , parallel versus antiparallel,  $p > 0.05$ , antiparallel versus control,  $p < 0.001$ ). There was an overlap between the departure dates of the parallel and antiparallel groups (figure 5), but less between controls and the two experimental groups.

### 4. DISCUSSION

The present experiments indicate that a magnetic pulse, designed to manipulate the perception of the Earth's magnetic field by an iron-mineral-based magnetic sense, affects the behaviour of naturally migrating songbirds. The perpendicular pulse deflected the orientation of both robins and reed warblers in a

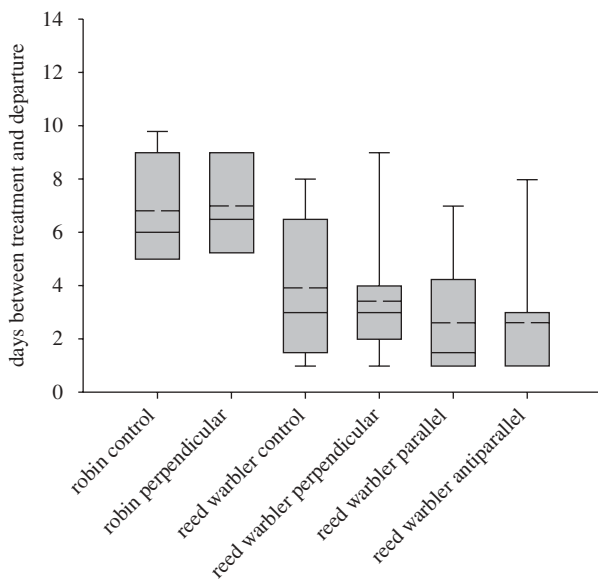


Figure 5. Box plots of mean times between tagging and departure. Lower and upper box limits represent the 25th and 75th percentiles and error bars represent the 10th and 90th percentiles. Dashed line represents the mean and solid line the median value.

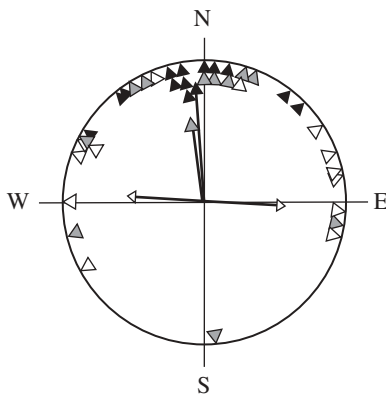


Figure 6. Circular diagram of departure bearings of control (black triangles,  $n = 13$ ), parallel pulse-treated (grey triangles,  $n = 11$ ) and antiparallel pulse-treated (open triangles,  $n = 13$ ) reed warblers. The arrows represent the mean bearings and vector lengths of each group.

clockwise direction relative to controls. This is consistent with remagnetization in the direction of the applied pulse. The parallel/antiparallel treatment produced a differential effect depending on the alignment of the pulse to the biasing field, with the parallel treatment being not significantly different from controls, and the antiparallel treatment producing orientation that was bi-modal in the east–west direction. These results are consistent with the hypothesis that an iron-mineral-based magnetoreceptor plays a role in the natural migratory navigation of these two species. The magnetic pulse is thought to be diagnostic of an iron-mineral-based sense as it should remagnetize remanence-bearing material. The fact that the pulse has led to reorientation rather than disorientation suggests that it has altered, rather than disrupted, the birds' perception of the Earth's magnetic field. While the pulse would potentially affect the radical-pair-based magnetic

sense at the moment of treatment, it is not thought that this sense would be disrupted after this point. The fact that the magnetic compass orientation of juvenile passerine birds is not affected by magnetic pulses (Munro *et al.* 1997*a,b*) would seem to support this statement. The effects of such a strong electromagnetic pulse on proteins or other electrically charged processes in an organism are also not known. However, the fact that the parallel group is not significantly different from controls argues against the effect being non-specific. A similar result in bats (Holland *et al.* 2008) and also the lack of an effect on juvenile migrants (Munro *et al.* 1997*a,b*) also argue against the pulse causing a non-specific effect, but it is still unknown exactly how the iron minerals in the sensory cells are affected by the pulse. While the results of Fleissner *et al.* (2003, 2007) indicate iron mineral deposits in sensory dendrites innervated by the trigeminal nerve, it is not yet conclusively demonstrated that these structures play a role in magnetoreception. The removal of the effect of a pulse by local anaesthesia to the beak provides circumstantial evidence that these iron-mineral-based deposits may play a role in this effect (Wiltschko *et al.* 2009), but the crucial trigeminal nerve lesion study remains to be performed. Further ultra-structural analysis of birds that have received magnetic pulses is also required to examine exactly how or whether the pulse affects the iron minerals in the sensory dendrites.

It has been argued that the parallel/antiparallel treatment could be diagnostic of a polarity-based sense. However, if this were the case, then as well as no effect in the parallel group reversal of orientation would be expected in the antiparallel group leading to a southerly heading during northward spring migration. The bi-modal distribution of this group does not easily support this explanation. It has been proposed that it cannot be ruled out that there is an interaction of the iron-mineral-based sense with the radical-pair system to provide a magnetic compass direction in birds (Mouritsen & Ritz 2005; Kirschvink *et al.* 2010). However, since we do not currently know what exact effect a strong magnetic pulse would have on the iron mineral deposits found in the upper beak of European robins (Falkenberg *et al.* 2010) and reed warblers, it is too early to speculate exactly why these birds reacted in the way they did. Nevertheless, the reaction of the birds observed here provides new information that can help in the search for the exact functional mechanism of the iron-mineral-based receptors, and the addition of a measure of behaviour in natural conditions may be able to add insights into how the two proposed magnetic senses interact in the wild.

The finding of this experiment is in contrast to a study on the Australian silvereye (*Z. l. lateralis*). Application of a pulse parallel to the biasing field resulted in a change in the orientation of those birds compared with controls (Wiltschko *et al.* 2002). Why reed warblers and Australian silvereyes have reacted differently to the pulse with biasing field can only be speculated on at this stage. Possible differences to be explored include the ecology of the species (silvereyes are a dawn/dusk migrant, reed warblers are a night migrant), the treatment (this study administered a 0.1 T pulse,

the silveryeye study a 0.5 T pulse) or the methodology (silveryeyes in an orientation cage versus natural migration in reed warblers).

Given that the mean time between tagging and departure in the region was 4 days, this suggests that one possible explanation for the lack of an effect in the previous study in natural conditions (Holland *et al.* 2009) may have been because the birds in that study departed later than this (8–10 days to departure, 14–20 days to final bearing). Birds treated with a pulse in an orientation cage have been shown to return to normal orientation after 10–14 days (Wiltschko *et al.* 1998). It is likely therefore that the birds treated in the present experiments would recover their magnetic sense. The site of treatment was within the known breeding range of both species.

It should also be noted that the controls of both European robins and reed warblers have a mean departure direction that does not match with data from ringing recoveries, which report a general northeasterly direction of birds from this area. This may be the result of local factors influencing the departure direction. Such effects are known in homing pigeons where so-called release site biases are known to affect the vanishing bearings of birds (Keeton 1973). Such biases have been proposed to be the result of variation in local factors causing misreading of the navigational map (Walker 1998; Mora & Walker 2009). The fact that in three of the four experimental cases the mean orientation was significantly different from controls suggests that these local factors are not masking an orientation decision. The influence of wind on orientation should also not be ruled out as a contributing factor here, given that the winds at the altitude the birds would rise up to were unknown. The lack of overlap between the control and experimental birds in the European robin group means that the difference between these groups should also be viewed with caution as while surface winds do not indicate any differential effect of wind exposure, the birds may have faced different winds at the altitude they took off to migrate.

Taken together these results are consistent with the hypothesis that an iron-mineral-based magnetic sense plays a role in the migratory navigation behaviour of naturally migrating passerine birds. The methodology used here represents a way to complement the laboratory-based studies used to investigate the behavioural aspects of the magnetic sense in migration with field-based studies in a natural setting. It should be married to further ultra-structural analysis of the putative iron-mineral-based sense of migratory birds to understand how this sense functions to provide navigational information from the magnetic field. This field-based method finally allows the study of magnetoreception in animals to span and integrate the contrasting disciplines of quantum physics, molecular biology and field-based ecology.

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