# Navigation and Orientation of Long-Distance Migratory Bats

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1. Lindecke, O.[1,2,*], Voigt, C.C.[1,2], Pētersons, G.[3], and Holland, R.A.[4] (2015) **Polarized skylight does not calibrate the compass system of a migratory bat.** Biology Letters, 11, 20150525.

2. Lindecke, O.[1,2,*], Elknse, A.[5], Holland, R.A.[6], Pētersons, G.[3], and Voigt, C.C.[1,2] (2019) **Orientation and flight behaviour identify the Soprano pipistrelle as a migratory bat species at the Baltic Sea coast.** Journal of Zoology, *in press*.

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[1] Prof. Friedrich W. Merkel, *Orientierung im Tierreich*, Gustav Fischer Verlag 1980.

# Content

## ZUSAMMENFASSUNG

Tierisches Leben zeichnet sich vor allem durch einen hohen Grad an individueller Mobilität aus. Sollen die Bewegungen im Raum jedoch gerichtet erfolgen, bedarf es eines endogenen Systems, das sowohl die Einordnung der subjektiven Position im Raum (und Zeit) ermöglicht, als auch die Orientierung auf ein fernes Ziel richtet. Der Weg zum Ziel wird in Folge durch Navigation aufrechterhalten bzw. kontrolliert. Exogene Informationen, die hierfür verarbeitet werden müssen, werden über die Sinnesorgane physiologisch wahrgenommen. Mit steigender Anzahl von Sinnesorganen, gewinnt das System der Navigation dabei an Komplexität und bietet zudem die Einordnung in Hierarchien, je nachdem wie aufwendig die Bewegung sich gestaltet. Die Erforschung des Navigationsvermögens bei Säugetieren in freier Natur steht im Vergleich zu Forschung bei Vögeln noch am Anfang, besonders was Langstreckenbewegungen betrifft. Die Wanderungen von Tieren gehören allerdings zu den komplexesten Phänomenen, die in der Natur beobachtet werden können. Da die Möglichkeiten zur Langstrecken-Verfolgung und experimentellen Manipulation von wandernden Säugetieren bisher zudem äußerst limitiert sind, blieben Untersuchungen der Navigations- und Orientierungsleistungen aus, sodass die der Wegfindung zugrunde liegenden Mechanismen bisher nur eingeschränkt identifiziert worden sind. Einzig von nicht-wandernden Fledermäusen sind Ergebnisse gewonnen worden, die belegen, dass Fledermäuse einen Magnetsinn basierend auf Eisenpartikel haben müssen, den sie zur Orientierung benutzen, und darüber hinaus dass dieser in der Dämmerungsphase kalibriert wird (Holland et al. 2006, 2008, 2010). Außerdem konnte für Flughunde die Existenz einer kognitiven Karte belegt werden, die ihnen effiziente Navigation innerhalb ihres angestammten Streifgebietes (home range) ermöglicht (Tsoar et al. 2008).

Diese Arbeit widmet sich deshalb vorranging der Frage (1): „**Können wandernde Fledermäuse als Modell für die Erforschung von Säugetiernavigation dienen?**"
Unter der Annahme, dass sich nicht-wandernde und wandernde Fledermausarten bzgl. ihrer Navigationssysteme nicht extrem voneinander unterscheiden, stellen sich im Anschluss folgende Fragen (2): „**Welche Richtungsreferenz dient Fledermäusen während der Dämmerung zur Kalibrierung ihres Kompasssystems?**" und (3) „**Nutzen wandernde Fledermäuse womöglich einen Magnetsinn zur Orientierung und für die Navigation?**"
Unter den wandernden Arten eignen sich besonders die Rauhautfledermaus, *Pipistrellus nathusii* (Keyserling und Blasius, 1839), und ihre Schwesterart, die Mückenfledermaus,

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*Pipistrellus pygmaeus* (Leach, 1825), für experimentelle Untersuchungen. Beide Arten treten im Spätsommer in großer Zahl (mehrere tausend Tiere) entlang der europäischen Ostseeküste auf. Im Baltikum beringte Rauhautfledermäuse wurden inzwischen u.a. in Südfrankreich wiedergefunden (~2.000 km), sodass von einem gut ausgeprägten Navigationssystem ausgegangen werden kann. Ich untersuchte deshalb die Fähigkeiten zum orientierten Weiterflug von Rauhaut- und Mückenfledermäusen nach einer Verfrachtung weg vom Migrationskorridor.

In **Kapitel I** untersuchte ich, ob polarisiertes Himmelslicht die Kalibrierungsreferenz für das Kompasssystem von Rauhautfledermäusen sein könnte. Eine kurz zuvor erschienene Studie an nicht-wandernden Großen Mausohren, *Myotis myotis*, hatte dies für diese Art berichtet (Greif et al. 2014). Ich benutzte exakt diese Käfige, inklusive Polarisationsfiltern, aus der Studie und verglich die Flugrichtungen einer Kontroll- und einer Experiment-Gruppe von Rauhautfledermäusen nach einer Verfrachtung um 11 km. Dabei hatte die Kontrollgruppe den Sonnenuntergang beobachten können, allerdings erlaubte ihnen die Ausrichtung der Polarisationsfilter die Sicht auf das natürliche Polarisationsband am Firmament. Die Käfige der Experimentgruppe waren allerdings so ausgerichtet, dass Fledermäuse ein 90° gedrehtes Band polarisierten Lichtes am Firmament sehen würden. Die Tiere wurden mit Radiosendern bestückt und die Abflugwinkel wurden notiert.

Nach der Verfrachtung flogen die Tiere beider Gruppen in Zugrichtung nach Süden, sodass nicht von einer Kalibration anhand des polarisierten Lichtes am Himmel ausgegangen werden kann; eventuell fehlt sogar die Fähigkeit zum Erkennen von polarisiertem Licht.

In **Kapitel II** brachte ich direkt an der Küste einen selbstgebauten, runden Auflaßkäfig (*circular release box*) mit acht Ausgängen zum Einsatz, um unter Zuhilfenahme von Nachtsichttechnik zu prüfen, ob die Abflugrichtung aus dem Käfig auch der Weiterflugrichtung entsprechen würde – Ein Grundbedingung für eine mögliche Weiterverfolgung des Ansatzes. Dabei verglich ich die als wandernde Art anerkannte Rauhautfledermaus mit der vor Ort ebenfalls häufig fliegenden Mückenfledermaus.

Für beide Arten konnte ich die Übereinstimmung von Abflug und Weiterflugrichtung feststellen. Zusätzlich waren die Weiterflugrichtungen geographisch so gerichtet, dass sie dem

Zuggeschehen vor Ort entsprachen. Damit erwies sich die Abflugmessung als praktisches Maß zur Untersuchung von Orientierungsverhalten.

In **Kapitel III** untersuchte ich mit Hilfe eines Metall-Spiegel-Experiments, ob die Position der Sonne bei Sonnenuntergang (anstatt das Band polarisierten Lichts) die Kalibrierungsreferenz für das Kompasssystem von Mückenfledermäusen sein könnte. Ich verglich dafür mutmaßlich migrationserfahrene Alttiere mit naiven Jungtieren der Mückenfledermaus um zu überprüfen, ob der Kalibrierungsleistung eine Lernleistung vorausgehen könnte. Für die Messung des Orientierungsverhaltens später in der Nacht habe ich den Auflaßkäfig derart weiterentwickelt, dass ein Tier in jede beliebige Richtung (omnidirektional) abheben kann.

Die adulten Mückenfledermäuse, die den Sonnenuntergang 180°-gedreht im Spiegel beobachten konnten, flogen entsprechende 180° in die der Kontrollgruppe entgegengesetzten Richtung ab. Die Jungtiere zeigten in keiner der beiden Testgruppen gerichtetes Abflugverhalten. Es lässt sich also schlussfolgern, dass die untergehende Sonne bzw. der Azimuth der Sonne als Kalibrierungsreferenz genutzt wird. Allerdings scheinen junge Mückenfledermäuse dies erst lernen zu müssen.

In **Kapitel IV** testete ich an Rauhautfledermäusen die Hypothese eines möglichen Vorkommens von magnetischen Partikel (Magnetitkristallen) in der Hornhaut (Cornea) des Auges von Säugetieren (Wegner et al. 2006). Unter der Annahme, dass diese magnetischen Partikel einen magnetischen Kompasssinn darstellen, sollten Tiere, die im Versuch mit beiderseits betäubten Hornhäuten aufgelassen werden, in zufällige Richtungen abfliegen. Tiere jedoch, die keine oder nur eine betäubte Hornhaut haben, sollten wie die Tiere in **Kapitel I** nach Süden orientiert weiterfliegen.

In Einklang mit der Hypothese von Wegner et al. (2006) flogen Fledermäuse mit beiderseits betäubten Hornhäuten in alle Richtungen davon, Tiere der Kontrollgruppen, bzw. Tiere mit nur einer betäubten Hornhaut flogen wie erwartet in Richtung Süden.

**Zusammenfassend** zeigen die Daten meiner Dissertation, dass wandernde Fledermäuse, d.h. *P. nathusii* und *P. pygmaeus* geeignete Modelle für Orientierungsversuche zur Erforschung von Navigationsverhalten und Sinnesphysiologie von Säugetieren sind. Sowohl die Methode der VHF-Telemetrie, als auch der neue Verhaltenstest basierend auf einem omnidirektionalen Auflaßkäfig erwiesen sich als praktisch einsetzbar. Zudem erbrachten sie

replizierbare Daten. Im Gegensatz zu einer nicht-wandernden Art, *M. myotis*, scheint das Band des polarisierten Lichts am Firmament keine Rolle für Rauhautfledermäuse bei der Kompasskalibration zu spielen. Im Gegensatz zu der Hypothese, nach der sich des Kompasssystem der Fledermäuse am polarisierten Himmelsband kalibrieren lässt (Greif et al. 2014), konnte gezeigt werden, dass Mückenfledermäuse die Sonne selbst, bzw. den Sonnenazimuth zur Kalibration ihres Kompasssystems benutzen. Allerdings trifft dies nur auf vermutlich (im Wandern) erfahrene Alttiere zu. Es lässt sich darüber spekulieren, dass dieser Prozess nicht nur bei wandernden Arten auftritt. Während des nächtlichen Fluges selbst, erfüllt nach den Daten dieser Studie die Hornhaut des Auges eine bedeutende Rolle in der Orientierungskapazität von Rauhautfledermäusen. Die zufälligen Abflugrichtungen der Tiere mit beiderseits betäubten Hornhäuten lassen den Schluss zu, dass eine orientierungsrelevante Information über die Nerven der Hornhaut an das Gehirn weitergeleitet wird. Zum gegenwärtigen Stand, d.h. ohne Manipulation eines künstlich erzeugten Magnetfeldes um die Fledermaus herum, lässt sich ein nicht-spezifischer Effekt der Hornhaut-Behandlung jedoch nicht gänzlich ausschließen. Es sind unbedingt Anschlussversuche durchzuführen. Am vielversprechendsten wäre prinzipiell die Verwendung des runden Auflaßkäfigs innerhalb einer entsprechend großen Magnetfeldspule, in der die Feldparameter genau kontrolliert werden können.

# SUMMARY

Animal life is largely characterized by movement and high levels of individual mobility. However, an endogenous system for egocentric and allocentric orientation is crucial if any movement is supposed to be goal-directed. Any goal can be reached by the help of more or less advanced navigational capacities. As part of this process, environmental cues are integrated by the available sensory organs. However, the more sensory organs are integrated, the more complex navigation will be due to the weighing of cues. Hierarchies of cues may be established for efficient navigation.

Wild mammal navigation research is still in its infancy compared to its avian counterpart, in particular when it comes to long-range navigation and movements of long distances. Animal migration belongs to one of the most complex phenomena, we can observe in nature. To date, observation of long-distance moving mammal migrants is still technically limited, in particular if experimental manipulation of the moving individual is envisioned. Therefore, the navigational capacities and orientation mechanisms of wild species are virtually unknown. A promising example however, are bats. For non-migratory bat species, evidence of a magnetic sense has been provided (Holland et al. 2006, 2008). Further, we know that the calibration of a nocturnal compass system in bats happens at dusk.

In this work I address the question of whether migratory bats have the potential for a mammalian model in navigation research. Assuming that non-migratory and migratory bats do not differ significantly in their navigational systems, two further questions can be deduced: **Which directional reference or cue calibrates the compass system of bats at dusk?** And further: **Do migratory bats possess a magnetic sense which they could use for orientation and navigation?**

Among the migratory species, the Nathusius' bat, *Pipistrellus nathusii* (Keyserling und Blasius, 1839), and its sister species the Soprano pipistrelle, *Pipistrellus pygmaeus* (Leach, 1825), make ideal models for experimental work, as they are relatively small and widely abundant in Europe. Every late summer, thousands of individuals from both species migrate along the Baltic Sea shore. Individuals ringed in the Baltics have been recovered in southern France (~2,000 km) which indicates that they might possess a well-advance navigation system.

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Here, I studied the orientation capacity of Nathusius' bats and Soprano pipistrelles after translocation away from their coastal migration corridor.

In **chapter I**, I investigated of polarized skylight calibrates the compass system in Nathusius' bats. A recently published paper about non-migratory greater mouse-eared bats, *Myotis myotis*, revealed that these bats would use polarized light for exactly this purpose at dusk. Here, I used the exact same cages including polarization filter windows from that study and compared the departure flight directions of a control and a treatment group after translocation for 11 km. In contrast to the control, the experimental group watched the sky with any polarization cue 90° shifted due to the polarization filters. Animals were later fitter with VHF-tags, released and tracked until they vanished from tracking range. However, after translocation both groups continued flight in a southerly direction. Therefore a calibration based on the band of polarized light at dusk appears non-existent.

In **chapter II**, I applied a self-made *circular release box* for bats based on eight exits to test whether takeoff orientation would be a reliable proxy for departure flight direction.

Using advanced night vision scopes, I found that both Nathusius' bats and Soprano pipistrelles depart in the same direction as they chose for takeoff.

In **chapter III**, I conducted a mirror-experiment to test whether Soprano pipistrelles would use the solar azimuth (or a 180° deflection of it) at sunset to calibrate their compass system. However, I discriminated between naïve subadult migrants and experienced adult bats. For the measurement of takeoff at night, I built a novel version of the *circular release box* which enabled bats to take of in any direction.

I found that adult Soprano pipistrelle takeoff orientation was dependent on the solar azimuth, with the experimental group departing in the opposite direction compared to the control group. However, naïve subadult migrants took off in random directions suggesting that calibration of a compass system using the solar azimuth bears a learning component.

In **chapter IV**, I used Nathusius' bats to investigate the hypothesis that the cornea could carry magnetic particles which could be part of a magnetic compass system (Wegner et al. 2006). If so, then bilateral topical anaesthesia using oxybuprocaine would impair magnetic compass-based navigation resulting in random departure flights of bats.

In accordance with the predictions, animal with bilateral corneal anaesthesia departed in random directions while controls and unilateral treated animals vanished in seasonally appropriate southerly direction.

**In conclusion** the data collected over the course of my dissertation demonstrate that migratory bats, i.e., *P. nathusii* and *P. pygmaeus* are highly suitable model species for studies of mammal navigation and sensory physiology. Both VHF tracking after translocation and release, and the application of the *circular release box for bats* are useful assays which also generate data that is reproducible. In contrast to findings from non-migratory *M. myotis*, the band of polarized skylight does not play a role for Nathusius' bats' compass calibration at dusk. However, in light of the data collected with Soprano pipistrelles, the solar azimuth appears to be the prevailing calibration reference. Yet, this could only be demonstrated in adult, experienced bats. Data from this thesis further support the assumption that the cornea plays an important role for the orientation capacity of Nathusius' bats. Observed randomly directed departure flights in bats with bilateral corneal anaesthesia argue for a corneal sense contributing to orientation and navigation which could be a magnetic sense based on iron particles *sensu* Wegner et al. (2006).

# GENERAL INTRODUCTION

Many mammals, such as whales, ungulates and bats, are capable of long-distance migration, yet it is poorly understood what specific cues they use to find their way. Compared to our knowledge of navigation in migratory birds, navigation research in mammals is in its infancy. Bats represent an excellent study system for investigating the importance of specific cues for navigation and also the underlying mechanisms, because they are capable of long-distance migration and because their relatively small size facilitates experimental manipulation. Nonetheless, only a few studies have thus far focused on the navigational skills of bats, and previous studies have worked exclusively with non-migratory species.

**The annual cycle of European migratory bats** is characterized by two sedentary phases (breeding in summer and hibernating in winter) and two periods of migratory movements (spring and autumn). Sedentary and migratory periods involve different navigational tasks. When migrating, I expected bats to follow a track and to adjust their nocturnal movements and stopover behaviour depending on weather conditions. During summer, when maternal and mating roosts are formed, a high resolution of a bat's spatial map is required. In fact, it has been documented that individual Nathusius' bats, *Pipistrellus nathusii*, may return to the same roost during subsequent years, indicating a high level of fidelity to their place of summer residence (Haensel 1994). For sedentary bats, environmental cues relevant for navigation do not change largely, at least less pronounced than during migration. Therefore, homing after hunting at feeding grounds might be based on other orientation and navigational skills than during migration. However, the lack of data on migratory bats' navigational capacities will let me focus here entirely on migratory species.

In general, navigation of animals is based on a compass-map system (Rozhok 2008, Holland 2014). Such a system involves an orientational map followed by integration of environmental cues to facilitate the precise navigation towards the destination (Kramer 1953; Wiltschko & Wiltschko 2003; Wallraff 2005; Bingman & Cheng 2005). The map is assumed to be established by referring to sensory cues that vary predictably along latitudinal and longitudinal gradients. In some bird species, this might cover the entire globe. The compass step is the interpretation of one or several cue(s) that indicates the bearing to reach the goal. In bird studies, the map and compass system is a well-established fact (Wiltschko & Wiltschko 2003). Only in first year migrants the map step seems to be replaced by a genetically imprinted orientation mechanism (Berthold 2001).

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Bats may use vision, magnetoreception, olfaction or hearing for orientation and navigation during migration. Echolocation calls emitted by bats are only effective over relatively short distances, depending on the ultrasonic frequencies used and on ambient conditions such as habitat clutter, ambient temperature and humidity. Therefore, the acoustical domain seems unsuitable when bats have to navigate over long distances. However, the relevance of some of the other sensory modalities for navigation has already been documented, at least for non-migratory species.

**Magnetoreception**, Minute magnetite particles in sensory cells have been documented in a variety of bat species (Tian et al. 2010; Fig. 1). It was argued that these particles may facilitate the perception of the Earth's magnetic field via mechanisms similar to those observed in mud-dwelling magnetotactic bacteria (Fig. 2).
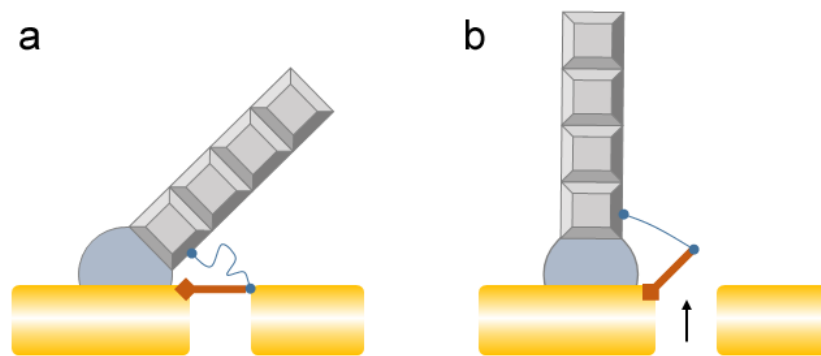


**Fig. 1** | The currently favoured theoretical model of neural signalisation via opening ion channels. A hypothetical representation of how single-domain grain magnetite crystals may rotate according to the external field (a ➤ b) resulting in the filament transferring the torqueto the channel (b). Based on Shaw et al. (2015).

The underlying compass may then be calibrated in the same way as in bird magnetoreception, i.e. bats are referencing it to the patterns of polarised ambient light at sunset (Greif et al. 2014). When doing so, bats recognize a bright band of light in the sky, which represent the natural maximum of polarised light projected to the sky at sunset from north to south. Yet, we lack any information whether or not polarized light is relevant for migratory bats as well, particularly we do not know if migratory bats may use polarized light for calibration on a daily basis, or only once before migration as it has been suggested for some bird species (Muheim et al. 2006, Fig. 3).
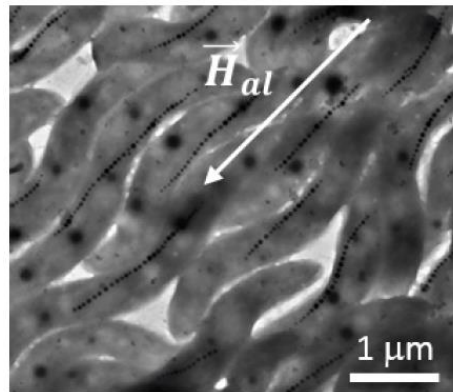
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**Fig. 2** | An example of magnetite-mediated navigation in *Magnetospirillum gryphiswaldense*. TEM image of magnetically oriented bacteria deposited onto a Si substrate under an aligning field $H_{al}$ (from Orue et al. 2018, electronic suppl. Opensource CC-BY).

Besides the aforementioned sensory modalities, lifetime experience might be an additional factor relevant for the accuracy and precision of navigation in migratory bats. Possibly, migrants develop a hierarchy of cues during their lifetime that helps in fine-tuning and thus accomplishing the navigational task. A possible difference in navigational performance between naïve, juvenile and experienced, adult migratory bats might help in answering the question which cue perception is based on innate and which on learned mechanisms.
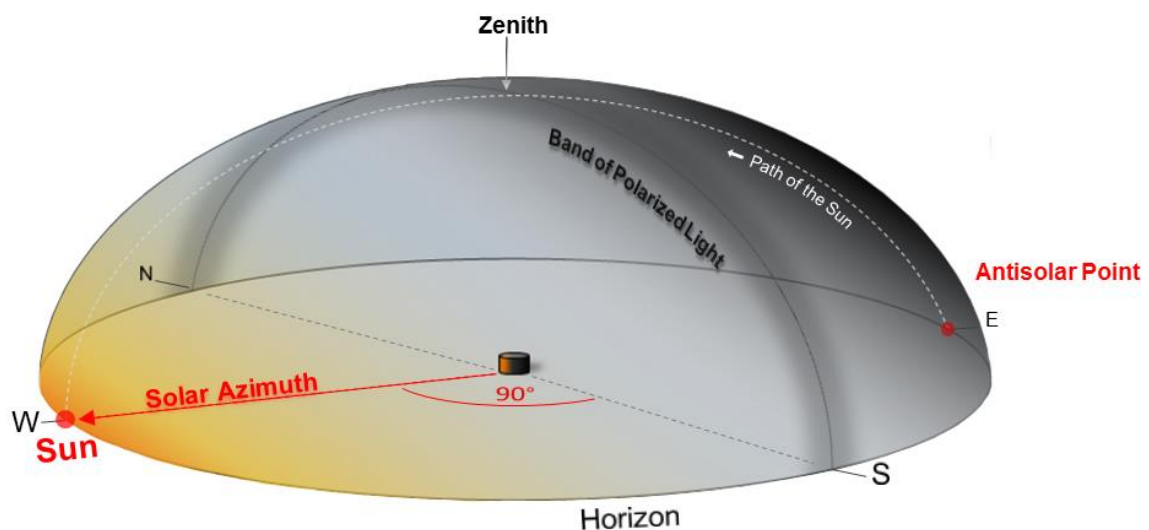


**Fig. 3** | Potential light cues for animal orientation and/or compass calibration at dusk. Note, the band of polarized sky light provides bimodal directional information while the solar azimuth is unimodal.

# THESIS OUTLINE

With more than 1,300 species described to date and a global distribution, bats bear strong potential as a study system for questions on animal navigation and movement ecology. With this thesis, I aimed to elucidate whether European migratory bats of the *Pipistrellus* genus could be used as a study model for vertebrates, and mammals in particular. Whether their navigation system is different to that of sedentary bat species, and how it compares to that of migratory birds, are further key questions raised in this thesis. In line with this, I aimed at widening the methodological spectrum of bat migration research by borrowing the recently established model for testing orientation and navigation in non-migratory bats based on radio tracking of their departure directions after displacement. Further, I focused on developing an orientation assay to enable the measurement of departure flight directions on a small scale for future studies in laboratory-sized environments. My fieldwork was conducted in a coastal bat migration corridor, and in the landscape in the immediate vicinity of it. In **CHAPTER I**, I investigated both the role of polarized skylight at dusk for calibration of the compass system in Nathusius' bats' (*P. nathusii*) and how bats would principally orientate after translocation away from the coastal migration corridor. In **CHAPTER II**, I investigated whether Soprano pipistrelles (*P. pygmaeus*) that show a similar phenology of abundance in the same migration corridor, could be classified as a migratory species. For this, I built a prototype of an orientation cage designed for recording bat takeoff directions. In **CHAPTER III**, I investigated the role of the solar disc at sunset for nightly navigation in experienced adult and naïve first-year Soprano pipistrelles applying a mirror experiment and an advanced model of the previous orientation cage for behavioural testing. In **CHAPTER IV**, I applied radio tracking and translocation in Nathusius' bats again for an investigation on whether corneal sensation could be an integral component of their orientation system. In example, I asked whether Nathusius' bats would make a suitable model organisms for the search of magnetoreceptors in the corneal epithelia.

# CHAPTER I – Navigation after translocation and the role of polarized skylight for compass calibration

as

**Polarized skylight does not calibrate the compass system of a migratory bat**

by

Lindecke, O., Voigt, C.C., Pētersons, G., Holland, R.A.

## Abstract

In a recent study, Greif et al. (Greif et al. Nat Commun 5, 4488. (DOI: 10.1038/ncomms5488)) demonstrated a functional role of polarized light for a bat species confronted with a homing task. These non-migratory bats appeared to calibrate their magnetic compass by using polarized skylight at dusk, yet it is unknown if migratory bats also use these cues for calibration.

During autumn migration, we equipped Nathusius' bats, *Pipistrellus nathusii*, with radio transmitters and tested if experimental animals exposed during dusk to a 90° rotated band of polarized light would head in a different direction compared with control animals. After release, bats of both groups continued their journey in the same direction.

This observation argues against the use of a polarization-calibrated magnetic compass by this migratory bat and questions that the ability of using polarized light for navigation is a consistent feature in bats. This finding matches with observations in some passerine birds that used polarized light for calibration of their magnetic compass before but not during migration.

**Keywords**: *mammalian navigation, orientation, migration, Chiroptera, sensory ecology*

## Introduction

Animals from a broad taxonomic background extract directional information from a variety of environmental cues (Papi 1992), including the sun (Kramer 1953), polarized light (Muheim et al. 2006), stars (Emlen 1967) and the Earth's magnetic field (Wiltschko & Wiltschko 1972, 2006). In migrating passerine birds, these cues appear to be used hierarchically, in which one provides an absolute geographical reference that calibrates others, which are then used as a compass to take up the desired direction of orientation (Bingman et al. 2003). Disagreement persists as to whether the magnetic field (Wiltschko & Wiltschko 1975, Sandberg et al. 2000, Wiltschko et al. 2008, Chernetsov et al. 2011, Schmaljohann et al. 2013), or polarized light (Muheim et al. 2006, 2009) provides this absolute geographical reference.

For bats, evidence from two species, one from Europe and one from North America, has indicated that non-migratory bats use a magnetic compass for orientation during homing (Holland et al. 2006). Surprisingly, for a strictly nocturnal mammal, this magnetic compass appears to be calibrated by sunset cues (Holland et al. 2010). Also, it has been shown for one of these species that polarization cues at dusk are the crucial geographical reference for calibrating the magnetic compass (Greif et al. 2014). A number of bat species migrate more than 1000 km between summer and wintering areas (Cryan 2003, Hutterer et al. 2005). Such journeys inevitably require a suite of navigational cues to allow bats to locate their specific breeding grounds, stopover sites along the migratory route, and the preferred wintering roosts. Yet, to date studies of the navigational skills of migratory bats are lacking (Holland 2007).

Here, we test for the first time, we believe, the orientation of bats during autumn migration, specifically investigating whether they use the same mechanisms of compass calibration as non-migratory bats.

## Material and methods

*Pipistrellus nathusii* is a long-distance European migratory bat (Dietz et al. 2009). Extensive banding studies have produced evidence of southwesterly migratory movements from northeastern Europe during autumn and distances of up to 2000 km (Pētersons 2004). At the peak of the migratory season in Latvia (between 13 and 22 August 2014), we caught 16 males and 24 females (all adult) at Pape Biological Station (56°09' N 21°03' E, Rucava Municipality, Latvia), using a funnel trap. Bats were kept in wooden boxes over periods of 7–16 days to avoid unsuitable release conditions under periods of poor weather. We did not anticipate any effect of this resting period on the outcome of our experiment, because all

animals were exposed to the same conditions. Captive bats were fed individually with mealworms and had access to ad libitum water.

**Experimental procedure.** On the day of the experiment, any handling of test subjects was performed indoors in order to avoid exposure to polarized skylight. Prior to the treatment at dusk, bats were fed with up to five mealworms and water to promote activity and to counteract re-entering torpor.

To test whether changing the polarization pattern of skylight affects heading of migratory flights, we fitted VHF radio transmitters (PicoPip AG379, BioTrack Ltd,Wareham, UK or LB-2N, Holohil Systems Ltd, Ottawa, Canada; 0.42 g) to the back of bats, using skin glue (Manfred Sauer GmbH Hautkleber, Lobbach, Germany). We hypothesized that experimental animals exposed during dusk to a 90° rotated band of polarized light would head in a different direction compared with control animals. Previous experiments have indicated that such a shift results in a bimodal distribution, shifted +90° from controls (Muheim et al. 2006, Greif et al. 2014). During the experiment, bats were placed in the original experimental polarization boxes as described in (Greif et al. 2014), but see the online supplementary material for a detailed description [*Appendix 1.1*]. Holding boxes were placed 50 m away from the funnel trap on a meadow offering a 360° free view of the horizon from 30 min before until 90 min after sunset, i.e. until the last visible post-sunset glow had vanished. Experiment evenings had stable weather with a light to moderate breeze (2–8 m s-1) and 15–60% cloud cover and always a visible sunset. Boxes were oriented either with the vertically polarized windows 90° away from the sun, corresponding to the natural polarization direction (PN, in a North–South axis) or they were shifted 90° so that horizontally polarized windows were oriented North–South, thus generating a shifted polarization direction (PS; Fig. 1a in (Greif et al. 2014)). To avoid integration of any other visual cues, bats were kept in cotton bags individually after the treatment and until release.

At 23:15 h, we translocated them to the release site (RS), which was approximately 11 km east of the capture site in the coastal migration corridor. We assume that the test individuals did not know this site in the very east of the capturing site. Similar to homing studies, we hereby evade the interference of previously experienced landmarks, e.g. the seashore, which could bias any departure direction. The area chosen for RS was a flat field offering a 360° free view of the horizon. Experiments were conducted during six nights (between 22 August and 1 September; see the electronic supplementary material for details [*Appendix 1.2*]. At the RS, bats were fed and offered water to prompt migration instead of foraging. The person who measured the direction of vanishing bearings was blind to the treatment conditions. Before

releasing bats, we surveyed the vicinity of the RS for the presence of any other bats (Echometer EM3+,Wildlife Acoustics, Inc., Maynard, MA, USA). If any bat would have been recorded, releases of subjects would have been paused to avoid confounding via eavesdropping. After midnight, bats were released individually from the roof of a car with a randomly chosen release direction and with a random order between treatments. Then, bats were tracked at about 4 m above ground using both a handheld three element Yagi antenna attached to an AR8200 III receiver (AOR) and another antenna attached to an Australis 26k receiver (Titley Scientific). When the signal of the radio transmitter vanished, we noted the bearing of the fading signal and the time elapsed since the release. Two minutes after the signal disappeared, we confirmed the absence of bats by monitoring the area with the radio-tracking equipment. During a given night, the last bats were released between 02:25 h and 04:55 h (more than 1 h before sunrise, six nights). After having released all bats during a given experimental night,we surveyed the area for radio transmitter signals to confirm that all experimental bats had disappeared. A further complete scan for all frequencies was repeated the following day prior to further release events.

All work was conducted under permit no. 5/2014 to the Institute of Biology, University of Latvia.

**Statistical analysis of orientation behaviour.** Vanishing bearings were analysed using Oriana 4.0 (Kovach Computing Services, Pentraeth, UK). The Rayleigh test was used to test for non-uniformity of each dataset and the Mardia–Watson–Wheeler test analysed for angular differences in the groups (22). A test for significant difference between vanishing times of groups was performed using the t-test (SigmaPlot 11.0, Systat Software Inc., Chicago, IL, USA).

## Results

The radio signals of all bats vanished after departing from the RS. Eight individuals spent a short time foraging or perching after release ($t_{mean}$ = 14 min; see the electronic supplementary material [*Appendix 1.2*]). Vanishing bearings of both groups were significantly oriented (Rayleigh's test, PN: n = 20, r = 0.507, Z = 5.138, p = 0.005; PS: n = 20, r = 0.629, Z = 7.922, p < 0.0001, Fig. 4). Mean bearing of bats with PN (control) was 200° (south–southwest) and 183° (south) in the PS group. There was no significant difference between the groups (Mardia–Watson–Wheeler test, W = 2.199, p = 0.333). There was no significant difference between the lengths of vanishing times of bats (PN = 15.8 min, PS = 18.4 min; t = 20.967, d.f.

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= 38, p = 0.339). Acoustic monitoring at the RS revealed no echolocation calls of any other bat during release events (see the electronic supplementary material for details [*Appendix 1.2*]).
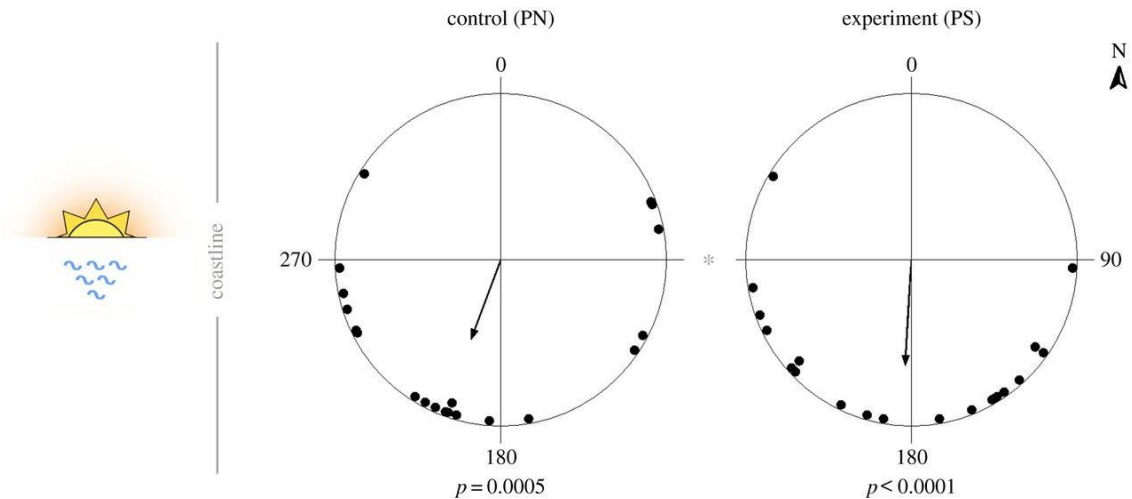


**Fig. 4** | Vanishing bearings of bats translocated to the release site (asterisk) in a presumed unfamiliar area 11 km away east from the coastal migration corridor. The natural coastline, where bats were caught and treated, follows the line of longitude. North (0°) is the top of the circular plots. Arrows depict the mean and vector length of all individual migratory flights after departure of the control group tested for natural polarization direction (PN) and the experimental group (PS) treated with a 90° shifted polarization direction (n $_{PN}$ = 20, n $_{PS}$ = 20). *p*-values from the Rayleigh tests are shown.

## Discussion

Understanding of the orientation and navigation mechanisms of migratory bats has lagged behind other comparable taxa (Holland 2007). In this paper, we demonstrate that departure directions measured by VHF telemetry are comparable with the seasonally appropriate direction of migratory *P. nathusii* in a nearby migration corridor (Pētersons 2004). Vanishing bearings of bats treated with a 90° shifted polarization field did not differ from controls exposed to natural patterns. Thus, our results contradict the hypothesis that *P. nathusii* use polarized light as their primary calibration reference.

An additional observation supports the fact that a 90° shift of polarization at sunset had no effect on subjects: we did not observe any evidence of bimodality in the vanishing bearings, such as was observed in homing experiments with non-migratory greater mouse-eared bats (*Myotis myotis*, Greif et al. 2014). This bimodality is typical for experiments with effective

PS (Muheim et al. 2006, Beltrami et al. 2010), as the polarization pattern is non-directional, i.e. without any polarity.

Our data suggest that the environmental cues used to calibrate the compass system may depend on the migratory status of bats, with non-migratory bats using cues of polarized skylight and a migratory bat not appearing to calibrate its compass system. This has parallels in bird migration, with some studies suggesting that celestial cues dominate in the pre-migratory period but not during the migratory period (Wiltschko & Wiltschko 1975, but see Muheim et al. 2006).

However, to date the use of polarized light has only been demonstrated for a single bat species (*M. myotis*, Greif et al. 2014), and so caution is warranted in interpreting ecological differences between these two single species. Further experiments are necessary and it remains to be tested if *P. nathusii* depend on polarized light for orientation during the non migratory period. It should also be noted that in birds, results are inconsistent, with some data supporting the role of polarization as a primary calibration reference for the magnetic compass during migration (Muheim et al. 2006, 2009), while others do not (Wiltschko & Wiltschko 1975, Wiltschko et al. 2008, Chernetsov et al. 2011, Schmaljohann et al. 2013). A review of the published literature indicated that methodological differences, namely, access to a view of the horizon at sunset, may explain these differences (Muheim et al. 2006). One recent study has also indicated an apparent difference depending upon the method by which orientation was measured (Giunchi et al. 2015). Our study used the same methodology as (Greif et al. 2014) in the view of the horizon at sunset, the nature of the experimental boxes to shift polarization and the method of measurement of orientation, and so methodological differences would seem an unlikely explanation for our results. Geographical position en route of migration or species specific differences could also explain varying compass calibration systems. For our experiments, we can exclude any acoustic orientation in the sense of eavesdropping as an effective cue for navigation towards the coastline. The migration corridor was more than 10 km from the RS and no bats were recorded when our test subjects were released.

We conclude that polarization of the sky is not a necessary daily calibration cue for navigation during migratory flights of bats. Further experiments are required to test for putative hierarchies of orientation cues or ecological factors influencing choice of the most reliable cues during bat migration.

## Acknowledgements

## Author contributions

O.L. carried out the fieldwork, participated in its design, analysed the data and drafted the manuscript. G.P. supported fieldwork. C.C.V. and R.A.H. supervised the project, designed the experiment, provided material and helped draft the manuscript. R.A.H. performed the statistical analysis on bearings. All authors contributed to the final form of the article.

## References Chapter I

Beltrami G, Bertolucci C, Parretta A, Petrucci F, Foà A. 2010. A sky polarization compass in lizards: the central role of the parietal eye. *J. Exp. Biol.* **213**: 2048–2054.

Bingman VP, Budzynski CA, Voggenhuber A. 2003. Migratory systems as adaptive responses to spatial and temporal variability in orientation stimuli. In: Berthold P, Gwinner E, Sonnenschein E, editors. *Avian Migration*. Berlin: Springer. p457-70.

Chernetsov N, Kishkinev D, Kosarev V, Bolshakov CV. 2011. Not all songbirds calibrate their magnetic compass from twilight cues: a telemetry study. *J. Exp. Biol.* **214**: 2540-2543.

Cryan PM. 2003. Seasonal distribution of migratory tree bats (*Lasiurus* and *Lasionycteris*) in North America. *J. Mammalogy* 84, 579–593.

Dietz C, von Helversen O, Nill D. 2009. Handbook of bats of Europe and Northwest Africa. A C Books. 400pp.

Emlen ST. 1967. Migratory Orientation in Indigo Bunting *Passerina Cyanea*. Part I: evidence for Use of Celestial Cues. *Auk* **84**: 309-318.

Giunchi D., Vanni L., Baldaccini NE., Spina F, Biondi F. 2015. New cue-conflict experiments suggest a leading role of visual cues in the migratory orientation of Pied Flycatchers *Ficedula hypoleuca*. *J. Orn.* **156**: 113-121.

Greif S, Borissov I, Yovel Y, Holland RA. 2014. A functional role of the sky's polarization pattern for orientation in the greater mouse-eared bat. *Nat Commun* **5**. doi: 10.1038/ncomms5488.

Holland RA. 2007. Orientation and navigation in bats: known unknowns or unknown unknowns? *Behav. Ecol. Sociobiol.* **61**: 653-660.

Holland RA, Wikelski M. 2009. Studying the migratory behavior of individual bats: current techniques and future directions. *J. Mamm.* **90**: 1324–1329.

Holland RA, Borissov I, Siemers B. 2010. A nocturnal mammal calibrates a magnetic compass by the sun. *Proc. Nat. Acad. Sci. U.S.A.* **107**: 6941-6945.

Holland RA, Thorup K, Vonhof M, Cochran W, Wikelski M. 2006. Bat orientation using Earth's magnetic field. *Nature* **444**: 702.

Hutterer RA, Teodora I, Meyer-Cords C, Rodrigues L. 2005. Bat migrations in Europe. Bonn, Germany: Naturschutz und Biologische Vielfalt.

Kramer G. 1953. Wird die Sonnehöhe bei der Heimfindeorientierung verwertet? *J. Orn.* **94**: 201-219.

Muheim R, Moor FR, Phillips JB. 2006 Calibration of magnetic and celestial compass cues in migratory birds—a review of cue-conflict experiments. J. Exp. Biol. 209, 2–17.

Muheim R, Phillips JB, Akesson S. 2006. Polarized light cues underlie compass calibration in migratory songbirds. *Science* **313**: 837-839.

Muheim R, Phillips JB, Deutschlander ME. 2009. White-throated sparrows calibrate their magnetic compass by polarized light cues during both autumn and spring migration. *J. Exp. Biol.* **212**: 3466-3472.

Papi F, (editor), 1992. *Animal Homing*. London: Chapman and Hall. 390pp.

Pētersons G. 2004. Seasonal migrations of north-eastern populations of *Pipistrellus nathusii*. Myotis 41–42: 29–56.

Sandberg R, Backman J, Moore FR, Lohmus M. 2000. Magnetic information calibrates celestial cues during migration. Anim. Behav. 60: 907-907.

Schmaljohann H, Rautenberg T, Muheim R, Naef-Daenzer B, Bairlein F. 2013. Response of a free-flying songbird to an experimental shift of the light polarization pattern around sunset. *J. Exp. Biol.* **216**: 1381-1387.

Schmidt-Koenig K, Ganzhorn JU, Ranvaud R. 1991. The sun compass. *Experientia Supplementum (Basel)* **60**: 1-15.

Wiltschko R, Wiltschko W. 2006. Magnetoreception. *Bioessays* **28**: 157-168.

Wiltschko R, Munro U, Ford H, Wiltschko W. 2008. Contradictory results on the role of polarized light in compass calibration in migratory songbirds. *J. Orn.* **149**: 607-614.

Wiltschko W, Wiltschko R. 1972. Magnetic compass of European Robins. *Science* **176**: 62-64.

Wiltschko W, Wiltschko R. 1975. The interaction of stars and magnetic field in the orientation system of night migrating birds. I. Autumn experiments with European Warblers (Gen. Sylvia). *Z. Tierpsychol* **37**: 337–355.

# CHAPTER II – Takeoff orientation and the circular release box

as

**Orientation and flight behaviour identify the Soprano pipistrelle as a migratory bat species at the Baltic Sea coast**

by

Lindecke, O., Elknse, A., Holland, R.A., Pētersons, G., Voigt, C.C.

## Abstract

Migration routes of bats remain largely unknown, as previous orientation studies have been challenging even with newly developed techniques in tracking, genetic and stable isotope studies. However, a lack of knowledge about migrations poses problems for species conservation, especially in newly described species for which ecological information is not yet available.

Here, we aimed to test flight orientation behaviour in the Soprano pipistrelle, *Pipistrellus pygmaeus*. This species has been described only 22 years ago but is now known to have a wide geographic distribution in Europe, yet virtually no information exists about seasonal movements of *P. pygmaeus*. In large parts of the continent, seasonal occurrence of *P. pygmaeus* matches with that of long-distance migratory Nathusius' bats (*P. nathusii*). To shed light on the migratory behaviour of both species, we investigated their orientation decisions at the Latvian Baltic Sea coast which is well-known for summer bat migration along a north-south axis.

We developed an arena-based assay designed to measure orientation of take-offs. The arena was installed in the natural flight path of *Pipistrellus nathusii* and *P. pygmaeus*, and after take-off, bats chose the direction freely.

We detected bearing fidelity between take-off and departure flights, suggesting bats used cues within the arena, putatively geomagnetic information, which allowed them to set a course prior to take-off. Further, our results show *P. pygmaeus* orientates in a southerly, seasonally appropriate direction, similar to *P. nathusii* during on-going migration.

Therefore, our findings are consistent with true migratory behaviour of *P. pygmaeus* in the northern part of its range. Predicting flight directions of bats based on take-off direction offers a simple test for orientation studies, and could further be used to test senses of bats under varying treatments, thereby facilitating a comparison of navigational skills across taxa, e.g. bats and birds.

## Introduction

Bats living in seasonal habitats migrate to reach mating and wintering grounds, yet orientation behaviour and spatial movements of bats remain poorly described for most species owing to the cryptic nature of bat migration (Holland 2007; Voigt et al. 2018). The traditional method of ringing bats with metallic bands uncovered some of the longest continental mammal migrations in the world (Barclay & Bell 1988; Hutterer et al. 2005). While ringing has been shown to be an effective solution for the study of spatial movements, it needs to be applied to vast numbers of bats to ensure reliable data because of often low recovery rates (Barclay & Bell 1988; Steffens et al. 2004; Ellison 2008; Holland & Wikelski 2009). Also, some species are sensitive to ringing which led to a reduction of ringing efforts based on ethical reasons (Baker et al. 2001; Dietz et al. 2006). Due to these limitations, our understanding of regional bat movements and migration has been largely restricted to selected species and study locations harbouring stable populations.

However, migratory behaviour has been newly described or revisited for many bat species over the past decades based on traditional ringing and rapid developments of other techniques in both field and laboratory (Wilkinson & Fleming 1996; Russell et al. 2005; Morales-Garza et al. 2007; Racey et al. 2007; Bryja et al. 2009; Moussy et al. 2013). For example, genetic techniques have revealed migratory directions could be inferred from pairwise comparisons of haplotype frequencies of common noctules, *Nyctalus noctula,* sampled in nursery roosts and hibernaculas (Petit & Mayer 2000). However, both dispersal and migration affect gene flow which makes exact delineations of migratory behaviours based on genetic patterns vulnerable to subadult dispersal movements (Moussy et al. 2013). Further, mating on migration, a common behavioural strategy observed in European and North American bats, e.g. *Tadarida brasiliensis*, can hamper assignments of individuals to geographically distinct subpopulations (McCracken & Gassel 1997).

Alongside this, automated acoustic recordings of bat echolocation calls represent an alternative technique to detect spatiotemporal occurrences of bats, often suggestive of migratory movements (Heim et al. 2016). Such acoustic monitoring is highly valuable for delineating the phenology of mass occurrences of bats at distinct locations and enable us to identify putatively migratory species by call characteristics (Jarzembowski 2003; Furmankiewicz & Kucharska 2009; Johnson et al. 2011; Rydell et al. 2014). In combination with visual observations, such acoustic monitoring might even enable determination of the direction from which bats arrive at specific locations (Furmankiewicz & Kucharska 2009).

Yet, acoustic monitoring alone does not reveal the direction of migratory movements of individual bats, and therefore the observation of seasonal presence of bats using acoustic methods fails to provide convincing evidence for migration

Lastly, stable isotope approaches have revealed the geographical origins of sedentary and migratory bats based on isoscape origin models (Cryan et al. 2004; Ossa et al. 2012; Popa-Lisseanu et al. 2012; Voigt et al. 2012; Lehnert et al. 2014; Voigt et al. 2016), yet the accuracy of these geographic assignments suffers from variability of isotope ratios in consumer tissues and source waters (Voigt & Lehnert 2018). Finally, our understanding of regional and long-distance migration is hampered in bats because conventional approaches are largely incompatible with their small size, which presents tagging bats with long-lasting loggers or GPS units (Holland & Wikelski 2009).

Yet, we recently used the direction of departure flights of bats tagged with radio transmitters to infer migratory orientation (*Pipistrellus nathusii*; Lindecke et al. 2015). These bats showed a consistent southern departure flight direction even when translocated 11 km far away from the migration corridor where they were captured (Lindecke et al. 2015). Based on that, we aimed to develop a method to study bat orientation and eventually migration.

Here, we investigated the migration behaviour of a cryptic bat species, the Soprano pipistrelle, *Pipistrellus pygmaeus* (Leach, 1825). Soprano pipistrelles are particularly interesting with respect to their movement ecology, because this species was once considered to be identical to *P. pipistrellus* (Schreber, 1774), a sister species which lacks any pronounced migration behaviour (Steffens et al. 2004, Hutterer et al. 2005). At the turn of the last century, *P. pygmaeus* was described as a new species based on genetic, morphological and acoustic parameters (Barratt et al. 1997; Mayer & von Helversen 2001). Therefore, banding data preceding this date accidentally lumps recapture data of the two species, confounding our understanding of the movement ecology of both species. Recent field studies demonstrated both species prefer distinct habitats, exhibit contrasting foraging behaviours and show different phenologies in large parts of their distribution range (Davidson-Watts & Jones 2006; Davidson-Watts et al. 2006; Nicholls & Racey 2006; Sattler et al. 2007). While *P. pipistrellus* is widely accepted as a sedentary, or eventually regionally migratory bat (Hutterer et al. 2005; Voigt et al. 2012; Voigt et al. 2016; but cf. Bryja et al. 2009), the status of *P. pygmaeus* as a potentially migratory species is still under debate, albeit in-depth genetic studies implied some degree of migratory behaviour for this species (Racey et al. 2007; Bryja et al. 2009; Dietz et al. 2009). Further genetic analysis suggested that migratory behaviour of *P. pygmaeus* is more

pronounced in continental European populations than in populations of the British Isles (Sztencel-Jablonka & Bogdanowicz 2011).

Here, we focused on the orientation behaviour of individual *P. pygmaeus* caught between August and September at Pape Bird Ringing Station (PBRS) at the Latvian Baltic Sea coast. Previous acoustic studies demonstrated a regular occurrence of the species at PBRS during summer migration (Rydell et al. 2014; Voigt et al. 2017, 2018), yet it is unknown if this reflects the seasonal occurrence of *P. pygmaeus* in this region without pronounced directional migration flights. Interestingly, similar to other bats passing PBRS on migration, *P. pygmaeus* only exceptionally displays foraging behaviour (Voigt et al. 2017, 2018). In our study, we aimed to compare directionality of flights, here estimated as the orientation behaviour of departing *P. pygmaeus* with that of a well-known European long-distance migrant, *P. nathusii*. Nathusius' bats have been extensively studied at the same location for many years (Pētersons 2004; Steffens et al. 2004, Hutterer et al. 2005) and show pronounced southward orientation when released close to the coastal line of PBRS (Lindecke et al. 2015).

In order to investigate the migratory behaviour of *P. pygmaeus* and *P. nathusii* without the use of tracking devices, we aimed to first identify a movement behaviour which is performed by all bats and which is measurable at a relatively small scale, yet with sufficient accuracy and precision. In the past, numerous attempts to measure orientation behaviour of bats in a confined space failed (e.g. Mueller 1966), however Wang et al. (2007) demonstrated that Chinese noctules (*Nyctalus plancyi)* aligned themselves to the magnetic field when resting in a round plastic basket. In studies of non-migratory bats, Holland and colleagues also demonstrated that bats use a magnetic compass during active flight (Holland et al. 2006; Holland et al. 2010). Based on these studies, we hypothesized that wild bats could use environmental cues, putatively the Earth's magnetic field, for orientation and also directional takeoff from the perimeter of a circular arena. Thus, we predicted that if a bat oriented towards a relatively distant (migratory) goal from inside a circular-shaped test apparatus, we would find bearing fidelity between measures. That means, we would observe persistence between repeated measures of direction, i.e. maintenance of a compass bearing while the focal bat is moving freely. In theory, high bearing fidelity indicates high relevance of the particular movement direction to the individuals' navigational decisions, sometimes despite elevated sensory noise or dynamically changing environmental information. To test our prediction on bearing fidelity in pipistrelles, we developed a simple, easy-to-build setup which we termed a *circular release box* (CRBox) for bats enabling us to register a bats' takeoff orientation (TOO). We observed the directionality of flights, i.e., initial departure flight orientation (DFO)

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of free flying bats after takeoff from the CRBox. In bird orientation studies, recording of initial movement bearings is standard (Wallraff, 2005), and it has been successfully used in hand-released bats in the past (e.g. Mueller 1966; Buchler & Childs 1982; Serra-Cobo et al. 2000). If *P. pygmaeus* oriented departure flights easterly towards inland instead of the Baltic Sea shoreline, i.e. in stark contrast to *P. nathusii*, we would reconsider our hypothesis of a true migratory motivation in *P. pygmaeus*.

Aside from spatial orientation measures, we also timed latency to takeoff. Latency, the delay between a stimulus and a behavioural response, is a standard measure in behavioural assays, e.g., in studies of spatial learning and escape responses (Sousa et al., 2006; Domenici et al., 2011). Conceptual aspects of both of these established research fields will be integral for future work applying an experimental assay to study bat orientation. Therefore we aimed to record baseline latency data with our CRBox setup. Our study is the first investigating wild migratory mammals with the help of an orientation arena.

## Material and Methods

**Study location and animals.** Field work was conducted between 24 August and 10 September 2016 at Pape Bird Ringing Station (56°09' N 21°03' E, Rucava Municipality, Latvia) under permit Nr. 31/2016-E issued by the Latvian Nature Conservation Agency. Using a Heligoland funnel trap, we caught in total 64 adult bats. We aged bats according to the closure of epiphyseal gaps of the phalanges; *P. nathusii*: 19 males, 16 females, *P. pygmaeus*: 12 males, 15 females, 2 individuals with undetermined sex. Bats were kept in wooden boxes in small groups of 3 to 4 individuals until tested.

**Test apparatus.** All components for construction of the orientation cage for bats, i.e. the CRBox, were purchased from hardware stores. As a measure of precaution, we only used non-magnetic materials to avoid interference with a putative magnetic sense of bats. The same rationale applied to the mechanism for remote release which is operated manually, as any electronics could also potentially interfere with magnetoreception due to electromagnetic noise (Engels et al. 2014). To ensure geomagnetic field intensity at the experimental site (50.8 µT, measured on 24 Aug 2016) was not altered inside the CRBox, we used an Apple iPhone 5 equipped with a 3D magnetometer (Asahi Kasei Microdevices AK8963, Tokyo, Japan; resolution: 0.15 µT/LSB, 16-bit) for control measurements. The CRBox consisted of

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two circular shaped elements, a lid made out of wood, including the mechanism for remote release, and the arena with eight openings for directional choice as the bottom (Fig. 5.1).
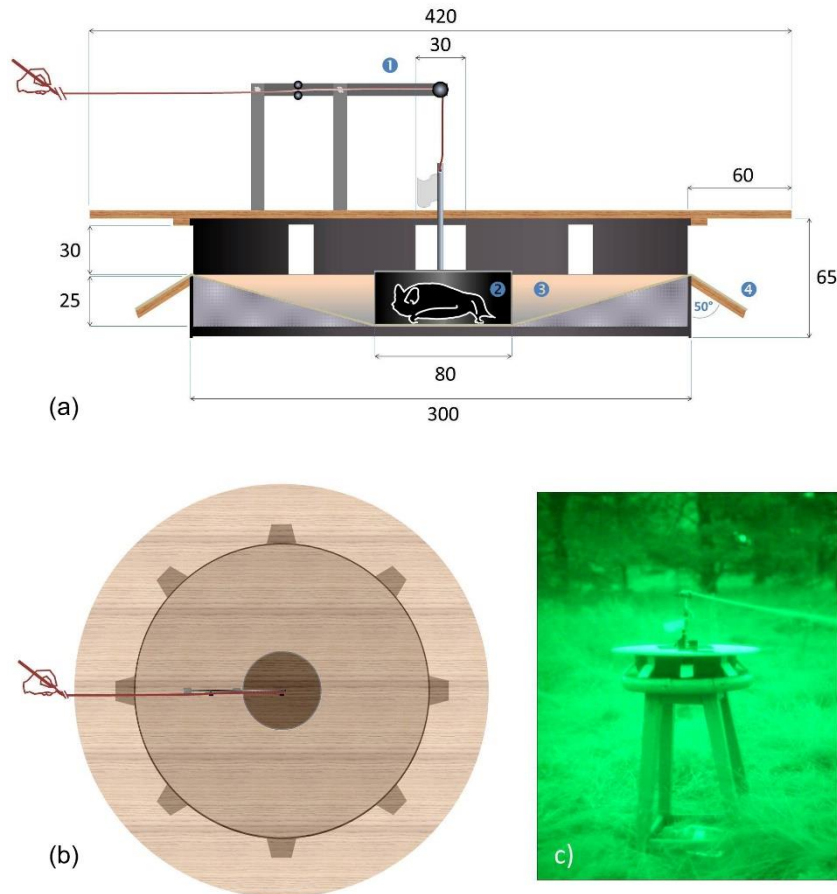


**Fig. 5.1** | The circular release box for bats. (a) Cross-section view, with dimensions (in mm) adapted for small bat species of < 80 mm body length. 1 = pulley for remote release, 2 = acclimatisation box with rod and white signal flag, 3 = funnel, 4 = takeoff platform. (b) Top view of the CRBox with takeoff platforms oriented in cardinal and ordinal directions. The roof board is shown transparent. (c) CRBox setup photographed through night vision goggles.

The arena part consisted of a shallow funnel, so that a bat started a test below the level of the wall openings, i.e. a focal animal was placed in the centre of the funnel, at its lowest part, and was required to crawl for ~12 cm upwards to leave the arena through one of eight exits. In this way, animals would not face the openings immediately, and thus the effect of a spontaneous escape response is minimized (Fig. 5.1a). To construct the funnel, we used extruded polystyrol (XPS, Jackodur, Jackon Insulation). The eight takeoff platforms were made out of wood and tilted downward 40° since an inclined platform would facilitate takeoff for bats. Remote

release of the focal bat is enabled through lifting the acclimatisation box (non-transparent PET) which was fixed to a stout cord running over a pulley. We attached a white stiff paper flag to the rod as a visual cue for the experimenter to stop pulling the chord when the acclimatisation box was lifted to the maximum. The lid of the CRBox extends beyond the arena (Fig. 5.1b). The brim serves to hinder the bat from seeing the night sky overhead and to curtail any emitted echolocation calls. If the bat is motivated to gather information from its surroundings, it is thus forced to takeoff. Finally, we used a stool to position the CRBox 1 m above ground to increase the likelihood for bats to takeoff (Fig. 5.1c). Using a spirit level, we adjusted the CRBox to the horizontal plane.

**Release procedure and orientation measures.** CRBox tests were conducted over the course of six nights on a meadow of rectangular shape (1,300 $m^2$) surrounded by trees, 100 m distant to the shoreline and only under calm wind conditions and clear sky. The CRBox was placed in the centre of the meadow with a minimum distance of 18 m to surrounding rows of trees. We used a bat detector (Pettersson D-100) tuned to the call frequency of the species under test (40 kHz for *P. nathusii* and 50 kHz for *P. pygmaeus* respectively) to control for any conspecific bat calls at the location of the CRBox. Bats are social animals and may orient towards or be repelled by echolocation or social calls emitted by conspecifics. This could affect exit latency, TOO and vanishing behaviour once a bat is airborne. We tested bats in the second half of the night, with the earliest release starting at 01:30 h and the latest at 05:30 h. We repeated acoustic checks for free flying wild bats between trials. Releases were paused when other bats were registered. Before any trial, the interior of the CRBox and takeoff platforms were cleaned with 70% ethanol to prevent animals from using olfactory cues left by previously tested bats. We manually placed individuals into the acclimatisation box varying the angle for insertion randomly by 90° between tests. Bats were restricted from viewing spatial details of the environment prior to release. We used night vision goggles (BIG25 Safran Vectronix AG) for behavioural observations, i.e., comparison of TOO and subsequent flight. Departure flight bearings were recorded in eight sectors with a width of 45°, i.e., cardinal and ordinal compass directions after the bat had disappeared from view. Final bat vanishing directions could be observed up to a distance of approx. 20 to 50 m, depending on whether a focal bat flew into or above the canopy of trees surrounding the meadow. Departure bearings were noted from the position of the CRBox. The observer moved to this position when a bat was about to vanish from viewing range in heights above canopy. If a bat disappeared in the canopy, the observer memorised that spot by means of tree characteristics and recorded the bearing from the position of the CRBox. The error introduced by measuring all departure

flight bearings from that single position is negligible considering the 45° wide sectors for recording of data. For analysis of bearing fidelity, we discarded trials in which we were not able to identify DFO due to rapid flight manoeuvres performed by these individuals, and thus could not calculate angular differences (*P. nathusii*, n = 13; *P. pygmaeus*, n = 5). However, we still used takeoff latency data from discarded trials as those were not compromised. Variances of bearing fidelity within and across animals can be evaluated based on angular differences between measures of directionality at different spatial scales, e.g., TOO and DFO. For intra- and inter-individual statistical comparison, the analysis of bearing fidelity requires normalisation of the initial orientation measure (here TOO) to a common direction. As a result, the second measure (DFO) can be visualised relative to the TOO of the bat. Measurement of takeoff latency started as soon as the acclimatisation box was lifted, i.e., when a bat was released inside of the arena, and stopped when the bat launched into air. Based on escape tests with several species of bats (Chase, 1981), we set a 3 minute cut-off time for cancelling a release trial. The experimenter at the other end of the cord remained motionless to avoid any noise which could hinder bats from taking off or motivate them to change flight paths over the meadow. For *P. pygmaeus*, we measured takeoff latency during 22 trials; in five trials however, we could not record the takeoff precisely enough to include these measurements in the statistical analysis.

**Statistical analysis of orientation behaviour and takeoff latency.** To test for persistence between TOO and DFO, we normalised TOOs (e.g. N, NE, E, etc.) of all individuals for which we observed departure flights to a common direction (0° ahead) and determined the individual angular difference of respective departure flight bearings by subtraction. If TOO was the consequence of a spontaneous escape-response dependant on direction of insertion or the result of stress by the procedure, we would expect uniform random distribution of departure flight bearings irrespective of normalisation, since we randomly inserted animals to the test apparatus. We determined unimodal deviation from circular uniformity by applying Rayleigh's test (Batschelet 1981). For both species, we compared mean DFO with normalised TOO (0° ahead) by inspecting 95% confidence intervals, which is a way to evaluate whether mean DFO would encompass TOO (confidence interval test), i.e. if bearing fidelity can be assumed after a bat takes off. We further aimed to evaluate how actual vanishing directions of both species matched with the topography around the release site, e.g. whether they were aligned with the coastline, or if orientations resemble migratory orientations previously recorded via capture-recapture (ringing) and radio tracking (Pētersons 2004; Lindecke et al. 2015). At our field station, the coastline runs in a straight line from NNW (339.0°) to SSE

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(159.0°) in both directions. We calculated mean bearing and mean vector length from DFOs of both species while using Rayleigh's test to control for deviation from circular uniformity. However, we observed bats taking off and vanishing in both, southerly and northerly directions, as well. Since orientations could eventually be bimodal, we analysed the data axially if $r_{axial} > r_{unimodal}$. Inspection of confidence intervals of bat data enabled us to compare it with coastline and site-specific migratory orientations in *P. nathusii*. All circular calculations were performed using Oriana 4.0 software (Kovach Computing Services, Pentraeth, UK).

We measured the time (seconds) a focal bat took to take off from the platforms after the acclimatisation box inside the CRBox got lifted. We compared takeoff latency among species by a Mann-Whitney U test (normality test failed, $P < 0.05$; SigmaPlot 11.0, Systat Software Inc., Chicago, IL, USA). We tested for differences between sexes using a Mann-Whitney U test for *P. nathusii* (normality test failed, $P < 0.05$) and an unpaired t-test for *P. pygmaeus* (normality test, $P = 0.164$).

## Results

**Bearing fidelity.** We measured movement directionality, i.e., TOO and corresponding DFO, in 45 released bats of two species *Pipistrellus nathusii* (n = 22), and *P. pygmaeus* (n = 23). Normalisation of individual TOOs to a common direction (0°), revealed a significantly unimodal distribution of departure flight bearings (Rayleigh test, *P. nathusii*: $r = 0.456$, $Z = 4.632$, $P = 0.008$; *P. pygmaeus*: $r = 0.605$, $Z = 8.427$, $P = 0.0001$; Fig. 5.2a, b). Further, in both species, mean vectors ($\mu$) of departure flights did not differ from takeoff bearings (95% confidence intervals for $\mu$, *P. nathusii*: 327.6° to 37.2° with $\mu = 2.4°$; *P. pygmaeus*: 354.7° to 51.0° with $\mu = 19.0°$; confidence interval tests for both species: $P > 0.05$). However after takeoff, we observed occasionally bats circling above the CRBox before disappearing from the experimental area in a straight line, i.e. in DFO.

**Orientation in the environmental context.** Bearings of *P. nathusii* showed a bimodal distribution (Rayleigh test, $r_{axial} = 0.502$, $Z = 5.552$, $P = 0.003$; Fig. 5.2c) with a mean N–S vector which did not differ significantly from the coast line orientation at our study site (95% confidence intervals for *P. nathusii*: 184.8° to 153.4° with $\mu_{axial} = 169.1° - 349.1°$; confidence interval test, $P > 0.05$). *Pipistrellus pygmaeus* were oriented unimodally ($r_{unimodal} = 0.702$, $Z = 11.322$, $P = 2.53*e^{-6}$; Fig. 5.2d) with a mean vector in SSW (209°) direction.
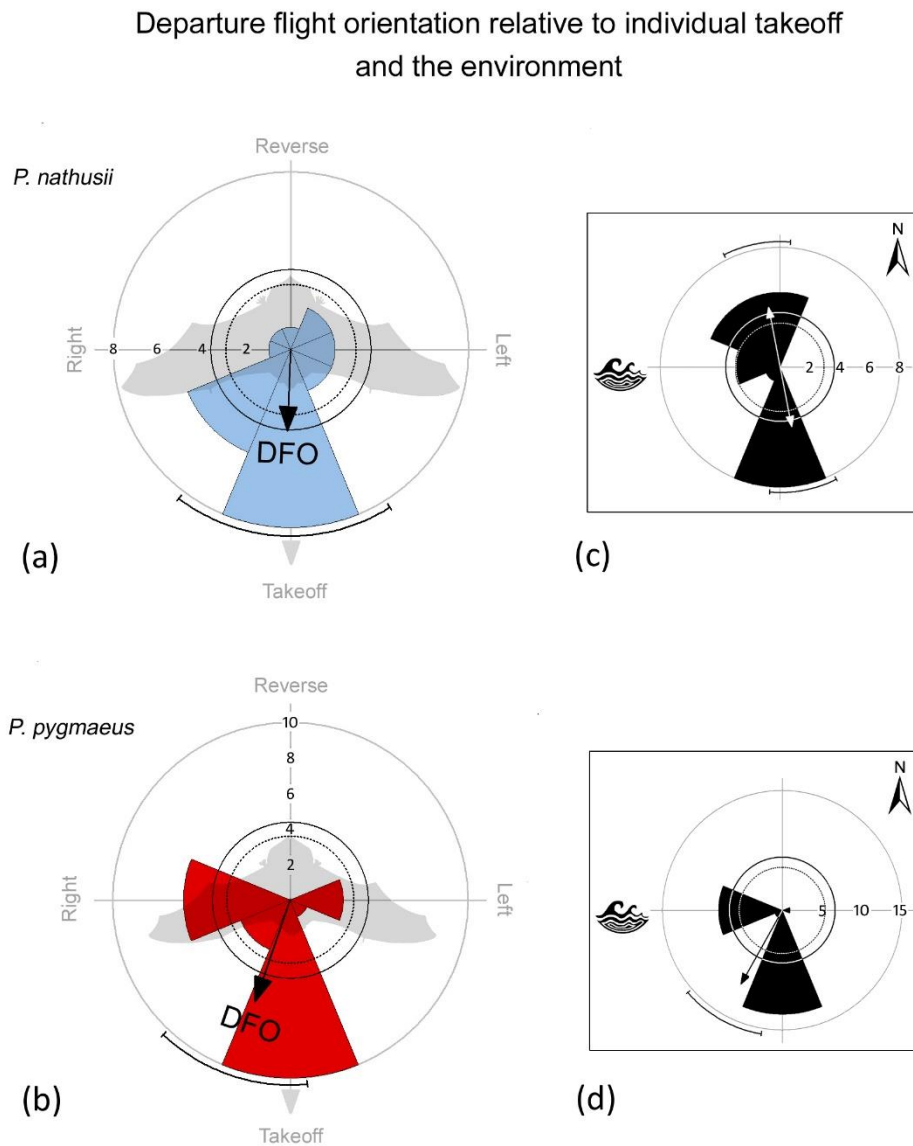
**Fig. 5.2** | Orientation behaviour of pipistrelle bats. Circular graphs on the left show normalised departure flight bearings (relative to individual takeoff orientation) of *P. nathusii* (a) and *P. pygmaeus* (b) which were significantly oriented unimodally in both species (*P. nathusii,* n = 22; *P. pygmaeus*, n = 23). Mean vectors (*μ*) of normalised departure flights were well within the orientation of takeoff (*P. nathusii*: *μ* = 2.4°; *P. pygmaeus*: *μ* = 19.0°). Directions of arrows depict the orientation of *μ* and the lengths reflect the *r* proportional to the radius of the circle. The 95% confidence intervals are indicated above the mean vector. The two inner circles mark the 5% (dotted) and the 1% significance border. Numbers on gridlines indicate sample sizes covered by the wedges. Circular graphs on the right show departure flight orientations in an environmental context with the Baltic Sea in the west. Vanishings of *P. nathusii* (c) were bimodal with a mean vector similar to the coastal orientation while the mean vector of *P. pygmaeus* (d) was oriented SSW.

**Takeoff latency.** Takeoff latency did not exceed 3 min in total for any of the tested bats (*P. nathusii*: n = 35, median: 55 s, range: 6–176 s; *P. pygmaeus*: n = 17, median: 17 s, range: 6–92 s; Fig. 5.3). We detected no difference in takeoff latencies between sexes (*P. nathusii*: U = 131.0, $P = 0.497$, $n_{males} = 19$, $n_{females} = 16$; *P. pygmaeus*: t = -0.183, $P = 0.858$, $n_{males} = 6$, $n_{females} = 9$, excluding two individuals with sex undetermined) and therefore we pooled data for interspecific comparison. *Pipistrellus nathusii* readily showed akinesia when carried and inserted into the CRBox, respectively. Consistent with our hypotheses, this may account for the significant difference in takeoff latency between the two species (Mann-Whitney U-Test, U = 154.0, $P = 0.004$).
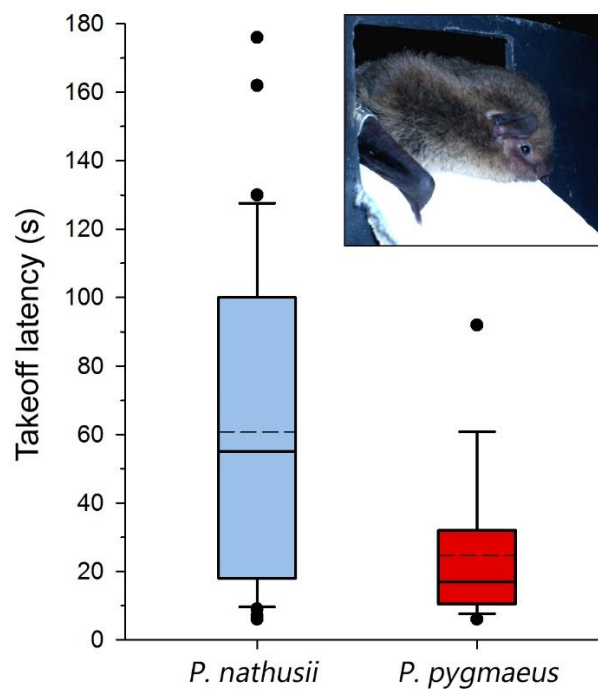


**Fig. 5.3** | Boxplots depict median and mean latency (dashed); outliers are indicated by points not covered by the whiskers (*P. nathusii*: n = 35; *P. pygmaeus*: n = 17). The inlet photograph shows a *P. pygmaeus* crawling onto a platform for takeoff. Takeoff latency in both species differed significantly ($P = 0.004$).

## Discussion

Close to a major migration corridor of bats at the coast of the Baltic Sea in Latvia, we compared the movement orientation of two congeneric bat species, one being a known long-distance migrant (*P. nathusii*) and the other with a putative migration behaviour

(*P. pygmaeus*). Our observation of consistent southern heading directions of *P. pygmaeus* is in line with migratory behaviour of this species. Indeed, orientation and takeoff direction were similar to that of *P. nathusii*, a species with known long-distance migration across Europe. The seasonal occurrence of *P. pygmaeus* at our study site in conjunction with orientation towards the south in departing individuals identifies *P. pygmaeus* as a migratory species, at least for its north-eastern distribution range (Voigt et al. 2017, 2018). Using our novel experimental setup, called the circular release box (CRBox), we here showed (1) initial movements of bats are goal-oriented on the level of takeoff, that (2) orientations are biologically meaningful, and that (3) the principal methodology enables comparison of different measures between species. Therefore, we established that takeoff directions of bats are a suitable measure for developing an experimental paradigm for studies of mammal navigation and sensory ecology using bats as a model organism.

**Bat orientation relative to the environment.** In both species, DFOs matched with the geographical orientation of the local coastline, i.e., the migratory corridor and direction, respectively (Pētersons 2004; Lindecke et al. 2015). For *P. nathusii*, we observed a bimodal orientation of movements in southern and northern direction, whereas *P. pygmaeus* flew in the direction in which bat migration is directed at PBRS in late summer (Lindecke et al. 2015). The observed orientations of *P. pygmaeus* would bring bats back to the flyway above the coastal dunes or 50-100 m parallel to it. In a study by Ahlén et al. (2009) conducted at southern shores of Scandinavia, *P. pygmaeus* showed behaviour similar to *P. nathusii* and *N. noctula*, as well, as it was observed in high numbers at departure sites of these migratory species leaving for offshore flights. However, regarding our subsample of northerly flying *P. nathusii*, we hypothesise that these bats reversed orientation to find a day roost in the coastal forest or to forage instead of continuing migration along the dunes. This reverse orientation could have been caused due to the timing of our experiments, which only covered the second part of a night. Species-specific orientation responses can principally change during the activity period of bats (Mistry 1990). Interestingly, in migratory bird studies, similar observations of bimodal orientations have been made, especially at coastal sites (Sandberg et al. 1988; Åkesson et al. 1996). It has been hypothesised that such differences in orientation depend on the stage of migration with reverse movements along the migratory route occurring in individuals from populations which cover shorter distances (Muheim et al. 2017).

In roe deer it has recently been found that disturbed individuals directed their initial movements depending on the initial alignment of the body axis (Obleser et al. 2017). In contrast to roe deer individuals aligned on the east-west axis, individuals aligned along the

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north-south axis escaped not randomly, but toward north or south, respectively. These findings routed in the magnetic alignment hypothesis (Begall et al. 2013; Obleser et al. 2017) deserve consideration in future work based on takeoff as initial movement being measured in departing bats.

**Takeoff as measure.** Comparisons of takeoff with respect to subsequent flight demonstrated directional persistence between these movements, i.e., bearing fidelity exists between individual TOO and DFO in migratory *P. nathusii* and *P. pygmaeus*. Thus, we inferred that TOO as measured by the CRBox is a suitable proxy to predict DFO after bats started flying. This observation supports our assumption that bats were able to acquire environmental information relevant for orientation and navigation, even in a relatively cue-deprived situation but with a multi-directional choice for takeoff, i.e., in the CRBox. Based on results gathered with a *resting assay* using a similar-sized setup in the laboratory (Wang et al., 2007), we speculate that the Earth's magnetic field might have played a functional role for our bats. Yet again, it is too early to draw further inferences about the role of magnetoreception in bat orientation on the level of takeoff. For example, this would require an experimental setup based on magnetic coils where the geomagnetic-field around a focal bat could be precisely manipulated. However, at the perimeter of the CRBox, echolocation would not have provided useful long-distance information about landmarks at the moment of release, and view of the nightly sky with stars, Milky Way or moon, was inhibited by the design of the CRBox as well (Fig. 1). However, environmental olfactory cues or distant low-frequency sounds, e.g. emitted by the Baltic Sea, might have influenced TOO of our bats as well (Childs & Buchler, 1981; Gagliardo, 2013). Previously, bat orientation studies measuring initial flight orientations visually were limited to sampling under twilight conditions at dusk and dawn (Davis, 1966; Buchler & Childs, 1981, 1982; Serra-Cobo, et al. 2000). In our study, application of night-vision technique for behavioural observations proved to be a solution to avoid effects of environmental light to which bats might be sensitive (Chase, 1981; Buchler & Childs, 1982; Holland et al. 2010; Greif, et al. 2014). However, we missed some flights due to rapid movements of animals even using advanced optical equipment.

We recorded sufficient baseline data about takeoff latencies for the two pipistrelle species and found differences, with *P. nathusii* performing significantly slower than *P. pygmaeus*. Compared to *P. pygmaeus*, *P. nathusii* readily shows a state of motionless inactivity, so-called akinesis, when being handled. We assume this behaviour of *P. nathusii* in response to handling was delaying takeoff. Yet, with respect to bearing fidelity, *P. nathusii*, like *P.pygmaeus*, qualifies as an appropriate model species for orientation tests with relatively short release

trials (< 3 min) which are practicable for testing multiple independent subjects over a short period. Previous research using crawling bats in Y-maze tests suggested a true escape response would take only two to three seconds for active bats in similar settings (Chase, 1981; Mistry 1990). Here observed latencies suggest that our bats did not escape spontaneously but took off after a delay putatively serving orientation in the confined space of the CRBox.

In principle, using an arena-based approach to elucidate preferred bat orientations adds to the methodological repertoire for the study of bat species. Eventually, this could be useful for identification of migration routes of other bats across their ranges. But primarily, testing directionality of takeoff represents a novel option for addressing a multitude of questions in bat physiology and sensory ecology where circular orientation responses have rarely been recorded before. We acknowledge that for certain bat families the current CRBox is eventually unsuitable, e.g. New World leaf-nosed bats (Phyllostomidae; but see Chase, 1981 & 1983 and Gröger & Wiegrebe, 2006) and Old world Horseshoe bats (Rhinolophoidae) which commonly do not use quadrupedal locomotion, i.e. crawling. However, for families like Vespertilionidae, Miniopteridae or Molossidae, which readily crawl on the ground or in crevices, TOO measures have high potential for orientation studies, as the principle CRBox design allows measurement of a natural behaviour in these species. Yet, for future works the CRBox should be improved to gain higher resolution of orientation measures, e.g. by offering more exits. This would allow for more sophisticated test procedures enabling statistical comparison between study species or experimental groups (Batschelet 1981).

**Conclusions and open questions.** Our results suggest *P. pygmaeus* from northern Europe behaves similar to long-distance migratory *P. nathusii* during times of summer migration at the Baltic Sea coast (Lindecke et al. 2015, and this study). The observed orientations would lead bats southwards and back to the flyway along the dunes of the shoreline. Based on our findings combined with previous results about population genetics, phenological occurrences and flight behaviour at sea sides, we argue that *P. pygmaeus* can be considered a true migratory species, at least in northern parts of its distribution range (Ahlén et al. 2009, Sztencel-Jabłonka & Bogdanowicz 2012; Rydell et al. 2014, Voigt et al. 2017, 2018). Quantitative studies based on tagged individuals are now needed to evaluate how regular *P. pygmaeus* migrate over longer distances and where individuals of northern populations are overwintering. Still, it remains unresolved which environmental cues contribute to the compass system bats and other mammals use for migration. Pipistrelle species appear to be a suitable future model to address this question in detail. With an easy-to-build experimental arena setup, we established a method to study initial orientation behaviour of bats, using their

natural takeoff behaviour as a proxy for directional choices. Future work is necessary to understand which environmental cues enable bats to orient in the small, confined space of a test arena. We speculate one of the most important cues to be the geomagnetic field (Holland et al. 2006; Wang et al. 2007).

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## Author contributions

O.L. conceived the study, designed and constructed the behavioural test, analysed the data and led the manuscript writing; O.L. and A.E. performed experiments; C.C.V. and G.P. supported fieldwork; C.C.V. and G.P supervised the students; O.L., R.A.H. and C.C.V. interpreted the data and wrote the manuscript.

## References Chapter II

Ahlén, I., Baagøe, H.J., Bach, L. (2009). Behavior of Scandinavian bats during migration and foraging at sea. J. Mammal. 90, 1318-1323.

Åkesson, S., Karlsson, L., Walinder, G., Alerstam, T. (1996). Bimodal orientation and the occurrence of temporary reverse bird migration during autumn in south Scandinavia. Behav. Ecol. Sociobiol. 38, 293-302.

Baker, G.B., Lumsden, L.F., Dettmann, E.B., Schedvin, N.K., Schulz, M., Jansen, L. (2001). The effect of forearm bands on insectivorous bats (Microchiroptera) in Australia. Wildl. Res. 28: 229–237.

Barclay, R.M.R., Bell, G.P. (1988). Marking and observational techniques. Pp. 59-76, in: Ecological and behavioral methods for the study of bats (ed. Kunz TH). 533 pp. Smithonian Institution Press.

Barratt, E.M., Deaville, R., Burland, T.M., Bruford, M.W., Jones, G., Racey, P.A., Wayne, R.K. (1997). DNA answers the call of pipistrelle bat species. Nature 387, 138-139.

Batschelet, E. (1981). Circular Statistics in Biology. London: Academic Press.

Begall, S., Malkemper, E.P., Červený, J., Němec, P., Burda, H. (2013) Magnetic alignment in mammals and other animals. Mammal. Biol. 78, 10-20.

Bryja, J., Kanuch, P., Fornuskova, A., Bartonicka, T., Rehak, Z. (2009). Low population genetic structuring of two cryptic bat species suggests their migratory behaviour in continental Europe. Biol. J. Linn. Soc. 96, 103-114.

Buchler, E.R., Childs, S.B. (1981). Orientation to distant sounds by foraging Big brown bats (*Eptesicus fuscus*). Animal Behaviour 29, 428-432.

Chase, J. (1981). Visually guided escape responses of microchiropteran bats. Anim. Behav. 29, 708-713.

Cryan, P.M., Bogan, M.A., Rye, R.O., Landis, G.P., Kester, C.L. (2004). Stable hydrogen isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. J. Mammal. 85, 995-1001.

Davidson-Watts, I., Jones, G. (2006). Difference in foraging behaviour between *Pipistrellus pipistrellus* (Schreber, 1774) and *Pipistrellus pygmaeus* (Leach, 1825). J. Zool. 268, 55-62.

Davidson-Watts, I., Walls, S., Jones, G. (2006). Differential habitat selection *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* identifies distinct conservation needs for cryptic species of echolocating bats. Biol. Conserv. 133, 118-127.

Davis, R. (1966). Homing performance and homing ability in bats. Ecol. Monogr. 36, 201-237.

Dietz, C., Dietz, I., Ivanova, T., Siemers, B. (2006). Effects of forearm bands on horseshoe bats (Chiroptera: Rhinolophidae). Acta Chiropterol. 8, 523-535.

Dietz, C., von Helversen, O., Nill, D. (2009). Bats of Britain, Europe and Nortwest Africa. 400 pp. London: A&C Black Publishers.

Dingle, H. (2014). Migration: the biology of life on the move. 2nd edn. New York: Oxford University Press.

Domenici, P., Blagburn, J.M., JP Bacon (2011) Animal escapology I: theoretical issues and emerging trends in escape trajectories. J. Exp. Biol. 214, 2463-2473.

Ellison, L.E. (2008) Summary and Analysis of the U.S. Government Bat Banding Program: U.S. Geological Survey Open-File Report 1363, 117 p.

Furmankiewicz, J., Kucharska, M. (2009). Migration of bats along a large river valley in southwestern Poland. J. Mammal. 90, 1310-1317.

Heim, O., Schröder, A., Eccard, J., Jung, K., Voigt, C.C. (2016). Seasonal activity patterns of European bats above intensively used farmland. Agr. Ecosyst. Environ. 233, 130-139.

Holland, R.A. (2007). Orientation and navigation in bats: known unknowns or unknown unknowns? Behav. Ecol. Sociobiol. 61, 653-660.

Holland, R.A., Borissov, I., Siemers, B. (2010). A nocturnal mammal, the greater mouse-eared bat, calibrates a magnetic compass by the sun. PNAS 15, 6941-6945.

Holland, R.A., Thorup, K., Vonhof, M.J., Cochran, W.W., Wikelski, M. (2006). Bat orientation using Earth's magnetic field. Nature 444, 653.

Holland, R.A., Wikelski, M. (2009). Studying the migratory behavior of individual bats: current techniques and future directions. J. Mammal. 90, 1324-1329.

Hutterer, R., Ivanova, T., Meyer-Cordes, C., Rodrigues, L. (2005). Bat migrations in Europe – a review of banding data and literature. Naturschutz Biologie Vielfalt 28.

Johnson, J.B.B., Gates, J.E., Zegre, N.P. (2011). Monitoring seasonal bat activity on a coastal barrier island in Maryland, USA. Envir. Monit. Assess. 173, 685-699.

Lehnert, L.S., Kramer-Schadt, S., Schönborn, S., Lindecke, O., Niermann, I., Voigt, C.C. (2014). Wind Farm Facilities in Germany Kill Noctule Bats from Near and Far. PLoS ONE 9, e103106.

Lindecke, O., Voigt, C.C., Pētersons, G., Holland, R.A. (2015). Polarized skylight does not calibrate the compass system of a migratory bat. Biol. Lett. 11, 201505125.

Mayer, F., von Helversen, O. (2001). Sympatric distribution of two cryptic bat species across Europe. Biol. J. Linn. Soc. 74, 365-374.

McCracken, G.F., Gassel, M.F. (1997). Genetic structure in migratory and nonmigratory populations of Brazilian free-tailed bats. J. Mammal. 78, 348-357.

Mistry, S. (1990). Characteristics of the visually guided escape response of the Mexican free-tailed bat, *Tadarida brasiliensis mexicana*. Anim. Behav. 39, 314-320.

Morales-Garza, M.R., del C. Arizmendi, M., Campos, J.E., Martínez-Garcia, M., Valiente-Banuet, A. (2007). Evidences on the migratory movements of the nectar-feeding bat *Leptonycteris curasoae* in Mexiko using random amplified polymorphic DNA (RAPD). J. Arid. Envir. 68, 248-259.

Moussy, C., Hosken, D.J., Mathews, F., Smith, G.C., Aegerter, J.N., Bearhop, S. (2013). Migration and dispersal patterns of bats and their influence on genetic structure. Mamm. Rev. 43, 183-195.

Mueller, H.C. (1966). Homing and distance orientation in bats. Z. Tierpsychol. 23, 403-421.

Muheim, R., Åkesson, S., Bäckman, J., Sjöberg, S. (2017). Magnetic compass orientation research with migratory songbirds at Stensoffa Ecological Field Station in southern Sweden: why is it so difficult to obtain seasonally appropriate orientation? J. Avian Biol. 48, 6-18.

Nicholls, B., Racey, P.A. (2006). Habitat selection as a mechanism of resource partitioning in two cryptic bat species *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*. Ecography 29, 697-708.

Obleser, P., Hart, V., Malkemper, E.P., Begall, S., Holá, M., Painter, M.S., Červený, J., Burda, H. (2016). Compass-controlled escape behaviour in roe deer. Behav Ecol Sociobiol (2016) 70: 1345-1355..

Ossa, G., Kramer-Schadt, S., Peel, A.J., Scharf, A.K., Voigt, C.C. (2012). The movement ecology of the Straw-colored fruit bat, *Eidolon helvum*, in Sub-Saharan Africa assessed by stable isotope ratios. PLoS ONE 7, e45729.

Pētersons, G. (2004). Seasonal migrations of northeastern populations of *Pipistrellus nathusii*. Myotis 41-42, 29-56.

Petit, E., Mayer, F. (2000). A population genetic analysis of migration: the case of the noctule bat (*Nyctalus noctula*). Mol. Ecol. 9, 683-690.

Popa-Lisseanu, A.G., Sörgel, K., Luckner, A., Wassenaar, L.I., Ibáñez, C., Kramer-Schadt, S., et al. (2012). A triple-isotope approach to predict the breeding origins of European bats. PLoS ONE 7, e30388.

Racey, P.A., Barratt, E.M., Burland, T.M., Deaville, R., Gotelli, D., Jones, G., Piertney, S.B. (2007). Microsatellite DNA polymorphism confirms reproductive isolation and reveals difference in population genetic structure of cryptic pipistrelle bat species. Biol. J. Linn. Soc. 90, 539-550.

Russell, A.L., Medellín, R.A., McCracken, G.F. (2005). Genetic variation and migration in the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*). Mol. Ecol. 14, 2207-2222.

Rydell, J., Bach, L., Bach, P., Diaz, L., Furmankiewicz, J., Hagner-Wahlstein, N., Kyheröinen, E.-M., Lilley, T., et al. (2014). Phenology of migratory bat activity across the Baltic Sea and the south-eastern North Sea. Acta Chiropterol. 16, 139-147.

Sandberg, R., Pettersson, J., Alerstam, T. (1988). Why do migrating robins, *Erithacus rubecula*, captured at two nearby stop-over sites orient differently? Anim. Behav. 36, 865-876.

Sattler, T., Bontadina, F., Hirzel, A.H., Arlettaz, R. (2007). Ecological niche modelling of two cryptic bat species calls for a reassessment of their conservation status. J. Appl. Ecol. 44, 1188-1199.

Serra-Cobo, J., López-Roig, M., Marquès-Bonet, T., Lahuerta, E. (2000). Rivers as a possible landmarks in the orientation fligh of *Miniopterus schreibersii*. Acta Theriol. 45, 347-352.

Sousa, N., Almeida, O.F.X., Wotjak, C.T. (2006). A hitchhiker's guide to behavioral analysis in laboratory rodents. Genes, Brain and Behavior 5 (Suppl. 2), 5-24.

_____

Steffens, R., Zöphel, U., Brockmann, D. (2004). 40th Anniversary Bat Marking Centre Dresden - Evaluation of Methods and Overview of Results. 132 pp. Dresden: Sächsisches Landesamt für Umwelt und Geologie.

Sztencel-Jabłonka A, Bogdanowicz W (2012). Population genetics study of common (*Pipistrellus pipistrellus*) and soprano (*Pipistrellus pygmaeus*) pipistrelle bats from central Europe suggests interspecific hybridization. Can. J. Zool. 90, 1251-1260.

Voigt, C.C., Frick, W.F., Holderied, M.W., Holland, R.A., Kerth, G., Mello, M.A.R., Plowright, R.K., Swartz, S., Yovel, Y. (2017). Principles and patterns of bat movements: From aerodynamics to ecology. Quart. Rev. Biol. 92, 267-287.

Voigt, C.C., Lehnert, L.S. (2018). Tracking bats and other terrestrial mammals: Considerations of physiology. In *Tracking animal migration with stable isotopes* (eds. Hobson KA, Wassenaar LI). Academic Press.

Voigt, C.C., Lindecke, O., Schönborn, S., Kramer-Schadt, S., Lehmann, D. (2016). Habitat use of migratory bats killed during autumn at wind turbines. Ecol. Appl. 26, 771-783.

Voigt, C.C., Popa-Lisseanu, A., Niermann, I., Kramer-Schadt, S. (2012) The catchment area of wind farms for European bats: A plea for international regulations. Biol. Conserv. 153, 80-86.

Voigt, C.C., Röleke, M., Marggraf, L., Pētersons, G., Voigt-Heucke, L. (2017) Migratory bats respond to artificial green light with positive phototaxis. PLoS ONE 12, e0177748.

Voigt, C.C., Rehnig, K., Lindecke, O., Pētersons, G. (2018) Migratory bats are attracted by red light but not by warm-white light: Implications for the protection of nocturnal migrants. Ecol. Evol. 00, 1-9.

Wang, Y., Pan, Y., Parsons, S., Walker, M., Zhang, S. (2007). Bats respond to polarity of a magnetic field. Proc. R. Soc. B 274, 2901-2905.

Wilkinson, G.S., Fleming, T.H. (1996). Migration and evolution of lesser long-nosed bats *Leptonycteris curasoae*, inferred from mitochondrial DNA. Mol. Ecol. 5, 329-339.

# CHAPTER III – Use of the sun's azimuth for compass calibration

as

## Experienced migratory bats integrate the sun's position at dusk for navigation at night

by

Lindecke, O., Elknse, A., Holland, R.A., Pētersons, G., Voigt, C.C.

## Abstract

From bats to whales, millions of mammals migrate every year. However, their navigation capacity for accomplishing long-distance movements remains remarkably understudied and lags behind by five decades compared to other animals (Holland 2007, Nathan *et al.* 2008); partly because, unlike for other taxa, such as birds and sea turtles, no small scale orientation assay has so far been developed. Yet recently, bats became a model to investigate the nature of the cues mammals use for long-range navigation, and surprisingly for nocturnal animals, sunset cues, and particularly polarized light cues, appear to be crucial for calibration of the magnetic-compass system in non-migratory bats (Holland et al. 2006, 2010, Greif et al. 2014). This does not appear to hold for a species of migratory bat, however (Lindecke et al. 2015), and thus the nature of the information used by migratory bats for navigation remains unclear.

Here, we asked whether the position of the solar disk per se is relevant for compass orientation in a migratory bat, *Pipistrellus pygmaeus*.

Using a new experimental assay that measures takeoff orientation, we tested the orientation of bats exposed to a shifted sunset azimuth using a mirror at dusk.

Bats exposed to a 180°-rotated azimuth of the setting sun and released after translocation during the same night shifted their heading direction by ~180° compared to control bats. However, first-year migrants had no clear orientation either as controls or following the same

treatment. This suggests that learning the migratory direction is a key component in the navigational system of naive bats in this species. Our study provides rare evidence for the specific cues and mechanisms migratory mammals use for navigation.

## Introduction

Migratory animals show an ability to navigate over vast distances with remarkable precision returning to the same area year after year (Mouritsen 2018). Whilst some aspects of this behaviour remain a mystery, advances in our understanding have been made in taxa such as birds and reptiles, by using small scale correlates of orientation that have allowed controlled manipulation of the cues animals are using (Kramer 1949, Lohmann et al. 2004, Chernetsov et al. 2017). One taxon, mammals, remains remarkably understudied with regard to navigation during migration however. Whilst the logistics of experiments on large terrestrial and aquatic migrants contributes to this dilemma, migratory insectivorous bats also remain understudied. A key reason for the lack of research on bats is the absence of an assay that measures a correlate of migratory orientation such as those that exist in birds and reptiles (Holland 2007). Therefore, a thorough understanding of the senses and environmental cues used by mammals for navigation over hundreds or even thousands of kilometres is lacking (Horton et al. 2011). Bats have emerged as a widely studied model in movement ecology because they combine both high ecophysiological diversity with a variety of movement behaviours (Voigt et al. 2017a). Bat eyes evolved to sense a wide range of light and a broad spectrum of wavelengths (Suthers et al. 1970, Zhao et al. 2009) and, presumably, bats of the family Vespertilionidae, and possibly also others, rely heavily on vision when orienting over long distances since echolocation and path integration are ineffective and error-prone at distances larger than a few dozen meters (Williams et al. 1966, Holderied & von Helversen 2003, Stilz et al. 2012, Boonman et al. 2013, Aharon et al. 2017).

Generally, the sun is considered to be the most prominent celestial cue for compass orientation, since it is key for bird navigation during homing and migration (Moore 1978, Guilford & Taylor 2014). Surprisingly, even nocturnal mammals, such as bats, integrate solar cues to successfully find their home roost at night, which appears counterintuitive because of bats' strictly nocturnal behaviour (Holland et al. 2010, Greif et al. 2014, Muheim et al. 2006). Furthermore, it has been demonstrated that adult non-migratory bats calibrate a magnetic compass to cues at sunset (Holland et al. 2006, 2010). Like some migratory birds (Muheim et al. 2006), a non-migratory bat species was demonstrated to use the maximum band of polarized sunlight at sunset for this calibration, which forms a band across the sky at 90° to the setting sun (Greif et al. 2014). In contrast, however, the only experiment so far to test the cues used for navigation by a migratory bat species, namely Nathusius' bats (*Pipistrellus nathusii*), failed to find an effect of manipulation of the polarized light pattern on heading

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directions (Lindecke et al. 2015). Therefore the cues and mechanisms used by migratory bats during navigation remain entirely unknown (Genzel et al. 2018).

Here, we tested whether the position of the setting sun on the horizon (solar disc azimuth) could be the key reference for bats to efficiently calibrate their compass system for navigation at night.

## Material and methods

**General overview.** We caught fifty-four Soprano pipistrelles (*Pipistrellus pygmaeus*) migrating towards their wintering grounds along the Latvian Baltic Sea shore. Our experimental approach was based on two-steps: (1) exposing bats to a shifted position of the sun at sunset created by using a polished steel-mirror and (2) subsequent translocation of experimental animals further inland with an orientation test at the release site during the same night. For the sunset treatment, we chose a location in the dunes with an open view of the Baltic Sea. On the day of treatment, bats were randomly assigned to two treatment groups and individually put in cylindrical cages with lids limiting their free view to the natural (C) or mirrored (M) sunset from 30 min before until 30 min after sunset, i.e., until the sun's disk disappeared below the horizon (-5°; Fig. 6.1). For each treatment group, the opposite view of the horizon was blocked from vision for 180° by the taped sidewall of the cage. Thus, bats of the M group faced the mirror 50 cm in front of them instead of a free view of the forest to the east opposite to sunset.
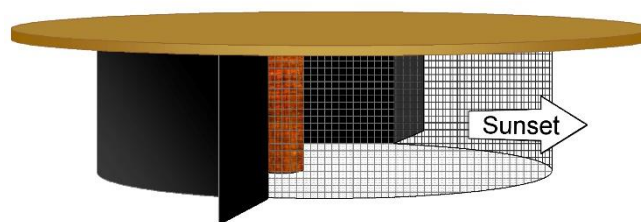


**Fig. 6.1** | Experimental cage used to expose bats to the natural or mirrored sunset. The cage design offers a 180° free panoramic view.

At the treatment site, we took utmost care that bats were not exposed to any other light source than defined by the experimental condition, i.e., we abstained from using artificial lights when collecting the animals from their cages again. We hypothesized that migrating bats use the sun, i.e. the directional information provided by the solar disk at sunset, to calibrate their

orientation system. Accordingly, we expected bats of the M group to orient in the opposite direction compared to bats of the C group when released. To mirror the sunset, we used pure metal plate mirrors (stainless steel, $60 \times 60$ cm) as recommended for deflector studies by Horváth & Pomozi (1997). Using metal plate mirrors is crucial since common glass mirrors generate unwanted optical stimuli with qualitative and quantitative changes of the natural skylight polarization and near ultra-violet light patterns which have been shown to bias animal orientation in deflector studies (Horváth & Pomozi 1997, Phillips & Waldvogel 1988). When handling bats, we ensured that they neither saw the sky nor the true horizon before and after the experimental treatment. After exposure to the sunset-treatment, we translocated the bats 5.1 km inland for nightly release on a large meadow surrounded by trees.

For recording of the heading directions of released bats, we designed a behavioural assay to quantify the departure flight direction based on measures of takeoff using a novel apparatus, a circular release box for bats (CRBox). The CRBox allowed us to remotely release bats and record their takeoffs in every possible compass direction (Fig. 6.2).
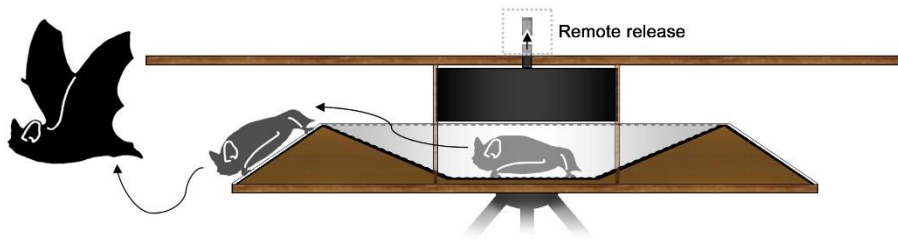


**Fig. 6.2** | The bat takeoff orientation assay. Scheme showing a bat departing from a circular release box. After the acclimatisation box is lifted, the animal may leave from the apparatus at the time and in the direction of choice. A thin layer of chalk tracks the movements of bats while orienting and crawling to the opening, enabling an accurate measure of takeoff orientation. The input of environmental cues is reduced until the bat takes off for free flight.

The CRBox consists of a circular-shaped arena with a lid including the remotely controlled mechanism for release of a bat from the centre of the arena. The apparatus is positioned in the field 1 meter above ground. By construction and due to translocation away from the treatment site, the CRBox reduces the amount of environmental information which could inform the navigation system of a bat, e.g., cues provided by the night sky, visual landmarks and winds. From the moment of release, the echolocation behaviour of the bat in the CRBox was monitored with the aid of a bat detector from 10 m distance. When echolocation calls indicated

flight by characteristic regular pulses, we noted the timing of takeoff. A 360° thin layer of chalk on the annulus of the arena enabled us to record the animals' orientation based on tracks, i.e., the footprints left after crawling to the edge for takeoff. This experimental behavioural assay is based on the recent finding that takeoff orientation is a reliable proxy for departure flight orientation in adult bats (Lindecke et al. 2019).

**Details on experimental bats and site.** Soprano pipistrelles (*Pipistrellus pygmaeus*) of both sexes were captured at Pape Bird Ringing Station (PBRS; 56°09' N 21°03' E, Rucava Municipality, Latvia) between 15 and 24 August 2017, using a Heligoland trap aligned to the Baltic Sea shore (dimensions at the entrance of the funnel $50 \times 15$ m). Soprano pipistrelle populations around the Baltic Sea move towards central Europe in August and September (Rydell et al. 2014, Voigt et al. 2017b). On the night of capture, bats were controlled for site-specific, seasonally appropriate body condition, aged according to the closure of the epiphyseal gaps of the phalanges, and afterwards transferred to a dark keeping room where wooden boxes served as cages for small groups for the subsequent day-roosting. We discriminated between first-year migrants and adult bats, assuming that subadults are inexperienced and adults experienced migrants. Bats were experimentally treated on the subsequent evening. The station and experimental sites are located in the Pape Nature Reserve.

All work adheres to the ASAB guidelines for Use of Animals in Research and was carried out under the permission of the responsible Latvian authorities to the Institute of Biology, University of Latvia and Latvia University of Agriculture (Nr. 33/2017-E from 19.07.2017 and Nr. 3.6/85/2017-N-E issued by the Latvian Nature Conservation Agency), in accordance with the guidelines and regulations of the institutional animal care and ethics committee. The bats were released directly back into the wild when they were set free in the CRBox.

**Details on the mirror experiment.** On the day of the experiment, any handling of test subjects was performed indoors in a wooden hut in order to avoid bats seeing the sun and any directional solar cues respectively, prior to the treatment phase. Experiments were only carried out during evenings with stable weather conditions such as zero wind to light breeze (0–2 m s$^{-2}$) and 0–60% cloud cover and a visible sunset. The treatment site was 60 m from the research station, about 100 m from the shoreline and 150 m from the funnel trap. For transportation to the treatment site, bats were put singly into cotton bags (Ecotone, Gdynia, PL) and additionally into dark plastic buckets. For the experiment, bats were individually placed in cylindrical cages (20 cm diameter, 7 cm height; Fig. 6.1) positioned on tables on the dune. For construction of these cages we chose materials which had no effects on the polarity or intensity of the local geomagnetic field, measured from inside the cage and compared with immediate

surroundings of the treatment site using a conventional compass (Suunto A-30 NH Metric, Vantaa, FIN) and a smartphone (Apple iPhone 5) equipped with a Hall-Sensor (3D Magnetometer, Asahi Kasei Microdevices AK8963, Tokyo, JP) which was read out via *Phyphox* software (RWTH Aachen University, Aachen, GER). For this location, we measured the geomagnetic field intensity at 50.8 µT on 15 Aug 2017. The sidewall of a cage consisted of conventional gauze mesh (8 mm aperture) and was partly taped so that a bat inside would have a 180° panoramic view of the surroundings. The lid was made of cardboard and fitted with a wooden stick in the centre (2 cm diameter) oriented down to the bottom of the cage. Thus, a bat could observe the environment from inside a cage in either horizontal body position or hanging heads down while clinging to the wood. We avoided using artificial light (flashlight or forehead lamps) for any procedure at the treatment site, so that the bats experienced natural sunlight, only. Further, according to the recently released atlas of artificial night sky brightness (Falchi et al. 2016), our study site can be considered particularly dark at night, i.e., the natural nightscape is less polluted by anthropogenic light than the average European sky (ratio artificial to natural brightness < 0.05). The mirrors (stainless steel, 60 × 60 cm) were aligned perpendicular to the sunset bearing daily and positioned vertically, 50 cm in front of the caged bats. In general, when animals were handled, great care was taken that they neither saw the sky nor the true horizon until inserted into the cages and after completion of the treatment until they were put back in cotton bags to wait for translocation and release.

**Details on the circular release box assay.** All components for the construction of the CRBox were purchased from hardware stores. The experimental setup consists of two circular shaped elements: an arena (42 cm diameter) as the bottom part and a lid (60 cm diameter; textured coated board) including the mechanism for remote release of the bat from a smaller acclimatisation box (8 cm diameter, 3 cm height; black plastic) in the centre (8 cm diameter) of the arena. The release mechanism is based on miniature electric motor (mounted onto the lid of the CRBox) which lifts the acclimatisation box for 3 cm when triggered remotely by radio control. For construction of the CRBox we only applied non-magnetic materials to avoid interference with a putative magnetic sense of bats. We made use of a flat funnel-shape for the arena (extruded polysterol, XPS, Jackodur, Jackon Insulation), so that a bat starts a test below the level of the edge. The edge is 17 cm distant from the centre point of the CRBox. At the edge, the arena surface was again tilted downward 40° to facilitate takeoff for bats. The arena is coated with synthetic leather because the texture of this material supports crawling of bats and because it can be cleaned easily. A thin layer of chalk on the annulus around the

acclimatisation box enables recording of the final takeoff direction based on tracks of a bat. The lid extends 9 cm beyond the arena, creating a brim that hinders the bat from seeing the night sky overhead and consequently impairs any celestial orientation at the RS. If the bat is motivated to gather information from the surroundings, it is thus forced to take off from the CRBox. For each trial the track of the previous test bat was eliminated with a renewed layer of chalk on the arena surface, and additionally, the CRBox was randomly rotated. Finally, the CRBox was positioned 1 m above ground. The horizontal position of the apparatus was adjusted using a spirit level.

**Details on the geographical translocation and orientation testing.** On the day of treatments after 2230 hours, we translocated the bats 5.1 km to the release site (RS) 98° east of the capture site away from the coastal migration corridor. By translocating the animals, we aimed to evade any bias on orientation behaviour which could arise from cues indicating the direction of the seashore, e.g. marine noise and visual landmarks provided by the dunes. The RS was on an open flat meadow (~3.1 ha) in the forested area of Pape Nature Reserve. Releases started at 2255 hours, the last trials during a given night were started between 0011 and 0239 hours. Before releasing bats individually, and during trials, we surveyed the vicinity of the RS for the presence of any other bats using a handheld ultrasound detector (D100, Pettersson Elektronik, Uppsala, SW). In the presence of other bats, experimental releases were paused to avoid confounding effects when released bats might have been biased in their vanishing direction. To measure the effect of our treatment on the bats' directional movements, we used a CRBox assay designed to record takeoff orientation on a full-circle. Previously, we identified takeoff orientation of as a useful proxy for departure flight direction of individually released adult bats (Lindecke et al. 2019). Bats were randomly chosen for testing and were unable to explore the spatial details of the environment in which they could head prior to release from the CRBox. Therefore, we took care to keep bats naïve to the surroundings, i.e., we covered the individuals with cotton bags when they were carried to the CRBox. The direction for insertion of bats into the acclimatisation box was changed constantly between trials. The experimenter position relative to the CRBox was changed between trials in 90° steps, as well. We waited 15 sec before carefully lifting the acclimatisation box from 10 m distance using a remote control. The activity of the focal bat, i.e. its echolocation behaviour, was constantly monitored using a bat detector. Based on a pilot study in adult pipistrelles, we set a threshold time of 3 min until a trial was cancelled in case no echolocation indicated a lack of movement activity (Lindecke et al. 2019). One bat took longer than this time threshold and consequently was ignored in the analysis. Free flight after

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takeoff was indicated by a sequence of echolocation calls leading away from the CRBox. However, we misinterpreted echolocation calls in three cases and exposed these individuals to light prior to takeoff. We excluded these trials. If a takeoff could be clearly identified by means of echolocations calls, we noted the time (sec), i.e. the takeoff latency a focal bat took to depart from the CRBox after the acclimatisation box was lifted. Takeoff latency potentially indicates behavioural differences between test groups (Lindecke et al. 2019). We recorded takeoff latencies in 37 trials (C=18; M=19). After a bat had disappeared from the RS, we measured the bearing for takeoff, i.e. takeoff orientation, using a compass which was placed in the centre of the CRBox. The experimenter who recorded orientation data was blind to the treatment of bats. Over the course of nine nights, we recorded takeoff orientations of 26 adult (10 male, 16 female) and 24 subadult (11 male, 11 female, 2 sex undetermined) bats.

**Statistical analysis of orientation behaviour and takeoff latency.** Takeoff bearings were analysed using the Oriana 4.0 circular statistics software package (Kovach Computing Services, Pentraeth, UK). We calculated mean bearings and vector lengths, and then Kuiper's omnibus test was used for each treatment group as a goodness-of-fit test against uniform circular distribution, i.e., to detect randomness of data (Batschelet 1981). If Kuiper's test statistic indicated non-random orientation of data, the Rayleigh test was used to test for unimodal departure from circular uniformity, i.e. a preferred takeoff orientation (Pewsey 2013). We compared variances in group-specific orientation scatter by applying the Levene's test based on the deviations of individual takeoff bearings from the group-specific mean (Levene 1960). The Mardia–Watson–Wheeler test was selected to test for differences in distribution across groups (Batschelet 1981). Testing for differences in takeoff latency was performed using t-tests for adult and subadult groups, and the treatments, respectively (SigmaPlot 11.0, Systat Software Inc., Chicago, USA).

**Data availability.** The heading data used in the statistical analysis for this study and a map showing the study location and mean orientations of adult test groups can be found in the supplementary materials associated with this article, available online (Data S1 file [*Appendix 3.1*] and Figure S2 [*Appendix 3.2*]).

## Results

We found that translocated adult bats took off for departure in a direction that depended on the experimental treatment. The heading direction depended on the sunset azimuth that bats had been exposed to, and which they apparently integrated for navigation decisions. Mean

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takeoff orientation of bats previously watching the natural sunset averaged 256° (west-southwest) in the C group, and 79° (east) in the M group which matched the 180° difference in the perceived azimuth of the sun during the treatment. Orientation data of both groups were significantly different from a uniform circular distribution (Kuiper test, adult C: $p < 0.01$, V = 2.04, n = 11; adult M: $p < 0.025$, V = 1.977, n = 15; Fig. 6.3), both being significantly unimodal (Rayleigh test, adult C: r = 0.591, p = 0.018, Z = 3.848; adult M: r = 0.511, p = 0.017, Z = 3.915). The variance of individual orientations around the group-specific means did not differ significantly, and thus can be considered equal (Levene's test: $F_{1,24} = 0.061$, p = 0.806), which is an additional indicator of the effectiveness of the mirror treatment. Distributions were significantly different between groups (Mardia–Watson–Wheeler test, W = 11.238, p = 0.004) while both groups took off in opposite directions (177° difference of mean angles). During 19 releases, unambiguous echolocation behaviour could be observed, indicating the exact moment when focal bats took off for flight. There was no significant difference in latencies between groups (adult C: mean = 88 sec, median 72 sec n = 7; adult M: mean = 85 sec, median = 81 sec, n = 12; t = 0.177, d.f. = 17, p = 0.908).

In contrast to adult takeoff orientations, subadult bats (n = 24) did not show any directional preference, displaying random orientation in both control and experimental groups. Takeoff data distributions from subadult control (subadult C) and mirrored (subadult M) bats did not differ from a uniform circular distribution (Kuiper test, subadult C: $p > 0.15$, V = 1.422, n = 14; subadult M: $p > 0.15$, V = 0.952, n = 10; Fig. 6.3), and thus any unimodal orientation could be ruled out a priori, which is supported by Rayleigh test results (subadult C: r = 0.313, p = 0.258, Z = 1.369; subadult M: r = 0.801, p = 0.801, Z = 0.232). In accordance with the above tests, we found no significant difference in either the variance of individual headings around group-specific means (Levene's test: $F_{1,22} = 1.328$, p = 0.262), nor the distributions of both groups (Mardia–Watson–Wheeler test, W = 0.813, p = 0.666). Thus, first year migrants were not oriented irrespective of treatment.

Again, takeoff latencies did not differ between treatment groups (subadult C: mean = 74 sec, median = 68 sec, n = 11; subadult M: mean = 77 sec, median = 64 sec, n = 7; t = -0.139, d.f. = 16, p = 0.891), nor was there a difference when we compared mean latencies among the age groups, suggesting that in principal subadults behave similarly when compared with adult test bats (adult C & M: mean = 86 sec, median = 72 sec, n = 19; subadult C & M: mean = 75 sec, median = 66 sec,  n = 18; t = 0.693, d.f. = 36, p = 0.493).
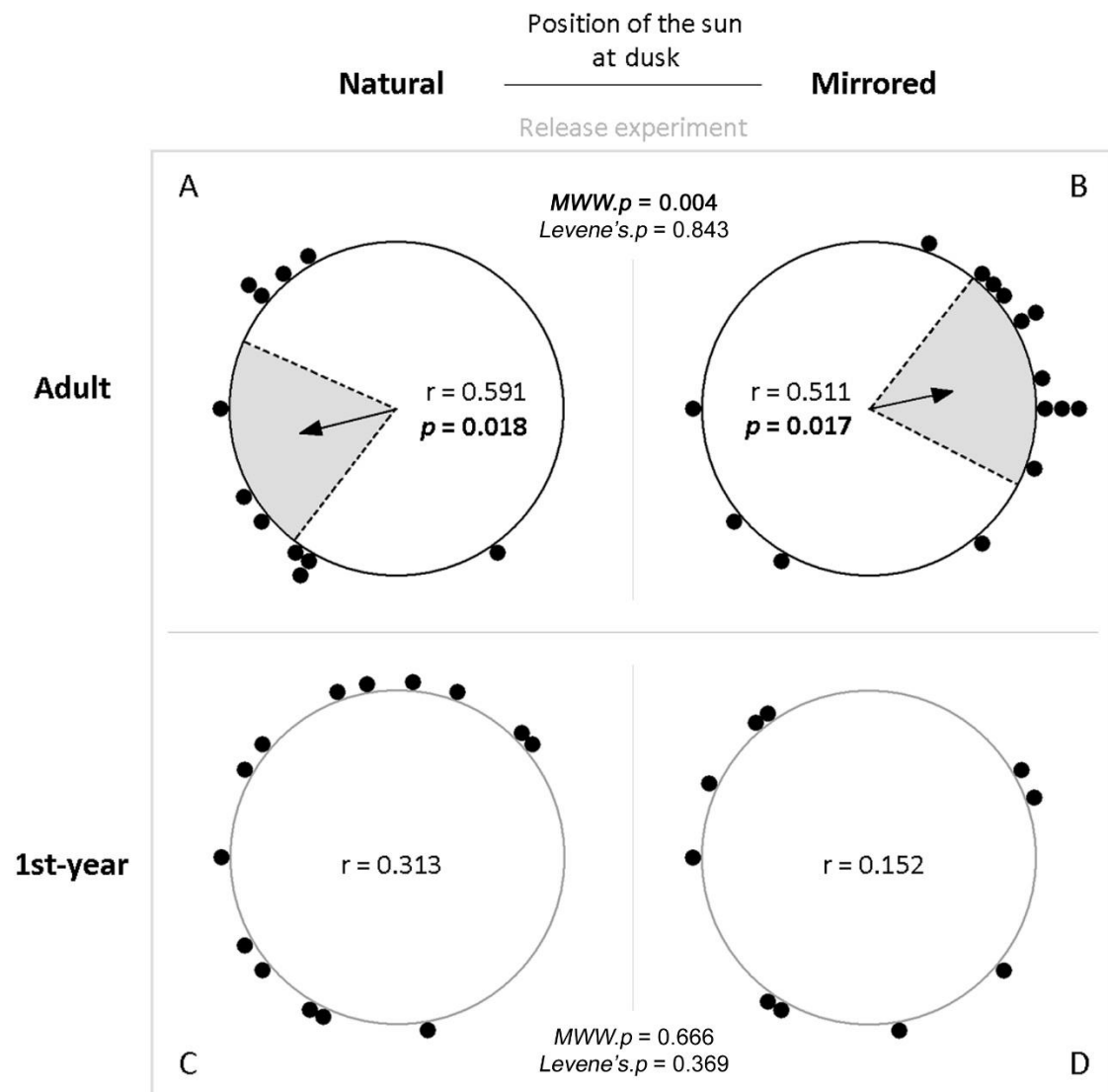
**Fig. 6.3** | Nightly takeoff orientation of adult and 1st-year bat migrants (*Pipistrellus pygmaeus*) at release depending on preceding sunset direction. Arrow and dashed lines (95% confidence intervals) highlight significant unimodal directionality in orientations of adult groups (A, B; n = 26) according to the Rayleigh test (5%, p-values are shown in the circular graph). The length of the black arrows corresponds to the vector length, r, in each graph. Orientation data of both subadult groups (C, D; n = 24) were indistinguishable from random distributions (Kuiper test results: p > 0.15). *MWW.p* and *Levene's.p* are p-values of the Mardia-Watson-Wheeler and the Levene's tests respectively, performed between neighbouring treatment groups. While adults but not subadults differed in distributions (*MWW*), the scatter of data around the means was equal (Levene tests). Significant differences are highlighted in bold. Total sample size n = 50.

## Discussion

Interestingly, in other taxa time-compensated sun compass orientation is possible at an early stage of ontogeny, as it supports long-distance dispersal movements of diurnal fish larvae and juvenile sea turtles (Mott & Salmon 2011, Mouritsen et al. 2013). If so, this suggests that unlike many first year migratory passerines which migrate alone, and are able to count on genetically controlled population specific migratory orientation in the wild (Thorup et al. 2007), and in an orientation cage (Berthold 1991), these bats do not appear to have an inherited migratory direction calibrated by the sun, or at least are not able to express it in the apparatus we use. This suggests that young *P. pygmaeus* need to learn long-distance orientation en route of migration from conspecifics, similar to some long lived bird species (Chernetsov et al. 2004, Mueller et al. 2013).

In conclusion, our study provides the first evidence to show unambiguously that a migratory bat species integrates the direction of the setting sun's disc at dusk to calibrate a compass system for nocturnal orientation, a capability which to our knowledge has not been shown for any other animal species. This complements recent results which did not support a calibration based on polarized light cues in migrating pipistrelle bats (*P. nathusii*), suggesting that magnetic compass calibration is dependent on the solar azimuth at sunset. However, we did not track the migratory transit flight as such and therefore actively migrating bats could also use different navigation strategies, like for example route-following instead of map-and-compass navigation. Thus, it cannot be excluded that bats also use solar azimuth-based orientation in a more general way, e.g., to head back to the coastal flight corridor as it would be the consequence of orientations we observed here, which is similar to the navigational challenge of locating foraging grounds or home roosts outside the migration season.

To date, it remains unknown how subadult *P. pygmaeus* navigate on their first migration. We speculate that subadults may learn by social factors since we regularly observe groups of 2 to 3 bats passing by the migratory corridor at our study site (Voigt et al. 2017a). Our results highlight for the first time in a migratory mammal that first year migrants appear to lack an inherited migratory direction and thus may rely on travelling with experienced conspecifics when making their first migratory journey – an intriguing difference to other migrants, such as juvenile passerine birds and sea turtles (Lohmann et al. 2004, Thorup et al. 2007, Wiltschko & Gwinner 1974).

## Acknowledgements

## Author contributions

O.L. developed the methodology and conceptualised the study. O.L. and A.E. carried out the experiment; C.C.V. and G.P. supported fieldwork. O.L. conducted the analyses, and drafted the original manuscript. C.C.V. and R.A.H. reviewed and edited the manuscript; all authors commented on the manuscript.

## References Chapter III

Aharon, G., Sadot, M., and Yovel, Y. (2017) Bats use path integration rather than acoustic flow to assess flight distance along flyways. Curr. Biol. 27, 3650–3657.

Batschelet, E. (1981). Circular Statistics in Biology. London, UK, Academic Press.

Berthold, P. (1991). Spatiotemporal programmes and genetics of orientation. In: Orientation in Birds, (ed. P. Berthold), pp. 86–104. Basel, CH, Birkhäuser.

Boonman, A., Bar-On, Y., Cvikel, N., and Yovel, Y. (2013). It's not black or white – on the range of vision and echolocation in echolocating bats. Front. Physiol. 4, 248.

Buchler, E.R., and Childs, S.B. (1982). Use of the post-sunset glow as an orientation cue by big brown bats (*Eptesicus fuscus*). J. Mammal. 63, 243–247.

Chernetsov, N., Berthold, P., and Querner, U. (2004) Migratory orientation of first-year white storks (Ciconia ciconia): inherited information and social interactions. J. Exp. Biol. 207, 937–943.

Chernetsov, N., Pakhomov, A., Kobylkov, D., Kishkinev, D., Holland, R.A., and Mouritsen, H. (2017). Migratory Eurasian reed warblers can use magnetic declination to solve the longitude problem. Curr. Biol. 27, 2647-2651.e2642.

Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C.C.M., Elvidge, C.D., Baugh, K., Portnov, B.A., Rybnikova, N.A., and Furgoni, R. (2016). The new world atlas of artificial night sky brightness. Science Advances 2, e1600377.

Genzel, D., Yovel, Y., and Yartsev, M.M. (2018) Neuroethology of bat navigation. Curr. Biol. 28, PR997-R1004.

Greif, S., Borissov, I., Yovel, Y., and Holland, R.A. (2014). A functional role of the sky's polarization pattern for orientation in the greater mouse-eared bat. Nat. Commun. 5, 4488.

Guilford, T., and Taylor, G.K. (2014). The sun compass revisited. Anim. Behav. 97, 135–143.

Holderied, M.W., and von Helversen, O. (2003). Echolocation range and wing beat period match in aerial-hawking bats. Proc. Biol. Sci. 270, 2293–2299.

Holland, R.A. (2007). Orientation and navigation in bats: known unknowns or unknown unknowns? Behav. Ecol. Sociobiol. 61, 653–660.

Holland, R.A., Borissov, I., and Siemers, B. (2010). A nocturnal mammal calibrates a magnetic compass by the sun. Proc. Natl. Acad. Sci. USA 107, 6941–6945.

Holland, R.A., Thorup, K., Vonhof, M.J., Cochran, W.W., and Wikelski, M. (2006). Bat orientation using Earth's magnetic field. Nature 444, 653.

Horton, T.W., Holdaway, R.N., Zerbini, A.N., Hauser, N., Garrigue, C., Andriolo, A., and Clapham, P.J. (2011). Straight as an arrow: humpback whales swim constant course tracks during long-distance migration. Biol. Lett. 7, 674–679.

Horváth, G., and Pomozi, I. (1997). How celestial polarization changes due to reflection from deflector panels used in deflector loft and mirror experiments studying avian orientation. J. Theor. Biol. 184, 291–300.

Kramer, G. (1949). Über Richtungstendenzen bei der nächtlichen Zugunruhe gekäfigter Vögel. [Directional tendencies of nocturnal restlessness in caged birds] In: Ornithologie als biologische Wissenschaft, pp. 269-283. Heidelberg, D: Carl Winter Verlag.

Levene, H. (1960). Robust tests for equality of variances. In: Contributions to probability and statistics: essays in honor of Harold Hotelling (ed. I Olkin, SG Ghurye, W Hoeffding, WG Madow, HB Mann), pp. 278–292. San Fransisco, CA: Stanford University Press.

Lindecke, O., Elksne, A., Holland, R.A., Pētersons, G., and Voigt, C.C. (2019). Orientation and flight behaviour identify the Soprano pipistrelle as a migratory bat species at the Baltic Sea coast. J. Zool., in press.

Lindecke, O., Voigt, C.C., Pētersons, G., and Holland, R.A. (2015). Polarized skylight does not calibrate the compass system of a migratory bat. Biol. Lett. 11, 201505125.

Lohmann, K.J., Lohmann, M.F., Ehrhart, L.M., Bagley, D.A., and Swing, T. (2004). Geomagnetic map used in sea-turtle navigation. Nature 428, 909–910.

Moore, F.R. (1978). Sunset and the orientation of a nocturnal migrant bird. Nature 274, 154–156.

Mott, C.R., and Salmon, M. (2011). Sun compass orientation by juvenile Green sea turtles. Chelonian Cons. Biol. 10, 73–81.

Mouritsen, H. (2018). Long-distance navigation and magnetoreception in migratory animals. Nature 558, 50–59.

Mouritsen, H., Atema, J., Kingsford, M.J., and Gerlach, G. (2013). Sun compass orientation helps coral reef fish larvae return to their natal reef. PLoS ONE, 8, e66039.

Mueller, T., O'Hara, R.B., Converse, S.J., Urbanek, R.P., and Fagan, W.F. (2013). Social learning of migratory performance. Science 341, 999.

Muheim, R., Phillips, J.B., and Akesson, S. (2006) Polarized light cues underlie compass calibration in migratory songbirds. Science 313, 837–839.

Nathan, R., Getz, W.M., Revilla, E., Holoyoak, M., Kadmon, R., Saltz, D., and Smouse, P.E. (2008). A movement ecology paradigm for unifying organismal movement research. Proc. Natl. Acad. Sci. USA 105, 19052–19059.

Pewsey, A., Neuhäuser, M., and Ruxton, G.D. (2013). Circular Statistics in R. Oxford, UK, Oxford University Press.

Phillips, J.B., and Waldvogel, J.A. (1988). Celestial polarized light patterns as a calibration reference for sun compass of homing pigeons. J. Theor. Biol 131, 55–67.

Rydell, J., Bach, L., Bach, P., Guia Diaz, L., Furmankiewicz, J., Hagner-Wahlsten, N., Kyheröinen, E.-M., Lilley, T., Masing, M., Meyer, M.M., et al. (2014). Phenology of migratory bat activity across the Baltic Sea and the south-eastern North Sea. Acta Chiropterol. 16, 139–147.

Stilz, W.-P., and Schnitzler, H.-U. (2012). Estimation of the acoustic range of bat echolocation for extended targets. J. Acoust. Soc. Am. 132, 1765–1775.

Suthers, R.A., and Wallis, N.E. (1970). Optics of the eyes of echolocating bats. Vision Res. 10, 1165–1173.

Thorup, K., Bisson, I.-A., Bowlin, M. S., Holland, R. A., Wingfield, J. C., Ramenofsky, M., and Wikelski, M. (2007). Evidence for a navigational map stretching across the continental U.S. in a migratory songbird. Proc. Natl. Acad. Sci. USA 104, 18115–18119.

Voigt, C.C., Frick, W.F., Holderied, M.W., Holland, R.A., Kerth, G., Mello, M.A.R., Plowright, R.K., Swartz, S., and Yovel, Y. (2017a). Principles and patterns of bat movements: From aerodynamics to ecology. Quart. Rev. Biol. 92, 267–287.

Voigt, C.C., Röleke, M., Marggraf, L., Pētersons, G., and Voigt-Heucke, L. (2017b). Migratory bats respond to artificial green light with positive phototaxis. PLoS ONE 12, e0177748.

Williams, T., Williams, J., and Griffin, D. (1966) The homing ability of the neotropical bat *Phyllostomus hastatus*, with evidence for visual orientation. Anim. Behav. 14, 468–473.

Wiltschko, W., and Gwinner, E. (1974). Evidence for an innate magnetic compass in garden warblers. Naturwissenschaften 61, 406.

Zhao, H.S., Rossiter, S.J., Teeling, E.C., Li, C., Cotton, J.A., and Zhang, S. (2009). The evolution of color vision in nocturnal mammals. Proc. Natl. Acad. Sci. USA 106, 8980–8985.

# CHAPTER IV – Magnetic sense and the role of corneal sensitivity

Manuscript in preparation for submission

as

**Navigational deficit in migratory bats with impaired trigeminal input: Support for a corneal magnetic sense**

by

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## Abstract

The exact anatomical location for an iron particle-based magnetic sense of vertebrates remains enigmatic. It has been hypothesised that the cornea carries the primary sensors for magnetoreception in mammals. Yet, this has never been tested in free-ranging animals.

Here, we investigated whether intact corneal sensation is crucial for navigation in migrating Nathusius' bats, *Pipistrellus nathusii*, translocated from their migratory corridor.

We found that bats treated with corneal anaesthesia in both eyes flew in random directions, contrasting bats with a single eye treated, and the control group, which both oriented in the seasonally appropriate direction.

We also tested retinal function which remained unaffected by anaesthesia and therefore our results are consistent with the corneal magnetoreception hypothesis. Furthermore, we demonstrate that corneal compass (or map) sense is of bilateral nature but can function in a single eye if necessary.

**Key-words**: *animal navigation, compass orientation, cornea, magnetoreception, mammal migration, map sense, sensory physiology*

## Introduction

While the capacity for magnetoreception among mammals is evident from a number of behavioural experiments (Mather & Baker 1981, Burda et al. 1990, Kimchi & Terkel 2001, Deutschlander et al. 2003, Holland et al. 2006, Muheim et al. 2006, Martini et al. 2018), the anatomical location of the involved receptors, remain as enigmatic as in any other animal to date (Mouritsen 2018, Malkemper et al. 2019). For most species studied so far, it is hypothesized that a magnetic sense is based on intra-cellular iron oxide, i.e., magnetite nanoparticles ($Fe_3O_4$) which are sensitive to a magnetic field's horizontal polarity, enabling animals to distinguish between magnetic north and south. Intra-cellular iron oxide seems to be also responsible for magnetic signal transmission through control of ion channels depending on the alignment of animals in the magnetic field (Kirschvink & Gould 1981, Walker et al. 1997, Thalau et al. 2006, Mouritsen 2018). Wegner and colleagues postulated that the cornea may be the location of the primary magnetoreceptors in mammals (Wegner et al. 2006). Specifically, they showed that in mole-rats, *Fukomys anselli*, bilateral anaesthesia of the cornea resulted in randomly oriented nest-building contrary to usually polarity-dependent nesting behaviour (Marhold et al. 1997, Wegner et al. 2006). According to the innervation of the cornea, the ophthalmic branch of the trigeminal nerve would transmit the magnetic signal to the midbrain where magnetic stimuli could be processed (Němec et al. 2001). Yet to date, the hypothesis of a corneal magnetic sense has never been challenged nor expanded from laboratory conditions onto freely moving animals performing an actual navigation task in the field, e.g., during seasonal migration.

Non-migratory bats are known to possess a polarity-sensitive magnetic compass which they use for homing tasks (Holland et al. 2006, 2010, Wang et al. 2007). Furthermore, results from a classic 'Kalmijn-Blakemore' pulse re-magnetization experiment in big brown bats (*Eptesicus fuscus*) revealed that the magneto-sensory cells somewhere in the body of bats carry freely rotating magnetite (Holland et al. 2008). In contrast, the compass cues and sensory structures migratory bats use for long-range navigation in-flight still remain undetermined. Only recently, it was demonstrated that bats calibrate their compass system to the solar azimuth at sunset and could take up a seasonally appropriate migratory heading after moderate displacement from their migration corridor (Lindecke et al. 2015, 2019). In order to study the role of the cornea in vertebrates that adapted to long-range navigation, we performed translocation experiments with 80 adult Nathusius' bats (*Pipistrellus nathusii*) during late summer migration season. Half of the bats received either unilateral or bilateral topical cornea

anaesthesia prior to release, while the other half was treated with saline solution to create a sham control condition. Importantly, we also conducted tests of retinal function in another 76 bats using a Y-maze choice experiment to validate that corneal anaesthesia would not affect the retinal function, specifically the vision of bats.

We hypothesized that migratory bats depend on corneal magnetoreception for navigation. If the cornea would play a role for orientation, we predicted that after translocation when bats were tracked during their departure flights, individuals with a topical anaesthetic applied on both eyes would vanish in random directions. However, bats with a single eye treated would be able to navigate irrespective of that because the other eye's cornea would still be functional, i.e., transmit sensory stimuli trough the ophthalmic branch of the trigeminal nerve, and enable these bats to fly in migratory direction similar to a sham-treated group.

We found that freely flying Nathusius' bats orient in a seasonally appropriate migratory direction, if at least one cornea is sensitive. While vision is retained unchanged after corneal anaesthesia, a navigational deficit was evident in bats with insensitive corneas in both eyes, however.

Our findings suggest that the cornea plays a functional role for the compass orientation of bats during migration, and that this sense is of bilateral nature but may function in a single eye, if necessary. Thus, the corneal layers are a promising target region in the ongoing search for mammalian magnetoreceptors signalling through the ophthalmic branch of the trigeminal nerve, in particular because the cornea belongs to the most densely innervated sensory tissues of the body.

## Material and methods

**Animal subjects.** Over the course of three field seasons, a total of 156 adult Nathusius' bats (*P. nathusii*) were caught at the shoreline of the Baltic Sea, using a custom made directional funnel trap (35 × 50 × 15 m; length × width × height) at Pape Bird Ringing Station (PBRS; 56°09' N 21°03'E, Rucava Municipality, Latvia). Capturing was performed all night at the peak of the late summer migration season (between 14 Aug to 1 Sep 2015, 19 Aug to 23 Aug 2016, and 18 Aug to 4 Sep 2017). Bats were aged based on the closure of the epiphyseal gaps. While bats assigned to the retina function test (n=76) were only controlled for seasonally appropriate body condition (≥7.0 g body mass), individuals assigned to the

translocation experiment (n=80) were also transitionally ringed and measured for body mass and forearm length. Subsequently, the animals were transferred to a keeping facility, where they were kept in groups of up to five individuals in wooden boxes ($38 \times 19 \times 13$ cm) in a dark and quiet environment until evening feeding or experimental release, respectively. Periods of keeping encompassed 2 to max. 5 days to avoid unsuitable release conditions under periods of poor weather and unfavourably strong winds. The retinal function experiments were conducted indoors and on the night subsequent to the capture of bats. Animals were housed in small groups and had no access to the natural night sky before release. Captive bats were fed individually with mealworms (larval stages of *Tenebrio molitor*, Coleoptera) during the evening hours and provided *ad libitum* water. Prior to feeding on experimental evenings, bats also received three small drops of saline solution (NaCl) into the nostrils, as they served as a control group for another study. We do not expect an effect on visual performance and corneal sensation from this nasal treatment. All work was conducted under the permits #10/2015, #31/2016, #33/2017-E and #3.6/85/2017-N-E issued by the Latvian Nature Conservation Agency to the Institute of Biology, University of Latvia.

**Topical anaesthesia of the cornea.** Bats were gently hold in an upright position and treated with one drop of oxybuprocaine hydrochloride (0.4%, Novesine®, Novartis, Germany) to the central cornea using a pipette. We chose this topical anaesthetic over lidocaine which is commonly applied in studies of orientation physiology, because lidocaine is known to cause sometimes ophthalmic side effects in birds and mammals, including visual impairment, because it may penetrate deep into tissues where it may even pass the blood-brain barrier (Pardridge et al. 1983, Wallraff 1988, Judge et al. 1997, Engels et al. 2018). Oxybuprocaine is different, as it numbs only the outermost layers of the cornea, however, its anaesthetic efficiency decreases after 30 min and thus the sensory impairment is quickly reversible (Giudici et al. 2015, Little et al. 2016). Oxybuprocaine is therefore routinely used in human and veterinary ophthalmology (Lytle & Thomas 1992, Furrer et al. 1999, Tonnu et al. 2005, Nam et al. 2006, Rosa et al. 2011, Sandström et al. 2011, Douet et al. 2013, Wieser et al. 2013, Giudici et al. 2015, Little et al. 2016). As a control, i.e. for a sham-treatment, we used eye drops of sterile saline solution (NaCl 0.9%, B. Braun Melsungen AG, Germany) which is a standard in both human and veterinary ophthalmology, and eye care (Little et al. 2016).

The bilateral treatment group received corneal anaesthesia to both eyes. Bats from the unilateral, i.e., single-eye treatment group and control bats (sham-control groups 1 and 2)

received a drop of the sham treatment to the contralateral or both eyes, respectively. After 20 s exposure time, any supernatant was gently sucked off from the surface of the eye using sterile tissue, and only then the contralateral eye was treated. It is noteworthy that bats did not blink during this procedure, nor did they show any behaviour indicating discomfort, such as emitting of distress calls or spontaneous movements. The choice for the individual cornea treatment was made in a blinded fashion, with different generations of students assisting the experimenter. The experimenter received two identical unlabelled pipettes and a note on lateral allocation for the application of eye drops. The left-right ratio of the unilateral treatment was kept at balance over the course of the study period, yet lateral allocations followed a randomized order in each night. Behavioural testing started immediately after the eye drops were applied. Bats assigned to the navigation experiment received the treatment only after translocation, just before individual releases.

**Testing retinal function and phototactic behaviour.** To make sure topical corneal anaesthesia did not affect visual capability, we tested 76 bats for phototaxis responses in a Y-maze task. Bats are known to choose lit exits over dark ones for emergence from Y-mazes (Chase 1981). We compared the bilateral anaesthesia treatment (n=22; 14/8 males/females) with a sham-control (n=22; 10/12; "sham-control 2") in 2015, and the unilateral treatment (n=16; 5/11) with another sham-control (n=16, 5/11, "sham-control 1") in 2017, respectively. Tests were performed indoors at PBRS, at room temperature, and went from 0400 to 0600 hours over the course of two nights in both years. Experimental individuals were kept in wooden boxes until tested. The maze-apparatus was constructed using plywood, and was inclined towards the exits 10° following recommendations of Chase (1981). The Y-maze had an arm length of 200 mm; cross-sectional dimensions of the runway were $80 \times 60$ mm (width $\times$ height). All surfaces were coloured dark-brown to minimize light reflections. For the floor, an easy to clean PVC coating with a structured surface was used, which was not slippery for crawling bats. The entrance of the Y-maze had a light level of 0.02 lx. Dim light (120 lx) was provided at the exit of one arm using three commercial white torch LEDs indirectly illuminating the space behind the exit, while the exit of the other arm was kept dark (0.01 lx). The area of the bifurcation inside the Y-maze was illuminated indirectly (0.12 lx) via the lit arm. Each bat was transferred manually to the acclimatisation compartment of the Y-maze, directly after corneal anaesthesia or sham treatment, respectively. Besides the Y-maze illumination, the testing room was kept dark. After 20 s for acclimatisation, a bat had to crawl a 100 mm runway to reach the bifurcation. We timed emergence latency. Bats of both groups

were tested in alternate order, with the lit arm of the maze changed after the first half of bats has been tested. Ethanol (70%) was used to clean the runways between trials. Individuals were tested only once and released in the nearby coastal forest after 1h to ensure that anaesthesia had ceased before bats were free again. When it was already daylight outside, bats were kept for the next day, fed and watered in the evening and released immediately after that at the site of capture. Emergence latency was compared using the Mann-Whitney U test since data were not normally distributed (P<0.05). Directional choices for exits of each group were analysed for a preference using a test of goodness of fit (Chi-squared test; R version 3.2.1, package *shiny*).

**Testing navigational performance after translocation and corneal anaesthesia.** For the release experiment 80 adult *P. nathusii* (36 males, 44 post-lactating females) were used. On the day of the translocation, bats were fed and watered from 1800 to 2000 hours. Subsequently, they were equipped with VHF radio transmitters (LB-2XT, Holohil Systems Ltd., Ottawa, Canada, 0.31 g; V1 and V3, Telemetrie-Service Dessau, Dessau-Roßlau, Germany, 0.35 g; Pip Ag337 and PicoPip Ag379, BioTrack Ltd., Wareham, United Kingdom, 0.35 and 0.43 g). These were glued onto the fur of the lower dorsum using skin glue (Manfred Sauer GmbH Hautkleber, Lobbach, Germany). Transmitters were selected so that the mass of the tag was always smaller than 5% of the individual body mass. Until translocation to the release site, bats were kept individually in large cloth bags to allow acclimatisation to the tag. Translocation and releases were performed between 2300 and 0400 hours of a given night and over the course of 26 nights. The release site was a flat field approx. 11 km east of the capture site and outside the coastal migration corridor where bats were caught. The location offered a clear line of sight for 360°. To increase the motivation to continue migratory transit flights, bats were offered water and mealworms again just prior to release but before any cornea treatments. The person who tracked the animals was blind to the treatment conditions. In order to achieve that, the assisting personnel randomly chose the substances to be applied, i.e., chose the test group, and consequently provided the experimenter with one pipette per eye for applications. Thereby, we ensured unbiased measuring of vanishing bearings. We aimed to release an even number of bats per group and night. Only the assisting personnel tracked the sequence of experimental and control bat releases of a given night and could balance the number and succession of releases of both groups of treatments. Before treatment and release from the roof of the car, we surveyed the vicinity of the site for the presence of any other bats using a bat detector (Echometer EM3+, Wildlife Acoustics, Inc., Maynard, MA, USA). If any

bat would have been recorded, the experiment would have been paused to avoid confounding via eavesdropping. After the cornea treatment prior to releasing, the surrounding was surveyed for bats again for 1 min and if no bat activity was measured test bats were offered to takeoff in their own speed while the release direction was chosen randomly. Bats were then tracked at about 4 m above ground using a handheld three element Yagi antenna attached to an Australis 26k receiver (Titley Scientific). When the signal of the radio transmitter vanished, the bearing of the fading signal and the time elapsed since the release were noted. After two minutes, we confirmed the absence of bats by monitoring the area for the individual radio signal again. This was also repeated for all individuals of the given night after the last bat has vanished. The next night, a complete scan for all frequencies was repeated before any new bat was released. For statistical comparisons we did not consider bats that took >30 min for vanishing (n=4) because full efficiency of the corneal anaesthesia lasts for half an hour (Douet et al. 2013; Furrer et al. 1999; Giudici et al. 2015; Little et al. 2016; Rosa et al. 2011). In our previous study, *P. nathusii* vanished from the tracking range in less than 20 min from the same release site (Lindecke et al. 2015), indicating that significantly longer vanishing times most likely represent outliers.

Mean bearings and vector lengths of each group were calculated using the Oriana 4.02 circular statistics software package (Kovach Computing Services). Groups were tested for departure from a uniform circular distribution using the Rayleigh's test (Batschelet 1981). Tests for significant differences between group orientations were performed using the Mardia-Watson-Wheeler test. Navigational accuracy between groups was assessed by testing for homogeneity of variances across groups, i.e. the scatter of the bearings. For this, the original bearings were transformed to absolute residuals from the group-specific orientation mean. With these we computed a Levene's test which does not assume underlying normality of the data (Levene 1960; R version 3.3.2, package *car* version 2). Departure flight times were compared using an analysis of variance (ANOVA; normality test passed, P=0.083).

## Results

**Retinal function is unimpaired after topical cornea anaesthesia.** In previous choice experiments it was shown that bats released in a Y-maze with one dark and one lit exit preferred the lit exit, and if deprived of retinal photoreception through blindfolding, bats choose exits randomly (Chase 1981). We performed similar tests for photoreceptive function, i.e. unimpaired vision, to rule out any effect on the retina through topical cornea anaesthesia

by oxybuprocaine eye drops. When we tested the unilateral and bilateral treatment groups which received the topical corneal anaesthetic, and the two sham control groups which received eye drops of saline solution bilaterally, our tests did not indicate a differential effect between these applications on the bats' behaviour, and animals of both the treatment and sham control groups significantly preferred the lit exit of the Y-maze (sham control 1: n=22, 77% (proportion of bats choosing the lid exit in %), $\chi^2$=6.55, W=0.55, P=0.011; single eye treated: n=16, 81.3%, $\chi^2$=6.25, W=0.625, P=0.0124; sham control 2: n=16, 75%, $\chi^2$=4.0, W=0.5, P=0.046; both eyes treated: n=22, 86%, $\chi^2$=11.64, W=0.727, P<0.001). Further, exit latency did not differ between bats with bilateral cornea anaesthesia and the respective sham control (both eyes treated, mean ± SD: 11.9 s ± 18.9 SD, median: 4.0 s; sham control 1, mean ± SD: 11.1 s ± 10.7 SD, median: 7.5 s; Mann-Whitney U-test: n= 44, U=199.5, P=0.321).

**Cornea sensation is crucial for accurate navigation after translocation**. Nathusius' bats with their eyes untreated were previously shown to spontaneously vanish in a southerly, seasonally appropriate direction after experimental translocation during migration (Pētersons 2004, Lindecke et al. 2015). Here, under the same conditions, vanishing bearings of the two sham control groups were also unimodally oriented towards the south (Rayleigh's test, sham control 1, Fig. 7a: 183° ± 34° (95% confidence intervals), n=20, r=0.495, Z=4.91, P=0.006; sham control 2, Fig. 7b: 187° ± 34°, n=19, r=0.502, Z=4.78, P=0.007). There was no difference between the mean orientations of the two control groups (Mardia-Watson-Wheeler test, W=0.189, P=0.91), nor did the variances of individual orientations around the sham group-specific means differ (Levene's test, $F_{1,36}$=0.008, P=0.931). Bats of the experimental group that received corneal anaesthesia in one eye and sham treatment for the other also vanished in a southerly direction (Rayleigh's test, single eye treated, Fig. 7c: 199° ± 37°, n=19, r=0.469, Z=4.183, P=0.013). Hence, the group mean vector did not differ from the mean of the respective sham control group (Mardia-Watson-Wheeler test, W=1.011, P=0.603). The variance of individual orientations around the mean did also not differ between the unilateral treatment group and the sham control from the same migration season (Levene's test, $F_{1,37}$=0.224, P=0.639). In contrast to all other groups, bats released with bilateral topical cornea anaesthesia departed in random directions (Rayleigh's test, both eyes treated, Fig. 7d: 240°, n=18, r=0.061, Z=0.066, P=0.937). Consequently, the variance of individual orientations between bats that received bilateral anaesthesia and the respective sham control group differed significantly (Levene's test, $F_{1,35}$=5.824, P=0.021). In addition, the variances around the means of the two groups that received corneal anaesthesia differed (Single eye

treated vs. both eyes treated: Levene's test, $F_{1,35}=5.310$, P=0.027). However, tests for differences between the two experimental groups, and between the bilateral treatment group and the respective sham control, that did not detect any differences in data distributions are of limited explanatory value due to the underlying scatter of data (Mardia-Watson-Wheeler tests: single eye vs. both eyes treated, W=3.513, P=0.173; sham control 2 vs. both eyes treated, W=3.524, P=0.172).

Experimental and sham control bats vanished promptly from the release site (mean values ± SD, single eye treated: 19.3 min ± 6, median=20 min; sham control 1: 16 ± 6 min, median=14.5 min; both eyes treated: 17.3 min ± 7, median=19.5 min; sham control 2: 16.1 min ± 6, median=16 min). No significant difference in the lengths of vanishing times of bats could be detected between groups (ANOVA, F=1.203, d.f.= 3, P=0.135).
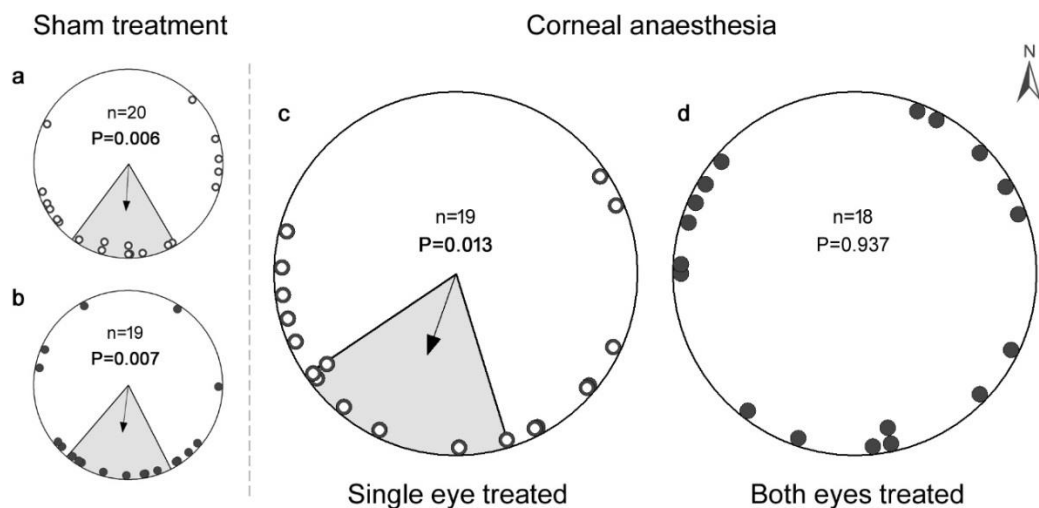


**Fig. 7 | Migratory Nathusius' bats vanish in random directions if corneal sensation is inhibited in both eyes**. (**a**) and (**b**) show control bats that received eye drops of saline solution as a sham treatment before release. Empty and filled dots indicate which groups were tested in the same trial. (**c**) shows experimental bats that randomly received a topical anaesthetic to the left or right eye's cornea and sham treatment for the other eye, accordingly. (**d**) shows bats with bilateral topical cornea anaesthesia. Arrows depict the group mean vectors with North (0°) always as top of the circles. Grey sectors encompassing the group mean vectors indicate the 95% confidence intervals for the mean. P-values from Rayleigh tests are shown. Total sample size: n=76.

## Discussion

To our knowledge, these experiments are the first to have evoked a response in the navigation behaviour of a freely moving mammal migrant without manipulation of any environmental cues. Further, these data support the hypothesis of a cornea-based orientation system in bats for the first time, which has been suggested to be the location for a magnetic sense in mammals (Wegner et al. 2006). When bats of our sham treatment groups were released after translocation from their migration corridor, they were significantly oriented in a seasonally appropriate migratory direction, which is in line with previous data from the same study location (Lindecke et al. 2015). This was also true, when bats were deprived of corneal sensation in either one of their eyes. Yet, bats vanished in random directions but in the same pace as other bats, when the cornea was temporally insensitive in both eyes. Our study clearly shows that topical corneal anaesthesia does not neutralize the photoreceptive function of bats' retina, and therefore the visual capacity can be assumed unimpaired. Thus, upon takeoff bats could have used any visual cue for orientation and navigation, yet bats seemed to not have relied on such cues.

Similarly disoriented flights, independent of retinal impairment, have also been observed in migrating birds and experiments with homing pigeons, when these encountered magnetic anomalies or stronger fluctuations of the Earth's magnetic field (Keeton et al. 1974, Alerstam 1987, Walcott 1978, Dennis et al. 2007, Mora & Bingman 2013). Also in dogs, directional preferences for body alignment were abolished when the Earth's magnetic field conditions fluctuated so that polarity changed (Hart et al. 2013). Such disorientation responses are associated not only with impaired compass orientation but also with a malfunction of the "map sense" in animals, i.e., when they cannot obtain positional information (Kirschvink & Gould 1981), which are further not compensated for by other intact compass systems (e.g. a sun compass, Keeton et al. 1974, Walcott 1978). Accumulating evidence supports a "magnetic map sense" based on freely-rotating magnetic iron particles that transmit magnetic field information through the trigeminal system (Mora et al. 2004, Kishkinev et al. 2013, Pakhomov et al. 2018). Indeed, such magnetic particles (magnetite) have been found in the heads of different bat species (Buchler & Wasilewski 1985, August et al 1989, Tian et al. 2010), yet no physical link to any sensorial neuronal network has been established so far. However, magnetic pulsing which should trigger re-magnetization of any magnetite-based sensor, and thus provide directionally reversed magnetic compass (or map) information, lead to deflections in adult homing bats that have established a map of their home range (Holland et

al. 2008). In migratory songbirds however, disruption of the magnetic map sense can reliably be elicited by bilaterally cutting the ophthalmic branch of the trigeminal nerve (Kishkinev et al. 2013, 2015, Pakhomov et al. 2018), which is the same nerve we anaesthetized here. Further, magnetite-particles are considered to support a light-independent magnetic polarity compass, which was found to be used by mammals, and in bats in particular (Burda et al. 1990, Wang et al. 2007, Mouritsen 2018)

Now, further experiments are needed since a disorientation response is open to criticism of a nonspecific affect, and the physiological composition and location of any trigeminal magnetosensor are generally elusive (Engels et al. 2018). Physiological and histological investigations using next-generation microscopy are essential for ultrastructural identification of any putative magnetic sensors in the corneal epithelia (Mouritsen 2018). However, as nocturnal animals, bats have particularly large corneal surfaces and the cornea ranks among the most densely innervated tissues in the mammalian body, which renders it a promising organ for the search of biological "compass needles" (Suthers & Wallis 1970, Roszà et al. 1982, de Castro et al. 1998, Müller et al. 2003, Wegner et al. 2006).

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## Author contributions

O.L. designed the experiments and carried out the fieldwork, conducted the data analysis and drafted the initial manuscript. G.P. and C.C.V. supported fieldwork and administration. C.C.V. and R.A.H. supervised the project; C.C.V. and R.A.H. provided material, reviewed and edited the manuscript; all authors commented on the manuscript.

## References Chapter IV

Alerstam, T. (1987) Bird migration across a strong magnetic anomaly. Journal of Experimantal Biology 130, 63-86.

August, P. V., Ayvazian, S. G. & Anderson, J. G. T. (1989) Magnetic orientation in a small mammal, *Peromyscus leucopus*. Journal of Mammalogy 70, 1-9.

Batschelet, E. (1981) Circular Statistics in Biology. London, UK, Academic Press.

Burda, H., Marhold, S., Westenberger, T., Wiltschko, R. & Wiltschko, W. (1990) Magnetic compass orientation in the subterranean rodent *Cryptomys hottentotus* (Bathyergidae). Experientia 46, 528-530.

Buchler, E.R. & Wasilewski, P. J. (1985) Magnetic remanence in bats. In: Kirschvink JL, Jones DS, MacFadden BJ, eds. Magnetite biomineralization and magnetoreception in organisms: a new biomagnetism. New York: Plenum Press. pp 483-488.

de Castro, F., Silos-Santiago, I., Lopez de Armentia, M., Barbacid, M. & Belmonte, C. (1998) Corneal innervation and sensitivity to noxious stimuli in trkA knockout mice. European Journal of Neuroscience 10, 146-152.

Chase, J. (1981) Visually guided escape responses of microchiropteran bats. Animal Behaviour 29, 708-713.

Dennis, T. E., Rayner, M. J. & Walker, M. M. (2007) Evidence that pigeons orient to geomagnetic intensity during homing. Proc. R. Soc. Lond. B 274, 1153–1158.

Deutschlander, M. E., Freake, M. J., Borland, S. C., Phillips, J. B., Madden, R. C., Anderson, L. E. & Wilson, B. W. (2003) Learned magnetic compass orientation by the Siberian hamster, *Phodopus sungorus*. Animal Behaviour 65, 779-786.

Douet, J.-Y., Michel, J. & Regnier, A. (2013) Degree and duration of corneal anesthesia after topical application of 0.4% oxybuprocaine hydrochloride ophthalmic solution in ophthalmically normal dogs. American Journal of Veterinary Research 74, 1321-1326.

Engels, S., Treiber, C. D., Salzer, M. C., Michalik, A., Ushakova, L., Keays, D. A., Mouritsen, H. & Heyers, D. (2018) Lidocaine is a nocebo treatment for trigeminally mediated magnetic orientation in birds. Journal of the Royal Society Interface 15, 20180124.

Furrer, P., Mayer, J. M., Plazonnet, B. & Gurny, R. (1999) Ocular tolerance of preservatives on the murine cornea. European Journal of Pharmaceutics and Biopharmaceutics 47, 105-112.

Giudici, V. Baeza, S., Douet, J.-Y. & Regnier, A. (2015) Corneal anesthesia following application of 0.4% oxybuprocaine hydrochloride ophthalmic solution to normal feline eyes. Veterinary Ophthalmology 18, 141-146.

Hart, V., Nováková, P., Malkemper, E. P., Begall, S. et al. (2013) Dogs are sensitive to small variations of the Earth's magnetic field. Frontiers in Zoology 10, 80.

Holland, R. A., Borissov, I. & Siemers, B. M. (2010) A nocturnal mammal, the greater mouse-eared bat, calibrates a magnetic compass by the sun. Proceedings of the National Academy of Sciences USA 107, 6941-6945.

Holland, R. A., Kirschvink, J. L., Doak, T. G. & Wikelski, M. (2008) Bats use magnetite to detect the Earth's magnetic field. PLoS ONE 3, e1676.

Holland, R. A., Thorup, K., Vonhof, M. J., Cochran, W. W. & Wikelski, M. (2006) Bat orientation using Earth's magnetic field. Nature 444, 702.

Judge, A. J, Najafi, K., Lee, D. A. & Miller, K. M. (1997) Corneal endothelial toxicity of topical anesthesia. Ophthalmology 104, 1373-1379.

Keeton, W. T., Larkin, T. S. & Windsor, D. M. (1974) Normal fluctuations in the Earth's magnetic field influence pigeon orientation. Journal of Comparative Physiology 95, 95-103.

Kimchi, T. & Terkel, J. (2001) Magnetic compass orientation in the blind mole rat *Spalax ehrenbergi*. Journal of Experimental Biology 204, 751-758.

Kirschvink, J. L. & Gould, J. L. B. (1981) Biogenetic magnetite as a basis for magnetic field detection in animals. BioSystems 13: 181-201.

Kishkinev, D., Chernetsov, N., Heyers, D. & Mouritsen, H. (2013) Migratory reed warblers need intact trigeminal nerves to correct for a 1,000 km eastward displacement. PLoS One 8, e65847.

_____

Kishkinev, D., Chernetsov, N., Pakhomov, A., Heyers, D. & Mouritsen, H. (2015) Eurasian reed warblers compensate for virtual magnetic displacement. Current Biology **25**, R822-R824.

Levene, H. (1960) Robust tests for equality of variances. In: Contributions to probability and statistics: essays in honor of Harold Hotelling (ed. I. Olkin, S. G. Ghurye, W. Hoeffding, W. G. Madow, H.B. Mann), pp. 278–292. San Fransisco, CA: Stanford University Press.

Lindecke, O., Voigt, C.C., Pētersons, G. & Holland, R.A. (2015) Polarized skylight does not calibrate the compass system of a migratory bat. Biology Letters 11, 20150525.

Lindecke, O., Elksne, A., Holland, R.A., Pētersons, G. & Voigt, C.C. (2019) Experienced migratory bats integrate the sun's position at dusk for navigation at night. Current Biology, 29, *in press*.

Little, W. B., St. Jean, G., Sithole, F., Little, E. & Yvorchuck-St. Jean, K. (2016) Degree of corneal anaesthesia after topical application of 0.4% oxybuprocaine hydrochloride and 0.5% proparacaine hydrochloride ophthalmic solution in clinically normal cattle. Australian Veterinary Journal 94, 181-185.

Lytle, J. & Thomas, N.F. (1992) Haemodynamic stability during general anesthesia for intraocular surgery: the effect of topical oxybuprocaine. Anesthesia 47, 616-617.

Malkemper, E. P., Kagerbauer, D., Ushakova, L., Nimpf, S., Pichler, P., Treiber, C. D., de Jonge, M., Shaw, J. & Keays, D. A. (2019) No evidence for a magnetite-based magnetoreceptor in the lagena of pigeons. Current Biology, 29, R1-R15.

Marhold, S., Wiltschko, W. & Burda, H. (1997) A magnetic polarity compass for direction finding in a subterranean mammal. Naturwissenschaften 84, 421-423.

Martini, S., Begall, S., Findeklee, T., Schmitt, M., Malkemper, E. P. & Burda, H. (2018) Dogs can be trained to find a bar magnet. PeerJ 6, e6117.

Mather, J. G. & Baker, R. R. (1981) Magnetic sense of direction in woodmice for route-based navigation. Nature 291, 152-155.

Mora, C. V., Davison, M., Wild, J. M., Walker, M. M. (2004) Magnetoreception and its trigeminal mediation in the homing pigeon. Nature 432, 508-511.

Mora, C. V., Bingman, V. P. (2013) Detection of magnetic field intensity gradient by homing pigeons (*Columba livia*) in a novel ''Virtual Magnetic Map'' conditioning paradigm. PLoS ONE 8: e72869.

Mouritsen, H. (2018) Long-distance navigation and magnetoreception in migratory animals. Nature 558, 50-59.

Müller, L.J., Marfurt, C.F., Kruse, F. & Tervo, T.M.T. (2003) Corneal nerves: structure, contents and function. Exp. Eye Res. 76, 521–542.

Muheim, R., Edgar, N. M., Sloan, K. A. & Phillips, J. B. (2006) Magnetic compass orientation in C57BL/6J mice. Learning & Behavior 34, 366-373.

Nam, S. M., Lee, H. K., Kim, E. K. & Seo, K.Y. (2006) Comparison of corneal thickness after the instillation of topical anesthetics – Proparacaine versus oxybuprocaine. Cornea 25, 51-54.

Němec, P., Altmann, J., Marhold, S., Burda, H. & Oelschläger, H. H. A. (2001) Neuroanatomy of magnetoreception: the superior colliculus involved in magnetic orientation in a mammal. Science 294, 366-368.

Pakhomov, A., Anashina, A., Heyers, D., Kobylkov, D., Mouritsen, H. & Chernetsov, D. (2018) Magnetic map navigation in a migratory songbirds requires trigeminal input. Scientific Reports 8, 11975.

Pardridge, W. M., Sakiyama, R. & Fierer, G. (1983) Transport of propranolol and lidocaine through the rat blood-brain barrier. Primary role of globulin-bound drug. Journal of Clinical Investigation 71, 900-908.

Pētersons, G. (2004) Seasonal migrations of north-eastern populations of Nathusius' bat *Pipistrellus nathusii* (Chiroptera). Myotis 41-42, 29-56.

Rosa, N., De Bernardo, M., Borrelli, M., et al. (2011) Effect of oxybuprocaine eye drops on corneal volume and thickness measurements. Optometry and Vision Science 88, 640-644.

Rózsa, A.J. & Beuerman, R.W. (1982) Density and organization of free nerve endings in the corneal epithelium of the rabbit. Pain 14, 105-120.

Sandström, J., Heiduschka, P.,  Beck, S. C.,  Philippar, U., Seeliger, M. W., Schraermeyer, U. & Nordheim, A. (2011) Degeneration of the mouse retina upon dysregulated activity of serum response factor. Molecular Vision 17, 1110-1127.

Suthers, R. A. & Wallis, N. E. (1970) Optics of the eyes of echolocating bats. Vision Research 10, 1165-1173.

Thalau, P., Ritz, T., Burda, H., Wegner, R. E. & Wiltschko R. (2006) The magnetic compass mechanisms of birds and rodents are based on different physical principles. Journal of the Royal Society Interface 3, 583-587.

Tonnu, P. A., Ho, T., Sharma, K. et al. (2005) A comparison of four methods of tonometry: method agreement and interobserver variability. The British Journal of Ophthalmology 89, 847-850.

Walcott, C. (1978) Anomalies in the Earth's magnetic field increase the scatter of pigeon's vanishing bearings. In: Animal Migration, Navigation and Homing, Schmidt-Koenig and Keeton (eds.), pp. 143-151, Springer, New York.

Walker, M. M., Diebel, C. E., Haugh, C. V., Pankhurst, P. M., Montgomery, J. C. & Green, C. R. (1997) Structure and function of the vertebrate magnetic sense. Nature 390, 371-376.

Wallraff, H.G. (1988) Olfactory deprivation in pigeons: examination of methods applied in homing experiments. Comparative Biochemistry and Physiology A - Molecular and Integrative Physiology. 89, 621-629.

Voigt, C. C., Röleke, M., Marggraf, L., Pētersons, G. & Voigt-Heucke, L. (2017) Migratory bats respond to artificial green light with positive phototaxis. PLoS ONE 12, e0177748.

Wang, Y., Pan, Y., Parsons, S., Walker, M. & Zhang, S. (2007) Bats respond to polarity of a magnetic field. Proceedings of the Royal Society B 274, 2901-2905.

Wegner, R. E., Begall, S. & Burda, H. (2006) Magnetic compass in the cornea: local anaesthesia impairs orientation in a mammal. Journal of Experimental Biology 209, 4747-4750.

Wieser, B., Tichy, A. & Nell, B. (2013) Correlation between corneal sensitivity and quantity of reflex tearing in cows, horses, goats, sheep, dogs, cats, rabbits, and guinea pigs. Veterinary Ophthalmology 16, 251-262.

# GENERAL DISCUSSION

The overall goal of this thesis was to gain insight into whether migratory bats could be studied in a systematic way to address questions of animal navigation in free-ranging, wild mammals. I was able to study two species of migratory bats in two different but similar assays based on natural behaviour, i.e. takeoff and flight: Radio tracking after translocation, a technique borrowed from studies in non-migratory homing bats, and measuring the takeoff orientation of bats with a circular release box setup which is a novel experimental assay developed over the course of this thesis. In future studies of animal navigation both methods will from now on enable comparison with other taxa, most importantly with avian migrants.

### How do migratory bats calibrate their compass system?

The data from **chapter I** suggest that polarized light cues play no role in pipistrelle bats' compass calibration. To the contrary, the solar azimuth appears to be the main reference (**chapter III**). However, we also gain indirect insight about another mechanism at work from **chapter IV**: Upon capture, bats in that study did not see any solar information until release after translocation at night, 2 to 5 days later. This strongly suggests that bats do not necessarily need to calibrate their compass system on a daily basis which would provide them with a robust mechanism against overcast inhibiting view of the setting sun at dusk.

### Do migratory bats possess a magnetic sense?

In **chapter IV**, I presented the results of behavioural experiments conducted with adult Nathusius' bats (*Pipistrellus nathusii*), conceived to provide direct empirical evidence of a corneal sense for orientation by physical manipulation of the eyes' surface via reversible sensory impairment, i.e. topical anaesthesia of the cornea, to test the hypothesis that receptors for magnetosensation rest in corneal tissue layers. The observed effect on navigation cannot be disputed. The cornea carries multiple sensory receptors, i.e., mechano-nociceptors, polymodal nociceptors and cold receptors. However, it appears unlikely that any of these could make up a compass system in bats (Belmonte et al. 2003). Therefore data from this experiment strongly support the argument of Wegner et al. (2006). The unidirectional southerly vanishing of migratory now established assay of tracking migratory bats (**chapters I and IV**) provides a basis for further investigations of a putative magnetic sense in bats.

Yet, the findings from **chapter IV** call for replications not only in other study locations and/or migratory species but also in non-migratory bats, or even non-migratory populations

of otherwise migratory bats, to enable an evaluation of the generality of the observation and the presented hypothesis of a magnetite-based orientation mechanism. When performing such replication experiments (using a blinded protocol), it must be kept in mind that similar to the findings on the role of polarized light for orientation in bats (**chapter I**), seasonally effects have to be considered, i.e., seasonally dependent expression of enzymes as for example in birds' magneto-sensitive cryptochromes (Mouritsen 2018).

I believe, however, that for any human investigator magnetoreception research will always bear certain difficulties, because as a species, we lack the capacity to control a magnetic sense at will, similar to how we can control and focus on, e.g., vision or hearing, because we consciously sense relevant cues. Indeed, evidence is accumulating that humans have a magnetic sense (Wang et al. 2019), however, our capacity to use it could be analogue to how a cave fish uses its photoreceptive capacity for vision.

However, in case of bats, it is not surprising that the cornea was never associated with a function in bat orientation. Previous homing experiments investigating the role of eye-sight in orientation of bats severely affected the visual apparatus as a whole: Bats were either blinded by cauterization or the eyes were covered with caps that touched the cornea directly causing sensory irritation (and most likely pain) due to direction surface contact (Davis 1966).

However, a repetition of the study performed by Wegner et al. (2006) appears necessary as well. Mainly, to exclude the possibility of a false-positive finding that could have caused the observed disorientation in nesting mole rats. An increasing number of records about (potentially harmful) side-effects, e.g., prolonged iris anaesthesia is described for lidocaine, the anaesthetic of choice by Wegner and colleagues (Behndig & Linden 1998, Behndig 2007, Ahmed & Wieraszko 2015). According to the experience made in this work, (1) any such work should follow a blind study protocol, and (2) oxybuprocaine could be recommended, and would actually be an alternative to otherwise maximally invasive surgery for bilateral nerve sections of the trigeminal nerve (Mouritsen 2018).

Cornea-related research (in humans and domestic animals) is well-advanced due to its clinical relevance. This is because beyond corneal treatment itself, any other ocular surgery also affects the cornea. Despite this importance of cornea research for clinical applications, and although the cornea is a transparent and slim organ, the exact role of its neurons in processes of healing, scarring, health and immunology are not yet fully understood (Müller et al. 2003, Meek & Knupp 2015). Also, the involved molecular and cellular mechanisms, have

yet to be determined (Shaheen et al. 2014, Meek & Knupp 2015). Therefore any hypothesis about a corneal magnetoreception pathway cannot be easily refuted to date, and therefore should be studied further in bats.

# GENERAL REFERENCES

Ahmed, Z. & Wieraszko (2015) Pulsed magnetic stimulation modifies amplitude of action potentials modifies amplitude of action potentials in vitro via ionic channels-dependent mechanism. Bioelectromagnetics 36, 386-397.

Behndig, A. & Linden, C. (1998) Aqueous humor lidocaine concentrations in topical and intracameral anesthesia. Journal of Cataract Refraction Surgery 24, 1598-1601.

Behndig, A. (2007) Effects on pupil size and accommodation from topical lidocaine hydrochloride and tetracaine hydrochloride. Journal of Ocular Pharmacology and Therapeutics. 23, 591-598.

Berthold P. (2001) Bird migration: a general survey. 2nd edn. Oxford University Press, Oxford.

Beuerman, R.W. & Schimmelpfennig, B. (1980) Sensory denervation of the rabbit cornea affects epithelial properties. Exp Neurol. 69:196e201.

Burger, T.A., Lucová, M., Moritz, R.E., Oelschläger, H.H.A., Druga, R., Burda, H., Wiltschko, W., Wiltschko, R. & Němec, P. (2010) Changing and shielded magnetic fields suppress c-Fos expression in the navigation circuit: input from the magnetosensory system contributes to the internal representation of space in a subterranean rodent. Journal of the Royal Society Interface 7, 1275-1292.

Davis, R. (1966) Homing performance and homing ability in bats. Ecological Monographs 36, 201-234.

Greif, S., Borissov, I, Yovel,Y, Holland RA. 2014. A functional role of the sky's polarization pattern for orientation in the greater mouse-eared bat. *Nat Commun* **5**. doi: 10.1038/ncomms5488.

Haensel, J. (1994) Altersrekord einer in Italien verunglückten Rauhhautfledermaus (*Pipistrellus nathusii*) aus Deutschland. Nyctalus N.F. 5, 103.

Heyers, D., Zapka, M., Hoffmeister, M., Wild, J.M. & Mouritsen, H. (2010) Magnetic field changes activate the trigeminal brainstem complex in a migratory bird. Proceedings of the  National Academy of Sciences USA 107, 9394-9399.

Holland RA, Borissov I, Siemers B. (2010) A nocturnal mammal calibrates a magnetic compass by the sun. Proc. Nat. Acad. Sci. U.S.A. 107: 6941-6945.

Holland, R. A., Kirschvink, J. L., Doak, T. G. & Wikelski, M. (2008) Bats use magnetite to detect the Earth's magnetic field. PLoS ONE 3, e1676.

Holland RA, Thorup K, Vonhof M, Cochran W, Wikelski M. (2006) Bat orientation using Earth's magnetic field. Nature 444: 702.

Kramer, G. (1953) Wird die Sonnenhöhe bei der Heimfindeorientierung verwertet? J. Orn. 94, 201-219.

Kirschvink, J.L., Winklhofer, M. & Walker, M. (2010) Biophysics of magnetic orientation: strengthening the interface between theory and exeperimental design. Journal of the Royal Society Interface 7, S179-S191.

Meek, K.M. & Knupp, C. (2015) Corneal structure and transparency. Progress in Retinal and Eye Research 49, 1-16.

Mouritsen, H. (2018) Long-distance navigation and magnetoreception in migratory animals. Nature 558, 50-59.

Müller, L.J., Marfurt, C.F., Kruse, F. & Tervo, T.M.T. (2003) Corneal nerves: structure contents and function. Experimental Eye Research 76, 521-542.

Němec, P., Altmann, J., Marhold, S., Burda, H. & Oelschläger, H. H. A. (2001) Neuroanatomy of magnetoreception: the superior colliculus involved in magnetic orientation in a mammal. Science 294, 366-368.

Nishida, T., Chikama, T., Sawa, M. et al. (2012) Differential contributions of impaired corneal sensitivity and reduced tear secretion to corneal epithelial disorders. Japanese Journal of Ophthalmology 56, 20e5.

Orue, I., Marcano, L., Bender, P., García-Prieto, A. et al. (2018) Configuration of the magnetosome chain: a natural magnetic nanoarchitecture. Nanoscale, 10, 7407.

Rhozok, A. (2008) Orientation and navigation in vertebrates. Springer-Verlag Berlin Heidelberg, 166 p.

Shaw, J., Boyd, A., House, M., Woodward, R., Mathes, F., Cowin, G., Saunders, M. & Baer, B. (2015) Magnetic particle-mediated magnetoreception. Journal of the Royal Society Interface 12, 20150499.

Tsoar, A., Nathan, R., Bartan, Y., Vyssotski, A. Dell'Omo, G. & Ulanovsky, N. (2008) Large-scale navigational map in a mammal. Proceedings of the National Academy of Sciences 108, E718-E724.

Wallraff H.G. (2005) Avian navigation: pigeon homing as a paradigm. Springer-Verlag,

Wang, C.X., Hilburn, I.A., Wu, D.-A., Mizuhara, Y., Cousté, C.P. et al. (2019) Transduction of the geomagnetic field as evidenced from alpha-band activity in the human brain. eNEURO.0483-18.2019.

Wiltschko R., Wiltschko W. (2003) Avian navigation: from historical to modern concepts. Anim. Behav. 65: 257-272.

Wegner, R. E., Begall, S. & Burda, H. (2006) Magnetic compass in the cornea: local anaesthesia impairs orientation in a mammal. Journal of Experimental Biology 209, 4747-4750.

Wu, Le-Q. & Dickman, J.D. (2012) Neural correlates of a magnetic sense. Science 336, 1054-1057.

# CURRICULUM VITAE

The curriculum vitae is not part of the online version due to reasons of data protection.

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

# APPENDICES

## Appendix Chapter I

**Appendix 1.1** | Electronic supplementary material and methods.

*Capture of animals, schedule of treatment and experimental box description*

We caught bats using a funnel trap, also known as Heligoland trap, with entrance dimensions of 65 m in width and 13 m in height. The trap is open towards the North and thus enables catching bats or birds migrating southwards along the coastline. Days chosen for experimental treatment were 22, 24, 25, 28, 29 of August and 1 September, when the weather was favourable and bat migration was expected to occur at the coastline. We tested 24 females ($n_{PN} = 14$, $n_{PS} = 10$) and 16 males ($n_{PN} = 6$, $n_{PS} = 10$).

The experimental boxes consist of two card boxes each. The inner box has large meshed windows (keeping box). The outer box (polarization box) has windows made out of filters (1) a polarizing filter on the inside and (2) a depolarizing filter on the outside. A detailed description of the dimensions of the boxes and properties of the filter used for construction is given in Greif et al. (2014).

*Release procedure*

Prior to any release, an EM3+ bat detector was used to control for other bats at the site of release. According to the manufacturer (Wildlife Acoustics, Inc., Maynard, United States) the estimated detection radius for *P. nathusii* call frequencies ranges from 18 to 19 m. One bat was released at a time to continuously record its movement and to avoid interference with previously released conspecifics. The distance for radio transmission was 3.5 km. Since releases took place successively, evaluation of wind direction and estimation of cloud cover as well as specific notes were taken prior to each bat release. Maximum wind speed accepted for release was a moderate breeze (breezes: calm = 0 m/s; light = 1-3 m/s; gentle = 4-6 m/s; moderate = max. 8 m/s).

In Figure S1 we show potential outcomes for vanishing directions integrating species specific information.

**Figure S1** | Hypothetical mean vectors for vanishing bearings of bats translocated to the release site (asterisk) in a presumed unfamiliar area 11 km east of the coastal migration corridor. The natural coastline, where bats were caught and treated (+), follows the line of longitude. North (0°) is the top of the circular plots. Arrows depict the vectors (grey) if animals return to the capture site, (white) if animals would depart in the species specific southwesterly migratory direction for autumn movements in Europe and (black) if animals continue migratory flights in parallel to the coastal migration corridor.

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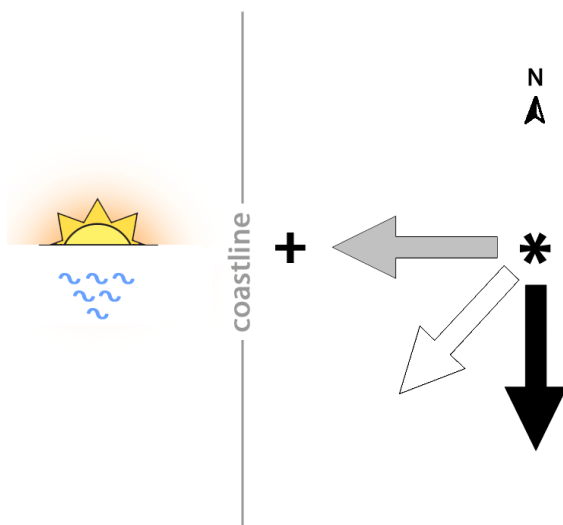
**Appendix 1.2 |** Raw data of bat releases including subjects individual information, treatment and release circumstances, radio telemetry data and bat activity. (*Online access via* http://dx.doi.org/10.1098/rsbl.2015.0525)

| treatment | sex | animal ID (ring) | body mass before feeding | body mass when released | tag type | capture date | Treatment site | | | | | | | Release site | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | date of treatment | treatment start | sunset | treatment time stop | wind speed (m/s) | wind direction | cloud cover (%) | lunar phase | fraction of Moon illuminated midnight | time of release | time (min) perching/foraging | time to vanish (min) | time vanished | vanishing bearings | proportion of time of radio sign in cardinal direction before vanishing (%) | cardinal directions | sunrise | wind speed (m/s) | wind direction | cloud cover (%) |
| PS | male | SA0144 | 7.2 | 8.3 | BioTrack | 2014-08-13 | 2014-08-22 | 20:27:00 | 20:57:00 | 22:30:00 | 7 | SW | 50 | waning | 0.07 | 01:15:00 | 0 | 4 | 01:19:00 | 150 | 100 | SSE | 06:21:00 | 7 | SSE | 10 |
| PS | fem | SA0139 | 7.3 | 7.4 | Holohil | 2014-08-13 | 2014-08-22 | 20:27:00 | 20:57:00 | 22:30:00 | 7 | SW | 50 | waning | 0.07 | 01:34:00 | 15 | 26 | 02:00:00 | 226 | 58 | SW | 06:21:00 | 7 | SSE | 10 |
| PN | fem | SA0124 | 7.2 | 8.0 | BioTrack | 2014-08-13 | 2014-08-22 | 20:27:00 | 20:57:00 | 22:30:00 | 7 | SW | 50 | waning | 0.07 | 02:44:00 | 0 | 5 | 02:49:00 | 118 | 100 | ESE | 06:21:00 | 7 | SSE | 10 |
| PS | fem | SA0158 | 6.9 | 7.7 | BioTrack | 2014-08-13 | 2014-08-22 | 20:27:00 | 20:57:00 | 22:30:00 | 7 | SW | 50 | waning | 0.07 | 03:05:00 | 0 | 5 | 03:10:00 | 158 | 100 | SSE | 06:21:00 | 7 | SSE | 10 |
| PN | male | SA0136 | 6.8 | 6.9 | Holohil | 2014-08-13 | 2014-08-22 | 20:27:00 | 20:57:00 | 22:30:00 | 7 | SW | 50 | waning | 0.07 | 03:30:00 | 0 | 3 | 03:33:00 | 170 | 100 | S | 06:21:00 | 7 | SSE | 10 |
| PS | fem | SA0113 | 7.0 | 7.4 | BioTrack | 2014-08-13 | 2014-08-22 | 20:27:00 | 20:57:00 | 22:30:00 | 7 | SW | 50 | waning | 0.07 | 04:00:00 | 15 | 28 | 04:28:00 | 93 | 82 | E | 06:21:00 | 7 | SSE | 10 |
| PN | fem | SA0156 | 6.8 | 7.7 | BioTrack | 2014-08-13 | 2014-08-22 | 20:27:00 | 20:57:00 | 22:30:00 | 7 | SW | 50 | waning | 0.07 | 04:55:00 | 5 | 13 | 05:08:00 | 196 | 100 | SSW | 06:21:00 | 7 | SSE | 10 |
| PS | fem | SA0153 | 6.6 | 7.1 | BioTrack | 2014-08-13 | 2014-08-24 | 20:07:00 | 20:52:00 | 22:20:00 | 2 | WNW | 50 | waning | 0.01 | 02:00:00 | 26 | 36 | 02:36:00 | 228 | 28 | SW | 06:25:00 | 2 | WNW | 80 |
| PS | male | SA0157 | 6.8 | 7.9 | BioTrack | 2014-08-13 | 2014-08-24 | 20:07:00 | 20:52:00 | 22:20:00 | 2 | WNW | 50 | waning | 0.01 | 03:00:00 | 0 | 16 | 02:56:00 | 260 | 100 | W | 06:25:00 | 2 | WNW | 80 |
| PN | fem | SA0112 | 7.6 | 8.2 | BioTrack | 2014-08-13 | 2014-08-24 | 20:07:00 | 20:52:00 | 22:20:00 | 2 | WNW | 50 | waning | 0.01 | 04:00:00 | 0 | 8 | 03:08:00 | 252 | 100 | WSW | 06:25:00 | 2 | WNW | 80 |
| PS | fem | SA0107 | 7.1 | 8.0 | Holohil | 2014-08-13 | 2014-08-24 | 20:07:00 | 20:52:00 | 22:20:00 | 2 | WNW | 50 | waning | 0.01 | 05:00:00 | 0 | 10 | 03:38:00 | 244 | 100 | WSW | 06:25:00 | 2 | WNW | 80 |
| PN | fem | SA0149 | 6.1 | 7.3 | BioTrack | 2014-08-13 | 2014-08-24 | 20:07:00 | 20:52:00 | 22:20:00 | 2 | WNW | 50 | waning | 0.01 | 06:00:00 | 0 | 14 | 04:14:00 | 267 | 100 | W | 06:25:00 | 2 | WNW | 80 |
| PN | fem | SA0150 | 6.5 | 7.9 | Holohil | 2014-08-13 | 2014-08-24 | 20:07:00 | 20:52:00 | 22:20:00 | 2 | WNW | 50 | waning | 0.01 | 07:00:00 | 0 | 5 | 04:30:00 | 244 | 100 | WSW | 06:25:00 | 2 | WNW | 80 |
| PS | fem | SA0131 | 6.5 | 7.5 | BioTrack | 2014-08-13 | 2014-08-24 | 20:07:00 | 20:52:00 | 22:20:00 | 2 | WNW | 50 | waning | 0.01 | 08:00:00 | 0 | 12 | 05:00:00 | 125 | 100 | SE | 06:25:00 | 2 | WNW | 80 |
| PS | fem | SA0126 | 7.1 | 7.5 | Holohil | 2014-08-13 | 2014-08-25 | 20:05:00 | 20:50:00 | 22:18:00 | 8 | W | 60 | New moon | 0.00 | 09:00:00 | 0 | 15 | 01:19:00 | 228 | 67 | SW | 06:27:00 | 5-8 | WSW | 50 |
| PN | fem | SA0146 | 6.5 | 7.3 | Holohil | 2014-08-13 | 2014-08-25 | 20:05:00 | 20:50:00 | 22:18:00 | 8 | W | 60 | New moon | 0.00 | 10:00:00 | 0 | 17 | 01:57:00 | 208 | 100 | SSW | 06:27:00 | 5-8 | WSW | 50 |
| PS | male | SA0101 | 6.7 | 7.0 | Holohil | 2014-08-13 | 2014-08-25 | 20:05:00 | 20:50:00 | 22:18:00 | 8 | W | 60 | New moon | 0.00 | 11:00:00 | 0 | 19 | 02:49:00 | 145 | 27 | SE | 06:27:00 | 5-8 | WSW | 50 |
| PS | fem | SA0114 | 6.7 | 6.9 | Holohil | 2014-08-13 | 2014-08-25 | 20:05:00 | 20:50:00 | 22:18:00 | 8 | W | 60 | New moon | 0.00 | 12:00:00 | 0 | 12 | 03:17:00 | 206 | 100 | SSW | 06:27:00 | 5-8 | WSW | 50 |
| PS | male | SA0104 | 7.0 | 7.3 | Holohil | 2014-08-13 | 2014-08-25 | 20:05:00 | 20:50:00 | 22:18:00 | 8 | W | 60 | New moon | 0.00 | 13:00:00 | 0 | 8 | 03:53:00 | 250 | 100 | WSW | 06:27:00 | 5-8 | WSW | 50 |
| PN | fem | SA0105 | 7.6 | 7.9 | Holohil | 2014-08-13 | 2014-08-25 | 20:05:00 | 20:50:00 | 22:18:00 | 8 | W | 60 | New moon | 0.00 | 14:00:00 | 15 | 23 | 04:22:00 | 258 | 100 | WSW | 06:27:00 | 5-8 | WSW | 50 |
| PN | male | SA0116 | 7.2 | 7.3 | Holohil | 2014-08-13 | 2014-08-25 | 20:05:00 | 20:50:00 | 22:18:00 | 8 | W | 60 | New moon | 0.00 | 15:00:00 | 15 | 23 | 04:48:00 | 70 | 35 | ENE | 06:27:00 | 5-8 | WSW | 50 |
| PN | male | SA0143 | 7.0 | 7.8 | BioTrack | 2014-08-13 | 2014-08-28 | 19:57:00 | 20:42:00 | 22:12:00 | 4 | NW | 40 | waxing | 0.10 | 16:00:00 | 0 | 10 | 02:07:00 | 204 | 100 | SSW | 06:33:00 | 2 | WNW | 5 |
| PN | fem | SA0122 | 7.1 | 7.8 | BioTrack | 2014-08-13 | 2014-08-28 | 19:57:00 | 20:42:00 | 22:12:00 | 4 | NW | 40 | waxing | 0.10 | 17:00:00 | 0 | 6 | 02:17:00 | 243 | 100 | WNW | 06:33:00 | 2 | WNW | 5 |
| PN | fem | SA0106 | 6.9 | 7.9 | Holohil | 2014-08-13 | 2014-08-28 | 19:57:00 | 20:42:00 | 22:12:00 | 4 | NW | 40 | waxing | 0.10 | 18:00:00 | 0 | 19 | 00:21:00 | 124 | 21 | SE | 06:33:00 | 2 | WNW | 5 |
| PS | male | SA0127 | 6.7 | 7.7 | Holohil | 2014-08-13 | 2014-08-28 | 19:57:00 | 20:42:00 | 22:12:00 | 4 | NW | 40 | waxing | 0.10 | 19:00:00 | 0 | 30 | 01:11:00 | 125 | 100 | SE | 06:33:00 | 2 | WNW | 0 |
| PN | fem | SA0111 | 7.2 | 8.1 | Holohil | 2014-08-13 | 2014-08-28 | 19:57:00 | 20:42:00 | 22:12:00 | 4 | NW | 40 | waxing | 0.10 | 20:00:00 | 0 | 12 | 02:43:00 | 199 | 75 | SSW | 06:33:00 | 2 | WNW | 5 |
| PN | fem | SA0151 | 6.7 | 7.4 | Holohil | 2014-08-13 | 2014-08-28 | 19:57:00 | 20:42:00 | 22:12:00 | 4 | NW | 40 | waxing | 0.10 | 21:00:00 | 0 | 31 | 00:56:00 | 79 | 35 | E | 06:33:00 | 2 | WNW | 0 |
| PS | fem | SA0160 | 6.5 | 7.2 | Holohil | 2014-08-13 | 2014-08-28 | 19:57:00 | 20:42:00 | 22:12:00 | 4 | NW | 40 | waxing | 0.10 | 22:00:00 | 0 | 16 | 01:36:00 | 190 | 100 | S | 06:33:00 | 2 | WNW | 0 |
| PN | fem | SA0108 | 6.3 | 7.1 | Holohil | 2014-08-13 | 2014-08-28 | 19:57:00 | 20:42:00 | 22:12:00 | 4 | NW | 40 | waxing | 0.10 | 23:00:00 | 0 | 12 | 01:54:00 | 199 | 100 | SSW | 06:33:00 | 2 | WNW | 0 |
| PN | fem | SA0359 | 7.3 | 8.1 | Holohil | 2014-08-22 | 2014-08-29 | 19:54:00 | 20:39:00 | 22:09:00 | 3 | NNW | 15 | waxing | 0.17 | 00:00:00 | 0 | 22 | 03:46:00 | 302 | 100 | WNW | 06:35:00 | 1 | W | 0 |
| PN | male | SA0363 | 7.0 | 7.5 | Holohil | 2014-08-22 | 2014-08-29 | 19:54:00 | 20:39:00 | 22:09:00 | 3 | NNW | 15 | waxing | 0.17 | 01:00:00 | 0 | 28 | 02:23:00 | 69 | 100 | ENE | 06:35:00 | 0 | - | 0 |
| PS | male | SA0154 | 6.6 | 7.3 | Holohil | 2014-08-13 | 2014-08-29 | 19:54:00 | 20:39:00 | 22:09:00 | 3 | NNW | 15 | waxing | 0.17 | 02:00:00 | 0 | 19 | 03:17:00 | 138 | 42 | SE | 06:35:00 | 1 | W | 0 |
| PN | male | SA0135 | 6.1 | 6.7 | Holohil | 2014-08-13 | 2014-08-29 | 19:54:00 | 20:39:00 | 22:09:00 | 3 | NNW | 15 | waxing | 0.17 | 03:00:00 | 10 | 24 | 00:52:00 | 212 | 50 | SSW | 06:35:00 | 0 | - | 0 |
| PS | male | SA0109 | 7.0 | 7.9 | Holohil | 2014-08-13 | 2014-08-29 | 19:54:00 | 20:39:00 | 22:09:00 | 3 | NNW | 15 | waxing | 0.17 | 04:00:00 | 0 | 18 | 01:18:00 | 148 | 100 | SSE | 06:35:00 | 0 | - | 0 |
| PS | male | SA0130 | 6.9 | 7.5 | Holohil | 2014-08-13 | 2014-08-29 | 19:54:00 | 20:39:00 | 22:09:00 | 3 | NNW | 15 | waxing | 0.17 | 05:00:00 | 0 | 17 | 02:50:00 | 149 | 100 | SSE | 06:35:00 | 1 | W | 0 |
| PS | male | SA0152 | 6.8 | 7.5 | Holohil | 2014-08-13 | 2014-08-29 | 19:54:00 | 20:39:00 | 22:09:00 | 3 | NNW | 15 | waxing | 0.17 | 06:00:00 | 0 | 23 | 01:47:00 | 170 | 100 | S | 06:35:00 | 0 | - | 0 |
| PS | fem | SA0626 | 8.0 | 8.9 | BioTrack | 2014-08-22 | 2014-09-01 | 19:46:00 | 20:31:00 | 22:01:00 | 2 | ENE | 50 | waxing | 0.44 | 07:00:00 | 10 | 22 | 02:57:00 | 301 | 69 | WNW | 06:41:00 | 2 | NE | 0 |
| PN | fem | SA0614 | 8.6 | 9.3 | Holohil | 2014-08-22 | 2014-09-01 | 19:46:00 | 20:31:00 | 22:01:00 | 2 | ENE | 50 | waxing | 0.44 | 08:00:00 | 0 | 20 | 01:32:00 | 200 | 100 | SSW | 06:41:00 | 2 | NE | 0 |
| PS | male | SA0601 | 7.6 | 8.4 | Holohil | 2014-08-22 | 2014-09-01 | 19:46:00 | 20:31:00 | 22:01:00 | 2 | ENE | 50 | waxing | 0.44 | 09:00:00 | 0 | 21 | 00:46:00 | 196 | 71 | SSW | 06:41:00 | 2 | NE | 0 |
| PN | male | SA0611 | 7.3 | 8.2 | BioTrack | 2014-08-22 | 2014-09-01 | 19:46:00 | 20:31:00 | 22:01:00 | 2 | ENE | 50 | waxing | 0.44 | 10:00:00 | 0 | 20 | 01:10:00 | 184 | 100 | S | 06:41:00 | 2 | NE | 0 |

# Appendix Chapter III

**Appendix 3.1** | Raw data of bat releases including subjects individual information, treatment and release circumstances. (*Online access via http://dx.doi.org/10.1016/j.cub.2019.03.002*)

| treatment | age | sex | date of treatment | sunset time | release site date of experimental release | time of release | takeoff orientation | observer position | temperature at release site (°C) | wind speed (m/s) | wind direction |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | adult | male | 2017-08-16 | 21:10 | 2017-08-16 | 23:19 | 310 | 160 | 19.2 | 0 | - |
| C | adult | male | 2017-08-16 | 21:10 | 2017-08-16 | 23:45 | 230 | 240 | 19.2 | 0 | - |
| M | adult | male | 2017-08-16 | 21:10 | 2017-08-17 | 00:20 | 230 | 120 | 15.5 | 0 | - |
| M | adult | fem | 2017-08-16 | 21:10 | 2017-08-17 | 00:45 | 140 | 20 | 12.9 | 0 | - |
| C | adult | fem | 2017-08-16 | 21:10 | 2017-08-17 | 00:45 | 240 | 270 | 14.8 | 0.2 | NW |
| M | adult | male | 2017-08-16 | 21:10 | 2017-08-17 | 00:50 | 60 | 180 | 15.5 | 0 | - |
| M | adult | fem | 2017-08-16 | 21:10 | 2017-08-17 | 01:16 | 60 | 280 | 14.7 | 0 | - |
| M | adult | fem | 2017-08-16 | 21:10 | 2017-08-17 | 01:21 | 50 | 270 | 14.8 | 0.2 | N |
| M | adult | fem | 2017-08-17 | 21:08 | 2017-08-17 | 23:04 | 40 | 250 | 14.9 | 0 | - |
| M | adult | male | 2017-08-18 | 21:05 | 2017-08-18 | 23:31 | 80 | 80 | 18.8 | 0 | - |
| M | subad | male | 2017-08-18 | 21:05 | 2017-08-18 | 23:43 | 295 | 100 | 18.8 | 0 | - |
| M | subad | fem | 2017-08-18 | 21:05 | 2017-08-19 | 00:02 | 130 | 290 | 18.8 | 0 | - |
| M | adult | fem | 2017-08-18 | 21:05 | 2017-08-19 | 00:16 | 110 | 330 | 15.4 | 0 | - |
| M | adult | fem | 2017-08-18 | 21:05 | 2017-08-19 | 00:46 | 90 | 160 | 15.0 | 0 | - |
| C | subad | fem | 2017-08-18 | 21:05 | 2017-08-19 | 01:04 | 270 | 250 | 14.8 | 0.1 | SE |
| C | adult | fem | 2017-08-18 | 21:05 | 2017-08-19 | 01:16 | 210 | 100 | 14.8 | 0.1 | S |
| M | subad | fem | 2017-08-18 | 21:05 | 2017-08-19 | 01:30 | 215 | 290 | 14.8 | 0.1 | SE |
| C | subad | fem | 2017-08-18 | 21:05 | 2017-08-19 | 01:40 | 170 | 20 | 15.7 | 0 | - |
| C | subad | male | 2017-08-18 | 21:05 | 2017-08-19 | 02:03 | 240 | 100 | 15.7 | 0 | - |
| C | adult | fem | 2017-08-18 | 21:05 | 2017-08-19 | 02:19 | 145 | 360 | 16.2 | 0.1 | S |
| C | subad | male | 2017-08-18 | 21:05 | 2017-08-19 | 02:39 | 350 | 200 | 16.2 | 0.1 | S |
| C | subad | male | 2017-08-19 | 21:03 | 2017-08-19 | 23:56 | 230 | 200 | 17.4 | 0.4 | W |
| C | subad | fem | 2017-08-19 | 21:03 | 2017-08-20 | 00:23 | 210 | 360 | 17.4 | 0.4 | SW |
| M | subad | - | 2017-08-19 | 21:03 | 2017-08-20 | 00:31 | 60 | n/a | 17.4 | 0 | - |
| C | adult | fem | 2017-08-20 | 21:00 | 2017-08-21 | 00:35 | 215 | 100 | 17.4 | 0 | - |
| M | adult | fem | 2017-08-20 | 21:00 | 2017-08-21 | 01:33 | 270 | 200 | 13.9 | 0 | - |
| C | subad | male | 2017-08-20 | 21:00 | 2017-08-21 | 01:48 | 50 | n/a | 13.4 | 0 | - |
| C | subad | fem | 2017-08-20 | 21:00 | 2017-08-21 | 02:00 | 340 | n/a | 13.3 | 0 | - |
| M | subad | fem | 2017-08-20 | 21:00 | 2017-08-21 | 02:14 | 270 | 290 | 13.0 | 0 | - |
| M | subad | fem | 2017-08-20 | 21:00 | 2017-08-21 | 02:33 | 320 | 100 | 13.0 | 0 | - |
| M | adult | fem | 2017-08-22 | 20:55 | 2017-08-22 | 22:55 | 90 | 270 | 14.2 | 0 | - |
| M | adult | male | 2017-08-22 | 20:55 | 2017-08-22 | 23:08 | 210 | 190 | 14.2 | 0 | - |
| C | adult | fem | 2017-08-22 | 20:55 | 2017-08-22 | 23:21 | 270 | 100 | 14.2 | 0 | - |
| M | subad | fem | 2017-08-22 | 20:55 | 2017-08-22 | 23:48 | 210 | 270 | 12.0 | 0 | - |
| M | subad | fem | 2017-08-22 | 20:55 | 2017-08-23 | 00:17 | 325 | 90 | 7.3 | 0 | - |
| M | subad | male | 2017-08-22 | 20:55 | 2017-08-23 | 00:38 | 170 | 270 | 7.3 | 0 | - |
| C | subad | - | 2017-08-22 | 20:55 | 2017-08-23 | 00:54 | 5 | 180 | 6.9 | 0 | - |
| C | subad | male | 2017-08-22 | 20:55 | 2017-08-23 | 01:19 | 300 | 330 | 6.9 | 0 | - |
| C | subad | male | 2017-08-22 | 20:55 | 2017-08-23 | 01:31 | 310 | 270 | 4.7 | 0 | - |
| C | subad | male | 2017-08-22 | 20:55 | 2017-08-23 | 01:45 | 45 | 100 | 4.7 | 0 | - |
| C | subad | fem | 2017-08-22 | 20:55 | 2017-08-23 | 01:53 | 205 | 360 | 4.7 | 0 | - |
| C | subad | male | 2017-08-23 | 20:53 | 2017-08-24 | 00:17 | 20 | 200 | 13.1 | 0.4 | N |
| C | adult | fem | 2017-08-23 | 20:53 | 2017-08-24 | 00:57 | 320 | 10 | 12.0 | 0.8 | NW |
| C | adult | fem | 2017-08-24 | 20:50 | 2017-08-24 | 23:16 | 310 | 270 | 14.8 | 0 | - |
| M | subad | male | 2017-08-24 | 20:50 | 2017-08-24 | 23:27 | 70 | 180 | 14.8 | 0 | - |
| M | adult | fem | 2017-08-24 | 20:50 | 2017-08-24 | 23:51 | 20 | 360 | 9.9 | 0 | - |
| C | adult | male | 2017-08-24 | 20:50 | 2017-08-25 | 00:11 | 330 | 180 | 9.9 | 0 | - |
| M | adult | male | 2017-08-25 | 20:48 | 2017-08-25 | 23:04 | 90 | 180 | 15.0 | 0 | - |
| M | adult | male | 2017-08-25 | 20:48 | 2017-08-25 | 23:18 | 45 | n/a | 15.0 | 0 | - |
| C | adult | male | 2017-08-25 | 20:48 | 2017-08-25 | 23:27 | 210 | n/a | 14.9 | 0 | - |

**Appendix 3.2** | Map of the study location in the Pape Nature Reserve, Latvia, indicating the sites of animal capture, experimental treatment and release after translocation. Arrows depict the mean group orientation of the adult control group (C) and the mirror group (M).

## DECLARATION OF AUTHORSHIP

I hereby declare that I prepared this thesis independently under the guidance of my supervisor. All direct or indirect sources used are given as references. All contributions of co-authors are acknowledged.

Berlin,                                                    Oliver Lindecke

 

 

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