Magnetic orientation of garden warblers (Sylvia borin) under 1.4 MHz radiofrequency magnetic field

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We report on the experiments on orientation of a migratory songbird, the garden warbler (Sylvia borin), during the autumn migration period on the Courish Spit, Eastern Baltics. Birds in experimental cages, deprived of visual information, showed the seasonally appropriate direction of intended flight with respect to the magnetic meridian. Weak radiofrequency (RF) magnetic field (190 nT at 1.4 MHz) disrupted this orientation ability. These results may be considered as an independent replication of earlier experiments, performed by the group of R. and W. Wiltschko with European robins (Erithacus rubecula). Confirmed outstanding sensitivity of the birds’ magnetic compass to RF fields in the lower megahertz range demands for a revision of one of the mainstream theories of magnetoreception, the radical-pair model of birds’ magnetic compass.

1. Introduction

The question as to how migrating animals find their way over great expanses of land and sea is one of the hottest topics of modern science [1]. The development of tracking technologies, including satellite-based, uncovers details of these seasonal movements that were beyond imagination just a decade ago—such as the 8 day non-stop flight of bar-tailed godwits (Limosa lapponica baueri) across the Pacific Ocean [2]. Such long-distance travels require a precise and reliable navigation system. Biophysical, physiological and neural bases of animal navigation are so far rather poorly understood, though, in certain directions, remarkable progress has been achieved. One of those is related to compass orientation in migratory birds.

Owing to conspicuous changes in physiology and behaviour, demonstrated by many bird species during the migration period, and to their obvious motivation for direction finding, birds can be used as an object for studying navigation abilities under controlled experimental conditions in the laboratory. This way, birds were shown to have at least three mechanisms of direction finding, namely the sun compass [3,4], stellar compass [5–8] and the magnetic compass [9]. The latter is especially interesting, because the well-documented existence of the magnetic compass in birds remains one of few unambiguous demonstrations of the elusive magnetic sense of animals, the physical and physiological origins of which are still unclear [10]. Based, again, mainly on laboratory experiments with birds, two theoretical models of magnetoreception in terrestrial animals were singled out as the most viable: one involving magnetic nanocrystals of iron oxides [11] and the other based on magnetic-sensitive photochemical reactions in the eye [12,13] (the radical-pair model, RPM).

While within the iron-oxide model magnetic fields are supposed to be detected through the torque they exert on the nanocrystal having a huge magnetic moment (largely like the magnetic needle in a technical compass), the physics behind the RPM is more subtle [14]. Here, the magnetic sensitivity is
provided by extremely short-lived (within the microsecond range) directional correlation of magnetic moments of just two electrons. These two electrons reside in radicals, which can be formed by splitting of a specific molecule (or by an electron transfer within the molecule) upon absorption of a photon. If the magnetic field affects these two magnetic moments in different ways (this may happen if hyperfine [15] or spin–orbit [16] interactions in the two radicals are different), then their mutual orientation changes as a function of the field, and this change becomes more pronounced with time elapsed after separation of the radicals. Because, as known from quantum chemistry, covalent chemical bonds are formed by electrons with antiparallel magnetic moments (singlet spin state), the conversion of the pair of magnetic moments from antiparallel (singlet) to parallel (triplet) configuration and vice versa strongly affects the probability of recombination of the two radicals, and ultimately the yield of the entire photochemical reaction. The sensitivity of radical-pair reactions to magnetic fields is well known in chemistry [17]; recently, such a sensitivity was demonstrated in vitro for cryptochrome, the photosensitive protein that is considered as the candidate magnetoreceptor molecule of the bird compass [18]. However, the field intensities, required to considerably affect the photochemical process in the cryptochrome, were two orders of magnitude higher than that of the geomagnetic field. Much higher sensitivity was reached with an artificial molecule comprising porphyrine, carotenoid and fullerene, the use of which realized a model ‘chemical compass’ [19].

Although the results of many behavioural experiments suggested that the bird’s compass actually worked on radical-pair reactions (for example, its sensitivity to the spectrum of the ambient light [20]), the crucial evidence was lacking. The situation has apparently changed with the demonstration of the effect of weak oscillating magnetic fields (OMFs) on the orientation ability of European robins (Erithacus rubecula) by the group of R. and W. Wiltschko in Frankfurt [21–24]. These experiments were specially designed to test the radical-pair theory of magnetoreception, which predicted sensitivity not only to static, but also to RF magnetic fields in the low-MHz frequency range (close to the electron spin resonance in typical geomagnetic fields). Their overwhelming success was, and often continues to be, considered as an indication that the orientational magnetoreception of birds is based on the radical-pair mechanism. However, it soon turned out that the experiments had been, in a sense, too successful; in fact, birds appeared to be RF field-sensitive to the extent that the radical-pair theory was unable to explain [25]; see also discussion in [26].

To understand the origin of this problem, one needs to recall the abovementioned time-dependence of the magnetic-field effect on the mutual orientation of electron magnetic moments. In the extreme and most favourable for the magnetic sensitivity case when the direction of one of the electron moments is fixed by strong and anisotropic hyperfine interaction, whereas the other one is free (the ‘reference–probe’ model [23]), the probabilities of finding the pair of electrons in the spin-singlet state oscillates at the Larmor frequency $f_L = \gamma_B B$, where $B$ is the magnetic field intensity and $\gamma_B \approx 28$ Hz nT$^{-1}$ is the electron gyromagnetic ratio. The amplitude of the probability oscillation depends on the direction of the magnetic field as $A(1 - \cos 2\theta)$, where $\theta$ is the angle between the magnetic field and the anisotropy axis of the ‘reference’ radical, and $A < 1/2$ is a constant depending on the details of the radical-pair formation process. A considerable difference in the singlet yield for radical pairs with different directions of the anisotropy axis with respect to the magnetic field can develop if both the lifetime of the radical pair and disorientation time of electron moments (spin relaxation time) are comparable with or longer than $(2\pi f_L)^{-1}$. For $B = 50$ µT, this time amounts to 0.1 µs. With the typical radical-pair lifetimes of the order of a few microseconds, this would give a very good directional sensitivity (provided controllable orientation of the molecules in the magnetoreceptor; see discussion of this issue in [26]). Comparison with the results of in vitro experiments performed on plant cryptochrome [18] shows, however, that RP systems in cryptochromes may be very far from optimal, most likely because spin relaxation times are short and hyperfine couplings in both radicals are of comparable strength. Because similar experiments with bird cryptochromes have not been done, it remains an open question whether and to what extent their sensitivity to magnetic fields could be improved by evolution (see [27] for a discussion of possible ways for such improvements).

Now, if we turn to the effect of RF fields, the situation looks much less optimistic even in the ideal ‘reference–probe’ model. If a weak RF field with the amplitude $B_{RF}$ and the frequency $f_{RF}$ is applied at the angle $\phi$ to the static field $B$, then, even at $\theta = 0$, oscillations between the singlet and triplet configurations will occur at the Rabi frequency $f_R = 1/2 \gamma_B B_{RF} \sin \phi$. If all the characteristic times in the system were long enough, this would equalize the singlet yield for all orientations of the molecule and, therefore, disrupt the work of the radical-pair compass. However, because now both the lifetime and spin relaxation time must be longer than $(2\pi f_{RF})^{-1}$, and $f_R \ll f_{RF}$ (because $B_{RF} \ll B$), the criterion becomes much more severe: for the lowest RF amplitude that was reported to disrupt orientation of European robins, i.e. 15 nT applied at the angle of 24° to the static field [22], this would mean 2 ms that exceeds the most daring estimates of electron spin relaxation time in organic radicals. Further, one must take into account that the static magnetic field varied from 46 to 47.4 nT for different experimental cages in [22], but the RF field with the amplitude $B_{RF} \geq 15$ nT and frequency 1.315 MHz always caused disorientation, while for most cages it was, in fact, off the resonance. The theory of magnetic resonance says that the expected effect of the RF field, in this case, is at least 100 times weaker that one of the strictly resonant RF field; therefore, the required spin relaxation time shifts to utterly unbelievable 0.2 s. A similar estimate (about 1 s) was obtained in reference [25] from the observed effect of a broadband (0.1–10 MHz) RF field with the amplitude of 85 nT [24]. No need to say that any remotely realistic model of the radical pair would give worse sensitivity to the RF field than the ‘reference–probe’ model considered above. Several theoretical works published in recent years have attempted to reproduce some of the experimental results [28–30], involving various quantum and statistical effects to suppress decoherence and enhance sensitivity. With rather liberal choice of relaxation times, the effect of a strictly resonant RF field with the amplitude of 150 nT could be reproduced [28]. No one has ever tried to explain the effect of 15 nT monochromatic or of 85 nT broadband fields. The scattering of static field intensities (and therefore of resonance frequencies) in the experiment of reference [22] has never been taken into account either.

An alternative approach to this puzzle is to say that the change of the reaction yield under the RF field might be very
small, should the bird’s sensory system be so sensitive to this signal that at the final stages of neural transduction it could interfere with the signal from the static field. This explanation is, however, no less problematic (see discussion in reference [26])—for instance, it would require an unrealistically large number of cryptochrome molecules to provide the required sensitivity. Ultimately, it is unclear why it could be advantageous in evolutionary terms to develop an outstanding sensitivity to tiny signals from RF fields which birds apparently never met in nature.

To summarize this long introduction, experimental works [21,22,24] and consequent theoretical studies aimed at their explanation [25,28] have led to a dilemma: either the experiments of the Frankfurt group on the RF field effect upon orientation of European robins were flawed and their results are incorrect, or the RPM in its present form does not correctly reproduce the physics of the bird’s magnetic compass. As the RPM is one of the mainstream theories of magnetoreception, and there exists a considerable body of experimental evidence supporting the idea that compass magnetoreception is based on the vision system of birds, independent experimental studies aimed at confirmation or negation of the results of previous studies [21,22,24] are of key importance for the further development of the science of magnetoreception [10].

Here, we report the results of our experiments which were aimed to independently replicate the claims that small-amplitude RF field disrupts magnetic orientation in migratory songbirds.

2. Material and methods

We performed orientation tests in autumn 2013 with garden warblers (S. borin) captured during their first autumn migration on the Courish Spit. Experimental birds were kept in an outdoor aviary, they experienced natural photoperiod, natural geomagnetic field and had access to astronomical orientation cues (sun, sunset polarization patterns and stars) during their time in captivity. They were provided food (mealworms) and water ad libitum.

In the first series of tests, migratory orientation of garden warblers was studied in the natural magnetic field (NMF) in Emlen funnels [31] under simulated total overcast. On top of Emlen funnels, we put lids made of milky glass which completely obscured star and any other patterns but let enough light in. Thus, the only orientation cue available to experimental birds during the test was geomagnetic field. Each test lasted for 40 min and started at the beginning of astronomical twilight. We used modified Emlen funnels made of aluminum (top diameter 300 mm, bottom diameter 100 mm, slope 45° with the top opening covered by netting). The directionality of the birds’ activity was recorded as scratches left by their claws as they hopped in the funnels on a print film covered with a dried mixture of whiting and glue. Two researchers (N.C. and A.P.) independently determined each bird’s mean direction from the distribution of the scratches. In most cases, the mean direction could be very precisely identified using the simple visual estimation method [32]. If a pattern of scratches was not clear, both persons independently counted scratches in each of 36 × 10° sectors (some birds leave so many scratches that an exact number of scratches cannot be counted, but must be estimated) and used circular statistics software to assess the directionality based on the numbers of scratches. The mean of the two observer’s determined directions was recorded as the orientation result. If both observers considered the scratches to be randomly distributed or if the two mean directions deviated by more than 30°, then the bird was considered to be not oriented in the given test. The same counting procedure was used also in all the tests with applied magnetic fields (see below). In view of the limited number of birds we could test in parallel during our outdoor testing sessions, we opted for not using the fully double-blind procedure, which would not have allowed us to complete the experiments within one field season; this choice was justified, in our view, by absence of obvious preferences in our group as to the expected outcome of the tests: both confirmation and non-confirmation of the results of Frankfurt experiments was considered equally interesting.

After testing the birds for at least three and up to five times and receiving at least two and up to five results when the birds were sufficiently active (i.e. left at least 40 and nearly always more than 100 scratches on underlying paper) and showed orientation sufficiently concentrated (i.e. it would have passed the Rayleigh test [33] at the 5% significance level had the movements in the funnel been independent events), the birds were transferred to the second phase of the experiment. Inactive (fewer than 40 scratches) tests and not sufficiently concentrated trials were excluded from analysis. From the active and oriented tests, the mean directions of each individual were calculated using vector addition.

In the second series of tests, the same birds were tested in RF OMF. The field was created by coils 0.75 m in diameter, fed from a commercial stabilized high-frequency generator and a custom-made broadband power amplifier, which served to match the generator output with the 50 Ohm coaxial cable 40 m in length. Each coil comprised three wraps of insulated copper wire 1 mm in cross section, fixed to a wooden frame. The cable transmitted the RF current to coils and allowed us to place them at a distance from the RF source assembly (generator, amplifier, power supply). The source assembly was put into a wooden case, placed on a clearing in reeds approximately 25 m along the straight line from the centre of another clearing, where two wooden tables for experimental cages were build. The sound of working devices was not discernible already 2 m apart from the case, and near experimental tables it was by far below natural environmental noises. On the tables, up to four coils could be installed, two on each. Coils were connected in parallel by small lengths of coaxial cable to the main RF cable and mounted, so that one side of the coil was raised above the table top to increase the angle between OMF and NMF, which as a result was approximately 30°. Emlen funnels were placed on tables, one in the centre of each coil. In all the experiments, monochromatic RF fields with the amplitude 190 nT and frequency 1.403 MHz (matching the Larmor frequency of a free-standing electrons spin in the local GMF of 50 100 nT) were used. The parameters of the OMF were controlled before and after each test. To this end, we used a single wire loop 20 cm in diameter, connected to a digital oscilloscope. The loop was placed consequently in the centre of each coil. The periodic in time induction EMF was visualized, its frequency and amplitude measured, and the latter was calculated into the amplitude of RF magnetic field. Using this device and applying real-time fast Fourier transform to the measured signal, we also analysed the spectrum of background noise in the frequency range 0.1–100 MHz. Its rms amplitude was found to be always below 0.5 nT.

The tests were again performed under milky glass, so that experimental birds had no access to astronomical orientation cues. After the birds were tested in OMF at least three times (and up to five times) and produced at least two and up to three usable directions in individual tests (see above for criteria for usability), they were tested again in stationary magnetic field, which was however rotated 120° anticlockwise in the horizontal plane (rotated magnetic field, RMF). The total intensity and vertical component of this rotated field were identical to the natural values. To produce the artificial rotated field, electric current flowed through the magnetic coils (Helmholtz coil system with two coils per axis) custom built by Pushkov Institute of Terrestrial Magnetism, Ionosphere and Radiowave Propagation (Russian Academy of Sciences, St Petersburg, Russia). The
coils were managed by custom-written software. These coils were the same as the ones used in reference [34]. This last experiment was performed to test (i) whether RF fields had any lasting effect on the birds’ orientation performance and (ii) whether they indeed used the magnetic information and not anything else for their orientation (even though the use of celestial cues was ruled out by putting milky glass lids on top of the funnels).

We tested a total of 19 garden warblers in NMF and in OMF. One bird never showed directed and sufficiently concentrated orientation in OMF, in spite of being tested five times. Unfortunately, we managed to test only eight garden warblers in RMF before the end of September when migratory activity and orientation sharply decreased. The reason for this could be that our experimental birds remained in the Baltic area, whereas in the wild, they should have been in the Iberian peninsula or already crossing the western Sahara [35]. They may have lacked some crucial cues and sharply decreased their activity because of that (cf. [34,36,37]). Thus, here we report the data on eight individuals that were successfully tested in all three conditions, NMF, OMF and RMF. To avoid pseudo-replication, the mean orientation of one individual bird. The triangle and letters mN show the position of the magnetic north in each testing condition. Arrowheads show group mean directions and vector lengths.

Table 1. Orientation behaviour of garden warblers during autumn migration on the Courish Spit in three testing conditions: natural magnetic field, radiofrequency oscillating magnetic field (OMF), and rotated magnetic field (RMF). RMF was rotated $120^\circ$ anticlockwise in the horizontal plane as compared to the NMF, i.e. to make the results in RMF comparable with the results of the same birds in NMF, $120^\circ$ should be added to each value. All tests were performed without access to celestial cues.

<table>
<thead>
<tr>
<th>ring no</th>
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<th>mean direction in NMF</th>
<th>results of successful tests in OMF</th>
<th>mean direction in OMF</th>
<th>results of successful tests in RMF</th>
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<td>552</td>
<td>229°, 249°, 313°</td>
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<td>40°, 130°</td>
<td>85°</td>
<td>69°, 234°</td>
<td>154°</td>
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<tr>
<td>528</td>
<td>260°, 240°, 310°</td>
<td>270°</td>
<td>270°, 0°</td>
<td>315°</td>
<td>0°, 110°, 99°</td>
<td>75°</td>
</tr>
<tr>
<td>716</td>
<td>300°, 205°, 216°</td>
<td>237°</td>
<td>85°, 100°, 320°</td>
<td>48°</td>
<td>100°, 172°, 210°</td>
<td>163°</td>
</tr>
<tr>
<td>625</td>
<td>151°, 140°, 210°, 258°, 001°</td>
<td>191°</td>
<td>170°, 330°</td>
<td>250°</td>
<td>159°, 328°, 60°</td>
<td>59°</td>
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<td>280°, 120°</td>
<td>200°</td>
<td>85°, 160°, 324°</td>
<td>93°</td>
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<tr>
<td>809</td>
<td>220°, 310°</td>
<td>265°</td>
<td>330°, 280°, 200°</td>
<td>273°</td>
<td>155°, 110°</td>
<td>132°</td>
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<tr>
<td>881</td>
<td>0°, 222°, 205°</td>
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<td>290°, 340°</td>
<td>315°</td>
<td>80°, 240°, 35°</td>
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<td>689</td>
<td>40°, 270°, 210°</td>
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<td>98°, 169°</td>
<td>134°</td>
<td>310°, 91°, 60°</td>
<td>34°</td>
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<td>second-order</td>
<td>$r = 0.89$,</td>
<td>$r = 0.16$,</td>
<td>$r = 0.71$,</td>
<td>$p = 0.012$</td>
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<tr>
<td>mean</td>
<td>$p &lt; 0.001$</td>
<td>n.s.</td>
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Second-order mean circular distributions were tested for directionality by the Rayleigh test [33]. Differences in mean direction between experimental groups were analysed using the parametric Watson–Williams F-test in the cases where the assumptions underlying this test were fulfilled. When the concentrations of the samples to be compared were too low to use the Watson–Williams F-test, the non-parametric Mardia–Watson–Wheeler test was used [33].

3. Results

Garden warblers tested in NMF without access to celestial cues showed the mean orientation towards the WSW (table 1; $\alpha = 256^\circ$, 95% confidence interval (CI) = 233–280°, $n = 8$, $r = 0.89$, $p < 0.001$, figure 1a). This value is identical to the mean direction shown by garden warblers in nocturnal tests at the same site with full access to celestial and magnetic cues: $\alpha = 256^\circ$, 95% CI = 224–288° [38] and somewhat more westerly than the direction of autumn migration from the Courish Spit as suggested by ring recoveries: 212°, 95% CI = 204–221° (calculated from data in [39]).

The same birds tested in OMF showed no consistent orientation: $\alpha = 281^\circ$, $n = 8$, $r = 0.16$, $p = 0.83$ (figure 1b).
confirmed by us, is difficult to explain within the existing radical-pair theory. The reason is that the time needed for the RF field to change the state of an electron spin cannot be shorter than the period of rotation of the same spin in a static field of the same amplitude (assuming all the other interactions nullified). This period is determined by the field amplitude and the electron gyromagnetic ratio, which for organic radicals cannot strongly deviate from its value for free electrons (given by a combination of fundamental constants). For this simple reason, the time needed to change the spin state by applying the RF field with the amplitudes known to disrupt the birds’ compass appears to be on the scale of milliseconds, exceeding by two orders of magnitude maximum electron spin lifetimes known for organic radicals. The presence of various spin–spin and spin–orbit interactions can only diminish the effect of the RF field, making the observed sensitivities even more difficult to explain.

It may be argued that we tested our birds only for a resonance effect and made no tests in an off-resonance or broadband RF oscillating field; therefore, we cannot distinguish between a specific Zeeman resonance effect of a relatively weak (190 nT) RF field and a putative effect of an off-resonance or broadband RF field of the same intensity. However, as emphasized in the Introduction, neither the resonance nor off-resonance RF field effect have so far found a satisfactory theoretical explanation. Our main point is that a RF field of roughly the same amplitude as used in the experiments of [22,24] is indeed able to disrupt the magnetic orientation of a migratory songbird, and thus it is very unlikely that the findings of aforementioned studies resulted from some systematic error or a hidden flaw of the experimental procedure. This, in its turn, means that the radical-pair theory of avian magnetoreception in its current form may need revision to account for this RF field effect.

More experimental work is needed to refine the spectral width of the observed Zeeman resonance in the sensitivity of the avian compass to RF fields [22] and to verify the reported sensitivity threshold at the exact resonance (about 15 nT). Only after obtaining these data (which may take several field seasons), one would be able to obtain a reliable experimental estimate of the spin lifetimes in the radical pair or whatever physical system is responsible for the compass magnetoreception of birds. However, already these previous results demand the renewed attention of theoreticians to the physical and chemical models of magnetoreception.

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Endnote

1 An inhomogeneous broadening (e.g. owing to weak hyperfine coupling with several distant nuclei) could apparently diminish this effect by bringing RPs back to resonance; however, the inhomogeneous broadening, in fact, shifts the resonant frequency of each given RP by
some random value, that is, it does the same thing as the deviation of static field in the Frankfurt experiments. The transition would have been brought back to resonance only if the frequency shifts, internal and external, were correlated in such a manner that they exactly compensated each other, which is obviously not possible to achieve for the entire ensemble of RPs with random hyperfine couplings.

References