Long-distance navigation and magnetosensory mechanisms in migratory songbirds

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Summary of the Ph.D. thesis

The question how migratory birds can find the way to their wintering grounds and back has been puzzling researchers for decades. Migratory birds travel thousands of kilometres over apparently featureless landscape, and some species even fly alone at nighttime. Since the 1950s, it has become clear that, to find and maintain their headings, migratory birds are able to use rather sophisticated mechanisms to derive orientation information from different natural cues: the sun and the star compass use respective celestial cues (the Sun: e.g., Kramer 1950a, 1950b; and stars: e.g., Sauer 1956, 1957a, 1957b; Emlen 1967a, 1967b, 1975) and the magnetic compass uses the Earth's magnetic field (e.g., Merkel and Wiltschko 1965; Wiltschko and Wiltschko 1972).

Despite significant progress in our understanding of the orientation and navigation mechanisms of migratory birds, there are still many open questions. For example, the mechanisms underlying long-distance navigation, i.e., the ability to reach goals without perceiving any direct information from them or to compensate for huge geographical displacements, still remain poorly understood. Particularly, we still do not know which natural cues migratory birds can use as surrogates for geographical coordinates. Since the 1960s, there is evidence that birds are able to use the Earth's magnetic field as a directional reference (e.g., Wiltschko and Wiltschko 1972; Cochran *et al.* 2004). But only recently, researchers started understanding the neurophysiological mechanisms underlying magnetoreception. Nowadays, there is a growing body of facts strongly suggesting that birds possess two different magnetosensory systems: i) a chemical sensor in the bird's eye based on a radical pair mechanism (Ritz *et al.* 2000; see Ritz *et al.* 2010 and Liedvogel and Mouritsen 2010 for reviews), and ii) iron mineral containing sensors in the upper beak (Fleissner *et al.* 2003, 2007). However, the neurophysiological substrates and interaction between these two putative magnetosensory systems are still the subjects of research. Magnetoreceptive mechanisms, in turn, may be closely related to navigational abilities of migratory birds. It has been proposed that natural cue(s) used to determine position on the globe must meet the following requirements: they must provide consistent information, must vary systematically so that single points on the surface of the Earth can be identified uniquely, must be sufficiently stable over time to permit natural selection for navigation, must be detected and used to determine position with sufficient resolution to meet needs of the animal (Walker *et al.* 2002). The parameters of the Earth's magnetic field, at least in part, meet these requirements and, therefore, understanding magnetoreception may help us answer the question how birds can navigate.

In my PhD work, I mainly focus on the following questions: i) are migratory birds able to detect a geographical displacement along east-west axis?; ii) if they are, which mechanism(s) may underlie this ability?; iii) which properties do the two putative magnetosensory systems possess? Specifically, what is the function of Cluster N and the beak organ?; and, finally, iv) is the avian magnetic compass strongly lateralized?

Because human navigation techniques are based on two coordinates (latitude and longitude), it is not surprising that most authors assume that migratory birds should also use bi-coordinate navigation (e.g., Berthold 1991, 1996; Rabøl 1978). However, this assumption may be too anthropocentric and, therefore, has to be experimentally tested. Theoretically, it is much easier to propose a mechanism detecting position along north-south axis. For instance, this mechanism may measure the height of starry sky's rotation center above the horizon (Sauer and Sauer 1960; Able 1980; Mouritsen 2003; Gould 2004, 2008), magnetic inclination and/or magnetic intensity. However, it is much harder to imagine which natural parameters may serve for detection of east-west position – the analogue of longitude (Åkesson and Alerstam 1998; Mouritsen 2003; Gould 2004, 2008). Therefore, it was plausibly hypothesized that migratory birds, particularly young birds on their first spring migration yet having no experience with finding their natal area, may use an *one-coordinate navigation strategy* (Mouritsen 2003). It implies that the birds may remember and identify latitude, but not longitude, of their natal area as well as landmarks around it before their first autumn migration. Next spring, young birds may travel north (situation for the northern hemisphere considered) until they reach latitude of their natal site destination. If a bird has made a small navigational mistake, but reached an area with visually known landmarks, it may easily pinpoint the natal area using landmark-based map. If a larger navigational mistake has been made, a bird may start searching for the goal moving back and forth along latitude of the natal site and trying to find known landmarks (Mouritsen 2003).

Using Eurasian reed warblers (*Acrocephalus scirpaceus*) as model long distance migrants, I together with my co-workers tested the hypothesis of one-coordinate navigation. We caught migrating Eurasian reed warblers in East Baltic during spring migration, tested their control orientation at a capture site and displaced them approximately 1,000 km due east to Moscow region. After the displacement, the birds were tested again. Their orientation strongly suggests that displaced Eurasian reed warblers are able to compensate for a 1,000 km displacement (Paper I). These results are in line with another recent study where adult white-crowned sparrows (*Zonothrichia leucophrys gambelii*) on their autumn migration along the west coast of USA were cross continentally displaced over 3,700 km to the east and were able to detect and compensate for this displacement (Thorup *et al.* 2007). Our results together with data presented in the study of Thorup *et al.* (2007) strongly suggest that migratory birds do use at least two, not one, coordinates for navigation.

In my PhD work, I also addressed the question which mechanism may enable Eurasian reed warblers to detect the 1,000 km displacement to the east in the aforementioned study (Paper I). There is a variety of hypotheses trying to explain how birds can detect east-west position. Because humans invented precise chronometers to detect longitude, most of the proposed hypotheses imply time-keeping effects. For example, it has been suggested that birds may have one biological oscillator ("clock") set at a "home time" (e.g., time of breeding area) and another clock, which is easily reset by local time (e.g., Rabøl 1980, 1998; Mouritsen and Larsen 2001). However, according to the literature, a fixed time clock has never been found in birds (see Gwinner 1986 for a review). On the contrary, the internal clocks of animals are known to become quickly adjusted to a local time (e.g., Gwinner 1996a; Gwinner *et al.* 1997; Albus *et al.* 2005; Piggins and Loudon 2005). During my PhD work, I proposed and tested a plausible variant of time-keeping hypotheses – *a double clock hypothesis*. This hypothesis assumes the existence of two coupled, re-synchronizable clocks. The first clock is slowly synchronized to a local light-dark (LD) regime, whereas the second clock, the fast-entraining one, is the well-known biological oscillator that becomes quickly synchronized to a local LD cycle. The time difference between these two clocks would enable birds to determine their east-west position after displacement on the basis of time zone or "jetlag" effects. To test this hypothesis, we caught Eurasian reed warblers during spring migration in East Baltic, tested their control direction at a capture site, and simulated the time difference which they would have been exposed to if they have been displaced 1,000 km to the east (Paper II). Our results suggest that reed warblers are unlikely to use the time zone effect for detection of east-west displacements. This, in turn, may indicate that mechanism(s) enabling Eurasian reed warblers to detect their east-west position is/are independent of time-keeping but rather relies on time-independent natural cue(s).

Not only adult avian migrants have to reach distinctive goals, young birds also have to find the species-specific wintering grounds, even though they do it for the first time. How can naïve migrants reach their wintering quarters without any previous experience? It has been suggested that first-year avian migrants use the inherited so-called *vector navigation or clock-and-compass programme*. This programme guides a first-year bird towards the wintering grounds by a series of leaps in genetically distinctive directions for distinctive period of time until the programme stops. The concept of the clock-and compass programme implies that, regardless of inevitable orientation mistakes and influence of weather conditions, most naïve avian migrants at the end of their first migration will find the species-specific wintering region. There are, however, species whose breeding ranges are extremely elongated in east-west direction though birds from all populations of a given species share common wintering grounds (e.g., the willow warbler, *Phylloscopus trochilus*, the yellow-breasted bunting, *Emberiza aureola*, and the pied flycatcher, *Ficedula hypoleuca*). It means that young birds coming from far separated populations of the same species have to be guided by very different clock-and-compass programmes on their first migration. In my PhD, I together with my colleagues compared the development of clock-and-compass programmes in first-year pied flycatchers born in East Baltic (the Courish Spit, Kaliningrad region) and Western Siberia (Alaevo, Kemerovo region). Birds from both the populations share the same wintering grounds in sub-Saharan West Africa. We took nestlings from nest boxes, hand raised them and tested their orientation during autumn migration (Paper III). All Baltic pied flycatchers were hand raised and tested at their natal site at the Courish spit, but Siberian pied flycatchers were divided into two groups – one was left at the natal site at Alaevo, and another was displaced to the Courish Spit. Our results suggest that Siberian pied flycatchers tested at their natal site during the beginning of autumn migration orient due west. This orientation would lead them first to Europe from where they most probably turn south/southwest to reach their wintering grounds in West Africa. The results obtained from the pied flycatchers hatched at the Courish Spit indicate that these birds are initially western-southwesterly oriented, and then significantly shift their orientation towards the southwest. Interestingly, the Siberian birds transported to the Courish Spit at an early age performed the southwestern orientation that was significantly different from the orientation of their Siberian conspecifics raised and tested at the natal site. This indicates that some local external cues at the Courish Spit might modify orientation programme of the displaced Siberian pied flycatchers (Paper III).

We suspect that the geomagnetic cues are essential not only for compass orientation, but also for determining east-west position. Therefore, the second main subject of my PhD thesis was devoted to understanding of magnetoreception in birds. More specifically, I asked the following three questions:

i) Are/Is an intact Cluster N (a specialized, night-time active, light-processing forebrain area discovered in nocturnal migratory birds) and/or an intact ophthalmic branch of the trigeminal nerve (the nerve that innervates the upper beak where iron-mineral clusters are found) crucial for orientation when the magnetic field is the only available orientation cue?

To do this, we compared the magnetic orientation capabilities of four groups of European robins (*Erithacus rubecula*) during migration: a group with bilateral chemical lesion of Cluster N, a group with bilateral section of the trigeminal nerves and two groups with equivalent sham surgeries (Paper IV). Magnetic orientation showed that only the group with bilateral lesion of Cluster N was unable to use magnetic field for orientation, whereas the three other groups, including the group with sectioned ophthalmic branches of the trigeminal nerves, were able to use the geomagnetic field for compass orientation (Paper IV). Further experiments strongly suggested that the inability to orient by the magnetic field was not due to general visual deficits because the Cluster N lesioned birds were successfully conditioned to visually stimuli. Neither was this dysfunction of the magnetic compass due to lack of motivation to perform orientation because the Cluster N lesioned birds were able to use a setting sun and stars for orientation. Thus, Cluster N is the first brain region demonstrated to be involved in processing information obtained from magnetic compass cues (Paper IV).

ii) Is the avian magnetic compass strongly lateralized and located only in the bird's right eye as it has been previously suggested by Wiltschko *et al.* (2002)?

Several recent findings (Mouritsen *et al.* 2004, 2005; Liedvogel *et al.* 2007a; Hein *et al.* 2010) seriously question whether it could really be true that the avian magnetic compass is very strongly lateralized. Therefore, we tested the magnetic orientation capabilities of European robins – the same species in which the strong lateralization of the magnetic compass to the right eye was previously reported (Wiltschko *et al.* 2002) – during autumn migration when either the right or the left eye was covered. Our results showed that the birds were able to use their magnetic compass for orientation irrespective of which of their eyes was covered (Paper V and VI). Thus, our results strongly suggest that European robins have the magnetic compass in both eyes, and magnetic compass sensing is not strongly lateralized.

iii) Can the operant conditioning approach successfully established in homing pigeons (*Columba livia domestica*, Mora *et al.* 2004) be transferred to a night-migratory songbird species to study magnetic discrimination behaviour?

In this work (Paper VII), Dr. Cordula Mora and I attempted to adapt the operant conditioning paradigm developed in the study of Mora *et al.* (2004) in homing pigeons to a migratory bird - the European robin. Despite more than 2 years of a dedicated work, we did not reach the point where the European robins' behaviour was obviously under the control of magnetic stimuli used. The general adequacy of our setup used was proven by a successful conditioning of the same birds to an auditory stimulus.

In summary, my PhD thesis provides evidence that:

(1) Eurasian reed warblers – typical long-distance songbird migrants – are able to compensate for a 1,000 km displacement due east. This result together with other similar recent works strongly suggests that migratory birds do use a few, at least two, natural cues as surrogates of geographical coordinates. At the same time, testing of the double clock hypothesis indicates that the mechanism(s) enabling Eurasian reed warblers to detect longitudinal displacements is/are independent of time-keeping;

(2) First-year Siberian pied flycatchers have an innate spatiotemporal programme that leads them to Europe before migration to West African winter quarters. Siberian pied flycatchers displaced to the Baltic area as nestlings, raised and tested there during their first autumn migration showed the southwestern orientation that is significantly different from their conspecifics raised and tested at their natal site in Western Siberia;

(3) An intact Cluster N is crucial for the magnetic compass in European robins whereas an intact ophthalmic branch of the trigeminal nerve, which presumably innervates iron-mineral-containing cells in the upper beak, is neither necessary nor sufficient for magnetic compass orientation in a given species. This indicates that Cluster N is involved in processing of directional magnetic information in migratory birds whereas the function of the putative magnetosensitive cells in the upper beak is still to be uncovered;

(4) The avian magnetic compass of the European robin is not strongly lateralized because birds of this given species are able to use the magnetic compass irrespective of which eye is covered;

(5) The magnetic operant conditioning paradigm successfully established in homing pigeons (Mora *et al.* 2004) does not seem to be optimal for a migratory songbird – the European robin.

Zusammenfassung der Dissertation

Seit Jahrzehnten hat die Fähigkeit der Zugvögel ihre Winterquartiere zu finden die Wissenschaft vor viele Rätsel gestellt. Zugvögel legen tausende von Kilometern über Landschaften ohne offensichtliche Orientierungspunkte zurück. Manche Arten wandern sogar solitär, während der Nacht.

In den 1950er Jahren verdichteten sich die Hinweise, dass Zugvögel über hoch entwickelte Sinnessysteme verfügen um natürliche Orientierungspunkte (wie den Stand der Sonne und der Sterne) zu nutzen, und die richtige Richtung des Zuges zu finden und aufrechtzuerhalten. Das Erdmagnetfeld bietet dabei die Möglichkeit, magnetische Informationen zur Navigation zu verwenden.

Trotz erheblicher Fortschritte in unserem Verständnis der Navigationsmechanismen von Zugvögeln verbleiben viele Fragen unbeantwortet. Beispielsweise ist die Navigation über lange Strecken oder die Fähigkeit ein Ziel zu erreichen ohne konkrete Richtungshinweise des Zieles selbst zu empfangen sowie nach geographischen Versetzungsexperimenten die Flugrichtung zu Kompensieren, noch immer schlecht verstanden. Insbesondere ist dabei die Nutzung handfester geographischer Koordinaten bis dato unerklärt.

Seit den 1960er Jahren bestehen konkrete Hinweise, dass Vögel das Erdmagnetfeld als Referenzrichtung benutzen. Die Wissenschaft geht heute davon aus, dass Vögel über zwei unterschiedliche Sinnessysteme zur Magnetperzeption verfügen: i). ein biochemischer Sensor im Auge des Vogels, basierend auf einem Radikal-Paar-Mechanismus; und ii). eisenhaltige Sensoren im Oberschnabel. Diese beiden Systeme könnten die physiologischen Grundlagen für die Navigationsfähigkeiten von Zugvögeln darstellen.

Folgende Vorraussetzungen müssten natürliche Referenzsysteme (Sonne, Sterne etc.) für eine globale Navigation erfüllen: Sie müssen konsistent sein und systematisch variieren, sodass eine eindeutige Lokalisation auf dem Globus möglich ist. Zudem müssen sie über hinreichende Zeiträume stabil bleiben, um evolutive Adaption durch natürliche Selektion zu gewährleisten. Freilich müssen sie von Tieren detektierbar und nutzbar bzw. in für die jeweilige Tierart geeigneter Auflösung zur Positionsbestimmung vorhanden sein. Die Eigenschaften des Erdmagnetfelds erfüllen diese Vorraussetzungen zumindest teilweise, weshalb eine gründliche Erforschung der Magnetperzeption zu großen Erkenntnisgewinnen bezüglich der Zugvogelorientierung führen kann.

Im Rahmen meiner Doktorarbeit habe ich mich im Wesentlichen mit der Untersuchung folgender Fragestellungen beschäftigt: i). Sind Zugvögel in der Lage eine experimentelle geographische Versetzung entlang der Ost-West Achse zu registrieren? ii). Ist dies der Fall, welche Sinnessysteme könnten sie dazu befähigen? iii). Welche für die Navigation relevanten Informationen liefern die oben genannten magnetperzeptiven Sinnessysteme, speziell Cluster N und das Schnabelorgan? iv). Ist der Magnetkompass von Zugvögeln deutlich lateralisiert?

Weil menschliche Navigationssysteme auf zwei Koordinaten (Längengrad und Breitengrad) basieren, ist es nicht überraschend dass die meisten Autoren ein ähnliches Prinzip bei Zugvögeln proklamieren. Diese Annahme ist recht antropozentrisch und muss experimentell geprüft werden. Theoretisch ist die Annahme, Zugvögel könnten ihre Position anhand der Nord-Süd Achse bestimmen, viel simpler. Hierbei könnte die Höhe des Rotationszentrums des Sternenhimmels relativ zum Horizont, die Inklination bzw. die Intensität des Erdmagnetfeldes gemessen werden. Die Vorstellung, dass Zugvögel die Ost-West-Achse, analog zur geographischen Länge detektieren, ist wesentlich unwahrscheinlicher.

Daher wurde prognostiziert, dass Zugvögel auf ihrer ersten Frühjahrsmigration ein Einkoordinatensystem nutzen, da sie noch keine Erfahrungen bezüglich des Weges zu ihren Brutgebieten haben.

Dies impliziert das die Vögel vor der ersten Herbstmigration vermutlich ihre Zugrichtung nach der geographischen Breite und anhand prominenter Landmarken ihrer Brutregion ausrichten und diese speichern. Im nächsten Frühjahr ziehen die Jungvögel dann nach Norden (Situation der nördlichen Hemisphere) bis sie die geographische Breite ihrer Brutregion erreicht haben. Im Falle eines größeren Richtungsfehlers während der Navigation, könnten die Vögel entlang der geographischen Breite ihrer Brutregion nach ihnen bekannten Landmarken suchen.

Weil der Teichrohrsänger (*Acrocephalus scirpaceus*) ein geeigneter Modellorganismus zur Untersuchung der Langstreckennavigation ist, fiel die Wahl des Modellorganismus auf diese Art, um die Einkoordinatensystem-Hypothese zu testen. Es wurden Teichrohrsänger im östlichen Baltikum während ihres Frühjahrszuges gefangen und ihre Zugrichtung vor Ort als Kontrollrichtung getestet. Im Anschluss wurden die Versuchstiere etwa 1000 Kilometer ostwärts in die Region um Moskau versetzt. Nach dieser Versetzung wurde die Zugrichtung in Moskau erneut getestet. Es stellte sich heraus, dass die Vögel die räumliche Versetzung kompensierten, um zu ihren angestammten Brutgebieten zu fliegen. Diese Resultate deuten stark daraufhin, dass die versetzten Teichrohrsänger tatsächlich in der Lage waren, die Versetzung zu bemerken.

Diese Ergebnisse entsprechen Ergebnisse aus anderen Studien, in denen adulte Dachsammern *(Zonothrichia leucophrys gambelii)* auf ihrem Herbstzug entlang der Westküste der USA über 3700 km an die Ostküste versetzt wurden. Auch hier waren sie in der Lage, diese Replatzierung zu bemerken und entsprechend ihre Zugrichtung zu ändern, um zu ihren Brutgebieten zu gelangen (Thorup *et al.* 2007). Zusammen mit diesen Daten legen unsere Ergebnisse die Schlussfolgerung nahe, dass Zugvögel mindestens zwei, und nicht nur eine Koordinate, für die Navigation nutzen.

In meiner Doktorarbeit habe ich mich ebenfalls mit der Frage beschäftigt, welcher Mechanismus es den Teichrohrsängern ermöglicht, die in der oben genannten Studie (Paper I) beschriebene Versetzung 1000 km nach Osten zu detektieren. Eine Vielzahl von Hypothesen versucht Erklärungen zu finden, wie Vögel die Ost-West-Position wahrnehmen können. Da die Menschen präzise Chronometer nutzen können, um Längengrade zu detektieren, beinhalten die meisten aufgestellten Hypothesen Zeitmessungs-Effekte. Eine Theorie besagt beispielsweise, dass Vögel eventuell einen biologischen Oszillator („clock“) haben, der auf eine „Heimat-Zeit“ festgelegt ist (z.B. Zeit des Brutgebietes) sowie eine weitere Uhr, die sich an die lokale Zeit anpasst (z.B. Rabøl 1980, 1998; Mouritsen und Larsen 2001). In Übereinstimmung mit der Literatur wurde eine fixierte innere Uhr in Vögeln nicht gefunden (siehe Gwinner 1986 als Übersichtsartikel). Im Gegensatz dazu weiß man, dass die internen Uhren von Tieren schnell an die lokale Zeit angepasst werden (z.B. Gwinner 1996a; Gwinner *et al.* 1997; Albus *et al.* 2005; Piggins und Loudon 2005). Während meiner Doktorarbeit testete ich eine plausible Variante der Zeitmessungs-Hypothese – die Doppeluhr-Hypothese. Diese Hypothese setzt die Existenz zweier gekoppelter, re-synchronisierbarer Uhren an. Die erste Uhr wird langsam an den örtlichen Hell-Dunkel-Rhythmus (HD) angepasst, während die zweite schnell-synchronisierte Uhr als biologischer Oszillator bekannt ist, der schnell an den lokalen HD-Rhythmus angeglichen wird. Die Zeitdifferenz zwischen diesen beiden Uhren würde es den Vögeln ermöglichen, ihre Ost-West-Position nach der Versetzung auf der Basis von Zeitzonen- oder „Jetlag“-Effekten zu bestimmen. Um diese Hypothese zu überprüfen, wurden Teichrohrsänger während des Frühlingszugs gefangen, ihre Kontrollrichtung am Fangort überprüft und die Zeitdifferenz simuliert, der sie ausgesetzt gewesen wären, wenn sie 1000 km östlich versetzt worden wären (Paper II). Unsere Ergebnisse weisen darauf hin, dass Teichrohrsänger nicht in der Lage sind, den Zeitzoneneffekt zur Detektion der Ost-West-Versetzung zu nutzen. Das wiederum weist eventuell darauf hin, dass der Mechanismus bzw. die Mechanismen, der/die den Teichrohrsängern die Feststellung ihrer Ost-West-Position ermöglicht, unabhängig von der

Zeitmessung ist/sind und im Gegenteil eher auf zeitunabhängigen natürlichen Markern, zum Beispiel der Messung des Erdmagnetfeldes, beruht (Paper II).

Nicht nur erwachsene, sondern ebenfalls einjährige Vögel (ohne Kenntnis der Flugstrecke) müssen ihre artspezifischen Ziele erreichen, auch einjährige Vögel, die erstmals auf Zugreise sind, müssen ihre artspezifischen Winterquartiere finden. Wie können diese jungen Vögel ihr Winterquartier ohne jegliche Vorerfahrung finden? Es wird vermutet, dass ein Jahr alte Zugvögel die angeborene, sogenannte Vektornavigation oder das Uhr-und-Kompass-Programm nutzen. Dieses Programm führt einen einjährigen Vogel in Richtung seines Winterquartiers über eine Serie von Sprüngen in genetisch determinierten Richtungen für eine bestimmte Dauer, bis das Programm endet. Das Konzept des Uhr-und-Kompass-Programms impliziert, dass trotz unvermeidbarer Orientierungsfehler und des Einflusses von Wetterbedingungen am Ende des ersten Vogelzugs die meisten jungen Zugvögel ihr artspezifisches Überwinterungsgebiet finden. Es gibt jedoch Arten, deren Brutbereiche extrem in die Ost-West-Richtung ausgedehnt sind, obwohl Vögel aller Populationen einer bestimmten Art ein gemeinsames Winterquartier teilen (z. B. Fitis, *Phylloscopus trochilus*, Weidenammer, *Emberiza aureola* oder der Trauerschnäpper, *Ficedula hypoleuca*). Dies bedeutet, dass einjährige Vögel, die aus weit entfernten Populationen derselben Spezies stammen, von sehr unterschiedlichen Uhr-und-Kompass-Programmen geführt werden müssen. In meiner Doktorarbeit habe ich zusammen mit meinen Kollegen die Entwicklung der Uhr-und-Kompass-Programme in einjährigen Trauerschnäppern verglichen, die im Ostbaltikum (Kurische Nehrung, Region Kalininingrad) und in Westsibirien (Region Kemerovo) geboren wurden. Die Vögel beider Populationen teilen sich das Winterquartier im subsaharischen Westafrika. In unseren Versuchen wurden Nestlinge aus Nistkästen genommen, mit der Hand aufgezogen und ihre Orientierung während des Herbstzugs getestet (Paper III). Alle baltischen Vögel wurden mit der Hand aufgezogen und am Fangort auf ihre Zugrichtung getestet. Die sibirischen Vögel wurden in zwei Gruppen geteilt: Eine wurde am Geburtsort freigelassen, die andere wurde an die Kurische Nehrung versetzt. Unsere Ergebnisse weisen darauf hin, dass Sibirische Trauerschnäpper, die am Geburtstort während des Beginns der Herbstzugsaison getestet wurden, sich in westliche Richtung orientieren. Diese Orientierung würde sie zunächst nach Europa führen, von wo aus sie wahrscheinlich in süd-/südwestliche Richtung orientieren würden, um ihr Winterquartier zu erreichen. Die Ergebnisse aus den Versuchen mit den an der Kurischen Nehrung gefangenen Trauerschnäpper deuten darauf hin, dass diese Vögel ursprünglich west-/südwestlich orientiert waren und ihre Orientierung dann signifikant in Richtung Südwest geändert haben. Interessanterweise zeigten die sibirischen Vögel, die zu einem frühen Zeitpunkt an die

Kurische Nehrung transportiert wurden, eine Südwest-Orientierung, die sich signifikant von der Richtung ihrer sibirischen Artgenossen unterschied, die am Geburtsort aufgezogen und getestet worden waren. Dies weist darauf hin, dass bestimmte lokale, externe Reize an der Kurischen Nehrung möglicherweise das Orientierungsprogramm der versetzten sibirischen Teichrohrsänger modifizieren (Paper III):

Wir vermuten, dass geomagnetische Reize nicht nur für die Kompassorientierung essentiell sind, sondern auch für die Bestimmung der Ost-West-Position. Aus diesem Grunde war das zweite Hauptthema meiner Doktorarbeit dem Verständnis der der Magnetrezeption zugrunde liegenden Mechanismen in Vögeln gewidmet. Im speziellen habe ich mit den folgenden drei Fragen beschäftigt:

i). Ist entweder das visuelle System (und seine angeschlossenen Hirnregionen – Cluster N) oder das Schnabelorgan für die Navigation vonnöten, wenn ein Zugvogel lediglich das Magnetfeld zur Orientierung zur Verfügung hat?

Um dies zu erreichen, haben wir die Orientierungsfähigkeiten von Rotkehlchen (*Erithacus rubecula*) am Erdmagnetfeld während der Zugzeit in vier Gruppen getestet: eine Gruppe von Vögeln mit bilateraler chemischer Läsion von Cluster N, eine Gruppe von Vögeln mit bilateraler Sektion des ophthalmischen Astes des Trigeminalnervs und jeweils zwei Gruppen, die der gleichen Operation unterzogen wurden, ohne jedoch die Funktionalität des einen oder anderen Sensors zu unterbinden (Paper IV). Diese Studie konnte zeigen, dass nur Vögeln mit bilateraler Läsion von Cluster N nicht in der Lage waren, sich am Erdmagnetfeld zu orientieren. Die drei anderen Gruppen hingegen, einschließlich der Gruppe von Vögeln mit durchtrenntem Trigeminalnerv, hatten die Fähigkeit, das Erdmagnetfeld zur Orientierung zu nutzen (Paper IV). Zusätzliche Versuche zeigten, dass die Unfähigkeit der Cluster N läsionierten Vögel, sich am Erdmagnetfeld zu orientieren, nicht auf allgemeinen visuellen Defiziten beruhte, da dieselben Vögel erfolgreich auf die Unterscheidung visueller Reize konditioniert werden konnten. Des Weiteren konnte ein generelles Fehlen der Zugmotiviation der Cluster N läsionierten Vögel ausgeschlossen werden, da diese Vögel dazu in der Lage waren, den Sonnenuntergang und den nächtlichen Sternenhimmel zur Orientierung zu nutzen. Demzufolge ist Cluster N die erste Hirnregion, bei der gezeigt werden konnte, dass sie höchstwahrscheinlich zur Verarbeitung von Magnetkompassinformation genutzt wird (Paper IV).

ii). Ist der Magnetkompass bei Vögeln stark lateralisiert und nur im rechten Auge der Vögel vorhanden, wie bereits zuvor von Wiltschko *et al.* (2002) proklamiert?

Die Ergebnisse aus einigen anderen aktuellen Veröffentlichungen (Mouritsen *et al.* 2004, 2005; Liedvogel *et al.* 2007a; Hein *et al.* 2010) geben Anlass zum Zweifel, dass der Magnetkompass bei Vögeln stark lateralisiert ist. Deshalb haben wir an Europäischen Rotkehlchen, derselben Vogelart, die bei der Studie von Wiltschko *et al.* (2002) verwendet wurde, untersucht, wie sich die Vögel während ihres Herbstzuges am Erdmagnetfeld orientieren können, wenn entweder ihr rechtes oder ihr linkes Auge abgedeckt war. Unsere Ergebnisse zeigen, dass die Vögel ihren Magnetkompass zur Orientierung unabhängig davon nutzen konnten, welches Auge bedeckt war (Paper V und VI). Daher legen unsere Ergebnisse die Schlussfolgerung nahe, dass Rotkehlchen ihren Magnetkompass mit beiden Augen nutzen können und dass der Magnetkompass-Sinn nicht stark lateralisiert ist.

iii). Ist der Versuchsansatz zur operanten Konditionierung von Brieftauben (*Columba livia domestica*, Mora *et al.* 2004) auf eine nachts ziehende Singvogelart übertragbar, um die Fähigkeit zur Diskrimination von Magnetreizen zu untersuchen?

In dieser Arbeit (Paper VII) haben Dr. Cordula Mora und ich versucht, das Paradigma zur operanten Konditionierung, das in der Studie von Mora *et al.* (2004) an Brieftauben entwickelt wurde, auf einen Zugsingvogel, das Europäische Rotkehlchen, anzuwenden. Trotz mehr als 2 Jahren Arbeit gelang es uns nicht, den Punkt zu erreichen, an dem das Verhalten der Rotkehlchen offensichtlich durch die angewendeten Magnetstimuli gesteuert wurde. Die generelle Anwendbarkeit unseres Versuchsansatzes konnte jedoch dadurch gezeigt werden, dass dieselben Vögel erfolgreich auf einen auditorischen Stimulus konditioniert werden konnten.

Zusammenfassend zeigt meine Doktorarbeit, dass:

(1) Europäische Teichrohrsänger, typische Langstrecken-Zugsingvögel, dazu in der Lage sind, eine 1000 km weite Versetzung nach Osten hin zu kompensieren. Dieses Ergebnis zeigt einerseits zusammen mit ähnlichen aktuellen Arbeiten, dass Zugvögel einige, mindestens aber zwei, natürliche Referenzgeber als Abbild geographischer Koordinaten nutzen. Andererseits zeigt die Untersuchung der „Double-Clock“ Hypothese, dass die Mechanismen der Teichrohrsänger zur Wahrnehmung longitudinaler Versetzung entlang eines Breitengrades unabhängig von einer inneren Uhr sind;

(2) einjährige Sibirische Trauerschnäpper ein angeborenes räumlich-zeitliches Programm haben, das sie auf ihrem Zug zunächst nach Europa führt, bevor sie in

ihre Westafrikanischen Winterquartiere ziehen. Sibirische Trauerschnäpper, die, nachdem sie als Nestlinge in den Ostseeraum transferiert und dort aufgezogen wurden, während ihres ersten Herbstzuges auf ihre Orientierung getestet wurden, zeigten eine südwestliche Orientierung. Diese Richtung unterscheidet sich signifikant von der ihrer Artgenossen, die in ihrer natürlichen Umgebung in West-Sibirien aufgewachsen sind und dort getestet wurden;

(3) ein intaktes Cluster N für den Magnetkompass-Sinn bei dem Rotkehlchen entscheidend ist, wobei der ophthalmische Ast des trigeminalen Nerven, der vermutlich die eisenmineralhaltige Strukturen im Oberschnabel innerviert, weder notwendig noch ausreichend für Magnetkompass-Orientierung ist. Dies zeigt, dass Cluster N in die Verarbeitung von richtungsgebender Magnetinformation bei Zugvögeln involviert ist, wobei die Funktion des Schnabelorgans nicht geklärt werden konnte;

(4) der Magnetkompass bei Vögeln nicht stark lateralisiert ist, da das Rotkehlchen in der Lage sind, ihren Magnetkompass unabhängig davon, welches Auge abgedeckt ist, zu nutzen;

(5) das Paradigma von Mora *et al.* (2004) zur operanten Konditionierung bei Brieftauben nicht auf einen Zugsingvogel, das Europäische Rotkehlchen, übertragbar ist, um die Unterscheidungsfähigkeit von Magnetreizen zu untersuchen.

Aims of my Ph.D. project

The main focus of my PhD research was to investigate mechanisms underlying navigational abilities in migratory songbirds. Additionally, I examined neurophysiological backgrounds of magnetoreception in migratory birds. In my PhD work, I addressed the following questions:

1. Can experienced avian migrants compensate for longitudinal displacement? (Paper I)

2. Can the time zone or jetlag effect explain navigational ability in migratory birds? (Paper II)

3. Do Siberian pied flycatchers, typical long distance bird migrants, have an inherited orientation programme that leads juvenile birds from their natal place in Western Siberia to the wintering quarters in West Africa by a detour around the Central Asia? (Paper III)

4. Is magnetic compass information processed in Cluster N? (Paper IV)

5. Is the magnetic compass of migratory birds located in both or only in the right eye? (Paper V and VI)

6. Can the operant conditioning approach that was successfully used in homing pigeons (Mora *et al.* 2004), be applicable in a migratory songbird? (Paper VII)

The results are presented as five published papers (Paper I-V), one submitted paper (Paper VII) and one manuscript (Paper VI).

Own contribution to each of the seven papers

Paper I

Title: A long-distance avian migrant compensates for longitudinal displacement during spring migration.

Authors: Chernetsov, N., Kishkinev, D. and Mouritsen, H. (2008).

Status: *published in Curr. Biol. 18, 188-190.*

This study was one of the main projects of my PhD work. Dr. Nikita Chernetsov and I were 50/50 engaged in the practical experimental design performance, data collection and data analysis. Dr. Nikita Chernetsov wrote the first draft of the paper, which the other co-authors commented on. H. Mouritsen supervised and designed this study.

Paper II

Title: A double clock or jetlag mechanism is unlikely to be involved in detection of east-west displacement in a long-distance avian migrant.

Authors: Kishkinev, D., Chernetsov, N. and Mouritsen, H. (2010).

Status: *published in The Auk 127, 773-780.*

One of the main studies of my PhD work where I together with N. Chernetsov was a co-principal investigator (co-PI). I proposed the double clock hypothesis. In 50/50 proportion with Dr. Nikita Chernetsov, I designed the experiment, collected and analyzed data. I wrote the first draft of the paper, which the other co-authors commented on. H. Mouritsen supervised the study and contributed to the design and structure of the paper.

Paper III

Title: Orientation programme of first-year pied flycatchers *Ficedula hypoleuca* from Siberia implies an innate detour around Central Asia.

Authors: Chernetsov, N., Kishkinev, D., Gashkov, S., Kosarev, S. and Bolshakov, C (2008).

Status: *published in Anim. Behav. 75(2): 539-545.*

In this study I was a co-PI taking 50% part in the design of the study, hand raising of the Siberian pied flycatchers at Alaevo (Kemerovo region), testing their orientation, and analysing data. N. Chernetsov was another co-PI, who did 50% of the study in Siberia, collecting data from the Siberian pied flycatchers at their natal site, and analysing orientation data. N. Chernetsov wrote the first draft of the paper, which I then provided a significant input to, and the other co-authors commented on. S. Gashkov assisted us to organize work in Siberia. V. Kosarev hand raised the displaced Siberian pied flycatchers and then tested their orientation during autumn migration at the Courish Spit (Kaliningrad region). C. Bolshakov was the supervisor of this study.

Paper IV

Title: Visual, but not trigeminal, mediation of magnetic compass information in a migratory bird.

Authors: Zapka, M., Heyers, D., Hein, C.M., Engels, S., Schneider, N.-L., Hans, J., Weiler, S., Dreyer, D., Kishkinev, D., Wild, M. and Mouritsen, H. (2009).

Status: *published in Nature 461, 1274-1277.*

In this study, I together with S. Weiler designed and performed an operant conditioning paradigm, which showed that the Cluster N lesioned European robins did not have generally impaired vision capabilities compared to the sham operated birds. M. Zapka, C.M. Hein, S. Engels and J. Hans conducted orientation tests. H. Mouritsen, N.-L. Schneider and M. Zapka designed the study and developed the setups used. D. Heyers and M. Wild performed the surgeries. D. Dreyer performed brain analysis of Cluster N lesion using Amira Software. H. Mouritsen wrote the first draft of the paper, which I, among the other co-authors, commented on.

Paper V

Title: Robins have a magnetic compass in both eyes.

Authors: Hein, C.M., Engels, S., Kishkinev, D. and Mouritsen, H.

Status: *published in Nature, 471, E11.*

In this study I conducted ca. 10 % of orientation tests and data analysis. The PIs of this study were C.M. Hein and S. Engels. H. Mouritsen supervised and wrote the first draft of the paper, which the rest of authors including myself commented on.

Paper VI

Title: Robins possess a magnetic compass in both eyes.

Authors: Hein, C.M., Engels, S., Kishkinev, D. and Mouritsen, H.

Status: *Manuscript.*

In this study, I conducted ca. 10 % of orientation tests and data analysis. The PIs of this study were C.M. Hein and S. Engels. H. Mouritsen supervised and wrote the first draft of the paper, which the rest of authors including myself commented on.

Paper VII

Title: An attempt to develop an operant conditioning paradigm to test for magnetic discrimination behaviour in a migratory songbird.

Authors: *Kishkinev D., Mora, C. and Mouritsen, H.*

Status: *submitted to Learning & Behavior.*

One of the main studies of my PhD work, where I was supervised by the initiator and the first designer of this study – Dr. Cordula Mora – and H. Mouritsen. The project lasted the most part of my PhD study (between November 2006 and February 2009). The setup and operant conditioning paradigm used in Experiment I (see Paper VII) were designed by Dr. C. Mora. I together with C. Mora re-designed setups and conditioning protocols in Experiment II, III and in most of intermediate pilot experiments (data are not shown in Paper VII), collected and analyzed most of the data and wrote the first draft of the paper, which the other authors commented on.

Introduction

The migration of birds – a regular travelling of numerous individuals across the globe – is not only a fascinating performance *per se* but also represents a phenomenon that raises many challenging and long lasting scientific questions. For instance, how can young migratory birds find their way to wintering grounds, even if they have never been there before? How can birds precisely find the place where they were born or bred last year? How do migratory birds perceive, process and integrate information from different reference cues, particularly from the Earth's magnetic field that people cannot perceive? These questions have inspired thousands of scientific works and discoveries, but there are still many questions unanswered.

In my PhD thesis, I will summarize the current state of bird navigation research and report my own contributions mainly related to the following topics:

1. *Bi-coordinate navigation in adult migratory songbirds and navigational mechanisms that enable birds to compensate for longitudinal geographical displacements;*
2. *Navigational mechanisms that enable naïve migratory songbirds to reach their wintering quarters;*
3. *Neurobiological mechanisms that underlie magnetoreceptive abilities of migratory songbirds.*

1. Orientation and navigation – terminology

Orientation is the ability of the subject to detect a specific compass direction and to stick to it while moving along the route. To find a direction, the subject can use one or several reference systems or compasses.

Navigation implies that the subject has the ability to determine its position on the globe and the direction towards a wished distant goal even when it is crossing unfamiliar landscape and not perceiving any information emanating from the goal (Baker 1981).

2. Methods to study and quantify orientation and navigation in captive avian migrants

It is known that captive migratory birds demonstrate a higher level of locomotor activity, which is especially noticeable in form of wing whirring and/or flapping in nocturnal migrants at nighttime, approximately during the time when their conspecifics in the wild migrate. This activity, called migratory restlessness or *Zugunruhe*, is driven by endogenous mechanisms and represents a behavioral part of migratory syndrome that also includes hyperphagia, fattening and orientation (see Berthold 1996 and Piersma *et al.* 2005 for reviews). A bird performing *Zugunruhe* in captivity is not randomly moving in all directions but shows a directional preference roughly corresponding to its migratory direction in the wild at a given time of the year (e.g., Kramer 1949, 1952; Emlen and Emlen 1966). Such an orientation component of *Zugunruhe* enabled the avian scientists to establish a behavioral paradigm based on round orientation cages (Kramer 1950a; Emlen and Emlen 1966). This method is still widely used to study orientation of migratory birds in captivity.

The first version of the orientation cage was proposed by Gustav Kramer in the end of the 1940s (e.g., Kramer 1949). Different modifications of Kramer cage became widespread in the 1950s and 1960s. At that time, avian researchers were trying to automate the procedure of oriented activity registration by using different kinds of automatic perches. But after all, Kramer cages were difficult to transport and keep running because of their complex and heavy constructions. That is why this cage was substituted by a funnel cage method invented by Stephen Emlen in the mid-1960s (Emlen and Emlen 1966). The advantages of Emlen cage are that this cage is much easier to make, transport and operate because it consists of a blotting paper funnel, an ink pad base and a screen top (Fig. 1). In a later modification of Emlen cage (e.g., Rabøl 1979, Mouritsen *et al.* 2009), which is now used as a gold standard in the vast majority of orientation studies, a funnel is made of aluminum or plastic (non-magnetic material not to disturb magnetic orientation) and covered with different types of special paper that can be easily scratched when a bird performing migratory restlessness during an orientation test jumps onto it. After the test, the orientation of an individual bird can be quantified by counting or assessing the number of scratches in different sectors of the paper (Fig. 1).

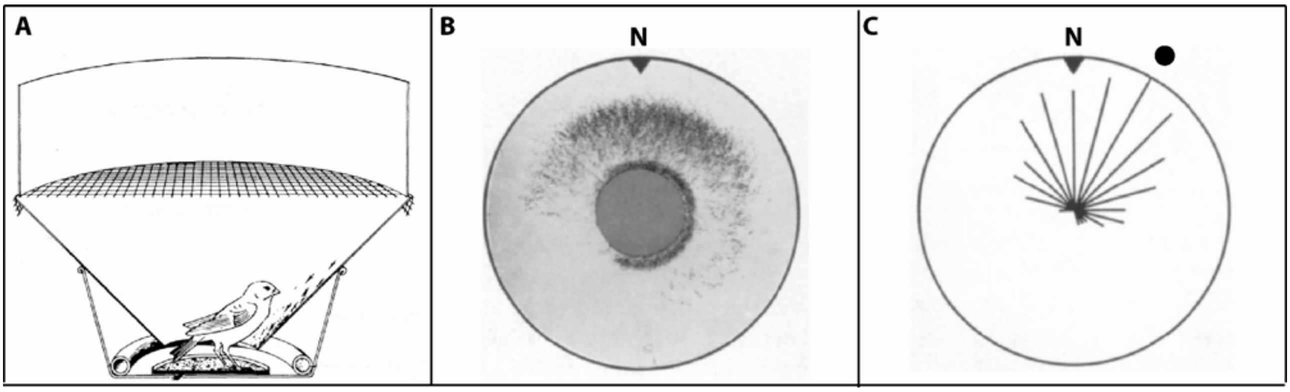

Figure 1. **Method of orientation cage**. **(A):** Diagrammatic side view of the original Emlen cage ("Emlen funnel"). An experimental bird is put in the centre of this funnel-shaped cage. An ink pad is situated on the bottom of the cage. The walls of the cage are covered with white blotting paper. A bird jumping on the walls leaves ink-marks. The currently used modification of the Emlen funnel (Rabøl 1979; Mouritsen *et al.* 2009) uses easy-to-scratch covering (e.g., typewriter correction paper or similar); **(B):** Sample footprint record; **(C):** Result of bird footmarks' quantification. *Length of each line* represents number of footmarks in a given sector. *A black dot* shows the mean direction of a given sample. *N* means north (geographical or magnetic) (*after Emlen and Emlem 1966 with modifications*).

Despite its simplicity, the Emlen funnel method has several drawbacks:

- *Noise in orientation data*. Not every pattern of scratches is relevant to orientation behavior. Some patterns of scratches represent escape behavior or result in directions which are irrelevant to migratory orientation of a bird. To increase signal to noise ratio in orientation data obtained by the Emlen funnel method, one has to take a large enough group of birds in migratory state and conduct numerous tests with each individual bird;

- *No temporal resolution.* Each pattern of scratches represents all the bird's movements during an orientation test that lasts, as a rule, from 40 to 60 min. Thus, one cannot say which scratches were made at which time. So far, numerous attempts have been undertaken to construct online automatic registration (e.g., Mouritsen and Larsen 2001; H. Mouritsen - pers. comm.; A. Mukhin – pers. comm.; M. Vorotkov and N. Chernetsov – pers. comm.). Unfortunately, such modifications have usually turned out to be not reliable enough for outdoor experiments and, therefore, are still not a widespread technique;

- *Applicable mostly to nocturnal migrants*. The Emlen funnel method, or any other round arena, is well-suited to test orientation only in nocturnal, not diurnal, migrants because they perform migratory restlessness during the night when this kind of locomotor activity can be easily separated from other kinds of locomotor activity like feeding, displaying or exploring activity.

3. Which reference systems do migratory birds use?

To date, it is well established that migratory birds are able to use both celestial (the Sun: e.g., Kramer 1949, 1950a, 1950b; and stars: e.g., Sauer 1956, 1957a, 1957b; Emlen 1967a, 1967b, 1975) and magnetic cues (e.g., Merkel and Wiltschko 1965; Wiltschko and Wiltschko 1972) as references for orientation. The physiological and behavioral mechanisms that provide birds with constant directions and a non-changing directional system are defined as biological compasses (Berthold 1996). Here I will first introduce the celestial compasses (the sun and the star compass), and then pay more attention to the magnetic compass because a significant part of my dissertation was focused on this compass.

3.1 Sun compass

The first evidence showing that migratory birds can use the Sun for orientation appeared in the late 1940s in works by Gustav Kramer and his colleagues (e.g., Kramer 1949). They used Kramer cages and mirrors to show that daytime songbird migrants - European starlings (*Sturnus vulgaris*) - are able to use the Sun's position to orient, and that European starlings' sun orientation can be manipulated by changing a visible position of the Sun (Kramer 1950a, 1950b, 1952). It was also shown that European starlings can use the position of the Sun in a conditioning experiment to find a food location in a round arena (Kramer and Saint Paul 1950).

To date, the sun compass has been demonstrated in more than 10 bird species including homing pigeons (Schmidt-König 1958a, 1958b), mallards (*Anas platyrhynchos,* Matthews 1968), European starlings (Kramer 1949, 1950a, 1950b), chaffinches (*Fringilla coelebs*, Shumakov 1965), and meadow pipits (*Anthus pratensis*, Schmidt-Koenig 1990). Most of them are migratory birds migrating during the daytime and/or at twilight. Interestingly, the European robin - a typical nocturnal migrant, can also use the sun compass even without a direct view of the sun disk after sunset (Helbig 1991a).

Clock shift experiments with European starlings (Hoffmann 1965) and homing pigeons (Schmidt-König 1958a, 1958b, 1960, 1961) demonstrated that the sun compass is time dependent meaning that birds compensate for the sun disk's movement by approximately 15° per hour. Whether the birds compensate for the apparent movement of the sun by relying only on the sun azimuth, as it was suggested in some works (e.g., Schmidt-König 1958a, 1958b, 1961; Neuss and Wallraff 1988), or by additionally taking into account the height of the sun disk above the horizon, as it was suggested in a few others (e.g., Wiltschko *et al.* 2000a), is still a matter of debate.

The sun compass is not inherited. It was demonstrated in the works by Wolfgang and Roswitha Wiltschko in young homing pigeons that the birds have to learn how to use the sun for orientation by observation and memorization of the apparent sun movements across the sky (Wiltschko and Wiltschko 1981, 1998). Interestingly, a sun compass established at one latitude seems to be not applicable at another because it was shown that homing pigeons trained at temperate northern latitudes are able to compensate for the sun's apparent movement north of the Arctic Circle, but not in equatorial and trans-equatorial latitudes (Schmidt-Koenig 1963).

Since the 1980s, it has been clearly shown that a setting sun plays an important role for choosing direction at least in some nocturnal migrants because they calibrate their compasses daily around the time of sunset and before the onset of migratory flight using both the view of the sun disk and the pattern of polarization light (e.g., Moore 1980, 1982; Able 1982; Helbig and Wiltschko 1989; Helbig 1991a; Cochran *et al.* 2004; Muheim *et al.* 2006, 2007, 2009; but see Sandberg *et al.* 2000; Gaggini *et al.* 2010).

3.2 Star compass

In the 1950s, Franz and Eleonore Sauer conducted a series of works with caged warblers (songbirds from the *Sylviidae* family) in a planetarium and found that the birds can use the stars for orientation. However, the Sauers' data were mostly based on the experiments with either a single or very few individuals and, therefore, were somewhat anecdotal (Sauer 1956, 1957a, 1957b; Sauer and Sauer 1955, 1959, 1960).

Later, in his classical planetarium experiments, Stephen Emlen unequivocally showed that juvenile migratory indigo buntings (*Passerina cyanea*) – common North American night-migrating songbirds – first have to learn the position of the rotational center of the starry sky by observing the rotating stars and then can use a remembered rotational center as a directional reference (Emlen 1967a, 1967b, 1975). In a planetarium, one can even make juvenile birds learn to use any point of the starry sky (or even of an artificial pattern composed from light dots) as a reference by simply rotating the starry sky (or its dot substitute) around an arbitrarily chosen rotational point (Emlen 1972, 1975; Wiltschko *et al.*

1987). If hand-raised birds had not been exposed to a rotating stellar sky (or to an artificial pattern of star-like dots) before their first autumn migration, they were unable to use the stars for orientation later during their migration periods (Emlen 1969, 1975).

To date, the use of the stars for orientation has been demonstrated in many other nocturnal songbird migrants, e.g., blackcaps (*Sylvia atricapilla*, Viehmann 1982); garden warblers (*Sylvia borin*, Wiltschko *et al.* 1987) and European redstarts (*Phoenicurus phoenicurus*, Mouritsen 1998). It was also shown in a few studies that the star-orientation does not involve the internal time sense meaning that the star compass is based on a time independent mechanism in contrast to the sun compass (Emlen 1967a, 1967b, 1972, 1975; Wiltschko *et al.* 1987; Mouritsen and Larsen 2001, but see Sauer and Sauer 1960; Rabøl 1998).

3.3 Magnetoreception and the magnetic compass of birds

As early as in the mid-1800s, Alexander von Middendorff proposed that migratory birds may use the geomagnetic field for orientation (von Middendorff 1859). But probably due to the lack of magnetosensitivity in humans, it took more than hundred years for researchers to demonstrate that birds possess magnetoreceptive abilities. Before I describe how magnetic field perception in birds was demonstrated and how it functions, I will first summarize the properties of the geomagnetic field.

3.3.1 The magnetic field of the Earth

The geomagnetic field is a magnetic field which can be closely approximated by the field of a magnetic dipole situated in the center of the Earth. The origin of the Earth's magnetic field can be described by the dynamo theory proposed by Joseph Larmor (1919). This theory states that the electroconductive fluid consisting of liquid iron located in the outer core of the Earth functions as a huge generator of the magnetic field (the so-called geodynamo), which can continuously regenerate the geomagnetic field. The North Geomagnetic Pole differs from the Geographic North Pole, and is nowadays situated in the northern Canada. The South Geomagnetic Pole differs from the Geographic South Pole and now lies in Adélie Land (Antarctica). The exact positions of the geomagnetic poles are permanently drifting with different speeds so that annual changes may compose up to 40 km (Skiles 1985). Occasionally, the Earth's magnetic field has completely flipped so that the north and the south poles swap places. Such reversals, detected by the magnetization of igneous rocks from paleovolcanic events, seem to be unpredictable and come at irregular intervals (on average every 300,000 years). The last one, called the Brunhes–Matuyama reversal, happened ca. 780,000 years ago and was lasting from 1,200 to 10,000 years depending on geomagnetic latitude (Bradford 2004).

The main parameters of the Earth's magnetic field are inclination, declination and total intensity. The inclination of the geomagnetic field is the angle between the magnetic vector and the Earth's surface. It varies from ±90º at the magnetic poles to 0º along the magnetic equator, which does not coincide with the geographic equator. The declination is the angle between the direction towards the magnetic north and true (geographical) north. The strength of the geomagnetic field is measured by total intensity, which nowadays varies from ca. 30,000 nT along the magnetic equator to ca. 60,000 nT at the magnetic poles (Fig. 2).

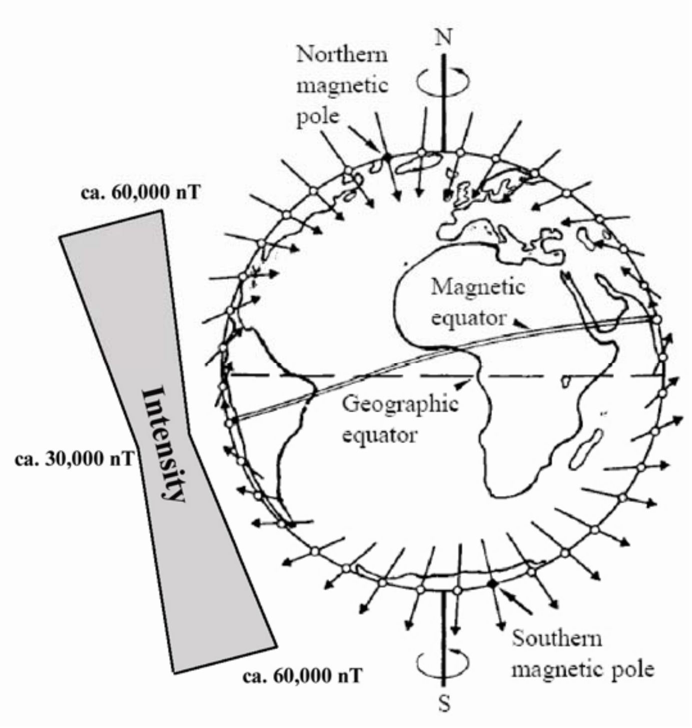

Figure 2. **The Earth's magnetic field.** *The arrows crossing the Earth's surface* represent the direction of the geomagnetic field at a given surface point. The length of these arrows is drawn relative to the intensity of the geomagnetic field. The angle between an arrow and the Earth's surface represents inclination. Intensity is at maximum at the magnetic poles (~ 60,000 nT), and it is gradually decreasing towards the magnetic equator (~ 30,000 nT) (*after Wiltschko and Wiltschko 1996 with modifications*).

The Earth's magnetic field is not constant: currents in the ionosphere caused by solar winds and disturbances from the Earth's interior permanently produce alterations of the geomagnetic field so that the mean daily changes during a quiet day on the Earth's surface comprise about one degree in inclination and declination, and about 25-50 nT in total intensity (Stepišnik J. 2006). The so-called magnetic storms can cause much bigger daily disturbances of the magnetosphere reaching 500 nT.

3.3.2 The magnetic compass in birds

The first data showing that caged migratory birds – European robins – are able to choose and maintain directions similar to their natural migratory directions even without access to celestial cues were obtained by Friedrich Merkel, Hans Fromme and Wolfgang Wiltschko in the late 1950s and early 1960s (Merkel and Fromme 1958, Merkel *et al.* 1964). Later, they found that the preferred direction of caged European robins and garden warblers can be deflected by shifting the horizontal component of the magnetic field with the help of the Helmholtz coils (e.g., Merkel and Wiltschko 1965, 1966; Wiltschko 1968, 1972, 1974; Wiltschko *et al.* 1971; Wiltschko and Wiltschko 1972). Despite initial skepticism about non-celestial orientation in birds due to lack of independent replications (e.g., Perdeck 1963; Emlen 1967c; Shumakov 1967; Matthews 1968), the ability of migratory birds to use the geomagnetic field for orientation became a well established fact by the mid-1970s (Shumakov and Vinogradova 1970; Wallraff 1972; Wiltschko and Wiltschko 1972).

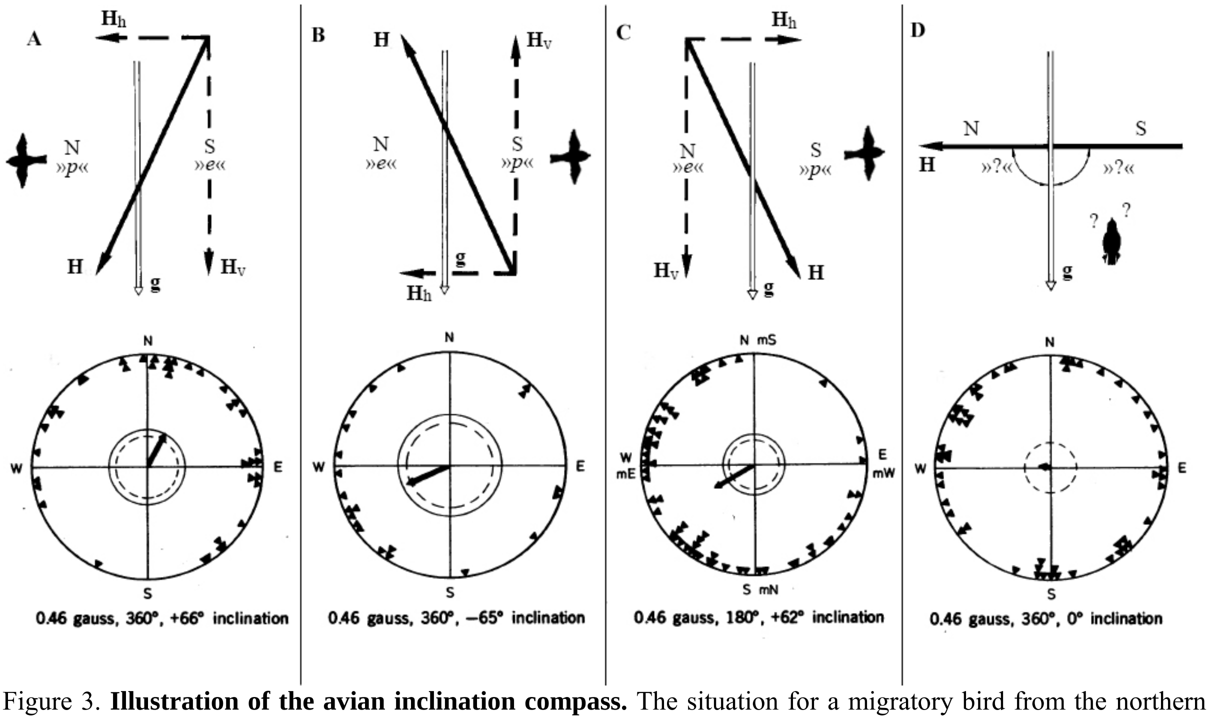

Figure 3. **Illustration of the avian inclination compass.** The situation for a migratory bird from the northern hemisphere during spring migration is shown. The *upper row* - vertical cross sections through the magnetic field. *H* - vector of an experimental magnetic field (H_h/H_v – horizontal and vertical components); *g* – gravity vector; *N*, *S* - geographical north and south; *»p«* - heading poleward, i.e., towards a magnetic pole; *»e«* - equatorward, i.e., towards the magnetic equator. The *lower row* – the results of spring orientation tests with European robins. *N, S, W, E* – geographical north, south, west and east, correspondingly; *mN, mS, mW, mE* – magnetic north, south, west and east, correspondingly; *triangles* at the periphery of circles – mean directions after one test night from individual birds; *arrow* – mean group direction; *inner dashed and solid circles* – 5% and 1% significance borders of the Rayleigh test, correspondingly. **(A):** the natural magnetic situation in Frankfurt-am-Main; **(B):** the vertical component of the magnetic field is inverted so that inclination is changed from +66° to -65°; **(C):** the horizontal component of the magnetic field is inverted, so that the magnetic north is now in the geographical south; **(D):** inclination is zero. In spring and in the northern hemisphere, the “bird” is always heading polewards, unless inclination is zero (*after Wiltschko and Wiltschko 1972, 1996 with modifications*)

To date, it is well established that the avian magnetic compass functions differently from the humans' magnetic compass. The bird magnetic compass is an ***inclination compass***. It means that the birds do not distinguish between the north and south magnetic poles but rather between a poleward and an equatorward direction (Wiltschko and Wiltschko 1972, Fig.3).

Furthermore, the avian magnetic compass does not work at all total intensities but is rather fine-tuned to the ***functional window*** lying roughly between 30,000 and 60,000 nT where it can normally be used in the wild (Wiltschko 1978). If a value of total intensity of the magnetic field, to which the bird is exposed, exceeds or lies below that functional window, caged birds without celestial cues become initially disoriented but may regain their orientation ability after being kept under such magnetic conditions for a few days (Wiltschko 1978).

It has been shown in numerous studies in two migratory songbird species – the Australian silvereyes (*Zosterops lateralis*) and the European robin – that the avian magnetic compass is ***functionally dependent on both the wavelength and light intensity*** to which the birds were exposed to. Birds can use the magnetic field for orientation under light from the short wavelength part of their visual spectrum (blue and green) as well as under ultraviolet light, but not under light from the long wavelength part of their visual spectrum (yellow and red) (Wiltschko *et al.* 1993, 2001, 2007; Wiltschko & Wiltschko 1995a, 1999, 2001; Munro *et al.* 1997a, Rappl *et al.* 2000; Muheim *et al.* 2002). However, it has also been suggested that ***increasing of the intensity of monochromatic light leads to a variety of unexpected orientation responses***. For instance, Australian silvereyes showed the so-called "fixed direction" orientation: an orientation with a fixed azimuth irrespective to the migratory season under a very bright green light (Wiltschko *et al.* 2000b). Later, it was suggested that the "fixed direction" orientation in European robins exposed to a bright turquoise light (502 nm, $54 \cdot 10^{15}$ quanta $s^{-1}m^{-2}$) is not related to the inclination compass but rather to a polar compass because the birds did not flip their orientation in the magnetic field with an inverted vertical component and shifted their orientation if the horizontal component was deflected (Wiltschko *et al.* 2005). Results obtained from European robins tested under different monochromatic conditions have suggested that birds can use normal compass orientation under low intensity lights (blue, turquoise and green light of $0.8 \cdot 10^{15}$ quanta $s^{-1}m^{-2}$ and ultraviolet light of $0.8 \cdot 10^{15}$ quanta $s^{-1}m^{-2}$) but show a variety of different orientation responses including axial orientation and disorientation under light of higher intensities (Muheim *et al.* 2002; Wiltschko *et al.* 2007) or "fixed direction" responses with the polarity compass under a dim red light (645 nm, 1mWm^{-2}, Wiltschko *et al.* 2008) and in full darkness (Stapput *et al.*

2008). Interestingly, magnetic orientation of European robins tested under a dim dichromatic (turquoise-and-yellow) light as well as in full darkness, where the fixed direction responses had been reported before (Wiltschko *et al.* 2007; Stapput *et al.* 2008), was not affected by the magnetic fields of strong total intensities (92,000 nT and 138,000 nT) as it was found under a dim white or monochromatic green light (Wiltschko 1978). As for the sensory basis of the "fixed direction" responses, there are data indicating that a putative magnetosensitive organ in the upper beak (the so-called upper beak organ, see more information in Section 4.2 below) plays a crucial role in such orientation (Wiltschko *et al.* 2005, 2007).

In general, the aforementioned data may suggest that either the "fixed direction" responses are based on a fundamentally different sensory mechanism than the normal magnetic orientation that birds show under a dim full-spectrum light, or the avian magnetic compass's peculiarities are drastically changed under unnatural light conditions (Wiltschko and Wiltschko 2009). One should mention here that all Wiltschkos' control orientation experiments in Emlen funnels since, at least, 2001 have been performed under unnatural 565 nm green light (e.g., Wiltschko *et al.* 2001, 2002, 2010; Ritz *et al.* 2004, Thalau *et al.* 2005). Therefore these data should be independently tested under more natural light conditions.

3.3.3 Lateralization of the bird magnetic compass: own contribution

It has been shown that the crossing of the optic nerve in adult birds is very close to 100% (McLoon and Lund 1982; Weidner *et al.* 1985). Additionally, birds are lacking a structure analogous to the corpus callosum in mammals, which allows immediate interhemispheric transfer of information (e.g., Nottelmann *et al.* 2002). Thus in birds, visual input from one eye is processed in only one (the contralateral) brain hemisphere. Therefore, the method of monocular occlusion is widely used to study hemispheric asymmetries (see Prior 2006 for a review).

In 2002, it was reported by Wolfgang Wiltschko and his co-workers (2002) that European robins with one eye occluded are able to use their magnetic compass when their right eye open only but are completely disoriented when their left eye open only. The authors of this study suggested that the ***avian magnetic compass is strongly lateralized and located only in the right eye***. Later, similar results were reported in Australian silvereyes (Wiltschko *et al.* 2003) and domestic chickens (*Gallus gallus domesticus*; Rogers *et al.* 2008). Recently, it was suggested that eye-covered European robins do not magnetically orient if they wear goggles over both their eyes, and their left eye has a goggle equipped with clear foil and the right eye wears an opaque goggle of the same translucence (70% of the ambient illumination level) so that a blurred vision through the right eye occurs (Stapput *et al.* 2010). In the opposite situation, i.e., when the right eye has clear vision and the left eye – a blurred vision, the birds in this study (Stapput *et al.* 2010) did perfectly use the magnetic field for orientation. The authors of this work (Stapput *et al.* 2010) suggested that the bird magnetic compass may require not only light propagation onto the right retina but also a non-degraded image formation so that a sharp perception of contours from the right eye is assured. This, in turn, may indicate that processing of directional magnetic information could be closely coupled with processing of ordinary visional information.

Despite the fact that the statement that all birds have a magnetic compass only in the right eye has become widespread in the animal navigation literature since the first work of Wolfgang Wiltschko and colleagues (Wiltschko *et al.* 2002), no independent replications of this strong lateralization of the bird's magnetic compass had been published until recently (Hein *et al.* 2010). Last year, the paper of Hein *et al.* (2010) for the first time showed that, in a truly double-blind experiment (*sensu* Kirschvink 1992 and Kirschvink *et al.* 2010), eye covered migratory garden warblers do not show strong lateralization of their magnetic compass and can perform magnetic orientation using either of their eyes (Hein *et al.* 2010). This finding drastically contradicts to the Wiltschko *et al.* data (Wiltschko *et al.* 2002, 2003; Rogers *et al.* 2008; Stapput *et al.* 2010) but could reflect species differences. However, there are other data fitting poorly with the idea of a strong lateralization of the avian magnetic compass. Below I summarize them:

i) Even though general lateralization is a common feature of the avian brain and has been reported in many studies (e.g., Prior *et al.* 2002, 2004; see also Güntürkün 2003 and Prior 2006 for reviews), usually only a modest preference to one or another side exists. An "all or nothing" or any strong lateralized behavioral response, like the magnetic compass lateralization that has been reported in European robins, Australian silvereyes, and domestic chickens by the Wiltschkos and their co-workers (Wiltschko *et al.* 2002, 2003; Rogers *et al.* 2008; Stapput *et al.* 2010), is a very rare feature (but see Gagliardo *et al.* 2005a);

ii) Homing pigeons were capable of learning magnetic compass directions in the operant task both after left and right eye occlusion, though some slight difference between these two treatments was detected (Wilzeck *et al.* 2010). The capability to successfully use both or only the left eye in the magnetic conditioning was also recently suggested in the Pekin duck (*Anas platyrhynchos domestica*; Freire and Birch 2010);

iii) The avian cryptochromes - the most promising magnetosensory candidate molecules (see Part 4.1 for details) - are expressed in both eyes, with no obvious difference in expression or in neuronal activity and/or connectivity during a magnetic compass orientation task (Mouritsen *et al.* 2004; Heyers *et al.* 2007);

iv) Neuronal activation in Cluster N – a specialized light-processing forebrain region, which has been shown to be necessary for birds to be able to perform magnetic compass orientation (Paper IV; see Section 4.1 below) – shows a slight preference towards the right brain hemisphere and the left eye (Liedvogel *et al.* 2007a), i.e., the opposite side to that suggested by the Wiltschkos and co-workers (Wiltschko *et al.* 2002).

Taken together, there is contradictory evidence related to a strong lateralization of the avian magnetic compass. Therefore, the need for a double-blind and independent test of the magnetic compass lateralization of European robins – the model bird species for magnetic orientation research – was overdue.

Therefore, during my PhD work, I took part in the behavioral tests of the study that was aimed to test the hypothesis of a strong lateralization of the magnetic compass in European robins (see Papers V and VI below).

We tested 27 European robins equipped with hoods allowing them to use the left, the right or both eyes for magnetic orientation in two magnetic conditions: i) a magnetic field with magnetic north turned by 120º counter-clockwise (changed magnetic field – CMF); ii) a magnetic field that very closely resembled the ambient magnetic field (normal magnetic field - NMF). The magnetic fields were produced with double-wrapped, three-dimensional Merritt four-coil systems (Kirschvink 1992) with average coil diameter of about two meters. To produce the CMF condition, current ran through the two subsets of windings of the four-coil system in the same direction. Under the NMF condition, the same current that we used to produce the CMF condition ran through the two subsets of windings but in opposite directions so that no significant changes (<10 nT) to the Earth's magnetic field were produced by the coils. We used the same double-blind behavioural protocol as in Hein *et al.* (2010) meaning that, first, in both our treatments current ran through the magnetic coils, and, second, the experimenters did not know which bird was tested in which condition before all orientation data were analyzed.

We found that our European robins are able to use their magnetic compass for orientation no matter which eye was covered, and can orient in the expected directions relative to magnetic north (Fig. 4). These results contradict to the data of the Wiltschkos and their co-workers (Wiltschko *et al.* 2002, 2003; Rogers *et al.* 2008; Stapput *et al.* 2010). Thereby, the species difference explanation seems not to be plausible. We suggest that the disagreement between our data and those of Wiltschkos and their co-workers may arise either from different behavioural paradigms used in orientation tests or from season (or migratory experience) dependent differences.

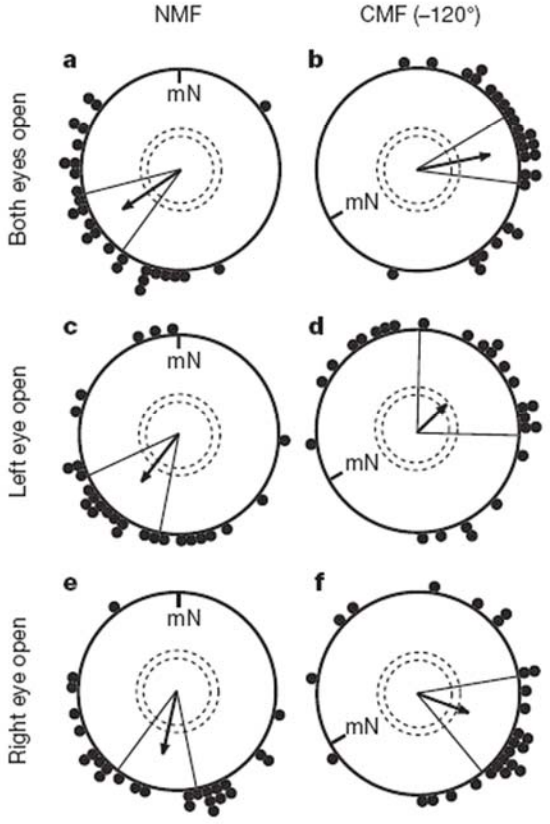

Figure 4. **European robins wearing eye covers can use their magnetic compass if light and/or visual input reaches any one eye**. Each dot at the circle periphery represents the mean orientation of one individual bird tested several times with the given type of hood. *mN*, magnetic North. The *arrows* indicate the group mean vectors. The *inner and outer dashed circles* indicate the radius of the group mean vector needed for significance according to the Rayleigh Test ($P < 0.05$ and $P < 0.01$, respectively). The lines flanking the group mean vectors indicate the 95% confidence intervals for the group mean direction (*after Paper VI*).

In our present study, the eye-cap treatments were equally often done with the left or right eye involved. In the study of Wiltschko *et al.* (2002), however, there were twice as many tests with the right eye open as with the left eye open. This might cause a bias towards the right eye. No information is available about numbers of tests with either eye covered in the study of Stapput *et al.* (2010), where European robins were also used. The discrepancies might also appear as a result of the non-blinded evaluation of the “raw” behavioural data in the study of Wiltschko *et al.* (2002) or be an artefact of the unnatural green light used by Wiltschko *et al.* (produced by green LEDs with peak frequency 565 nm, half bandwidth is 12 nm; Wiltschko *et al.* 2002) instead of the white light from incandescent bulbs more closely resembling the natural sunlight spectrum that we used (Papers V and VI).

As mentioned above, it has been reported in numerous recent studies that migratory birds tested under abnormal light regimes show a variety of strange “fixed direction” orientation responses (see Section 3.3.2 above and Wiltschko and Wiltschko 2009 for reviews). Moreover, in the study of Muheim *et al.* (2002), it was suggested that European robins can not use their magnetic compass for orientation and seem to be disoriented when tested under a dim green light (1 mW/m^2, peak frequency at 567.5 nm, half bandwidth is 10 nm). This light is extremely close to the light used in Wiltschko *et al.* (2002). At the same time, in the study of Muheim *et al.* (2002), the birds were able to use the magnetic field for compass orientation under a dim light with a slightly shorter wavelength (peak frequency 560.5 nm; half bandwidth is 9 nm). Thus, one may suggest that inability of European robins to use magnetic compass with only their left eye open in the work of Wiltschko and his co-workers (Wiltschko *et al.* 2002) may be one more example of strange and so far poorly understood orientation responses caused by exposition of experimental birds to unnatural light conditions.

The only potential explanation for the disagreement between our current results and those of Wiltschko *et al.* (2002) that does not imply technical differences may relate to the fact that we tested our birds during autumn migration, whereas the birds in the study of Wiltschko *et al.* (2002) were tested during spring migration. It is well known that during their first autumn migration, young birds orient only with the help of an innate clock-and-compass or vector navigation (e.g., Perdeck 1958; Mouritsen and Larsen 1998; Thorup *et al.* 2007; see Mouritsen 2003 and Section 6 below for reviews). It means that, during their first migration, young migratory birds travel into an innate population specific compass direction for a genetically coded time period so that, at the end of the migration, they most probably reach their wintering grounds even without having visited them previously. Few displacement studies (e.g., Mewaldt 1964; Thorup *et al.* 2007; Paper I) and numerous site fidelity data (see Sokolov 1997 and Newton 2008 for reviews) strongly suggest that, from their first spring migration onward, migratory birds can use previously gathered information to precisely reach their destinations (breeding site and wintering quarters) and, therefore, must possess navigation abilities, i.e., map information (Mouritsen 2003). Moreover, there is some evidence that map-like information is stored preferentially in the left brain hemisphere (e.g., Gilbert *et al.* 1991; Rogers *et al.* 2004; Gagliardo *et al.* 2005a, 2005b; see Vallortigara 2000 for a review). It is therefore possible that compass information derived from the right eye being transmitted only to the left brain hemisphere can be easier combined with map information lying in the same brain hemisphere than information obtained with the left eye. The latter would require interhemispheric transfer, which is time-consuming and usually goes hand in hand with loss of information (Nottelmann *et al.* 2002). Thus, one may suggest that experienced (performed at least one migration journey) birds, tested in spring as in the study of Wiltschko *et al.* (2002), are disoriented with only the left eye open because, in this case, they could fail to combine map and compass information. If so, we should expect that there is a difference in orientation of birds depending on age (and experience) both in spring and autumn. However, we cannot test this hypothesis on our autumn data because amongst our birds there were only 6 adult individuals, and orientation data from them show no significant mean direction due to low numbers of data points. Nevertheless, a more detailed comparison on lateralization patterns of spring and autumn migrants as well as of juvenile and adult migrants may provide for several very interesting studies in the future.

Whatever the explanation for the differences in the experimental outcomes is, it is certain that more independent, double-blind studies with monocular occlusions are needed before a reliable conclusion about the amount of lateralization of the avian magnetic compass can be drawn. It is well possible that some smaller degree of lateralization of magnetic information processing exists in birds (e.g., Ulrich *et al.* 1999; Prior 2006; Liedvogel *et al.* 2007a; Wilzeck *et al.* 2010). However, the data presented here and published earlier (Hein *et al.* 2010) show that the magnetic compass of night-migratory songbirds is not strongly lateralized and certainly not located in only one eye.

4. The two magnetosensory systems in birds

To date, the vast majority of the available experimental data supports the notion that, in birds, *two different magnetosensory mechanisms co-exist*: i) a chemical sensor in the bird's eye based on a radical pair mechanism, and ii) an iron-mineral-containing sensor in the upper beak (the so-called beak organ). Below, I summarize the current state of magnetoreception research and show my contribution to this topic done during my PhD study.

4.1 The chemical magnetoreceptor: radical pair mechanism in the eye

The idea that a chemical reaction may be used to detect the direction of the Earth's magnetic field was first suggested by Klaus Schulten and his co-authors in the late 1970s (Schulten *et al.* 1978; Schulten 1982; Schulten and Windemuth 1986). In the 1980s, it was well established that strong magnetic fields (10 - 100 G) can change product yields of biradical reactions (e.g., Steiner and Ulrich 1989). Later, it was shown *in vitro* that even weak magnetic fields that are comparable with the geomagnetic field (ca. 0.5 G) can result in changes of product yields (Batchelor *et al.* 1993). But only around ten years ago, a comprehensive model of chemical magnetoreception in animals – the *Radical Pair Model* – was proposed by Ritz, Adem and Schulten (Ritz *et al.* 2000).

In this model, Ritz *et al.* (2000) hypothesized that birds may possess light-sensing magnetoreceptive molecules that change their oxidation state upon light absorption. According to the model, light photons with energy large enough to induce an electron transfer in a magnetoreceptive molecule should bring it to an intermediate state, in which a magnetosensitive pigment (donor - D) and its electron transfer partner (acceptor - A) form a radical pair ($D^+ + A^-$), i.e., a pair of molecules with an unpaired electron each. This intermediate radical pair decays into reaction products on the time scales from microseconds to milliseconds. The two electrons on the donor and acceptor radicals possess the electron spin, which can be thought of as a small magnetic moment. Chemical reactivity of the radical pair is determined by the relative alignment of the two electron spins at any given time. Electron spins can be in two different relative orientations: singlet or triplet spin states. Depending on the spin state, different reaction products will be formed, and at different rates (Fig. 5A). The radical pair should be sensitive to external magnetic fields because they affect the electron spins in both radicals. Even weak magnetic fields such as the geomagnetic field can alter the dynamics of spin state conversion, and, eventually, the relative concentration of product states. If, for example, the singlet state products function as a signal for further downstream receptors, then increasing or decreasing the concentration of singlet products will change the signal.

If the proposed radical pair mechanism exists, where can we find it in the bird's body? The model of Ritz *et al.* (2000) suggests that, for detecting directions of magnetic fields, the alignment of the detecting molecule plays an essential role. Therefore, motion of the radical pair forming molecules has to be, at least to some extent, restricted, i.e., these molecules must somehow be fixed in the cell, perhaps by attachment to some cytoskeletal proteins and/or cellular membranes. There are, however, some recent modelling studies claiming that a radical pair does not have to be rigidly fixed in the cell because even a moderate extent of a radical pair's immobilization may be sufficient for a radical-pair-based magnetic compass to function (e.g., Hill and Ritz 2010; Lau et al. 2010; Solov'yov et al. 2010). Nevertheless, Ritz *et al.* (2000) proposed that the avian retinal cells seem to be the most suitable locations for the radical pair mechanism because sensory transduction in the retina involves cellular membranes that often assume an ordered structure with large-scale orientational preferences. For example, the rod and cone receptor cells hold the visual pigments in membranes that are oriented tangentially to the retina's surface. The putative magnetoreceptive molecules, if they are situated in the retina cells, may be oriented in a similar way.

As candidate molecules, Ritz *et al.* (2000) proposed the cryptochromes (CRYs) – a family of blue-light, flavoprotein photoreceptors with a molecular weight between 50 and 70 kDa known from plants and animals including birds (Ahmad and Cashmore 1993; Sancar 2003; Möller *et al.* 2004; Mouritsen *et al.* 2004; Liedvogel *et al.* 2007b; Lin and Todo 2005; Partch and Sancar 2005; Weber 2005). The cryptochromes were suggested as potential magnetic field sensors because they represent the only so far known class of vertebrates' molecules that form radical pair intermediates upon light excitation. CRYs are able to absorb photons because they contain light photoreceptive cofactors: a flavin (in the form flavin adenine dinucleotide) and a pterin (in the form of 5,10-methenyl-6,7,8-tri-hydrofolic acid). It is well known that CRYs play a key role in the generation and maintenance of the circadian rhythm in plants and animals (Klarsfeld *et al.* 2004).

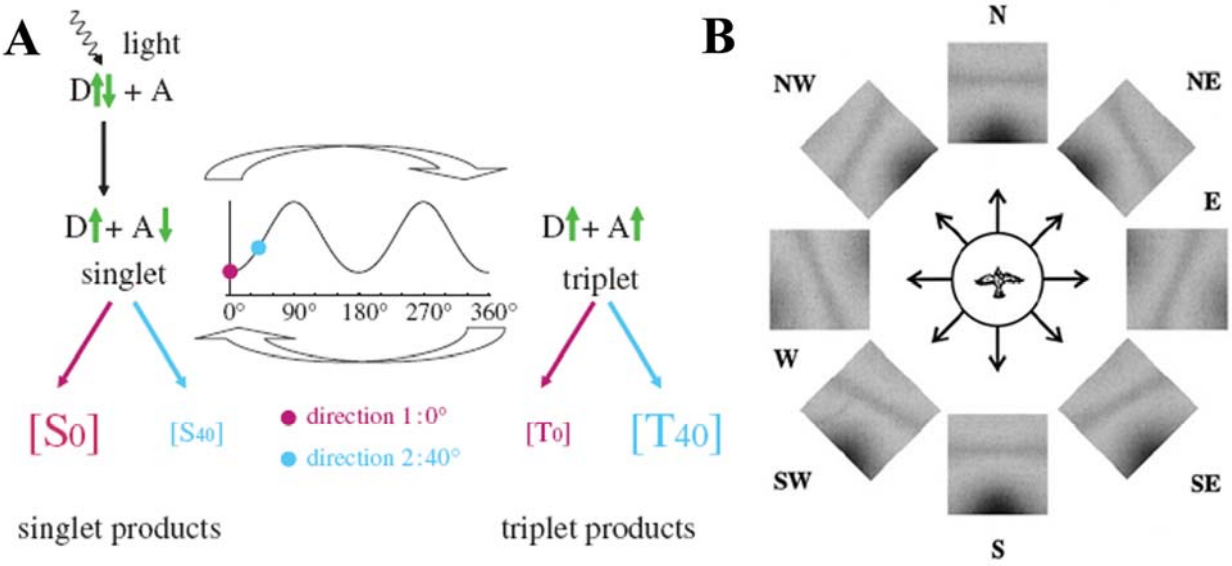

Figure 5. **The radical pair model**. **(A):** Illustration of a radical pair mechanism with a light-sensing molecule. *Upper left* - a light photon induces electron transfer (*D* – donor; *A* - acceptor) in a magnetoreceptive molecule resulting in a radical pair ($D^+ + A^-$), i.e., a pair of molecules with a unpaired electron each. *Green arrows* are electron spins. The spins occur either in parallel (triplet state) or antiparallel (singlet state) relative orientation. The Earth's magnetic field affects the dynamics of spin state interconversion (*black curved arrows*), and, eventually, the relative concentration of product states. *The curves in the centre* and *the letters of different sized* (S and T) below indicate that the relative yields of singlet (S) and triplet (T) products depend on the orientation of the magnetic field relative to the radicals (S_0 and T_0 for 0°, or S_{40} and T_{40} for 40° in this case). **(B):** Illustration of visual modulation patterns caused by the geomagnetic field (inclination of 68°) for a bird looking parallel to the horizon toward *N, NE, E, SE, S, SW, W* and *NW* (A&B: *after Ritz et al. 2010 and 2000, correspondingly*).

But how can birds perceive magnetic field by the radical pair mechanism? Ritz *et al.* (2000) hypothesized that different concentration of radical pair reaction products may modulate either sensitivity of photoreceptors or affect the light response, for instance, in ganglion cells, so that light responses from different parts of the retina would be different depending on alignment of a cryptochrome-containing cell relative to the magnetic field vector. For example, if alignment of the CRY is (anti)parallel then magnetically modulated light response derived from this cell would be at its maximal or minimal level. If the CRY is oriented perpendicularly to the magnetic vector then the light response would be at the opposite extreme. In all intermediate cases of the CRY alignment, the light responses would be gradually increasing or decreasing as the magnetic vector is changing its alignment to the CRY axis from (anti)parallel to perpendicular (Fig. 5B). As a result, the bird may literally ”see” magnetic field direction as a visual pattern overlaid on the ordinary visual input. One may imagine that the bird sees the world through “magnetosensitive glasses with heads-up display” showing the direction of the geomagnetic field vector as a hazy darkish or lightish but transparent spot in the visual field (Fig. 5B).

Since the time when Ritz *et al.* proposed their model (Ritz *et al.* 2000), a great deal of evidence has been collected supporting the idea that a chemical magnetoreception mechanism can actually work in different animals including birds. The data supporting the hypothesis of Ritz *et al.* (2000) are summarized below:

i) The model of Ritz *et al.* (2000) predicts that animals with such a light dependent magnetosensor would be sensitive to the axis, but not to the polarity, of the magnetic field lines, i.e. the birds should have an inclination compass. This is in agreement with the behavioural data from all the so far tested bird species (see Wiltschko and Wiltschko 1995a for a review);

ii) The absorption spectrum of isolated avian CRYs matches, at least for the most part, the range of light spectrum where birds are able to perform magnetic compass orientation (Liedvogel *et al.* 2007b). Also, it was suggested that, in a binary-choice behavioural assay, *Drosophila* flies are able to show choice responses only under the full-spectrum light but not under the light of long wavelength part of the visual spectrum (>420 nm). Moreover, CRY-deficient *Drosophila* flies do not seem to show choice responses to magnetic field under the full-spectrum light (Gegear *et al.* 2008);

iii) It was shown *in vitro* that avian CRYs after photoexcitation form radical pairs, and that these radicals live much longer (~10 ms) than the minimal lifetime (1 μs) that such radical pairs should live to theoretically be affected by the Earth’s magnetic field (Liedvogel *et al.* 2007b). It was also recently established that, under green light, a cryptochrome-like triad compound (caratenoid, porphyrin and fullerene groups) produces a radical pair with a long lifetime. This radical pair was both sensitive to the magnetic fields of <50,000 nT and responded anisotropically to the fields’ rotation (Maeda *et al.* 2008). This finding represents a proof of principle that a radical pair could work as a magnetic compass;

iv) As the theory predicts, magnetic fields oscillating in the range of 1-100 MHz should change the yields of radical pair reactions by resonance of an applied magnetic field with a singlet-triplet interconvertion frequency (Timmel and Hore 1996). At the same time, any magnetite particles of sufficient size to function as a compass are too large to reorient in a magnetic field of frequency higher than 1 MHz (Kirschvink 1996). Thus, a disruptive resonance effect of oscillating magnetic field was suggested to be a diagnostic tool for radical-pair-based magnetoreception. Indeed, European robins tested in the broad-band (0.1 - 10 MHz) 85-nT radio frequency field as well as in the weak (470 nT) single-frequency fields of 1.3 MHz (480 nT) and 7 MHz seem to be disoriented (Ritz *et al.* 2004; Thalau *et al.* 2005);

v) To date, four forms of CRYs - CRY1a, CRY1b, CRY2 and CRY4 – have been found in the avian retina (Möller *et al.* 2004; Mouritsen *et al.* 2004; Liedvogel *et al.* 2007b; Liedvogel and Mouritsen 2010). CRY 1 was found in photoreceptors, neurons of the innernuclear layer and ganglion cells of two migratory birds: the European robin and the garden warbler (Mouritsen *et al.* 2004). Garden-warbler CRY2 has been found only in the cell nucleus but garden-warbler CRY1 is cytosolic (Mouritsen *et al.* 2004). Because of the theory requesting that a magnetosensitive molecule must be, at least to some extent, fixed in space (Rodgers and Hore 2009; Hill and Ritz 2010; Solov'yov *et al.* 2010), it is most probable that magnetosensitive CRYs are not situated in the cell nucleus but rather in cytosol and anchored to cytosolic skeleton proteins and/or cellular membranes. CRY1 expression in both non-migratory zebra finches (*Taeniopygia guttata*) and migratory garden warblers is similar at daytime. But at night-time, CRY1 expression drastically drops only in zebra finches while it stays at a high level in migratory garden warblers (Mouritsen *et al.* 2004). Moreover, it was found that the expression of neuronal activity markers (ZENK and c-fos) colocalizes with high expression of CRY1 in all garden-warbler ganglion cell, at night during magnetic orientation (Mouritsen *et al.* 2004);

vi) It was established that, in nocturnal and twilight migrants (garden warblers, European robins and meadow pipits), there is a specialized light-processing forebrain region, which is a part of the visual Wulst, called Cluster N (N from nocturnal), which is highly active only during the night and/or under dim light (Mouritsen *et al.* 2005; Feenders *et al.* 2008; Zapka *et al.* 2010). The high neuronal activation of Cluster N requires visual input because it disappears when both eyes are covered. In contrast, neuronal activation of Cluster N in non-migratory zebra finches is not increased during the night (Mouritsen *et al.* 2005). It was also demonstrated by neuronal tracing that Cluster N is anatomically connected with retinal neurons via the visual thalamus, i.e., the neuronal pathway to Cluster N is a part of an ascending visual processing stream - the thalamofugal pathway (Heyers *et al.* 2007). Finally, it was recently shown that lesion of Cluster N specifically leads to disorientation of birds with the magnetic field as a single available orientation cue, but not when other orientation cues are available (Paper IV, see Section 4.3 below for more details). Thus, Cluster N is most likely to be a processing centre for visually mediated magnetic compass information.

4.2 The iron mineral containing magnetoreceptor: the upper beak organ

The first evidence showing that birds have permanently magnetized material in their bodies appeared in two studies that were done in homing pigeons and published around 1980 (Walcott *et al.* 1979; Presti and Pettigrew 1980). But neither of these works managed to detect individual magnetic particles or to find the exact location and structural arrangement in the birds' tissue. Later, in the early 1990s, it was reported that exposure of birds to a short strong magnetic pulse causes deflection of magnetic orientation, albeit this effect can be transient (e.g., Wiltschko *et al.* 1994, 1998; Beason *et al.* 1995, 1997). These data could imply that birds may have magnetosensors containing iron mineral particles. Short strong magnetic pulses can overcome the coercivity of the iron minerals and remagnetize them in the direction of the applied pulse and thus change the orientation of the bird. However, the works where the deflective effects of strong magnetic pulses on birds' orientation were observed do not provide a strong evidence of the existence of iron mineral containing magnetosensors because these findings may be side effects of the treatment. Even if we accept the hypothesis that strong magnetic pulses affect a putative magnetoreceptor in bird's body, the question of the receptor's location has been not addressed by this method.

In the mid-1980s, the electrophysiological studies of Semm *et al.* (1984) in homing pigeons and Beason and Semm (1987) in bobolinks (*Dolichonyx oryzivorus*, a common New World migratory songbird species) suggested that the ophthalmic branch of the trigeminal nerve (the 5th cranial nerve that innervates mechanoreceptors in the skin of the upper beak (Bubien-Waluszewska 1981)) might transfer magnetic information into the brain. This finding is in line with the study of Walker *et al.* (1997) in rainbow trouts (*Oncorhynchus mykiss*), where the authors found ferromagnetic material in the trout's nose and reported that single neurons in the superficial ophthalmic ramus of the trigeminal nerve – the nerve that innervates the anterior part of the head – respond to magnetic stimuli. Despite the fact that the studies of Semm *et al.* (1984) and Beason and Semm (1986) have never been replicated, they inspired several later works in birds testing the hypothesis that a putative iron-mineral-containing magnetoreceptor is situated in the upper beak.

Several studies of the late 1990s and early 2000s (Holtkamp-Rötzler *et al.* 1997; Hanzlik *et al.* 2000; Winklhofer *et al.* 2001) localized Fe3+ concentration in the subcutis of the upper beak of the homing pigeon, and identified this iron containing material as aggregates of magnetite nanocrystals with sizes of 1-5 nm that form clusters of 1-3 µm in diameter. Such small magnetite particles are supposed to be superparamagnetic particles, that is, these particles' magnetization can randomly flip direction under the influence of temperature. The tracing study of Williams and Wild (2001) suggested an anatomical association between iron-containing structures in the upper beak of homing pigeons and zebra finches with the ophthalmic branch of the trigeminal nerve. In the studies of Fleissner *et al.* (2003, 2007), a detailed histological and physiochemical analysis of a putative magnetosensor in the upper beak of the homing pigeon was presented (Fig. 6). Recently, the same structures were described in the upper beaks of three more avian species: the domestic chicken, the European robin and the garden warbler (Falkenberg *et al.* 2010). Because iron-mineral-containing magnetoreceptors in the upper beak (also called the beak organ) were found in species belonging to three evolutionary and ecologically distant orders (the domestic chicken - Gallifromes, the homing pigeon – Columbiformes; the garden warbler and the European robin - Passeriformes), it was suggested that the beak organ is a common avian characteristic, which appeared at an early stage of avian evolution (Falkenberg *et al.* 2010).

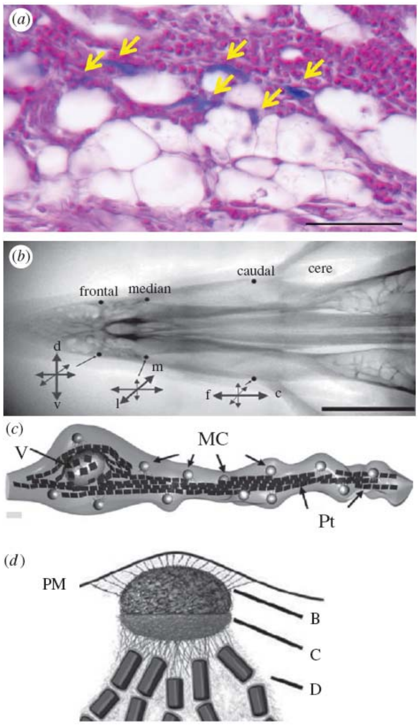

Figure 6. **The putative upper beak organ.** (***a***): Iron (Fe3+) concentrations (*yellow arrows*) in the skin of the putative upper beak of homing pigeons visualized by Prussian blue staining. It was suggested that iron-containing material is situated in the dendrites (Fleissner *et al.* 2003, 2007). Scale bar: 50 µm. (***b***): Underneath view of the homing pigeon's upper beak. Black dots show three pair of fields (*d* – dorsal, *m* – median, and *c* - caudal) where iron-mineral-containing cells were found by Fleissner *et al.* (2003, 2007). It was suggested that the caudal Prussian blue positive cells are mostly aligned along the frontal-caudal (*f* – *c*) axis; the median cells are aligned along the lateral-medial (*l* - *m*) axis, and the frontal cells are mostly aligned along the dorsal-ventral (*d* - *v*) axis. Scale bar: 0.5 cm. (***c***): A 3D-reconstruction of an iron-mineral-containing cell, probably a dendrite, illustrating the super paramagnetic magnetite-containing clusters (MC) connected by maghemite-containing platelets (Pt). (***d***): Reconstruction of a magnetite cluster. The cluster is nested in a fibrous basket (C) and connected to the cell membrane as well as to the platelets (D) by filaments (B). (a-d: *after Fleissner et al. 2003, 2007 with modification*).

It was suggested that the putative beak organ in the homing pigeon consists of iron-mineral-containing structures supposedly situated in unmyelinated dendrites supported by bundles of axons in the inner skin of the upper beak (Fleissner *et al.* 2003, 2007). It was also suggested that, in homing pigeon, iron-mineral-containing cells of the upper beak are grouped in six fields (Fig. 6c, Fleissner *et al.* 2007), which comprise three pairs bilaterally symmetrically arranged near lateral rim of the upper beak: one pair next to the tip of the beak (distal patches), one pair next to the glandular base of the beak (proximal patch) and the last pair just between the other two (middle patch). The distal patches were found in all the homing pigeons inspected in the study of Fleissner *et al.* (2003), but the proximal and middle pairs were missing in some pigeons (Fleissner *et al.* 2003). In homing pigeons, it was also suggested (Fleissner *et al.* 2007) that for each pair of patches enriched with iron-mineral-containing cells there is one prevailing orientation: the two proximal patches seem to be have mostly caudal-to-rostral orientation, the two middle patches seem to have a predominant median-to-lateral orientation, and the distal patches seem to be mostly oriented in dorsal-to-ventral direction. On the subcellular level, three structures containing two different types of iron minerals have been described: i) superparamagnetic magnetite (Fe_3O_4) nanoparticle spherules attached by a fiber network to the cellular membrane (Fig. 6d); ii) maghemite (Fe_2O_3) platelets (each of them is ca. 1 μm wide and long and less than 0.1 μm thick) most probably consisting of single-crystalline straight chains, which preferentially extend along the cell (very little is known about the magnetic properties of these platelets); and iii) one vesicle with diameter of 3 - 5 μm that is either spherical or elongated and covered by a crust of non-magnetite iron mineral. The vesicle seems to contact the cellular membrane (Fleissner *et al.* 2003; Fig. 6c).

The mechanism that converts magnetic stimuli into neuronal signals in the beak organ is still elusive, but theoretical considerations suggest that the beak organ structures may serve as a magnetic field amplifier that enhances the weak geomagnetic field and leads to deformation of the spherules built from magnetite nanoparticles and attached to the cellular membrane by protein fibers. This, in turn, may lead to opening of specific mechanosensitive ion channels in the membrane and, thus, initiate a neuronal response (Fleissner *et al.* 2007; Solov'yov and Greiner 2009).

Recently, it was reported that, in European robins, an intact ophthalmic branch of the trigeminal nerve is necessary for magnetically induced neuronal activation in and near the principal and spinal brain nuclei of the trigeminal brainstem complex, which are the two brain regions that receive primary inputs from the trigeminal nerve (Heyers *et al.* 2010). These data strongly suggest that the ophthalmic branch of the trigeminal nerve in European robins innervates a putative magnetosensitive organ in the upper beak, and support the idea that the iron-mineral-containing structures in the upper beak can sense the magnetic field.

4.3 Integration of magnetic information from the eye and the upper beak: own contribution

As shown above, there is a growing body of data supporting the idea that birds have at least two different magnetosensory systems: one is based on cryptochrome(s) in the bird's eye and another is mediated by iron-mineral-containing cells located in the upper beak. But why do birds need two types of magnetoreceptors? During my PhD work, I took part in the study that aimed to uncover the functions of two different magnetosensitive systems (Paper IV).

In this study, we asked the following questions: i) does Cluster N play a crucial role in the visually mediated magnetoreceptive mechanism that underlies the magnetic compass? and ii) is the upper beak organ innervated by the ophthalmic branch of the trigeminal nerve involved in detection of the direction of the geomagnetic field?

To investigate these two questions, we did orientation tests with the following four groups of European robins: i) a group that had bilateral Cluster N lesions performed by injection of ibotenic acid into Cluster N (Cluster N lesion group); ii) a group that underwent the same bilateral surgeries as the previous group but without injection of ibotenic acid (sham Cluster N lesion group); iii) a group with bilateral sections of the ophthalmic branch of the trigeminal nerves (trigeminal section group); and iv) a group that underwent the same surgery as by the trigeminal section group but without sectioning of the trigeminal nerve (trigeminal sham section group). The orientation of all the groups was tested in Emlen funnels during the spring migratory season in two different magnetic field conditions: a normal magnetic field (NMF) and a changed magnetic field (CMF) with the magnetic north turned horizontally 120º counterclockwise. After double-blinded orientation tests, i.e., tests when experimenters did not know which birds belong to which group, we found that birds with sham Cluster N lesion, real and sham trigeminal section could use their magnetic compass for orientation in both the NMF and CMF conditions. In contrast, the birds from the Cluster N lesion group were disoriented. This finding strongly suggests that an intact Cluster N is crucial for magnetic orientation. But taken alone this cannot prove that Cluster N is functionally connected with the magnetic compass. Alternatively, one may hypothesize the following explanations of our results:

i) The chemical lesion of Cluster N deteriorates all kinds of visual performances because Cluster N is a part of the visual Wulst, the highest center of the avian retinothalamofugal pathway and a close functional analogue of the visual cortex of mammals (e.g., see Jarvis *et al.* 2005 for a review). In this case we should expect that Cluster N lesioned birds should perform poorer than the sham Cluster N lesioned birds in any visually based tasks;

ii) Cluster N could be a compass integration center in the brain, and, therefore, lesioning it may unspecifically disturb integration and processing of directional information from all kinds of orientation cues. In this case we should expect that the birds with Cluster N lesions cannot use other orientation cues such as sunset and/or stellar cues.

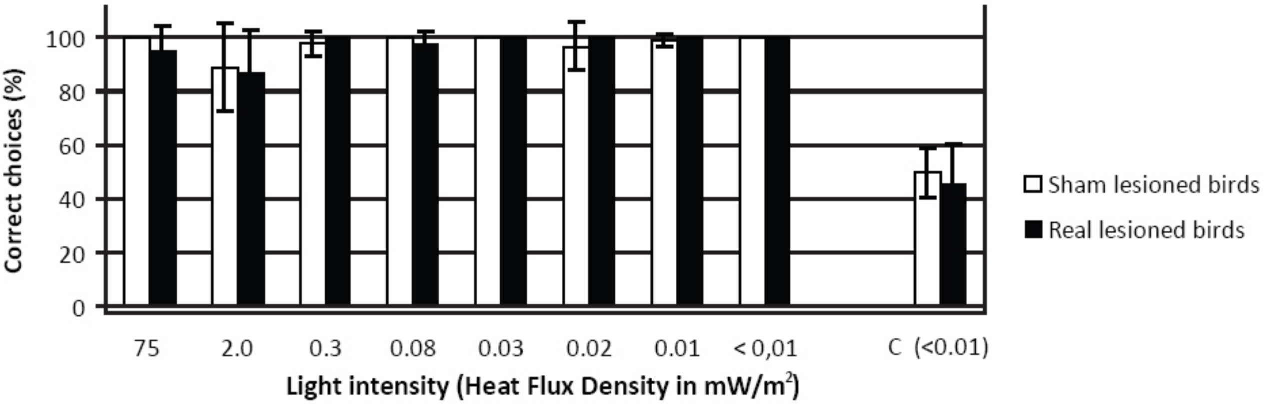

Figure 7. **Light spot detection capabilities of the Cluster N lesioned European robins were not affected by Cluster N lesion. Explanation is in the text**. The percent correct choice at each light intensity is represented by the bars. In the control experiment ("*C*"), both diodes were turned on at the <0.01 mW/m2 intensity, and the birds performed at levels close to chance level of 50%. The experimental chamber's dim house lights were always turned on. The two lesioned birds (*black bars*) and the two sham lesioned birds (*white bars*) could all detect the conditioned light stimulus irrespective of the light intensity used at a success rate close to 100%, including when the light stimulus which was 400 times dimmer than the light present in the wooden huts during the orientation experiments. Error bars represent SD. (*after Paper IV*).

To test the above mentioned explanations, additional tests were conducted. My part of the work was to address the first alternative explanation suggesting that Cluster N lesioned birds may have deficits in vision guided tasks. We therefore tested whether Cluster N lesioned birds show deficits compared to the sham Cluster N lesion birds in an operant conditioning test, where birds have to discriminate a very dim point of light. To do this, I together with Simon Weiler designed and performed a set of simple, two choice operant conditioning experiments that required the birds to hop from a choice perch onto either of two response perches each associated with a white LED being turned on in order to receive a food reward. In the pre-training phase, the birds learned the task at the highest light intensity (75 mW/m^2). Each of the four birds (2 real lesioned and 2 sham lesioned) took part in 10 - 20 sessions consisting of 10 - 12 choices per session. Once the birds had learned the task, the real experiment began. We reduced the intensity of both white LEDs in steps until their intensity was approximately 400 times dimmer than the light present during the orientation experiments (the visual detection limit was ~0.01 mWm^{-2} for both groups). For these critical tests, each bird was tested for, in average, 8 sessions each consisting of 10 choices. The two lesioned birds and the two sham lesioned birds could all detect the conditioned light stimulus irrespective of the light intensity used at a success rate close to 100%, even when the light stimulus was 400 times dimmer than the light present during the orientation experiments (Fig. 7). In control experiments, both white LEDs were turned on at the 0.01 mW/m^2 intensity. In this case both groups performed indistinguishable from chance level of 50% (Fig. 7). We therefore concluded that the observed difference in magnetic orientation between the Cluster N lesioned and sham Cluster N lesioned birds is unlikely to be due to reduction of general visual capabilities caused by the chemical lesion.

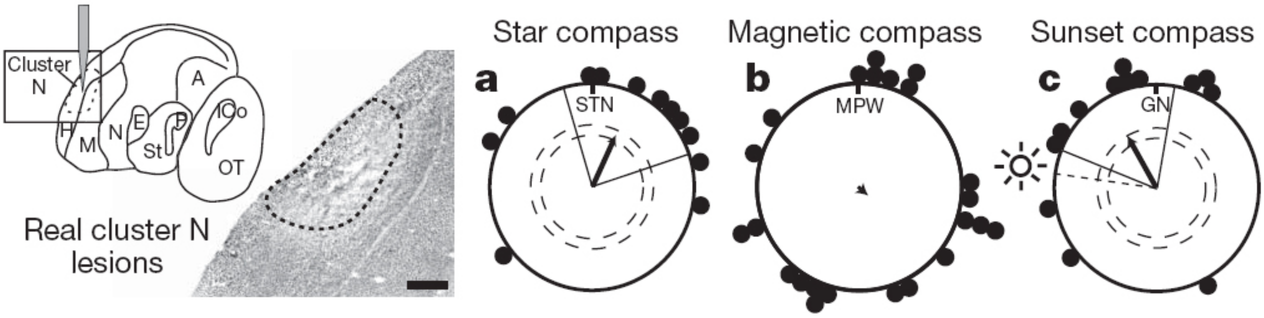

Figure 8. **Birds with Cluster N lesions can use their star and sun compasses, but not their magnetic compass.** *The left photo* shows example of brain section from an actually lesioned European robin: a saggital cut through the centre of Cluster N stained with anti-HuC/HuD (a neuronal marker). The tissue where Cluster N is situated is at a large part destroyed. The drawing indicates where in the brain the photo was taken: *A*, arcopallium; *E*, entopallium; *H*, hyperpallium; *DNH*, dorsal nucleus of the hyperpallium; *ICo*, intercollicular complex; *M*, mesopallium (*MD*, mesopallium dorsale; *MV*, mesopallium ventrale); *N*, nidopallium; *OT*, optic tectum; *P*, pallidum; *St*, striatum. Rostral, left; caudal, right. Scale bar, 500 µm. (**a**): Birds with Cluster N lesions tested in a planetarium simulating the local starry sky (ten tests per bird; STN, star north) oriented in the typical north-northeast spring migratory direction (a = 27° ± 44° (± CIs), r = 0.55, N = 12, P = 0.02); (**b**): Birds with Cluster N lesions could not orient (a = 132°, r = 0.12, N = 23, $P > 0.07$) using their magnetic compass; (**c**): Birds with Cluster N lesions could also orient during sunset (a = 331° ± 39°, r = 0.57, N = 13, P = 0.01), presumably using their sun compass. (*after Paper IV with modifications*)

The second alternative explanation suggesting that lesion of Cluster N may lead to a general orientation deficit, so that the birds could not use any of their compasses, was tested by performing orientation tests under two additional conditions: i) outdoor orientation tests under clear skies during sunset, and ii) indoor orientation tests under a stationary planetarium sky simulating the local starry sky. After these tests, it was found that both the sham Cluster N lesioned and real Cluster N lesioned robins can use sun related cues as well as planetarium stars for orientation (Fig. 8). These findings clearly show that the Cluster N lesioned birds can use their sunset and star compasses. Thus, lesion of Cluster N only disrupts the birds' ability to use their magnetic compass.

All in all, our data are consistent with the hypothesis that magnetic compass input is processed in Cluster N – a part of visual system of night-migratory songbirds. This, in turn, strongly supports the suggestion that the avian magnetic compass is based on receptors located in the bird's eye. Simultaneously, our data clearly show that the putative iron-mineral-based receptors in the upper beak connected to the brain via the ophthalmic branch of the trigeminal nerve are neither necessary nor sufficient for magnetic compass orientation in European robins. Thus, the function of the upper beak organ still remains a mystery. It has been suggested by different authors that the upper beak organ may play an important role in navigation of migratory songbirds and in homing of homing pigeons – phenomena that are supposedly based on a magnetic map (e.g., Munro *et al.* 1997a, 1997b; Fleissner *et al.* 2003; Wiltschko *et al.* 1998, 2006, 2009; Holland *et al.* 2010; but see Gagliardo *et al.* 2006, 2008, 2009; Patzke *et al.* 2010). One may propose that the beak organ functions as a "magnetic map organ" in birds. However, this hypothesis has not been proven yet.

5. An attempt to develop an operant conditioning paradigm to test for magnetic discrimination behaviour in a migratory songbird: own contribution

As described above (see Sections 4.2 and 4.3 above), the function of the beak organ is still elusive. What is known now is that innervation of the beak organ via the ophthalmic branch of the trigeminal nerve is neither necessary nor sufficient for magnetic compass orientation of the European robin. At the same time, there is a strong evidence that lesion of Cluster N makes the European robin's magnetic compass dysfunctional (Paper IV). Thus, the magnetic compass of European robin is not dependent on functionality of the beak organ. Therefore, the magnetoreceptive abilities of the beak organ cannot be investigated in Emlen funnel, which only tests for compass responses. So the question arises: how can we investigate the function of the beak organ in an experiment?

One possible approach would be operant conditioning, where experimental birds have to learn to respond to magnetic stimuli, e.g., by jumping onto an automatic perch or pecking a key. A response of the animal can be followed either by a positive reinforcer (food) or a negative reinforcer (time penalty during which access to food is denied). In operant conditioning, reinforcement will occur in the presence of a specific stimulus if, and only if, the conditioned response occurs. As a rule, the animal performs the rewarded response and avoids performing the punished response as a consequence of experience and, over time, increases behavior leading to rewards and decreases behavior leading to punishments. The approach of operant conditioning is based on the principle that the animal is only able to respond differently to different sensory stimuli if it is able to detect these stimuli and discriminate among them. However, the opposite is not always true because ability to discriminate any stimuli not obligatory means that the animal would show discrimination of these stimuli in an experiment.

Between the 1950s and 2000s, many attempts to train birds to discriminate changes in magnetic stimuli have been undertaken, but most of them failed (e.g., Orgel and Smith 1954; Meyer and Lambe 1966; Reille 1968; Kreithen and Keeton 1974; Beaugrand 1976; Alsop 1987; Moore *et al.* 1987; Couvillon *et al.* 1992). Bookman's study (Bookman 1977) was the first one where success with operant conditioning in birds (homing pigeons) was reported. Homing pigeons in this study (Bookman 1977) were rewarded for going into one or another food box depending on the magnetic field. Bookman suggested that homing pigeons in his study (Bookman 1977) were able to discriminate magnetic stimuli only if they have fluttered, i.e., performed sustained hovering, jumping or short flights before to make a choice. Bookman, however, did not use individual pigeons but rather mated pairs. Thus, Bookman's behavioural observations were not obtained from independent individuals. Carman and Mahowald attempted to replicate the experiment of Bookman (1977) using a duplicated setup and similar magnetic stimuli but reported negative results (data reported in Carman *et al.* 1987). Only recently, a few studies reporting positive results obtained from birds were published (e.g., Freire *et al.* 2005; Voss *et al.* 2007 and Wilzeck *et al.* 2010 – conditioning at magnetic direction in the chicken, the zebra finch and the homing pigeon, respectively; Mora *et al.* 2004 - conditioning at a magnetic anomaly in the homing pigeon).

Mora *et al.* (2004) demonstrated for the first time that individual homing pigeons, not mated pairs (Bookman 1977), can be conditioned to discriminate between the presence and absence of a magnetic anomaly, that is, a stimulus that provides change in both total intensity and directional components. In this study, Mora and her co-workers developed their design based on the analysis of previously successful studies with both vertebrates (the homing pigeon: Bookman 1977; the yellow-fin tuna, *Thunnus albacares*: Walker 1984; the rainbow trout: Walker *et al.*, 1997; Haugh and Walker 1998; the short-tailed stingray, *Dasyatis brevicaudata*: Walker *et al.* 2003) and invertebrates (the honey bee, *Apis mellifera*: Walker and Bitterman, 1985). Mora *et al.* (2009) found that all the previously successful studies fulfilled two requirements: i) the magnetic stimuli used were spatially distinctive, i.e., their parameters were significantly changing inside the space of an experimental chamber where the animal was sampling the magnetic field before it had to respond, and ii) the behavioural responses included movement of the animal. These two characteristics were taken into account in the study of Mora *et al.* (2004), where pigeons were successfully conditioned to discriminate a magnetic anomaly (a peak value of total intensity of the magnetic field used was in the centre of a chamber, and was approximately 189,000 nT, i.e., about four times higher than the background magnetic field level of 44,000 nT) from the absence of the anomaly. Mora *et al.* (2004) reported that intact ophthalmic branches of the trigeminal nerves are crucial for discrimination of magnetic stimulus used. This finding strongly suggests that this conditioning was mediated by the beak organ. One should, however, take into account that homing pigeons have been domesticated long time ago, and are being artificially selected for good homing performance in every generation. Therefore, it could be that the beak organ of homing pigeons may considerably differ from the beak organs of migratory songbird species. Thus, it is important to test whether the paradigm successfully used in the study of Mora *et al.* (2004) can be adapted to a migratory songbird, e.g., the European robin - a medium-distance migrant and a model species in orientation studies.

During my PhD work, Dr. Cordula Mora and I, as a co-principle investigator, did a study where a serious attempt was undertaken to adapt the operant conditioning approach used in Mora *et al.* (2004) to European robins (Paper VII). In this study, we attempted to train six European robins to discriminate magnetic anomalies with different peak values of total intensity from the background magnetic field. The study was divided into three experiments (Fig. 9).

In Experiment I and II, we used experimental setups very similar to the study of Mora *et al.* (2004, see Fig. 9A). The experimental birds used in these two experiments had to discriminate between the presence of the magnetic anomalies with peak total intensities of 85,000 nT and 52,000 nT in Experiment I and II, respectively, from their absence. Despite a dedicated effort, we did not reach the point where our experimental birds showed a clear discrimination of the presented stimuli (Fig. 10).

In Experiment III, we tested the general adequacy of our setup for operant conditioning by introducing an additional auditory discriminative stimulus – a pure tone of 2 kHz (sound pressure level (SPL) = 90 dB) alternating every 0.5 s with pauses of silence (Fig. 9B, C). Experiment III was divided into three phases. During Phase 1, the experimental birds had to discriminate an acoustic stimulus presented together with a strong magnetic anomaly (peak value was 177,000 nT, i.e., about four times the total intensity of the background magnetic field and very similar to the magnetic anomaly successfully used to condition homing pigeons in the study of Mora *et al.* (2004)) from the background acoustic environment together with the background magnetic field in the experimental room. Over 42 consecutive daily conditioning sessions, the birds developed obvious discrimination of the auditory stimulus (Fig. 11, Phase 1). Then Phase 2 started. During this phase, the birds had to discriminate the same stimuli as during Phase 1 but the loudness of the auditory stimulus was decreasing in a stepwise fashion until the SPL of the acoustic stimulus became comparable with the background level of acoustic noise in the experimental room. During Phase 2, i.e., over 28 consecutive daily conditioning sessions, the birds' discrimination performance was rapidly declining so that by the end of this phase it was not different from chance level (Fig. 11, Phase 2). Finally, in Phase 3, that is, over the last 17 consecutive daily conditioning sessions when only the magnetic discriminative stimulus was presented, mean performance resided around chance level without any uprising tendency (Fig. 11, Phase 3).

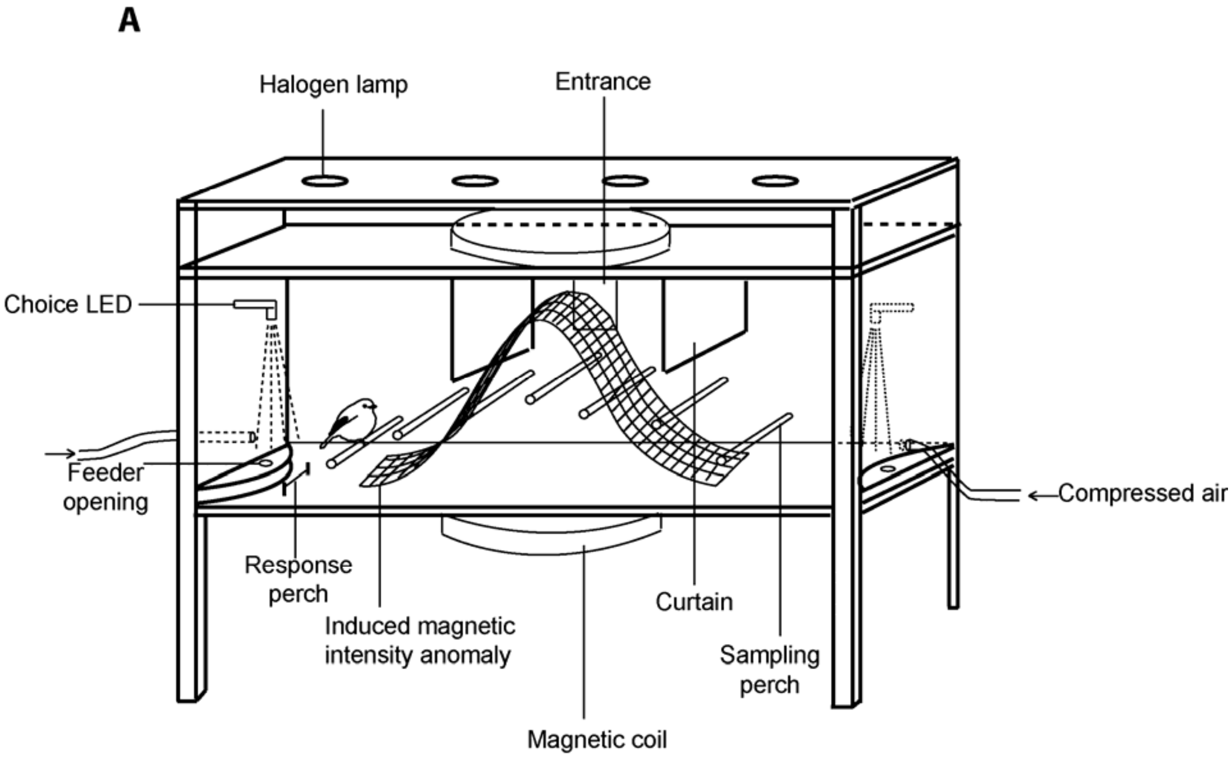

Figure 9A (s*ee the full legend on the next page).* **Experimental setups used in the conditioned choice discrimination**.

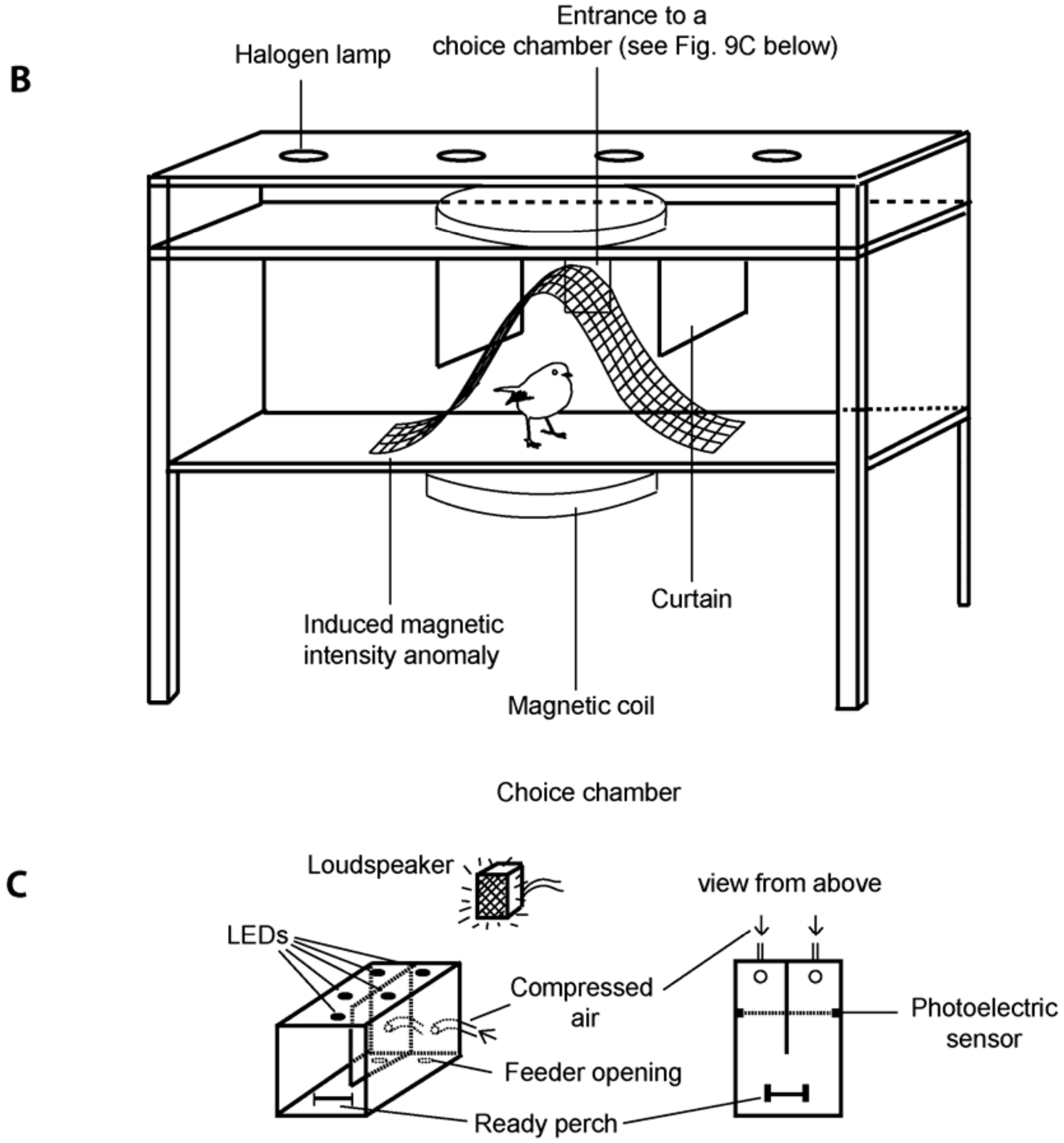

Figure 9B, C (*see Fig. 9A on the previous page*). **Experimental setups used in the conditioned choice discrimination**. **(A):** Setup used in Experiment I and II. In Experiment I individually trained European robins were required to discriminate between the presence and absence of a magnetic field anomaly, which from the bird's perspective was wave-shaped in its intensity profile. The anomaly was located centrally in the experimental chamber with its peak intensity and inclination varying respectively from 42,000 nT and 67.5º (at the bird's head level at the two outermost sampling perches on either end of the experimental chamber) to 85,000 nT and 73.0 º (at the central sampling perch). The birds were required to mount one of two response perches located at either end of the experimental chamber depending on the magnetic field stimulus presented during the sampling phase of a given discrete trial. Correct choices were rewarded with food (piece of meal worm) from a feeder opening next to each response perch whereas incorrect choices resulted in a 20 s time penalty. In Experiment II the same apparatus was used but i) the peak total intensity of the magnetic anomaly was lower (52,000 nT), and ii) a puff of air was introduced as a secondary negative reinforcer (in addition to the time penalty). **(B and C):** Setup used in Experiment III. In B, the sampling chamber is shown. Sampling and response perches as well as feeder openings used in Experiments I & II were removed. In C, a new choice chamber (general view, left, and view from above, right) as well as a loudspeaker are shown. At the entrance of the choice chamber, a ready perch was situated. Further inside, the choice chamber was divided into two tunnels, with a feeder opening located at the end of each tunnel. A photoelectric sensor detected the bird entering one of the two tunnels to permit delivery of the food reward from that tunnel's rotating feeder or administration of a puff of air for a correct and incorrect choice respectively. In addition to the magnetic anomaly (peak total intensity of 177,000 nT), a secondary discriminative stimulus was introduced in form of a pure tone (2 kHz, 90 dB) alternated every 0.5 s with pauses of silence (for further details see the text and Paper VII). A&B adapted from Mora *et al.* (2004). (*after Paper VII*).

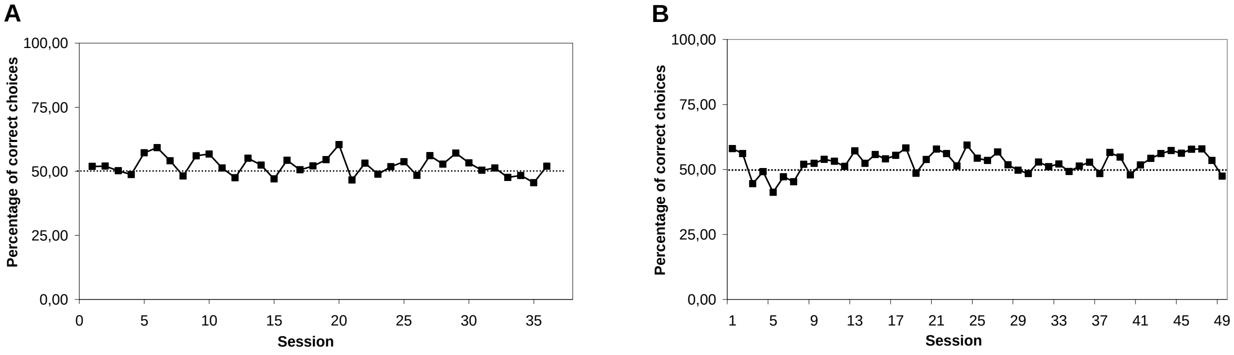

Figure 10. **Mean daily percentage of correct choices for all experimental birds in Experiment I (A) and II (B).** The horizontal lines at 50% indicate the level of chance performance. (*after Paper VII*).

When stimulus control over the animal's behaviour cannot be demonstrated for a particular set of discriminative stimuli, it is very difficult to determine the exact reasons for lack of discrimination. One may suggest that our European robins could not perceive the magnetic stimuli presented. However, not only has the magnetic compass of the European robin been well documented by numerous previous orientation studies (e.g., Paper IV; see Wiltschko and Wiltschko 1995b for a review), but more recently it was demonstrated that, in this species, changing magnetic field induces neuronal activity in two brainstem complexes innervated via the ophthalmic branch of the trigeminal nerve (Heyers *et al.* 2010). This is the same nerve that was shown to be crucial for homing pigeons to be able to detect magnetic anomaly (Mora *et al.* 2004).

Our European robins were also exposed to a changing magnetic field while sampling through the magnetic anomalies used, and they had intact trigeminal nerves. Thus, we suggest that explanation of our negative results by a physiological inability to perceive magnetic stimuli seems to be very unlikely.

Alternatively, one may suggest that our combination of stimuli, responses and reinforcement was not optimally chosen. For instance, one may speculate that European robins may have shorter primary or short-term memory than homing pigeons do. If that is the case, the spatial and thus temporal separation of stimulus, behavioural response and reinforcement is crucial. Our robins might simply have forgotten which stimulus had been presented by the time they reached the choice phase after a long sampling phase. Similarly, the European robins' attention may have been easily distracted during the sampling and/or choice phase by the complex requirements to hop onto various sampling and response perches.

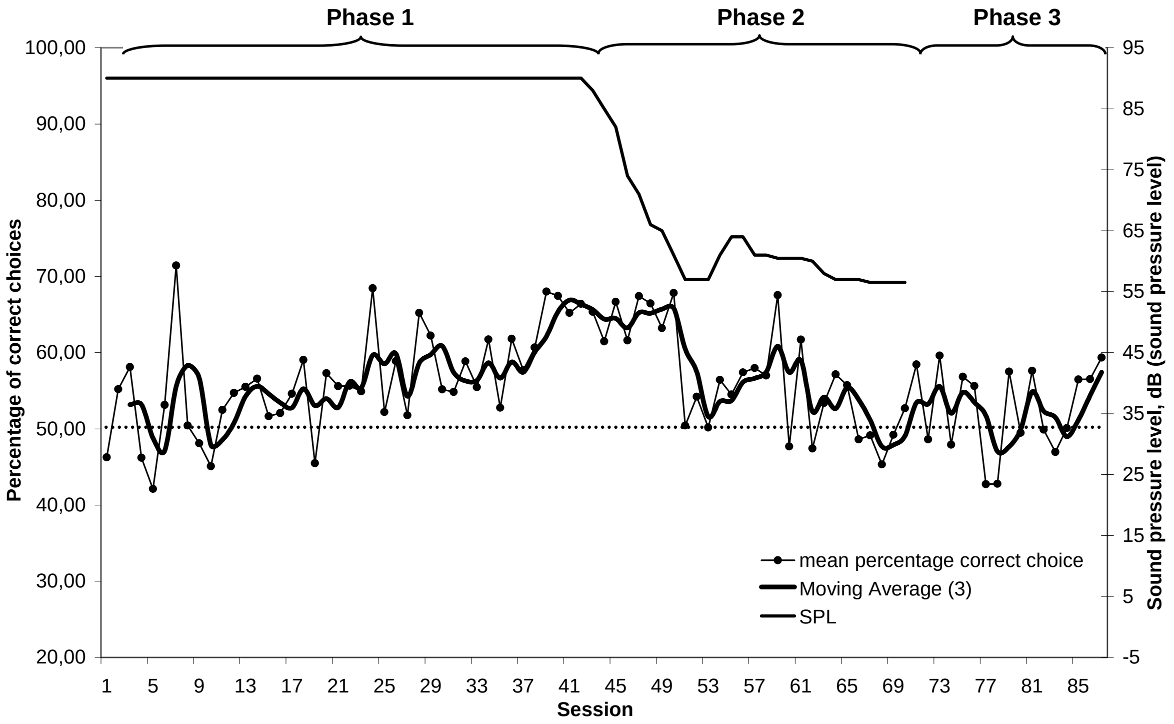

Figure 11. **Mean daily percentage of correct choices for four individually trained robins over consecutive sessions testing for the discriminating of auditory and magnetic stimuli in Experiment III**. **Phase 1**: discrimination of an auditory stimulus (a pure tone of 2 kHz alternated every 0.5 s with 0.5 s pauses of silence, Sound Pressure Level (SPL) = 90 dB) coupled with a strong magnetic anomaly (peak intensity value of 177,000 nT in the centre of the sampling chamber) from the absence of the auditory stimulus with only the magnetic background field present. **Phase 2:** discrimination of the same auditory stimulus with SPL stepwise decreasing to approximately 55 dB coupled with the same strong magnetic anomaly as used for Phase 1 from the absence of the auditory stimulus with only the magnetic background field present. **Phase 3**: discrimination of the presence and absence of the strong magnetic anomaly in the absence of any auditory cues. The horizontal line indicates the level of chance performance (50%). A simple moving average (3 session blocks) was added to reduce variability of the mean daily percentage of correct choices. (*after Paper VII*).

In conclusion, the approach successfully applied in pigeons (Mora *et al.* 2004) occurred to be suboptimal for a migratory songbird. Our study adds another example reporting negative results of magnetic conditioning in birds. This indicates that operant conditioning of birds to magnetic stimuli is an extremely challenging task. So far, even though positive results have been reported in non-migratory avian species (e.g., Bookman 1977; Mora *et al.* 2004; Freire *et al.* 2005; Voss *et al.* 2007; Wilzeck *et al.* 2010), few, if any, successful conditioning paradigms have turned out to be routinely replicable in other laboratories by other research groups (e.g., Carman *et al.* 1987 - a failure to replicate Bookman 1977; A. van Hettinga and H. Mouritsen (Liedvogel and Mouritsen 2010) – a unsuccessful attempt to replicate Freire *et al.* 2005). Moreover, due to the difficulty of publishing equally important but less exciting negative results, we suspect that a significant number of additional negative results has never been published. What the field of animal navigation strongly needs is a conditioning design using magnetic cues as discriminative stimuli, which can be easily replicated independently. Such a design would become as important at facilitating magnetic navigation research as the Emlen funnel (Emlen and Emlen 1966) has been to magnetic compass research. At present, however, no such paradigm is available.

6. How can juvenile birds find their way to wintering quarters?

6.1 Reviewing the literature

The question how migratory birds, especially juvenile individuals during their first migration, can find way to their wintering grounds has been challenging naturalists for centuries. Only in the 20th century, data appeared suggesting that birds have rather sophisticated navigational abilities. It was found that juvenile and adult birds use very different strategies to get to their desired destinations.

Juvenile migrants without migratory experience can use, at least in theory, three different strategies: guiding, clock-and-compass orientation programmes and travelling between signposts (Mouritsen 2003; Fransson *et al.* 2001, Kullberg *et al.* 2003, 2007). Below I summarize these strategies:

1). ***Guiding.*** Some juvenile birds can travel in flocks and possibly follow adult individuals – the so-called *guiding*. Such a strategy is known in ducks, geese, storks, cranes, birds of prey and other, mostly soaring non-passerine, bird migrants. Data from studies where juvenile white storks (*Ciconia ciconia*) were captured, kept at a capture region and released several weeks after their young conspecifics in the wild had left on their autumn migration (ringing recoveries in Schüz 1949, 1950; and satellite tracks in Chernetsov *et al.* 2004) strongly suggest that naïve white stocks, which are normally guided by adults, do have an inherited knowledge about their migratory direction(s). Interestingly, displaced naïve white stocks in the study of Chernetsov *et al.* (2004) performed very broad scatter of chosen migratory directions that may suggest that juvenile white stocks, when travelling alone, choose their migratory direction very inaccurately;

2). ***Clock-and-compass orientation***. Juvenile birds belonging to nocturnal migratory species (e.g., species of *Sylviidae*, *Muscicapidae*, *Turdidae*, *Parulidae*), as a rule, travel alone and/or in instable and sparse flocks, and frequently depart later than their adult conspecifics. Nevertheless, these migrants are still able to reach their species specific wintering grounds. Thus, it has been suggested that such juvenile birds may rely exclusively upon an inherited behavioral programme that gives the birds an idea of what migratory direction(s) they have to choose and maintain during their first migration as well as how long they should fly in order to reach their species specific wintering quarters. This is the so-called *clock-and-compass* or *vector navigation* strategy. In other words, such an inherited programme could be verbalized as follows: "At the beginning of the migratory journey, keep the course α for X days (or weeks), then choose the course β for another Y days (or weeks). Once these instructions are executed, you are supposed to end up at a site lying inside wintering range."

Indeed, since the 1930s, there is a growing body of evidence, both from field based and lab studies, supporting the *clock-and-compass* or *vector navigation* hypothesis in naïve bird migrants. Below, these data are summarized:

i) *Displacement experiments*

Drost (1938) caught juvenile migrating Eurasian sparrowhawks (*Accipiter nisus*) during autumn migration, divided the birds into two groups, ringed, displaced and released part of the captured birds southeasterly, and the rest of the birds was released at the capture site. Ringing recoveries strongly suggested that the displaced sparrowhawks had not compensated for the displacement and kept travelling parallel to the normal direction that the undisplaced birds chose.

Later, the classical large-scale experiments of Perdeck (1958, 1967) in European starlings and chaffinches again confirmed the idea that naïve avian migrants do not notice displacement and keep travelling along the course they were flying before displacement. The results obtained in displaced juvenile birds by release-and-capture method (Mewaldt 1964), orientation cage method (e.g., Hamilton 1962; Mouritsen and Larsen 1998) and even by radio telemetry in naturally migrating birds (Thorup *et al.* 2007) are consistent with this idea. In contrast, birds that completed at least their first migratory journey are able to compensate for displacements and, therefore, can successfully reach their wintering grounds despite being displaced (Thorup *et al.* 2007; Paper I; see Sections 7 and 8);

ii) *Inherited timing of migration*

It is well known that birds, like all other animals, have biological clocks – the genetic and molecular mechanisms that regulate an animal's activity in a rhythmical manner (see Gwinner 1986 for a review). There is strong evidence that timing of migration relies on genetically based mechanisms. For example, it has been shown that the duration of migratory restlessness in captive juvenile birds is generally correlated with the distance migrated by their free living conspecifics (e.g., Gwinner 1968; Berthold and Querner 1981, 1982; Berthold 1988). It means that the longer it takes for birds from a given population to get to their wintering grounds in the wild, the longer their captive conspecifics from the same population demonstrate migratory restlessness in cages. Moreover, it is possible to change the duration of the performed migratory restlessness in captive songbirds by directed artificial selection (Berthold *et al.* 1990). Thus, most researchers now agree that endogenous circannual clocks are under direct genetic control that determines initiation, duration and termination of migration, and thus distance of migration (e.g., Gwinner 1977, 1996a, 2003; Holberton and Able 1992; see also Berthold 1996, 2001 for reviews).

But are duration and distance of migration really rigidly controlled or there is some degree of flexibility added to the system? We still do not have a clear answer for this question. In the laboratory, the studies on migratory warblers showed that the timing of the performed migratory restlessness is strictly controlled by a time programme that is little affected by a bird's energy turnover or actual performance, that is, how much a bird is allowed to perform migratory restlessness in the cage (see Gwinner 1996a for a review). At the same time, there are doubts that such a rigid migratory clock-and-compass programme alone can lead naïve birds to winter quarters because delays and deflections along the migratory route are difficult to avoid (see Newton 2008 for a review). Therefore, it has been suggested that juvenile birds on their first migration can fine-tune duration of their clock-and-compass programmes to real life (e.g., Ramenofsky and Wingfield 2007). Indeed, it has been showed that there is adaptive responsiveness of the migratory time programme to the photoperiodic, nutritional and energetic situation along the migratory routes. For instance, in garden warblers wintering in central and southern Africa, the onset of autumn migratory restlessness can be advanced by short photoperiod (see Gwinner 1989, 1996b for reviews). This accelerating effect is extremely important for young birds from late clutches because, in order to be on time, they have to depart in a younger age than their conspecifics hatched from earlier clutches. Furthermore, in garden warblers, the end of autumn migratory restlessness and the onset of spring restlessness can be advanced by long photoperiods (see Gwinner 1989, 1996b). This may be important for individuals that happened to migrate too far into the southern hemisphere and, therefore, have to cover a longer distance next spring than other garden warblers wintering closer to the equator. Little is known, however, about the factors that control termination of migration in free-living birds. It has been suggested that finding a suitable place towards the end of migration may induce termination of the migratory state

(Perdeck 1964; Klein 1980). Even during winter, bird migrants can perform facultative movements (Terrill 1990), i.e., this termination may be reversed for a while;

iii) *Inherited migratory directions*

One of the early studies suggesting the existence of spatiotemporal orientation programmes in juvenile bird migrants was published by Gwinner and Wiltschko (1978). They hand raised and subsequently kept juvenile garden warblers at their natal site situated in the southern Germany under constant temperature and photoperiodic conditions. The orientation of the birds was regularly tested during the whole migratory season (from August to October) and the beginning of wintering time (from November to December) indoors without celestial cues but only with magnetic cue available. The authors expected that captive garden warblers should perform roughly the same changes in their orientation as garden warblers in the wild do. According to ringing recoveries, garden warblers from Germany initially head southwest until they reach Spain and/or Morocco, and from there onwards they proceed due south to south-southeast to reach Western Africa where some of the warblers stop and overwinter (Zink 1973-75, 1977). Indeed, the study of Gwinner and Wiltschko (1978) suggests that captive juvenile garden warblers change their orientation from southwestern to southeastern approximately at the same time when their conspecifics do the same in the wild. Later, the genetic control of migratory directions was proven in the study of Helbig (1991b), where songbird migrants from populations with different migratory orientations were crossbred and their F1 hybrids demonstrated averaged orientation.

Can juvenile migrants always perform the whole orientation programme when they are being kept at their natal place and exposed only to local cues? There are data suggesting that it is not a case. For example, in the studiy of Beck and Wiltschko (Beck and Wiltschko 1982, 1988), juvenile pied flycatchers (long-distance migrants breeding in most of Europe and Western Asia, and overwintering in Western Africa) from the southern Germany performed seasonally appropriate changes in orientation only when they were permanently exposed to the magnetic conditions (inclination and total intensity) that were altered during autumn migratory season so that movement along the presumed migratory route was imitated. These results contradict to the study of Gwinner and Wiltschko (1978) where shift in orientation was seen in juvenile birds exposed only to the magnetic conditions of their natal region during the whole autumn migration (but see also the critical re-analysis of these data performed by Rabøl: www.jorgenrabol.dk/files/gwinner2.pdf). Moreover, the data obtained by myself together with my co-authors (Kishkinev *et al.* 2006; Paper III) in juvenile pied flycatchers from the Eastern Baltic, which were kept at their natal site and tested in orientation cages provided with all orientation cues, indicate that the birds initially performed southwesterly orientation corresponding to their migratory direction at the beginning of autumn migration. However, later in the migratory season, the birds seem not to change this initial orientation to southerly or southeasterly orientation, as one should expect on the base of ringing recoveries from actually migrating pied flycatchers, but rather kept showing the same orientation until the end of migratory season. Thus, captive juvenile birds seem to need some additional external factors to adequately perform the whole orientation programme.

In summary, there are data supporting the hypothesis that juvenile bird migrants on their first migration are driven by inherited behavioral mechanisms providing them with information when and which way one should head to reach wintering quarters. Currently, most of avian migration researchers explain orientation behavior of naïve bird migrants by the notion of clock-and-compass genetic programmes;

3). ***Travelling between signposts***. If we suggest that juvenile avian migrants use a simple clock-and-compass strategy to find their winter quarters, we should expect a parabolic spatial distribution of birds departed from a natal region and flying towards their wintering quarters as a function of migratory distance. In other words, the further the location of juvenile migrating bird from departure site is, the broader expected deviation from the species specific mean migratory direction has to be (Mouritsen and Mouritsen 2000). Indeed, it was shown that ringing recoveries of juvenile birds of two songbird species (the European robin – a medium-distance migrant, and the pied flycatcher - a long-distance migrant) ringed in Scandinavian countries and recovered the same year in Western Europe are distributed close to the parabolic mathematical prediction (Mouritsen and Mouritsen 2000; but see discussion Thorup *et al.* 2000 and Mouritsen 2000).

It is difficult, however, even theoretically, to explain how a simple clock-and-compass inherited programme can lead first-autumn marsh warblers (*Acrocephalus palustris*), barred warblers (*Sylvia nisoria*) or spotted flycatchers (*Muscicapa striata*) to their relatively small wintering quarters in Africa (Thorup and Rabøl 2001), unless the birds are able to increase accuracy of their migratory directions by using some external cues. Similarly, it was found by satellite telemetry that first-year Eleonora's falcons (*Falco eleonorae*) migrating from Sardinia to Madagascar through Western Africa demonstrate unexpectedly high concentration of tracks in the last part of their migration, so that they can precisely reach the west coast of the Mozambique Channel and then cross it in the narrow part (Gschweng *et al.* 2008). As the juvenile birds in the study of Gschweng *et al.* (2008) most probably migrated independently from adults, it is again difficult to explain how the birds can perform such a precise first migration by a clock-and-compass programme alone.

Thus, it has been proposed that juvenile birds can combine clock-and-compass programmes with some external cues associated with species or population specific regions situated along migratory route ("signposts") to increase the precision of their migration. In other words, one may hypothesize that juvenile birds keep one course until they reach signpost A. Once this goal has been reached, birds change their migratory direction to reach next intermediate goal region (signpost B) and so on. The last signpost may be the species specific wintering region.

Indeed, during the recent decade new lab-based data supporting the idea of signposts were made available. For example, juvenile thrush nightingales (*Luscinia luscinia*) captured in Sweden and kept during autumn migration in magnetic coils, where they were exposed to the magnetic parameters (total intensity and inclination) simulating their magnetic displacement from Sweden to Northern Egypt, showed a larger increase in body mass than control birds exposed only to the local Swedish geomagnetic field during the same time (Fransson *et al.* 2001, Kullberg *et al.* 2003). Thrush nightingales are long distance migrants travelling from Sweden to sub-equatorial Africa during their autumn migration. On their way to wintering grounds, thrush nightingales congregate in northern Egypt, presumably in preparation for crossing the Sahara Desert - a severe ecological barrier (Frannson *et al.* 2005). Thus, the data of Fransson *et al.* (2001) and Kullberg *et al.* (2003) suggest that juvenile nightingales use the geomagnetic parameters of northern Egypt as a signpost to accumulate sufficient reserves before they start crossing an extensive ecological barrier. Interestingly, first year Swedish European robins – medium-distance migrants wintering in southern Spain and crossing no large ecological barriers during their migration – seem to show no refueling effect of the magnetic displacement from Sweden to Spain (Kullberg *et al.* 2007). These results may indicate that the fuelling reaction depends on the relevance for a given species or even population. Finally, in the study of Åkesson *et al.* (2005), juvenile white-crowned sparrows after passive longitudinal displacement from their natal site in Alaska eastward to the magnetic North Pole (Northern Canada) and then across the 0° declination line abruptly shifted their orientation from the migratory direction to a direction that would lead back to the breeding area or to the normal migratory route. Åkesson *et al.* (2005) suggested that the juvenile birds, once they had crossed the 0° declination line, began compensating for the displacement by using geomagnetic cues alone or together with solar cues. If so, one should consider the possibility that juvenile white-crowned sparrows from Alaska may use, along with a simple clock-and-compass genetic programme, a simple navigation system based on a combination of celestial and geomagnetic information (possibly declination) to correct for extended eastward longitudinal displacements.

In summary, there are data suggesting that first-time migrants use not only a simple clock-and-compass strategy but rather are able to adaptively adjust their spatiotemporal migratory programmes to external factors and, therefore, reach their winter quarter destination more accurately than it has been previously thought. In the next part of my PhD thesis, I will present my own data supporting the idea that juvenile songbird migrants – pied flycatchers – may use a signpost mechanism during their first migration.

6.2 The development of a migratory programme in Siberian pied flycatchers implies a detour around the Central Asia: own contribution

Some long-distance songbird migrants in northern and temperate Palearctic, for example the willow warbler, the yellow-breasted bunting or the pied flycatcher, have lengthy breeding ranges that are extremely extended along the east-west axis, probably due to gradual colonization of cross continental geographical zones in the postglacial time. At the same time, wintering quarters of these species can occupy relatively small regions in the tropics. In such species there are populations breeding near to their wintering grounds and others that breed much further (up to several times) from their wintering grounds. Therefore, juvenile birds from different populations of the same species should have very different inherited migratory programmes because they face very different challenges on their migratory routes in terms of distance, time and food availability.

The cases when the farthest (relative to wintering grounds) population overwinters together with populations breeding much closer to the wintering grounds, despite the fact that other potentially suitable wintering areas exist closer to the breading grounds (Fig. 12A, B), are of a great interest for understanding the evolution of migratory programmes. For example, how could such a *conservatism of wintering quarters* emerge? One may suggest that, in this case, the evolution of spatiotemporal orientation programmes went by small genetic changes that influenced migratory timing and directions as a given species was colonizing new regions. These genetic changes together with natural selection may have resulted in a state when juvenile birds born in newly colonized regions first migrated along the colonization route and then followed the same route that the first colonizers used (e.g., Schüz *et al.* 1971; Fiedler 2003). Thus, nowadays, the initial part of the migratory route of new populations may reflect the route of colonization in the past (Fig. 12A).

But why do juvenile birds from new populations not change their migratory routes? Do changes of migratory programme take a long time? There is a growing body of data showing that genetic changes of migratory programmes resulting in the establishment of new wintering ranges can occur very fast, i.e., during a few decades (Berthold *et al.* 1992; Surtherland 1998; Bearhop *et al.* 2005; Rolshausen *et al.* 2009; see Fiedler 2003 and Newton 2008 for reviews; Fig. 12C). Therefore, the study of orientation programmes in newly established populations breeding very far from the wintering grounds may shed light on the evolution of spatiotemporal orientation programmes in migratory species and help us understand why conservatism of wintering range exists in some species but not in others.

One such case where the farthest (from the wintering quarters) population overwinters with the other populations is European and Siberian populations of pied flycatchers. It is well known that pied flycatchers colonized Western Siberia only in the early 1900s (Rogacheva 1992), Nevertheless, all pied flycatchers overwinter together in sub-Saharan West Africa (Lundberg and Alatalo 1992). Siberian pied flycatchers from Tomsk and Kemerovo regions demonstrate a very high level of site fidelity even after first winter (Grinkov and Gashkov 2003), and, therefore, their orientation programmes must be adaptively tuned to lead juvenile birds across much longer distances than those travelled by their European conspecifics. At the same time, there are almost no data about the migratory route of Siberian pied flycatchers.

It was recently suggested that Siberian pied flycatchers in autumn do not fly through Central Asia or Caucasus (that would be close to the shortest route) but rather through Western Europe as their European conspecifics do. This hypothesis is supported by the following facts: i) during autumn migration, very low numbers of ringing recoveries and captures of this species in central Asia have been reported (Bolshakov 2002), ii) moon-watching data collected in autumn showed that in northwestern Kazakhstan a very high density of migrating small passerine head towards African winter quarters (Bulyuk and Chernetsov 2005), and iii) there are a few ringing recoveries from a juvenile bird ringed in summer in Siberia and found later the same year in Western Europe (Fig. 13).

For my PhD, I took part in the first study (Paper III) aimed to compare orientation programmes in European and Siberian populations of pied flycatchers and to test the hypothesis that Siberian pied flycatchers detour the Central Asia on their autumn migratory route. To do this, we took nestlings from nest boxes at the Courish Spit, the southeastern Baltic coast, and at Alaevo (Kemerovo region, Western Siberia), hand-raised them at their natal sites and tested their orientation with Emlen funnels during autumn migration at least once every 10 days.

In this study, we also addressed the question how geographical location, where juvenile birds are raised and kept before and during their first autumn migration, can influence and/or modify their orientation programme. To test this, we transported siblings of our experimental Siberian pied flycatchers to the Courish Spit, where they were hand-raised and where their orientation was tested alongside of the Baltic pied flycatchers.

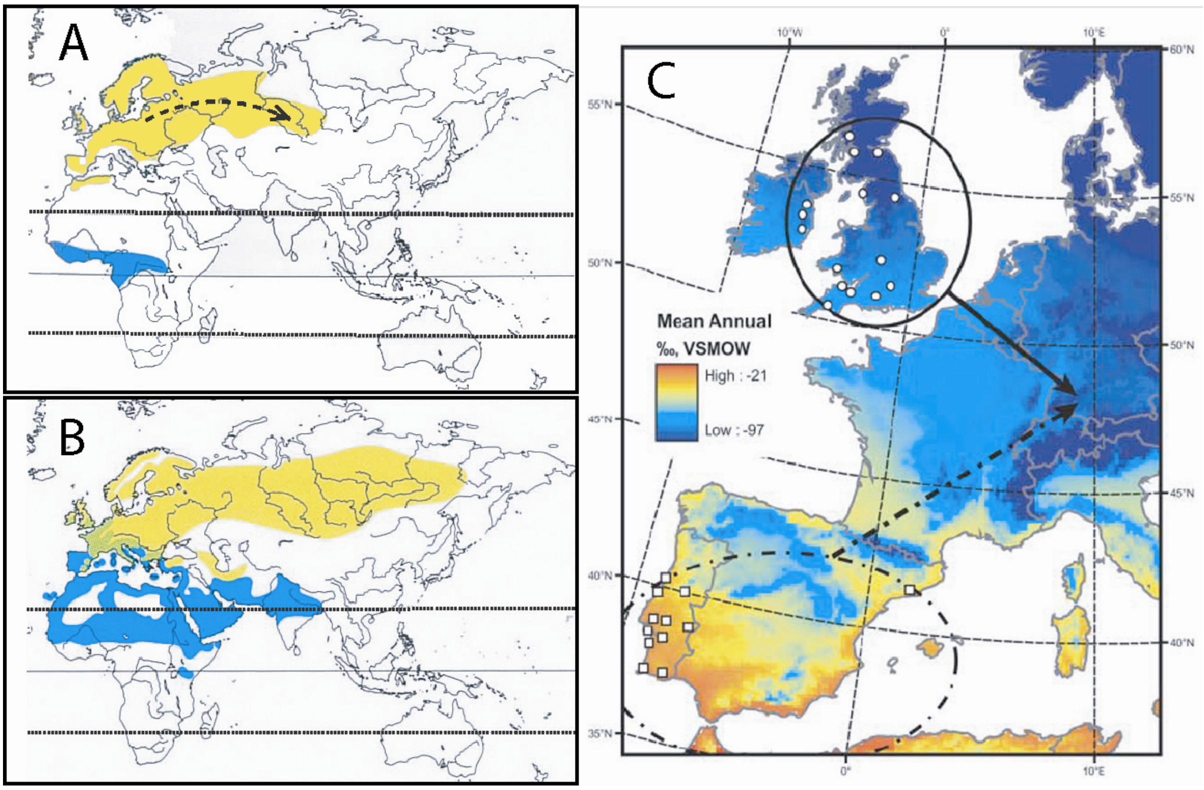

Figure 12. (**A-B**): **Wintering grounds conservatism in pied flycatchers from Europe and Western Siberia compared to more east-west extended wintering region of the common chiffchaff, *Phylloscopus collybita.*** Both the species have breeding ranges extremely elongated in the east-west direction as a result of colonization in the postglacial time. (**A**): *The distribution of the pied flycatcher*. Hereafter, a breeding range is in *yellow*, and a wintering ground is in *blue*. A dashed line arrow shows most probable route of colonization of the eastern most part of the pied flycatcher's breeding range. Interestingly, Siberian pied flycatchers may theoretically overwinter much closer to the natal region (for instance, in India, see Fig. 12B), but they still share the wintering quarter in sub-Saharan West Africa with conspecifics from Western Europe. (**B**): *The distribution of the common chiffchaff*. Birds from the eastern most populations migrate to the Near East and India to overwinter. (A & B are taken from the *Handbook of the birds of the world (2006)* with modifications); **(C): Illustration of a rapid microevolution of orientation behaviour in a songbird species.** Black caps breeding in Germany and Austria, which were previously known to overwinter only in southwestern Europe, recently started establishing new wintering grounds in Ireland and UK *(taken from Bearhop et al. 2005).* An increasing number of black caps began overwintering in Britain and Ireland only since the 1960s. In the study of Bearhop *et al.* (2005), it was found that the black caps overwintering in Spain and Portugal (the wintering range in *a dashed oval*; *white squares* represent sample sites) and the black caps overwintering in Britain and Ireland (the wintering range in *a solid oval*; *white circles* represent sample sites) have significantly different stable isotope (δ^2H) signatures in claws. *Colour gradients* represent distribution of δ^2H in Western European rainfall measured by the international Vienna standard mean ocean water (VSMOW) for δ^2H. This clear difference of δ^2H values in bird's body was used to detect wintering places of black caps breeding in Germany and Austria. It was found that black caps, which are sympatric during summer but wintering in different regions, prefer to mate with conspecifics wintering in the same region where they do. This finding strongly supports the idea that the recently appeared orientation programme leading first-year black caps from Central Europe to the north-west can be genetically fixed in the Central European population by a mechanism of breeding isolation. The *arrows* represent approximate spring migration directions.

Our data suggest that the non-displaced Siberian pied flycatchers orient due west during the first part of their first autumn migration (from late August to mid September, Fig. 13). Severe weather conditions from the beginning of October onward in Alaevo prevented us from testing orientation of the Siberian birds hand raised at Alaevo during the late part of their migration. Thus, our results from Alaevo imply that Siberian pied flycatchers detour around the Central Asia on their autumn migration. Ringing recoveries suggest that Siberian flycatchers most probably travel through Western Europe despite the fact that such a route would increase the length of their journey by up to 15% in comparison with great circle course - the shortest way (Fig. 13).

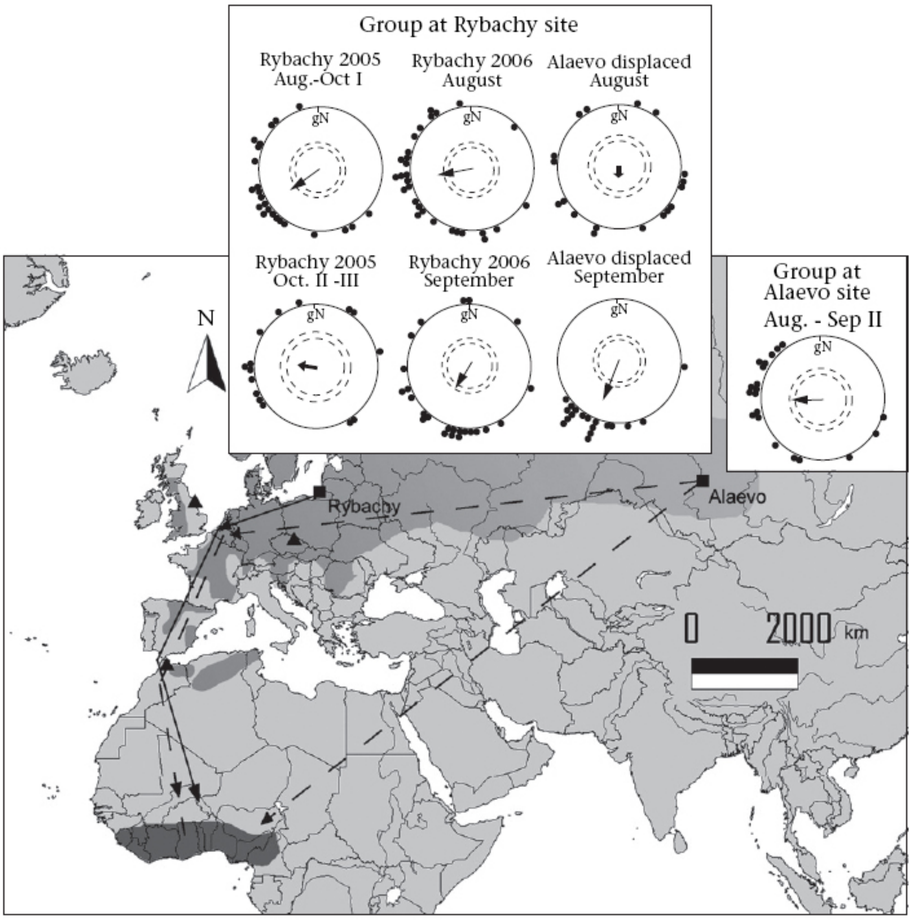

Figure 13. **Orientation of Baltic and Siberian pied flycatchers during their first autumn migration in the Baltic region (Rybachy, Kaliningrad region) and Western Siberia (Alaevo, Kemerovo region).** *The filled symbols* at the periphery of the circles represent the mean headings of individual birds for the given period of time; *arrows* represent grand mean vectors, with their length proportional to the radius of the circle; *gN* means geographical North. *The two inner circles* are the 5% (broken) and 1% (solid) significance borders of the Rayleigh test. *The triangles* indicate recoveries of the birds ringed in Siberia and found later during the same year (UK) or later (Czech Republic and Morocco). *The solid line arrow from Rybachy* indicates migratory route of Baltic birds based on ring recoveries. *The two dashed line arrows from Alaevo* indicate hypothesized migratory routes of Siberian birds. The breeding range is shaded *light grey*; the wintering range is shaded *darker grey*. (*after Paper III*).

The non-displaced, local birds, which hatched and were tested at the Courish Spit, were west-southwesterly oriented in the second half of August, and significantly shifted their orientation towards the southwest later in September (Fig. 13). Interestingly, we found that the birds hatched in Alaevo and displaced to the Courish Spit did not show orientation during August. In September, the displaced birds from Siberia were southwesterly oriented (Fig. 13). This direction differed from the direction shown at the same time by their non-displaced conspecifics at the natal site in Siberia. This result may suggest that our experimental birds displaced at an early age from Western Siberia to Europe might detect the displacement based on some local cues (celestial, geomagnetic, or both) and, thus, modified their migratory programme so that they skipped the first part of their orientation programme leading them due west in the wild, and started performing southwestern orientation at the time when their conspecifics normally reach Europe. Further studies are needed to qualify the proposed hypothesis, and, if confirmed, to find which exogenous cues can modify the orientation programme of pied flycatchers.

7. True navigation in experienced migratory songbirds - terminology

It is now generally accepted by most of avian migration biologists that experienced birds (i.e. birds that have accomplished at least one migratory journey) are able to perform *true navigation,* i.e. they are able to find the direction towards their desired goal from any unknown place even when no cues from the goal are detectable at the unknown location. One should distinguish between *dead reckoning, piloting* and *true navigation* (Fig. 14).

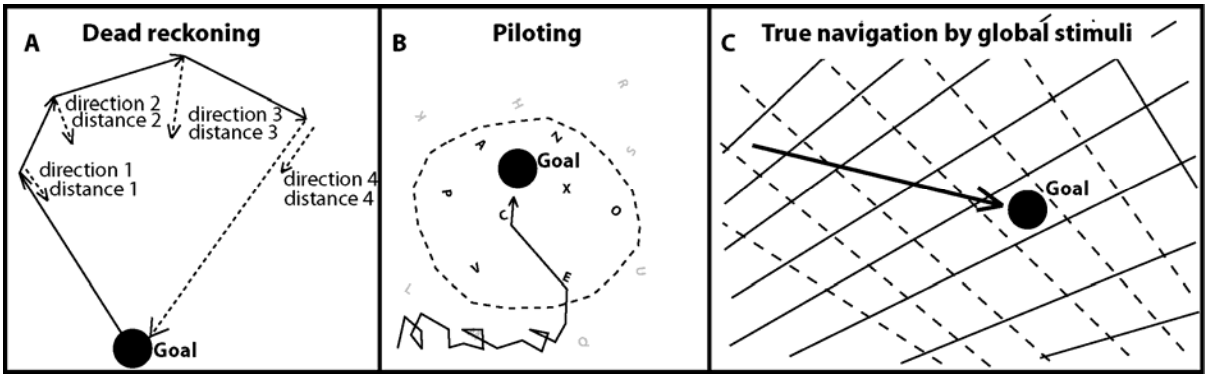

Figure 14. **Alternative navigational strategies**. (**A**): Dead reckoning or path integration strategy. *Solid line arrows* represent the path of the animal off the goal. The *short dashed line arrows* represent an integrated direction and distance needed to reach the goal from each turn point as the animals is moving away from the goal. *A long dashed arrow from the last turn point* represents the homeward path; (**B**): Piloting strategy. *A dashed broken line with arrow head* represents the path of the animal searching for the area with remembered landmarks (*dashed circle*). *Letters* represent visual landmarks: *faded letters* are the unknown landmarks; *bold letters* are the known landmarks; (**C**): True navigation using globally distributed stimuli. *An arrow* represents the path of the animal. *Solid and dashed lines* represent isoclines of some global stimuli. For this kind of navigation, the animal does not to know landmarks around the goal or permanently calculate distance and direction towards the goal, but rather the animal has to learn the patterns of global stimuli's distribution around the goal. (*B&C are adapted from Åkesson 2003*).

Piloting is the method in which an individual retraces a route by the use of fixed references (mostly visual and/or olfactory landmarks) leading towards a destination. In order to perform piloting, one has first to build a landmark-based map during previous journey(s). It has been shown, but only in homing pigeons, that birds are able to use a piloting strategy if they are flying back along familiar routes (Biro *et al.* 2002, 2004; Meade *et al.* 2006). However, piloting does not help if a bird is displaced to the region where it has never been before and, therefore, could not establish a landmark-based map previously.

Dead reckoning or path integration is a process in which an individual determines its current position based on movements made since its last known location. It has been shown that some animals, such as *Cataglyphis* ants (e.g., Wittlinger *et al.* 2006) and rodents (Maaswinkel and Whishaw 1999), are able to use dead reckoning for navigation. However, it has been shown in a few displacement experiments that birds are able to navigate even if it is impossible or very difficult to use path integration (e.g., Matthwes 1951; Wallraff 1980; Wallraff *et al.* 1980).

The main evidence supporting the existence of true navigation abilities in migratory birds has come from displacement experiments. In the late 1950s, it was shown in the classical displacement experiments by Perdeck that adult European starlings are able to compensate for displacement from the Netherland to Switzerland (Perdeck 1958). Later, Mewaldt displaced both first-year and adult golden-crowned sparrows (*Zonotrichia atricapilla*) and white-crowned sparrows of two subspecies (*Z. leucophrys gambelii* – medium-distance migrant and *Z. l. pugetensis* – a short-distance migrant) from their wintering quarters in California across North America to Baton Rouge (Louisiana) and Laurel (Maryland) by jet aircraft. At least some of the displaced birds compensated for the displacements most probably next spring and were recaptured next winter at the same wintering site in California. Interestingly, this returning included a greater percentage of the adults expected to return than of the immature birds expected, and a greater proportion of the medium-distance migrants, *Z. 1. gambelii* and *Z. atricapilla*, than of the short-distance migrant, *Z. 1. pugetensis* (Mewaldt 1964). Recently, the first displacement study was published where true navigation abilities in naturally migrating and radio tracked adult birds (white-crowned sparrows) after the cross-continental displacement was clearly shown (Thorup *et al.* 2007).

In addition to the displacement data, adult birds are known to tend to return to small territories (in some seabirds and raptors even to the same nest-sites) where they have bred and/or overwintered a year before. As numerous studies based on ringing recoveries have shown, return rates of adult birds in successive years can be close to 100% considering the yearly survival rates estimated to be around 30 - 60% in different songbird species (see Sokolov 1997; Newton 2008 for reviews). Without assuming that experienced bird migrants have navigation abilities, it is hard to explain how birds can pinpoint so small areas on the basis of a simple clock-and-compass strategy. Thus, the phenomenon of very precise breeding and/or wintering site fidelity can also be considered, although indirectly, as evidence for the existence of navigation abilities in adult birds. We should keep in mind, however, that not only true navigation, but also a piloting strategy, may explain precise site fidelity.

But how can birds navigate? The most accepted conceptual suggestion came from Gustav Kramer (1953, 1957), who hypothesized that birds use a two step process to reach their destination from any unfamiliar start position: first, the bird detects its current location and direction leading it from the location towards the desired destination (map step), and, second, the bird chooses and maintains the chosen direction using any of its known compass systems or a combination of them until the bird reaches its destination (compass step). By the 1970s, it became known which references birds can use for compass step and how they use compass mechanisms (the sun compass – Kramer 1949, 1950b; the star compass – Emlen 1967a, 1967b, 1975; the magnetic compass – Wiltschko 1968, 1972, 1974; Wiltschko *et al.* 1971; Wiltschko and Wiltschko 1972; see Section 3). The main question challenging avian migration researchers now is: what kind of information do birds use as the basis of their map?

8. The map of birds: a question of coordinates

8.1 Reviewing the literature

Nowadays, to find any location on the globe, people use two geographical coordinates. However, it took several thousands years before humans developed the current bi-coordinate system with longitudes and latitudes, produced detailed maps, and invented precise navigational techniques including global navigation satellite systems allowing us to detect our position with an accuracy down to a few metres. But how can birds having no GPS receiver or detailed maps return to a small breeding or nest-site with an accuracy of, at least, a few kilometres even after travelling several thousands kilometres?

As human navigation techniques are based on two coordinates (latitude and longitude), it is not surprising, although anthropomorphical, that most authors assume that migratory birds should also use bi-coordinate navigation (e.g., Wallraff 1974; Rabøl 1978; Berthold 1991, 1996). However, it is still the matter of debate which natural parameters birds can use as coordinates.

It has been suggested that birds can use the height of starry sky's rotation center above the horizon (Sauer and Sauer 1960; Able 1980; Mouritsen 2003; Gould 2004, 2008), magnetic inclination and/or intensity (Wiltschko and Wiltschko 1995b; Walker *et al.* 2002; Mouritsen 2003) to detect their north-south position – the analogue of latitude.

It is much harder, however, to imagine which natural parameter(s) may serve to detect the east-west position, i.e., as a surrogate for longitude (Åkesson and Alerstam 1998; Mouritsen 2003; Gould 2004, 2008). It should be mentioned here that measuring longitudes was a long lasting challenge for sailors until the 18th century, when marine chronometers that were able to maintain precise time at sea despite variations in motion, temperature, and humidity, were invented (Hutson 1974; Sobel 1997).

It has been proposed that geomagnetic inclination and intensity serve as two coordinates (Lohmann et al. 1994, 2001; Fransson *et al.* 2001; Fischer *et al.* 2003; Freake *et al.* 2006; Kullberg *et al.* 2007; Henshaw *et al.* 2010; Putman *et al.* 2011), but these geomagnetic parameters are not consistently distributed across the surface of the Earth, and in many parts of the Earth's surface they do not make a proper grid (Åkesson and Alerstam 1998). For example, in Western Europe isolines of both total intensity and inclination run almost parallel to geographic latitudes (see the maps here: http://geomag.usgs.gov). Thus, it is hard to imagine how the bi-coordinate map based on these two geomagnetic parameters could function in such regions.

It has been theoretically and experimentally considered that birds may use celestial cues for detection of east-west position (Rabøl 1998). To do this, a bird would need a dual time sense, i.e., two internal clocks, one of which is fixed on home time (Mouritsen and Larsen 2001; Mouritsen 2003). However, the internal clock is known to adapt quickly to local time (Gwinner *et al.* 1997; see Gwinner 1986 for a review), and a fixed-time internal clock has never been shown to exist in any animal (see the double clock hypothesis in Section 8.3 below).

Considering the aforementioned difficulties with establishing a putative bi-coordinate map, it was plausibly hypothesized that migratory birds, particularly young birds one their first spring migration yet having no experience with finding their natal area, may use a *one-coordinate navigation strategy* (Mouritsen 2003, Fig. 15). It means that the birds may remember and identify latitude, but not longitude, of their natal area as well as landmarks around it before their first autumn migration. Next spring, young birds may use a simple clock-and-compass strategy now modified for spring situation, and fly north (situation for the northern hemisphere considered) until they reach the latitude of their natal site destination. If a bird has made a small navigational mistake, but reached an area with visually known landmarks, it may easily pinpoint the natal area using landmark-based map. If a larger navigational mistake has been made, a bird may start searching for the goal moving back and forth along latitude of the natal site and trying to find known landmarks (Fig. 15).

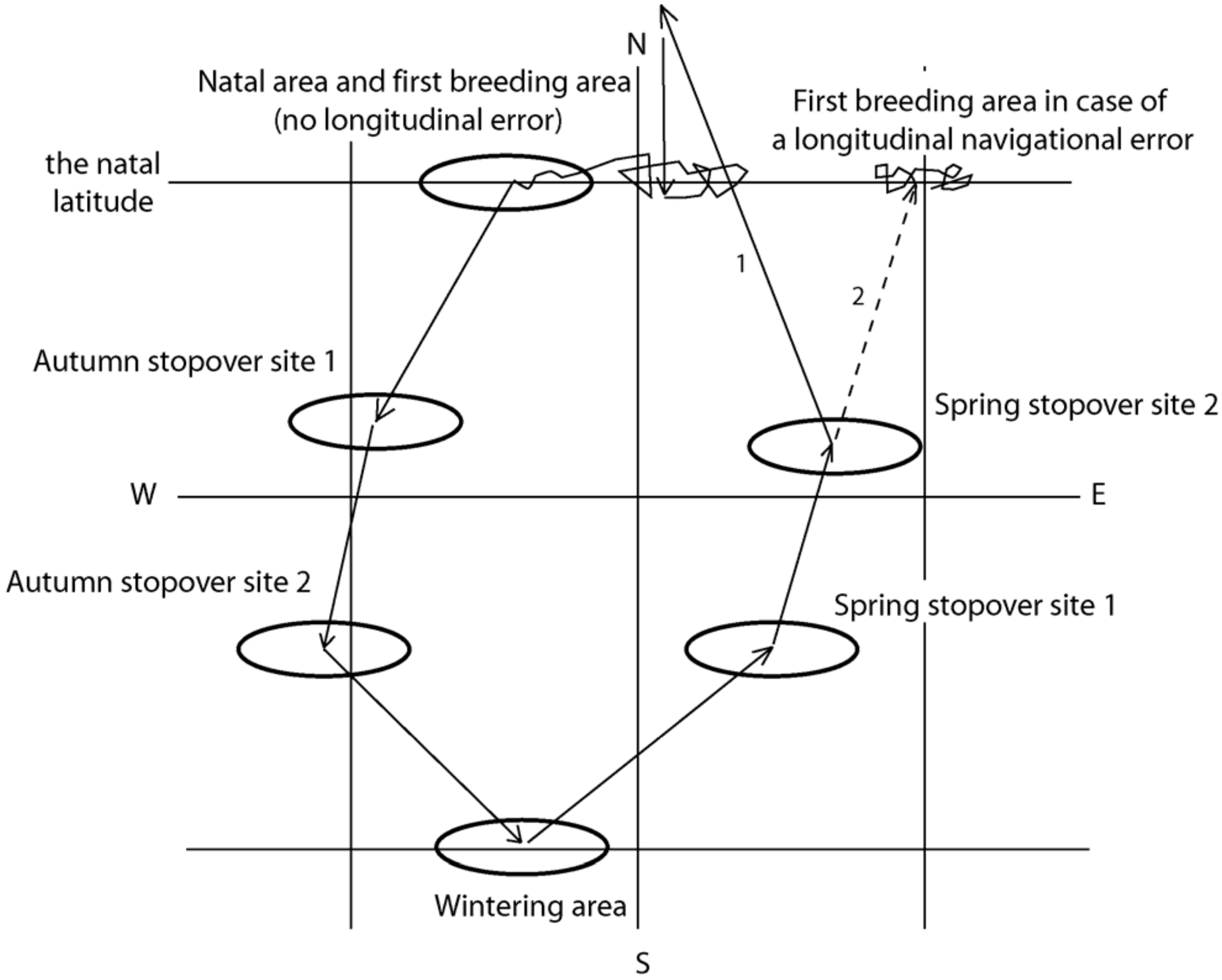

Figure 15. **Principal figure illustrating the one-coordinate hypothesis for a first-year bird migrant**. A first-year bird is able to compensate for a latitudinal navigational error (two *solid arrows*, route 1), but are not able to compensate for a longitudinal error (*a dashed arrow*, route 2). Upon arrival at the remembered natal latitude, a bird starts searching for known landmarks (*solid broken lines*). If a longitudinal error is relative small, a bird can eventually encounter the remembered landmarks around the natal area and can pinpoint the natal area to start breeding (*the left broken line*). If a bird made a large longitudinal error to the east, the searching for the natal area can end up without success and a bird would breed somewhere to the east of the natal area (*the right broken line*). *N* is north, *S* is south, *W* is west, and *E* is east. *Solid horizontal lines* represent geographical latitudes, and *solid vertical lines* represent geographical longitudes. *Ellipses* represent areas with visually remembered landmarks: the natal area becomes remembered between fledging time and the onset of the first autumn migration; stopover sites become remembered during the first autumn and spring migration; winter area becomes remembered during wintering time. *Arrows* represent migratory routes.

Of course, some birds may end up still too far from a familiar area, and, after an unsuccessful search, they have to stay in some suitable area to breed. Later, from the second year onward, the migratory bird has full year migration experience and may visually know landmarks not only around the natal/breeding and wintering sites but also along both the autumn and spring migration routes. There are data indicating that migratory birds possess enhanced spatial memory capabilities during migration (Healy *et al.* 1996; LaDage *et al.* 2010). The one-coordinate hypothesis suggests that from the second autumn migration onward, the experienced migratory bird uses a strategy consisting of a series of simple clock-and-compass migratory steps together with one-coordinate navigation to detect longitude.

The proposed one-coordinate navigation hypothesis (Mouritsen 2003) may actually explain navigational abilities of migratory birds on the basis of a well known clock-and-compass vector navigation combined with one-coordinate (latitudinal) navigation. Latter may be easily based on a celestial cue or/and a geomagnetic parameter. The hypothesis predicts that first-year migrants on their first spring migration should be not able to compensate for east-west displacements but only when a large north-south displacement took place.

There are data from displacement studies that may support this idea (Rüppell 1944; Perdeck 1958). However, there are songbird migrants that have to travel, at least on some extended parts of their migratory routes, along east-west axis (e.g., Siberian pied flycatchers travelling from Western Africa to Western Siberia, see Section 6.2 and Paper III). According to the one-coordinate hypothesis, Siberian pied flycatchers should have very low level of breeding/natal site fidelity, but in contrast they do demonstrate very high site fidelity rates (Grinkov and Gashkov 2003). Thus, it was proposed to test the one-coordinate navigation hypothesis by a properly designed displacement experiment, to which I significantly contributed during my PhD work.

8.2 Testing the navigational abilities in a long-distance migrant, Eurasian reed warbler, after longitudinal displacement: own contribution

In this study (Paper I), Dr. Nikita Chernetsov and I tested the hypothesis whether migratory birds displaced along east-west axis are able to compensate for the displacement. To do this, we mist netted Eurasian reed warblers during spring migration in the Eastern Baltic region (Courish Spit, Kaliningrad region), tested their orientation in Emlen funnels outdoors providing the birds with all known orientation cues (geomagnetic and celestial), and displaced them by aircraft 1,000 km almost due east to Moscow region. After this displacement, we tested the orientation of the birds with the same method again and compared the results of our orientation tests before and after the displacement. We repeated the experiments in three different years.

One should stress here that the Eurasian reed warbler is particularly well suited for such a displacement experiment because of the geographical aspects of its breeding range. Eurasian reed warblers migrate through the Eastern Baltic in spring and breed northeasterly of the capture site in the Baltic countries, Finland and northwestern Russia (Bolshakov *et al.* 2001, 2002; Popelnyukh 2002), but they do not breed in Moscow region. Our displacement due east from the capture site was done intentionally because, in this case, the birds were displaced to, for them, a certainly unknown region southeasterly of any known breeding location (Fig. 16).

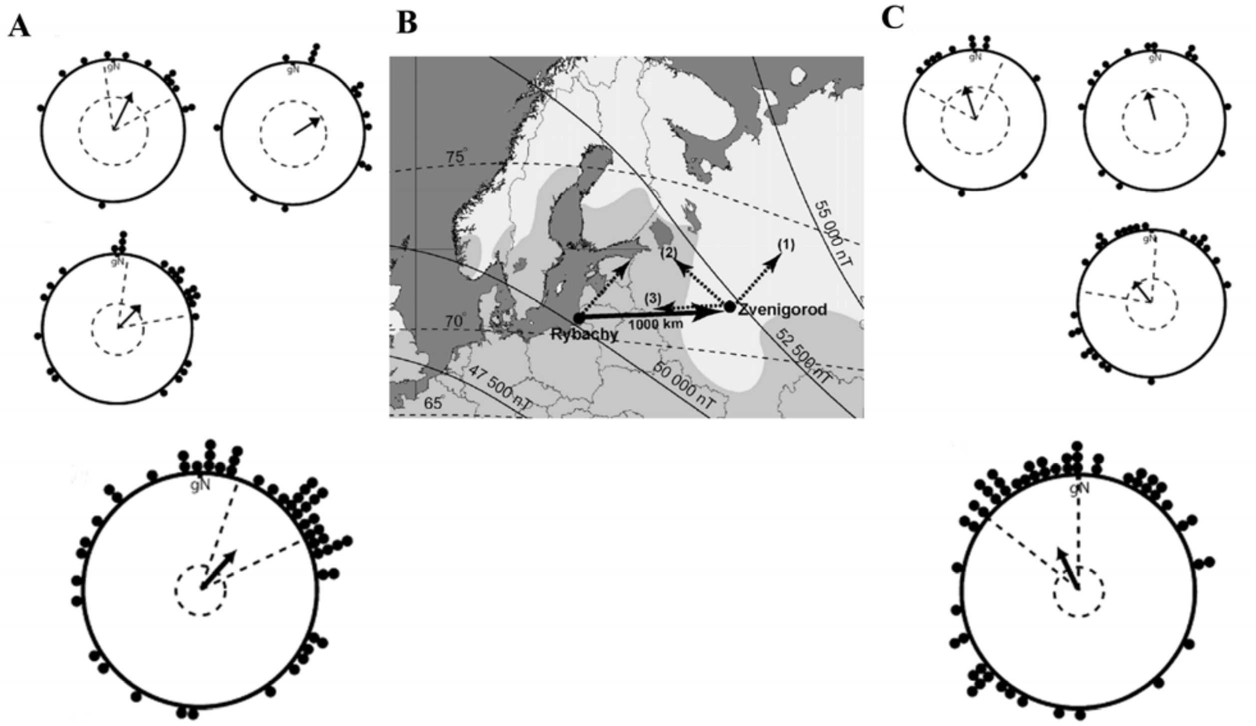

Figure 16. **Test of the one-coordinate navigation hypothesis in Eurasian reed warblers during spring migration**. (**A**): *Upper three small circular diagrams*: orientation of wild caught Eurasian reed warblers tested at the capture site on the Baltic coast (Rybachy, Kaliningrad region) in three different years (*the left diagram in the first row*: α = 26°, r = 0.59, n = 13, and $p = 0.008$, data from 2004; *the right diagram in the first row*: α = 57°, r = 0.45, n = 14, and $p = 0.06$, data from 2005; *thediagram in the second row*: α = 44°, r = 0.42, n = 25, and $p = 0.012$, data from 2007). *A lower large circular diagram*: pooled orientation data of the experimental birds at the capture site: α = 42°, 95% confidence intervals (CIs) 19° - 64°, r = 0.41, n = 52, and $p < 0.001$. Hereafter, *each dot at the circle periphery* indicates the mean orientation of one individual bird, *arrows* show group mean directions, *the dashed circle* indicates the radius of the group mean vector needed for significance ($p < 0.05$) according to the Rayleigh test of uniformity, and *dashed radial lines* show 95% confidence intervals of group mean vectors; (**B**): Position of the study sites with isolines of total intensity and inclination of the geomagnetic field. Map of capture (Rybachy, Kaliningrad region) and displacement (Zvenigorod, Moscow region) sites and the breeding range of the Eurasian reed warbler in the region (*shaded light gray*). *Solid arrow* shows the displacement direction. *The broken arrow at the capture site* shows the mean migratory direction of a given species according to the ring recoveries (Bolshakov *et al.* 2001, 2002), and *the broken arrows* at the displacement site show our working hypotheses: (*1*) no compensation, (*2*) compensation toward the breeding destinations, and (*3*) compensation toward the capture site. *Solid and broken curves* represent the isolines of geomagnetic field total intensity and inclination, respectively (the magnetic data were taken from http://geomag.usgs.gov/ and calculated according to the US / UK World Magnetic Model 2005). (**C**): *Upper three small circular diagrams*: orientation of the same individuals tested after a 1,000 km displacement due east to the vicinity of Moscow (Zvenigorod) in three different years (*the left diagram in the first row*: α = 342°, r = 0.5, n = 13, and $p = 0.035$, data from 2004; *the right diagram in the first row*: α = 345°, r = 0.42, n = 14, and $p = 0.08$, data from 2005; *the diagram in the second row*: α = 322°, r = 0.36, n = 25, and $p = 0.036$, data from 2007). *A lower large circular diagram*: pooled orientation data of the experimental birds after the displacement: α = 334°, 95% CIs 308° - 360° (do not overlap with the CI of the direction shown at the capture site), r = 0.41, n = 52, and $p < 0.001$. (*after Paper I with modifications*)

Our data obtained after three seasons from 52 different individuals were surprisingly consistent and strongly suggested that the displaced Eurasian reed warblers were able to compensate for the east-west displacement even when being tested in the limited space of an Emlen funnel (Paper I). The results of our orientation tests after the displacement indicate that the birds shifted their orientation in such a way that, in the wild, they would be headed towards the breeding range, not the capture site (Fig. 16). Thus, we can conclude that Eurasian reed warblers, typical nocturnal long-distance songbird migrants, are able to detect their east-west location on spring migration.

Our results are at variance with those by Rüppell (1944) who captured hooded crows (*Corvus corone cornix*) during spring migration at the Courish Spit (the Eastern Baltic), displaced them across the Baltic Sea to Flensburg and released. Rüppell's ringed crows were later found northeasterly of the release site in Scandinavia and seemed not to compensate for the east-west displacement but rather proceeded parallel to their migratory route in the Eastern Baltic. One may explain this discrepancy between our and Rüppell's results, first, by the Baltic sea that could serve as an ecological barrier for the Rüppell's crows and, therefore, biased their migratory route, and, second, by the fact that hooded crowns are short-distance daytime migrants, whereas our experiment involved a long-distance nocturnal migrant that is supposed to have superior navigational abilities due to higher pressure of natural selection. At the same time, our data are in line with those of the recent study by Thorup *et al.* (2007) where the adult, but not juvenile, long-distance migrants, white-crowned sparrows, displaced during autumn migration approximately 3,700 km to the east were able to compensate for this displacement.

Taken together, our data and those of Thorup *et al.* (2007) strongly suggest that experienced migrants are able to perform bi-coordinate navigation.

8.3 The problem of longitude and a test of the double-clock hypothesis: own contribution

The data from the above mentioned study with displaced Eurasian reed warblers (Paper I) raises the following question: which mechanism underlies bi-coordinate navigation in migratory birds? In particular, it is still a great mystery how migratory birds can detect longitude.

One of the possibilities would be to use differences in position of the same celestial cues (stars and/or the sun) at the same standard "home" time between two sites (e.g., Matthews 1955; Pennycuick 1960; Dolnik 1981). In the late 1950s, Franz and Eleonore Sauer performed experiments that might be interpreted as evidence that migratory garden warblers and blackcaps use an internal clock running in phase with a local time to compensate for an advanced or delayed planetarium starry sky (e.g., Sauer and Sauer 1960). However, the Sauers' results could not be replicated (e.g., Emlen 1967b; Mouritsen and Larsen 2001). The common drawback of all so far proposed celestial-based true navigation mechanisms is the assumption that birds have a fixed biological clock. However, numerous studies of circadian and circannual rhythms in birds (see Gwinner 1986 for a review) have shown that there are no fixed biological clocks. In contrast, all known biological oscillators relatively quickly synchronize to a local light-dark cycle, which serves as a *Zeitgeber* (from German for "time giver", or "synchronizer"). The strongest Zeitgeber is light-dark regime. No biological substrate for a fixed time clock has been found so far in any living organism.

Nevertheless, we suggested that birds may still use time differences as a longitudinal navigational cue without using non-realistic biological structures like a fixed time clock. During my PhD work, I together with my co-workers proposed and experimentally tested *the hypothesis of a double-clock or jetlag mechanism* (see Paper II). This hypothesis assumes the existence of an ensemble of two biological oscillators ("clocks") in the bird's body, coupled in such a way that one of these clocks is slowly synchronized to the local light-dark (LD) regime, whereas another is the well-known biological oscillator that quickly synchronizes to the local LD cycle. The time difference, albeit transient, between the two clocks would enable birds to determine their east–west position after displacement on the basis of the time-zone (or jetlag) effect. If, for instance, a bird with two such oscillators was displaced eastward and exposed to the local LD regime for some time, long enough to synchronize the fast-entraining clock, that clock would run ahead of the slow-entraining clock until the latter became synchronized with the local photoperiod. The different synchronization speeds of these two oscillators could give the bird navigational information to correct its orientation more towards the west and, thus, at least partly compensate for longitudinal displacement (Fig. 17). This hypothesis has turned out to be of a particular interest in recent years, because recent neurophysiological and molecular evidence suggest that at least some animals possess multiple biological oscillators that become synchronized at different speeds (e.g., de la Iglesia *et al.* 2004, Piggins and Loudon 2005). To test this hypothesis, I together with my co-workers did the experiment in migratory Eurasian reed warblers.

We captured reed warblers during spring migration on the Courish Spit and tested their orientation in Emlen funnels under the capture site photoperiodic conditions and without access to celestial cues (not to give time-related cues).

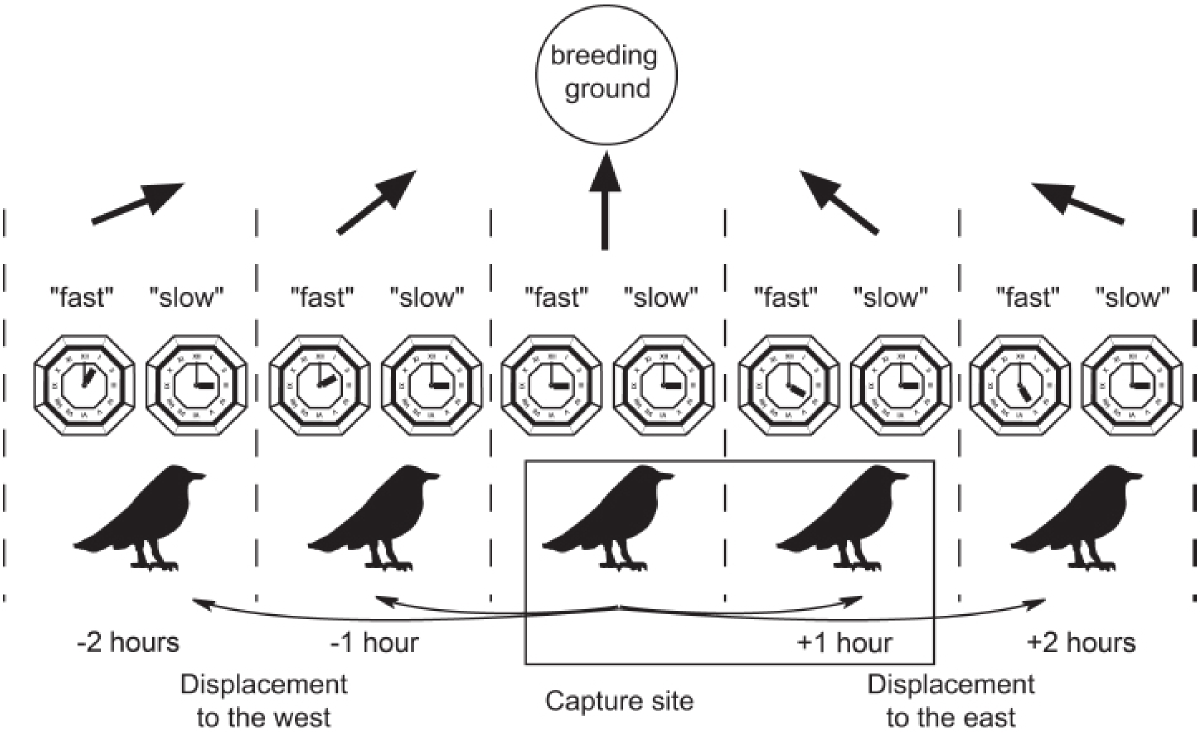

Figure 17. **Schematic illustration of the double-clock hypothesis for spring migrants in Europe in which the breeding ground is a navigational goal**. The bird in the centre stayed at a stopover site for a while and therefore starts out with both its biological clocks — ”fast” and “slow” — entrained by a local light-dark regime. The direction leading the bird to the breeding ground is pointed directly north (*arrow in the centre*). Once we displace a captured bird either to the east or the west to the neighbouring time zones (+1, +2 and −1, −2 h, respectively) discrepancies between the fast- and the slow-entraining clocks will occur until both clocks become resynchronized with each other. The larger the time-zone difference between a capture and a displacement site, the larger the desynchronization between the clocks will be. The magnitude of this desynchronization may help the bird shift its orientation and thereby compensate for displacement (*four tilted arrows* at different angles and pointed toward the breeding ground). In the rectangle the situation simulated in our experiment (Paper II) is shown. (*after Paper II*).

After these northeasterly oriented control tests, we exposed the birds to a LD regime that simulated a 1,000-km eastward displacement to Moscow region from which we have shown that actually displaced Eurasian reed warblers on spring migration compensate for their displacement by orienting northwestwardly (Paper I). As a result, the exposure to the Moscow LD regime did not affect the birds’ orientation when tested without celestial cues, and the birds were still showing northeastern orientation (Fig. 18). We conclude that LD regime effects alone are unlikely to trigger compensation for the longitudinal displacement in long-distance migratory Eurasian reed warblers.

Thus, the question of what cue(s) Eurasian reed warblers used to compensate for east-west displacement in our study (Paper I) seems to be still open. We suggest that our actually displaced Eurasian reed warblers might use some variables related to geomagnetic fields (e.g., total intensity, inclination, or both) as coordinates along both north-south and east-west axes.

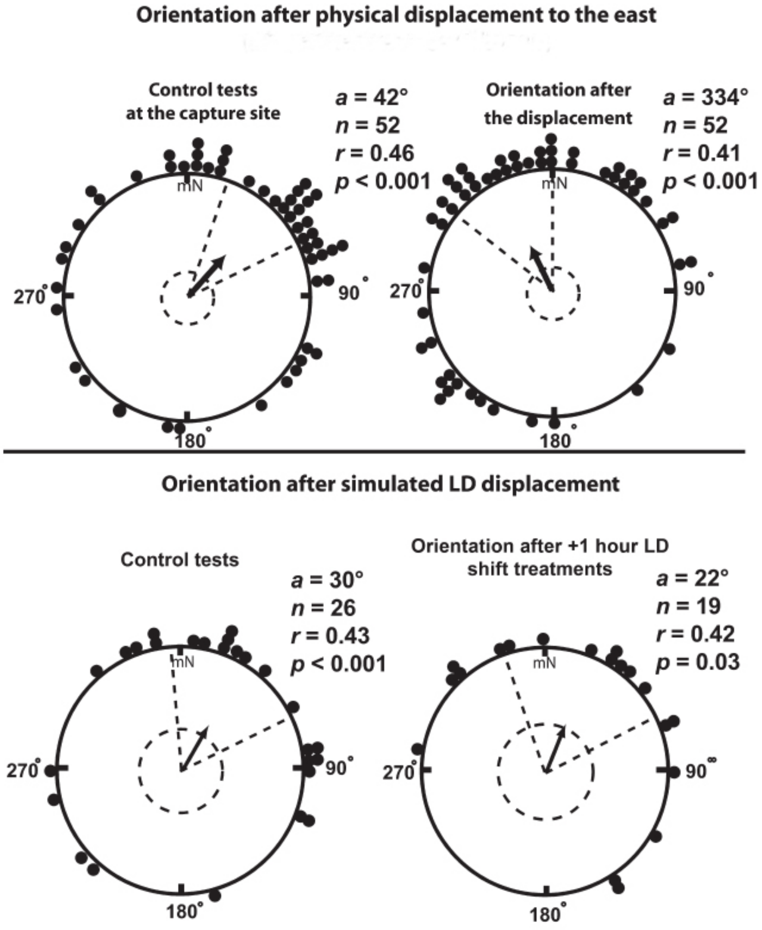

Figure 18. **Test of the double clock hypothesis**. Results of orientation tests before and after physical displacement (data taken from Paper I) compared to results obtained before and after light-dark (LD) regime treatments simulating the same displacement to the east but with the birds remaining at the capture site. Circular diagrams: *each dot at the circle periphery indicates* a mean direction of one bird; *arrows* represent the group mean vectors; *the dashed circle* indicates the radius of the group mean vector needed for significance (*P* < 0.05) according to the Rayleigh test of uniformity; *dashed radial lines* indicate 95% confidence intervals of group mean vector directions; *mN* represents magnetic north; *a* = group mean direction; *n* = number of active birds in given group; *r* = length of a group mean vector; *p* = probability of Rayleigh test. (*after Paper II with modifications*).

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In this case, however, birds should be able to detect rather small difference in intensity and inclination (~3%). Whether such small differences in the geomagnetic parameters are detectable by the avian magnetosensory system is still a matter of debate. Another option may be olfactory-based navigation. Olfactory cues seem to play a significant role in the map component of some homing pigeons (e.g., Gagliardo *et al.* 2006, 2008, 2009) and in determination of the experience-based migratory direction of adult gray catbirds (*Dumetella carolinensis*), a North American medium-distance migrant (Holland *et al.* 2009). Further studies are needed to understand the proposed mechanisms of bi-coordinate navigation.

Conclusion

During my PhD, I was mainly focused on two topics – how migratory birds of different ages can perform long-distance navigation and how migratory birds can perceive the Earth's magnetic field. Both of them are parts of the big question: how migratory birds can solve large-scale spatial tasks such as migration and/or homing over hundreds or even thousands of kilometres?

By using the classical Emlen funnel method to study orientation in night-migratory songbirds, I together with my co-workers provided strong evidence that experienced (i.e., performed at least one migration) Eurasian reed warblers can compensate for a 1,000 km east-west displacement on their spring migration. Hence, these birds should be able to detect longitude – a much more challenging navigational task than detection of latitude. These results suggest that experienced migratory birds use a sophisticated navigational system using at least two different natural cues as surrogates of latitude and longitude to detect their position on the globe (bi-coordinate navigation). At the same time, our data present a new intellectual challenge to bird migration researchers because cue(s) and sensory system(s) that enable avian migrants to determine their east-west position are yet to be found. During my PhD, I together with my colleagues proposed and tested one hypothesis attempting to explain the mechanism of longitudinal navigation.

One of the most discussed hypotheses of longitudinal navigation implies a dual time sense mechanism that includes two internal clocks: one is fixed on home time and another becoming quickly adjusted to a local time. The results of our study suggest that, even if the proposed double clock mechanism exists in Eurasian reed warblers, it is unlikely to be sole cue used for detection of east-west position. In other words, our results indicate that Eurasian reed warblers, and maybe other migratory birds, possess a mechanism for detecting longitude that is independent of time-keeping.

Another navigational study done during my PhD study was devoted to studying the navigational programmes of naïve migratory birds, that is, birds during their first autumn migration. The current consensus in the bird migration researchers' community implies that first-year avian migrants use the clock-and-compass programme to reach their wintering grounds. I allowed myself to question this conclusion because there are avian species whose populations have breeding ranges situated very far from each other but having a common wintering range. Birds from such populations, despite their common ancestors, are faced with very different migratory challenges in sense of time and distance. One of such examples is

European and Siberian pied flycatchers both overwintering in sub-Saharan West Africa. During my PhD, my co-workers and I compared the development of clock-and-compass programmes in first-year pied flycatchers hatched at the Courish Spit (East Baltic) and in Kemerovo region (Western Siberia). Our results suggest that i) Siberian pied flycatchers demonstrate due western orientation at the beginning of their first autumn migration that indicates that they detour the Central Asia on their first autumn migration, and ii) Siberian pied flycatchers displaced as nestlings to East Baltic, raised and tested there during their first autumn migration perform an orientation programme that differs from that of their first year conspecifics raised and tested in Western Siberia. This finding suggests that displaced Siberian juvenile pied flycatchers might modify their orientation programme by using some still unknown external natural cues. This finding indicates that the currently established idea of a simple clock-and-compass strategy must be updated by adding simple signposts, which can modify the basic clock-and-compass strategy.

With respect to magnetoreception in migratory birds, I took part in the study those results strongly suggest that Cluster N – a specialized, night-time active, light-processing forebrain region found in migratory birds – is crucial for performing magnetic compass orientation in European robins. These data support the hypothesis that birds perceive the direction of the Earth's magnetic field with the help of a visually based magnetosensory system, and that Cluster N is a high level processing center of visually based magnetic compass information. At the same time, the beak organ – the putative iron mineral containing receptors in the upper beak – is neither necessary nor sufficient for performing magnetic compass orientation in European robins.

In another study devoted to magnetoreception of migratory songbirds, we showed that European robins are able to use their magnetic compass using any one of their eyes. This result, together with previous data of our group, strongly suggests that the magnetic compass of migratory songbirds is not strongly lateralized to the right eye, as it has been previously proposed (Wiltschko *et al.* 2002).

Last but not least, during my PhD, my co-workers and I undertook a serious attempt to develop an operant conditioning approach based on a previous work made in homing pigeons (Mora *et al.* 2004). We wanted to test for magnetic discrimination behaviour in a migratory songbird – the European robin – using three magnetic anomalies with different peak values of total intensity as discriminative stimuli. Despite a prolonged effort, we did not reach the point when performance of our experimental birds was under control of the used magnetic stimuli. Our negative results probably do not mean that European robins are unable to sense changes of the magnetic field, because not only has the magnetic compass of the

European robin been well documented by numerous previous orientation studies (e.g., Paper IV; see Wiltschko and Wiltschko 1995b for a review), but more recently it was demonstrated in the same species that changing magnetic field induces neuronal activity in two brainstem complexes innervated by the ophthalmic branches of trigeminal nerves (Heyers *et al.* 2010). This is the same nerve that was shown to be crucial for homing pigeons to be able to detect magnetic anomaly (Mora *et al.* 2004). The general adequacy of our setups for operant conditioning with European robins was proven in a control experiment, where our experimental birds developed an obvious discrimination of an auditory stimulus. This indicates that the lack of magnetic conditioning in European robins might be due to a suboptimal combination of stimuli, responses and reinforcement used. Considering a great numbers of previous failures to establish widely used operant conditioning paradigm with magnetic stimuli and/or replicate positive operant conditioning results in other laboratories by independent research groups, we conclude that an operant conditioning paradigm for migratory songbirds, which can be routinely replicable, is yet to be found.

Outlook

Despite several new contributions to our understanding of long-distance navigation in migratory birds made during my PhD, many aspects of the mechanisms underlying this phenomenon are still poorly understood. Particularly, there is a vivid discussion amongst specialists whether migratory birds use olfactory or magnetic cues (or, alternatively, combination of these cues) for long-distance navigation (olfactory vs. magnetic map). On the one hand, recent works made in homing pigeons displaced for 20-60 kilometres (Gagliardo *et al.* 2006, 2008, 2009; Patzke *et al.* 2010) support the hypothesis of an olfactory map rather than the existence of a magnetic map. One the other hand, it is hard, even theoretically, to explain navigation in the cases when birds are displaced across thousands kilometres. Therefore, future studies should thoroughly test the proposed hypotheses not only in the laboratory but also in the wild both in domesticated and wild avian species. The paradigm established in our two studies (Paper I and Paper II) using displaced Eurasian reed warblers seems to be a helpful tool to address these issues. Even if our results do not support the hypothesis of double clock mechanism, they narrow the field of possible hypotheses. In the coming years, we plan to test alternative hypotheses of long-distance navigation using this system.

For the last decade, plenty of new data regarding magnetoreception in birds have appeared. One of my PhD studies supports the idea that the visually based magnetosensory system represents the physiological substrate for the avian magnetic compass. However, the function of the beak organ, to a large extent, remains a riddle. The hypothesis claiming that the beak organ enables a bird to use the magnetic map (e.g., Fleissner *et al.* 2003) has to be tested in future experiments and may shed light upon the mechanism underlying long-distance navigation mentioned above. The recent publication of our group (Heyers *et al.* 2010) strongly suggests that the beak organ, which is neither necessary nor sufficient for the magnetic compass, still transfers magnetically induced signals into the brain in the European robin. Thus, the question arises whether this information gets integrated with visually based magnetic information? If yes, the next question is where in the brain this integration takes place?

The studies in my PhD testing whether the avian magnetic compass is strongly lateralized indicates that the strength of this lateralization might have been overestimated (Wiltschko *et al.* 2002). In fact, we could conclusively show that the claim that the avian magnetic compass is only located in one of bird's eyes is almost certainly wrong. However, due to the conflicting results of Papers V and VI and Wiltschko *et al.* (2002), other research groups should perform independent replications of the lateralization experiments.

The negative outcomes of our operant conditioning study with European robins, along with other unsuccessful attempts to condition birds at magnetic stimuli undertaken before, should be taken into account when one wants to proceed with further attempts to establish an operant conditioning in migratory birds in the future. Particularly, a simpler conditioning design bringing magnetic stimulus, behavioural response and reinforcement in closer spatial and temporal proximity may be a promising approach.

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List of abbreviations

A	arcopallium
B	magnetic field intensity, in Tesla
c	caudal
Cb	cerebellum
CMF	changed magnetic field
CRY	cryptochrome
D	dorsal
DNH	dorsal nucleus of the hyperpallium
E	entopallium
ER	European robin
f	frontal
FAD	Flavin adenine dinucleotide
G	Gauss, unit of measurement of a magnetic field
gN	geographical North
H	hyperpallium
HuC/D	anti-human neuronal protein
I	inclination, in degrees
IEG	immediate early gene
ICo	intercollicular complex
l	lateral
LD	light-darkness
LED	light-emitting diode
m	medial
M	mesopallium
MD	mesopallium dorsal
MV	mesopallium ventrale
mN	magnetic North
MPW	orientation data depicted relative to the magnetic direction towards the pole
N	(on anatomical figures) - nidopallium
N	(on circular diagrams) - north
NMF	normal magnetic field
nT	nano Tesla (10^{-9} T) – SI unit for magnetic flux density
OT	optic tectum
P	pallidum
PI	principal investigator
r	rostral
RL	real lesion
RA	real ablation
S	south
SA	sham ablation
SD	standard deviation
SE	standard error
SL	sham lesion
SP	sampling perch
SPL	sound pressure level
St	striatum
stN	starry sky North
v	ventral

CURRICULUM VITAE

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Education

10/2006 - present	Ph.D. study in AG Neurosensorik (Animal Navigation), at the University of Oldenburg, Oldenburg, Germany. International PhD program “Neurosensory Science and Systems”
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08/1998 - 06/2003	Diploma study (teacher of biology and chemistry) at the Ulyanovsk State Pedagogical University, Ulyanovsk, Russia. Subjects: Biology and chemistry
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Professional experience

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2004 - 2006	Research assistant at the Biological Station “Rybachy” (Rybachy, Kaliningrad region), Zoological Institute of Russian Academy of Sciences, St. Petersburg, Russia
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02/2004	4-week internship in AG Animal Navigation, University of Oldenburg. Acquaintance with scientific projects and laboratory assistance
1999 - 2005	Summer internships at the Biological Station “Rybachy” (Rybachy, Kaliningrad region), Zoological Institute of Russian Academy of Sciences, St. Petersburg, Russia

Scientific publications (11)

2011	Hein, C. M., Engels, S., Kishkinev, D. & Mouritsen H. Robins have a magnetic compass in both eyes. *Nature*, 471, E11
2010	Kishkinev D., Chernetsov N. & Mouritsen H. A double clock or jetlag mechanism is unlikely to be involved in detection of east-west displacements in a long-distance avian migrant. *Auk*, 127, 773-780
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2006	Kishkinev, D.A. Current trends in study of avian orientation and navigation. *Zoologichesky zhurnal*, 85, 342 - 368 (in Russian)
2005	Mukhin, A.L., Chernetsov, N.S. & Kishkinev, D.A. Song of Reed Warbler *Acrocephalus scirpaceus* (Aves, *Sylviidae*) as an acoustic marker of wetland habitat during migration. *Zoologichesky zhurnal*, 84, 995 - 1003 (in Russian)
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Chernetsov, N., Kishkinev, D., Kosarev, V., & Bolshakov, C.V. Not all songbirds calibrate their magnetic compass from twilight cues: a telemetry study. Submitted to *J. Exp. Biol.*

Kishkinev, D., Mouritsen, H. & Mora, C.V. An attempt to develop an operant conditioning paradigm to test for magnetic discrimination behaviour in a migratory songbird. Submitted to *Learn. & Behav.*

Meetings and conferences

2011	*Talk* at the 7th International Conference on Animal Navigation (RIN 11) “Orientation & Navigation of Birds, Humans & Other Animals” of the Royal Institute of Navigation, 6th-9th April, Reading, UK
2009	*Attendance* to the 7th European Ornithologists’ Union Conference, 21st - 26th August, Zurich, Switzerland
2008	*Poster* (the 1st author) at the 6th International Conference on Animal Navigation (RIN 08) “Orientation & Navigation of Birds, Humans & Other Animals” of the Royal Institute of Navigation, 2nd - 4th April, Reading, UK
2007	*Talk* at the 6th European Ornithologists’ Union Conference, 24th - 29th August, Vienna, Austria
2006	*Poster* (the 1st author) at the 12th International Ornithological Conference, 31st January - 3rd February, Stavropol, Russia
2003	*Co-author of a poster* at the 4th European Ornithologists’ Union Conference, 16th – 21st August, Chemnitz, Germany
2001	*Co-author of a talk* at the 11th International Ornithological Conference, Kazan, Russia

Participation in research grants

2005 - 2006	*Research assistant* in the grant: “Populational specificity of an inherent orientational behavior in Pied Flycatcher (*Ficedula hypoleuca*) from the Eastern Baltic (Kaliningrad region) and Tomsk Region (Western Siberia)” funded by the Russian Foundation for Basic Researches. The grant is headed by Dr. hab. Casimir Bolshakov, Biological Station “Rybachy”, Zoological Institute of Russian Academy of Sciences, St. Petersburg, Russia

Fellowships, scholarships, awards

05/2010 - present	Individual PhD Stipends from the Volkswagen-Stiftung
05/2007 – 04/2010	3 year PhD Fellowship from the International Graduate School for Neurosensory Science and Systems (funded by DFG and the University of Oldenburg)
2007	12 month Stipend from Heinz Neumüller-Stiftung (was not accepted)
2006	10 month DAAD Research Fellowship to work at the University of Oldenburg, AG Animal Navigation, supervised by Prof. Dr. Henrik Mouritsen
2006	St. Petersburg Government Award for Young Scientists
2006	Malchevsky Award (St. Petersburg Society of Naturalists) for Master Thesis
2004	St. Petersburg Government Award for Students
2004	Malchevsky Award from the St. Petersburg Society of Naturalists
2002	1 year Russian President Scholarship
2001	Soros Student Award from the George Soros Foundation

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PAPER I

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“A long-distance avian migrant compensates for longitudinal displacement during spring migration”

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Report

A Long-Distance Avian Migrant Compensates for Longitudinal Displacement during Spring Migration

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Summary

In order to perform true bicoordinate navigation, migratory birds need to be able to determine geographic latitude and longitude. The determination of latitude is relatively easy from either stellar or magnetic cues [1–3], but the determination of longitude seems challenging [4, 5]. It has therefore been suggested that migrating birds are unable to perform bicoordinate navigation and that they probably only determine latitude during their return migration [5]. However, proper testing of this hypothesis requires displacement experiments with night-migratory songbirds in spring that have not been performed. We therefore displaced migrating Eurasian reed warblers (*Acrocephalus scirpaceus*) during spring migration about 1000 km toward the east and found that they were correcting for displacements by shifting their orientation from the northeast at the capture site to the northwest after the displacement. This new direction would lead them to their expected breeding areas. Our results suggest that Eurasian reed warblers are able to determine longitude and perform bicoordinate navigation. This finding is surprising and presents a new intellectual challenge to bird migration researchers, namely, which cues enable birds to determine their east-west position.

Results and Discussion

Many displacement experiments have been performed with young birds on their first autumn migration [4, 6–10]. The vast majority of these studies suggest that young birds on their first autumn migration use a very simple spatiotemporal navigation strategy, namely, simple vector navigation, also called the clock-and-compass or, better, the calendar-and-compass strategy [11–13]. In contrast to young birds on their first autumn migration, adult birds and young birds returning in spring have personal experience with their goals and might thus use information collected through experience to refine the orientation strategies [5, 7, 10–12]. Unfortunately, very few displacement experiments have been performed in spring. Consequently, our knowledge about the spatiotemporal navigation strategies of experienced migrants in spring is very sparse and rather speculative. Do birds on return migration in spring perform true navigation toward a specific goal area?

We performed cage experiments under clear natural skies to test whether long-distance avian migrants captured during spring passage and displaced about 1000 km toward the east (Figure 1) are able to compensate for an east-west displacement. Experienced avian migrants are usually assumed to be able to perform true navigation toward the goal of their migration. This notion is based on a few cases of rather high breeding and natal philopatry ([14], but see [5]) and several predominantly north-south displacement experiments during spring passage ([15–17], but see [8]). The hypothesis of true bicoordinate navigation [11, 12] assumes that night-migrating passerine migrants are able to identify at least two coordinates (roughly corresponding to geographic latitude and longitude) globally. Theoretically, migrants can determine geographic latitude from the height of the celestial center of rotation above the horizon and/or from the angle of magnetic inclination or total geomagnetic-field intensity [1–3]. However, the identification of longitude is a problem [5, 18]. Geomagnetic inclination and total intensity could potentially be good map cues [19–21], but in many parts of the world, geomagnetic inclination and total intensity's isolines are almost parallel with latitudes. To use celestial cues for navigation, a bird would need a dual time sense, i.e., two internal clocks, one of which is fixed on home time [4, 5]. However, the internal clock is known to adapt to local time quickly [22–24], and a fixed-time internal clock has never been shown to exist in any animal. Therefore, returning migrants in spring have been suggested to perform one-coordinate navigation, i.e., they know the latitude, but not the longitude, of their migratory destination [5]. Thus, if birds perform one-coordinate navigation on the basis of latitude alone, displaced Eurasian reed warblers (*Acrocephalus scirpaceus*) should not be able to correct for an east-west displacement, whereas they should correct for an east-west displacement if they perform true bicoordinate navigation.

The mean directions shown by spring migratory Eurasian reed warblers on the Baltic coast before displacement were $\alpha = 26^\circ$, $r = 0.59$, $n = 13$, and $p = 0.008$ in 2004; $\alpha = 57^\circ$, $r = 0.45$, $n = 14$, and $p = 0.06$ in 2005; and $\alpha = 44^\circ$, $r = 0.42$, $n = 25$, and $p = 0.012$ in 2007 (Figures 2A–2C). The 3 years were not significantly different from each other (Mardia-Watson-Wheeler [MWW] test: $W = 2.02$, $p = 0.73$), and the pooled sample had a mean direction of $\alpha = 42^\circ$ (95% confidence interval 19°–64°), $r = 0.46$, $n = 52$, and $p < 0.001$ (Figure 2D).

The mean vectors shown by the same individuals after displacement to the vicinity of Moscow were $\alpha = 342^\circ$, $r = 0.50$, $n = 13$, and $p = 0.035$ in 2004; $\alpha = 345^\circ$, $r = 0.42$, $n = 14$, and $p = 0.08$ in 2005; and $\alpha = 322^\circ$, $r = 0.36$, $n = 25$, and $p = 0.036$ in 2007 (Figures 2E–2G). The 3 years were not significantly different from each other (MWW test: $W = 1.88$, $p = 0.76$), and the pooled sample showed a mean direction of $\alpha = 334^\circ$ (95% confidence interval 308°–360°), $r = 0.41$, $n = 52$, and $p < 0.001$ (Figure 2H). The 95% confidence intervals of the pooled samples before and after the displacement did not overlap, and MWW test also indicated a highly significant difference between the orientation of the birds before and after displacement ($W = 14.16$, $p < 0.001$).

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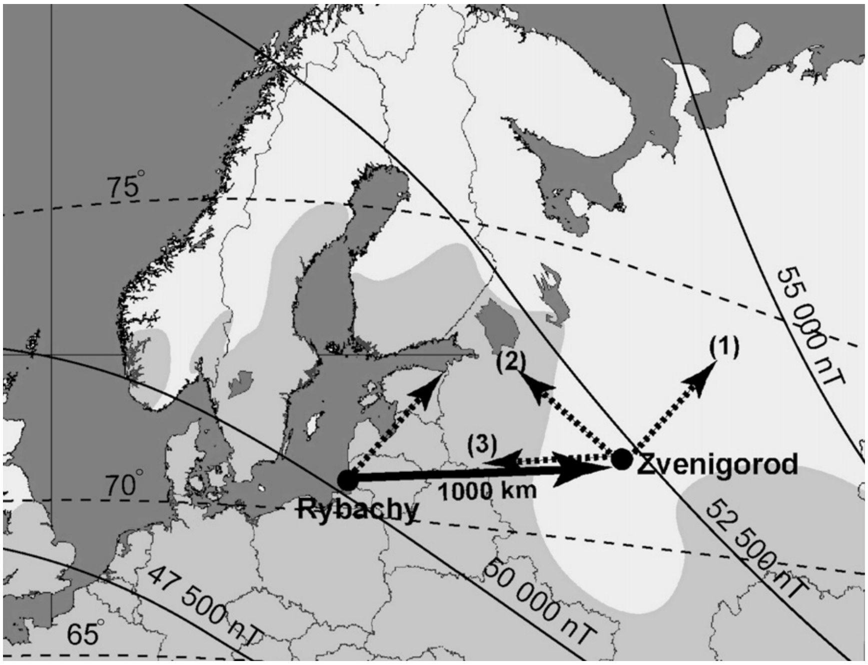

Figure 1. Position of the Study Sites with Isolines of Geomagnetic-Field Total Intensity and Inclination

Map of capture (Rybachy, Kaliningrad region) and displacement (Zvenigorod, Moscow region) sites and the breeding range of Eurasian reed warblers in the region (shaded light gray). Solid arrow shows the displacement direction. The broken arrow at the capture site shows the mean migratory direction, and the broken arrows at the displacement sites show our working hypotheses: (1) no compensation, (2) compensation toward the breeding destinations, and (3) compensation toward the capture site. Solid and broken lines represent the isolines of geomagnetic-field total intensity and inclination, respectively.

Eurasian reed warblers displaced toward the east from their migratory route during spring migration and located southeast of their presumed migratory destinations apparently compensated for the displacement when tested under the natural starry sky in the natural magnetic field. In other words, Eurasian reed warblers seem to be able to perform true bicoordinate navigation during spring migration, i.e., to find their goal without a direct sensory contact with it [25]. By transporting the birds by air, we excluded the possibility that they might have used some positional information for path integration. Our findings refer to birds that were completing their return journey in spring and therefore do not challenge the concept of clock-and-compass strategy in naive first-autumn migrants [5, 12, 26]. Our results are at variance with those by Rüppel [8], who displaced hooded crows (*Corvus corone cornix*) captured during spring migration toward the west. Recoveries suggested that crows did not correct for displacement but moved in parallel to their normal spring migratory route. However, it should be emphasized that hooded crows are short-distance daytime migrants, whereas our experiments involved a long-distance nocturnal migrant. In contrast, our data are in agreement with recent study performed on experienced white-crowned sparrows during autumn migration [27]. This work strongly suggested that adult migrants in the wild can navigate toward their wintering ground even after an east-west, crosscontinental displacement of about 3000 km toward the east. Taken together, this study and that of Thorup et al. [27] suggest that experienced, night-migratory songbirds can perform true navigation. One possibility is that they use the geomagnetic information. In western Russia, where our experiment was performed, it could potentially be used for longitude identification: total intensity and inclination of the geomagnetic-field changed by approximately 3% between Rybachy and Zvenigorod (Figure 1). Another possibility is that birds determine their east-west position on the basis of time lag: Piggins and Loudon [23] have shown that the circadian clock in the suprachiasmatic nucleus

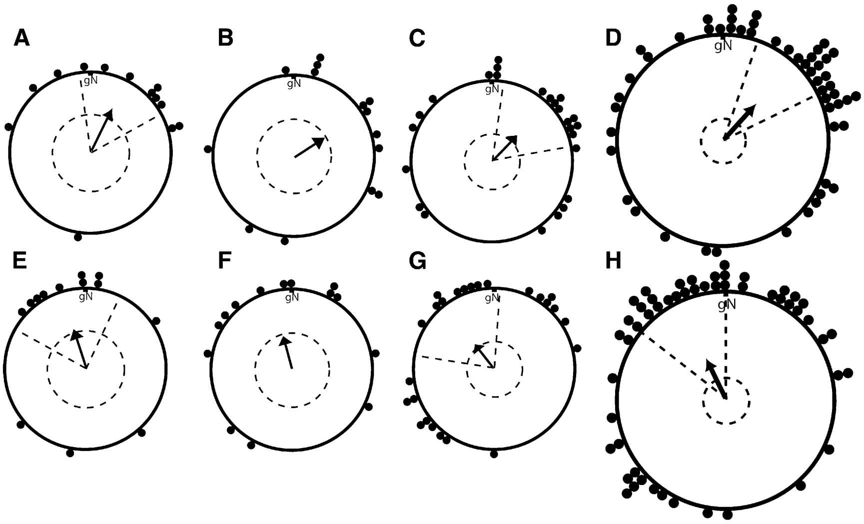

Figure 2. Orientation of Eurasian Reed Warblers during Spring Migration Before and After West-East Displacement

Orientation of Eurasian reed warblers tested at the site of capture during spring migration (A–D) and after displacement 1000 km to the east of the capture site (E–H). All birds were tested in the local geomagnetic field under starry sky.

(A–C) Orientation of the experimental birds at the Courish spit in 2004 (A), 2005 (B), and 2007 (C).

(D) Pooled orientation of the experimental birds at the capture site.

(E–G) Orientation of the experimental birds displaced to the Moscow region in 2004 (E), 2005 (F), and 2007 (G).

(H) Pooled orientation of the experimental birds after displacement. Each dot at the circle periphery indicates the mean orientation of one individual bird, arrows show mean bearings, the dashed circle indicates the radius of the group mean vector needed for significance ($p < 0.05$) according to the Rayleigh test of uniformity, and dashed lines give 95% confidence intervals of group mean vectors.

of mammals consists of two substructures, with a ventral core shifting almost immediately after a clock shift and a dorsal shell lagging behind. This difference, if it were possible for individual animals to measure it, would provide the necessary information on the direction and amount of time change and thus indirectly work as a dual-clock system would do. To show what cues the birds use to determine their east-west location will be a big challenge for researchers in this field in the years to come.

Experimental Procedures

We captured Eurasian reed warblers at Rybachy (southeastern Baltic coast), tested them in cage experiments, and subsequently displaced them by air to Zvenigorod near Moscow, 1004 km toward the east. We recorded the birds' orientation at the capture site and at the displacement site with Emlen cages. Further information on experimental sites and procedures is given in Figure 1 and in the Supplemental Data available online.

Supplemental Data

Experimental Procedures are available at http://www.current-biology.com/cgi/content/full/18/3/188/DC1/.

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Supplemental Data

A Long-Distance Avian Migrant Compensates for Longitudinal Displacement during Spring Migration

Nikita Chernetsov, Dmitry Kishkinev, and Henrik Mouritsen

Supplemental Experimental Procedures

We captured 56 Eurasian reed warblers (*Acrocephalus scirpaceus*) during their spring migration of 2004, 2005, and 2007 at Rybachy on the southeastern Baltic coast (55°09′ N, 20°52′ E). The birds were not aged, so the sample probably included both yearlings and older individuals. Their orientation was tested in Emlen funnels [S1]; after that, the individuals that showed a significant vector were displaced by air to Zvenigorod Biological Station of Moscow State University, some 40 km west of Moscow (55°42′ N, 36°45′ E). The displacement distance was 1004 km (Figure 1). Eurasian reed warblers that migrate through Rybachy in spring have their breeding destinations in the Baltic countries, Finland, and northwestern Russia [S2, S3], and they do not breed further east than the southeastern coast of Lake Ladoga, which is 33°–34° E [S4]. Therefore, the birds displaced to Zvenigorod were most probably southeast of their migratory destinations, and if they compensate for the displacement, they should show northwesterly orientation when tested in Emlen funnels.

In Rybachy, the birds were kept in an indoor aviary with large windows, so that they had a good view of sunset. In Zvenigorod, the birds were kept in cages in a room with fewer windows, so 1 hr before sunset, the cages were put outdoors so that the birds could be provided with the view of the sunset. This was done for the facilitation of a potentially important transfer of directional information from sunset to magnetic cues [S5, S6]. For tests, we used only the birds that had at capture a fat index of 3 or higher on a scale of 0–8 [S7]. Only birds that showed significant orientation vectors at the capture site were selected for displacement.

The Emlen funnels were identical to those used by other authors [S8]. The top opening was covered by cotton netting, allowing a clear view of the sky. The directionality of the birds' activity was recorded as claw marks on typewriter Tipp-Ex correction paper attached on the sloping walls of the funnels. We counted all scratches in each of the 36 10° sectors. Inactive birds (less than 40 scratches) and disoriented individuals (the mean vector not significant) were excluded from analysis. In some cases, thin, barely visible lines ("weak scratches" by [S9]) were much more numerous than "good" (countable) scratches. Because these lines were only visible in a side light and it did not appear possible to count all of them, we fitted the preferred direction by eye. In all cases when both good and weak scratches were visible, the preferred direction derived from them was roughly the same.

The tests were performed in the darkness when the glow from the setting sun had vanished or was as weak as possible and was exactly in the north (in tests after displacement, when it never vanished completely). All tests were performed when at least 50% of a starry sky was visible; in most tests, it was 95%–100% clear. In Rybachy, tests were performed at the earliest 2 days after capture. In Zvenigorod in 2004 and 2005, the birds were tested at the earliest 2 days after the displacement, so that they could synchronize their time sense with the local time, which differed from the local time in Rybachy by 1 hr.

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PAPER II

Kishkinev, D., Chernetsov, N. & Mouritsen, H.

“A double clock or jetlag mechanism is unlikely to be involved in detection of east-west displacement in a long-distance avian migrant”

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A DOUBLE-CLOCK OR JETLAG MECHANISM IS UNLIKELY TO BE INVOLVED IN DETECTION OF EAST–WEST DISPLACEMENTS IN A LONG-DISTANCE AVIAN MIGRANT

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Abstract.—Migratory birds are known to be able to navigate—to determine their position on the globe and the direction toward their distant goal—even if they cannot perceive any information emanating from the goal. One hypothesis, that of true bicoordinate navigation, claims that birds should be able to sense and use a grid of two natural parameters as coordinates. Some indirect data support the idea that migratory birds can determine their north–south position, and several recent studies have suggested that at least long-distance migrants, including the Eurasian Reed Warbler (*Acrocephalus scirpaceus*), can determine their east–west position. How they do this remains a mystery. Birds could theoretically detect the magnitude of east–west displacements if they have two clocks, one synchronizing faster to local time than the other. We tested whether this putative "double-clock" mechanism may serve as a navigational tool for detecting east–west position. We captured Eurasian Reed Warblers during spring migration and tested their orientation in Emlen funnels under capture-site photoperiodic conditions. After these northeasterly oriented control tests, we exposed them to a light–dark regime that simulated a 1,000-km eastward displacement to the Moscow region from which we have shown that actually displaced Eurasian Reed Warblers on spring migration compensate for their displacement by orienting northwestwardly. Exposure to the Moscow light–dark regime did not affect the birds' orientation. Our results suggest that light–dark regime effects alone are unlikely to trigger compensation for the longitudinal displacement in long-distance migratory Eurasian Reed Warblers. *Received 12 February 2010, accepted 4 June 2010.*

Key words: *Acrocephalus scirpaceus,* double-clock hypothesis, Eurasian Reed Warbler, migration, navigation.

Es Poco Probable que un Mecanismo de Doble Reloj o de *Jet Lag* esté Involucrado en la Detección de Desplazamientos Este–Oeste en un Ave Migrante de Larga Distancia

Resumen.—Se conoce que algunas aves migratorias pueden navegar—determinar su posición en el globo y su dirección con respecto a su destino lejano—aún sin percibir información emanada por el destino. Una hipótesis, la de la navegación verdaderamente bicoordinada, propone que las aves estarían en capacidad de percibir y emplear una cuadrícula de dos parámetros como coordenadas. Algunos datos indirectos apoyan la idea de que las aves migratorias pueden determinar su posición norte–sur y varios estudios recientes han sugerido que, al menos los migrantes de larga distancia (incluyendo a *Acrocephalus scirpaceus*), pueden determinar su posición este–oeste. Cómo hacen esto es todavía un misterio. Teóricamente, las aves podrían detectar la magnitud de deplazamientos este–oeste si tuvieran dos relojes, uno de los cuales se sincroniza con la hora local más rápidamente que el otro. Evaluamos si este mecanismo de doble reloj podría actuar como una herramienta de navegación para detectar la posición este–oeste. Capturamos individuos de *A. scirpaceus* durante la migración y evaluamos su orientación en embudos de Emlen bajo las condiciones de fotoperíodo del sitio de captura. Después de esas pruebas de control orientadas al noreste, expusimos a las aves a un régimen de luz y oscuridad que simulaba un desplazamiento hacia el este de 1000 km, a la región de Moscú en donde hemos demostrado que individuos de esta especie efectivamente desplazados durante la migración de primavera compensan el desplazamiento orientándose hacia el noroeste. La exposición al régimen de luz y oscuridad de Moscú no afectó la orientación de las aves. Nuestros resultados sugieren que es poco probable que los efectos de luz y oscuridad por sí solos provoquen la compensación por el desplazamiento longitudinal en esta especie migratoria de larga distancia.

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Each fall, billions of birds migrate from high and temperate latitudes to tropical areas, enduring journeys that cover thousands of kilometers. How do they find their way? Over the past 50 years it has been debated whether migratory birds can perform true navigation (e.g., Kramer 1953; Rabøl 1978, 1998; Berthold 1991, 1996; Mouritsen and Mouritsen 2000; Mouritsen 2001, 2003; Wiltschko and Wiltschko 2003; Alerstam 2006; Chernetsov et al. 2008a). True navigation implies that birds have the ability to determine their position on the globe and the direction toward their distant goal even if they cannot perceive any information emanating from the goal (i.e., across an unfamiliar landscape; Baker 1981). Navigational abilities of migratory birds are supported by both numerous displacement experiments with adult birds (e.g., Perdeck 1958) and by the widespread phenomenon of site fidelity (i.e., the return to natal, breeding, or wintering places; e.g., Sokolov 1997). Given that human navigation techniques are based on two geographic coordinates, it is not surprising that most authors assume that migratory birds also use bicoordinate navigation (e.g., Rabøl 1978; Berthold 1991, 1996).

It has been proposed that birds use the height of the celestial center of rotation above the horizon (Able 1980; Mouritsen 2003; Gould 2004, 2008), the angle of magnetic inclination, or total geomagnetic field intensity (Wiltschko and Wiltschko 1995, Walker et al. 2002, Mouritsen 2003) to detect north–south position.

By contrast, identification of east–west position is a nontrivial task (Mouritsen 2003, Åkesson et al. 2005, Gould 2008). Geomagnetic inclination and total intensity could potentially function as bicoordinate cues (Fransson et al. 2001, Lohmann et al. 2001, Fischer et al. 2003, Freake et al. 2006, Kullberg et al. 2007, Henshaw et al. 2008), but in many parts of the world geomagnetic inclination and total intensity isolines run almost parallel to geographic latitude (e.g., Freake et al. 2006: fig. 2). Another possibility would be to use celestial cues for east–west navigation. In the late 1950s, Franz and Eleonore Sauer performed experiments that might be interpreted as evidence that migratory Garden Warblers (*Sylvia borin*) and Blackcaps (*S. atricapilla*) use an internal clock running in phase with a local time to compensate for an advanced or delayed planetarium starry sky (e.g., Sauer and Sauer 1960). However, the Sauers' results could not be replicated (e.g., Emlen 1967, Mouritsen and Larsen 2001). To use celestial cues such as sunrise and sunset times for navigation, a bird would need a dual time sense, that is, two internal clocks: one, for instance, is fixed on a home time and another one quickly becomes adjusted to a local time (Rabøl 1980, 1998; Mouritsen and Larsen 2001; Mouritsen 2003). The internal clock of animals is known to adapt quickly to local time (e.g., Gwinner 1996, Gwinner et al. 1997, Albus et al. 2005, Piggins and Loudon 2005), but a fixed-time internal clock has never been shown to exist in any animal. Thus, understanding the east–west positioning mechanism in migratory birds remains a challenge for researchers.

Therefore, a parsimonious hypothesis was suggested that during their homeward migration, birds might perform one-coordinate navigation (i.e., they know the latitude, but not the longitude, of their migratory destination; Mouritsen 2003). If so, displaced long-distance migrants such as the Eurasian Reed Warbler (*Acrocephalus scirpaceus*) should not be able to correct for an east–west displacement. However, our previous work showed that during spring, migrating Eurasian Reed Warblers can correct their orientation after being physically displaced 1,000 km east of their migratory routes (Chernetsov et al. 2008b). These data are in agreement with the results of a recent study of adult White-crowned Sparrows (*Zonotrichia leucophrys gambelii*) during fall migration (Thorup et al. 2007). That study strongly suggested that adult migrants in the wild can navigate toward their wintering ground even after an eastward cross-continental displacement of ~3,000 km. Taken together, our displacement study and that of Thorup et al. (2007) suggest that experienced night-migratory songbirds can perform true navigation using at least two natural parameters as coordinates. These coordinates should be ubiquitous or at least widely spread across the globe, predictably changeable across thousands of kilometers, and physiologically accessible for migratory birds.

We suggested that the use of a "double-clock" or jetlag mechanism might be a mechanism for east–west positioning in migratory songbirds (Chernetsov et al. 2008b). Our hypothesis assumed the existence of an ensemble of two biological oscillators ("clocks") in the bird's body, coupled in such a way that one of these clocks, the slow-synchronizing one, is not synchronized (or is synchronized slowly) with the local light–dark (LD) regime, whereas the other, the fast-entraining one, is the well-known biological oscillator that quickly synchronizes with the local light–dark cycle. The time difference between the two clocks would enable birds to determine their east–west position after displacement on the basis of time-zone effects. If, for instance, a bird with two such clocks was displaced eastward and exposed to the local LD regime for some time, long enough to synchronize the fast-entraining clock, that clock would run ahead of the slow-entraining clock until the latter became synchronized with the local photoperiod. The different synchronization speeds of these two oscillators could give the bird navigational information to correct its orientation more toward the west and, thus, at least partly compensate for its orientation mistake (Fig. 1). This hypothesis has turned out to be of particular interest in recent years, because recent neurophysiological and molecular evidence suggest that at least some animals possess multiple biological oscillators that synchronize at different speeds (e.g., de la Iglesia et al. 2004, Piggins and Loudon 2005).

To test whether a double-clock mechanism may enable migratory birds to detect east–west displacements, we captured Eurasian Reed Warblers, which are typical long-distance migrants, during their spring migration at stopover sites and tested their orientation in Emlen funnels after exposing them to either the local LD regime or the LD regime that they would experience after a 1,000-km displacement toward the east. The latter experimental LD treatment was intentionally chosen to simulate photoperiodic and timing aspects of the actual geographic displacement that we performed with birds of the same species in our previous work (Chernetsov et al. 2008b). We did not allow the birds to see any celestial cues from the moment of capture until their release because we wanted to prevent the birds from seeing astronomical cues, which are at variance with their inner clocks and could have therefore informed the birds that they had not been physically moved. We hypothesized that if a double clock was the only mechanism responsible for the detection of an east–west displacement, migratory Eurasian Reed Warblers should show a counterclockwise shift in their orientation after exposure to the LD regime of the displacement site near Moscow, as they did after the physical displacement (Chernetsov et al. 2008b).

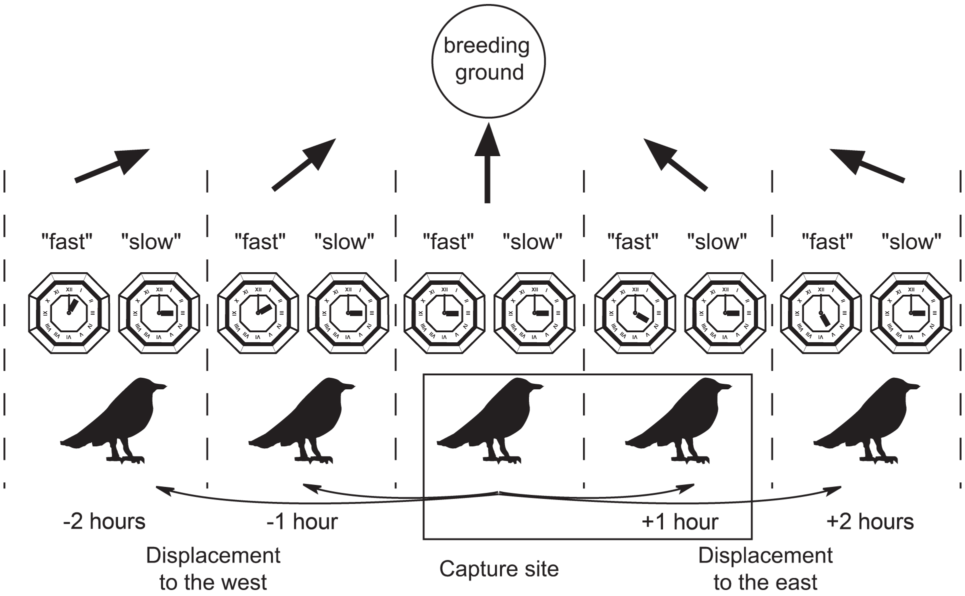

Fig. 1. Schematic illustration of the double-clock hypothesis for spring migrants in Europe in which the breeding ground is a navigational goal. The bird in the center stayed at a stopover site for a while and therefore starts out with both its biological clocks—"fast" and "slow"—entrained by a local light–dark regime. The direction leading the bird to the breeding ground is pointed directly north (arrow in the center). Once we displace a captured bird either to the east or the west to the neighboring time zones (+1, +2 and −1, −2 h, respectively) discrepancies between the fast- and the slow-entraining clocks will occur until both clocks become resynchronized with each other. The larger the time-zone difference between a capture and a displacement site, the larger the desynchronization between the clocks will be. The magnitude of this desynchronization may help the bird shift its orientation and thereby compensate for displacement (four arrows tilted at different angles and pointed toward the breeding ground). In the rectangle the situation simulated in our experiment is shown.

Methods

Study species and site.—The Eurasian Reed Warbler is a common migratory songbird species that breeds across Europe into temperate western Asia and overwinters in sub-Saharan Africa (Cramp and Brooks 1992). We mist netted 37 birds on the Courish Spit in the Kaliningrad Region (Russia) during their spring migration between 16 and 30 May 2008. The birds were kept and tested at the Biological Station Rybachy on the southeastern Baltic coast (55°09′N, 20°52′E; Fig. 2). Eurasian Reed Warblers that migrate through Rybachy in spring breed to the northeast of the study site in the Baltic countries, Finland, and northwestern Russia (Bolshakov et al. 2001, 2002).

The main reason for our use of this particular species is that a 1,000-km displacement due east from the capture site moves the birds southeast of any known breeding location of Eurasian Reed Warblers migrating through Rybachy (Popelnyukh 2002). When exposed to a 1-h advanced LD regime, the birds will experience photoperiodic conditions of the Moscow region, which was the displacement site of our previous study, in which we found compensation for a physical displacement in the same species (Chernetsov et al. 2008b).

Groups of experimental birds, test conditions, and light–dark treatments.—In order to select birds that were probably in a migratory state, we chose individuals with a subcutaneous fat score ≥2 (Kaiser 1993). The birds were not aged, but the sample probably included both yearlings and older individuals. All captured

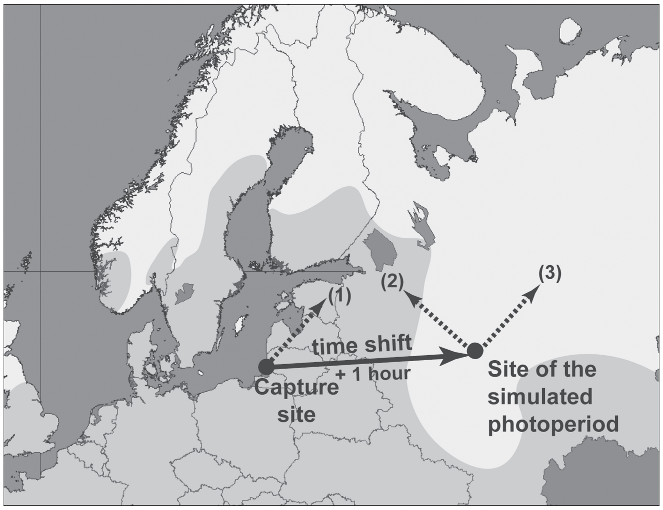

Fig. 2. Map of the capture site (Rybachy, Kaliningrad region, Russia) and the site of simulated photoperiod (Moscow region, Russia). The breeding range of Eurasian Reed Warblers is shaded light gray. The broken arrow (1) at the capture site shows the mean migratory direction of locally captured Eurasian Reed Warblers in spring and, thus, the expected control direction of captured birds. The broken arrows at the site of the simulated photoperiod show our working hypotheses: (2) counterclockwise shift in birds' orientation may indicate the effect of a double-clock mechanism and (3) no change in orientation would indicate no effect of a photoperiod shift on birds' orientation.

birds were kept indoors in a windowless room in individual cages (60 × 20 × 20 cm or 40 × 20 × 20 cm) and were provided with food (mealworms) and water ad libitum.

Each cage was illuminated by both fluorescent (General Electric F18W/33-640 Cool White 1150 Lm) and glow lamps (Philips Classictone 25W, 230V), but not simultaneously. Illumination control was provided by an automatic dimmer (ACD, model 04; Bird-Box Patrick Enger, Viersen-Duelken, Germany). Between the phases of bright daylight and darkness we added twilight periods to simulate transition between light and darkness in a more natural way. As break points between onset and end of each of the three illumination phases, we chose the beginning and end of the civil twilights (i.e., when the position of the sun was either 6° above or 6° below the horizon line). Once the sun disk at a site at which we wanted to simulate the local LD regime was raised higher than −6° below the horizon, the darkness phase ended and the glow lamps began gradually getting brighter until they reached their brightest level at the moment when the sun disk's position was 6° above the horizon (dawn phase). At that moment glow lamps switched off and, simultaneously, fluorescent lamps switched on and lit up the room until the sun's position became lower than 6° above the horizon (daylight phase). At that moment fluorescent lamps switched off. Simultaneously, glow lamps switched on and began gradually getting dimmer until darkness was reached at the time the position of the sun was −6° below the horizon (sunset phase). The orientation tests were performed under dim light (0.01–0.04 lx) because it never gets completely dark in June in Rybachy or Moscow.

We first conducted control tests with all the captured birds. After capture the birds were exposed to the photoregime corresponding to the local one at Rybachy. During the control tests, we obtained significant orientation directions from 26 birds. We kept these 26 birds for further tests and released the other 11 captured birds into the wild.

We divided all the birds with significant control directions into two experimental groups with equal numbers of individuals and tested them during the following 10 days. To avoid any directional biases in our experimental groups, we ranked all the birds' azimuths on a circular diagram from 0° to 360° clockwise and assigned each bird with an odd rank to one group and each bird with an even rank to another one. Once the two experimental groups were formed on 1 June 2008, one group of birds (group 1) was kept on the local LD regime, and the other (group 2) was brought into another room and was kept under the LD regime in which both sunset and sunrise times advanced by 1 h to simulate displacement to Moscow. Group 2 with advanced photoperiodic regime was tested in Emlen funnels for the first time during the second darkness phase on 3 June 2008. The birds that were not active or that showed no significant orientation were tested for the second time 2 days after a time shift on 4 June 2008.

Three days after the beginning of experimental tests (on 4 June) we swapped group 1 and group 2, so that group 1 was now exposed to the advanced LD regime whereas group 2 was brought back to and kept under the local LD regime. After one night (on 6 June), we tested the birds' orientation in the same manner described above. Birds that were not active or that showed no significant orientation were tested again on 7 June.

For the third and the last time we swapped both groups so that group 2 was brought to the advanced LD regime for the second time. This happened on 7 June, six days after the beginning of the experimental tests. After having kept them under the new LD conditions one night, we tested the orientation of group 2 with the advanced photoperiodic regime on 9 and 10 June in the manner described above. The second test of group 2 involved a backshift of the birds by 1 hour, 3 days before the second forward shift. The first test of group 1 and the first test of group 2 did not involve any backshift. If an effect of the first forward shift is observed, one must take a very careful look at the orientation of the backshifted birds. However, if no effect is observed of the first 1-h forward shift, a 1-h backshift is very unlikely to affect the behavior of the birds during the second forward shift.

Group 2 was tested twice, from 3 to 4 June and from 9 to 10 June, whereas group 1 was tested only once, from 6 to 7 June. It was not possible to do a second set of tests with group 1 because of the diminishing migratory directedness of the birds. Care was taken that the birds had no access to any local celestial cues at any time from capture and until all birds were released back into the wild on 11 June.

Splitting of the control group into two experimental groups and swapping of the experimental groups occurred during the daylight phase because the physical displacement to the Moscow region in the work of Chernetsov et al. (2008b) was also done during the day.

Orientation tests and statistics.—We used modified Emlen funnels (Emlen and Emlen 1966) made of aluminium to test the birds' orientation. Emlen funnels are circular orientation cages (top diameter 300 mm, bottom diameter 100 mm, slope 45° with the top opening). To diffuse the light illuminating the funnels and to prevent the birds from seeing celestial cues, we covered the funnels with milky glass (3 mm thick). Each test lasted 40 min and started at the beginning of astronomical twilight. The cages were placed on a wooden table inside a tent placed at Rybachy, so that the birds had access to local geomagnetic cues but no celestial cues. The cages were illuminated by an indirect light from several small incandescent bulbs placed on the ground inside the tent so that the level of illumination inside the cages was 0.01–0.04 lx. Testing of each bird continued until a complete test yielded a significant orientation. This first significant orientation direction was included in our analysis, irrespective of direction. The birds that were inactive or showed random circular activity were tested again. If this occurred two or three times in succession during the control test, no further tests were done and those individuals were released and excluded from analysis. 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. The entire wall of the funnels was covered with the print film, and scratches were counted in each of the thirty-six 10° sectors.

The result of a given test was included only if at least 40 scratches were visible on the print film and a unimodal mean direction was statistically significant (Rayleigh test). From the mean directions of each individual, a sample mean direction and vector length were calculated using vector addition (Rayleigh test, according to Batschelet 1981). Differences in mean direction between experimental groups were analyzed using the overlap of 95% confidence intervals (CI) and the nonparametric Mardia-Watson-Wheeler test. We did not use a powerful but parametric

Watson-Williams test because the r values for our group mean vectors are <0.75. An r value >0.75 is a crucial assumption for this test (Batschelet 1981). Results were regarded as significant if $P < 0.05$. Statistical tests were performed with the ORIANA, version 2.02, statistical package (see Acknowledgments).

RESULTS

Control tests.—The group mean direction of the birds in the control tests was toward north-northeast: $\alpha = 30°$, $r = 0.43$, 95% CI: 355° to 64°, $P < 0.01$; Fig. 3). This direction corresponded to the migratory direction of Eurasian Reed Warblers in the eastern Baltic region, based on ringing recovery data ($\alpha = 19°$, $n = 17$, data from Bolshakov et al. 2001, 2002).

Directions after photoperiodic regime treatments.—From the birds that were exposed to the advanced LD regime, we obtained three mean group directions for the periods from 3 to 4 June (group 2), from 6 to 7 June (group 1), and from 9 to 10 June (group 2 for the second time). Taken separately, all individual mean group directions were nonsignificantly directed because of the few individuals tested in each period. However, after pooling all data and using each individual's mean direction, the experimental birds that experienced an LD-regime to simulate displacement to the Moscow region showed significantly directed orientation toward north-northeast ($\alpha = 22°$, $r = 0.42$, $n = 19$, 95% CI: 341° to 63°, $P = 0.03$; Fig. 3). The 95% CI of this group's mean direction overlapped broadly with that of the mean orientation of the control group, and the Mardia-Watson-Wheeler test showed no significant difference

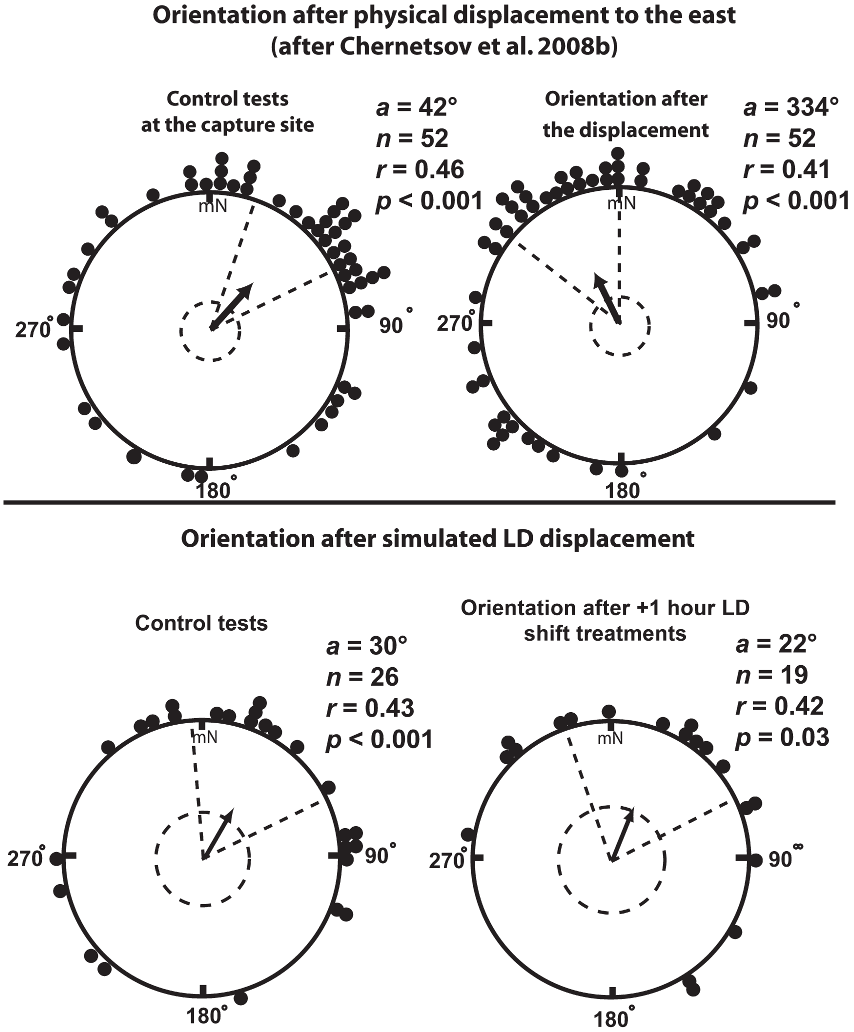

FIG. 3. Results of orientation tests before and after physical displacement (data from Chernetsov et al. 2008b, reproduced with permission) compared to results obtained before and after light–dark (LD) regime treatments simulating the same displacement to the east but with the birds remaining at the capture site. Circular diagrams: each dot at the circle periphery indicates the mean direction of one bird; arrows represent the group mean vectors; the dashed circle indicates the radius of the group mean vector needed for significance ($P < 0.05$) according to the Rayleigh test of uniformity; dashed lines indicate 95% confidence intervals of group mean vector directions; mN represents magnetic north; a = group mean direction; n = number of active birds in given group; r = length of a group mean vector; p = probability of Rayleigh test.

($W = 0.07$, $P = 0.96$). Furthermore, the CI for the mean orientation did not include the 334° mean direction shown by the birds that were actually displaced to the Moscow Region in 2004–2007 (Chernetsov et al. 2008b). Thus, our LD treatments (i.e., exposure of the birds to an advanced photoregime) did not seem to shift the birds' orientation.

Discussion

Our study aimed to test the double-clock or jetlag-mechanism hypothesis for detection of longitudinal displacements in migratory birds. Two earlier studies, which are at first glance similar to ours, were performed by Moore (1980) with Savannah Sparrows (*Passerculus sandwichensis*) and by Able and Cherry (1986) with White-throated Sparrows (*Z. albicollis*), with birds caught and tested during spring. Moore subjected his birds to a 6-h delayed shift and tested them outdoors at an ambient sunset but during the middle hours of the light phase of the artificial photoregime, which the birds were exposed to in captivity. The results showed no influence of the time shift on the birds' orientation. Able and Cherry exposed their birds to a 3-h advanced clock shift. After exposure the birds were tested during their subjective night just after sunset for 45 min and before the appearance of the first stars. The mean orientation of the advanced clock-shifted birds was shifted counterclockwise as compared with the orientation of the control group. These results were in agreement with the hypothesis of a time-compensated sun compass.

Unlike earlier authors who were studying which orientation cues their birds used and therefore exposed their birds to large time-shifts that the birds were allowed to adjust to for 6–8 days (Moore 1980, Able and Cherry 1986), we tested our birds within 36 h after they were subjected to a smaller time-shift, which is the relevant stimulus to determine whether birds can use time effects to perform east–west navigation. This testing shortly after the time change is likely to be important because the hypothetical slow- and fast-entraining clocks would probably become synchronized after a few days. Most importantly, our design simulated the light–dark treatments that our Eurasian Reed Warblers were exposed to during our actual 1,000-km springtime geographic displacement to the east, during which the birds corrected for the displacement (Chernetsov et al. 2008b). Thus, east–west navigational ability during the birds' first spring migration has been demonstrated previously with the same time-shift and the same species captured at the same place during the same season (Chernetsov et al. 2008b). So, if the time-zone difference between the capture and displacement sites was used to perform the east–west navigation in the real displacement, our stimulus should have been able to elicit this compensatory orientation behavior.

That our birds did not seem to use the LD information raises the question of what cues did Eurasian Reed Warblers use to compensate for the real displacement (Chernetsov et al. 2008b)? In theory, the birds may have used some variables related to geomagnetic fields (e.g., total intensity, inclination, or both) as coordinates along both the north–south and the east–west axes. However, Eurasian Reed Warblers would need to detect rather small difference in the intensity and inclination of the geomagnetic field (~3%) to navigate on the basis of geomagnetic field parameters. Another option is olfactory-based navigation. Olfactory cues seem to play a significant role in the map component of some homing pigeons (*Columba livia*; e.g., Gagliardo et al. 2006, 2008, 2009) and in determination of the experience-based migratory direction of adult Gray Catbirds (*Dumetella carolinensis*), a North American medium-distance migrant (Holland et al. 2009).

Our procedures were designed to mimic as precisely as possible the conditions experienced by the birds that we actually displaced to Moscow (Chernetsov et al. 2008b), because this displacement led to compensatory orientation 3 years in a row. Therefore, we advanced the LD regime 1 h during the light phase (the displacement took place by aircraft during the day in Chernetsov et al. 2008b), kept birds one night and the next day under the changed LD conditions (after the actual displacement, we gave the birds one night and day of relaxation after the plane journey), and then tested them during the second and third nights after the shift of the LD regime. We cannot exclude the possibility that a double-clock mechanism, if it exists in nature, works best during the first night after displacement, when we did not test our birds. One should also take into account the fact that night-migratory birds such as Eurasian Reed Warblers in the wild would experience a change in LD regime over a night, not over a day, and that most passerine migrants never encounter such a fast LD regime change as we exposed them to because their natural migratory leaps rarely exceed 500 km (e.g., Cochran et al. 2004, Newton 2008). Thus, we cannot exclude the possibility that a double-clock mechanism might work under more natural conditions. However, we are faced with the fact that the birds that we actually displaced corrected for a 1,000-km eastward displacement under the conditions we simulated in our experiments. They must have used some kind of information to perform this correction. Our results strongly suggest that this information could not have been based on LD information alone. Moreover, there are other studies in which migratory birds displaced by plane across hundreds and thousands of kilometers were still able to navigate (e.g., Perdeck 1958, Thorup et al. 2007). It means that experienced (non-first-autumn) migratory birds are able to correct for displacement even if they have moved rapidly across time zones.

We conclude that LD regime effects alone are unlikely to explain why our physically displaced birds could correct for an eastward displacement to the Moscow region in spring, and it is therefore unlikely that LD regime effects alone can trigger compensation for longitudinal displacement in long-distance migratory Eurasian Reed Warblers. We cannot rule out the possibility that interactions between photoperiod and other sun-related cues such as skylight polarization patterns at different times of day or sunset–sunrise related cues (Cochran et al. 2004, Muheim et al. 2006) could play a role in the proper performance of a putative double-clock mechanism. However, a multitude of photoperiodic studies performed indoors (for reviews, see Gwinner 1986, 1996) showed that changes of LD regime per se are sufficient for various manipulations with biological clocks. Thus, we suggest that even if a double-clock mechanism exists in migratory birds, it is not likely to be the sole cue used when birds detect longitude.

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PAPER III

Chernetsov, N., Kishkinev, D., Gashkov, S., Kosarev, S. & Bolshakov, C.

“Orientation programme of first-year pied flycatchers *Ficedula hypoleuca* from Siberia implies an innate detour around Central Asia”

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Migratory programme of juvenile pied flycatchers, *Ficedula hypoleuca*, from Siberia implies a detour around Central Asia

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Field studies suggest that in autumn, passerine Siberian-African migrants make a detour around Central Asia. We tested whether it results from an innate spatiotemporal programme. We hand-raised juvenile pied flycatchers from Europe and western Siberia in captivity and studied their migratory orientation by testing in Emlen funnels. The birds were kept outdoors in the local natural magnetic field throughout the experiment. Siberian birds showed a purely westerly orientation in mid August–mid September, before changing direction in late September. These data suggest that juvenile Siberian pied flycatchers indeed have an innate spatiotemporal programme that brings them to Europe before migration to West African winter quarters. Siberian pied flycatchers displaced to the Baltic area as nestlings, raised and tested there showed no significant second-order orientation vector in August; in September their mean orientation direction was south-southwestern (202°) and differed significantly from the western direction shown by their conspecifics in Siberia in August–mid September. A possible explanation is that the displaced birds detected displacement on the basis of the innate knowledge of some signposts. They may have 'skipped' the section of the route from Siberia to Europe and 'switched on' their migratory programme when in Europe, already towards the south-southwest.

Keywords: *Ficedula hypoleuca*; migration; orientation; pied flycatcher; spatiotemporal programme

Some long-distance avian migrants, for example willow warblers, *Phylloscopus trochilus*, or yellow-breasted buntings, *Emberiza aureola*, have breeding ranges much extended across longitude, especially in temperate and northern Palaearctic (the so-called Rapoport's rule, Rapoport 1982). In the aforementioned species, all birds spend their winter in rather limited areas, even though some populations may have potentially suitable areas much closer (in SE Asia for willow warblers and in Africa for yellow-breasted buntings). Such evolutionary stasis might be explained by small genetic variation together with strong natural selection of spatiotemporal programmes, and by serious ecological barriers between breeding and potential wintering areas (Merilä et al. 2001; Pulido 2007). On the other hand, several cases of rapid evolutionary change of migratory routes have been reported from different species (Sutherland 1998; Fiedler 2003; Bearhop et al. 2005). For a better understanding of conservatism of some migratory programmes and rapid evolution of others, population studies of reaction norm in migratory traits are indispensable (van Noordwijk et al. 2006).

The pied flycatcher is a typical long-distance migrant with the breeding range extended from the west to the east. It colonized western Siberia in the early 1900s (Rogacheva 1992), but birds from all breeding populations still winter in West Africa (Fig. 1). Currently, migratory routes of the easternmost populations are unexplored. Two possible routes may be hypothesized: a shortcut across arid and mountainous regions of Central Asia or a detour to avoid them (Fig. 1).

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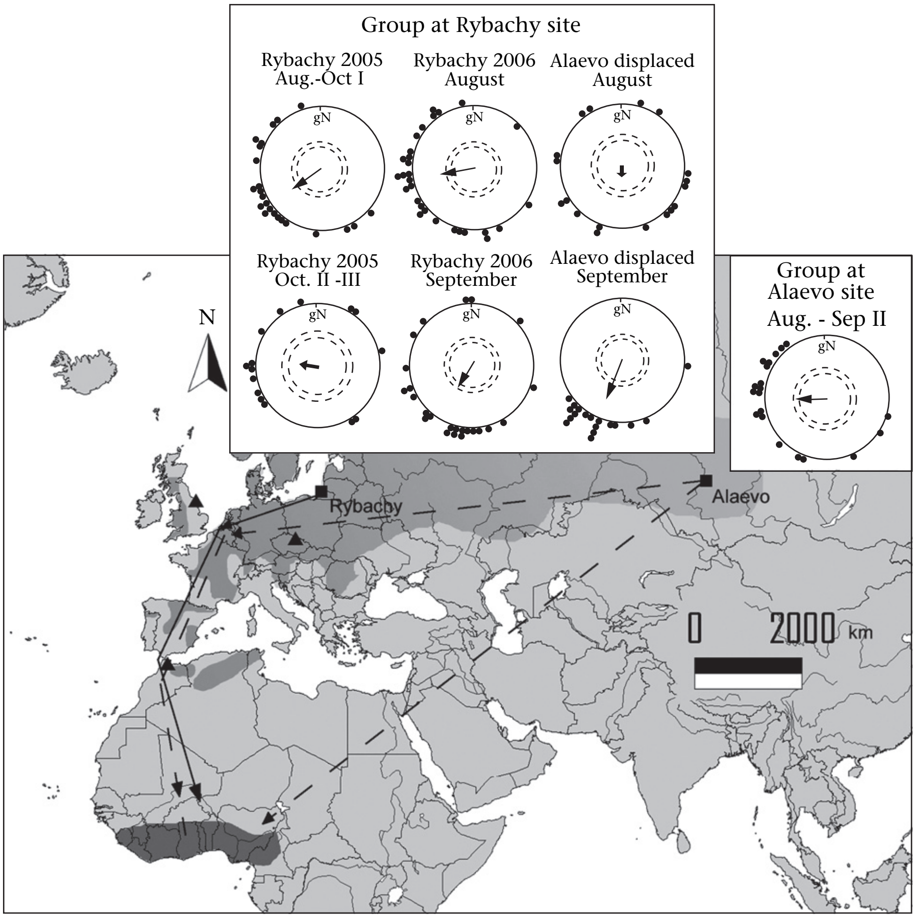

Figure 1. Orientation directions of first-autumn pied flycatchers during their migration in the Baltic region (Rybachy, 2005–2006) and western Siberia (Alaevo, 2006). The filled symbols at the periphery of the circles mark the mean headings of individual birds for the given period of time; arrows represent grand mean vectors, with their length proportional to the radius of the circle = 1; gN means geographical North. The two inner circles are the 5% (broken) and 1% significance borders of the Rayleigh test. The triangles indicate recoveries of the birds ringed in Siberia and found later during the same year (UK) or later (Czech Republic and Morocco). The solid line from Rybachy indicates migratory route of Baltic birds based on recoveries. The two dash lines from Alaevo indicate hypothesized migratory routes of western Siberian birds. The breeding range is shaded light grey; the wintering range is shaded darker grey.

It has been recently suggested that Siberian-African passerine migrants, the pied flycatcher including, do not cross Central Asia during autumn migration but rather make a detour from the north and northwest and migrate via Western Europe (Bolshakov 2002, 2003). This hypothesis is based on scarcity of these migrants in autumn captures in Central Asian oases and the very few ring recoveries (Bolshakov 2002; Fig. 1). However, ring recovery patterns should be treated with caution as reporting probability strongly depends on density and cultural traditions of human population that varies broadly across Eurasia. Moon-watching data from northwestern Kazakhstan showed a very high density of passerine nocturnal migrants heading towards African winter quarters in autumn (Bulyuk & Chernetsov 2005). Unfortunately, moon-watching data do not allow specific identification of small passerines (Bolshakov 1985).

In this paper we report the first experimental evidence that pied flycatchers make a detour around Central Asian deserts and highland that is a part of their innate spatiotemporal migratory programme. We studied autumn migratory directions in hand-raised birds from western Siberia (northern Kemerovo Region) by testing them in Emlen funnels. For comparison, we also tested migratory directions in pied flycatchers from the Baltic area (Kaliningrad Region), and in Siberian birds raised in the Baltic area. We tested the hypothesis that in August–September Baltic birds should show a southwesterly, and Siberian birds a westerly migratory direction. We also tested whether displacement to the Baltic area influences the development of migratory programme in Siberian pied flycatchers.

METHODS

Study Sites and Species

The pied flycatcher is a common passerine in the forest zone of Europe and Western Siberia (Lundberg & Alatalo 1992). Birds from the entire breeding range spend their winter in Western Africa (Borrow & Demey 2001). Pied flycatchers are nocturnal migrants (Bolshakov 1977) with a complex migratory route (Fig. 1). This species has several times been used in the studies of spatiotemporal programmes (Beck & Wiltschko 1988; Weindler et al. 1995, 1998) and is common at both our study sites: on the Baltic coast (Kaliningrad Region, Russia) and in northern Kemerovo Region (western Siberia). In both areas, pied flycatchers mainly breed in nestboxes provided by the Biological Station Rybachy and the Zoological Museum of Tomsk State University, respectively.

Study in the Baltic area was conducted at Fringilla field station of the Biological Station Rybachy (55°05′N, 20°44′E) in 2005–2006. In western Siberia, the study has been done in the village of Alaevo, Yurga district, Kemerovo Region (56°08′N, 84°53′E) in 2006. The distance between these sites is 4027 km (Fig. 1).

Experimental Birds

Siberian pied flycatchers were divided into two groups. Eighteen birds were tested in the natal area. Among them, nine birds, aged 9–11 days, were taken from nests near Alaevo on 28 June 2006 and hand-raised at the natal site. Nine more birds were mist-netted in the same area in August 2006 at early stages of body moult indicating their origin from the near vicinity. Hand-raised birds were kept indoors until 23–25 days old (until 11 July) when they were transferred to an outdoor aviary. They were kept there until the end of the experiment on 26 September. Eighteen more pied flycatchers from the same Siberian population were taken from nests on 25 June 2006 and on the next day, at the age of 10–13 days, transported by air to the Baltic area. During transportation, they were kept in an artificial nest. Food and drinking water were provided to the nestlings continuously during the flights (Tomsk–Moscow and Moscow–Kaliningrad) and when changing flights in Moscow. The displaced Siberian pied flycatchers were kept in a common garden experiment with Baltic birds.

In the Baltic area, we raised 51 birds in 2 study years. In 2005, nestlings were taken from their nests at the age of 8–12 days between 14 and 23 June ($N = 23$). They were hand-raised and kept indoors until 23–34 days old (until 7 July) when they were transferred to an outdoor aviary and kept there until late October. These birds had access to natural celestial and geomagnetic cues at their natal site. In 2006, Baltic nestlings were taken from nests when 7–12 days old between 20 and 22 June ($N = 28$). They were kept indoors until 35–40 days old (until 18 July) and then transferred to outdoor aviaries. All birds were kept in the experiment until late October.

The birds were fed ad libitum by mealworms and ant pupae. They always had access to fresh drinking water with vitamins added. Since fledging, the birds were kept in cages (120 × 100 × 100 cm) inside outdoor aviaries (3.5 × 3.0 × 2.2 m), with 7–10 birds per cage. In every cage, four perches were available to the birds. A part of cage roof was covered with transparent plastic that provided shelter from rain. In cases of heavy rain, the whole aviary was covered by plastic wrapping. Experimental birds never showed any signs of aggression caused by overcrowded conditions.

After the experiments, most birds were killed by asphyxia by quickly rising carbon dioxide concentration. These birds were hand-raised and therefore could not be released, and we had no funds and facilities available to keep them in captivity throughout their lives. At the moment of writing, five Siberian-born individuals were still kept in captivity at the Zoological Museum of Tomsk State University.

Orientation Tests and Statistics

We used modified Emlen funnels (Emlen & Emlen 1966) identical to those used by Mouritsen & Larsen (1998) made of PVC. Emlen funnels are circular orientation cages (top diameter 300 mm, bottom diameter 100 mm, slope 45°) with the top opening covered by a fine-meshed plastic net, allowing the birds to see the sky. The maximum view of the sky for the birds was 168°. Each test was performed during 1 h after the beginning of astronomical twilight, that is when the Sun was at least 12° below the horizon and no glow from the Sun could be seen. The tests were done when at least 50% of the starry sky was visible, in most cases it was 95–100%. The cages were placed on a sand dune (on the Baltic coast) or in a large forest clearing near Alaevo (in Siberia). The birds could not see any landmarks. Each bird was tested until a significant direction was obtained that was included into analysis irrespectively of direction. If some bird was inactive or showed random circular activity it was tested again; if this occurred 2–3 times in succession, no further tests were done during the given 10-day period.

The directionality of the birds' activity was recorded as scratches left by the birds' feet in the pigment of Tipp-Ex typewriter correction paper when they hopped in the funnels. The entire wall of the funnels was covered with typewriter correction paper or transparent plastic sheet, and scratches were counted in each of the 36 10° sectors. The result of a given experiment was only included only if at least 40 scratches were visible on the funnel paper and a unimodal mean direction was apparent. When put into the funnel, the birds showed no signs of distress. Their escape behaviour was limited to several (often none, always less than 10) hops in the funnel. After several minutes of rest, they started to hop towards the preferred migratory direction. When put back into their cage after testing, pied flycatchers showed no distress, either and immediately sat on the perches.

From the mean directions of each individual, a sample mean direction and vector length were calculated using vector addition (Batschelet 1981). When pooling the data across time windows, we calculated the mean direction and vector length from each bird's individual mean vector across this interval. This means that each individual bird contributed only one data point to the sample mean vector to avoid pseudoreplication. The graphical representations and calculations of the circular data were made using a custom-designed computer program. Differences in mean direction between experimental groups were analysed using the nonparametric Mardia–Watson–Wheeler (MWW) test. Difference from an a priori assumed direction was tested by *V* test (Batschelet 1981), results for which

$P > 0.05$ were regarded as nonsignificant. Statistical tests were performed with Oriana 2.0 (www.kovcomp.com).

RESULTS

Orientation of European Pied Flycatchers

In the Baltic area, direction of migratory activity was tested in each bird at least once in 10 days between 3 August–23 October 2005 and 9 August–25 September 2006. In 2005, we performed 10 test sessions (four in August, three in September and October each) with 23 birds and obtained 119 significant directions. In August–early October 2005, the mean direction across this season was 245° (95% confidence interval [CI] 223–268°, $N = 23$, $r = 0.64$, $P < 0.001$, Fig. 1). During the two last sessions in mid and late October, no significant second-order directions were obtained, even though nocturnal activity of individual birds was directed ($N = 15$, $r = 0.35$, $P = 0{,}16$, Fig. 1).

In 2006, we conducted four sessions (two each in August and September) with 28 local birds and recorded 85 significant directions. In August 2006, the mean direction was 252° (95% CI 230–273°, $N = 28$, $r = 0.61$, $P < 0.001$, Fig. 1). This was not significantly different from the direction shown in August–early October 2005 (MWW test: $W = 1.29$, $P = 0.52$). The mean direction shown in September 2006 was 212° (95% CI 183–241°, $N = 24$, $r = 0.52$, $P = 0.001$, Fig. 1) and differed from the one observed in August ($W = 6.57$, $P = 0.037$), despite overlapping 95% CIs.

Orientation of Siberian Pied Flycatchers

In Siberia, we performed six test sessions between 12 August and 25 September 2006 (two in August and four in September) and obtained 109 significant directions. Hand-raised pied flycatchers and birds mist-netted after fledging showed similar preferred directions (MWW test was not applicable because of small sample sizes; Watson U^2 test: $U^2 = 0.125$, $N_1 = N_2 = 8$, $P > 0.10$ for mid August–mid September; $U^2 = 0.037$, $N_1 = 8$, $N_2 = 7$, $P > 0.50$) and were pooled for analysis. The mean direction pooled over the period from mid August to mid September was 269° (95% CI 241–296°, $N = 20$, $r = 0.58$, $P < 0.001$, Fig. 1). The mean direction in late September was strikingly different: 80° (95% CI 37–123°, $N = 15$, $r = 0.48$, $P = 0.031$; MWW test: $W = 15.46$, $P < 0.001$). CIs did not overlap (Table 2).

The mean direction in Siberia during the most part of experiment (late September excluded) was not significantly different from directions shown by Baltic birds in August–early October 2005 (MWW test: $W = 5.22$, $P = 0.074$) and in August 2006 ($W = 1.95$, $P = 0.378$). When the Baltic birds changed their orientation in September 2006 as compared with August (see above), this new direction differed from the one shown by Siberian birds in Siberia ($W = 11.35$, $P = 0.003$).

Five test sessions were performed with Siberian pied flycatchers raised the Baltic area between 9 August and 25 September 2006 (two in August and three in September), with 63 significant directions obtained. The mean direction shown by these birds pooled over August was not significant ($r = 0.19$, $N = 18$, $P = 0.51$). The mean direction in September was 202° (95% CI 189–216°, $N = 18$, $r = 0.87$, $P < 0.001$) and differed significantly from the direction shown by their conspecifics in Siberia in August–mid September (MWW test: $W = 16.86$; $P < 0.001$; 95% CI did not overlap).

DISCUSSION

Migratory Orientation of Baltic Pied Flycatchers

Baltic pied flycatchers showed orientation of their nocturnal activity that was generally similar to flight directions of their free-living conspecifics within Europe, that is during the first half of they autumn migration. The ringing recoveries of birds marked in the eastern Baltic in subsequent years ($N = 4$) show the southwestern direction of 232° (Kishkinev et al. 2006). Orientation of Baltic experimental birds was not significantly different from that direction in either year (V test: 2005: $V = 0.618$; 2006: $V = 0.593$; $P < 0.001$ in both cases).

Mean orientation showed by pied flycatchers from the Courish Spit in August–early October 2005 and August 2006 (245° and 252°, respectively) was more western than the one shown by birds from Latvia raised in the local natural geomagnetic field under a rotating planetarium sky (215°, $r = 0.59$, $N = 18$; Weindler et al. 1995) or under the natural sky (232°, $r = 0.60$, $N = 16$; Weindler et al. 1995). The difference was not significant with our 2005 data (MWW test: $W = 1.91$, $W = 4.92$, respectively, $P > 0.05$) and marginally insignificant with 2006 data ($W = 5.77$, $P = 0.056$; $W = 1.07$, $P > 0.05$, respectively). It should be, however, stressed that unlike our birds, Latvian pied flycatchers were tested with the geomagnetic field as the only directional clue.

In 2005, experimental birds showed mean directions varying between the southwest (239°) and west-northwest (295°) in individual test sessions (mean 245°) until early October (Table 1). In mid and late October, no significant second-order direction was recorded, even through activity of individual birds remained directed. In 2006, pied flycatchers significantly changed their direction in September (south-southwest 212°) as compared with August (west-southwest 252°). We assume that this corresponds to changing flight directions of free-living conspecifics that at this time are making stopovers in the Iberian Peninsula before crossing the Sahara (Bibby & Green 1980). This shift was not observed in 2005.

It is worth noting that our birds throughout the experiment were kept in natural magnetic conditions of their natal site. In other studies, seasonally appropriate changes in orientation direction occurred only when magnetic conditions (field inclination and intensity) were gradually altered imitating movements along the presumed migratory route (Beck & Wiltschko 1982, 1988). It cannot be ruled out that our experimental birds lost

Table 1. Results of orientation tests in Emlen funnels of pied flycatchers hatched in the Baltic area

Group	Testing session	Dates	*N*	Mean vector (°)	*r*	95% CI	Significance (Rayleigh test)
Rybachy 2005	August 1	3–9 August	14	252	0.58	218–286°	0.007
Rybachy 2005	August 2	16–18 August	11				NS
Rybachy 2005	August 3	21–25 August	13	243	0.69	215–270°	0.001
Rybachy 2005	August 4	28 August–1 September	14				NS
Rybachy 2005	September 1	4–7 September	13				NS
Rybachy 2005	September 2	11–17 September	12	295	0.67	265–325°	0.003
Rybachy 2005	September 3	22–27 September	11	263	0.83	240–287°	<0.001
Rybachy 2005	Oct 1	2–7 Oct	11	239	0.72	207–271°	0.002
Rybachy 2005	Oct 2	11–17 Oct	10				NS
Rybachy 2005	Oct 3	18–21 Oct	10				NS
Rybachy 2006	August 2	9–10 August	12	244	0.61	209–279°	0.009
Rybachy 2006	August 3	21–28 August	25	256	0.65	235–277°	<0.001
Rybachy 2006	September 2	17–21 September	21	199	0.51	167–231°	0.003
Rybachy 2006	September 3	23–25 September	23	217	0.41	179–256°	0.02

N, sample size of birds that showed significant orientation in the given session; *r*, length of the mean vector; NS, orientation does not differ significantly from the random circular one.

orientation in October 2005 because they had not reached a natural (e.g. magnetic) signpost. The innate orientation programme of pied flycatchers has been shown to depend on magnetic signposts more strongly than in, for example garden warblers, *Sylvia borin* (Gwinner & Wiltschko 1978; Beck & Wiltschko 1988).

Migratory Orientation of Siberian Pied Flycatchers in Siberia

Siberian pied flycatchers showed in their natal area autumn migratory orientation that indicated passage through Western Europe (Fig. 1). In late September, they abruptly changed direction of their activity from a westerly direction to an easterly one. This could either be a somewhat distorted reflection of a directional change from west to south or south-southeast seen in their free-living conspecifics, or a reflection of the last part of migration when passerine migrants after crossing the Sahara might possibly fly towards the east or southeast along the West African coast (Hilgerloh 1989, 2001). Another possibility is that this behaviour resulted from their failure to reach a certain signpost on the migratory route (cf. disorientation of European birds in October). We could not continue our tests in Siberia into October because of weather conditions that did not permit to keep the birds outdoors.

Our data suggest that first-autumn pied flycatchers from western Siberian populations have a spatiotemporal programme including migration towards the west during August and most September. This programme brings them to Central or Western Europe from where they migrate to West Africa across the Sahara by the same way as European pied flycatchers. This is supported by the few available recoveries of Siberia-ringed pied flycatchers (Fig. 1). It should be stressed that this complex migratory route results from realization of an innate spatiotemporal programme, as shown from our tests with caged inexperienced migrants.

One may argue that this spatiotemporal programme results from evolutionary conservatism and recapitulates the route of expansion of pied flycatchers to Siberia. It has been claimed that migrants have more difficulty to colonize new breeding areas than sedentary species, possibly because of their rigid migratory programmes (Bensch 1999). However, rapid evolutionary changes of migratory

Table 2. Results of orientation tests in Emlen funnels of pied flycatchers hatched in Western Siberia

Group	Testing session	Dates	*N*	Mean vector (°)	*r*	95% CI	Significance (Rayleigh test)
Alaevo	August 2	12–18 August	14				NS
Alaevo	August 3	24–27 August	15				NS
Alaevo	September 1	1–10 September	16				NS
Alaevo	September 2	12–15 September	16				NS
Alaevo	September 3	16–19 September	17	260	0.55	228–292°	0.004
Alaevo	September 4	22–25 September	15	80	0.48	37–123°	0.031
Alaevo disp	August 2	9–16 August	8				NS
Alaevo disp	August 3	21–28 August	18				NS
Alaevo disp	September 1	2 September	6	171	0.72	122–220°	0.037
Alaevo disp	September 2	11–17 September	15	200	0.84	181–219°	<0.001
Alaevo disp	September 3	21–25 September	14	208	0.83	188–229°	<0.001

Group 'Alaevo' was tested at the natal site; group 'Alaevo disp' was displaced to the Baltic area and tested there.
N, sample size of birds that showed significant orientation in the given session; *r*, length of the mean vector; NS, orientation does not differ significantly from the random circular one.

directions causing the development of novel winter quarters have been reported in passerine migrants (Berthold et al. 1992; Hill et al. 1998; Fiedler 2003; Bearhop et al. 2005). Siberian pied flycatchers have not replaced their complex orientation programme by another one that might have enabled them to change their wintering range from West Africa into southern or southeastern Asia. Detours are costly (Alerstam 2001), and if they occur in extant populations, they must bring some benefits, that is making shortcuts must carry costs. In the case of Siberian pied flycatchers, this cost is probably crossing the deserts of western Central Asia that are a serious ecological barrier for migrants in autumn (Chernetsov et al., in press).

Orientation of Siberian Pied Flycatchers Displaced to Europe

Siberian birds displaced to the Baltic area showed no significant direction either in individual test sessions (Table 2) or when the data are pooled across August. In September, their south-southwestern direction differed from the western direction shown in Siberia. A possible explanation is that the displaced birds detected displacement on the basis of the innate knowledge of some astronomical and/or magnetic signposts along the migratory route. They may have 'skipped' the section of the route from Siberia to Europe and 'switched on' their migratory programme when in Europe, already towards the south-southwest. These triggers could be magnetic ones, as a similar mechanism was reported from a German pied flycatcher population (Beck & Wiltschko 1982, 1988); from thrush nightingales, *Luscinia luscinia*, that sharply increased their fuel stores when magnetic conditions typical of North Africa were simulated in the laboratory (Fransson et al. 2001; Kullberg et al. 2003); and from loggerhead turtles that corrected their orientation in a way that allows them to remain in North Atlantic gyre (Lohmann & Lohmann 1994, 1996; Lohmann et al. 2001). When travelling from Siberia to Europe, magnetic intensity changes in a regular pattern and may theoretically be used as a basis for one-coordinate magnetic map (Freake et al. 2006).

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PAPER IV

Zapka, M., Heyers, D., Hein, C.M., Engels, S., Schneider, N.-L., Hans, J.,
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“Visual, but not trigeminal, mediation of magnetic compass information in a migratory bird”

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Visual but not trigeminal mediation of magnetic compass information in a migratory bird

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Magnetic compass information has a key role in bird orientation[1–3], but the physiological mechanisms enabling birds to sense the Earth's magnetic field remain one of the unresolved mysteries in biology[2,4]. Two biophysical mechanisms have become established as the most promising magnetodetection candidates. The iron-mineral-based hypothesis suggests that magnetic information is detected by magnetoreceptors in the upper beak and transmitted through the ophthalmic branch of the trigeminal nerve to the brain[5–10]. The light-dependent hypothesis suggests that magnetic field direction is sensed by radical pair-forming photopigments in the eyes[11–15] and that this visual signal is processed in cluster N, a specialized, night-time active, light-processing forebrain region[16–19]. Here we report that European robins with bilateral lesions of cluster N are unable to show oriented magnetic-compass-guided behaviour but are able to perform sun compass and star compass orientation behaviour. In contrast, bilateral section of the ophthalmic branch of the trigeminal nerve in European robins did not influence the birds' ability to use their magnetic compass for orientation. These data show that cluster N is required for magnetic compass orientation in this species and indicate that it may be specifically involved in processing of magnetic compass information. Furthermore, the data strongly suggest that a vision-mediated mechanism underlies the magnetic compass in this migratory songbird, and that the putative iron-mineral-based receptors in the upper beak connected to the brain by the trigeminal nerve[6–8] are neither necessary nor sufficient for magnetic compass orientation in European robins.

Thirty-six European robins (*Erithacus rubecula*) were caught within 200 m of the testing site and their spontaneous migratory orientation was tested in modified Emlen funnels[20,21] inside wooden huts in the natural magnetic field (NMF) and in a magnetic field with geomagnetic north turned horizontally 120° anticlockwise (CMF). After these control tests confirmed well-oriented magnetic compass behaviour, one of the following surgeries was performed: bilateral section ($N = 7$; for details, see Methods) of the ophthalmic branch of the trigeminal nerve (V_1); trigeminal sham section ($N = 6$; the same treatment except that V_1 was not sectioned); chemical lesion of cluster N ($N = 13$; bilateral, focal injections of ibotenic acid were made into cluster N); or sham lesion of cluster N ($N = 10$; the same treatment but without injection of ibotenic acid). The surgeries were performed by two of us (J.M.W. and D.H.) without the others knowing which bird underwent which surgery. After the surgery and a recovery period of at least one week, the birds were retested under the same magnetic conditions as in the control tests (NMF and CMF) during spring migration. Subsamples of the cluster-N-lesioned or sham-lesioned birds were also tested in a natural magnetic field with an inverted vertical component (IMF). All orientation results were evaluated independently by two or three individuals who did not know which kind of surgery (real or sham) or which magnetic condition the birds had experienced.

The orientation results showed that in European robins the ophthalmic branch of the trigeminal nerve is not necessary (Fig. 1) for magnetic compass orientation, whereas cluster N is necessary (Fig. 2a–f). The sham-sectioned birds oriented north in the geomagnetic field ($\alpha = 10^\circ \pm 20^\circ$ (mean vector orientation angle; 95% confidence interval), $r = 0.95$ (mean vector length), $N = 6$, $P < 0.002$; Fig. 1a). When geomagnetic north was turned to 240°, the same birds oriented west-southwest ($\alpha = 245^\circ \pm 26^\circ$, $r = 0.90$, $N = 6$, $P < 0.005$; Fig. 1b). The trigeminal-sectioned birds also clearly oriented north in the geomagnetic field ($\alpha = 354^\circ \pm 20^\circ$, $r = 0.93$, $N = 7$, $P < 0.001$; Fig. 1d) and west-southwest when geomagnetic north was turned to 240° ($\alpha = 264^\circ \pm 34^\circ$, $r = 0.77$, $N = 7$, $P < 0.01$; Fig. 1e). The 95% confidence intervals in Fig. 1a, b and Fig. 1d, e do not overlap, so we conclude that both groups significantly changed their orientation in response to the turned magnetic field. Furthermore, the mean orientation of both groups under the CMF condition was not significantly different from

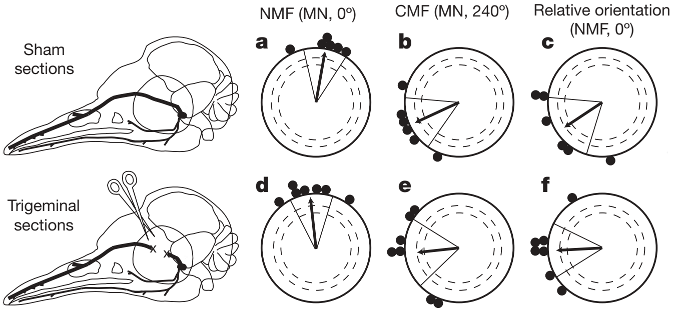

Figure 1 | Bilateral sectioning of the ophthalmic branch of the trigeminal nerve does not affect magnetic compass orientation in European robins. The drawings show the approximate locations of the three branches of the trigeminal nerve. The ophthalmic branch (V_1) is shown in bold. The crosses indicate the approximate locations at which the nerve was sectioned and a piece removed. **a**–**c**, Magnetic orientation of six sham-sectioned birds (MN, magnetic north). **d**–**f**, Magnetic orientation of seven trigeminal-sectioned birds. Each filled circle at the periphery indicates the mean orientation of an individual bird based on nine tests under the given magnetic condition. **c** and **f** compare the orientation of each bird in the turned magnetic field (CMF) with the same bird's orientation in the natural magnetic field (NMF, standardized to 0°). Arrows indicate the group mean vectors. The longer is the group mean vector, the more consistent are the orientation choices between individuals. Inner and outer dashed circles indicate the radii of the group mean vectors needed for directional significance according to the Rayleigh test (inner, $P < 0.05$; outer, $P < 0.01$). Radial lines flanking the group mean vector mark the 95% confidence interval for the group mean direction.

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that expected to result from a magnetic field turn of $-120°$, but was significantly different from the same birds' orientation under the NMF condition (the 95% confidence intervals of Fig. 1c, f include 240° but do not include 0°).

The sham-lesioned birds oriented north-northeast ($\alpha = 25° \pm 21°$, $r = 0.90$, $N = 10$, $P < 0.001$; Fig. 2a) in the geomagnetic field. When geomagnetic north was turned to 240°, the same birds oriented southwest ($\alpha = 219° \pm 26°$, $r = 0.83$, $N = 10$, $P < 0.001$; Fig. 2b), and when the vertical component of the geomagnetic field was inverted, the same birds oriented south-southeast ($\alpha = 154° \pm 37°$, $r = 0.69$, $N = 10$, $P < 0.01$; Fig. 2c). None of these 95% confidence intervals overlap, so we conclude that the birds oriented significantly differently under the three magnetic conditions. Judging from the 95% confidence intervals, the orientations of the CMF and IMF groups were not significantly different from those expected to result from magnetic field turns of $-120°$ and 180°.

In contrast to the well-oriented, sham-lesioned birds, the birds with a chemical lesion of cluster N oriented randomly under all of the three magnetic field conditions (NMF: $\alpha = 355°$, $r = 0.33$, $N = 13$, $P = 0.25$; CMF: $\alpha = 318°$, $r = 0.40$, $N = 13$, $P = 0.13$; IMF: $\alpha = 120°$, $r = 0.07$, $N = 7$, $P > 0.90$; Fig. 2d–f). Furthermore, the consistency of the birds' directional choices between tests was significantly poorer in the cluster-N-lesioned birds than in the sham-lesioned birds (comparing the r values for the individual mean directions in NMF: t-test, $N_{\text{lesion}} = 13$, $N_{\text{sham}} = 10$, $t = 3.160$, $P < 0.01$). Consequently, birds with lesions of cluster N cannot perform magnetic compass orientation in an orientation cage. The fact that the cluster-N-lesioned birds, which possessed intact trigeminal nerves, did not orient indicates that information transmitted through the ophthalmic branch of the trigeminal nerve is not sufficient for magnetic compass orientation in European robins.

After completion of the experiments, all birds that had undergone real (non-sham) surgery were killed for anatomical/histological analysis. In all trigeminal-sectioned specimens, the nerves were found not to have rejoined. Brains from cluster-N-lesioned birds were sectioned and stained for anti-human neuronal protein[22] (anti-HuC (also known as anti-ELAVL3) or anti-HuD (anti-ELAVL4), Molecular Probes), which enabled us to determine the extent of the lesions. Most birds had well-placed lesions covering at least 66% of cluster N on both sides of the brain (mean ± s.d., 78 ± 9%). In three of the 13 lesioned birds, less than 50% of cluster N was lesioned (no. 14: 63% of left side, 33% of right side; no. 16: 17% of left side, 24% of right side; no. 18: 35% of left side, 29% of right side). We note that these three birds oriented well under all three magnetic field conditions ($N = 3$; NMF: $\alpha = 27°$, $r = 0.82$; CMF: $\alpha = 249°$, $r = 0.97$; IMF: $\alpha = 184°$, $r = 0.81$; mean vectors lie within the 99% confidence intervals of the mean orientation of the sham-lesioned birds under all magnetic field conditions; Fig. 2d–f, open circles).

Our results, and the fact that cluster N is part of the visual system[16–19], appear to strongly support the hypothesis that magnetic compass input is processed in the visual system of night-migratory passerines. However, we consider two alternative explanations. The first is reduced general night-vision capability. Considering that cluster N is part of the visual Wulst[18] and that it is known to process night-time light-dependent information[16,17], we tested whether a reduction of general night-vision capability could explain the difference in orientation performance between cluster-N-lesioned and sham-lesioned birds. First, we noticed that the cluster-N-lesioned birds also showed a high level of migratory restlessness in the funnels (346 ± 184 (mean ± s.d.) scratches per hour and per active test). In contrast, when we tested European robins in complete darkness, that is, when they were unable to see, they showed very little migratory restlessness (on average <20 scratches per hour). Second, Wulst lesions in pigeons (*Columba livia*) have been shown to affect the threshold for detecting the intensity of a dim point of light[23]. Therefore, we performed operant conditioning tests in which European robins, one group with cluster N lesions and one group without, were trained to detect a dim point of light. Both groups could perform this visual discrimination task at light intensities 400 times dimmer than the light level under which the magnetic compass orientation tests were

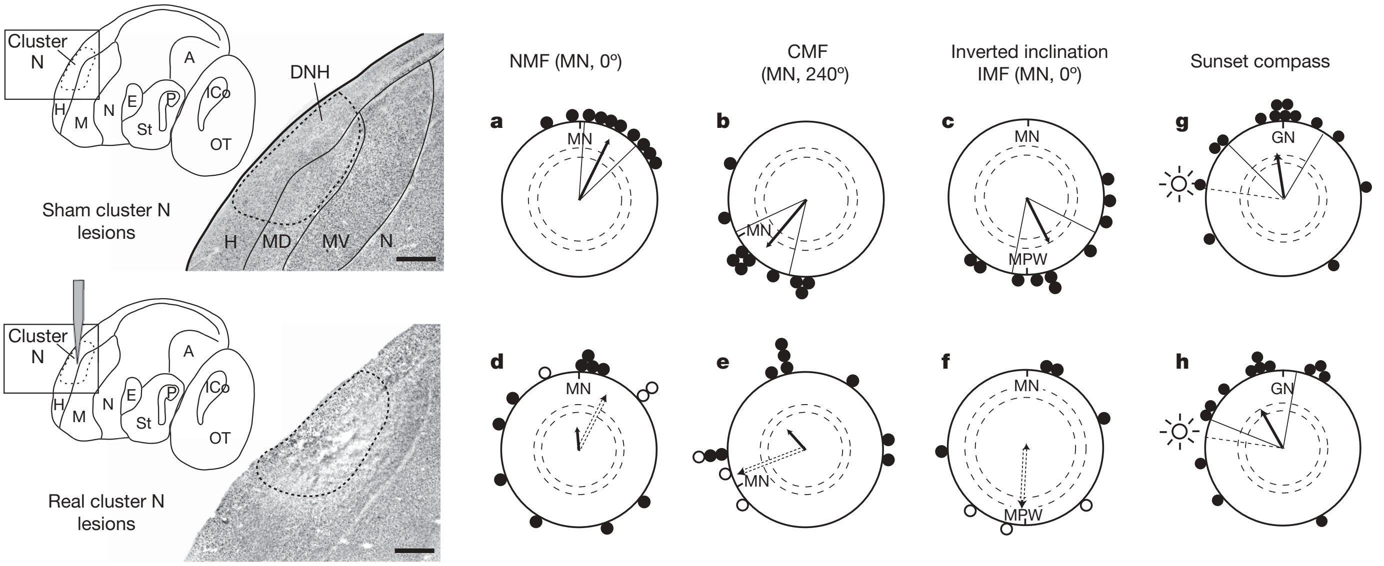

Figure 2 | Bilateral lesions of cluster N disrupt magnetic compass orientation in European robins. The photos show examples of brain sections from a sham-lesioned (top) and an actually lesioned (bottom) European robin, each saggitally cut through the centre of cluster N and stained with anti-HuC/HuD (a neuronal marker). The tissue where cluster N should have been in the lesioned bird is destroyed (compare with top photo). The drawings indicate where in the brain the photos were taken: A, arcopallium; E, entopallium; H, hyperpallium; DNH, dorsal nucleus of the hyperpallium; ICo, intercollicular complex; M, mesopallium (MD, mesopallium dorsale; MV, mesopallium ventrale); N, nidopallium; OT, optic tectum; P, pallidum; St, striatum. Rostral, left; caudal, right. Scale bar, 500 μm. **a**–**c**, Well-oriented, springtime, magnetic compass behaviour of the ten birds that received sham cluster N lesions (MPW, direction of magnetic pole for an inclination compass). **d**–**f**, Non-oriented magnetic compass behaviour of the 13 cluster-N-lesioned birds. The data are means of 14 tests of each individual in the NMF, 13 tests in the CMF and 8 tests in the IMF. The open circles in **d**–**f** show the mean orientations of the three birds in which only 20%, 32% or 47%, respectively, of cluster N was lesioned. The dashed arrows show the corresponding mean vectors. **g**, **h**, Sunset orientation (GN, geographical north) of the sham-lesioned (**g**) and lesioned (**h**) birds (14 tests per bird). The dashed radial line indicates the average sunset direction during the tests. Other details of the circular diagrams are as in Fig. 1.

performed (the visual detection limit was $<0.01\,\mathrm{mW\,m^{-2}}$ for both groups; Supplementary Fig. 1). We therefore conclude that a lesion-induced reduction in visual capability is unlikely to have caused the observed differences in orientation performance.

The second alternative explanation is that cluster N might not specifically be involved in the circuit processing magnetic compass information. Finding directions in a magnetic orientation experiment is a complex task involving coordination of information from multiple neural pathways and probably input from memory. Lesions of cluster N could have affected neural processes required for motivation to migrate or for solving orientation tasks in general, thus leading to disorientation even though magnetic sensing itself remains intact. To test the specificity of cluster N in magnetic compass orientation, we tested 13 cluster-N-lesioned European robins and 14 sham-lesioned European robins for their orientation abilities under two conditions: outdoors during sunset and indoors under a stationary planetarium sky simulating the local starry sky (Oldenburg, Germany; Figs 2 and 3).

During natural sunset, both the sham-lesioned and cluster-N-lesioned birds oriented significantly towards the north-northwest (sham-lesioned group: $\alpha = 353^\circ \pm 38^\circ$, $r = 0.57$, $N = 14$, $P < 0.01$; cluster-N-lesioned group: $\alpha = 331^\circ \pm 39^\circ$, $r = 0.57$, $N = 13$, $P = 0.01$; Fig. 2g, h). The mean orientation of both groups indicates a compromise direction between phototactic tendencies towards the setting sun and the birds' north-northeast migratory direction. This reaction is typical of outdoor sun compass orientation tests on migratory birds during sunset[24]. The point is that the birds' orientation was significantly more northerly than the sunset direction, meaning that pure phototactic orientation can be excluded (95% confidence intervals for the mean direction do not overlap with the sunset point, which on average was at 278° during our experiments).

In the planetarium, we simulated celestial north to be located at magnetic east so that we could determine whether orientation behaviour was guided by a star compass or a magnetic compass. To encourage the birds to use their star compass, we added more magnetic disturbance to the already significantly disturbed geomagnetic field inside the planetarium (Methods). The cluster-N-lesioned birds oriented significantly towards star north-northeast ($\alpha = 27^\circ \pm 44^\circ$, $r = 0.55$, $N = 12$, $P = 0.02$; Fig. 3a) and the mean orientation was almost identical to the direction chosen by the sham-lesioned birds using their magnetic compass ($\alpha = 25^\circ \pm 21^\circ$; Fig. 2a). These results show that birds with bilateral lesions of cluster N can use their star compass and their sunset compass, but cannot use their magnetic compass to perform appropriately directed migratory restlessness behaviour (Fig. 3). Therefore, a generally reduced motivation or inability to migrate cannot explain the disorientation of birds with cluster N lesions.

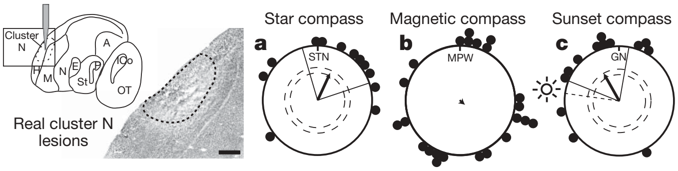

Figure 3 | Birds with cluster N lesions can use their star and sun compasses, but not their magnetic compass. Photo from a lesioned bird as in Fig. 2. **a**, Birds with cluster N lesions tested in a planetarium simulating the local starry sky (ten tests per bird; STN, star north) oriented in the typical north-northeast spring migratory direction ($\alpha = 27^\circ \pm 44^\circ$, $r = 0.55$, $N = 12$, $P = 0.02$). **b**, Birds with cluster N lesions could not orient ($\alpha = 132^\circ$, $r = 0.12$, $P > 0.70$) using their magnetic compass. Shown are the combined data of Fig. 2d–f, depicted relative to the magnetic direction towards the pole (MPW) as European robins use an inclination compass (data from the three poorly lesioned birds are not included). **c**, Birds with cluster N lesions could also orient during sunset ($\alpha = 331^\circ \pm 39^\circ$, $r = 0.57$, $N = 13$, $P = 0.01$), presumably using their sun compass.

Because the ophthalmic branch of the trigeminal nerve is the only nerve branch that innervates the candidate ferromagnetic, magnetosensory structures in the upper beak[7–9], our results show that these putative magnetoreceptors are neither necessary nor sufficient for magnetic compass orientation, and can therefore be excluded as the sole magnetic compass sensor in European robins. This conclusion is in line with results from other studies including, for instance, those on bobolinks, in which anaesthetic blockade of the trigeminal nerve also failed to affect compass orientation[25]. However, our findings do not rule out the possibility that these or other putative magnetoreceptors in other regions of the body can sense geomagnetic information[26,27]. In fact, in pigeons, the putative magnetosensors in the upper beak have been strongly implicated in the sensing of non-compass aspects of the geomagnetic field[10] (but see also refs 28, 29).

The results of the present study, together with those which show that cluster N is the most active forebrain region during magnetic compass orientation behaviour[16,17,19] and is a specialized part of the visual system[18] requiring light perceived through the eyes for its neuronal activation[16,17], specifically suggest that cluster N of European robins is an essential part of a circuit processing light-dependent magnetic compass information for night-time orientation. The exact role of cluster N within this circuit has not been determined, but the present results raise the distinct possibility that this part of the visual system enables birds to 'see' magnetic compass information.

METHODS SUMMARY

All magnetic field conditions were produced using double-wrapped, three-dimensional Merritt four-coil systems[30] with average coil diameters of 2 m. All experiments were performed within the central space of the coils, where the heterogeneities were <1% of the applied field. Current flowed through the coils in all magnetic conditions.

The operations were performed under general anaesthesia. To lesion cluster N, 50 nl 1% ibotenic acid in 0.9% NaCl were injected with a microinjector. To section V_1, a small cut was made through the skin just above the eye and the eyeball and muscles were gently pushed to the side, so that V_1 could be sectioned and a 2–3-mm piece of the nerve removed. The extent and degree of overlap between cluster N and the cluster N lesions were reconstructed post mortem using AMIRA software (Visage Imaging).

The magnetic compass experiments were performed in Emlen funnels inside four wooden huts lined with grounded aluminium shields to minimize electromagnetic disturbances. Immediately before the orientation tests, we exposed all test birds to parts of the local evening sky.

The sun compass experiments were performed on clear evenings around the time of sunset on an open field. The star compass experiments were performed in a planetarium simulating the local starry sky of Oldenburg. The magnetic field inside the planetarium was strongly disturbed.

Two to three independent observers determined the mean directions in the single tests, and all oriented and active tests were used to calculate the mean orientation of each individual bird under each experimental condition. These individual mean directions are depicted as circles on the peripheries of the large circles in Figs 1–3. The group mean vectors were calculated by vector addition of individual unit vectors in each of the individual bird mean directions and division by the number of birds tested.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions H.M. designed and supervised the study. M.Z., C.M.H., S.E. and J.H. performed and M.Z. and C.M.H. supervised the majority of the orientation experiments. M.Z., C.M.H., S.E., J.H. and H.M. analysed the orientation results. J.M.W. and D.H. performed the surgeries. D.H. did the post-mortem histological analyses. D.D. performed the lesion analyses using AMIRA. S.W. and D.K. performed and analysed the operant conditioning. N.-L.S. suggested and made crucial improvements to the experimental set-up. H.M., M.Z., J.M.W. and D.H. wrote most of the paper. All authors read and commented on the manuscript.

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METHODS

Magnetic fields. To produce the CMF condition, current flowed in the same direction through each subset of windings of the double-wrapped, three-dimensional Merritt four-coil systems[30]. To produce the natural geomagnetic field condition, the coils were turned on but the current ran through the two subsets of windings in opposite directions. Before each experiment, the actual magnetic field was measured (FVM-400, Meda) in the centre and at the edges of the experimental volume, within which nine Emlen funnels were placed simultaneously. The actual fields experienced by the birds under the three magnetic field conditions were as follows (mean ± s.d.): for the NMF the field strength was 48,620 ± 330 nT, the inclination was 67.6° ± 0.5° and the horizontal direction was 360° ± 1°; for the CMF the field strength was 48,800 ± 320 nT, the inclination was 67.8° ± 0.5° and the horizontal direction was 240° ± 2°; and for the IMF the field strength was 48,660 ± 500 nT, the inclination was −68.1° ± 0.5° and the horizontal direction was 1° ± 1°.

Cluster N lesioning and nerve sectioning. Birds were anaesthetized by intramuscular injection of ketamine (Pfizer)/Rompun (Bayer). The heads were then fixed in a custom-built stereotaxic apparatus. The scalp was additionally anaesthetized using a local surface anaesthetic (xylocaine, Astra Zeneca), incised and retracted. For cluster N lesions, a small part of the skull was carefully removed and 50 nl 1% ibotenic acid in 0.9% NaCl solution were stereotaxically injected using a microinjector. The coordinates used corresponded to the ones described in ref. 18. Sham-lesioned birds underwent exactly the same procedures except that ibotenic acid was not injected. For nerve sectioning, the scalp was retracted and the fascia along the rim of the orbit was incised to allow gentle depression and retraction of the globe. The ophthalmic branch of the trigeminal nerve (V_1) was revealed and sectioned behind the eyeball immediately before it left the orbit and 2–3 mm farther proximally; this piece was then removed and the cut ends of the proximal and distal stumps were sealed with surgical cyanoacrylate to prevent re-fusion. Sham-sectioned birds underwent exactly the same procedures except that the nerves were not sectioned. Finally, the skin edges were sealed and the birds were given 1–10 weeks to recover from surgery before taking part in any behavioural experiment.

Behavioural experiments. The experimental birds were caught within 200 m of the testing site at the University of Oldenburg, Germany, during autumn migration and tested during the following spring migratory season. The magnetic field orientation experiments were conducted in wooden huts placed on the university campus. The walls of the huts were lined with grounded aluminium shields, which acted as Faraday cages to shield non-stationary electromagnetic disturbances. One hour (±10 min) before the lights went out in the bird rooms (light for 14 h, dark for 10 h), the birds were placed outdoors in wooden transport cages that allowed them to see parts of the evening sky for 1 h to give them the opportunity to calibrate their magnetic compass from the local sunset sky[3]. Immediately thereafter, the birds were placed in aluminium Emlen funnels[20] (35 cm in diameter, 15 cm high, walls inclined at 45°) and tested for 1 h under dim light conditions (4 mW m^{-2}) produced by incandescent bulbs (for spectrum, see Supplementary Fig. 2).

The funnels were coated with thermal paper[21] on which the birds left scratches as they moved. Nine European robins were tested simultaneously in each hut. The birds were put into a randomized funnel position each night, and were put into the funnels from different sides. We observed no systematic differences between the nine funnel positions. The birds were tested twice each night under the same magnetic field condition. There were no statistically significant differences (Mardia–Watson–Wheeler tests, $P > 0.65$ for all comparisons) or even indications that the orientation was systematically different during the first test relative to the second test, and because any given bird was tested in different huts during the first and second tests, both values were entered into the calculation of the mean for that bird under the given magnetic field condition. The first test started 30 min after sunset and the second test started around 2 h after sunset. The magnetic field condition present in a given hut was switched every second night, and usually different magnetic fields were present in different huts on any given night.

Orientation experiments during sunset took place in spring on an open field near Gristede, 20 km north-northwest of Oldenburg. The tests were performed in the undisturbed local magnetic field with an open view of the sky on clear evenings and the test started 30 min before sunset and ended 30 min after sunset.

Orientation experiments in the planetarium of Elsfleth, 28 km east-northeast of Oldenburg, were conducted under a stationary simulation of the local sky. The starry sky was projected using a Zeiss ZKP-2 projector on a dome (9 m in diameter). Nine Emlen funnels were placed symmetrically around the projector 10 cm above the horizon plane, such that the projector was never visible from inside any of the funnels. To increase the likelihood that the magnetic field of the planetarium (already disturbed by the iron-containing projector and magnetic material in the walls) would not provide any useful magnetic compass information, heavy iron racks were used for stabilization of the set-up and strong neodymium magnets were attached under each funnel. The resulting magnetic field inside each funnel varied strongly in intensity (from ~44,000 nT to ~84,000 nT), direction (owing to the central placement of the magnet, the horizontal direction of the field within the funnels varied in all directions depending on exactly where the bird was located within the funnel) and inclination (from +59° to +88°). Other testing procedures were the same as in the magnetic field orientation experiments, except that the birds were only tested once per night. Before each testing session, the projector was adjusted to the time the birds were tested, and on every second day, the star pattern was adjusted to the actual date.

Orientation-data analysis. Two independent researchers, who did not know either the test condition or the operation a given bird had experienced, determined each bird's mean direction from the distribution of the scratches. If both observers considered the scratches to be randomly distributed or if the two mean directions deviated by more than 30°, a third independent researcher determined the mean direction. If this third individual determined a mean direction similar to one of the first two, and if the individual with the initially differing opinion also agreed with this direction, the mean of the two similar directions was recorded as the orientation result. If the three independent individuals could not agree on one mean direction, the bird's heading was defined as random and excluded from the analyses (only 10% of all tests were excluded on the basis of this criterion). Birds with fewer than 35 scratches on the paper were considered inactive and also excluded from the analysis (the birds were inactive in 18% of all tests).

Size and position of lesion analysis. To determine the exact extent and location of the lesions relative to cluster N, each brain slice was photographed with a digital camera (Leica DFC 320) through a stereo microscope (Leica M, Leica IM50). On these pictures, boundaries of the whole telencephalon, the injured tissue (lesion) and cluster N were marked, and the sections were aligned using Photoshop 6.0/Illustrator 10.0 (Adobe Systems).

The extent of cluster N was determined by comparison with ZENK in-situ hybridized brain slices from birds performing magnetic compass orientation in a funnel[16,17]. The stacks were aligned using the outline of the telencephalon. Stacks of each hemisphere were launched in AMIRA (Visage Imaging) and converted into AMIRA files (AMIRA mesh binary). The resolution of AMIRA files was reduced to 800 × 600 pixels and the physical distance between slides—the actual distance between each slide of each series—was set at 240 μm. The file sequence was fused into one data stack. 'Label fields' were created, in which the marked boundaries were labelled and interpolated into three three-dimensional bodies: the lesion, cluster N and the telencephalon. On the basis of the overlap in space between these three volumes, the percentage of the volumetric overlap between the lesion and cluster N was determined. This procedure was done for each hemisphere of each bird separately.

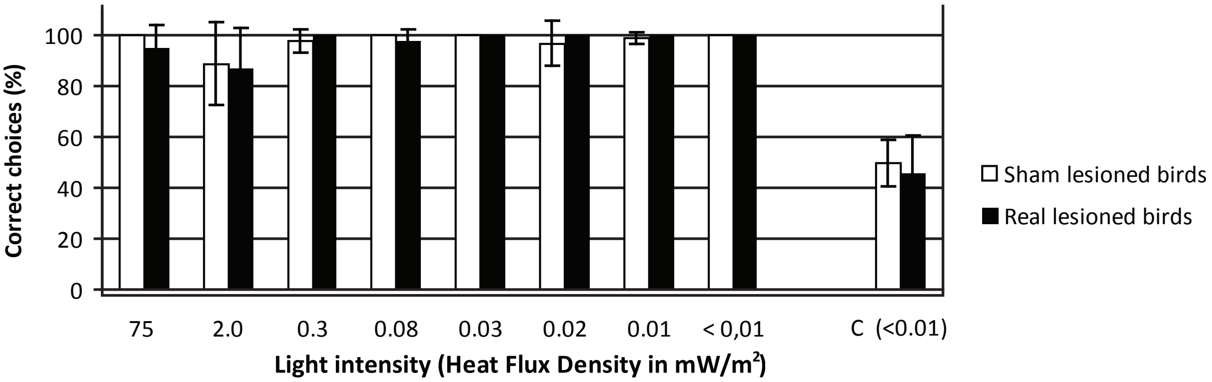

Fig. S1. Light spot detection capabilities of European robins were not affected by Cluster N lesions. We performed a set of simple, two choice operant conditioning experiments, which required the birds to jump from a choice perch onto the one of two response perches associated with a white diode being turned on in order to receive a food reward. Before the real tests, the birds learned the task at the highest light intensity (75 mW/m2). Each of the four birds (2 lesioned and 2 sham lesioned) took part in 10-20 sessions consisting of 10-12 choices per session. After they had learned the task, no further training at the other light intensities was done. In the experiments, we reduced the intensity of the diode in steps until its intensity was approximately 400 times dimmer than the light present in the wooden huts during the orientation experiments. For the critical tests, each bird was tested for an average of 8 sessions each consisting of 10 choices. The percent correct choice at each light intensity is represented by the bars. In the control experiment ("C"), both diodes were turned on at the <0.01 mW/m2 intensity. The experimental chamber's dim house lights were always turned on. The two lesioned birds (black bars) and the two sham lesioned birds (white bars) could all detect the conditioned light stimulus irrespective of the light intensity used at a success rate close to 100%, including when the light stimulus which was 400 times dimmer than the light present in the wooden huts during the orientation experiments. Error bars represent SD.

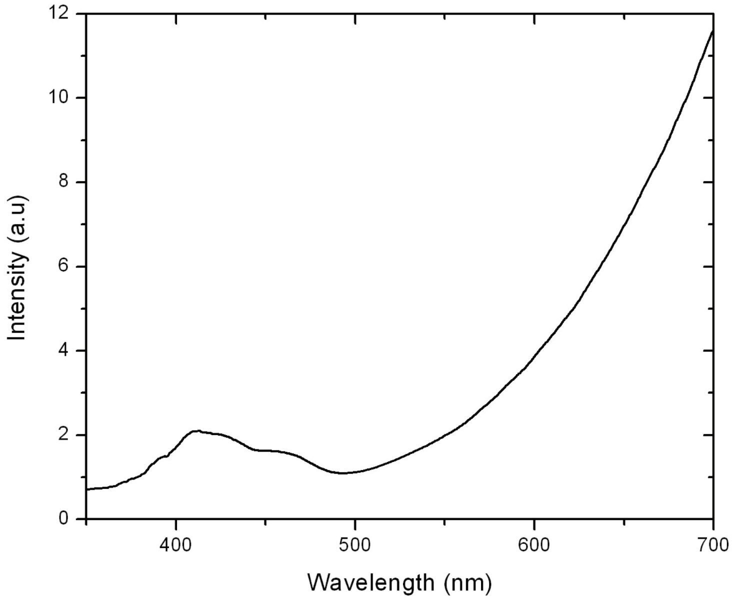

Fig. S2. Spectrum of light produced by the incandescent bulbs in the testing huts. The total intensity was 4 mW/m². The units on the y-axis are arbitrary, relative intensity units.

PAPER V

Hein, C.M., Engels, S., Kishkinev, D. & Mouritsen, H.

“Robins have a magnetic compass in both eyes”

Nature, 2011, 471, E11
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BRIEF COMMUNICATIONS ARISING

Robins have a magnetic compass in both eyes

ARISING FROM W. Wiltschko *et al.* *Nature* **419,** 467–470 (2002)

The magnetic compass of migratory birds is embedded in the visual system[1–3] and it has been reported by Wiltschko *et al.*[1] that European Robins, *Erithacus rubecula*, cannot show magnetic compass orientation using their left eye only. This has led to the notion that the magnetic compass should be located only in the right eye of birds[1,3–5]. However, a complete right lateralization of the magnetic compass would be very surprising, and functional neuroanatomical data have questioned this notion[2,6–8]. Here we show that the results of Wiltschko *et al.*[1] could not be independently confirmed using double-blind protocols. European Robins can perform magnetic compass orientation with both eyes open, with the left eye open only, and with the right eye open only. No clear lateralization is observed.

More or less pronounced lateralization is a common feature of the avian brain[9], but an all-or-nothing lateralization like the one reported by Wiltschko *et al.* in European Robins[1] and Silvereyes[4], *Zosterops lateralis*, would be highly unusual for any sensory system and seems evolutionarily counterproductive. A bird having a magnetic compass located exclusively in its right eye would be more easily affected by eye infection or monocular damage than a bird having functional magnetic compasses in both eyes.

We therefore tested 27 European Robins during autumn migration, when they use simple compass orientation[10], and equipped them with light tight[8,11] hoods enabling them to see with both eyes, their right eye only, or their left eye only. In all three conditions, the birds oriented in their expected autumn migratory direction towards the South-West in the unchanged geomagnetic field (normal magnetic field, NMF; both eyes open: 236° ± 20° (95% confidence intervals), $r = 0.69$, $N = 27$, $P < 0.001$, Fig. 1a; left eye open: 217° ± 27°, $r = 0.57$, $N = 27$, $P = 0.001$, Fig. 1c; right eye open: 192° ± 24°, $r = 0.65$, $N = 26$, $P < 0.001$, Fig. 1e) and towards the East in a magnetic field turned 120° counter-clockwise (changed magnetic field, CMF; both eyes open: 78° ± 20°, $r = 0.72$, $N = 27$, $P < 0.001$, Fig. 1b; left eye open: 47° ± 45°, $r = 0.38$, $N = 26$, $P < 0.03$, Fig. 1d; right eye open: 112° ± 30°, $r = 0.52$, $N = 27$, $P = 0.001$, Fig. 1f). In all cases, the CMF direction is significantly (no 95% confidence intervals overlap) turned in the expected direction compared to the NMF direction.

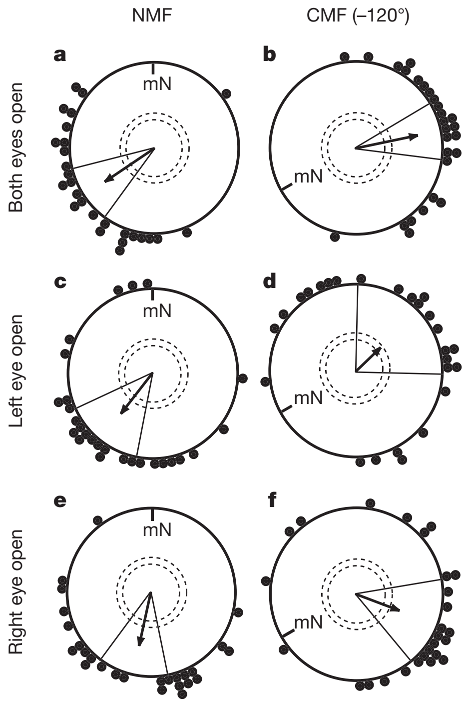

Figure 1 | European Robins wearing eye covers can use their magnetic compass if light and/or visual input reaches any one eye. a–f, Each dot at the circle periphery represents the mean orientation of one individual bird tested several times with the given type of hood. mN, magnetic North. The arrows indicate the group mean vectors. The inner and outer dashed circles indicate the radius of the group mean vector needed for significance according to the Rayleigh Test ($P < 0.05$ and $P < 0.01$, respectively). The lines flanking the group mean vector indicate the 95% confidence intervals for the group mean direction.

Our results showing that European Robins have a magnetic compass in both eyes are in line with other recent findings, which otherwise would be difficult to explain: (1) garden warblers have a magnetic compass in both eyes[11]; (2) the putative magnetoreceptive cryptochromes are located in both eyes[6]; (3) Cluster N[7,8], the brain area recently shown to be necessary for magnetic compass orientation in European Robins[2], shows similar activation in both brain hemispheres during magnetic compass orientation[7,12]. In fact, Cluster N activation in European Robins shows a slight but significant dominance of the left eye and right brain hemisphere[8], that is, lateralization in the opposite direction to that suggested by Wiltschko *et al.*[1,4]; (4) the neuronal pathways between the eye and Cluster N seem to be symmetrical[13]; (5) magnetic compass orientation is only weakly lateralized in pigeons[14,15]. We suggest that the Wiltschko *et al.*[1] data may have been artefacts of the unnatural green light conditions under which their birds were tested or of the non-blinded procedures. Alternatively, they might have resulted from the more complicated interaction of map and compass information potentially occurring in spring.

In conclusion, it is very possible that some smaller degree of lateralization of magnetic information processing exists in birds[8,14,15]. However, our data show that the magnetic compass of night-migratory songbirds is not strongly lateralized and certainly not located in only one of the birds' eyes.

METHODS

We tested the birds' magnetic compass orientation capabilities under broad spectrum white light[2] in the normal geomagnetic field (NMF) and in a changed geomagnetic field with magnetic North turned 120° counter-clockwise (CMF). We used a double-blind protocol and large, three-dimensional, double-wrapped, Merritt 4-coils to produce highly homogenous magnetic fields (for details see ref. 2). The same current ran through the coils in both magnetic field conditions. We tested all birds inside aluminium-lined wooden huts, where no cues other than the geomagnetic field were available. The mean directions are based on 4.11 ± 2.76 (s.d.) active and oriented tests per condition (six conditions).

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Wiltschko *et al.* reply

REPLYING TO C. M. Hein, S. Engels, D. Kishkinev & H. Mouritsen *Nature* **471,** doi:10.1038/nature09875 (2011)

Hein and colleagues[1] challenge our 2002 paper[2], claiming that they cannot replicate our findings. The paper had two conclusions: (1) magnetic compass information is mediated by the eyes, as had been proposed by Ritz and colleagues[3], and (2) the magnetic compass is lateralized in favour of the right eye. The new data do not contradict the first conclusion; in fact, this has been supported by a recent paper from the authors[4]. It is only the second conclusion they question, although it has been demonstrated not only in two species of migrants[2,5], but also in domestic chickens[6] and is the basis of a new paper indicating an interaction between contour vision and magnetoreception[7].

These obvious differences in findings require explanations, and offhand, three possibilities come to mind:

(1) The authors do not observe migratory orientation, but a 'fixed direction' response. 'Fixed direction' responses do not involve the inclination compass based on the radical pair mechanism, but are polar responses originating in the magnetite-based receptors in the beak[8]; they are not lateralized[9]. The observed scatter is in agreement with this interpretation, as 'fixed directions' are often more scattered than compass responses[8]. Critical tests to distinguish between the two types of responses, like inverting the vertical component of the magnetic field, are missing.

(2) The studies by Hein *et al.*[10] were autumn experiments, where young birds fly innate compass courses[11], whereas ours[2,5,7,8] involved spring experiments, where birds can use true navigation to head back to the familiar breeding regions[12]. There are indications that the navigational 'map' is lateralized in favour of the right eye/left brain system[13], which, in turn, could have led to a lateralized response.

(3) Another difference between the studies is the number of tests per bird. Whereas we tested the birds two[2,5] or three[7,9] times, the authors' means are based "on 4.11 ± 2.76 (s.d.) active and oriented tests per condition", which implies that the individual birds have been tested more often. Hence the total time the birds had their right eye covered was considerably longer than in our studies. In certain tasks acquired unihemispherically, an interhemispheric transfer is observed in animals that have to rely on the naive eye; in some cases, this takes just a few hours[14]. A similar transfer may have occurred when the right eye was covered for a longer period. The observation that the vectors of the birds with the right eye covered are the shortest in both magnetic conditions is in agreement with this interpretation. This could also explain the weaker lateralization observed in pigeons[15], where the total time of covering the right eye was also much longer. It would mean that although the avian magnetic compass is normally mediated by the right eye only, left-eye input is able to substitute the process after a critical amount of time.

In summary, there are considerable differences between the studies. Which of them or which possible combination of them caused the difference in findings cannot be decided at present, but will be determined by future experiments.

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PAPER VI

Hein, C., Engels, S., Kishkinev, D., Prior, H. & Mouritsen, H.

“Robins possess a magnetic compass in both eyes”

Manuscript

Robins possess a magnetic compass in both eyes

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Abstract

Previous studies on European robins, *Erithacus rubecula*, and Australian silvereyes, *Zosterops lateralis*, have suggested that magnetic compass information is being processed only in the right eye and left brain hemisphere of migratory birds. A lateralization in an all-or-none fashion would, however, be highly unusual for any sensory system and it would be hard to explain how a 100% lateralization towards one side would be evolutionary advantageous. After having demonstrated that garden warblers, *Sylvia borin*, are able to orient with any one eye, the possibility of species-specific lateralization effects still existed. In this study, we therefore independently tested European robins wearing eye covers using a double-blind protocol. Here, we show that European robins, too, are able to perform magnetic compass orientation with both eyes open, with the left eye open only, and with the right eye open only. These results are in line with neuroanatomical studies and recent findings in pigeons and songbirds and thus raise the question if the strong lateralization effect observed in earlier experiments might have arisen from artefacts or special experimental conditions instead of true all-or-none lateralization of the magnetic compass in European robins.

Introduction

Each year, migratory birds travel long distances between their breeding grounds and their wintering quarters, and it is well established that they use a light-dependent magnetic compass for orientation (Schulten et al. 1978; Wiltschko et al. 1993, 2005; Ritz et al. 2000; Muheim et al. 2002; Wiltschko & Wiltschko 2007; Mouritsen et al. 2004a; Mouritsen & Ritz 2005; Heyers et al. 2007; Zapka et al. 2009). The direction of the Earth's magnetic field is supposedly sensed by radical pair-forming, light-dependent photopigments in the birds´ eyes (Schulten et al. 1978; Schulten & Weller 1978; Ritz et al. 2000, 2004, 2010; Wiltschko & Wiltschko 2007; Möller et al. 2004; Mouritsen et al. 2004b; Maeda et al. 2008; Liedvogel et al. 2007a; Wiltschko et al. 2007; Rodgers & Hore 2009) and then processed in Cluster N, a specialized, night-time active, light-processing forebrain region (Mouritsen et al. 2005; Heyers et al. 2007; Liedvogel et al. 2007b; Feenders et al. 2008; Zapka et al. 2009).

In 2002, Wiltschko and colleagues published data on European robins, *Erithacus rubecula*, suggesting that these birds are unable to orient with the help of the geomagnetic field using their left eye only (Wiltschko et al. 2002). Subsequently, Wiltschko et al. (2003) suggested a corresponding all-or-none right lateralization of magnetic compass orientation in a diurnally migrating songbird, the Australian Silvereye, *Zosterops lateralis*. These findings have led to the notion that the vision-mediated magnetic compass is located only in the right eye of migratory birds, whereas input from the left eye only is not sufficient for magnetic compass orientation (Wiltschko et al. 2002, 2003; Wiltschko & Wiltschko 2002, 2010).

A complete right lateralization of the magnetic compass would however be very surprising, because although slight to moderate lateralization is a common feature of the avian brain, so far no other functions involving the visual system have been shown to be lateralized in an all-or-none modality (e.g. Güntürkün 1997; Rogers & Andrew 2002; Prior 2006). Usually, only a preference towards one side exists. A strong lateralization like the one reported by Wiltschko and colleagues in European robins (Wiltschko et al. 2002) and Australian silvereyes (Wiltschko et al. 2003) would also seem counterproductive from an evolutionary perspective. The survival of a bird having a magnetic compass located exclusively in its right eye would be more easily affected by eye-infection or monocular damage than a bird having a functional magnetic compass in both eyes. Likewise, the possibility that birds may show uni-hemispheric sleep during flight (Rattenborg 2006) would favour bilateral perception of magnetic compass directions in a night-migratory bird.

In addition, functional neuroanatomical data have questioned the right-lateralization of the magnetic compass: cryptochromes, the most promising candidates for primary sensory molecules involved in the radical pair mechanism are found in both eyes with no obvious difference in cryptochrome expression, neuronal activity, or connectivity during a magnetic compass orientation task (Mouritsen et al. 2004a; Heyers et al. 2007; Liedvogel et al. 2007a), and Cluster N, which has been shown to be involved in the magnetic compass information processing circuit (Mouritsen et al. 2005; Zapka et al. 2009) is active in both hemispheres of the brain of European Robins and garden warblers, *Sylvia borin*, when performing magnetic compass guided orientation (Mouritsen et al. 2005; Liedvogel et al. 2007b; Feenders et al. 2008). In fact, neuronal activation patterns in Cluster N of European Robins are slightly but significantly lateralized in the opposite direction to the one suggested by Wiltschko and colleagues (Liedvogel et al. 2007b). A quantification of neuronal activity revealed a dominance of the right brain hemisphere which – due to the almost complete crossover of the fibers of the optic nerve of birds (e.g. Cowan & Powell 1963; McGill et al. 1966) – gets its input mainly from the left eye (Liedvogel et al. 2007b).

Furthermore, a study on the magnetic compass performance of garden warblers, another night-migratory songbird species, found no lateralization effect (Hein et al. 2009). The birds could orient with either both eyes open, with the left eye open only, and with the right eye open only.

To sum up, the suggested all-or-none lateralization of magnetic compass orientation towards the right eye only is not as clear-cut as suggested previously. One remaining possible explanation for these apparently contradictory results was that there might be species-related differences concerning the lateralization of the magnetic compass of migratory birds. Therefore, the aim of the present study was an attempt to independently confirm the findings of Wiltschko et al. (2002) using the same species, namely European robins. The birds were tested under two different magnetic field conditions while being equipped with eye covers that had openings in front of either both eyes, the left eye only, or the right eye only.

Methods

Magnetic fields

Magnetic fields were produced with double-wrapped, three-dimensional Merritt four-coil systems (Kirschvink 1992) with average coil diameters of two meters. All experiments were performed within the central space of the coils where the heterogeneity was <1% of the applied field. Before the beginning of each experiment, the ambient magnetic field was measured in the centre and at the edges of the experimental volume within which the orientation cages were placed. Birds were tested in two different magnetic conditions: in a magnetic field resembling the natural one of Oldenburg (Natural Magnetic Field, NMF: MF strength=48.900nT±150nT [s.d.]; inclination=67,7°±0,6°; horizontal direction=360°±0,1°) and in a magnetic field turned 120° counter-clockwise (Changed Magnetic Field, CMF: MF strength=49.000nT±470nT; inclination=68,0°±1,1°; horizontal direction=-120°±0,5°). To produce the CMF condition, the current ran through the two subsets of windings of the four-coil system in the same direction. Under the NMF condition, the same current that we used to produce the CMF condition ran through the two subsets of windings but in opposite directions so that no significant changes (i.e. <10nT) to the magnetic field were produced by the coils.

Test Subjects

In our study, we tested 27 European robins, which had been caught on the campus of the University of Oldenburg, Germany. The birds were housed indoors in individual cages in a windowless room under a light regime simulating the local photoperiod. The behavioural experiments were performed during the autumn migratory season 2009 on the campus of the University of Oldenburg. All animal procedures were approved by the Animal Care and Use Committees of the LAVES (Oldenburg, Germany).

Behavioural experiments

The birds were tested in orientation cages inside wooden huts placed on the university campus, where no other cue than the geomagnetic field was available. The walls and ceilings of the huts were lined with aluminum shields, which acted as Faraday cages and shielded non-stationary electromagnetic disturbances by approximately two orders of magnitude. All technical supplies and equipment were placed in a separate room in a shelf that was also shielded with aluminum to minimize electromagnetic disturbances.

One hour (±10min) before the experiments started (i.e. half an hour before until half an hour after sunset), the birds were placed outdoors in wooden transport cages that allowed them to see parts of the evening sky to give them the possibility to calibrate their magnetic compass from twilight cues (Cochran et al. 2004; Muheim et al. 2006a, 2006b, 2009). Immediately thereafter, they were placed in modified aluminum Emlen funnels (35cm diameter, 15cm high, walls 45° inclined; Emlen & Emlen 1966), which were coated with thermal paper (Mouritsen et al. 2009) on which the birds left scratches as they moved. The overlap point of the paper was changed randomly between nights and huts. The birds were tested for one hour under dim light conditions ($2.1 mW/m^2$) produced by incandescent bulbs (spectrum in Zapka et al. 2009). In each hut, nine birds were tested simultaneously. A second test of a given night started 1.5 hours (±10min) after the first one, and each bird was tested in a different hut compared to the first test but under the same magnetic field condition (NMF or CMF). The orientation directions of the first and the second test can therefore be treated as independent and thus were both entered into the calculation of the mean direction of each individual bird. The magnetic field conditions applied in a given hut were switched approximately every second night, and usually both magnetic field conditions were tested in different huts on any given night.

Before the eye cover experiments started, we tested the birds without wearing eye covers for several nights to ensure that they were in migratory mood and to get a control direction. For the eye cover experiments, we used the same procedures as in the control experiments, except that the birds were fitted with eye covers just before they were placed outdoors for one hour in the wooden transport cages. The eye covers (<0.5g) were sewed of light-tight, artificial leather with tightly fitted openings left for the beak and the neck. In addition, they had openings of 8mm diameter in front of either both eyes (controls), the right eye only, or the left eye only. The eye covers reduced the ambient light by at least five orders of magnitude, which means that the light intensity under the hoods during the experiments was <$1*10^{-5}$mW/m^2, and neuronal activity of Cluster N was reduced to background level by the eye covers. The eye covers were removed every night immediately after the end of the behavioural tests. This technique of covering the eyes differed from the one used in Wiltschko et al. (2002). We considered our eye covers preferable because comparative control tests had shown that the technique used in the present study was considerably less stressful for the birds.

Orientation data analysis

Two researchers visually determined each bird's mean direction from the distribution of the scratches independently from each other (Mouritsen 1998), without knowing the direction of the overlap point of the paper, and without knowing the magnetic field condition experienced by the bird. If the two researchers considered the scratches to be randomly distributed or if the two independently determined mean directions deviated by more than 30°, a third independent researcher was asked to determine the mean direction. If this third individual determined a mean direction similar to one of the first two, and if the individual with initially differing opinion also agreed with this direction, the mean of the two similar directions was recorded as the orientation result. If the three independent researchers could not agree on one mean direction, the bird's heading was defined as random and excluded from the analyses (14% of all control tests; 11% of all eye cover tests). Birds with fewer than 30 scratches on the paper were considered inactive and were also excluded from the analysis (35% of all control tests; 34% of all eye cover tests). The average mean heading for each bird was calculated from all its oriented tests recorded under a given experimental condition. Based on these individual mean vectors, group mean vectors were calculated and the significance of the group mean vector was tested using the Rayleigh-test (Batschelet 1981).

Results

In the control experiments (Figure 1), i.e. without wearing any eye cover, the birds headed significantly into their seasonally appropriate south-westerly direction in the NMF condition (220°±20°, $r=0.69$, $p<0.001$, $N=27$; Fig. 1A) and into an appropriate corresponding easterly direction in the CMF condition (97°±18°, $r=0.75$, $p<0.001$, $N=27$; Fig. 1B).

FIGURE 1

After the control experiments, we performed identical experiments with birds equipped with eye covers, which enabled them to see either with the left eye only, with the right eye only, or with both eyes. Each bird was tested 7.6±3.8 [s.d.] times per condition (altogether 6 experimental conditions), which resulted in 4.11±2.76 active and oriented tests per bird. When the birds were equipped with eye covers enabling them to see with both eyes, they oriented significantly in a seasonally appropriate migratory direction towards south-west in the NMF condition (236°±20°, r=0.69, p<0.001, N=27; Fig. 2A). When the magnetic field was turned 120° counter-clockwise, the birds with both eyes open oriented towards east-north-east (78°±20°, r=0.72, p<0.001, N=27; Fig. 2B). The mean orientation in the CMF condition of the birds with both eyes open differed significantly from the same birds' orientation in the NMF condition (95% confidence intervals do not overlap; Mardia-Watson-Wheeler-Test (MWW): W=37.46, p<0.001)

FIGURE 2

When equipped with monocular eye covers that enabled them to see with their left eye only, the birds were again significantly oriented into their appropriate migratory direction under the NMF condition (217°±27°, r=0.57, p<0.001, N=27; Fig. 2C), as well as under the CMF condition (47°±45°, r=0.38, p<0.05, N=26; Fig. 2D). The north-easterly direction found in the CMF condition is not significantly different from the expected easterly migratory direction towards approximately 85°-95° (see Mouritsen & Mouritsen 2000), because the expected migratory direction lies within the 95% confidence interval of the group mean orientation direction (2°-92°). The mean orientation of the birds, which had only their left eye open, tested in the CMF condition differed significantly and in the expected direction from the same birds' orientation in the NMF condition (95% confidence intervals do not overlap; MWW: W=22.76, p<0.001).

When equipped with monocular eye covers that enabled them to see with their right eye only, the birds were also significantly oriented into the appropriate migratory direction under the NMF condition (192°±24°, r=0.65, p<0.001, N=26; Fig. 2E) as well as under the CMF condition (112°±30°, r=0.52, p<0.001, N=27; Fig. 2F). The mean orientation in the 120° turned magnetic field of the birds, which had only their right eye open, differed significantly and in the expected direction from the same birds' orientation tested in the NMF condition (95% confidence intervals do not overlap; MWW: W=17.71, p<0.001).

In order to test whether migratory experience might have influenced the degree of lateralization of the magnetic compass, we compared the directional preferences of the six birds captured as adults that were part of our experiments with the mean direction of the 21 birds captured as juveniles (see table 1). On average, the mean direction of the adult birds deviated by 22° from that of the juvenile birds, with a mean (NMF and CMF) of 37° deviation for the left eye open condition and a mean of 15° for the right eye open condition. The mean vector lengths of adult bird orientations were similar to those of the juvenile birds (V tests: NMF: both eyes open: p=0.02; left eye open: p=0.011; right eye open: p=0.012; CMF: both eyes open: p=0.013; left eye open: p=0.22; right eye open: p=0.14). Thus, experience does not seem to affect the orientation responses of birds wearing eye covers (c.f. discussion).

TABLE 1

While Wiltschko et al. (2002) tested each bird only twice, we did more tests to reduce the noise in the data and to secure that our results are consistent to internal replication. We do not believe that there is any good reason why more tests should affect the principle outcome of the experiments. However, to avoid any putative discussion at later stages, we also analysed our data based on the first two oriented (i.e. not random) and active tests of each bird in each of the six experimental conditions (see table 2). Based on the first two oriented tests only, the orientation was also significant in all test conditions except for the "right eye open condition" in the changed magnetic field (but this condition also showed a clear tendency in the expected direction). Thus, the capability to orient with both eyes, the left eye only, or the right eye only was present from the very beginning. Although - according to Wiltschko et al. - birds are supposed to lack a magnetic compass sense in the left eye, we already had significant results after only two tests per bird in this experimental condition. Thus, the difference in number of tests conducted per bird between our study and that of the Wiltschkos cannot be the cause for the differing results.

TABLE 2

In all studies analyzing effects of monocular occlusion on orientation in pigeons, there was a strong and reliable orientation bias into the direction of the open eye in monocular birds (e.g. Ulrich et al. 1999; Diekamp et al. 2002; Prior et al. 2002, 2004): The orientation of individuals with the right eye open only deviated in a clockwise direction and the orientation of individuals with the left eye open only deviated in a counterclockwise direction as compared to binocular controls. As this systematic bias might be diagnostic of how information from either eye is integrated (c.f. discussion), we analyzed whether such a bias would occur in eye-covered songbirds performing magnetic compass orientation in Emlen funnels. For each individual, the angular deviation with the left eye open only or with the right eye open only from the binocular mean (eye covers with holes for both eyes) of the same individual was calculated.

FIGURE 3

In neither of the two magnetic field conditions did we observe a significant difference between a birds´ orientation with the left eye open only and with the right eye open only when compared with the same birds´ orientation with eye covers allowing them to see with both eyes (Fig. 3; NMF condition: MWW: W=0.304, p=0.859; CMF condition: no difference in direction can be tested because one distribution is random). A comparable analysis on the orientation of garden warblers, which have also been shown to be able to use their magnetic compass using only their left eye or only their right eye (Hein et al. 2009), gave a similar result. Thus, during magnetic compass orientation, no systematic bias towards the side of the open eye seems to occur.

Discussion

The control experiments show that our birds were in migratory mood and that they were able to perform magnetic compass guided orientation (Fig. 1). When equipped with eye covers, the robins were significantly oriented into the expected directions in both magnetic field conditions, whether they were tested with both eyes open (Fig. 2A+B), with the left eye open only (Fig. 2C+D) or with the right eye open only (Fig. 2E+F). None of the 95% confidence intervals between any of the two magnetic field conditions of one eye cover condition overlap. Thus, European robins can orient using their magnetic compass with both eyes open, with their left eye open only, and with their right eye open only. There is no obvious difference between the eyes; i.e. we do not observe any clear lateralization.

The present results seem to contradict the findings of Wiltschko and colleagues in European robins (Wiltschko et al. 2002) and Australian silvereyes (Wiltschko et al. 2003), where it was suggested that magnetic compass orientation is possible only with the right eye, but not with the left eye. Our results are, however, in line with the following behavioural, molecular and neuroanatomical studies on magnetic compass sensing:

(1) Behavioural experiments obtained with garden warblers wearing eye covers revealed that this night-migratory bird species possesses a magnetic compass in both eyes with no obvious difference in orientation performance (Hein et al. 2009).

(2) Cryptochromes, the most promising and likely magnetosensory candidate molecules to be involved in the visual magnetic compass, are expressed in both eyes, with no obvious difference in cryptochrome expression or in neuronal activity and/or connectivity during a magnetic compass orientation task (Mouritsen et al. 2004a; Heyers et al. 2007; Liedvogel et al. 2007b).

(3) Cluster N, which has been shown to be required for the processing of magnetic compass information in migratory birds (Zapka et al. 2009), is active in both hemispheres of the brain of European robins and garden warblers when the birds perform magnetic compass orientation (Mouritsen et al. 2005). A more detailed study on Cluster N in European robins even revealed a slight but significant dominance of the right hemisphere (Liedvogel et al. 2007b): if European robins wore eye covers that covered either one eye, the activity level in the brain hemisphere contra-lateral to the open eye was higher when the right brain hemisphere was analysed (the visual input thus stemming mainly from the left eye) than it was in the left brain hemisphere when the right eye was open only (Liedvogel et al. 2007b). The same was true if the birds wore a control eye cover where both eyes were open: a slight right-dominance in Cluster N activity was found.

To sum up, molecular and physiological studies, which in several cases included data from European robins, observed no all-or-nothing lateralization but suggest a slight dominance of the left eye, i.e. pointing in the opposite direction to the one found by Wiltschko et al. (2002, 2003) and Rogers et al. (2008).

The potential explanation that species related differences might account for the discrepancy of previous orientation results – a strongly lateralized magnetic perception in European robins and Australian Silvereyes (Wiltschko et al. 2002, 2003) and a non-lateralized perception in garden warblers (Hein et al. 2009) - no longer seems plausible. This species-related explanation also seems unlikely because the ability to sense magnetic fields seems to have evolved long back in evolutionary history in a common ancestor of all present-day birds, since non-migratory zebra finches and domestic chicken also seem to possess a magnetic compass (e.g. Wiltschko & Wiltschko 1999; Keary et al. 2009). Evolutionary plausibility also speaks against the strong lateralization of the magnetic compass: For the evolution of a strong lateralization there would have had to be some kind of advantage for an unilateral mechanism. But why should evolution favour a sensory system that is very vulnerable to injuries? If the all-or-none-right-lateralization was true, even temporary damage or illness affecting only the right eye would be a major problem for a migratory bird, since it would no longer be able to sense the magnetic field and would thus have to rely only on sun and star compasses for orientation. It is therefore far more likely that the magnetic sense is located in both eyes. Slight to moderate lateralization effects might, of course, still arise through hemispheric differences in higher level processing (e.g. Güntürkün 1997; Prior 2006; Rogers et al. 2008; Wilzeck et al. 2010), such as for example a preference for the processing of directional information of the left brain hemisphere in birds (Prior 2006). But these hemispheric differences would not result in an all-or-none lateralization.

The absence of a systematic bias towards the side of the open eye in the monocular conditions (Fig. 3) also supports the view that magnetic compass information is perceived independently with either eye. Many orientation studies with pigeons in the field and in the laboratory revealed a strong and very reliable systematic bias: the birds deviated into the direction of the open eye (e.g. Prior et al. 2002, 2004). The origin of this bias is not fully understood yet, but since it can already be observed when the birds are sitting still before being released (Diekamp et al. 2002), a motoric or turning bias is unlikely. This observation rather suggests a representational bias. Such a representational bias is most likely to occur in a system where competing information from each side has to be integrated into a panoramic bilateral representation. If the input from only one side is sufficient for generating the normal behavioural output, removal of the input from one or the other side should not affect the overall balance of the system. Thus, the absence of a systematic angular deviation in birds having only one eye open (left or right) during magnetic compass orientation in songbirds suggests that each hemisphere can independently translate compass information from the contralateral eye into a valid migratory direction.

Given the clear presence of a magnetic compass in both eyes in European robins documented here, why did Wiltschko and colleagues find such a strong lateralization of the magnetic compass? One possible explanation for the differing results related to a lateralization of the magnetic compass is that they arose because of differences in the experimental paradigm or they may simply have been artifacts.

Lateralization of directional information might depend on details of the experimental conditions and treatments (e.g. Prior 2006). While the number of tests per conditions *per se* is unlikely to have caused the differences between Wiltschko et al. (2002) and the present study, a possibly crucial difference concerns the relative number of the different test conditions. In the present study, the number of tests with the right eye open only and the left eye open only was the same (1:1). In Wiltschko et al. (2002), there were twice as many tests with the right eye open as with the left eye open. This might have shaped the birds´ behaviour towards the preferred use of their magnetic compass with the right eye that was functional in two thirds of the monocular tests, while they avoided the use of the compass with the left eye, which was functional in only one third of the monocular tests. In addition to the small number of tests per condition in the case of Wiltschko et al. (2002), artifacts might also have arisen because of their non-blinded raw data evaluation procedures. Another possible explanation for the differing results is the fact that Wiltschko and co-workers (2002) tested their birds under unnatural green light conditions. In recent years, many difficult to explain orientation responses of birds tested under different combinations and intensities of coloured light of rather narrow wavelength ranges have been reported. Maybe the result of Wiltschko et al. 2002 is another such example.

The only potential explanation for the discrepancy between the present results and those of Wiltschko et al. (2002, 2003) does not involve artifacts and/or differences in experimental techniques is that we tested our birds during the autumn migratory period, whereas Wiltschko et al. (2002, 2003) tested their birds in spring (see Wilzeck et al. 2010). This could have resulted in the use of different navigational mechanisms: during their first autumn migration, young birds seem to orient only with the help of innate vector navigation, also called clock-and-compass orientation (e.g. Perdeck 1958; Helbig 1996; Mouritsen & Larsen 1998; Mouritsen & Mouritsen 2000, Thorup et al. 2007). Young birds fly in an innate population specific compass direction for a certain time period without using a map (for a review see Mouritsen 1999, 2003). Some displacement studies (Chernetsov et al. 2008) and numerous site fidelity data (for review see Newton & Brockie 2007) strongly suggest that from their first spring migration onwards, migratory birds can use previously gathered information to precisely reach their goals (breeding and wintering grounds) and, therefore, must possess navigation abilities (i.e. use map information). Data from displacement studies have shown that, while young autumn migrants cannot correct for displacements (e.g. Perdeck 1958; Mouritsen & Larsen 1998), spring migrants can correct for large east-west displacements (Thorup et al. 2007; Chernetsov et al. 2008). This correctional ability requires that the birds are able to determine their position relative to a distant goal and then correct their compass direction towards this goal even if they cannot perceive any information emanating from it, i.e. across unfamiliar landscape. Thus, during spring, the magnetic compass direction chosen by a bird in an orientation cage might potentially be the result of a navigational process based on a more complicated interaction of map and compass information than in first year migrants tested in autumn. As there is some evidence that map-like information is stored preferentially in the left brain hemisphere (e.g. in sun compass and olfaction-based homing, food-storage, etc.; e.g. Gilbert et al. 1991; Rogers et al. 2004; Gagliardo et al. 2005a, 2005b; for a review see Vallortigara 2000), it is conceivable that compass information derived with the right eye can be more easily combined with map-like information than compass information obtained with the left eye. The first might only require connectivity within the same hemisphere, while the latter might require interhemispheric transfer, which is time-consuming and usually goes hand in hand with loss of information.

Despite the small number of adult birds tested in our study, our adult birds seemed to show equal competence in compass orientation with the left eye only as with the right eye only, a difference in experience of the birds can hardly explain the discrepancies between the robin studies. Nevertheless, a more detailed comparison on lateralization patterns of spring and autumn migrants as well as of juvenile and adult migrants might be very interesting in future studies as more specific differences in hemispheric balance cannot be excluded and might be quite revealing with regard to the question of how navigational information is processed in the brain of migratory birds.

In conclusion, the notion of a strong right eye lateralization of the magnetic compass of migratory songbirds (Wiltschko et al. 2002, 2003) cannot be supported by double-blind, independent experiments performed in our lab. The data presented here, together with the results conducted with garden warblers (Hein et al. 2009) and pigeons (Wilzeck et al. 2010), suggest that potentially all bird species can perceive and process magnetic compass information with any single eye if they are forced to do so. In other words, birds can use the right eye and left brain hemisphere as well as the left eye and right brain hemisphere for visual magnetic compass orientation.

Whatever the explanation for the differences in the experimental outcomes, it is certain that more studies with monocular occlusions are needed, particularly independent studies of different groups involving the same species already tested as well as new ones, before a reliable conclusion about the amount of lateralization of the magnetic compass can be drawn. It is very possible that some small to moderate degree of lateralization of magnetic information processing exists in birds (e.g. Ulrich et al. 1999; Prior 2006; Liedvogel et al. 2007). However, our data show that the magnetic compass of night-migratory songbirds is not strongly lateralized and certainly not located in only one of the birds' eyes.

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Tables & Figures

age	magnetic field condition	Eye cover condition											
		control / both eyes open				left eye open				right eye open			
		α	r	N	p-value	α	r	N	p-value	α	r	N	p-value
juvenile birds	NMF	234	0.7204	21	0.001***	216	0.5477	21	0.01**	189	0.6505	20	0.001***
	CMF	75	0.7416	21	0.001***	69	0.3877	20	0.05*	115	0.5766	21	0.001***
adult birds	NMF	244	0.5918	6	0.12	221	0.6437	6	0.08	203	0.6543	6	0.07
	CMF	91	0.6566	6	0.07	360	0.6454	6	0.07	96	0.3421	6	0.52

Table 1: Orientation results of birds captured as juveniles and/or as adults presented separately. NMF = normal magnetic field; CMF = changed magnetic field; α = group mean direction; r = length of the vector (a low *r value* reflects high variability and vice versa; Batschelet 1981); N = number of individuals; * p<0.05; ** p<0.01; *** p<0.001.

	Eye cover condition											
magnetic field condition	control / both eyes open				left eye open				right eye open			
	α	r	N	p-value	α	r	N	p-value	α	r	N	p-value
NMF	241°	0.4466	27	0.007**	235°	0.4904	27	0.002**	180°	0.3494	27	0.054
CMF	47°	0.4414	25	0.007**	27°	0.3678	26	0.038*	109°	0.2906	27	0.105

Table 2: Orientation results of birds if only the first two active and oriented tests per bird are considered. For further details, see legend of table1.

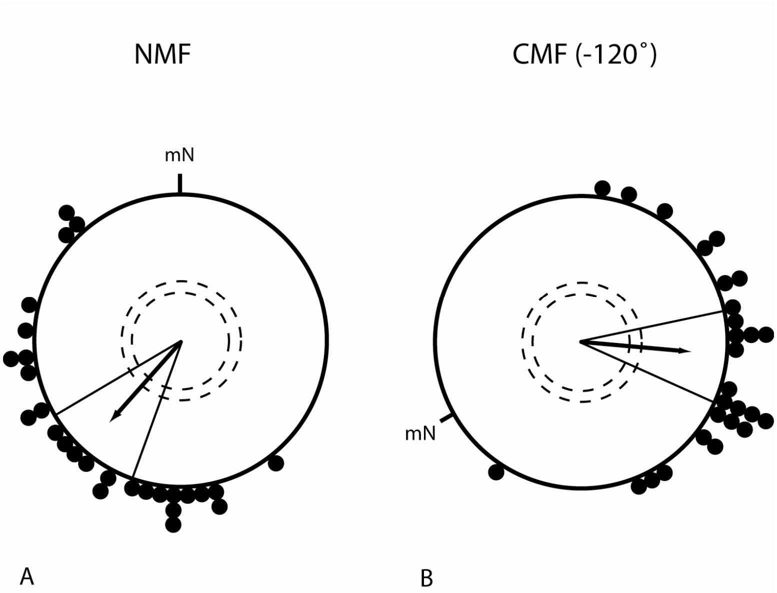

Figure 1

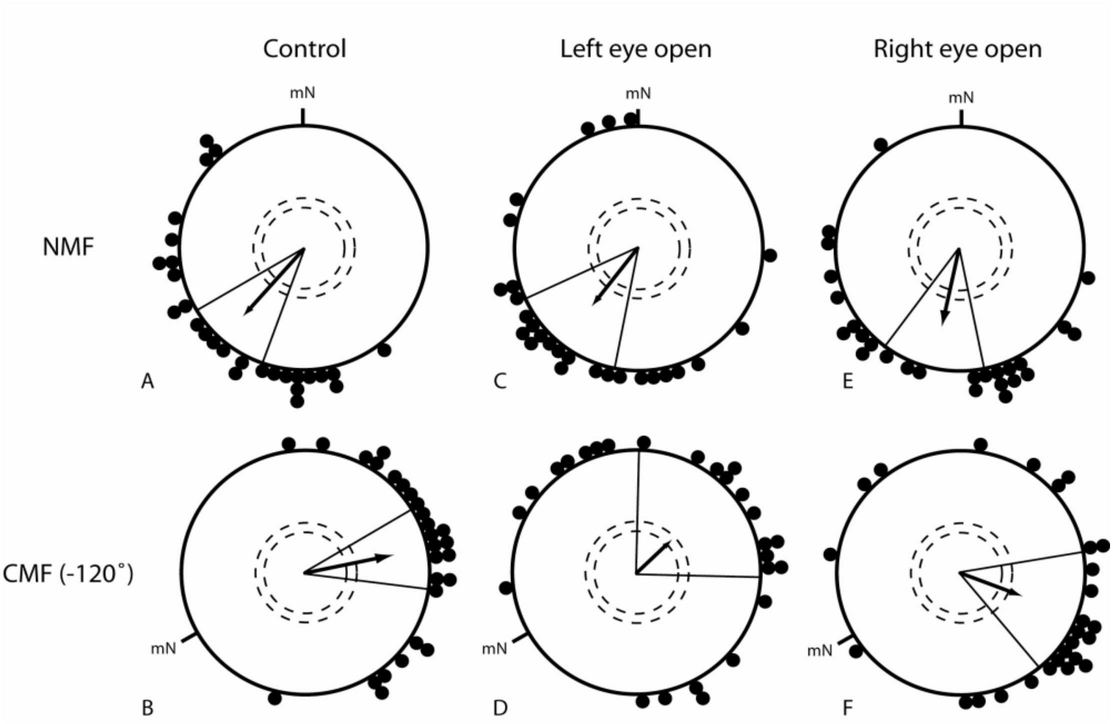
Control
Left eye open
Right eye open
NMF
CMF (-120°)
mN
mN
mN
A
C
E
mN
mN
mN
B
D
F

Figure 2

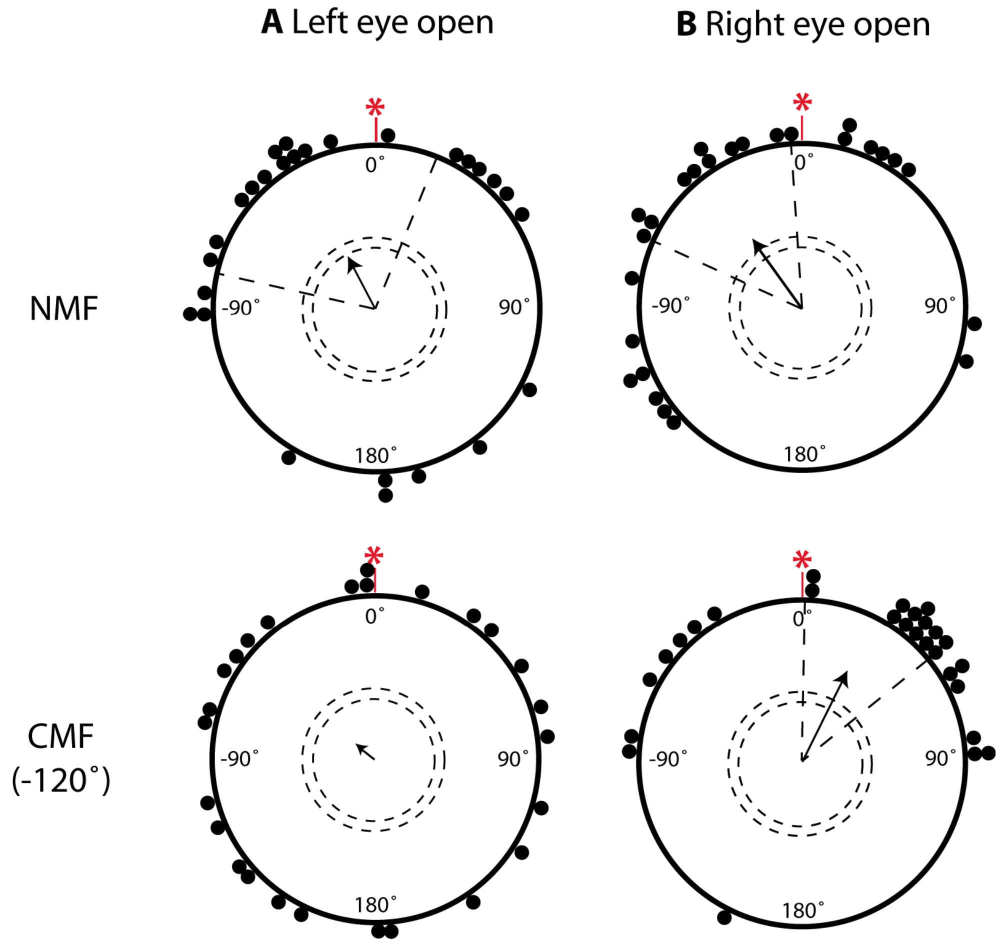
A Left eye open
B Right eye open
NMF
CMF
(-120°)
0°
-90°
90°
180°
0°
-90°
90°
180°
0°
-90°
90°
180°
0°
-90°
90°
180°

Figure 3

Figure legends

<u>Figure 1: Control experiments without eye covers show that the birds were in migratory mood and able to perform magnetic compass orientation.</u> The left diagram shows that our European Robins oriented in their typical south-westerly migratory direction in autumn in the natural magnetic field when no eye cover was attached to their head. The right diagram shows the same birds' orientation without eye covers in a magnetic field of the same intensity and inclination but turned 120° counter clockwise. mN = magnetic North. The arrows indicate the group mean vectors. The inner and outer dashed circles indicate the radius of the group mean vector needed for significance according to the Rayleigh Test ($p < 0,05$ and $p < 0,01$ respectively). The lines flanking the group mean vector indicate the 95% confidence intervals for the group mean direction.

<u>Figure 2: European Robins wearing eye covers can use their magnetic compass if light and/or visual input reaches any one eye.</u> **A-B** show the results from birds equipped with eye covers with an 8 mm diameter hole in front of both eyes. **C-D** show the results from birds equipped with eye covers allowing light and visual input to reach only the left eye. **E-F** show the results from birds equipped with eye covers allowing light and visual input to reach only the right eye. The data in A, C, and E were collected in an unchanged magnetic field (NMF). The data in B, D, and F were collected in an magnetic field turned 120° counter clockwise (CMF). mN = magnetic North. For description of the circular diagrams, see legend to Fig. 1.

<u>Figure 3: The orientation in each of the monocular conditions depicted relative to the same birds´ orientation when they wore eye covers with openings in front of both eyes (within-subject comparisons).</u> To produce these figures, we defined the orientation of each individual bird with both eyes open as 0° and depicted the orientation of the same individual in a given monocular condition relative to its binocular orientation (i.e. monocular mean direction – binocular mean direction). If the birds choose the same directions in a given monocular condition as they did in the binocular condition, the data should be centered around zero. We see no systematic and consistent differences between the angular deviations of the left eye open only condition and the right eye open only condition in neither the normal magnetic field condition (NMF, top), nor in the changed magnetic field condition (CMF, bottom). For description of the circular diagrams, see legend to Fig. 1.

PAPER VII

Kishkinev, D., Mouritsen, H. & Mora, C.V.

“An attempt to develop an operant conditioning paradigm to test for magnetic discrimination behaviour in a migratory songbird”

Submitted to Learning & Behavior

An attempt to develop an operant conditioning paradigm to test for magnetic discrimination behaviour in a migratory songbird

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Abstract

Birds are thought to possess two magnetosensory systems: i) a chemical sensor in the bird’s eye, and ii) a putative iron-mineral based sensor in the upper beak connected to the brain via the trigeminal nerve. It has been recently demonstrated that the visually mediated magnetosensory system is crucial for the magnetic compass of the European robin (*Erithacus rubecula*). In contrast, the trigeminal nerve system, despite neuronal responses to magnetic stimuli, is neither necessary nor sufficient for magnetic compass orientation in this species. Unfortunately, the potential role of the trigeminal organ is unlikely to be elucidated by the classical behavioral paradigm of the orientation cage, because it tests only for compass responses. An operant conditioning study by Mora *et al.* (2004) demonstrated that homing pigeons can be conditioned to discriminate the presence and absence of a magnetic anomaly, with the discrimination depending on the trigeminal nerve. Here, we report detailed attempts aimed at adapting the behavioral paradigm used by the above study for a model migratory bird, the European robin. We tested three different variants of the same conditioning procedure, which all fulfilled the criteria thought to be critical for successful magnetic conditioning. Despite extensive training, we were not able to demonstrate that our experimental birds were able to discriminate the magnetic stimuli presented to them. This was, however, not due to a general unsuitability of the conditioning setup for this species, because robins were able to successfully discriminate the presence and absence of an auditory stimulus in the same setup.

Keywords: European robin, magnetoreception, operant conditioning, magnetic stimulus, navigation

Introduction

It has been suggested that migratory birds possess two different magnetosensory systems: i) a chemical sensor in the bird's eye based on a radical pair mechanism (e.g., Ritz et al., 2000; see Liedvogel & Mouritsen, 2010 for a review) and ii) an iron-mineral-based sensor in the upper beak (the so-called beak organ; see Fleissner et al., 2003, 2007; Falkenberg et al., 2010). A recent study by Zapka et al. (2009) investigated the proposed functions of these two systems in a night-migratory songbird, the European robin (*Erithacus rubecula*). It demonstrated that the visually mediated magnetosensory system is crucial for the avian magnetic compass, whereas the putative iron-mineral-based receptors in the upper beak, which are connected to the brain via the ophthalmic branch of the trigeminal nerve (Heyers et al., 2010), are neither necessary nor sufficient for magnetic compass orientation in the European robin. Thus, the function of the beak organ remains yet to be elucidated. One likely role of this organ may be a magnetic intensity sensor related to a navigational map as it has been suggested for the beak organ in the homing pigeon (*Columba livia domestica*, Fleissner et al., 2003, 2007; Mora et al., 2004; Falkenberg et al., 2010). At the same time, the classical behavioral paradigm of the round orientation cage (the Emlen funnel), frequently used by avian navigation researchers since the 1950s (Emlen & Emlen, 1966), seems to be unsuitable to study the potential role of the beak organ as it tests only for compass responses (Zapka et al., 2009). There is therefore currently a need for establishing a new and easy-to-replicate behavioural paradigm, for which magnetic stimulus discrimination would be dependent on the functionality of the beak organ.

Between the 1950s and 2000s, many attempts to train birds to discriminate changes in magnetic stimuli were undertaken, but most of them failed (e.g., Orgel & Smith, 1954; Meyer & Lambe, 1966; Reille, 1968; Kreithen & Keeton, 1974; Beaugrand, 1976; Alsop, 1987; Moore et al., 1987; Couvillon et al., 1992; see Wiltschko & Wiltschko, 1995 for a review).

Bookman’s study (1977) achieved some success with a flight tunnel within which homing pigeons were rewarded for going into one of two available food boxes depending on the magnetic field stimulus presented. Bookman reported that his homing pigeons seemed to be able to discriminate the used magnetic stimuli only if they have fluttered, i.e., performed sustained hovering, jumping or short flights before making a choice (Bookman, 1977). However, Bookman did not use individual pigeons but rather mated pairs. Thus, Bookman’s behavioural observations were not obtained from independent individuals. Carman and Mahowald attempted to replicate the experiment of Bookman (1977) using a duplicated setup and similar magnetic stimuli but obtained negative results (data reported in Carman et al., 1987).

Only in the 2000s, a few conditioning studies made in birds reporting positive results were published (Freire et al., 2005; Voss et al., 2007, and Wilzeck et al., 2010 – conditioning of chickens, *Gallus gallus domesticus*, zebra finches, *Taeniopygia guttata*, and homing pigeons, respectively, to magnetic directions). Mora et al. (2004) demonstrated for the first time that homing pigeons, not mated pairs (Bookman, 1977), can be conditioned to discriminate the presence and absence of a magnetic anomaly, that is, a stimulus that provides spatial change both in total intensity and directional components, not only direction of magnetic field as in Freire et al. (2005), Voss et al. (2007), and Wilzeck et al. (2010). The stimulus used in the study by Mora et al. (2004) consisted of a magnetic anomaly, which peaked in the centre of a large tunnel-shaped experimental chamber at 189,000 nT, i.e., approximately four times the background level of 44,000 nT. In this work, Mora and her co-workers clearly showed that intact trigeminal nerves are crucial for the stimulus discrimination. This finding is in line with the hypothesis that this conditioned discrimination is mediated by the beak organ. This and other successful conditioning studies in a variety of species, including both vertebrates (the yellow-fin tuna, *Thunnus albacares*: Walker, 1984;

the rainbow trout, *Oncorhynchus mykiss*: Walker et al., 1997; Haugh & Walker, 1998; the short-tailed stingray, *Dasyatis brevicaudata*: Walker et al., 2003) and invertebrates (the honey bee, *Apis mellifera*: Walker & Bitterman, 1985) revealed two requirements seem to be critical to achieve success in magnetic conditioning (Mora et al., 2009): i) the magnetic stimulus used should be spatially distinctive, i.e., its parameters should significantly change within the area of the experimental chamber in which the animal is sampling prior to the response, and ii) the animal should move prior to responding.

Here we report the results of a detailed attempt aimed to adapt the behavioral paradigm used in the Mora et al. study (2004) for a typical nocturnal migratory bird, the European robin. In our work we used three different experimental approaches, each based on the experimental apparatus used in the Mora et al. study (2004). Despite extensive conditioning attempts lasting longer than two years, we were not able to demonstrate that our experimental birds were able to discriminate the magnetic stimuli presented. This was, however, not due to the unsuitability of the conditioning setup for this species, because the same experimental birds were able to discriminate successfully the presence and absence of an auditory stimulus in the same setup.

Method

Experimental birds

Six adult European robins (*ER)* were caught on the campus grounds at the University of Oldenburg. ER numbers 1, 3, 4, 6 and 7 took part in Experiment I, and ER numbers 1, 3, 4, 6 and 7 took part in Experiment II. ER numbers 1, 3, 7 and 10 took part in Experiment III (see details below). All the birds were older than 1 year when the experiments commenced. The birds were housed indoors in individual cages in a windowless room under an artificial light cycle simulating the local photoperiod adjusted for seasonality. The birds were given water *ad libitum* while their food intake was restricted such that each bird's weight at the start of its training session was 80-85% of its free-feeding body weight. To avoid handling stress, birds were trained to enter and exit the experimental chamber through a tunnel connecting a bird's cage and the chamber.

Experiment I

Experimental apparatus and conditioning protocol

The sessions were performed in a chamber made of polyvinyl chloride (1.6 m length x 0.4 m width x 0.47 m height). There were two identical response perches, each located at opposite ends of the experimental chamber in front of a feeder opening (Fig. 1A). Each response perch was fitted with several microswitches to detect the weight of a sitting bird. A rotating feeder disk controlled access to food through a feeder opening. Seven sampling perches, each also fitted with microswitches to detect the bird's weight, were arranged in the chamber such that sampling perch 4 (SP 4) was at the centre of the tunnel at the highest position while the other six sampling perches were symmetrically positioned on either side of SP4 (SP 1, 2, and 3 on one side and SP 5, 6, and 7 on the other side) in a downward-stepping fashion (Fig 1A). Two transparent curtains suspended from the ceiling of the chamber above SP 2 and SP 6 forced the birds to hop rather than fly across the sampling perches. The chamber's ceiling consisted of milky glass to diffuse main light from four full-spectrum halogen lamps (MR-16, Viva-Lite, Winterbach, Germany) located 0.3 m above the milky glass. Three fish-eye video cameras attached to the ceiling allowed observation of a bird in all parts of the chamber with the bird's behaviour being video recorded. One white light-emitting diode (LED), which was pointed downward and situated directly above each response perch, signalled whether or not the correct response had been made. Another LED above each response perch was pointed towards an opposite side of the chamber and showed the availability of the response perches for the choice response.

During an initial pre-training phase (21st November 2006 to 18th March 2007), the birds learned to obtain food rewards (meal worms) and to perform sampling movements in the experimental chamber (back and forth crossing of the sampling perches) without any changes in the magnetic background field. This was followed by a phase of response acquisition (19th March to 5th June 2007), during which the birds gained experience with the reinforcement contingencies associated with the response in the presence and absence of the magnetic field anomaly. Thereafter 36 daily conditioning sessions were conducted with each individual bird (6th June to 12th July 2007).

Each conditioning session was fully automated with the experimental equipment controlled by custom-written conditioning software. Each trial during a conditioning session consisted of two phases. During the first (sampling) phase, the main light from the halogen lamps was on and the bird was required to continue hopping back and forth across the sampling perches 4 to 11 times until a pseudo-randomly pre-chosen number of crossings was reached (maximal length of sampling period was 300 s). At the end of the sampling phase, a second (choice) phase began once the bird had reached SP 4. During this phase, the light from the halogen lamps was turned off whereas two white LEDs target-lit the location of each feeder opening and associated response perch. The bird had to make then a choice by crossing three sampling perches and hopping onto either response perch. In order to prevent choice bias toward the response perch, which the bird happened to be facing toward from SP4, the conditioning software pre-determined on a pseudo-random schedule for a given trial which direction the bird had to face in on SP4 at the end of the sampling phase for the choice phase to commence. If a correct choice was made, the LED lights above the correct side were left on, while the LEDs at the opposite end of the chamber were turned off (visual feedback).

Simultaneously, the feeder associated with the correct response perch was rotated to permit reward of the bird with a piece of meal worm. In case of an incorrect choice, a time penalty (darkness for 25 s) was delivered. In order to keep each bird motivated for as long as possible to perform the choice discrimination, only 50% of correct choices were rewarded (partial reinforcement schedule) to avoid early satiation. That is, which correct choices were rewarded was pseudo-randomly chosen by the conditioning software. The first correct choice for a given session was always rewarded and each session was finished on a rewarded correct choice. Each session was limited to a maximum number of 8 mealworm rewards. The inter-trial interval (ITI), during which any magnetic stimuli and all lights were turned off, was fixed at 5 s. The maximum session length was limited to 45 min.

Discriminative stimulus

A magnetic field intensity anomaly was produced by two identical coplanar, concentric, double-wrapped circular coils (0.60 m in diameter), located just above and below the centre of the experimental chamber at a distance of approximately 0.52 m (Fig. 1A). Current running through the double wiring of each coil in the same direction (parallel) produced a circular anomaly in the centre of the experimental chamber, but the side walls of the chamber prevented birds from sampling the entire anomaly so that, from the bird's perspective, the anomaly was wave-shaped (Fig. 1A and 1B, consequently hereafter such magnetic stimuli are referred to as wave-shaped anomalies). Within the anomaly's area, both magnetic total intensity and inclination changed rapidly from the background level (approximately 42,000 nT and 67.5° respectively) at either end of the tunnel to the maximum level (approximately 85,000 nT and 77° respectively) over the central sampling perch (SP 4) at the bird's head level. Current running in anti-parallel direction within each coil resulted in no significant changes (less than 10 nT) in the background magnetic field intensity.

The experimental birds were divided into two groups (2 and 3 individuals per group; unequal sample sizes due to one bird, whose data were excluded from this analysis, died prior to completion of this conditioning series). For one group, the right response perch (in relation to the entrance into the experimental chamber) was the correct choice in the presence of the magnetic anomaly, whereas the left response perch represented the correct choice in the anomaly's absence. The reinforcement contingencies were reversed for the other group of the birds.

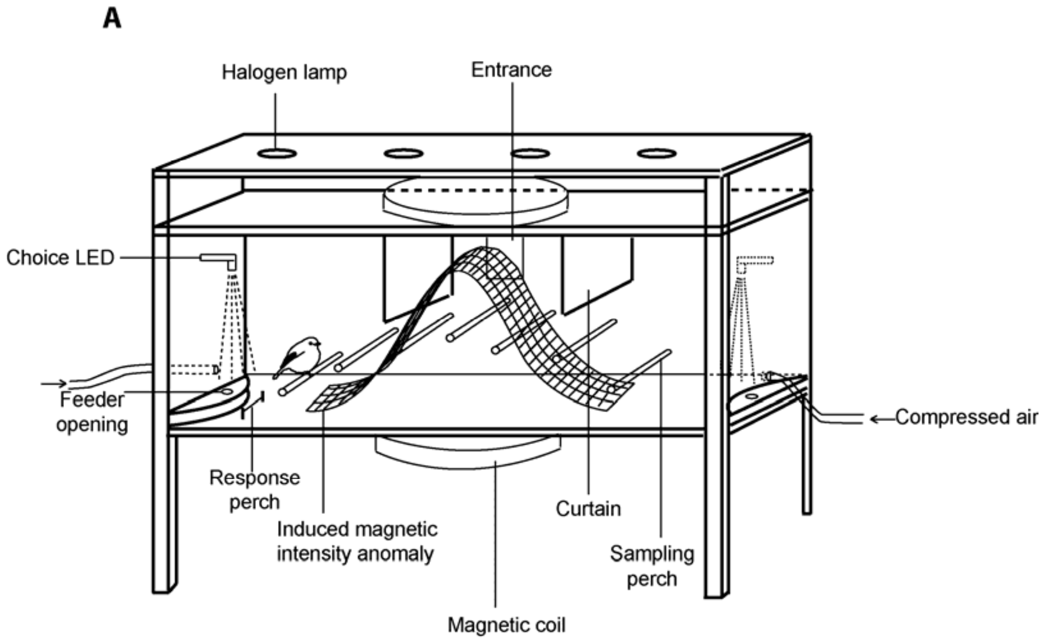
Experiment II

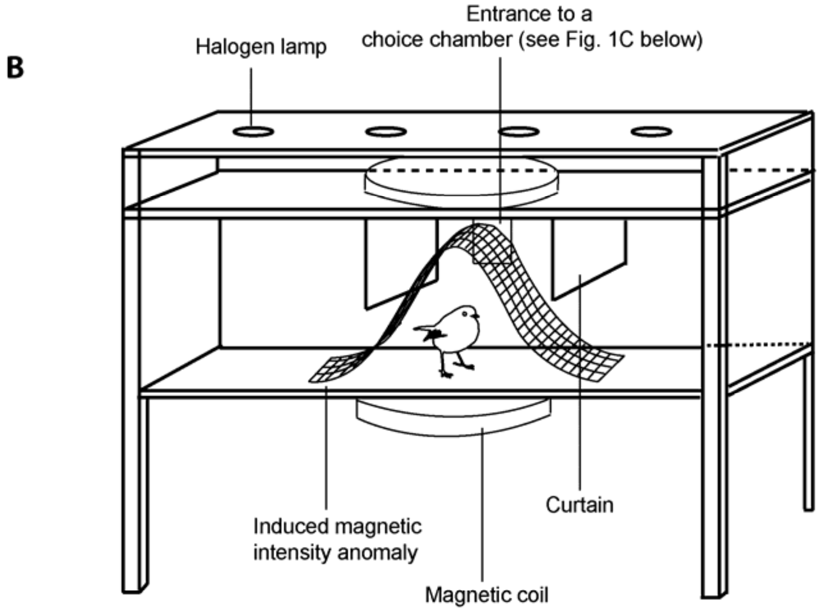
Experimental apparatus and conditioning protocol

For this experiment, 49 daily conditioning sessions were conducted for each individual bird daily (24th November 2007 to 11th January 2008). The only change made to the conditioning protocol was the addition of a second negative reinforcer, namely a puff of air, which occurred immediately after the bird had made an incorrect choice. The puff of air was delivered through tubes connecting a small hole in the end wall of the experimental chamber, situated just above each response perch at the bird's chest height, and a system of compressed air. Release of the compressed air through the hole was controlled via an electronic valve activated by the conditioning software. A 20 s time penalty, for which all lights were switched off, started 5 s after the incorrect choice had been made.

Discriminative stimulus

The birds were required to discriminate the background magnetic field (approximately 42,000 nT and 67.5°) from a wave-shaped magnetic anomaly of lower peak values than the anomaly in Experiment I (52,000 nT total intensity and 73° inclination at the central sampling perch). This peak total intensity was chosen because it more closely resembled the natural range of the Earth’s magnetic field as the total intensity of the Earth’s magnetic field varies from roughly 60,000 nT near the magnetic poles to around 30,000 nT at the magnetic equator. The experimental birds were again divided into two groups (2 and 3 individuals per group). Reinforcement contingencies were the same as in Experiment I.


A
Halogen lamp
Entrance
Choice LED
Feeder opening
Compressed air
Response perch
Induced magnetic intensity anomaly
Curtain
Sampling perch
Magnetic coil


B
Halogen lamp
Entrance to a choice chamber (see Fig. 1C below)
Induced magnetic intensity anomaly
Curtain
Magnetic coil

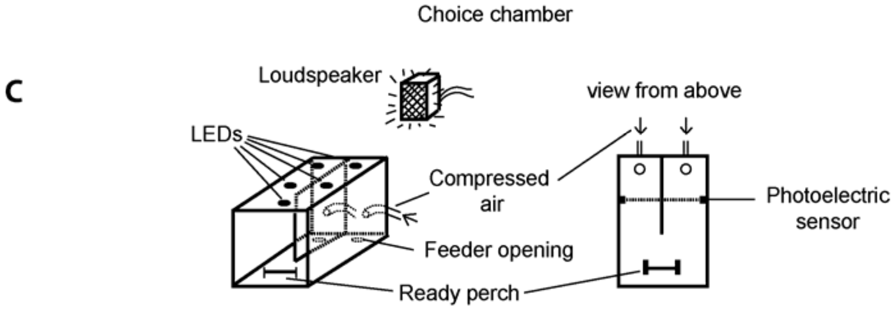

Choice chamber
C
Loudspeaker
LEDs
Compressed air
Feeder opening
Ready perch
view from above
Photoelectric sensor

Fig. 1 Experimental setups used in the conditioned choice discrimination. ***A:*** *Setup used in Experiment I and II. In Experiment I individually trained European robins were required to discriminate between the presence and absence of a magnetic field anomaly, which from the bird's perspective was wave-shaped in its intensity profile. The anomaly was located centrally in the experimental chamber with its peak intensity and inclination varying respectively from 42,000 nT and 67.5º (at the bird's head level at the two outermost sampling perches on either end of the experimental chamber) to 85,000 nT and 73.0 º(at the central sampling perch). The birds were required to mount one of two response perches located at either end of the experimental chamber depending on the magnetic field stimulus presented during the sampling phase of a given discrete trial. Correct choices were rewarded with food (piece of meal worm) from a feeder opening next to each response perch whereas incorrect choices resulted in a 20 s time penalty. In Experiment II the same apparatus was used but 1) the peak total intensity of the magnetic anomaly was lower (52,000 nT), and 2) a puff of air was introduced as a secondary negative reinforcer (in addition to the time penalty).* ***B and C:*** *Setup used in Experiment III. In B, the sampling chamber is shown. Sampling and response perches as well as feeder openings used in Experiments I & II were removed. In C, a new choice chamber (general view, left, and view from above, right) as well as a loudspeaker are shown. At the entrance of the choice chamber, a ready perch was situated. Further inside, the choice chamber was divided into two tunnels, with a feeder opening located at the end of each tunnel. A photoelectric sensor detected the bird entering one of the two tunnels to permit delivery of the food reward from that tunnel's rotating feeder or administration of a puff of air for a correct and incorrect choice respectively. In addition to the magnetic anomaly (peak total intensity of 177,000 nT), a secondary discriminative stimulus was introduced in form of a pure tone (2 kHz, 90 dB) alternated every 0.5 s with pauses of silence (for further details see text). A&C adapted from Mora* et al. *(2004).*

Experiment III

Experimental apparatus and conditioning protocol

Conditioning sessions were conducted between the 5th of September and the 3rd of December 2008. For the first seven days (5th to 11th of September 2008) conditioning sessions were performed daily, whereas thereafter (12th of September to 3rd of December 2008) two sessions per day were conducted.

During Experiment I, we noticed that birds sometimes developed side biases probably due to finishing sampling either closer to the left response perch or to the right one. To prevent development of such side biases, which may affect a bird's choice behaviour in addition to the discriminative stimuli, the following changes were made to the design of the apparatus:

1. The sampling and response perches as well as the feeder openings were removed from the experimental chamber. The bottom of the tunnel was raised by 0.21 m. This change was made to require the bird to walk on the floor instead of jumping across the perches, during the sampling phase;

2. A new plastic (choice) chamber (0.25 m length x 0.17 m width x 0.18 m height) was added to the old chamber that had comprised the experimental chamber in Experiments I and II. Thus, the bird was required to sample the discriminative stimuli whilst in the old chamber (hereafter, sampling chamber) and display its choice in the new chamber (hereafter, choice chamber, Fig. 1B and 1C). The entrance to the choice chamber was located opposite to the entrance of the sampling chamber, through which the bird entered and exited the experimental chamber at the start and end of each conditioning session respectively. A ready perch with a white LED overhead

> was situated at the entrance to the choice chamber. The ready perch was fitted with automated microswitches to register the bird's weight. The choice chamber was internally divided by a partition into two smaller elongated chambers (choice tunnels), each equipped with a photoelectric sensor to register the bird entering either tunnel during the choice phase. Several white LEDs were installed in the ceiling of each choice tunnel and a feeder opening was located at the end of each tunnel. Holes, through which a puff of air could be delivered as negative reinforcement, were situated in the wall at the end of each choice tunnel just above the feeder opening at the bird's chest level.

Some modifications were also made to the conditioning protocol. The same custom-written conditioning software was utilized but for Experiment III the conditioning sessions were not fully automated. Instead, the experimental equipment was manually controlled via the software's user interface panel. During the sampling phase of a given trial, the halogen lamps were switched on, and the bird was required to walk back and forth on the floor of the sampling chamber. Once the bird had crossed the centre of the sampling chamber 2 to 4 times within approximately 10 s (visually monitored through video cameras using a stop watch), the light from the halogen lamps was switched off and the white LEDs above the ready perch inside the choice chamber were turned on (ready phase). During this phase, the bird was required to jump from the sampling chamber onto the ready perch in the middle of the choice chamber's entrance. This phase was introduced prior to the choice phase in order to situate the bird immediately prior to the choice phase such that it was located neutrally between the two choice tunnels and thus, to prevent the development of any side biases. During the last phase, the bird was required to enter either tunnel in the choice chamber (choice phase). The bird’s choice was registered by the interruption of a light beam associated with a photoelectric sensor in each choice tunnel. In case of a correct choice, a feeder disk was rotated underneath the choice tunnel to reward the bird with a piece of meal worm through a feeder opening. To maintain the bird’s motivation to perform the discrimination task, a reward was not delivered, where it would have been the second reward in a row. Maximal number of rewards per session was limited to 8. In case of an incorrect choice, a puff of air was administrated. No time penalty was used in this experiment and the ITI was fixed at 5 s.

Discriminative stimuli

To test whether our experimental setup is generally suitable for operant conditioning with this species, we introduced a new discriminative stimulus, namely, an auditory stimulus, in the form of a pure tone (2 kHz, sound pressure level (SPL) = 90 dB) alternating every 0.5 s with pauses of silence. This auditory stimulus was produced by a loudspeaker, which was connected to a computer and placed on a table approximately 1 m from the choice chamber.

Experiment III was divided into three phases. During Phase 1 (Fig. 4), experimental birds had to discriminate the acoustic stimulus presented together with a strong magnetic anomaly (peak value in the middle of sampling chamber was 177,000 nT, i.e., about four times the total intensity of the background magnetic field and thus very similar to the magnetic anomaly successfully used to condition homing pigeons in the study of Mora et al. (2004)) from the background acoustic environment together with the background magnetic field in the experimental room. Once the experimental birds showed a clear and stable discrimination of the stimuli presented, Phase 2 was initiated. During this phase, the birds had to discriminate the same stimuli as during Phase 1, but this time the loudness of the auditory stimulus was gradually decreasing in a stepwise fashion (Fig. 4). Once the SPL of the acoustic stimulus had become comparable with that of the background level of acoustic noise in the experimental room, Phase 2 was finished and Phase 3 started (a standard procedure for transferring control of discriminative performance from one stimulus to another). During Phase 3, there was no acoustic stimulus (Fig. 4). We tested whether the experimental birds were able to discriminate between the presence of the magnetic anomaly and the background magnetic field (analogous to Experiments I and II).

This revised conditioning protocol allowed us to:

1. Test whether our experimental birds were able to achieve a sustained and sufficient level of discrimination performance with our experimental setup and conditioning protocol but using a non-magnetic discriminative stimulus that is well-known to be perceived by this species;

2. Increase the likelihood of obtaining a sustained and sufficient level of discrimination performance with a magnetic discriminative stimulus alone by i) increasing the strength of the discriminative magnetic stimulus (177,000 nT versus 85,000 nT and 52,000 nT in Experiments I and II, respectively), and ii) initially coupling the magnetic stimulus with a strong auditory stimulus. The latter permitted us, once a sustained and sufficiently high level of discrimination performance based on auditory discrimination had been achieved, to shift stimulus control from the auditory stimulus to the magnetic one by gradually decreasing the intensity of the former until it was not longer perceived by the birds.

Four experimental birds were divided into two groups (2 individuals per group). During Phases 1 and 2, for one group, the correct choice was to enter the left tunnel of the choice chamber in the presence of the combination auditory and magnetic stimulus. If no auditory stimulus and no magnetic anomaly were present, the correct choice was to enter the right tunnel. For the other group, the reinforcement contingencies were reversed. During Phase 3, for one group the correct choice was to enter the left choice tunnel of the choice chamber in the presence of the magnetic anomaly and to enter the right choice tunnel if the magnetic anomaly was absent (i.e., background magnetic field only). For the other group, the reinforcement contingencies were again reversed.

Other experiments

In addition to the three thoroughly tested experimental approaches described above, several pilot experiments were conducted with a number of alternative designs such as: i) a design similar to Experiment I (time penalty as a single negative reinforcer) and Experiment II (a puff of air as an additional negative reinforcer) but using an oscillating magnetic anomaly (0.25 Hz) with a peak intensity of 55,000 nT as the discriminative stimulus, ii) a design similar to Experiment III but requiring hopping across the sampling perches instead of walking across the bottom of the sampling chamber as the sampling behaviour in combination with a non-oscillating magnetic anomaly with a peak intensity of 82,000 nT over the middle sampling perch, and iii) a design similar to Experiment III but with colour LEDs lighting the choice tunnels so that green or blue light was coupled either with the magnetic anomaly (a peak intensity of 82,000 nT) or the magnetic background field. None of these experimental designs produced encouraging results.

Statistical analysis

A linear mixed model was fitted to each normally distributed data set using SPSS software (SPSS Inc.). The model permitted us to examine the data for the occurrence of learning, detected as changes in behaviour over time and assumed to be based on increased experience with the experimental setup and reinforcement contingencies, as well as estimate any autocorrelation between sessions. In cases where no changes in behaviour over time were detected, we applied two-sample binomial tests and two-sample t-tests to estimate whether the birds' mean performance was equally distributed around the chance level of 50% or not.

Results

Experiment I

Over 36 consecutive conditioning sessions, the percentages of individual correct choices varied considerably ranging from 30% to 80% without either any increasing tendency over sessions (type III test of fixed effects: $F_{session} = 1.22$, $p = .27$; Fig. 2A-E) or any systematic differences between performances of individual birds (type III test of fixed effects: $F_{subject} = 2.32$, $p = .058$; Fig. 2A-E). Interestingly, the mean percentage of correct choices was only slightly but statistically significantly higher than the level of chance performance of 50% (mean performance over all 36 session 52.13 ± 0.61 SE (standard error); two sample t-test with chance level as the expected value: $n = 36$, $t_{obs} = 3.50$, $p = .001$; Fig. 2F). Mean discrimination performance varied between 45% and 60% (Fig. 2F) and was greater than 50% for 26 out of 36 sessions (two-sample binomial test: n = 36, $p = .011$). Despite this, we cannot definitively conclude that a clear discrimination of the presence and absence of the magnetic anomaly was apparent in either the individual birds' performance or their mean values.

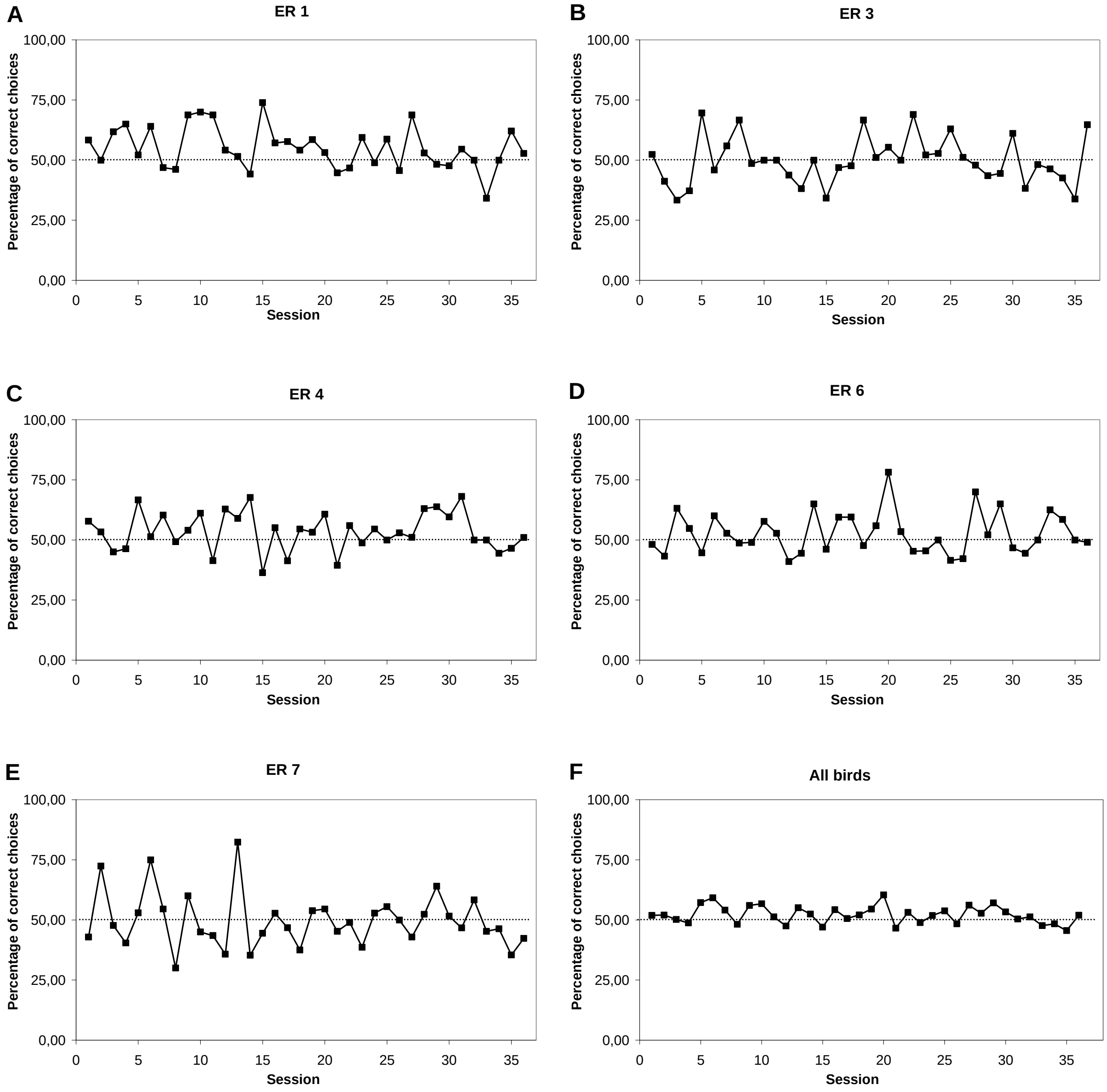


Fig. 2 Daily percentage of correct choices of individual experimental birds (A-E) and mean daily percentage of correct choices for all birds (F) in Experiment I. The horizontal lines at 50% indicate the level of chance performance.

Experiment II

As seen in Experiment I, the percentage of individual correct choices over 49 consecutive conditioning sessions also varied considerably ranging from 21% to 78% without any increasing tendency in any of the individuals (Fig. 3A-E). Similarly, the mean discrimination performance continued to be close to chance level (50%), as it had been observed for Experiment I, without any increasing tendency (type III test of fixed effects: $F_{session} = 2.49$, $p = .12$; Fig. 3F). Mean performance, however, was for most sessions slightly and again significantly above chance level (mean performance over all 49 sessions: 52.81 ± 0.57 SE; two-sample t-test with chance level as the expected value: $n = 49$, $t_{obs} = 4.94$, $p < .001$; Fig. 3F), and even continuously so for sessions 8 to 28 with only one session as exception (two-sample binomial test: n = 49, $p < .001$; Fig. 3F).

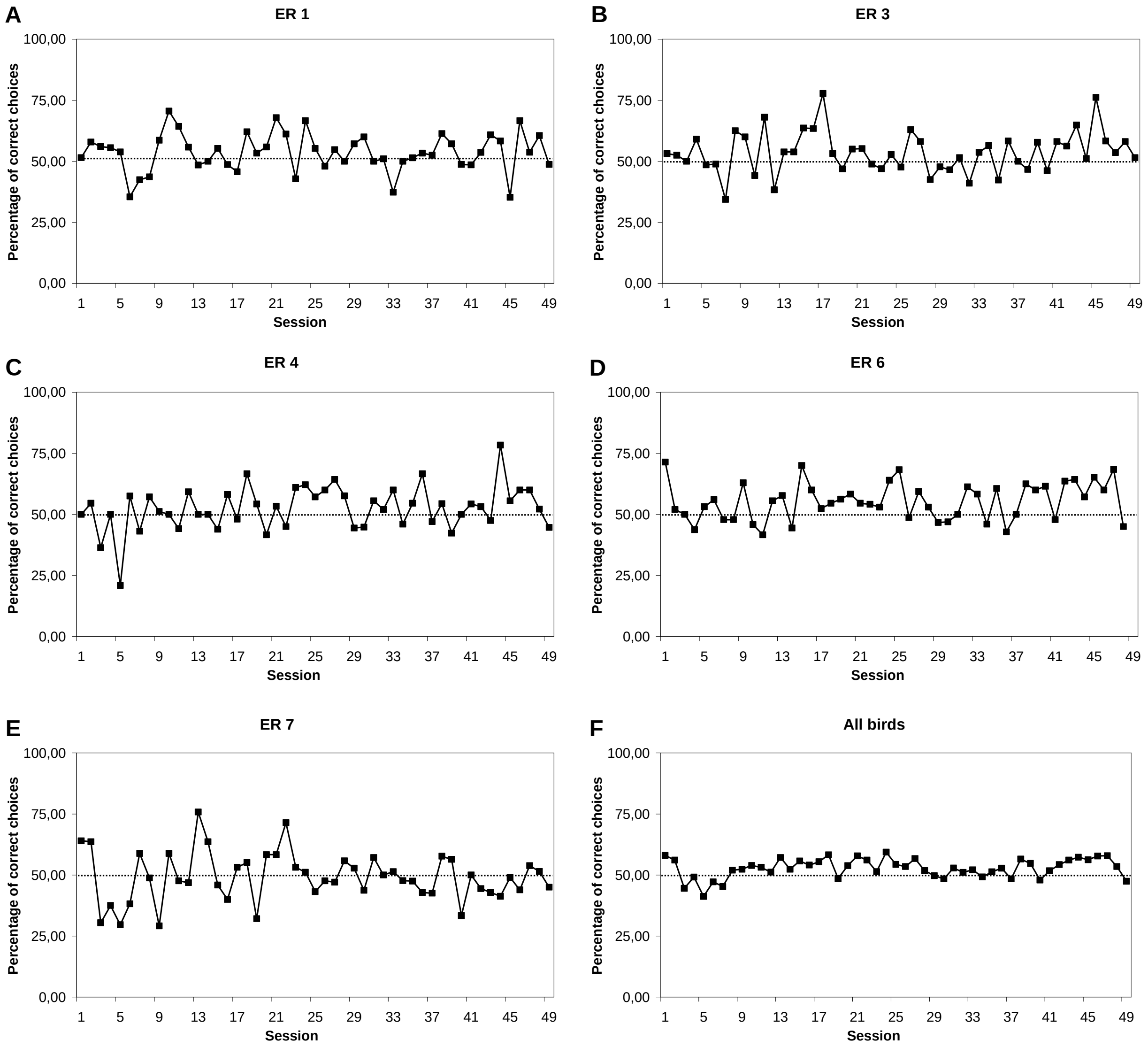


Fig. 3 Daily percentage of correct choices of individual experimental birds (A-E) and mean daily percentage of correct choices for all birds (F) in Experiment II. The horizontal lines at 50% indicate the level of chance performance.

Experiment III

During Phase 1, that is over the first 42 consecutive daily conditioning sessions, mean discrimination performance was increasing (type III test of fixed effects: $F_{session} = 22.11$, $p < .001$; the right half of Phase 1 on Fig. 4) so that in the second part of this Phase, it fell around 60% (59.78±1.20 SE) and was significantly different from the chance level of 50% (two sample t-test with chance level as the expected value: $n = 21$, $t_{obs} = 8.17$, $p < .001$; Phase 1 on Fig. 4). During Phase 2, that is over the next 28 consecutive daily conditioning sessions during which the auditory stimulus was being faded out, mean discrimination performance rapidly declined (Phase 2 on Fig. 4). By the end of this phase, performance was close to chance level (type III test of fixed effects: $F_{session} = 25.53$, $p < .001$; Phase 2 on Fig. 4). Finally, during Phase 3, that is over the last 17 consecutive daily conditioning sessions when only the magnetic discriminative stimulus was presented, mean performance resided around chance level without any uprising tendency (mean performance over 17 sessions was 52.73 ± 1.39 SE; two sample t-test with chance level as the expected value: $n = 17$, $t_{obs} = 1.97$, $p = .066$; Phase 3 on Fig. 4). Mean discrimination performance varied between 42% and 60% (Phase 3 on Fig. 4) and was greater than 50% for 10 out of 17 sessions, i.e., was equally distributed over and below chance level (two-sample binomial test: n = 17, $p = .63$).

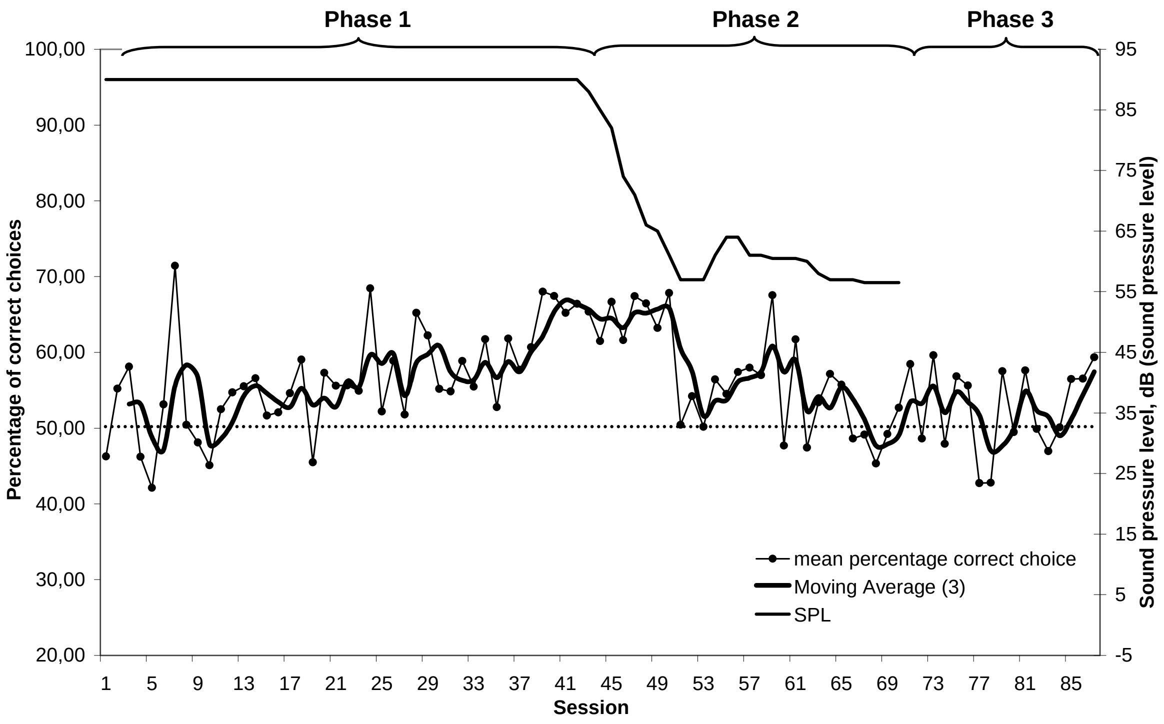


Fig. 4. Mean daily percentage of correct choices for four individually trained robins over consecutive sessions testing for the discriminating of auditory and magnetic stimuli. ***Phase 1****: discrimination of an auditory stimulus (a pure tone of 2 kHz alternated every 0.5 s with 0.5 s pauses of silence, Sound Pressure Level (SPL) = 90 dB) coupled with a strong magnetic anomaly (peak intensity value of 177,000 nT in the centre of the sampling chamber) from the absence of the auditory stimulus with only the magnetic background field present.* ***Phase*** *2: discrimination of the same auditory stimulus with SPL stepwise decreasing to approximately 55 dB coupled with the same strong magnetic anomaly as used for Phase 1 from the absence of the auditory stimulus with only the magnetic background field present.* ***Phase 3****: discrimination of the presence and absence of the strong magnetic anomaly in the absence of any auditory cues. The horizontal line indicates the level of chance performance (50%). A simple moving average (3 session blocks) was added to reduce variability of the mean daily percentage of correct choices.*

Discussion

To our knowledge, this study represents the first attempt at adapting the approach successfully used to establish operant magnetic conditioning in homing pigeons (Mora et al., 2004) to a nocturnal migratory songbird, namely the European robin. The magnetic compass sense of this species has been extensively studied in round orientation arena experiments (e.g., Wiltschko & Wiltschko, 1972, Wiltschko, 1978; Zapka et al., 2009; Hein et al., 2011), but little is known about the role of magnetoreception in relation to position determination ("map" sense) in this or any other migratory bird species.

During Experiment I, we used an experimental setup very similar to the one developed by Mora et al. (2004) except that the discriminative stimulus presented – the wave-shaped magnetic anomaly - had a lower peak value of total intensity (85,000 nT in our Experiment I vs. 189,000 nT in the study by Mora et al. (2004)). Over the course of 36 daily conditioning sessions, our experimental birds did not display any obvious discrimination ability indicating that they were able to distinguish between the presence and absence of the magnetic anomaly.

If an animal only performs at or around chance level during a discrimination task, there are several possible explanations. Firstly, the animal may be able to perceive the discriminative stimuli, but due to lack of motivation may nevertheless not be willing perform the discrimination task at all or in a sustained fashion. We used a food reward (meal worms) as a positive reinforcer, which the birds usually eagerly consumed upon being presented, and the birds were kept at approximately 80% - 85% of their free-feeding body weight. Another possible explanation for lack of stimulus control over the birds' behaviour might have been an unsuitable negative reinforcer. The purpose of the time penalty is to delay the next available opportunity to obtain a food reward. Such a delay, however, is most likely differentially effective in different species with different feeding habits. Furthermore, some species, particularly diurnal ones such as the homing pigeon, generally display a preference for being in lit surroundings rather than in the dark. For a nocturnal migrant, such as the European robin, a time penalty may thus be an ineffective negative reinforcer. Additionally, the discriminative stimulus used in Experiment I might have been unnaturally strong (almost twice the local background field) compared to the total intensity of the Earth's magnetic field, which varies with latitude between approximately 30,000 nT and 60,000 nT at the magnetic equator and the magnetic poles respectively.

Because of the above considerations, in Experiment II we introduced an additional negative reinforcement in form of a puff of air to the bird's chest region, which the birds clearly did not become accustomed to over the course of the experiment. In addition, we lowered the peak intensity of the magnetic anomaly to a more naturally encountered value of 52,000 nT. Despite these changes, the level of discrimination performance was only slightly, though significantly, higher than the chance level of 50% after 49 additional consecutive conditioning sessions. This may indicate that some learning of the discrimination task had occurred but that the behaviour had not come completely under stimulus control. That is, the birds' attention to the magnetic stimuli during the discrimination task was not sustained due to lack of sufficient motivation, the degree of difficulty of the discrimination task, or combination of the above. An alternative explanation for the lack of improved performance over time is that, despite our efforts to control for extraneous cues, some minute choice bias of unknown origin in our protocols may have moved chance performance of the experimental birds to slightly above 50%.

Finally, in Experiment III, we aimed to check the suitability of our experimental setup and conditioning protocol by first establishing conditioned discrimination of an easier-to-discriminate stimulus dimension, namely an auditory cue presented simultaneously with the magnetic stimulus. The auditory stimulus was then faded out until it was completely eliminated and only the magnetic discriminative cue remained. The results demonstrated that our European robins were able to develop stimulus discrimination for the auditory stimulus, but discrimination performance decreased again to around chance level when only the magnetic stimulus remained (with an even stronger peak intensity than in Experiments I & II, namely 177,000 nT).

Based on the results of the three experiments described above, we can conclude that the conditioned magnetic discrimination behaviour demonstrated for homing pigeons by the study of Mora et al. (2004) could not be replicated in our study for a migratory songbird with a very similar setup.

As in any case when stimulus control over the animal's behaviour cannot be demonstrated for a particular set of discriminative stimuli, it is very difficult to determine the exact reasons for such results. But absence of evidence is not evidence of absence, so that failure to demonstrate that European robins are able to discriminate between the presence and absence of a magnetic anomaly does not necessarily imply that this species lacks the sensory capacity to do so. Not only has the magnetic compass of European robins been well documented by numerous previous orientation studies (e.g., see Wiltschko & Wiltschko, 1995, for a review), but more recently it was demonstrated that i) European robins use a part of their visual system (Cluster N) to process magnetic compass information (Mouritsen et al., 2005; Zapka et al., 2009), and ii) changing magnetic field induces high neuronal activity in the European robin's brainstem complexes innervated by the ophthalmic branch of the trigeminal nerve (Heyers et al., 2010). That is the same nerve previously shown to be crucial to discriminate the presence and absence of a magnetic anomaly in homing pigeons (Mora et al., 2004) and which also seems to carry magnetic information in the European robin (Heyers et al. 2010).

Therefore, it is most likely that the experimental setup and/or conditioning procedure, rather than lack of magnetoreceptive abilities in European robins, led to the negative results reported here. This is despite the fact that the two requirements for successful magnetic conditioning previously proposed (Mora et al., 2004, 2009), namely spatial distinctiveness of the magnetic stimulus and movement of the animal as part of the behavioural response, were fulfilled in our study. The possibility that our experimental setup and/or conditioning procedure was/were not suitable to condition European robins in a two-choice discrimination task was eliminated by the successful discrimination of the auditory stimulus in Experiment III. Finally, we also considered whether bird numbers 1, 3, 4, 6 and 7 in Experiment II and bird numbers 1, 3 and 7 in Experiment III were not able to show discrimination of the magnetic stimuli due to having been "spoiled" by the experience obtained in the previous experiments. This explanation seems to be unlikely because a naïve bird (number 10) in Experiment III was also unable to show a significant discrimination, when only the magnetic stimulus was presented.

Our study adds another example suggesting that conditioning of birds to magnetic stimuli is an extremely challenging task. So far, even though positive results have been reported in non-migratory avian species (e.g., Bookman, 1977; Mora et al., 2004; Freire et al., 2005; Voss et al., 2007; Wilzeck et al., 2010), very few, if any, successful conditioning paradigms have turned out to be routinely replicable in other laboratories by other research groups (e.g., the data of Carman & Mahowald reported in the study by Carman et al. (1987) - a failure to replicate Bookman (1977); A. van Hettinga and H. Mouritsen (reported in Liedvogel & Mouritsen (2010)) – a unsuccessful attempt to replicate Freire et al. (2005)). Moreover, due to the difficulty of publishing equally important but less exciting negative results, we suspect that a significant number of additional negative results has never been published. What the field of animal navigation strongly needs is a conditioning design using magnetic cues as discriminative stimuli, which can be easily replicated independently. Such a design would become as important at facilitating magnetic navigation research as the Emlen funnel (Emlen & Emlen, 1966) has been to magnetic compass research. At present, however, no such paradigm is available.

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Erklärungen gemäß § 10 der Promotionsordnung

Hiermit erkläre ich gemäß § 10 der Promotionsordnung, dass ich mit dieser Dissertation den Titel Dr. rer. Nat. (Doktor) anstrebe.

Hiermit erkläre ich gemäß § 10 der Promotionsordnung, dass ich die Arbeit selbständig verfasst und nur die angegebenen Hilfsmittelbenutzt habe.

Hiermit erkläre ich gemäß § 10 der Promotionsordnung, dass ich meine Dissertation weder in ihrer Gesamtheit noch in Teilen einer anderen wissenschaftlichen Hochschule zur Begutachtung in einem Promotionsverfahren vorgelegt habe

Oldenburg,   Dezember 2011

Dmitry Kishkinev