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MAGNETORECEPTION AND NAVIGATION IN VERTEBRATES FROM QUANTUM MECHANICS TO NEUROSCIENCE AND BEHAVIOUR

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COVER ILLUSTRATION European robin (Erithacus rubecula) with soft pastel and its migratory route. Cryptochrome 4 model with the highlighted radiacal pair, interacting with the Earth's magnetic field. Everything is connected and integrated by the neuronal network acting in the background. Cover illustrated by Corinna Langebrake and Ilia Solov'yov.

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Editorial

Henrik Mouritsen*

Magnetoreception and navigation in vertebrates from quantum mechanics to neuroscience and behaviour

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Each year, billions of small songbirds, with "bird brains" often weighing a gram or less, leave their arctic and temperate breeding areas to overwinter in the tropics and subtropics (Mouritsen, 2018). For instance, northern wheatears (*Oenanthe oenanthe*) breeding in Alaska and wintering in Eastern Africa travel 30,000 km each year, alone and at night. At coral reefs around the globe, microscopic fish larvae are flushed out into the open ocean where they are at the mercy of currents for weeks. To survive, they must navigate back to the only survivable location, they can be sure exists, namely their natal reef. Thus, to persevere, many animals have developed exquisite navigation skills. The incredible accuracy of some of these navigation systems is illustrated by the fact that adult experienced migratory birds are able to navigate with an ultimate precision of centimetres after having migrated thousands of kilometers. How do they do it?

When these journeys are studied carefully in adult experienced animals, most of them consist of at least three different phases (Mouritsen, 2018):

A long-distance phase, during which global and regional reference cues provided by the Earth's magnetic field, the sun, and the stars, are predominantly used to get the animals into the approximate vicinity of the final goal; **a homing phase** during which compasses and gradient maps based on a variety of cues could help an animal to narrow-in on the final goal; and finally, **a pin-pointing-the-goal-phase**, where specific and familiar local, typically olfactory and/or visual landmarks, enable an animal to locate its nest or sleeping perch. It is therefore clear that no single cue, strategy, or mechanism is able to explain the extraordinary precision reached by adult migrants. Several senses, mechanisms and strategies need to work in concert (Figure 1).

While the general mechanisms underlying vision, olfaction and hearing are quite well understood, much less is currently known when it comes to magnetoreception. Therefore, one of the main goals of the DFG-funded Sonderforschungsbereich (SFB) 1372 is to understand magnetoreception. To understand how animals navigate, however, we also need to understand how navigation-relevant cues from all the senses are processed and integrated in the brain. Once the nervous system has integrated all available information, it will express these decisions as behavioural output. Thus, we need to study many different aspects if we want to understand magnetoreception and vertebrate navigation from the biophysics of primary sensor function via biochemistry, neuroanatomy and neurophysiology to the behaviour in the lab and global trajectories of freely moving animals. Furthermore, because we want to understand vertebrate navigation in general, we have specifically strived to choose the best suited species and systems available to answer each specific research question. We thus combine work on fish, bats, and birds.

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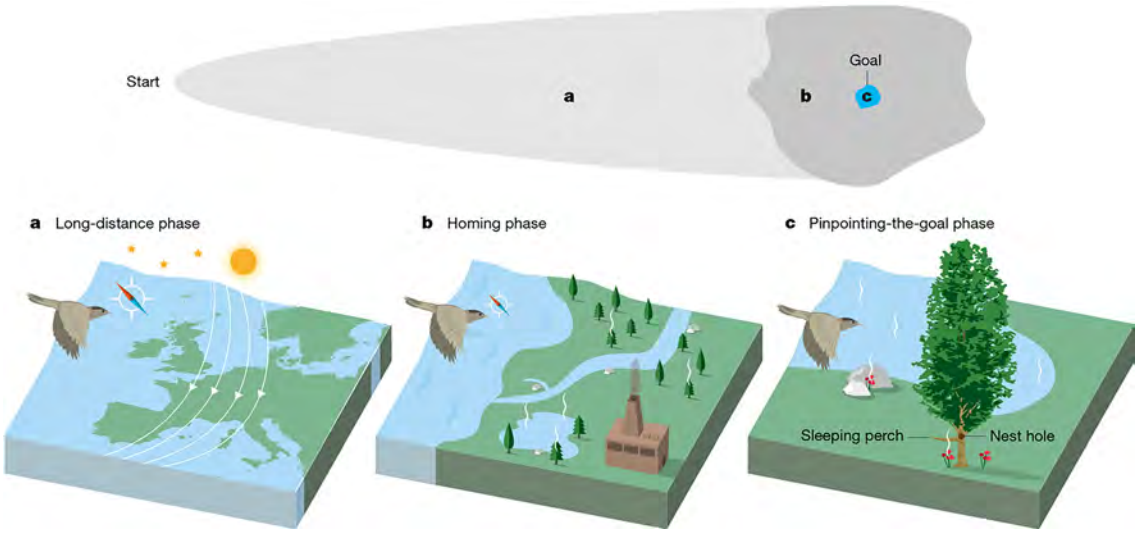

Figure 1: When long-distance navigational journeys back to a previously visited location are studied in detail, most of them consist of at least three different phases. For details see text (from Mouritsen, 2018).

This enormous diversity of approaches and scales is also reflected in the five papers written by SFB members for this issue of Neuroforum. We start out by introducing the natural phenomenon that we study in the first paper. In the next four papers, we will then go deep into the mechanisms, first at the quantum mechanical level, then at the biochemical level, and finally at the neurobiological level of the retina and the brain, respectively.

In the first paper, Spiecker et al. introduce the natural phenomenon of migration and navigation in birds and fish. They describe how long-distance navigation can be studied under meticulously controlled laboratory conditions, and how such studies have allowed us to understand how migratory birds and fish can use especially the sun, the stars, and the Earth's magnetic field for compass orientation. The authors then describe the recent discovery that human-made radio-frequency "electrosmog" in the environment can prevent night-migratory birds from using their magnetic compass, and they explain shortly why the effects of such fields strongly suggest that the magnetic compass of migratory birds must be based on a quantum mechanical, electron-spin-based, sensory mechanism. However, to return to a former breeding site, compass information is not sufficient, the animals must also have some kind of a map. Spiecker et al. give a few examples of recent work by SFB members showing how these maps can work in birds and fish. Since most juvenile birds and fish must navigate on their own without help or guidance from experienced conspecifics, the instructions of how and where to navigate to must be inherited. Even though a lot still remains to be discovered until we understand how this information is encoded in their genes, Spiecker et al. summarize the current state of the art on the genetic basis of migration and navigation in birds and fish.

In the second paper, Wong et al. introduce the reader to the fascinating world of quantum mechanics and why it likely plays a key role in understanding how the magnetic compass of night-migratory birds works at the primary sensory molecule level. Electron-spins in radical-pairs inside proteins called cryptochromes are sensitive to magnetic fields because of magnetic interactions between nuclear spins, electron spins, and the Earth's magnetic field. It is fascinating that by expressing cryptochromes in cell cultures and studying the purified proteins spectroscopically, we can "see" the electrons jump under different magnetic conditions and thereby get much closer to understanding how the magnetic compass works. Wang et al. also explain how and why key computational methods such as molecular dynamics and spin dynamics simulations provide a so-called "computational microscope" to study aspects of biophysics and biochemistry which are not yet approachable by experiments. Furthermore, the authors describe how theoretical predictions and experiments at the many scale levels studied within SFB 1372 can interact and mutually benefit from each other to better understand magnetoreception in animals. It is important to stress that, currently, biological sensory systems are not thought to be able to sense stimuli below the thermal noise limit (k_BT). However, if the radical-pair mechanism is responsible for magnetoreception in night-migratory

songbirds, it would indirectly move the lowest energy level limit that biological sensory systems can detect down by 6–7 orders of magnitude! This would be of fundamental importance.

Once the basic magnetic signal has been detected, this physical signal needs to be converted into a change in membrane potential. Therefore, the third paper by Bartölke et al. presents what we know about the biochemistry of cryptochromes in birds and summarizes some very recent SFB results demonstrating that Cryptochrome 4 from a night-migratory bird is magnetically sensitive and providing the first indications of which other proteins are potential interaction partners of bird cryptochromes. Based on these results, the authors make exciting suggestions on how the cryptochrome signal transduction cascade may be able to convert magnetic compass information into a change in membrane potential.

Because the cryptochrome-based magnetic compass requires light, it is likely to be embedded in the visual system. Therefore, after a change in membrane potential has taken place, the light-dependent magnetic signal needs to be processed within the retina. This is where the fourth paper by Seth et al. picks and summarizes what we know about the anatomy and neurophysiology of the retinal processing pathway of magnetic signals. The paper reports where the different cryptochrome types are located inside the retina of migratory birds, and what we know about the connectivity of the cryptochrome-containing cell types with other retinal neurons.

From the peripheral nervous system, the navigation relevant signals enter the brain for further processing. The fifth paper by Haase et al. summarizes our recent discoveries showing that several specific brain regions are involved in processing magnetic sensory information from the magnetic compass in the birds' eyes and from a magnetic map that seems to be associated with the ophthalmic branch of the trigeminal nerve. However, to understand how animals navigate, we also need to understand how and where navigation–relevant cues from all the senses are integrated in the brain. Haase et al., therefore, also summarize our knowledge about multisensory integration of navigation-relevant signals in the brain.

Once the nervous system has decided where the animal is and where it wants to go, it will express these decisions as behavioural output. Thus, we have come back up to the orientation behaviour summarized in the first paper by Spiecker et al. and the scientific concept loop of SFB 1372 is closed.

In the end, we want to take the opportunity to thank the German Neuroscience Society as well as the editorial board of Neuroforum for the invitation to compile a special issue. We hope that the five papers will convince you that, to achieve a holistic understanding of the fascinating subjects of magnetoreception and vertebrate navigation, a highly integrative and multidisciplinary approach is needed. We are very thankful that the DFG has given us this unique possibility to bring together scientists from quantum physics, biophysics, biochemistry, molecular biology, neuroscience, animal behaviour, genetics, and mathematical modelling with excellent funding and a 12-year perspective to solve some of the most intriguing mysteries related to how the magnetic sense of animals works and how exactly animals are able to find their way over thousands of kilometres across continents and oceans and back again.

Reference

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

Review article

Lisa Spiecker, Bo Leberecht, Corinna Langebrake, Malien Laurien, Shambhavi Rajendra Apte, Henrik Mouritsen, Gabriele Gerlach and Miriam Liedvogel*

Endless skies and open seas – how birds and fish navigate

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Abstract: Every year, billions of animals leave their home range and start seasonal migrations in order to find more favorable resources and to escape harsh environmental conditions. These round trips often span thousands of kilometers. To successfully navigate along their route, animals rely on various external references. While landmarks and celestial cues like stars or the sun are easy to imagine as guidance on these journeys, using the geomagnetic field for orientation is more elusive. The geomagnetic field is an omnipresent cue, which can be sensed and relied upon by many animals, even when visual cues are sparse. How magnetic fields can be perceived seems to vary between birds and fish. While birds seem to use a mechanism based on the quantum mechanical properties of electron spins, fish may have evolved a compass similar in its function to the technical devises developed by humans. How these mechanisms work precisely and how they are integrated are research questions addressed in SFB 1372.

Keywords: animal navigation; behavioral genomics; magnetoreception; orientation behavior; SFB 1372.

Zusammenfassung: Jedes Jahr verlassen Milliarden von Tieren ihre Heimat und beginnen saisonale Wanderungen, um optimale Ressourcen und Umweltbedingungen zu finden. Diese Reisen erstrecken sich oft über Tausende von Kilometern. Um auf ihrer Wanderung erfolgreich zu navigieren, sind Tiere auf externe Referenzen angewiesen. Während Anhaltspunkte wie Sterne oder die Sonne als Orientierungshilfe leicht vorstellbar sind, ist die Verwendung des Erdmagnetfelds schwerer nachzuvollziehen. Das Erdmagnetfeld ist allgegenwärtig und Tiere können es auch bei schlechten Sichtverhältnissen nutzen. Wie Magnetfelder wahrgenommen werden, scheint zwischen Vögeln und Fischen zu variieren. Während Vögel wahrscheinlich einen Mechanismus verwenden, der auf den quantenmechanischen Eigenschaften von Elektronenspins basiert, haben Fische möglicherweise einen Kompass entwickelt, der in seiner Funktion den vom Menschen entwickelten technischen Geräten ähnelt. Wie diese Mechanismen genau funktionieren wird im SFB 1372 erforscht.

Schlüsselwörter: Magnetrezeption; Orientierungsverhalten; SFB 1372; Tiernavigation; Verhaltensgenomik.

Migratory behavior has a strong heritable component and can be influenced by environmental factors. Little is known about the actual genes and regulatory pathways that shape these migratory traits. In the framework of our multidisciplinary SFB consortium, we are now able to approach these highly complex questions through complementary integration of scientists and state-of-the-art techniques from many different disciplines. In this article, we sketch the current state of knowledge within the fields of migration genomics and behavior and highlight key questions that we will approach within our SFB.

Migration in birds

Through the seemingly endless skies of our planet, birds migrate thousands of kilometers even crossing vast oceans and hostile deserts (Mouritsen 2018). Examples of

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impressive journeys include the arctic tern (*Sterna paradisaea*) that migrates up to 80.000 km each year, circling the globe with ease (Egevang et al. 2010), and the bar-tailed godwit (*Limosa lapponica*) that covers 10.000 km of nonstop flight in less than 8 days on its journey from Alaska to New Zealand (Gill et al. 2005). However, also common visitors in our gardens, such as the European robin (*Erithacus rubecula*) and the Eurasian blackcap (*Sylvia atricapilla*), perform exceptional migrations, facing different challenges. First, like most songbirds (passerines), they migrate at night, and second, they do this alone. That means that juveniles have to find their way without guidance through their parents' expertise (Merlin and Liedvogel 2019; Mouritsen 2018). How are all these birds able to find the right way to an area they have never been to before, sometimes across featureless oceans and in the gloomy night?

To investigate the mechanisms underlying a complex behavior like migration, it is of key importance to be able to precisely characterize and quantify the focal behavioral components (such as orientation). But how can this be done if a migratory journey covers thousands of kilometers? Here, we can utilize the fact that caged migratory birds are so eager to migrate that they become active at night during the migratory season and jump primarily towards their migratory direction (Kramer 1952). This phenomenon is called migratory restlessness and allows us to study migration under controlled laboratory conditions in circular orientation cages such as so-called Emlen funnels (Emlen and Emlen 1966) where birds leave scratch marks on the special paper coating the funnel walls that allow us to infer their intended migratory heading. When orientation-relevant cues are experimentally manipulated, the birds will adjust their jumping direction accordingly. This method allowed researchers to investigate different compass systems in migratory birds (Chernetsov 2016; Mouritsen 2018).

For example, birds can use the stars as a compass reference (Emlen 1975) and when young songbirds observe the rotating night sky for two weeks or more, they learn the location of the rotation center that corresponds to geographic North (Michalik et al. 2014). But, what do they do when the stars are not visible owing to overcast? Birds can sense another compass cue, the Earth's magnetic field, which is especially fascinating to humans, as we cannot perceive it.

Already in 1968, Wiltschko discovered that a night-migratory songbird possesses a magnetic compass (Wiltschko 1968). European robins were tested in orientation cages surrounded by a Helmholtz coil system that allowed controlled manipulation of the magnetic field conditions while keeping other parameters unchanged. Robins tested under local geomagnetic field conditions displayed the same directional preference as their wild conspecifics, and when magnetic North was turned, the birds rotated their orientation accordingly. A follow-up study demonstrated that the magnetic compass of robins is not based on field polarity like our compass, but on the inclination of the magnetic field lines, defined as the angle between the magnetic field lines and the Earth's surface that steepens as the bird move towards the poles (Wiltschko and Wiltschko 1972). This means that birds do not discriminate between North and South, but between equatorward and poleward.

A series of displacement and lesion studies discovered that night-migratory songbirds also possess a magnetic map sense that means that the individual bird knows something about its location with respect to a goal. That sense seems to be located in the trigeminal system of the birds' upper beak (Chernetsov et al. 2008; Haase et al. 2021; Kishkinev et al. 2013, 2015; Pakhomov et al. 2018). Birds that showed normal magnetic orientation behavior at the origin location were displaced 1000 km to the east either physically or virtually by testing them in the magnetic field conditions of the real displacement location 1000 km eastwards. In both cases, the birds compensated for the displacement by shifting their orientation towards their original goal. Birds with an ablated trigeminal nerve were unable to compensate for the physical or virtual displacement. Instead, they kept their original heading. It was thereby demonstrated that night-migratory songbirds can use magnetic information to locate their position relative to a goal and to navigate towards their goal.

The ability of night-migratory songbirds to perceive, orient, and navigate as per the magnetic field gives rise to the question of how they sense magnetic cues. Two prevailing magnetic sensing hypotheses exist: The first suggests that magnetite, small accumulations of iron oxide particles within the animal, are responsible for magnetoreception (Haase et al. 2021). The second hypothesis is called the radical-pair mechanism. It suggests that light absorption leads to the formation of radical pairs probably in the magnetically sensitive Cryptochrome 4 molecule (Xu et al. 2021) within the avian retina. This transient radical state can switch between two different quantum states (Wong et al. 2021) that differ in their chemical properties. The relative amount of these two states is affected by the direction of the Earth's magnetic field and by this could act as a chemical compass (Hore and Mouritsen 2016; Ritz et al. 2000).

Several studies reported effects of different light wavelengths on the orientation ability of night-migratory

songbirds. Such wavelength effects are expected for a light-dependent radical pair-based magnetic sense but are hard to explain by a magnetite-based mechanism (Hore and Mouritsen 2016). Furthermore, theoretical considerations predict that birds using a radical-pair mechanism should be disoriented by specific radio frequency fields, whereas a magnetite-based mechanism should be entirely unaffected (Hore and Mouritsen 2016; Ritz et al. 2000). Therefore, radio frequency exposures present a diagnostic test for the radical-pair mechanism.

A series of carefully controlled, double-blinded, behavioral tests revealed that man-made electromagnetic noise in the frequency range of 0.4–10 MHz can disrupt the magnetic compass orientation performance of night-migratory European robins (Engels et al. 2014). It was demonstrated that electromagnetic noise at much lower field intensities than the World Health Organization guideline limits emitted from buildings around the university testing site inhibited the bird's magnetic orientation capability. Once the wooden test buildings were shielded against this electromagnetic noise, the orientation performance of these birds was no longer impaired. An artificial reintroduction of the electromagnetic noise inside the shielded test buildings predictably disrupted the bird's orientation performance. Interestingly, the effects of anthropogenic electromagnetic noise seemed to be spatially limited because robins tested in a rural, less noisy area showed perfectly normal orientation behavior without any screening (Engels et al. 2014, Figure 1.1).

Man-made electromagnetic noise is widely distributed in our urban environment, but its intensity decreases with the distance to the source cubed (r^3). The important question thus arises, how severe do the radio frequencies affect bird orientation behavior in the wild, and does it have any serious influence on migratory bird conservation? To answer this question, SFB 1372 will investigate the effect of radio frequency magnetic fields on free-flying birds. Birds carrying radio tags will be subjected to radio frequencies that are known to have an inhibiting effect (Schwarze et al. 2016) and tracked along their migratory route.

Back in the laboratory, specific radio frequencies exposures can be used as a tool to assess different molecular and quantum biological properties of the radical-pair mechanism on the behavioral level. For example, how long does the magnetically sensitive radical-pair state exist? What is the chemical nature of the involved molecules? Both are important questions that are being addressed within SFB 1372.

For the first question, one has to consider that the transient radical state of the molecule needs to remain stable for the time of at least one entire radio frequency

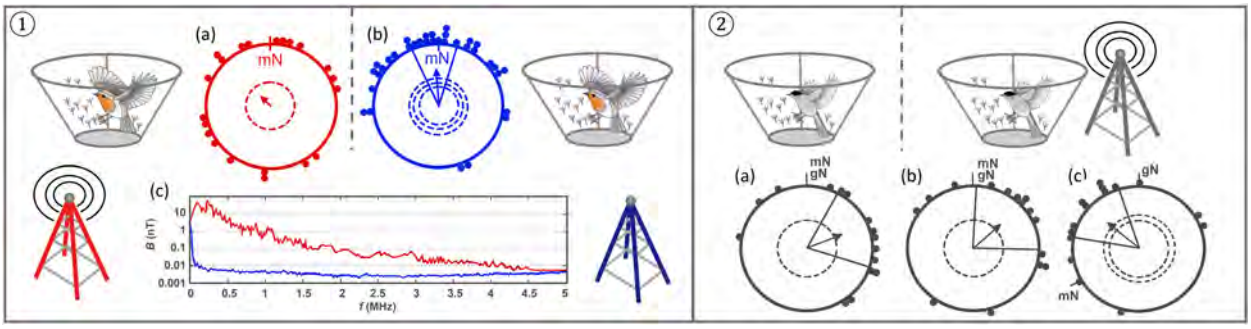

Figure 1: To study the orientation capabilities of night-migratory songbirds under controlled laboratory conditions, so-called Emlen funnels in which birds leave scratch marks in their intended migratory heading are used.
① In unshielded wooden test buildings, European robins were disoriented (a), but after shielding from radio-frequency electromagnetic noise, the birds oriented highly significantly towards North in spring (b). The antenna depiction color-coding should clarify the presence (red) and absence (blue) of electromagnetic noise. (c): The magnetic components ("B [nT]") of the anthropogenic electromagnetic noise in the huts before (red) and after (blue) shielding are shown, as a function of frequency (f). Modified after Engels et al. 2014.
② Eurasian Blackcaps show their normal spring heading towards North-east in a magnetic field equal to the geomagnetic field (a). When exposed to radio frequency noise from 0.1 to 100 kHz (indicated by antenna depiction), the birds group orientation is still heading Northeast (b). With the magnetic field rotated 120° counterclockwise and the radio frequencies on, the birds rotate their heading accordingly (c). Modified after Kobylkov et al. 2019. On the circular diagrams, each dot at the circle periphery indicates the mean heading of an individual bird in the given condition. The arrows show group mean vectors flanked by their 95% confidence interval limits for the mean heading (solid lines). The dashed circles indicate the minimum length of the group mean vector needed for significance according to the Rayleigh test (inner circle: $p = 0.05$; middle: $p = 0.01$; outer: $p = 0.001$). mN = magnetic North; gN = geographic North.

wave in order for such a wave to affect the radical-pair mechanism. Therefore, the lower the applied radio frequency (and thus the longer the wave period), the longer the lifetime of the magnetically sensitive radical-pair state is required in order to be affected by it. Following this logic, we exposed birds to radio frequencies up to 100 kHz (= 1/100,000 s = 10 µs wave period). Their magnetic orientation performance was unaffected (Kobylkov et al. 2019, Figure 1.2). Combined with earlier studies in which electromagnetic noise with a frequency of 50–450 kHz disrupted the magnetic compass orientation capabilities of night-migratory songbirds (Engels et al. 2014), we now have evidence that the magnetically sensitive radical pair probably persists between 2 and 10 µs.

For the second question, the diagnostic application of radio frequencies is used to investigate the organic properties of the two radical-forming molecules. Organic molecules usually contain several hydrogen and/or nitrogen atoms. In their most common naturally occurring isotopes, the nuclear spins of their uneven number of protons and/or neutrons generate strong internal magnetic fields at close distances (0.1 nm; Hore and Mouritsen 2016). Each of these "magnetic nuclei" doubles the number of possible energy level splittings. This will in turn affect radio frequencies that can disrupt the radical-pair mechanism because only radio frequencies corresponding to a present energy level splitting can disrupt the mechanism. Thus, the upper limit of the radio frequencies that affects the birds' magnetic orientation capabilities in behavioral experiments can provide important information about how many hydrogen and/or nitrogen atoms are in the immediate vicinity of the two radicals and thus gives hints to the chemical identity of the radicals (Hiscock et al. 2017; Hore and Mouritsen 2016).

While our understanding of the physical and behavioral mechanisms underlying the birds' ability to sense the magnetic field is improving, these mechanisms by themselves do not provide our young robin with a plan to find its way completely on its own. But, did the parents really provide no guidance? To answer this question, we can look at the migration behavior of cuckoos (*Cuculus canorus*) that never meet their parents. Young cuckoos are perfectly able to fly to their species-specific wintering quarter regardless of where their foster parents migrate to (Thorup et al. 2017). This natural example impressively shows that there must be another layer of information. This information is in their genes inherited from their parents (Liedvogel and Lundberg 2014).

The genetic basis of migratory traits, such as orientation, was demonstrated in selection and crossbreeding experiments in Eurasian blackcaps (Berthold 1999; Berthold et al. 1992). The blackcap exhibits different migratory strategies throughout its breeding range with respect to migratory distance, direction, and the propensity to migrate (Figure 2.1). Crossbreeding experiments between parents

with different migratory directions revealed an intermediate migratory orientation preference in the first generation offspring. A tracking study of blackcaps in the field was able to confirm these results in the wild across a natural migratory divide/hybrid zone (Delmore et al. 2020b). Migratory distance and propensity can also be altered by cross-breeding. These results convincingly demonstrated that migratory traits are under genetic control and probably inherited by few genes with major effect (Berthold 1999; Berthold et al. 1992).

Although a genetic basis of migratory behavior has been demonstrated through quantitative genetics approaches, the number and identity of the actual genes shaping the migratory phenotype (distance, direction, timing, propensity to migrate) and their regulation remain to be identified (Merlin and Liedvogel 2019). Rapidly developing sequencing techniques allow for genome-wide association studies that can identify genetic variation (such as single-nucleotide polymorphism (SNP); Figure 2.2) correlating with the migratory phenotype, and thus, candidate regions associated with migratory traits can be identified *de novo* (Delmore et al. 2016, 2020a; Lundberg et al. 2017). Genes involved in circadian rhythms, the nervous system, and cell signaling were associated with migration direction in Swainson's thrush (*Catharus ustulatus*; Delmore et al. 2016), whereas in the willow warbler (*Phylloscopus trochilus*), this trait might be controlled by inversion polymorphisms. In willow warblers, two distinct regions of the genome are completely reversed between two migratory phenotypes (Lundberg et al. 2017). Very recently, we could associate migratory propensity in blackcaps with variation in regulatory regions which might be associated with *de novo* identified candidate genes that could act as major regulators of migratory behavior (Delmore et al. 2020b).

Modulation of migratory behavior may not exclusively occur on the sequence level. Small changes in the sequence of regulatory regions may lead to large effects on the transcription level of genes (Figure 2.2). Thus, differential gene transcription approaches in focal tissues, such as the brain of migratory songbirds, and contrasting patterns between the migratory and nonmigratory season can also help to identify main regulatory pathways of migratory behavior and its neuronal organization (Boss et al. 2016; Frias-Soler et al. 2020; Johnston et al. 2016; Sharma et al. 2018). So far, however, studies struggle to identify the same key players but increasingly accumulate evidence for common pathways to be associated with

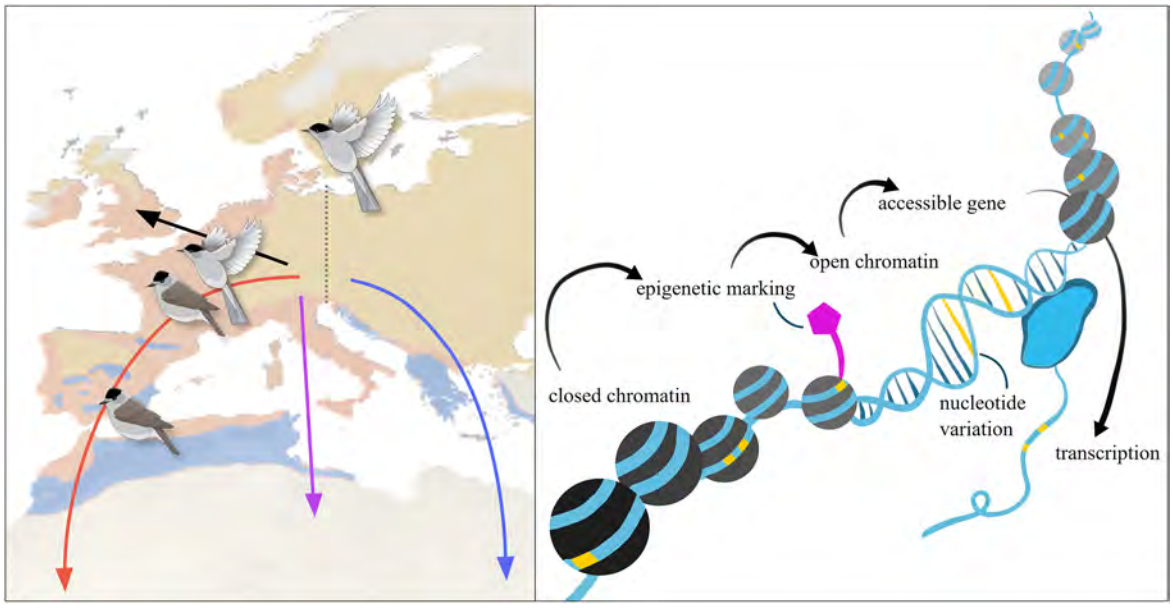

Figure 2: Several migratory traits including migratory direction and distance are genetically controlled.
① Migratory strategies of the Eurasian Blackcap. Blackcaps show highly variable migration patterns across their range regarding migration direction, distance, and propensity. By crossing South East (blue) with South West migrants (red) an intermediate phenotype was bred (purple). This demonstrated the genetic basis of migratory traits. Yellow = only summer, orange = year-round, blue = only winter. From Langebrake et al. 2021.
② Potential control mechanisms of migration and orientation behavior. Nucleotide variation (Single Nucleotide Polymorphism, SNP) can result in different functions of genes in migratory compared to resident phenotypes. The DNA is normally coiled up as chromatin, but epigenetic markings can open the structure and make genes accessible for transcription. Differences in the epigenetic marking pattern of phenotypes can lead to differentially expressed genes. Additionally, SNPs that are different between migrants and non-migrants in regulatory regions of the genome can also result in differential gene expression.

an upregulation of cellular energy demand, extensive neuronal remodeling, and neurogenesis during migration in the brain (Frias-Soler et al. 2020; Johnston et al. 2016; Sharma et al. 2018). Neurogenesis has been suggested to be associated with spatial learning and memory development, two crucial attributes for birds during migration, performing exceptional navigational tasks.

The transcription of genes does not solely depend on genetic constraints, but it is a dynamic process that is also influenced by environmental cues. One factor that might contribute to this environmental response by regulating transcription are epigenetic markings of the genome (Merlin and Liedvogel 2019). These markings are, for example, methylations or acetylations that can upregulate or downregulate the expression of their target genes (Figure 2.2). Epigenetic control mechanisms of migratory behavior are hardly studied in migratory birds yet; however, some data are available for other migratory systems such as the monarch butterfly. In this model species, a change in migratory direction for spring migration is a consequence of prolonged exposure to cold temperatures, representing the first evidence of an epigenetically reprogrammed sun compass in the brain of migratory animals (Guerra and Reppert 2013).

So, it is evident that the robin indeed was provided with some genetic guidance by its parents. But all in all, too little is known about the underlying genetics, transcriptomics, and epigenetics of migratory traits and how they result in the variable behavior we observe. Projects of SFB 1372 will address all three research fields to generate the most complete explanation for the complex question why the birds know when to leave, which direction to fly, and when to return.

Migration in fish

Long-distance migration is not limited to the endless skies, they also happen hidden from our view in the vast open oceans of our planet. Aquatic animals face different challenges compared to terrestrial animals when it comes to long-distance navigation with no prior migratory experience. Visual cues or landmarks are limited to the clarity of

water, and olfaction or auditory cues are limited to short-distance migration owing to attenuation and turbulences. Nonetheless, many fish species travel great distances back to their natal site. For instance, eels (*Anguilla anguilla*) start their life in the ocean and migrate several thousand kilometers to their freshwater feeding grounds, only to migrate back to their spawning grounds in the ocean (Righton et al. 2016). Another famous fish navigator, the salmon, hatches in freshwater river streams, then spends many years of its adult life in the open ocean, only to migrate back to its natal stream to spawn. Similar to the Eurasian blackcap, hybrid studies in the eel and salmon suggest that the direction of migration in fish has a genetic basis (Albert et al. 2006; Candy and Beacham 2000).

Magnetic orientation behavior has been demonstrated for several teleost fish species, and in consequence, they must be able to sense the Earth's magnetic field (Hart et al. 2012; Putman et al. 2013, 2014; Shcherbakov et al. 2005; Tesch, 1974). In one interesting study, Putman et al. (2013) showed that the salmon uses geomagnetic imprinting as a homing mechanism. The authors analyzed a 56-year fishery data set on the Fraser River sockeye salmon (*Oncorhynchus nerka*) that needs to swim along the northern or southern passageway around Vancouver Island in order to reach the Fraser River mouth. They could predict the proportion of salmon using each route by the drift of the geomagnetic field during that specific time period. A subsequent study suggested that an "inherited magnetic map" provides the juvenile Atlantic salmon (*Salmo salar*) with location information, which helps them to navigate (Putman et al. 2014).

Magnetic sensing abilities have also been indicated in conditioning experiments with the nonmigratory zebra fish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*). For instance, the zebra fish was trained to escape into another tank compartment upon magnetic field alterations (avoidance behavior). The fully automated experiment relied on negative reinforcement (weak electric impulses as punishment) (Figure 3). In another experiment, trouts were trained to receive food during a specific period of time when magnetic field alterations were applied (positive reinforcement). Both species showed significant learning effects, strongly suggesting that they can detect changes in the magnetic stimuli (Shcherbakov et al. 2005).

While it has been convincingly shown that fish can use magnetic fields to orient, the underlying molecular and

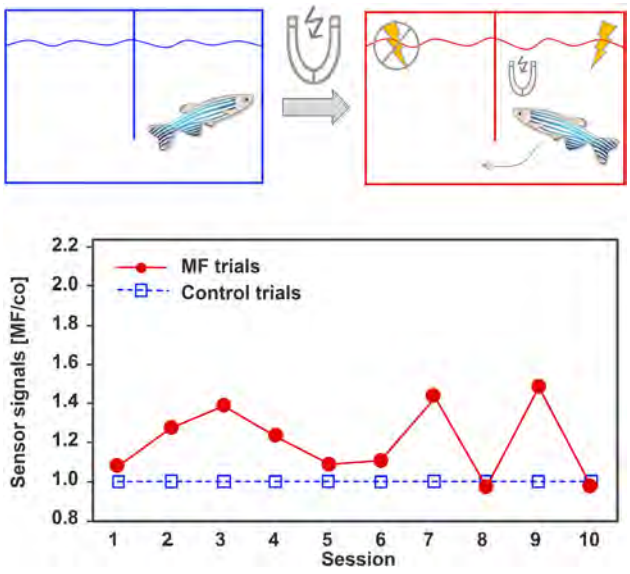

Figure 3: A magnetic field conditioning experiment in zebrafish. Within the experimental tank, the fish were trained to cross an infrared light barrier system that disables punishment and escape into another tank compartment upon magnetic field alterations. The graph shows relative response rates defined as the percentage of infrared sensor signals per magnetic field (MF) trial (or per control (Co) trial) during a training session with 10 trial pairs. Results were obtained using four fish trained as a group in 10 sessions of 10 trial pairs each. The fish are not drawn to scale. Modified after Shcherbakov et al. 2005.

cellular magnetoreception mechanism still remains unclear and debatable. In sharks and rays (elasmobranchs), it was suggested that the ampullae of Lorenzini, delicate sensory structures in their snouts that function as electroreceptors, can also be used as magnetic sensors (summarized in Kalmijn 1978). Bony fish do not have the ampullae of Lorenzini, so they must use a completely different sensory system to detect changes in the magnetic field. Magnetite has been found in a variety of different bony fish species (Formicki et al. 2019) but so far never in a consistent, independently reproduced, cellular location associated with the nervous system in a large number of conspecifics (Mouritsen 2018). A first transcriptomic approach focusing on brain tissue before and after exposure to a magnetic pulse identified 181 different candidate genes in the rainbow trout (Fitak et al. 2017), including genes coding for ferritin, which is believed to help with the biomineralization of magnetite (Hsu and Chan 2011). No significant differential expression was found in the trout's retina in a follow-up experiment (Fitak et al. 2018). Even if this study could not show any differential expression of genes that might be involved in the radical-pair mechanism, as it was shown in birds, it does not mean that a light-dependent magnetoreception can be ruled out as a magnetoreceptor in fish. Overall, relatively few studies have analyzed the molecular mechanism behind magnetoreception in fish, and scientists are still unsure about which proteins and genes are involved in their magnetic sense.

In addition to prominent long-distance migratory fish such as the eel and salmon, there are also a large number of short-distance movements in fish, which are just as interesting from a navigation perspective. Most coral reef fish hatch at a reef, but the larvae are subsequently swept into the open ocean and disperse up to 200 km (Bode et al. 2019). Mortality during this pelagic phase is assumed to be high, and dispersal was long considered to be a nondirectional event driven by currents or storms (Sale 1994), but more recent studies demonstrate that a significant number of juvenile coral reef fish return to their natal reefs (Robertson 2001). In fact, a recent genetic approach using microsatellite markers to characterize natal population destiny could assign up to 60% of settling juvenile fourline cardinalfish (*Ostorhinchus doederleini*) in a complex reef system to the adult population of the specific reef's population, suggesting natal homing capabilities (Gerlach et al. 2016). One theory, why coral reef fish larvae invest so much effort to return to their natal reef, is that the ocean is vast and unpredictable and the only suitable place to settle that the larvae know exists is their birthplace. But how do these tiny larvae orient themselves in the open ocean, an environment without any points of reference?

Atema et al. (2002) and Gerlach et al. (2007) showed that different species of settling coral reef fish larvae could differentiate between the olfactory cues of open ocean and reefs and also preferred the odor of their natal reef versus other nearby reefs. However, olfactory cues are only detectable within a few kilometers off the reef, depending on the currents. Within even shorter distances, reef sound could also guide larvae to a reef environment (Radford et al. 2008), but how do they navigate back to their natal reef from larger distances? In a study by SFB members, we could show that the juvenile fourline cardinalfish can also use a time-compensated sun compass to orient back towards their natal reef during the day (Mouritsen et al. 2013). In circular tanks with a clear view to the sky, fish oriented towards SE, opposing the main NW drift current present around their home reef (Figure 4a), and after the fish were clock-shifted for 6 h, they chose a NW direction (a 180-degree change fitting with the change in sun azimuth over 6 h at the testing location and time of year). Because *O. doederleini* larvae are presumed to be primarily night active, like recently shown in clownfish larvae (*Amphiprion ocellaris*) (Schalm et al. 2021), juvenile fourline cardinalfish were placed in circular

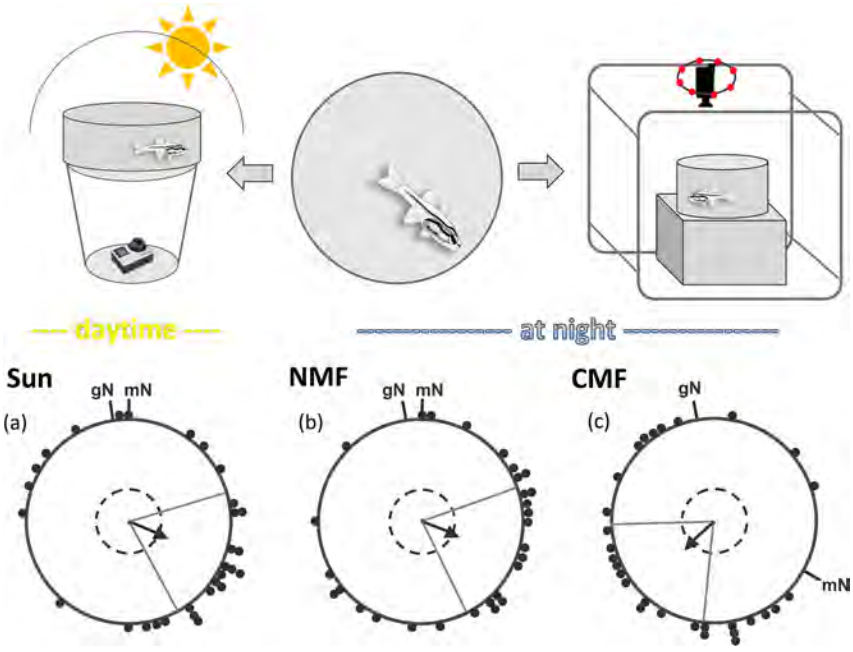

Figure 4: Spontaneous compass orientation of settlement-stage fourline cardinalfish in an orientation bowl. Juveniles were tested (a) under clear sunny skies during the day (Sun), and at night inside the 99% homogenous center of Helmholtz coils in the absence of any celestial cues (b) in the natural magnetic field (NMF). They oriented SE, thereby compensating the drift current around the test site. (c) When the magnetic field was rotated 120° clockwise (CMF), the fish rotated their orientation accordingly. Each dot indicates the mean orientation of one individual fish tested at least three times in the given condition. gN = geographic North, mN = magnetic North. The fish are not drawn to scale. Modified after Bottesch et al. 2016.

tanks inside Helmholtz coils during the night (Bottesch et al. 2016). In the unchanged, natural geomagnetic field, the fish oriented SE (Figure 4b), which corresponds to the sun compass data. When magnetic north was rotated by 120° clockwise, the fish adjusted their orientation accordingly (Figure 4c). Similar conclusions were drawn by another group testing individual black-axil chromis larvae (*Chromis atripectoralis*) in their presettlement phase in the absence of visual cues (O'Connor and Muheim 2017). These examples show that coral reef fish larvae/juveniles are able to orient themselves to the sun as well as to magnetic cues, but we still do not know whether this orientation approach is exclusively based on an inherited clock-and-compass system or on a map-based strategy that requires previous imprinting, like the geomagnetic imprinting of natal areas shown in salmon (Putman et al. 2013). Displacement experiments are the method of choice to answer this important question in our SFB.

Another question that remains to be answered is the question regarding the sensitivity thresholds of magnetoreception. Appropriate bioassays need to be developed because previously conducted conditioning experiments with field changes from 40 to 150 μT by far exceed the sensitivity of fish required to detect navigationally relevant magnetic field changes. Because coral reef fish disperse only a few kilometers up to 200 km from their natal reef, they would need to be able to discriminate differences in magnetic field intensity as small as 0.1–0.6 μT.

As presented in this short appetizer review, both birds and fish face great challenges when it comes to navigation over distances of various scales. Both birds and fish can use external references, like visual landmarks, celestial, olfactory and auditory cues, magnetic maps, and/or magnetic compasses to orient. However, birds and fish might sense the magnetic field differently. In birds, light-dependent radical pair–based magnetoreception seems to be a promising mechanism, while magnetite-based magnetoreception as well as indirect magnetic sensing using the ampullae of Lorenzini are more likely to be used in fish. In SFB 1372, we intend to clarify these mechanistic questions on many different levels from quantum chemistry to molecular biology, neurobiology, and genetics all the way to the behavior of the intact animals. A lot to do, but also a lot of fun ahead.

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Bionotes

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Review article

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Navigation of migratory songbirds: a quantum magnetic compass sensor

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Abstract: The remarkable ability of migratory birds to navigate accurately using the geomagnetic field for journeys of thousands of kilometres is currently thought to arise from radical pair reactions inside a protein called cryptochrome. In this article, we explain the quantum mechanical basis of the radical pair mechanism and why it is currently the dominant theory of compass magnetoreception. We also provide a brief account of two important computational simulation techniques that are used to study the mechanism in cryptochrome: spin dynamics and molecular dynamics. At the end, we provide an overview of current research on quantum mechanical processes in avian cryptochromes and the computational models for describing them.

Keywords: cryptochrome; magnetoreception; molecular dynamics; radical pair mechanism; spin dynamics.

Zusammenfassung: Zugvögel können mit Hilfe des Erdmagnetfeldes über tausende Kilometer hinweg akkurat navigieren. Es wird heutzutage angenommen, dass quantenmechanische Vorgänge im Cryptochromprotein diese Fähigkeit ermöglichen. Bei diesen Prozessen handelt es sich um Radikalpaar-Reaktionen. In diesem Artikel werden wir den Radikalpaarmechanismus erklären und erläutern, warum er momentan die vorherrschende Theorie ist. Des Weiteren werden wir kurz zwei wichtige, rechnerische Simulationstechniken vorstellen, die benutzt werden um Cryptochrome zu studieren: Spindynamik und Molekulardynamik. Abschließend geben wir einen Überblick über aktuelle Forschungsfragen.

Schlüsselwörter: Magnetsinn; Cryptochrom; Radikalpaarmechanismus; Molekulardynamik; Spindynamik.

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Introduction

To secure better environmental conditions, migratory birds fly thousands of kilometres every year. Their ability to navigate over vast distances with seemingly little effort has fascinated scientists and non-scientists alike. It is clear that birds use a multitude of cues for navigation over different geographic scales including the sun, stars, olfaction and landmarks (Mouritsen, 2018; Spiecker et al., 2021). In this article, we concentrate on the magnetic compass sense.

Given the low intensity of the Earth's magnetic field ($\sim 5 \times 10^{-5}$ T)—about 100 times weaker than the field experienced near a small fridge magnet—it is remarkable that living organisms have a magnetic sense. Although avian magnetoreception has been researched for the last 50 years, it is still unclear how this feat is achieved at the molecular level. The broad range of length scales (Figure 1) involved means that a highly multidisciplinary approach, as realised in SFB 1372, is essential for an understanding of the mechanism.

A functioning magnetic compass sense requires a receptor molecule to translate the direction of the geomagnetic field into a chemical signal which can be amplified and sent to the brain for processing. We focus here on the dominant hypothesis, known as the radical pair mechanism (RPM), in which the signal is derived from magnetically sensitive reactions of short-lived pairs of radicals (reactive molecules containing odd numbers of electrons) inside a protein called cryptochrome (Cry). A full description of this mechanism requires a quantum mechanical treatment (Fay et al., 2020). An additional hypothesis, based on biogenic magnetite (Fe_3O_4), is beyond the scope of this article (Winklhofer, 2010).

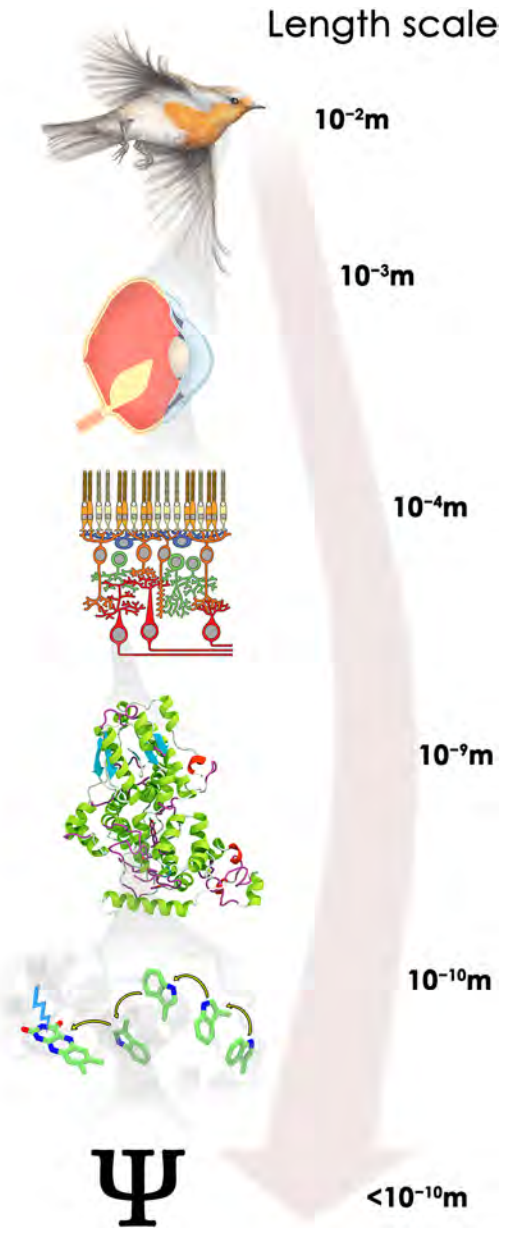

Figure 1: The range of distances involved demands a multidisciplinary approach to understand exactly how birds sense the direction of the Earth's magnetic field. Experiments to determine how birds ($\sim 10^{-2}$ m) respond to a magnetic field requires specialists in animal behaviour. The anatomy of the bird's eye ($\sim 10^{-3}$ m), and the cells in the retina ($\sim 10^{-4}$ m) where the magnetoreceptors are located, are studied by cell biologists. To sense the magnetic field, the cells must contain molecules that respond to magnetic stimuli having first been activated by light. The protein thought to fulfil this role is cryptochrome 4 ($\sim 10^{-9}$ m) whose properties are explored using biochemical and biophysical techniques. Inside the cryptochrome, light-induced magnetically sensitive radical pairs are formed by a series of electron-transfer reactions from tryptophan amino acid residues to a flavin chromophore ($\sim 10^{-10}$ m). The quantum nature of this 'radical pair mechanism' ($<10^{-10}$ m) takes us to the realm of quantum physics (Greek letter Psi, Ψ) in order to understand the primary events of compass magnetoreception.

To date, members of the cryptochrome class of proteins are the only candidate radical pair magnetoreceptors. Their ability to form radical pairs when activated by blue light is consistent with behavioural studies of migratory birds which clearly show that the magnetic compass sense is light dependent (Wiltschko et al., 2010). Of the various types of avian cryptochrome, the requirement for photo-excitation makes cryptochrome 4 (Cry4) the most likely to have a magnetic sensing function (Xu et al., 2021). Cry4 binds a flavin adenine dinucleotide (FAD) cofactor which enables it to absorb visible light and is present in the retina where the magnetoreceptors are known to be located (Günther et al., 2018). SFB 1372 has recently discovered that at least two forms of Cry4 exist in migratory birds (Einwich et al., 2020). Cryptochromes are found in many organisms, including plants where they were first reported (Ahmad and Cashmore, 1993; Yu et al., 2010). While plant, insect and avian cryptochromes *in vitro* are sensitive to magnetic fields (Maeda et al., 2012; Sheppard et al., 2017; Xu et al., 2021), this may only be of biological significance for long-distance migrants. One aspect of SFB 1372's work is therefore to compare cryptochromes from different species.

In the following pages, we outline the quantum mechanical basis of the radical pair mechanism (Hore and Mouritsen, 2016), summarise what we hope to learn from computational and biophysical studies of Cry4 in SFB 1372, and how it will complement behavioural studies.

Radical pair mechanism

The key to understanding the radical pair mechanism is the fundamentally quantum property known as 'spin'. Electrons and atomic nuclei such as hydrogen and nitrogen have spin and therefore behave like microscopic magnets. We represent electron spins here by means of arrows (↑ and ↓). Molecules containing an even number of electrons usually have them arranged in pairs (↑↓) such that their magnetic moments cancel. Radicals have an odd number of electrons, meaning that at least one electron cannot have a partner with opposite spin. In a radical pair, the two odd electrons, one in each radical, can either have their spins antiparallel (↑↓) or parallel (↑↑). These states are known as singlet and triplet, respectively.

Figure 2(A) shows a simplified radical pair reaction scheme in which A and D represent electron acceptor and electron donor molecules. When one of the reactants absorbs light an electron is transferred from D to A and a radical pair, $[A^{\bullet -}\ D^{\bullet +}]$, is formed where the superscripts denote the odd electrons (dots) and the charges on the radicals. $[A^{\bullet -}\ D^{\bullet +}]$ is formed exclusively in the singlet (↑↓)

state and subsequently interconverts spontaneously, rapidly and reversibly with the triplet state (↑↑). The magnetic interactions of the odd electrons with the spins of atomic nuclei and with the geomagnetic field drive this interconversion at frequencies of around a million times per second (Hore and Mouritsen, 2016). While this is going on, the radical pair can either (a) recombine to form the initial reactants (A and D), or (b) react to form a product (P). Since pathway (a) involves pairing up the electrons again, it can only occur from the singlet state. Pathway (b) may occur for both singlet and triplet states. If the radical pair spends less time in the singlet state, less recombination will occur and more product will be formed, and vice versa. Since the external magnetic field is one of the driving forces of the interconversion, its direction can influence the yield of the chemical product via its effect on the relative amounts of singlet and triplet radical pairs. It is the oscillatory interchange of singlet and triplet that is fundamentally quantum in nature.

Spin relaxation (of which more later) is the enemy of the radical pair compass. After it is born as a pure singlet, the pair starts to relax towards its equilibrium state in which the two electron spins are completely uncorrelated, at which point the geomagnetic field can no longer influence the spin dynamics. For a sensitive compass, it is therefore crucial to maintain a non-equilibrium state for as long as possible, which in practice means for at least a microsecond (Kattnig et al., 2016a,b). Normally, weak magnetic interactions like the ones discussed here have negligible influence on the outcome of chemical reactions because they pale in comparison to the random fluctuations in energy experienced by all molecules at physiological temperatures. Radical pair reactions, by contrast, can respond to minute magnetic interactions, more than a million times smaller than thermal energies (~k_BT, where k_B = Boltzmann's constant and T = temperature), because the electron spins are far from equilibrium. Having been created as a reaction intermediate using the large amount of energy (>>k_BT) carried by photons of blue light (Figure 2(A)), it only takes a tiny additional amount of magnetic energy to tip the singlet-triplet balance and so cause more pairs to proceed to the product and fewer to return to the reactants, or vice versa. Thereby, a radical pair–based magnetoreception mechanism could make animals sensitive to environmental stimuli more than a million times lower in energy than previously thought possible.

The RPM is a genuine mechanism solidly backed by theory and experiment, with hundreds of laboratory studies reporting effects of external magnetic fields on radical pairs (Rodgers and Hore, 2009; Steiner and Ulrich, 1989; Woodward, 2002). Several behavioural observations support the RPM hypothesis. One is the finding that birds require light to use their compass sense (Wiltschko et al., 2010). Another is that birds have an inclination compass: instead of distinguishing north from south, they detect the difference in the directions of the magnetic pole and magnetic equator (Wiltschko and Wiltschko, 1995, 1972). This accords precisely with the RPM in which the yield of the reaction product is unaffected by an exact inversion of the magnetic field vector. Finally, migratory birds are unable to use their magnetic compass in the presence of weak radiofrequency fields (Engels et al., 2014; Ritz et al., 2004; Schwarze et al., 2016; for more details, see Spiecker et al., 2021 in this issue). This, too, could be consistent with radical pairs which are known to be affected by electromagnetic fields in resonance with their singlet-triplet interconversion frequencies (Hiscock et al., 2017).

There is clear evidence that suggests that magnetoreception starts in the bird's retinas (for more details, see

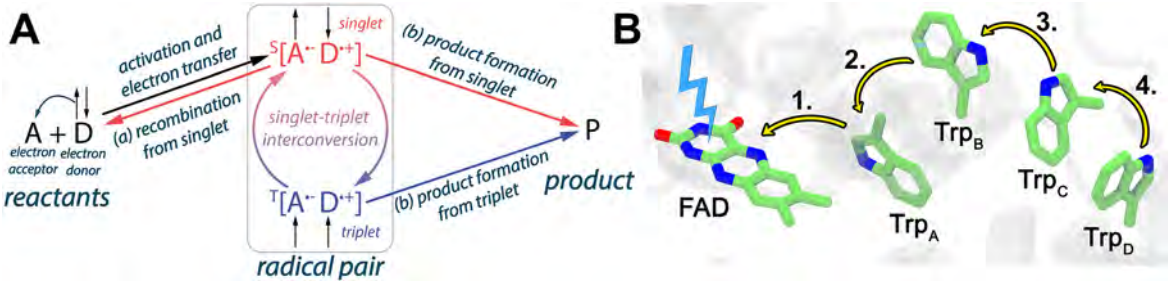

Figure 2: (A) A simplified scheme of the radical pair mechanism. The radical pair $[A^{\bullet-}\ D^{\bullet+}]$ is formed from reactants A and D by light-absorption (black arrow) and interconverts between singlet and triplet states (curved red/blue arrows). The singlet state can recombine to regenerate the reactants or react to form the product P (red arrows), while the triplet state can only form P (blue arrow). (B) Electron transfer in Cry4 to FAD along a chain of four tryptophan (Trp) residues. This process occurs after the protein has absorbed a photon of blue light and results in the formation of a radical pair comprising the FAD and the terminal tryptophan.

Haase et al., 2021 in this issue): the signal is carried to the brain along the optical nerve to Cluster N, a part of the brain region that processes visual information (Mouritsen et al., 2005). Cluster N has not been found in non-migratory songbirds, and chemical inactivation abolishes the ability of night-migratory birds to orient in a magnetic field (Zapka et al., 2009).

Cryptochrome

With strong experimental evidence pointing towards a radical pair–based magnetoreceptor in the birds' visual system, we turn our attention to cryptochromes, the only class of vertebrate proteins that can form long-lived radical pairs on exposure to light. More than 20 years ago, Ritz et al. (2000) suggested that the magnetically sensitive radical pair could be formed inside a cryptochrome by sequential electron transfers along a chain of three tryptophan (TrpH) residues to the FAD cofactor, triggered by blue-light activation of the FAD. Type-4 cryptochromes have four tryptophans (Einwich et al., 2020; Günther et al., 2018; Müller et al., 2015) all of which are involved in photoreduction of FAD (Hochstoeger et al., 2020; Xu et al., 2021).

Figure 2(B) shows the FAD excited by blue light (depicted by a lightning bolt) and the four TrpH residues in Cry4, where the yellow arrows indicate the sequential electron transfers. The wavelength of light required to excite FAD is around 450 nm, which is within the range of wavelengths (373–565 nm) the birds require to be able to use their magnetic compass in behavioural studies (Wiltschko et al., 2010). Depending on whether the fourth electron transfer occurs, the net result is the formation of either $[FAD^{\bullet-}\ Trp_CH^{\bullet+}]$ or $[FAD^{\bullet-}\ Trp_DH^{\bullet+}]$. Evidence of the sensitivity of these radical pairs to external magnetic fields comes from spectroscopic measurements of the photochemical yields of radicals in purified cryptochromes subject to millitesla magnetic fields (Maeda et al., 2012; Sheppard et al., 2017). Xu et al. (2021) have recently shown that both radical pairs contribute to the magnetic sensitivity of European robin Cry4 and that this protein, from a migratory bird, shows larger magnetic field effects than Cry4s from non-migratory chicken and pigeon.

It has not yet proved possible to detect effects of Earth-strength magnetic fields (~50 μT) on cryptochrome photochemistry. A demonstration of the sensitivity of cryptochromes to the direction of an external magnetic field would provide strong evidence to support the RPM hypothesis. Such a measurement would require the proteins to be aligned and immobilised (Lau et al., 2010), a challenge that has yet to be met experimentally.

The reactions of $[FAD^{\bullet-}\ TrpH^{\bullet+}]$ in cryptochromes are analogous to (a) and (b) in Figure 2(A) (Hore and Mouritsen, 2016): (a) is the spin-selective recombination of the singlet state of $[FAD^{\bullet-}\ TrpH^{\bullet+}]$, and (b) is the non-spin-selective deprotonation of $TrpH^{\bullet+}$ to form a neutral $Trp^{\bullet}$ radical. Ideally, these two processes should occur on similar timescales and result in a radical pair lifetime of a few microseconds (Rodgers and Hore, 2009). If the lifetime is too short, there will be insufficient time for the direction of the geomagnetic field to be encoded in the product yield, greatly impacting the sensitivity of the compass. Behavioural experiments in which birds are subjected to radiofrequency electromagnetic fields suggest that the lifetime of radical pairs in the bird's retinas is around 2–10 μs (Engels et al., 2014; Kobylkov et al., 2019).

The deprotonation of $TrpH^{\bullet+}$ to give $Trp^{\bullet}$ results in a stabilised, longer-lived form of the protein (corresponding to the product in Figure 2(A)), which can be monitored spectroscopically (Maeda et al., 2011; Neil et al., 2014). *In vivo*, this stabilised form of the protein is presumed to lead to the signalling state. As explored in Bartölke et al., 2021 in this issue, the signalling process is thought to be initiated by conformational changes in the C-terminal region of the cryptochrome molecule. Information on the C-terminal tail is therefore vital not just for an understanding of signal transduction but also because its interaction with binding partners may help optimise the structure of the cryptochrome for magnetic sensing. Unfortunately, no crystallographic data are available for this crucial part of the protein. As we now discuss, computer simulations can shed light on this and other issues.

Computational studies of cryptochrome

Modern computational techniques are powerful and versatile and form an integral part of the multidisciplinary approach of SFB 1372. Biophysicists aim to provide increasingly accurate estimates of various properties of proteins, allowing experimental observations to be rationalised and theoretical predictions to be tested in experiments. Using a 'computational microscope', it is

possible to address questions about the dynamics of the protein and the radical pairs within it. We start with molecular dynamics (MD) simulations.

Molecular dynamics simulations

The MD method involves solving Newton's equations of motion to obtain the instantaneous velocity and direction of movement of every single atom in the protein. This information is used to calculate where all the atoms would move to after a short time-step, typically ~10^{-15} s. Using these new positions, Newton's equations are solved again and the atoms moved accordingly. The whole process is repeated ~10^8 times to produce a 'trajectory' that is essentially a stop-motion movie of the structure of the protein at atomic resolution. Solvent molecules and ions can be included so that the protein is in an environment resembling that *in vivo*.

An example of the use of MD in the context of magnetoreception is our attempt to model the C-terminal tail of cryptochrome. As mentioned before, there is no crystal structure of any full-length avian Cry4 incorporating the C-terminal tail because the intrinsic disorder of this part of the protein has so far precluded crystallisation. To learn more about how the conformation and position of the C-terminal tail might change when the protein is photoreduced, we are trying to determine its structure computationally. One approach uses the software Pep McConst to randomly generate a multitude of structures (Schuhmann et al., 2021) on which we can perform MD simulations to investigate their properties.

One of the many properties that MD simulations provide is interatomic distances. The separation of the FAD radical and its partner, $TrpH^{\bullet+}$, can for example be used to estimate the fluctuations in the strength of the magnetic coupling of the two radicals. As these interactions affect singlet-triplet interconversion and spin relaxation of the radical pair, realistic estimates are important for an accurate description of the spin dynamics. Figure 3(A)(ii) and (iii) show an example for European robin Cry4. As well as studying intraprotein distances, one can use statistical tools to filter out the structural differences produced by changes in the redox state of the protein, for example when radical pairs are formed (Kattnig et al., 2018), to calculate the root-mean-square deviation (RMSD) in the positions of the atoms as a measure of the stability of the simulated structure (Figure 3(A)(i)), and to determine the energies of the different radical pair states. Using such methods, Xu et al. (2021) found evidence that the third radical pair, $[FAD^{\bullet-}\ Trp_CH^{\bullet+}]$, in robin Cry4 interconverts rapidly with the fourth, $[FAD^{\bullet-}\ Trp_DH^{\bullet+}]$. It was argued that such an equilibrium could allow migratory birds to evolve a magnetoreceptor in which the sensing and signalling functions were independently optimised.

Software for MD simulations is widely available. NAMD (Phillips et al., 2020, 2005), for example, allows the calculations to be automated and performed using parallel computing architectures. VMD (Humphrey et al., 1996) aids in the visualisation of trajectories and provides tools for analysis. The online platform VIKING (Korol et al., 2020), which is continuously developed and updated within SFB 1372, allows users to set up simulations with no need for in-depth computational knowledge.

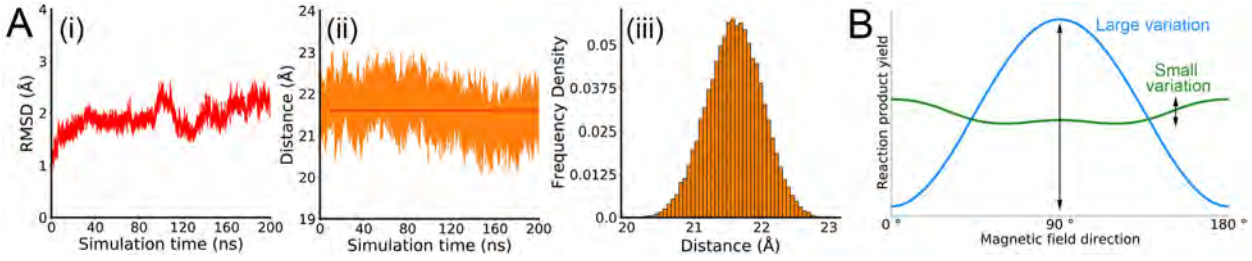

Figure 3: (A) Three graphs obtained from an MD simulation of European robin Cry4. (i) The root-mean-square deviation (RMSD) of the atomic positions over the course of the simulation. The stability of this structure is shown by the levelling out of the RMSD. (ii) and (iii) The centre-to-centre distance between FAD and Trp_DH: (ii) gives the variation as a function of time while (iii) shows the distribution of distances. (B) Calculated variation of the yield of the reaction of two radical pairs as a function of the direction of a 50 μT magnetic field. The green and blue lines are $[FAD^{\bullet-}\ Trp_CH^{\bullet+}]$ and $[FAD^{\bullet-}\ Z^{\bullet}]$, respectively, where the $Z^{\bullet}$ radical lacks the internal magnetic interactions present in $Trp_CH^{\bullet+}$. The much smaller signal for $[FAD^{\bullet-}\ Trp_CH^{\bullet+}]$ has been magnified tenfold to make the variation easier to see. Simulation parameters were taken from Lee et al. (2014), and the relative orientations of FAD and Trp_CH came from the crystal structure of pigeon Cry4 (Zoltowski et al., 2019). Further examples of such spin dynamics calculations can be found in (Hiscock et al., 2016).

Modelling protein dynamics using MD simulations is a valuable source of information on spin relaxation. Caused principally by the vibrations of chemical bonds within the protein, fast equilibration of the electron spins can drastically reduce the sensitivity of a radical pair to the geomagnetic field. How this process could be slow enough to make a protein-based radical pair viable as a geomagnetic sensor is one of the important research questions to be addressed in SFB 1372.

Spin dynamics simulations

The RPM has a strong theoretical foundation making it possible to simulate the responses of even quite complex radical pairs to the geomagnetic field (Hiscock et al., 2016; Lewis et al., 2014; Manolopoulos and Hore, 2013). Requiring quantum mechanical modelling of the singlet-triplet interconversion including chemical reactions and spin relaxation, these calculations have three motivations: (1) to interpret data from spectroscopic studies of purified cryptochromes (e.g. Maeda et al., 2012) and from behavioural experiments (e.g. Kobylkov et al., 2019); (2) to suggest and guide future experiments (e.g. Hiscock et al., 2017; Worster and Hore, 2018); and (3) to explore aspects of the radical pair hypothesis that are currently too challenging to study experimentally (e.g. Ren et al., 2021; Wong et al., 2021). Input parameters for these simulations typically come from crystallography and MD simulations (protein structure and dynamics) and from molecular orbital calculations (magnetic interactions). Available software includes MolSpin (Nielsen and Solov'yov, 2019) and Spinach (Hogben et al., 2011).

As outlined above, the influence of spin relaxation on radical pair magnetoreceptors can be explored by combining molecular and spin dynamics simulations. The seminal work in this area is Kattnig et al. (2016a,b) who used information derived from all-atom MD trajectories to model the magnetic sensitivity of [$FAD^{\bullet-}$ $TrpH^{\bullet+}$] radical pairs in a plant cryptochrome. Although, the relaxation caused by molecular vibrations could be compatible with geomagnetic sensing, it was clearly too fast to account for the disruptive effects of weak radiofrequency fields that have been observed in behavioural studies (Engels et al., 2014; Hiscock et al., 2017). The conclusion was that a cryptochrome sensor *in vivo* would need different dynamics from the isolated protein, e.g. as a result of binding to signalling partners and/or the structures required for its alignment and immobilisation. In SFB 1372, this work will be extended to European robin Cry4 with a focus on identifying any mechanisms that might shield the radicals from the dynamical modes of their environment. The long-term plan is to model the surroundings of the cryptochrome and develop a general picture of spin relaxation including all relevant magnetic and electronic interactions.

It is clear that the magnetic field effects that have been observed in spectroscopic experiments on purified cryptochromes derive from [$FAD^{\bullet-}$ $TrpH^{\bullet+}$] radical pairs (Maeda et al., 2012; Sheppard et al., 2017; Xu et al., 2021). *In vivo*, however, there could be contributions from the $FAD^{\bullet-}$ radical paired with a radical simpler than $TrpH^{\bullet+}$ (Ritz et al., 2009). Possibilities such as this can be explored by simulating the magnetic responses of a range of radicals. In this way, Lee et al. (2014) found that the magnetic properties of the $FAD^{\bullet-}$ radical are near-optimal for compass sensitivity. However, much of that sensitivity is lost if $FAD^{\bullet-}$ is partnered with $TrpH^{\bullet+}$ but could be regained if $TrpH^{\bullet+}$ were replaced by a hypothetical radical, denoted $Z^{\bullet}$, that is devoid of the internal magnetic interactions in $TrpH^{\bullet+}$. A simplified version of the calculation by Lee et al. (2014) is shown in Figure 3(B): even in this simple case, the reaction product yields of [$FAD^{\bullet-}$ $Z^{\bullet}$] are much more sensitive to the magnetic field direction than those of [$FAD^{\bullet-}$ $Trp_CH^{\bullet+}$]. However, as most biological radicals have multiple internal interactions, there are limited options for the identity of $Z^{\bullet}$. One candidate, superoxide ($O_2^{\bullet-}$), has the serious drawback that it probably undergoes extremely rapid spin relaxation (Hogben et al., 2009; Player and Hore, 2019). Other species that contain fewer magnetic nuclei than tryptophan have also been proposed, such as the radical form of ascorbic acid (Lee et al., 2014; Nielsen et al., 2017). As already mentioned, many of the predictions of these spin dynamics simulations can be tested experimentally by *in vitro* spectroscopic measurements on recombinantly expressed and purified proteins. In a typical experiment, changes in the yields and rates of formation and decay of potential signalling states are measured as a function of time after absorption of a short laser light pulse. Intermediate states of the proteins, including the radical pair forms, can be identified from their characteristic UV-visible absorption spectra. Observations with and without applied magnetic fields

give key clues to the factors that could make cryptochromes sensitive magnetic sensors *in vivo*. Such measurements allowed Xu et al. (2021) to claim that "Cry4 from the night-migratory European robin seems fit for purpose a magnetic sensor". The next step will be to see whether it is possible to manipulate Cry4 in the eyes of a migratory bird.

In conjunction with behavioural experiments (Engels et al., 2014; Kobylkov et al., 2019; Schwarze et al., 2016), extensions of these calculations can be used to explore the identities and properties of the radicals involved *in vivo*. This work exploits the effect of weak radiofrequency magnetic fields on birds' ability to use their magnetic compass. Spin dynamics simulations can predict the radiofrequency conditions in which these effects should occur for different radical pairs (Hiscock et al., 2017). Radiofrequency fields with cleverly chosen intensities and frequency ranges can then be used in behavioural studies to discriminate between different radical pair models (for details, see Spiecker et al., 2021 in this issue). The close collaboration within SFB 1372 will allow an efficient iterative process, where the first experimental findings will shape the next set of simulations, which in turn will allow informed choices for subsequent experimental design.

In summary, among the open questions we will attempt to solve within the SFB using computer simulations combined with multidisciplinary collaboration are: is the radical pair *in vivo* really [$FAD^{\bullet-}$ $TrpH^{\bullet+}$] and how might evolution have optimised the spin relaxation of the radical pair *in vivo*?

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Review article

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The secrets of cryptochromes: photoreceptors, clock proteins, and magnetic sensors

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Abstract: A class of light-activated proteins in the eyes of birds, called cryptochromes, are thought to act as the primary magnetic sensors allowing night-migratory songbirds to navigate over thousands of kilometers using the earth's magnetic field. Having evolved from DNA-repairing photolyases, cryptochromes have redirected the energy from light to fuel a variety of other functions: as photoreceptors, as regulators of the circadian clock – and, in some species, most likely as sensors of the magnetic field. While the quantum effects of magnetic fields on cryptochromes are already being studied in detail, almost nothing is known about the signaling cascade involving cryptochrome as the primary receptor protein. Two different screening methods have identified potential interaction partners that suggest an involvement of the visual phototransduction pathway, the visual cycle, potassium channels or glutamate receptors, but more pioneering research is needed to unravel the signaling cascade responsible for transducing the magnetic signal.

Keywords: FAD; interactome screening; magnetoreception; navigation; signaling.

Zusammenfassung: Es wird angenommen, dass eine Klasse von lichtaktivierten Proteinen, sogenannte Cryptochrome, in den Augen von Vögeln als primäre Magnetsensoren fungieren, welche es Vögeln ermöglichen mithilfe des Erdmagnetfelds über Tausende von Kilometern zu navigieren. Cryptochrome haben sich aus DNA-Reparaturenzymen, den Photolyasen, entwickelt und die Lichtenergie für andere Funktionen nutzbar gemacht: Cryptochrome wirken als Photorezeptoren und Regulatoren der circadianen Uhr – und in einigen Spezies wahrscheinlich auch als Sensoren des Magnetfelds. Während die Quanteneffekte von Magnetfeldern auf Cryptochrome im Detail untersucht werden, ist fast nichts über die Signalkaskade bekannt, in der Cryptochrom als primäres Rezeptorprotein fungiert. Zwei verschiedene Screening-Methoden haben potenzielle Interaktionspartner identifiziert, die auf eine Beteiligung des visuellen Phototransduktionsweges, des visuellen Zyklus, der Kaliumkanäle oder der Glutamatrezeptoren hinweisen. Um die für die Übertragung des magnetischen Signals verantwortliche Signalkaskade zu entschlüsseln, sind jedoch weitere grundlegende Forschungsarbeiten erforderlich.

Schlüsselwörter: FAD; Interaktom Screening; Magnetrezeption; Navigation; Signalübertragung.

Introduction

The evolution of life on the planet has equipped animals with some extraordinary senses, and perhaps the most fascinating and least understood of all is magnetoreception. Although there is compelling behavioral evidence that this sense exists and that the earth's magnetic field is used for orientation and navigation by migratory birds, fish, amphibians and reptiles (for details, see Spiecker et al., 2021 in this issue), we still have very little knowledge about the underlying molecular mechanism (Mouritsen, 2018). Two main hypotheses have been developed to explain the phenomenon: a light-dependent biochemical reaction, known as the radical pair mechanism, and/or magnetic particles (for details, see Wong et al., 2021 in this issue). A wealth of experimental evidence and theoretical considerations favor the first hypothesis, which is based on a photopigment in the eye that upon light excitation forms

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radical pairs, which are sensitive to the earth's magnetic field (Hore and Mouritsen, 2016). Cryptochromes are so far the only known candidate proteins that meet these requirements. They are, however, also involved in a number of different functions, and are best known for regulating the circadian clock (Liedvogel and Mouritsen, 2010; Ozturk, 2017; Sancar, 2003). The structure and function of these putative magnetosensors will be discussed in the first part of this article.

In order to allow birds to sense the geomagnetic field, the information gathered by the primary sensor molecule needs to be carried to the brain, where it can be interpreted. This is generally achieved by signaling cascades that result in the opening or closing of channels in the cell membrane, which in turn trigger a change in membrane potential and nerve cell firing. This signaling cascade for magnetoreceptive cryptochromes is still completely unknown and one way to discover it is to find all the proteins that can interact with the cryptochromes. We will also discuss how hypothetical signaling cascades could look like and what is known so far.

Cryptochromes as potential magnetoreceptors

Cryptochromes are found throughout the animal kingdom and share between 25 and 40% of their amino acid sequence with the evolutionarily related photolyases (Liedvogel and Mouritsen, 2010; Ozturk, 2017; Sancar, 2003). Photolyases are an ancient family of DNA-binding proteins that can harvest the energy of blue-light photons to fuel a radical-based DNA repair mechanism in a highly efficient manner (Mei and Dvornyk, 2015; Sancar, 2003). While cryptochromes lack the DNA-repairing ability, many of them still share the ability to use a flavin-based chromophore, namely FAD, to absorb blue light and are key regulators of the circadian system. The core structure of these proteins, the photolyase homology region (PHR), remains highly conserved to the ancestral photolyases. The PHR consists of an N-terminal α/β domain and a C-terminal α-helical domain that are connected by an extensive inter-region segment (Figure 1). The α-helical domain contains a highly conserved binding site for the flavin chromophore and makes up the active site of the enzyme. A second chromophore (either 5,10-methenyl-5,6,7,8-tetrahydrofolate (MTHF) or 8-hydroxy-5-deazaflavin (8-HDF)) can bind to the more variable α/β domain of at least some cryptochromes and functions as a light-harvesting antenna to aid FAD (Kavakli et al., 2017). Upon light excitation, FAD and a tryptophan chain form long-lived magnetically sensitive radical pairs via intramolecular electron transfer, which is suggested to be at the heart of the magnetic sense (for details, see Wong et al., in this issue). Plant and animal cryptochromes acquired an additional C-terminal tail, whose role will be discussed later. One of the goals of SFB1372 is to understand the structural changes of avian cryptochromes that take place when they are exposed to light and magnetic field stimuli, and thus to understand how the primary magnetic sensor works.

The animal CRYs can be grouped into three main types, of which type 1 are found in insects (for example, Drosophila), type 2 in vertebrates, and type 4 cryptochromes have so far been found in birds, frogs, sea turtles and fish. While type 1 CRYs are blue light receptors regulating the circadian rhythm, type 2 CRYs are light-independent transcriptional regulators of the clock (Takahashi, 2015). Whether the vertebrate type 2 CRYs can also act as photoreceptors is still under debate (Vanderstraeten et al., 2020).

In the bird retina, where the magnetosensor is thought to be located (Hein et al., 2010, 2011), five different cryptochromes have been described so far. Of the type 2 CRYs, Cry1a expression is found in UV cones (Bolte et al., 2021; Niessner et al., 2011), and Cry1b in photoreceptors and ganglion cells (Bolte et al., 2016; Niessner et al., 2016). Cry2 shows a rather broad nucleic expression, which would be in line with a role in the circadian clock rather than magnetoreception (Mouritsen et al., 2004). Additionally, two type 4 CRYs, Cry4a (Cry4) and its isoform Cry4b, have been found in a migratory bird (Einwich et al., 2020; Gunther et al., 2018; Liedvogel and Mouritsen, 2010). Cry4 was shown to localize in the outer

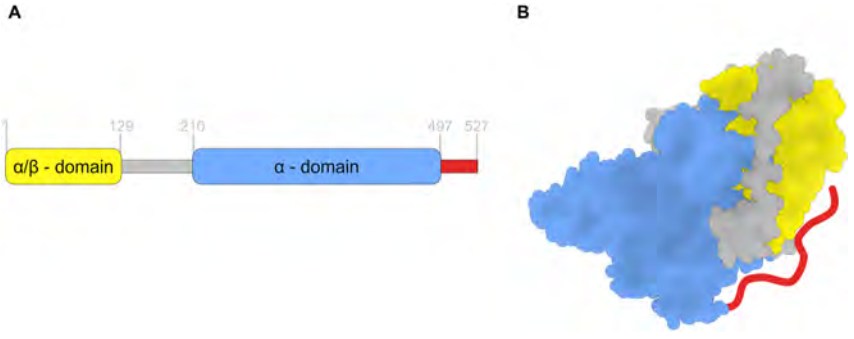

Figure 1: Architecture of cryptochromes. (A) Sequence of ErCry4, a representative of the type-4 class of cryptochromes, and (B) illustration of a hypothetical ErCry4 fold based on the crystal structure of pigeon Cry4 (PDB 6PU0). The PHR comprises an N-terminal α/ß domain (yellow) and a C-terminal α-helical domain (blue) connected by an extensive inter-region segment (grey). Additionally, plant and animal cryptochromes have acquired a C-terminal tail (red) of variable length that is believed to be mainly unstructured.

segments of double cones and long-wavelength single cones of night-migratory European robins (Gunther et al., 2018). This location could provide an ideal situation for a light-dependent magnetoreceptor, as the hundreds of parallel cell membranes in the outer segments could align the cryptochromes relative to each other, a prerequisite of a magnetic sensor (Hore and Mouritsen, 2016; Liedvogel and Mouritsen, 2010; Worster et al., 2017). In pigeons, however, Cry4 seemed predominantly expressed in retinal horizontal cells (Hochstoeger et al., 2020). A more detailed description of the retina is provided by Seth et al. (in this issue).

While Cry1a, Cry1b, Cry2 and Cry4b show a circadian expression rhythm, which would be expected for clock proteins, Cry4a expression shows no circadian expression rhythm and Cry4a expression is increased by a factor of 2.5 during the migratory season (Einwich et al., 2020; Gunther et al., 2018). At the present time, the localization of Cry4 in the retina, its seasonal expression pattern, as well as strong binding of FAD and a large magnetic sensitivity *in vitro* (Xu et al., 2021) are all reasons that make Cry4 the seemingly best-suited candidate for magnetoreception.

To sum up, cryptochromes have been found to act as timers of the circadian rhythm (Michael et al., 2017; Parico et al., 2020), as soluble receptors for blue-light (Cashmore et al., 1999), and as a potential receptor for magnetic cues (Gunther et al., 2018; Ritz et al., 2000). Thus, the functional diversity of cryptochromes is much larger than that of the single-purpose photolyases (Dodson et al., 2013; Kutta et al., 2017). On the sequence level, this broadening of functions is reflected not only by C-terminal, and partially by N-terminal, extensions, but also by more subtle adaptations of the core region. While the effects of the N-terminal extensions remain to be analyzed in detail, the diverse nature of the C-terminal extensions, differing clearly both in length and in sequence content (Ozturk, 2017), have sparked significant research interest.

Tales from flexible tails

At some point in evolution, the photolyase proteins appeared with a flexible and intrinsically disordered addition: the C-terminal extension or the C-terminal tail. Along with it came a new role for the proteins other than DNA repair: the cryptochrome light receptor family was born.

Structural data on these extensions are still lacking, with the notable exception of *Drosophila melanogaster* *Dm*Cry that features the shortest C-terminal tail reported to date, and that could be successfully crystallized as it adopts an alpha-helical fold under dark conditions, mimicking the DNA substrates of photolyases (Zoltowski et al., 2011). Light then drives the release of the C-terminal tail from the surface of the PHR core region (Berntsson et al., 2019; Vaidya et al., 2013). The initial residues of the pigeon *Cl*Cry4 C-terminal tail were equally found to form a helical structure, oriented towards the FAD-binding pocket (Zoltowski et al., 2019), which implies that blue light excitation of FAD may result in conformational changes of the C-terminal tail and trigger a signaling cascade (Figure 2). Experimental evidence from Cry variants of different species including chicken, pigeon and drosophila suggests that light-induced structural changes are associated with the C-terminal tail (Hochstoeger et al., 2020; Watari et al., 2012). Functional studies further highlight the importance of the C-terminal tail for the specific functions and roles cryptochromes can adopt. Its truncation can lead both to constantly active cryptochromes, as observed for *Dm*Cry1 (Dissel et al., 2004) or mouse *Mm*Cry1 (Chaves et al., 2006) cryptochromes, and to constantly inactive cryptochromes, as observed for human *Hs*Cry1 (Parico et al., 2020).

Intriguingly, the C-terminal tail is poorly conserved among groups of organisms, varying in length and composition (Franz-Badur et al., 2019), allowing the protein to play a role in a variety of functions. Furthermore, the high flexibility of this tail permits it to have more and different interaction partners because its flexibility allows it to adapt to diverse binding sites. Thus, the addition of the C-terminal tail has created light-dependent proteins with fundamentally different functions from their ancestors and is rightly considered the key to understanding the signaling of cryptochromes.

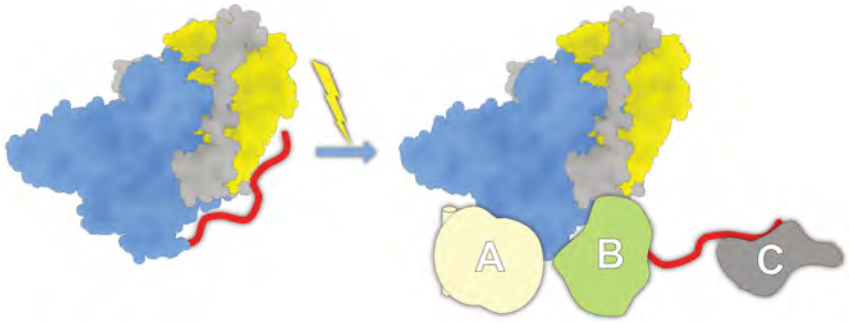

Figure 2: Model of the role of the C-terminal tail of cryptochromes. In a general model of cryptochromes, the C-terminal tail is embedded in a groove of the photolyase domain in the dark and adopts an open and active conformation upon light exposure. The exposed C-terminal tail is then thought to allow interactions with other proteins (hypothetical proteins A, B and C) and initiate signaling.

Cryptochrome signaling

Since several lines of evidence support the hypothesis that Cry4 is the primary magnetoreceptor molecule in the retina of navigating birds (see above), one of the key goals of SFB1372 is to identify a signaling pathway that connects the activation of Cry4 with an electrophysiological cell response. Up to date, we still lack a cellular readout from a bird's retina, for example, the recording of a magnetoreceptive electrophysiological response, another key endeavor pursued with the SFB. Concerning possible signaling routes however, two experimental approaches were published in 2020 to identify protein–protein interaction partners of Cry4 (Hochstoeger et al., 2020; Wu et al., 2020).

The SFB-related study (Wu et al., 2020) used the yeast-two-hybrid system to screen avian retina cDNA libraries for possible interaction partners of Cry4 in the European robin. We identified six proteins as particularly promising candidates. Interestingly, all genes are expressed in vertebrate retinae of different species and they can be categorized in three groups according to their described functions.

In this screen, Cry4 interacted with the long wavelength-sensitive opsin (also called red opsin or iodopsin), a G protein-coupled receptor that functions as the visual pigment present in the long wavelength sensitive single cones and in the double cones. Wu et al. (2020) further found that the α- and γ-subunit of the cone specific variant of a heterotrimeric G protein (Gtα and Gtγ) interacted with Cry4.

A second group of two proteins that seemed to interact with Cry4 are known to be components of the visual cycle. The retinol binding protein (RBP) takes up retinol (for example, from the liver) and transports it in the bloodstream to different targets. The second protein in this group is the retinal G protein-coupled receptor (RGR), a non-visual opsin that has photoisomerase activity converting all-*trans*-retinal to 11-*cis*-retinal (Palczewski and Kiser, 2020).

Finally, Wu et al. (2020) identified a subunit of the voltage-gated potassium channel (Kv8.2) that normally forms a functional channel in complex with another subunit (Kv2.1) as a potential interaction partner of Cry4.

A common feature of at least four (Figure 3) candidates is that they are expressed in the outer or inner segments of photoreceptor cells. Wu et al. (2020) discussed different intracellular scenarios, how Cry4 would trigger or participate in signaling making use of phototransduction. For example, Cry4 could form a complex with long-wavelength-sensitive opsin and this complex might trigger the activation of G protein-mediated phototransduction. Alternatively, Cry4 could interact with Gtα that could activate any downstream signaling proteins, which could be part of the well-known phototransduction pathway or a so far not identified signaling route (Figure 3).

A direct interaction of Cry4 with a K^+-channel would not involve intermediate signaling proteins. Such an interaction process might take place in the inner segments of photoreceptor cells. Interaction of Cry4 with the RBP and RGR proteins of the visual cycle seems counterintuitive at present and could point to the additional involvement of Cry4 in cellular processes other than magnetoreception. Putative involvement of European robin Cry4 in these processes is summarized in Figure 3.

Hochstoeger et al. (2020) characterized the molecular and biophysical properties of pigeon Cry4. These authors found evidence that pigeon Cry4 could operate as an ultraviolet-blue photoreceptor and form long-lived radical pairs. By combining co-immunoprecipitation with mass spectrometric analysis, they identified eight retina specific proteins as potential interactors of pigeon Cry4. Two of these candidates are scaffolding proteins (named GRIP1 and GRIP2) that harbor PDZ protein–protein interaction domains and target glutamate receptors in postsynaptic membranes. Hochstoeger et al. (2020) localized Cry4 in horizontal cells in the pigeon retina, where they further indicated that GRIP2 co-localizes with Cry4 in the horizontal cell/cone synapse. In their model of primary magnetoreceptor signaling, Cry4 interacts with a complex consisting of GRIP1 and GRIP2. Photoexcitation of Cry4 could trigger a conformational change leading to a signaling state of Cry4,

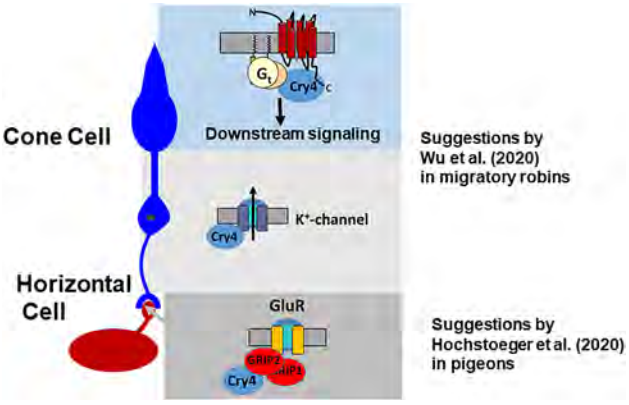

Figure 3: Hypothetical signaling of Cry4 in cone photoreceptor and horizontal cells. In cone outer segments Cry4 could transduce a magnetic signal by interacting with cone opsin (seven-transmembrane receptor protein in red, upper part) and/or with the cone specific G protein (Gtα, upper part). Cry4 interaction with a K+-channel in the inner segments or in the synaptic terminal could represent a transduction mode without intermediate signaling proteins (middle part). Interaction of Cry4 with the scaffolding GRIP protein complex could influence the gating properties of an ionotropic glutamate receptor in pigeons that is located in the postsynaptic membrane of horizontal cells.

which might then have an influence on the gating properties of an ionotropic glutamate receptor (Figure 3). These authors further discuss a possible mechanism existing in *Drosophila*, where a cryptochrome-mediated light response can influence neuronal depolarization of redox-dependent potassium channels (Fogle et al., 2011).

Open questions

It becomes apparent that there are more open than answered biochemical questions in the field of magnetoreception. Although more and more findings support a radical-pair based mechanism, there is no final proof that cryptochromes act as the magnetosensors, which is doubtless the biggest research challenge in the coming years and one of the key aims of the SFB1372. Coupled to that is the question which of the cryptochromes can act as magnetosensors – and which cannot. Which animals have a magnetically sensitive cryptochrome, and if so, are they also able to perceive and act upon these signals? Mixed behavioral results show that magnetic orientation can be very delicate and difficult to measure. A thorough biochemical analysis unraveling the signaling cascade from A to Z would, therefore, reduce the need to rely on tricky behavioral tests only. With the tools available today, we can tackle the controversial discussions about magnetoreception and aim to put them on a firm biochemical, biophysical and neuroscientific footing.

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Bionotes

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Review article

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The retinal circuitry for magnetoreception in migratory birds

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Abstract: Night-migratory birds use the Earth's magnetic field to determine the direction in which they want to migrate. Many studies suggest that this "magnetic compass sense" is light dependent and mediated by blue light sensors, called cryptochromes, which are expressed in the retina of night-migratory birds. In this review, we summarize the evidence that the avian retina processes not only visual information but also magnetic compass information. We also review the current knowledge on cryptochrome expression in the bird retina and highlight open questions which we aim to address within the framework of SFB 1372 *Magnetoreception and Navigation in Vertebrates*.

Keywords: cryptochrome; eye; magnetic field; magnetoreception; retina.

Zusammenfassung: Nachtziehende Vögel nutzen das Magnetfeld der Erde, um die Richtung zu bestimmen, in die sie ziehen möchten. Viele Studien legen nahe, dass dieser Magnet-Kompasssinn lichtabhängig ist und durch Blaulicht-Sensoren, sogenannte Cryptochrom-Moleküle, vermittelt wird, die in der Retina von nachtziehenden Vögeln gefunden wurden. In diesem Übersichtsartikel fassen wir das aktuelle Wissen über die Cryptochrom-Expression in der Netzhaut von Vögeln zusammen und heben offene Fragen hervor, die wir im Rahmen des SFB 1372 *Magnetoreception and Navigation in Vertebrates* beantworten möchten.

Schlüsselwörter: Auge; Cryptochrom; Magnetfeld; Magnetrezeption; Retina.

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Introduction and objectives

Night-migratory songbirds, such as European robins (*Erithacus rubecula*), cover thousands of kilometers between their breeding and wintering grounds each year. They do so with surprising precision and are even able to find back to their nesting hole or favorite sleeping perch (Mouritsen, 2018). Interestingly, these journeys are taken individually and at night time. Nocturnal migration has advantages because the birds can forage during daytime to refuel their energy reserves before traveling long distances at night (Kerlinger and Moore, 1989). The questions that naturally arise are: How do night-migratory songbirds find their way? How can they migrate such large distances with such brilliant precision?

From several studies, it has become clear that migratory birds use multiple cues during their migratory journeys (reviewed in Mouritsen, 2018): 1) celestial cues, including the sun (Kramer, 1950) and the stars (Alert et al., 2015; Emlen, 1967); 2) visual landmarks (Åkesson, 2003), like mountains; 3) odor gradients (Wallraff, 1980); and 4) magnetic cues (Cochran et al., 2004; Wiltschko and Wiltschko, 1972, 1996), which provide birds with positional (magnetic map sense; Heyers et al., 2017; Pakhomov et al., 2018) and directional information (magnetic compass sense). The advantage of magnetic cues over other cues is that they are ubiquitous, constantly available throughout the day and year (Mouritsen, 2018), and independent of weather conditions, such as an overcast sky. In this article, we focus on the magnetic compass sense. It is thought to be embedded in the bird's visual system, providing information on the inclination angle between the magnetic field lines and the land surface (Schwarze et al., 2016; Wiltschko and Wiltschko, 1972, 1996). The inclination angle is steep at the poles and becomes zero at the equator so that birds can differentiate poleward from equatorward directions. The inclination-based magnetic compass was shown to be light dependent (Wiltschko et al., 1993). It involves a radical-pair–based mechanism (Hore and Mouritsen, 2016; Ritz et al., 2000; Schulten et al., 1978), which is suggested to be mediated by sensor molecules (called cryptochromes) expressed in the retina of night-migratory birds.

In the following sections, we will discuss the pieces of evidence suggesting that light-dependent magnetoreception starts in the retina (Figure 1). We will then describe the gross anatomy of the avian retina and recent findings on cryptochrome expression. Finally, we will discuss some open questions and potential experiments to answer them.

Evidence that the retina is involved in light-dependent magnetoreception

Several lines of evidence point to the retina as the initiation point of magnetoreception: 1) the proposed radical-pair-based mechanism is light dependent; 2) night-migratory songbirds show magnetic compass orientation only under certain wavelengths of light; 3) a brain area in the visual wulst, which receives information from the retina via the thalamofugal pathway, is essential and sufficient for magnetic compass-based orientation behavior; and 4) the putative sensory molecules (cryptochromes) are abundantly expressed in the retinal neurons (Mouritsen, 2018).

The light-dependent radical-pair-based mechanism (Hore and Mouritsen, 2016; Ritz et al., 2000; Schulten et al., 1978) suggests that molecules called cryptochromes are able to form long-lived radical pairs when excited by light, which then interconvert between a singlet and a triplet state. The Earth's magnetic field can influence this interconversion and thereby the likelihood with which a signaling state is formed. This signaling state may represent the basis of the magnetic compass sense (Hore and Mouritsen, 2016; for more details, please see Wong et al., 2021). As light absorption seems necessary to start magnetoreception, light-sensitive organs of birds were investigated, such as the pineal gland and the eye. However, surgically removing the pineal gland did not affect the magnetic compass sense in birds (Schneider et al., 1994). This made the eye the only plausible location for magnetosensor molecules. As the visible spectrum of light consists of different wavelengths, magnetic compass-based orientation experiments were done under monochromatic light to understand if birds preferred a certain portion of the spectrum. European robins tested under blue and green light could orient in the appropriate migratory direction, whereas they were disoriented under yellow and red light (Wiltschko and Wiltschko, 1995, 1999; Wiltschko et al., 1993). This experimental data suggest that the putative magnetosensor molecules are located in the retina and act in a wavelength-dependent manner.

Another piece of evidence that magnetoreception starts off in the retina comes from neuroanatomical studies. A region called cluster N is the most active brain region during magnetic compass orientation in night-migratory birds

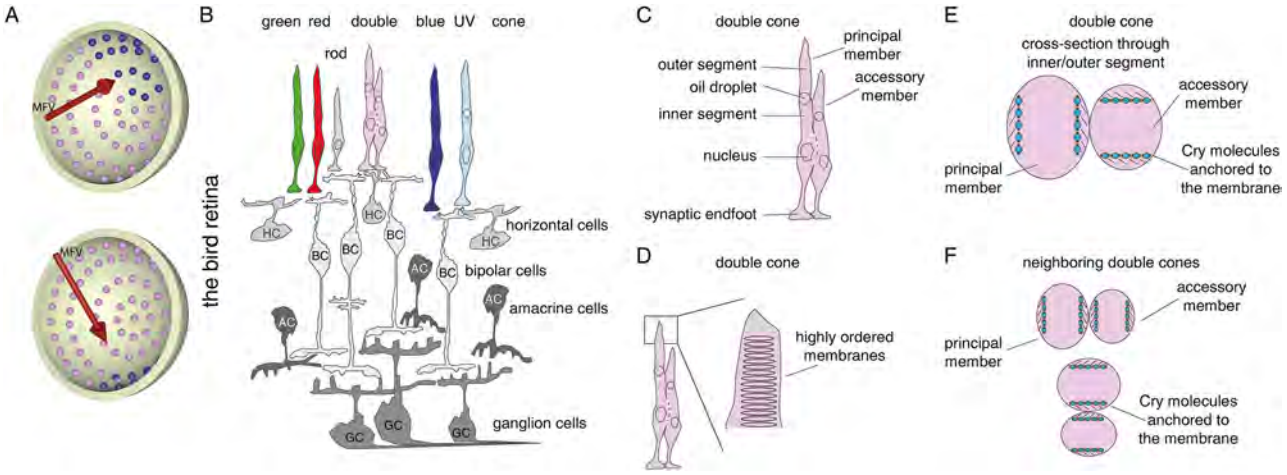

Figure 1: The avian retina.
(A) Distribution of the magnetosensor molecules across the retinal surface. Magnetic field vectors (MFVs) with different inclination angles will excite magnetosensor molecules in different areas of the retina differently. (B) Avian retina with rod and cone photoreceptors, bipolar cells (BC), horizontal cells (HC), amacrine cells (AC), and ganglion cells (GC). (C) The different cellular compartments of avian photoreceptors, shown for a double cone. (D) Photoreceptors contain highly ordered membrane structures in their outer segments. (E) Magnetosensor molecules may be aligned differently in the two members of the double cone. (F) Alternatively, neighboring double cones may have different orientations while the magnetosensor moleculare are aligned in the same way in both members. In both configurations shown in (E) and (F), the magnetic signal would be independent from the light intensity because adjacent or neighboring double cones receive similar amounts of light.

(Liedvogel et al., 2007; Mouritsen et al., 2005; Zapka et al., 2009, 2010). This brain area is part of the visual wulst (Heyers et al., 2007) and only gets activated when the bird's eyes receive light input (Liedvogel et al., 2007; Mouritsen et al., 2005). Using tracing experiments, Heyers et al. (2007) showed that cluster N receives input from the retina via the thalamus. When cluster N was lesioned in night-migratory birds, magnetic compass orientation was abolished, whereas the sun and star compass orientation were still intact (Zapka et al., 2009). These studies indicate that the retina transmits information on magnetic field inclination to the thalamus, which relays this information to cluster N.

Cryptochromes play a role in the circadian clock of vertebrates (Chaves et al., 2011; Hsu et al., 1996; Todo et al., 1996). They are the only known light-sensitive molecules in vertebrates that form long-lived radical pairs after the absorption of blue light (Ritz et al., 2000). This wavelength specificity aligns well with the behavioral data demonstrating that birds perform magnetic compass orientation only under blue/green light (Wiltschko and Wiltschko, 1999). In birds, six different cryptochrome isoforms have been identified. All of them are expressed in retinal neurons (Bolte et al., 2016, 2021; Einwich et al., 2020; Günther et al., 2018; Hochstoeger et al., 2020; Mouritsen et al., 2004; Nießner et al., 2013). We describe the retinal expression pattern of the individual isoforms in detail below.

Finally, the hemispherical shape of the eyeball is ideally suited for magnetoreception because retinal neurons have a fixed orientation with respect to the retinal surface (Figure 1(A)). In consequence, sensor molecules in retinal neurons could be aligned at different angles to the incident magnetic field lines (Hore and Mouritsen, 2016; Solov'yov et al., 2010) when the sensor molecules are restricted in diffusion or rotation, for example by fixed orientations in, or close to membranes (Figure 1(E) and (F)).

In summary, many studies from different scientific fields indicate that the magnetic compass sense originates in the retina. However, the precise identity of retinal cells taking part in this process is still unknown. Therefore, within the framework of SFB1372 "Magnetoreception and Navigation in Vertebrates" we aim to answer two important questions: Where are the putative sensor molecules localized? Through which retinal pathway do magnetic signals reach the only retinal output neurons, the ganglion cells?

The avian retina

Like all other vertebrate retinae, the avian retina is built from five primary classes of neurons (Figure 1(B); photoreceptors, bipolar, horizontal, amacrine, and ganglion cells), arranged in three nuclear and two synaptic (plexiform) layers. Photoreceptors (Figure 1(B)–(D)) transduce the incoming photons into electrical signals and mediate these signals to bipolar cells, which in turn contact the retinal ganglion cells (Figure 1(B)). On their way from photoreceptors to ganglion cells, electrical signals are modulated by horizontal cells in the outer retina and amacrine cells in the inner retina (Figure 1(B)).

Although this seems to be a rather simple neuronal circuit, there is great complexity because each of these five cell classes comprises many different cell types. The bird retina contains one type of rod photoreceptor, important for dim-light vision, four different types of single cone photoreceptors for color vision, and one type of double cone (Figure 1(B)–(F); Hart, 2001), whose function is not fully understood yet. Recent data from transcriptomics (Yamagata et al., 2021) suggest that the chicken retina contains additionally 22 types of bipolar cells, 4 types of horizontal cells, 59 types of amacrine cells, and 41 types of ganglion cells. For most of these neurons, the detailed morphology and function are still unknown. Like in other vertebrates (Field et al., 2007), bird retinal neurons are connected in different parallel pathways which transform the patterns of light into various streams of stimulus features, such as contrast, motion, color, edges, and possibly magnetic field inclination. This information is carried by the many types of retinal ganglion cells to the brain. They are the sole output neurons of the retina and project to two main brain areas: the tectum and the thalamus, from where signals are relayed to the entopallium and visual wulst, respectively. Data from pigeons suggests that most retinal ganglion cells project to the tectum, relaying visual information on brightness, color, patterns (Remy and Güntürkün, 1991), and object motion (Baron et al., 2007). Only 10–25% of all retinal ganglion cells project to the thalamus (Remy and Güntürkün, 1991). They are suspected to be involved in the perception of self-motion and in binocular vision (Murcia-Belmonte and Erskine, 2019). As mentioned above, data suggest that the thalamofugal pathway also sends information on the Earth's magnetic field from retinal ganglion cells to higher brain areas (Heyers et al., 2007; Zapka et al., 2009).

Cryptochrome expression in the avian retina

Cryptochromes are the putative sensor molecules for light-dependent compass orientation in night-migratory birds. Birds contain three cryptochrome genes (*Cry1, Cry2, Cry4*)

(Feng et al., 2020) which give rise to two cryptochrome isoforms each (Cry1a/b, Cry2a/b, Cry4a/b). All isoforms are expressed in the bird retina although the expression pattern seems to differ in some cases between species (Günther et al., 2018; Hochstoeger et al., 2020).

Cry1a is found in UV/violet cones of European robins and several other bird species (Bolte et al., 2021; Nießner et al., 2011). It is localized in the outer segments of the photoreceptors (Figure 1(D)), where also the phototransduction of visual signals takes place. Cry1b, in contrast, is expressed in the inner segments of photoreceptors (Figure 1(D)) and in ganglion cells (Bolte et al., 2016; Nießner et al., 2016). Recent data from our group showed that the Cry2 protein is expressed in most cell nuclei of the European robin retina. This localization strongly suggests that avian Cry2 plays a role in the circadian clock and not in magnetoreception because clock proteins affect DNA transcription in cell nuclei. Out of all known isoforms, Cry2b and Cry4b are the most recent additions. Hochstoeger et al. (2020) showed Cry2b expression in pigeon retina and we discovered Cry4b in European robin retina homogenates (Einwich et al., 2020). However, as we do not have antibodies yet, which exclusively identify Cry2b and Cry4b, their cell-specific retinal localization remains unknown.

However, using antibodies that recognize both Cry4 isoforms, Cry4 was detected in the outer and inner segments of double cones and red cones in the European robin and chicken retina (Günther et al., 2018; Figure 2(B)). In outer segments, it colocalized with iodopsin, the opsin molecule of red and double cones (Günther et al., 2018). This localization is highly interesting because 1) iodopsin was identified as an interaction partner of Cry4 in an independent study (Wu et al., 2020) and 2) double cones were hypothesized to be ideal locations for putative magnetosensors (Hore and Mouritsen, 2016; Worster et al., 2017). This is because Cry4 could be aligned in one direction in the principal member and in another in the accessory member of the double cone (Figure 1(C)). Such a differential alignment would allow the two members of the double cone to generate two different magnetic signals although they receive very similar light intensities (Hore and Mouritsen, 2016; Worster et al., 2017). To date, Cry4 seems to be the most plausible candidate as magnetosensor in the light-dependent magnetic compass of night-migratory birds, not least because it is known to bind FAD (Zoltowski et al., 2019), the light-absorbing co-factor which is required for the potential magnetoreceptive function of any Cry.

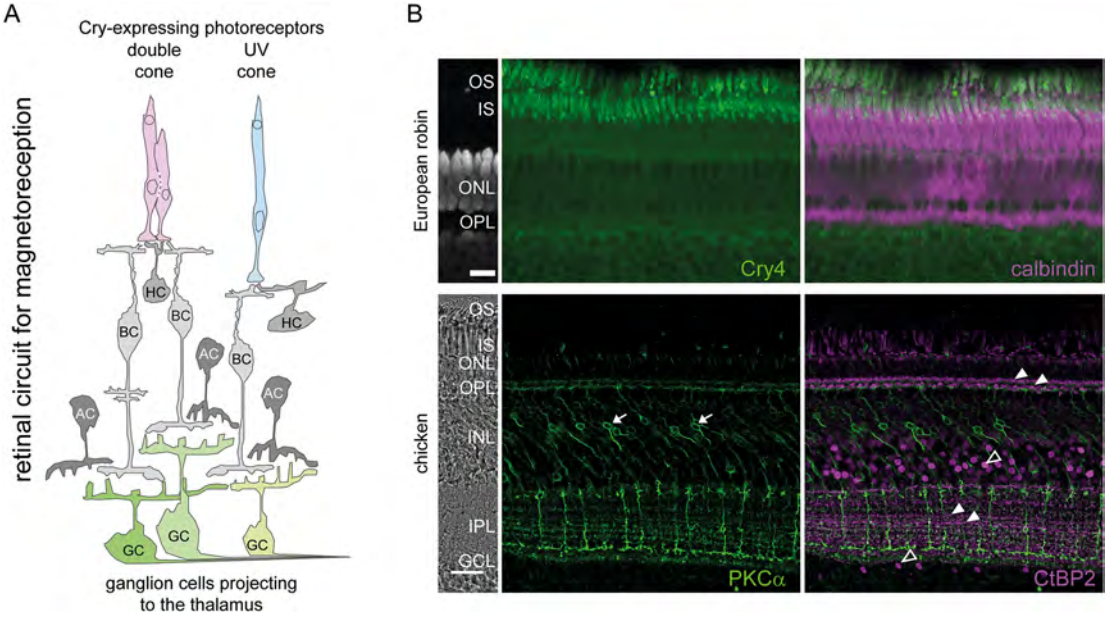

Figure 2: Studying magnetoreception in birds.
(A) Putative retinal circuit for magnetoreception in night-migratory birds. (B) Cry4 is expressed in calbindin-labeled double cones in the European robin retina (top panel). Bipolar cell markers and synaptic markers can be used to study the retinal circuitry for magnetoception (bottom panel). Here, antibodies for protein kinase C alpha (PKCα) and C-terminal binding protein 2 (CtBP2) were used as a marker for distinct populations of bipolar cells (arrow) and all ribbon synapses (filled arrow heads), respectively, in the chicken retina. Please note that CtBP2 also labels the somata of amacrine cells in the bird retina (open arrowheads). Scale: 10 µm (top); 20 µm (bottom). GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; IS, inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, outer segments.

Conclusions and open questions

In summary, there is substantial evidence that light-dependent magnetoreception starts in the retina of night-migratory birds, potentially in Cry4-expressing double cones. Magnetic information likely leaves the retina via ganglion cells that project to the thalamus. However, several questions remain unanswered:

Which bipolar cells/horizontal cells and amacrine cells are involved in magnetoreception?

So far, little is known about the detailed connectivity of the bird retina. Recent transcriptomic data from chicken provided a list of more than 120 retinal cell types (Yamagata et al., 2021) but no information on the morphology and—more importantly—the connectivity of these cell types. If we want to understand magnetoreception in night-migratory birds, we first need to study the different bipolar cells in the avian retina. This can be done by labeling these individual cell types with different markers (see an example for chicken retina in Figure 2(B)). This approach has been successfully employed in the mouse retina before (Wässle et al., 2009). However, as it is unlikely that we will find individual markers for all 15–22 bipolar cell types, a new and a more practical approach might be needed. Serial electron microscopy allows us to generate high-resolution three-dimensional images from a single piece of retina and can be used to reconstruct individual cells (Helmstaedter, 2013). This approach would elucidate the morphology and stratification pattern of bipolar cells (and horizontal cells) and their connections with photoreceptors. Specifically, it would enable us to identify the bipolar cells that contact double cones because they are likely to carry magnetic signals from the photoreceptors to the ganglion cells. Similar experiments can be used to label or reconstruct amacrine and ganglion cells (Haverkamp et al., 2021) and study their connectivity with candidate bipolar cells from the other end of the retina.

Are both Cry4 isoforms involved in magnetoreception?

To date, Cry4 is the most promising candidate as magnetosensor molecule. In European robins, it is upregulated during migratory season (Günther et al., 2018). It is also known to bind the co-factor FAD which is crucial for radical-pair formation and initiation of light-dependent magnetoreception (Xu et al., 2021; Zoltowski et al., 2019). In contrast, FAD binding by Cry1 and Cry2 is highly debated (Günther et al., 2018; Kutta et al., 2017). Cry4a proteins show a robust magnetic field effect *in vitro* (Xu et al., 2021; for more details, please see article by Bartölke et al., 2021). We recently identified a second isoform (Cry4b) which contains an additional exon (Einwich et al., 2020). When we analyzed the daily expression pattern of both isoforms, we found interesting differences between them because mRNA levels of Cry4a did not change throughout the day whereas Cry4b levels did (Einwich et al., 2020). This may point to a role for Cry4b in the circadian clock and suggest that the two Cry4 isoforms fulfill different functions in the European robin retina. Whether Cry4b is also expressed in the outer segments of double cones in the European robin remains to be seen. To solve this question, we need isoform-specific antibodies for all Cryptochrome proteins.

How is the magnetic sensor molecule aligned?

As mentioned above, one essential requirement for any magnetosensor molecule (also cryptochromes) is that it needs to be restricted in movement and rotation (Hill and Ritz, 2010; Lau et al., 2010; Solov'yov et al., 2010), i.e., it needs to be aligned and anchored to a cellular structure, like a membrane. The outer segments of photoreceptors are densely packed with membrane stacks (Figure 1(D)) which may keep cryptochrome molecules aligned and stable (Figure 1(E) and (F)). The expression of Cry4 in double cones may add another advantage: the two members of the double cone that get hit by the same light intensity can respond to different magnetic inclination angles when Cry4 is oriented differently in each member (see Figure 1(E); Hore and Mouritsen, 2016; Worster et al., 2017). Similarly, neighboring double cones may have different orientations in the retina, when the Cry4 molecules are oriented in the same way in the principal and accessory member of a double cone (Figure 1(F)). In both cases, such an arrangement (different orientation within one double cone or between neighboring double cones) would make the magnetic compass system independent from the actual light intensity. While it seems difficult to test for the orientation of molecules in individual double cones right now, comparing the orientation of double cones across the avian retina may be possible.

Do retinal neurons change their membrane potential in response to magnetic stimuli?

In every sensory system, the physical stimulus (here the change in inclination angle of magnetic field lines) needs to be transduced into an electrical signal, i.e., a change in membrane potential of the cell. How this is achieved in light-dependent magnetoreception is unclear so far (see also Bartölke et al., 2021) but it is likely that the magnetic field-dependent radical pair will (via some intermediate steps) eventually open some ion channels, causing a change in the membrane potential (Wu et al., 2020). To prove this, it will be necessary to demonstrate this membrane potential change in the retina following a combination of visual and magnetic stimuli. This might not only help us to understand how an inclination compass works in physiological terms, but also has the potential to create a breakthrough in investigating sensory detection limits.

In summary, we have reviewed the evidence that light-dependent magnetoreception has its origin in the retina. However, as many parts of this sensory processing pathway are still missing, we have highlighted exciting paths for future research.

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Bionotes

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Review article

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The neuronal correlates of the avian magnetic senses

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Abstract: In addition to other natural orientation cues such as the stars, the sun, landmarks and olfactory cues, migrating birds possess the ability to orient by the Earth's magnetic field. In recent years, neuroscientific research has pinpointed brain regions and connecting neuronal pathways that seem to be involved in processing magnetic information. To date, the most compelling neuroanatomical and behavioural evidence comes from the visual and trigeminal sensory systems. We expect that navigational information from both systems could be integrated in higher-order brain structures, such as the hippocampus and the "decision-making" caudolateral nidopallium. This review summarizes the current state of research on the neurosensory basis of magnetoreception in birds.

Keywords: magnetoreception; migratory birds; trigeminal system; visual system.

Zusammenfassung: Neben verschiedenen in der Natur vorkommenden Orientierungsreizen wie z.B. den Sternen, der Sonne, visuellen Landmarken sowie olfaktorischen Reizen, nutzen Zugvögel das Erdmagnetfeld zur Orientierung. Neurowissenschaftliche Forschungsansätze haben in den letzten Jahren dazu beigetragen, mögliche an der Magnetrezeption beteiligte Hirnstrukturen sowie ihre Verbindungsstrukturen zu identifizieren. Neurobiologische sowie Verhaltensstudien deuten aktuell auf eine Beteiligung des visuellen sowie trigeminalen Systems an der Magnetrezeption hin. Wir erwarten, dass Navigationsinformationen aus beiden Systemen in hippocampalen sowie präfrontalen Strukturen, wie dem caudolateralen Nidopallium, integriert werden. Dieser Übersichtsartikel bildet den aktuellen Stand der Wissenschaft zu den neurosensorischen Korrelaten der Magnetrezeption von Vögeln ab.

Schlüsselwörter: Magnetrezeption; Trigeminales System; Visuelles System; Zugvögel.

Introduction

Imagine being shipwrecked and stranded on a deserted island. How do you find home? Unlike humans, who rely on modern positioning technologies to cope with such unanticipated situations, migratory birds have proven to successfully navigate thousands of kilometres even over featureless terrain, during night and on their own without regular contact to any conspecifics (Berthold 2017). By now, it is a proven fact that, in addition to natural cues such as the sun, stars, visual landmarks and olfactory gradients, migratory birds can use the Earth's magnetic field as a source of both positional and directional information to help them master their biannual journeys between their breeding and wintering grounds (Mouritsen 2018).

Simplistically speaking, the Earth represents a giant bar magnet. Its magnetic field lines leave the southern magnetic pole and curve around the Earth to re-enter the northern magnetic pole. The steepness of the magnetic field lines relative to the Earth's surface, called "inclination", gradually changes from vertical at the poles to parallel at the magnetic equator. At the same time, the magnetic field intensity gradually increases from ~30 μT around the equator to ~60 μT at the poles (Mouritsen 2015; Wiltschko and Wiltschko 1995). Based on these cues, birds could theoretically derive both directional and positional information from geomagnetic parameters. But how do birds sense magnetic fields?

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Unlike well-known sensory modalities, such as touch, taste, smell, vision or hearing, whose sensing organs and processing brain regions are generally known, magnetoreception is an exception. Magnetic fields pass freely through biological tissue, thus making any sensor "a needle in a haystack" potentially located anywhere in the organism. To date, any proven primary magnetic sensor has remained elusive (Mouritsen 2018).

To serve as a reliable reference cue, magnetic fields have to be actively sensed and translated into a nervous signal. This information needs to be processed and integrated with other cues in the central nervous system (CNS) and, finally, translated into a resulting motor output. Its representation in the CNS could thus be instrumental in elucidating important aspects on the neuronal correlates underlying avian magnetoreception. Indeed, neuroscientific research has pinpointed primary brain relays likely involved in processing magnetic field information in the visual and trigeminal sensory systems (Frost and Mouritsen 2006; Mouritsen et al. 2016). On the contrary, our knowledge, how magnetic information from both systems is being further processed and integrated with other navigational information in the brain, has remained largely hypothetical (Mouritsen et al. 2016). The vestibular system has also been suggested to be potentially involved in processing magnetic cues (Nimpf et al. 2019; Wu and Dickman 2011), but evidence from migratory birds is still missing. In the following sections, we will summarize the current status of research on the neuroanatomical basis of magnetoreception and present some of the exciting open questions we intend to answer in SFB 1372.

Can birds "see" the reference direction provided by the Earth's magnetic field?

Similar to human navigators, who need to identify directions in space to maintain a constant heading while moving, a reliable compass sense ("*In which direction do I want to go*?") is of vital importance for migratory birds to reach their desired goal. Could the Earth's magnetic field serve as a compass cue? In theory, the direction of the magnetic field lines could be used as a reference relative to which birds could orient in any desired direction. However, given the fact that birds have exceptionally well-developed visual systems, birds using visually perceived, directional stimuli, such as the stars or the sun, for a long time sounded far more likely than them being able to orient by the Earth's magnetic field. The latter idea only became scientifically supported when night-migratory songbirds tested in orientation cages without having access to visual cues rotated their season-specific migratory direction accordingly when the magnetic field was rotated likewise. Curiously, the birds did not use the polarity but the inclination of the ambient magnetic field (Wiltschko and Wiltschko 1972, 1995). Later, it was demonstrated that night-migratory birds could only use their magnetic inclination compass when specific colours of light were available in the room (Wiltschko et al. 1993), thus providing the first behavioural evidence suggesting an involvement of the visual system in the perception of magnetic directional information.

Visual information in a bird's brain is processed along three pathways. From the retina, visual information is carried through the optic nerve to the optic tectum in the midbrain (tectofugal pathway), to the thalamic dorsolateral geniculate nucleus (GLd; thalamofugal pathway; Figure 1A, ochre), or to the nucleus of the basal optic root (accessory visual pathway). Currently, most evidence points toward an involvement of the thalamofugal pathway in magnetoreception. GLd projections terminate in the so-called "visual Wulst" in the telencephalic hyperpallium (Figure 1A). The visual Wulst resembles the primary visual cortex in mammals and contains various, retinotopically organised, receptive fields (Bischof et al. 2016), which comprise highly complex neuronal networks extracting various aspects of vision (Güntürkün 2000). A region in its lateral posterior part, called "Cluster N" (Figure 1A, ochre), was shown to display strongly increased neuronal activation in nocturnally orienting migratory birds. Because Cluster N was only activated under low-light conditions and could not be observed in a resident songbird species, Mouritsen et al. (2005) suggested that this night-vision-processing region could be related to the vision-mediated magnetic compass. Indeed, connectivity studies showed that Cluster N receives input from the contralateral eye via the GLd (Heyers et al. 2007; Figure 1A, ochre). The most compelling evidence demonstrating that Cluster N is involved in processing magnetic compass information is that chemical inactivation of Cluster N disrupted magnetic compass orientation in migratory European robins (*Erithacus rubecula*), whereas their sun and star compasses remained unaffected (Zapka et al. 2009; Figure 1B).

As part of our future work, we aim to answer the next obvious questions: "Which specific retinal ganglion cell types send magnetic information to Cluster N?", "Can we detect magnetic signals at the neurophysiological level?", and "Are there further upstream regions processing light-dependent magnetic compass information?"

While behavioural and neuronal activation studies have shown that night-migratory birds have a photo-induced

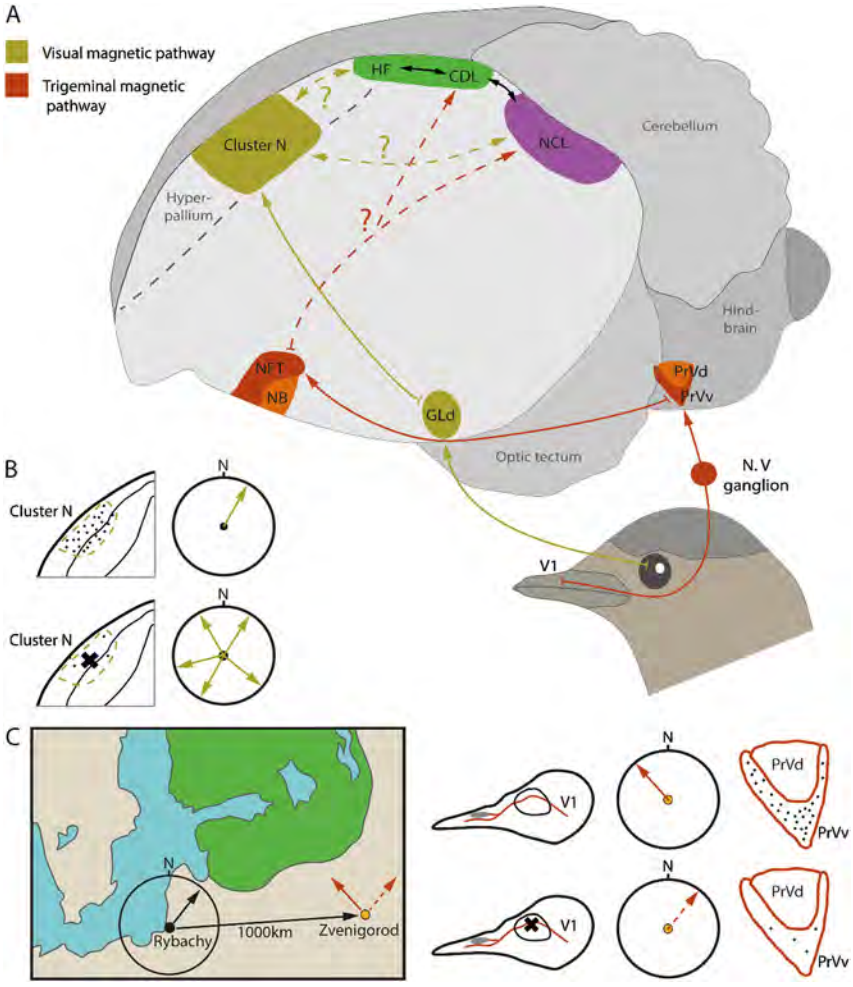

Figure 1: The neuronal correlates of avian magnetoreception.
(A) Schematic drawing of the known brain pathways (solid arrows) involved in magnetoreception and their currently unknown onward projections (dashed arrows with "?") to putative integration centres (updated based on Mouritsen et al. 2016). Light-dependent magnetic compass information from the eye reaches Cluster N via the thalamic GLd (ochre). Trigeminal magnetic information perceived by V1 is processed in PrVv and then passed onto the telencephalic NFT (red). We hypothesise that information from both senses could be integrated and stored in the HF (green) and translated into context-dependent motor output in NCL (violet). (B) Night-migratory songbirds with intact Cluster N show directed magnetic compass orientation (upper panel) but become random after chemical lesion of Cluster N (lower panel; modified from Zapka et al. 2009). (C) Birds display a north-easterly orientation during spring towards their breeding areas (green; modified from Kobylkov et al. 2020). Only birds with intact, but not surgically ablated, V1s compensate for a virtual magnetic/geographic displacement 1000 km eastwards by turning their migratory direction towards north-west (left panel; modified from Pakhomov et al. 2018). Magnetic fields activate the ventral part of PrV. This activation significantly drops after V1 ablation (right panel; modified from Heyers et al. 2010). Activated neurons in B and C are depicted as black dots. Abbreviations: CDL, dorsolateral corticoid area; GLd, dorsolateral geniculate nucleus; HF, hippocampal formation; N. V ganglion, trigeminal nerve ganglion; NB, nucleus basalis; NCL, caudolateral nidopallium; NFT, telencephalic frontal nidopallium; PrVd, dorsal part of the principal trigeminal brainstem nucleus; PrVv, ventral part of the principal trigeminal brainstem nucleus; V1, ophthalmic branch of the trigeminal nerve.

magnetic compass mechanism located in both of their eyes (Engels et al. 2012; Hein et al. 2010, 2011; Liedvogel et al. 2007), the underlying primary magnetic sensors remain unknown. Within the retina, various cell types have been suggested to harbour the primary magnetic sensors which are most likely to be a cryptochrome protein (Bolte et al. 2021; Einwich et al. 2020; Günther et al. 2018; Zoltowski et al. 2019). Cryptochromes are magnetically sensitive proteins (Xu et al. 2021) which are likely to form the molecular basis for the so-called "radical-pair mechanism" of magnetoreception, which represents one of the most frequently suggested theories on avian magnetoreception (Hore and Mouritsen 2016;

Maeda et al. 2012). A deeper insight into the molecular and quantum chemical details of the cryptochrome-based avian magnetic compass is provided in accompanying chapters of this issue.

Nature's GPS – a trigeminal-based "map" sensor in the upper beak?

While a compass mechanism can enable birds to define directions in space, it does not allow any conclusions about position ("*Where am I?*"). The birds' ability to determine their position is readily attested by their remarkable ability to return to the same breeding and wintering sites year after year even after dramatic geographical displacements away from their usual migration route (Chernetsov et al. 2008, 2017; Kishkinev et al. 2013, 2021; Thorup et al. 2007; Wynn et al. 2020). Visual "landmarks" or olfactory gradients can serve as a meaningful positional reference to navigate over at least short to middle distances (Gagliardo 2013). However, more globally available positional cues are needed when navigating over larger scales. Could the Earth's magnetic field provide this kind of information? Certain combinations of geomagnetic isolines in some regions on Earth form a reasonably consistent grid and thus could represent one possible source for positional information (Heyers et al. 2017; Mouritsen 2018; Wynn et al. 2020), but the exact combination of magnetic parameters being used by night-migratory songbirds has not yet been unequivocally documented (Chernetsov et al. 2017, 2020; Pakhomov et al. 2018).

On the level of the CNS, accumulating evidence suggests that the trigeminal system, and in particular, the ophthalmic branch of the trigeminal nerve (V1; Figure 1A, red) is involved in sensing magnetic *positional* cues. Early electrophysiological studies reported magnetic stimulus-dependent responses from V1 (Semm and Beason 1990). In a conditioned choice experiment, pigeons were shown to no longer be able to distinguish between the presence or absence of a strong magnetic anomaly, when their V1s were ablated (Mora et al. 2004). Direct evidence that V1 might actually be involved in sensing magnetic *positional* information comes from displacement experiments in which long-distance, night-migratory songbirds were either physically or virtually magnetically translocated from their migration route. While birds with intact V1s compensated for the displacement by reorienting towards their breeding grounds, individuals with bilaterally ablated V1s, which prevents any neuronal information from this nerve to reach the brain, failed to correct for the displacement and headed in the same direction as before the displacement (Kishkinev et al. 2013; Pakhomov et al. 2018; Figure 1C).

Within the brain, V1 projects to the principal trigeminal brainstem nucleus (PrV; Wild and Zeigler 1996). PrV is subdivided into an oval-shaped dorsal and a ventrally adjacent, crescent-shaped part (Figure 1A, red). Only the latter was shown to display significantly increased neuronal activation when migratory birds were exposed to a strongly changing magnetic field stimulus. This activation was significantly reduced after the ambient magnetic field was compensated or V1 was ablated (Elbers et al. 2017; Heyers et al. 2010; Lefeldt et al. 2014; Figure 1C, right panel). These findings indicate that V1 indeed carries magnetic information and that the ventral PrV part might represent the first relay station for trigeminally perceived positional information in the brain.

Recent neuronal tract tracing studies within the SFB discovered a specific, previously unknown connection between the magnetically activated ventral PrV and the telencephalic frontal nidopallium (NFT; Figure 1A, red). The NFT could thus represent the next upstream brain relay of a neuronal pathway, with which birds seem to process trigeminally perceived magnetic information (Kobylkov et al. 2020). At the moment, we are studying where this information is further processed.

Another key question is, "What type of sensor underlies trigeminally mediated magnetoreception?" The known magnetic properties of some naturally occurring materials, such as magnetite, led to the straightforward suggestion that the responsible magnetoreceptor could be based on biogenic iron. Indeed, as per theoretical considerations, iron-containing magnetic minerals, associated with nervous tissue, could serve as magnetosensory structures (Winklhofer and Kirschvink 2010). Active alignment of these structures in an external magnetic field could exert a torque that could lead to opening or closing of mechanosensitive ion channels and thus a nervous signal (Lohmann 2010). This idea was supported by behavioural experiments in which strong, directed, magnetic pulses that remagnetise any proposed iron-based magnetosensors led to temporary deflected orientation responses of night-migratory songbirds (Holland 2010; Munro et al. 1997; Wiltschko et al. 2009).

Yet another key question is, "Where is the trigeminal magnetic sensor located?" While iron-containing structures were described in multiple locations within various body parts of birds (Shaw et al. 2015), the claimed identification of iron particles at six specific locations in bilateral

symmetry within V1 terminals inside the birds' upper beak (Fleissner et al. 2003) made the magnetoreception community believe that it might have identified the long-sought trigeminal magnetic sensor. However, in an attempt to replicate these findings, the previously described iron-containing structures turned out to be diffusely distributed in the upper beak in highly variable numbers between individuals. Furthermore, the iron-containing structures were not consistently colocalized with neuronal tissue but rather with macrophage-specific markers. It is thus almost certain that the previously proposed magnetosensitive neurons were in fact macrophages (Engels et al. 2018; Mouritsen 2012; Treiber et al. 2012, 2013), which yet again has made the identification of any trigeminal-based magnetic sensors one of the most pressing questions in the magnetoreception community. We will use a multitude of techniques from neurobiology to biophysics with the aim to narrow down and eventually find the locations of any trigeminal-based magnetic sensors.

Where and how is multisensory navigational information integrated and translated into motor output?

Neither a directional sense nor a positional sense by itself will guide you home from an unknown location. Thus, it is clear that multifactorial input needs to be integrated at higher brain levels.

The hippocampal formation (HF; Figure 1A, green) is one of the best-studied brain integration centres in the context of spatial orientation and cognition. In mammals, the HF contains place, grid and head direction cells encoding for directional and spatial information on a mental grid. Although the existence of such neurons in birds has not been proven yet, the HF is almost certainly involved in navigational processes in birds. The HF is usually larger in species whose lifestyle requires a higher demand for spatial memorization processes (Krebs et al. 1989; Pravosudov et al. 2006), and the HF was shown to display increased neuronal activation in pigeons when navigating by familiar landmarks within their home range (Shimizu et al. 2004). Currently, we investigate whether the HF might receive magnetic compass information from Cluster N along the visual streams (Atoji and Wild 2004; Figure 1A, ochre) as well as trigeminal magnetic map information from NFT, probably via the dorsolateral corticoid area (CDL; Atoji and Wild 2012; Kobylkov et al. 2020; Figure 1A, red).

The final decision to take off in a set direction and to translate all needed multisensory navigation-relevant information into motor action not only requires the implementation of all available navigational information but also heavily depends on limbic input. The limbic system supports a variety of functions, such as aiding the formation of long-term memory and informs about nutrition, health, weather and thus the resulting emotional and motivational status of the animal (Güntürkün 2005). This could happen in the caudolateral nidopallium (NCL; Figure 1A, violet), which, based on histochemical, behavioural and electrophysiological evidence, is considered the avian analogue of the mammalian prefrontal cortex and, thus, responsible for setting executive orders (Güntürkün 2005). The NCL has been shown to receive input from various limbic structures, is connected to the visual and trigeminal systems in a strictly topographic manner in pigeons (Kröner and Güntürkün 1999) and closely interacts with the HF (Shanahan et al. 2013; Figure 1A). Finally, the NCL also seems to be of central importance for sensory to motor transition. "Motor clusters" in the mesopallium and nidopallium, shown to display neuronal activation during limb movement (Feenders et al. 2008), might function as a linking part between the NCL and the two main motor output pathways in birds (Mouritsen et al. 2016). The connectivities of the NCL and HF and if/how magnetic information from both the visual and trigeminal sensory systems is integrated are in the focus of our current studies.

Conclusion

Neuroanatomical studies and the analyses of behaviour-induced brain activation patterns have identified complex, multifactorial networks in the visual and trigeminal systems, which are likely to be involved in processing navigational information, but many open questions remain. Taking advantage of the interdisciplinary expertise of the collaborating groups, SFB 1372 "Magnetoreception and Navigation in Vertebrates" aims to identify and understand the neuronal basis of avian magnetoreception from the sensor level up to its entire representation in the brain.

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Book Review

Frank W. Stahnisch, *A new field in mind. A history of interdisciplinarity in the early brain sciences*, McGill-Queens University Press, Montreal, PQ and Kingston, ON (Canada) 2020, 600 pp, CAN$ 65, ISBN: 978-0-773-55932-5.

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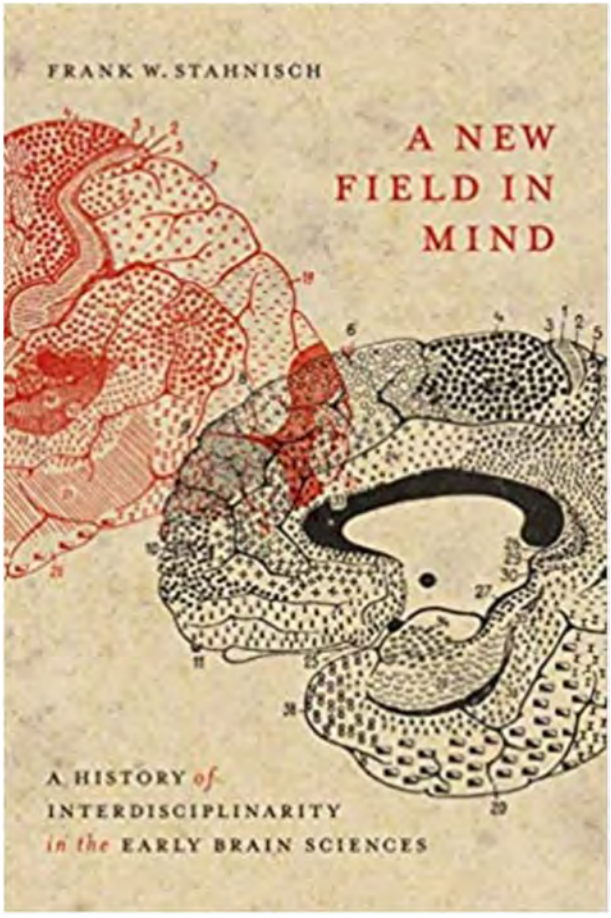

https://www.mqup.ca/new-field-in-mind–a-products-9780773559325.php.

On 14 December 1882, the Viennese Medical Faculty approved the inauguration of the Institute for the Anatomical and Physiological Study of the Central Nervous System by Heinrich Obersteiner (1847–1922) – the first interdisciplinary "neuroscience" institute worldwide. Frank W. Stahnisch, AMF/Hannah Professor in the History of Medicine and Health Care at the University of Calgary in Canada, offers in his new book a fascinating revisionary historical account of the development of modern neuroscience. Whereas it is commonly held that the new field of interdisciplinary neurosciences was formed in an Anglo-American context, Stahnisch shows the origins of the field in Europe and the formative influence of emigré scientists.

Stahnisch is both trained in history of medicine and in the clinical neurosciences. This allows him to bring to bear his personal experience on the historical material. And more importantly, it allows him to add a lot of oral history material provided by the interviews with neuroscientists, neurologists, and psychiatrists. They were conducted to elucidate the historical complexity of traditions and ideologies in the field.

In the published excerpts from interviews with German-speaking neurologists, the idea of modern neuroscience centers was rather connected to modern research institutes and centers in Germany since the 1990s. Stahnisch can show that many interviewees believe that the organization of such centers originated in models developed in twentieth-century American neuroscience. These models are seen to have only gradually made their way into German-speaking academia through international contacts and relations established during the Post-War Period.

Against this historical perception, Stahnisch emphasizes the pioneering collaborations in the 19th-century brain sciences at centers in Austria, Germany, and Switzerland. They had an important impact on the development of the modern neurosciences in the first half of the twentieth century, and specifically on the establishment of neuroscience institutes in North America, the new home of so many continental émigré neuroscientists during the Nazi period. These concepts made their way back only later and provided templates for contemporary neuroscience centers in Europe.

In terms of methodology, Stahnisch uses case studies and collective biographies for the examination of the evolving relationships between individual disciplines, such as anatomy, physiology, neurology, psychiatry and neurosurgery. These relationships created new philosophical and social contexts for brain research.

The book is organized in 9 chapters and provides interesting perspectives on how the changing political conditions in Central Europe (the German Empire, First World War, the Weimar Republic, National Socialism, the Second World War, and the immediate Post-war Period) have influenced the development of the neurosciences internationally. The final two chapters analyse the

enormous impact ("the brain drain") of the expulsion of so many neurologists and psychiatrists during the 30s and 40s of the last century.

Stahnisch critically examines the influences of changing political conditions in Germany, Austria and Switzerland and their neighboring countries on the research questions, technological and economic support for neuroscience research during the time period analyzed,

"… by drawing attention to the marginalization and later expulsion of a large number of Jewish and oppositional German-speaking émigré neuroscientists, and their move to North America. The forced migration wave of neurologists, psychiatrists, neuropathologists, and many other medical researchers in this field indeed provides us with ample perspective on the effect of culturally laden research styles, the enhancement of knowledge, and the advancement of particular areas of the neurosciences." (p. 66). Stahnisch highlights "that major reassessments were well underway from the 1910s to the 1930s in Central Europe. Their pace was accelerated, however, and they were thoroughly transformed through their integration into the pre-existing medical and scientific cultures of émigré neuroscientists' new host countries, the United States and Canada." (ibid.).

This well researched book provides many thought-provoking insights and gives intriguing examples of the changes in neurology and psychiatry, as they took place in the early Post-War Period and the changing research cultures in the lab and at the bedside. It emphasizes that the pioneers of modern neuroscience in fact had "*a new field in mind*."

This timely book is complemented by a substantial and up-to date bibliography of primary and secondary sources (66 pp.), a useful name and subject index (46 pp.) as well as graphic representations of institutes and research schools (13 pp.). It can be recommended to all neuroscientists interested in history and philosophy. Science administrators and young researchers will also profit from reading the book by better understanding the foundations of the neurosciences.

Presentation of scientific institutions

Christian Geis* and Stefan Hallermann

DFG research unit 3004: "Synaptic pathology in autoimmune encephalitis" (SYNABS)

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Keywords: autoimmune encephalitis; electrophysiology; LGI1; NMDA receptor; super-resolution imaging; synaptic pathology.

The concept of pathogenic autoantibodies mediating neurological disease was first established in the peripheral nervous system in the 1970s. The prototype of these disorders is Myasthenia gravis with antibodies to the nicotinic acteylcholin receptor leading to impaired neuromuscular transmission and muscle weakness (Toyka et al., 1975). In recent years, the discovery of autoantibodies against synaptic antigens in the central nervous system (CNS) in patients with severe neuropsychiatric disorders was a breakthrough in neurology (Dalmau et al., 2007). As of today, more than 15 target molecules have been identified to which specific autoantibodies are directed, each defining a subtype of disease (Dalmau et al., 2017). Interestingly, all of these target antigens are part of central synapses and are comprised of ionotropic and metabotrobic receptors (e.g. NMDA, Glycine and $GABA_B$ receptors) as well as adhesion and transsynaptic signaling molecules (e.g. LGI). The resulting diseases represent a novel entity of CNS disorders that has been termed "autoimmune encephalitis".

Antibodies targeting neuronal surface proteins can have diverse and multifaceted pathological mechanisms, e.g. internalization of target molecules, direct interaction with the target antigen function, or secondary effector activation. In addition, the consequence of any of those antibody-induced effect on neuronal function critically depends on the respective target antigen. So far, the underlying pathophysiology including the molecular interactions and the often detrimental impact of the antibodies on neurons, synapses, and consequently on network function are only partly understood. As a consequence, target-specific therapies are currently not available (Sell et al., 2021).

The DFG Research Unit (RU) *"Synaptic pathology in autoimmune encephalitis" (SYNABS)* brings together clinician scientists in the field of antibody-mediated immunological disorders with basic scientists in the field of neurophysiology, molecular neurobiology, and neuroimmunology jointly investigating autoantibody-mediated pathology in the CNS. The aim of SYNABS is to elucidate the pathophysiology of autoimmune encephalitis. We will apply new and state-of-the art technology, reaching from various passive transfer murine and natural feline models, to advanced electrophysiological methods, super-resolution imaging, and engineering of human antibodies (Haselmann et al., 2018; Kreye et al., 2016; Werner et al., 2021). Furthermore, as a proof of concept, the human immunopathology will also be assessed in autopsy material from deceased patients (Hoftberger et al., 2015).

The RU started its work in 2020 and consists of eight scientific projects (Figure 1). The RU is led by Christian Geis at the Jena University Hospital together with the deputy speaker Stefan Hallermann at the University of Leipzig. Further members are Manfred Heckmann (University of Würzburg), Markus Sauer (University of Würzburg), Ryuichi Shigemoto (Institute of Science and Technology – IST) Austria, Harald Pruess (Charité University Medicine and German Center for Neurodegeneative Disorders, DZNE Berlin), Hedda Wardemann (German Cancer Research Center, DKFZ Heidelberg), Romana Höftberger (Medical University of Vienna, Austria), Claudia Sommer (University Hospital Würzburg), Carmen Villmann (University Hospital Würzburg), Dietmar Schmitz (Charité University Medicine Berlin), Angela Kaindl (Charité University Medicine Berlin), Knut Kirmse (University of Würzburg), Michael Hust (Technical University of Braunschweig). Moreover, Joseph Dalmau as Mercator-Fellow from the University of Barcelona.

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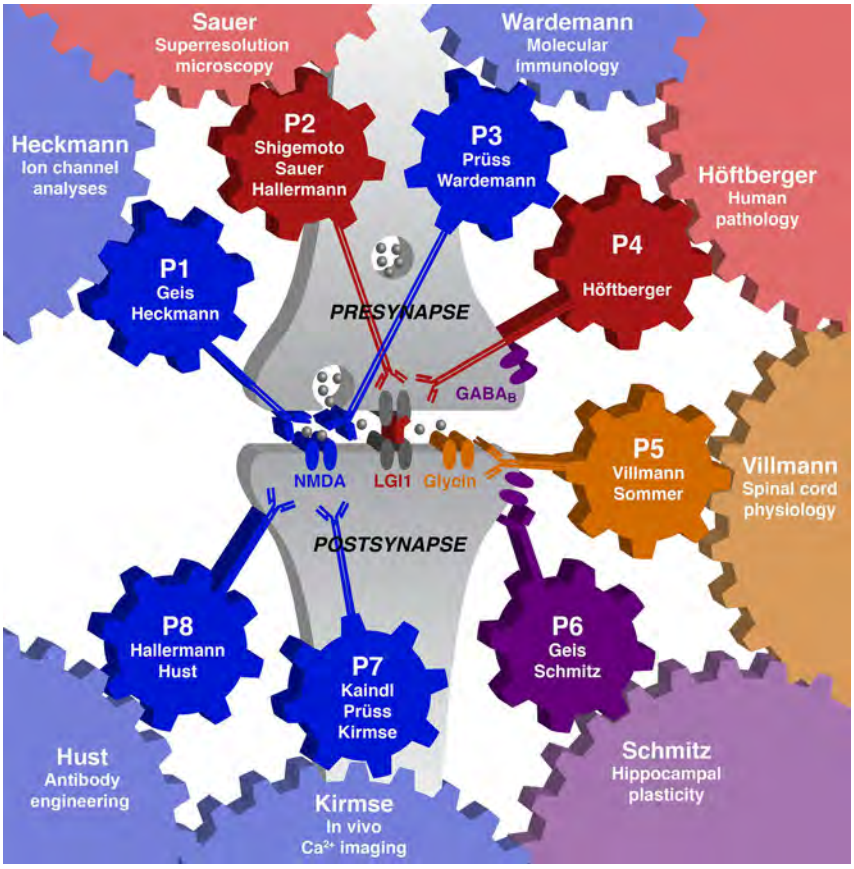

Figure 1: SYNABS FOR3004 scientific projects – Cooperating researchers and research focus: Each subproject is illustrated by a gearwheel. The stylized antibodies point towards the synaptic epitope on which the project focuses on. The outer gearwheels illustrate the cooperating researchers providing specific techniques and expertise that are essential for the project.

By using the synergistic experimental expertise of the consortium we aim to achieve the overarching goal of (1) in-depth understanding the basis of autoimmune encephalitis and (2) development of target- and tissue-specific therapeutic tools in animal models that hopefully may translate to the afflicted patients.

We hereby focus on the four most relevant synaptic targets within the spectrum of autoimmune encephalitides: the NMDA glutamate receptor, LGI1 which is a transsynaptic linker protein of excitatory synapses, the $GABA_B$ receptor, and the glycine receptor (Figure 1).

The NMDA receptor encephalitis is the most common subtype of autoimmune encephalitis and we will investigate antibody-mediated NMDA receptor dysfunction with several projects covering the entire spectrum from basic experiments investigating ion channel malfunction, neuronal and network disturbance, use of animal models of disease up to direct translational projects aiming at development of target-specific therapeutics.

The transsynaptic linker protein LGI1 is the target of the second most frequent subtype of autoimmune encephalitis with distinct clinical characteristics. Because of its mechanistic complexity and the unclear immunopathologic mechanisms in the central nervous system we will start with projects on the basic mechanisms and on the immunological aspects of LGI1 encephalitis.

The $GABA_B$ receptor is the most frequent antigen in autoimmune encephalitis with antibodies to metabotropic receptors. Applying the combined expertise of our consortium in investigating defective $GABA_B$ receptor signaling allows unraveling the exciting interplay of disturbed presynaptic function and autoimmune brain disease.

Finally, we study autoimmune disorders mediated by antibodies to glycine receptors because this prototypical spinal antigen offers the chance to understand the pathogenic sequence from synaptic dysfunction to neuronal network disturbance and to disease in an extraordinarily well-understood neuronal network.

The RU investigates fundamental questions such as:

- What are the pathomechanisms at the level of proteins and individual synapses?
- Can these pathomechanisms be tested in disease models?

- Is it possible to establish direct translational approaches by therapeutic antibody neutralization?
- Can autoantibodies to CNS antigens also cause neurodevelopmental defects in newborn children through transplacental transmission?

Overall, SYNABS is a multidisciplinary and comprehensive research unit with a high emphasis on translational approaches which aim to understand and develop novel therapeutics in neurobiological underpinnings of emotion dysfunctions and bridge the gap between clinical and basic neurosciences.

Homepage: https://www.uniklinikum-jena.de/synabs/en/.

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Nachrichten aus der Gesellschaft

https://doi.org/10.1515/nf-2021-0017

Göttingen went virtual

Albert C. Ludolph

Diese Ausgabe enthält bereits den Call for Symposia für die Göttinger Tagung 2023. Dabei ist der letzte Tag der diesjährigen Tagung, der 31. Mai 2021, an dem die Online-Stellung der Beiträge für die Tagung des Jahres 2021 endete, gerade einmal drei Monate her. Wir möchten an dieser Stelle auf die virtuelle Tagung der NWG 2021 zurückblicken.

Zuerst ein kurzer Blick auf die Zahlen der virtuellen Tagung. Sie übertrafen bei weitem die Erwartungen, denn verglichen mit dem Vorjahr waren zu Beginn der Planung große Einbußen an Registrierungen befürchtet worden. Tatsächlich lagen diese aber mit knapp über 1.100 registrierten Teilnehmenden nur um 24 % unter der Zahl der letzten Tagung im Jahr 2019. Auch die Entwicklung der Zahl der Teilnehmenden aus dem Ausland war sehr erfreulich: insgesamt nahmen 395 ausländische Wissenschaftler*innen aus 32 Ländern an der Tagung teil, das entspricht knapp einem Viertel aller Teilnehmenden, was sogar eine erhebliche Steigerung zur letzten Tagung bedeutet, bei der etwa ein Sechstel der Teilnehmenden aus dem Ausland kamen. Hier machte sich die Tatsache, dass nicht gereist werden musste, bemerkbar.

Leider ist die Beteiligung der NWG-Mitglieder an der Tagung im Vergleich zu 2019 zurückgegangen. Waren 2019 noch knapp die Hälfte der Teilnehmenden NWG-Mitglieder, so waren es 2021 nur noch ein Drittel. Das mag zum einen im gestiegenen Anteil an ausländischen Teilnehmenden begründet sein, spiegelt aber auch wider, was wir seit längerem beobachten: es gibt noch ein sehr großes Potential für mehr NWG-Mitglieder in der deutschen Neurolandschaft. Gerade Personen in Schlüsselpositionen sind oft noch keine NWG-Mitglieder. Diese zu gewinnen hat sich der neue Vorstand zur Aufgabe gemacht und wir möchten auch jedes Mitglied bitten, sich bei dieser Sache einzubringen. Werben Sie an Ihrem Standort für eine Mitgliedschaft in der NWG! Die Geschäftsstelle unterstützt Sie bei diesem Vorhaben gerne mit Material, Texten und Informationen.

Doch zurück zur Tagung: ebenso wie die Zunahme der Beteiligung aus dem Ausland ließ auch das Geschlechterverhältnis der Teilnehmenden mit 52 % weiblichen und 48 % männlichen Teilnehmenden nichts zu wünschen übrig.

Das wissenschaftliche Programm bestand aus: Symposien, Hauptvorträgen, Workshops und Postern. Im Rahmen der 33 Symposien fanden insgesamt 196 Vorträge statt, davon 65 Kurzvorträge von Studierenden. Weitere 10 studentische Kurzvorträge wurden im Breaking News Symposium gehalten, das ausschließlich Studierenden vorbehalten war. Wie bei der vergangenen Tagung hat das Programmkomitee versucht, damit der Altersstruktur der Tagung Rechnung zu tragen. Von den 131 Symposiumsredner*innen kam fast die Hälfte (63) aus dem Ausland, ebenso wie fünf der insgesamt sechs geladenen Hauptredner*innen, der siebte Hauptvortrag war die Schilling Forschungspreis Lecture.

Auch die traditionell angebotenen Workshops zu den Themen Tierschutz, wissenschaftliches Publizieren und DFG-Förderung konnten angeboten werden und wurden gut angenommen. Ergänzt wurden sie noch von einem Workshop des Bernstein Netzwerks zu dessen neuesten Initiativen und einem von NFDI organisierten Workshop zur Forschungsdateninfrastruktur. Neu war, dass auch Industriepartner Workshops durchführen konnten, als Alternative zur immer gut besuchten Industrieausstellung im Hörsaalgebäude.

Für die virtuellen Poster standen auf der Website mehrere Präsentationsmöglichkeiten zur Verfügung: als PPT-Video und PDF und fakultativ durch die Bereitstellung von Zusatzmaterial. Die Zahl der Poster war geringer als bei den vorherigen Tagungen: 348 statt 673 Poster in 2019.

Der Trend zu einer zunehmend „jungen“ Tagung“, der sich bei den letzten Meetings schon manifestiert hatte, hat sich bei der virtuellen Tagung nochmals verstärkt. 69 % der Teilnehmenden waren unter 40 Jahre alt, wobei die jüngste Gruppe (20–29 Jahre) mit 38 % aller Teilnehmenden die insgesamt stärkste ausmachte. Analog dazu war die Gruppe der Studierenden mit 49 % die stärkste Teilnehmergruppe, gefolgt von den Postdocs mit 33 %. Somit konnte sich die Tradition, dass Nachwuchswissenschaftler*innen dieses Forum nutzen, um eigene wissenschaftliche Arbeiten vorzustellen und sich über neueste Entwicklungen auf dem Gebiet der Neurowissenschaften zu informieren, mit der virtuellen Tagung fortsetzen.

Auf den ersten Blick können sich die Zahlen sehen lassen. Doch spiegelt sich dieser erste positive Eindruck auch in der Bewertung der Tagung durch die

Teilnehmenden wider? Das kann mit einem klaren Ja beantwortet werden.

An der Teilnehmerbefragung haben vorwiegend jüngere Personen im Alter unter 40 Jahren teilgenommen (240), also die Zielgruppe der Göttinger Tagung. Die Umfrage zeigt, dass das wissenschaftliche Programm und die Möglichkeiten zu einer aktiven Teilnahme an der Tagung durchschnittlich zu 70 % mit exzellent bis gut bewertet werden.

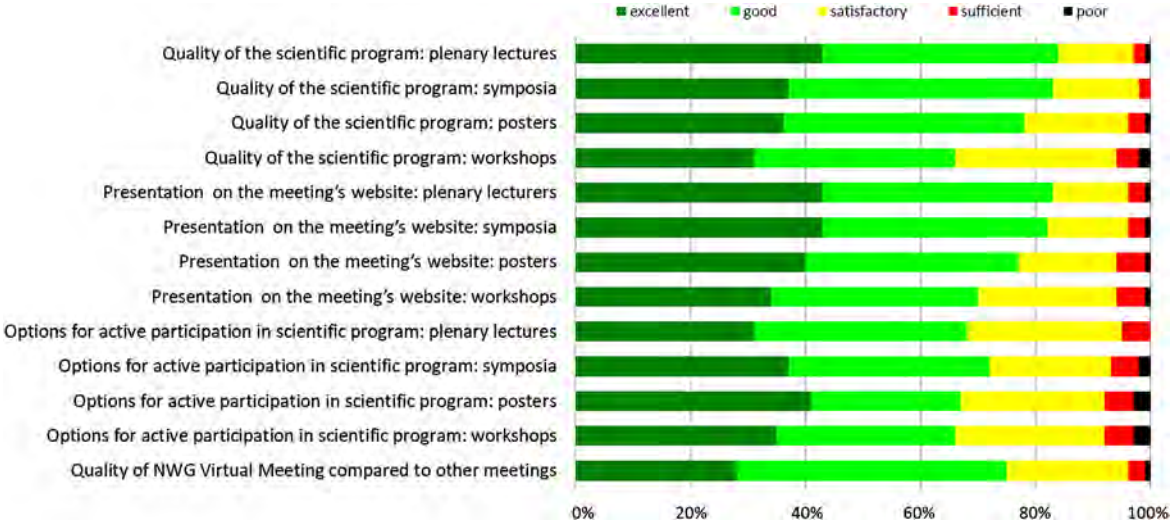


Abb. 1: Ergebnis der Umfrage zur Qualität des wissenschaftlichen Programms und zu den Möglichkeiten einer aktiven Teilnahme an der Tagung

Es ist sehr erfreulich, dass knapp die Hälfte der Befragten sich aktiv an den Diskussionsrunden beteiligt hat. Dieser hohe Anteil an Diskussionsbeiträgen erzeugt von aktivem Interesse. Dies zeigt, dass es auch in der Zukunft sinnvoll ist über das Format dieser Diskussionsrunden, auch bei den gewohnten Tagungen, nachzudenken.

Die Teilnehmerzahlen der Live-Lectures sind allerdings nicht mit denen eines Hauptvortrags im großen Göttinger Hörsaal mit 500 bis 600 Zuhörenden vergleichbar. Bei beiden Vorträgen waren jeweils um die 200 Personen zugeschaltet. Aber auch die Symposien konnten Diskussionsteilnehmer*innen z.T. im höheren zweistelligen Bereich verzeichnen. Eine besonders lebhafte, engagierte, ungezwungene und weit über die vorgesehene Zeit hinausgehende Diskussion erlebte das von der neuen Sektion Junge NWG (jNWG) organisierte Symposium. Auch die traditionell von der NWG organisierten Live-Workshops (Tierversuche verstehen, DFG und Erfolgreich Publizieren) waren mit zwischen 80 und 118 Teilnehmenden sehr gut besucht.

Die anfängliche Befürchtung, dass die Diskussionsrunden aus Mangel an Interesse nicht stattfinden würden, hat sich in keiner Weise bewahrheitet. Aus dieser Befürchtung heraus war eine vorherige Anmeldung für die Diskussionsrunden verpflichtend festgelegt worden. Dies war allerdings zum Teil auf Kritik bei den

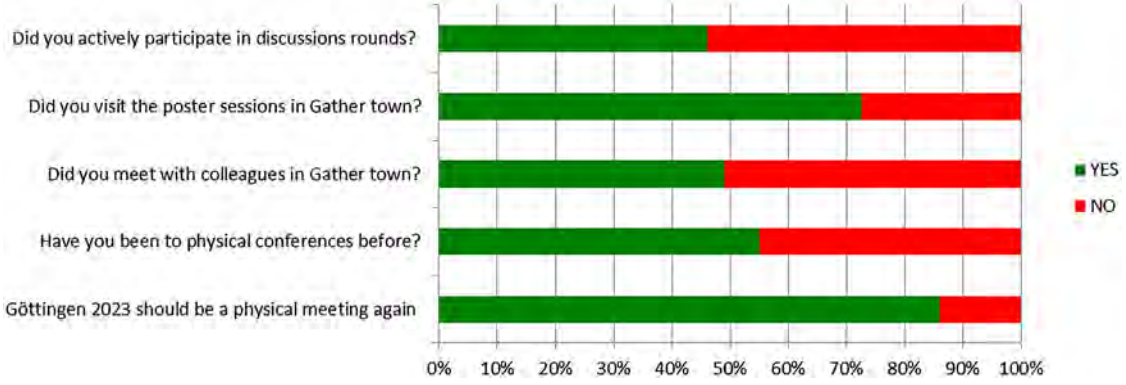


Abb. 2: Ergebnis der Umfrage zur Nutzung der interaktiven Möglichkeiten und zur zukünftigen Tagung.

Teilnehmenden gestoßen, weil dadurch eine kurzfristige flexible Teilnahme nicht mehr möglich war. Letztendlich hatten alle Diskussionsrunden mindestens 20 und manche bis zu 76 Teilnehmenden. Sollte es ein nächstes Mal geben, was keiner wirklich hofft, kann man optimistischer planen.

Auf die Symposienseiten gab es in der Vorschau- und Konferenzphase insgesamt knapp 8.000 Zugriffe, davon etwa 3.100 auf die Videos. Das bedeutet, jede*r Teilnehmende hat im Schnitt auf zwischen sieben und acht Symposienseiten zugegriffen und bei ca. drei dieser Symposien auch die Videos angeschaut. Die Poster hatten insgesamt knapp 6.600 Zugriffe, was bedeutet dass sich jeder durchschnittlich 6 Poster angeschaut hat. Dabei liegen die beiden am häufigsten aufgerufenen Poster bei 49 Zugriffen. In der dritten Phase, also in der Zeit nach der Tagung bis zum 31. Mai 2021, gab es auf die Poster noch 828, auf die Symposien noch 989 und auf die Plenarys noch 107 weitere Zugriffe.

Das Programm *Gather.town* bot an zwei vollen Tagen (23. und 29. März 2021) nicht nur die Möglichkeit zur Interaktion zwischen Poster-Autor*innen und Teilnehmenden. Hier konnten sich die Teilnehmenden auch „zufällig begegnen“ oder zu einem Chat zu verabreden. Dieses Tool stieß bei vielen, vor allem aber bei den vielen jungen Leuten, auf großen Zuspruch, da es auf sehr unkomplizierte Art Nähe schaffte und Diskussion und Austausch erleichterte.

Welches Fazit können wir ziehen? Ganz klar – allen anfänglichen Zweifeln und Vorbehalten zum Trotz ist der Plan der NWG für die virtuelle Tagung gut aufgegangen. Die NWG hat versucht, das Beste aus der Covid-19-bedingten Situation zu machen, und das ist gelungen. Sie hat der neurowissenschaftlichen Gemeinde mit der virtuellen Plattform einen fruchtbaren Boden für wissenschaftlichen Austausch geboten und der wurde gut bestellt. Zudem hat die virtuelle Tagung Anregungen gegeben, die auch für zukünftige, dann hoffentlich wieder physische Tagungen ausgelotet werden könnten, so z. B. die Online-Stellung von Beiträgen auch noch nach der Konferenz. Und, vielleicht die schönste Bestätigung im digitale Zeitalter: der Mensch ist ein soziales Wesen. Einhellig wurde festgestellt, dass eine virtuelle Tagung zwar auch ihre Vorteile hat – man muss nicht reisen, man hat kaum Kosten, man verbringt kein anstrengenden langen Tage im Hörsaalgebäude, man ist flexibler beim Anhören der Beiträge -, dass aber eine virtuelle Tagung keine dauerhafte Alternative zur traditionellen Tagung im Göttinger Hörsaalgebäude ist und dass die allermeisten hoffen, dort im Jahr 2023 Studienfreunde, Kollegen und Vorbilder von Angesicht zu Angesicht zu sehen.

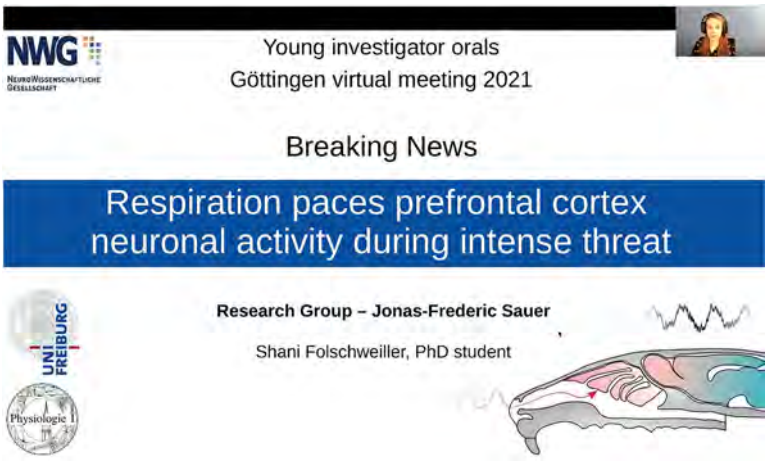
Der Termin steht schon fest: 22. – 25. März 2023. See you there!

Breaking News’ Best Paper Award 2021

Zum zweiten Mal wurden die drei Breaking News’ Best Paper Awards der NWG verliehen, und zwar im Rahmen der virtuellen Göttinger Tagung 2021. Preisträger*innen sind drei Studierende, die einen Kurzvortrag im Breaking News Symposium gehalten haben. Für einen Vortrag in diesen Symposien konnten sich Bachelor, Master und Promotionsstudierende bewerben. Mark Spehr als Chair des Symposiums wählte aus diesen Bewerbungen 10 Abstracts für das Breaking News Symposium aus. Aus diesen wiederum wählte eine Jury, der auch zwei Vertreter*innen der Sektion „Junge NWG“ angehören, die drei besten Vorträge aus. Kriterien für die Auswahl waren die Aktualität und der Neuheitswert der Ergebnisse und deren eventuelle Bedeutung für zukünftige Forschung sowie die Qualität der Darbietung als Video-Aufzeichnung, sowohl in Bezug auf die Folien der Präsentation als auch auf den Vortragsstil. Außerdem floss die Diskussionsrunde mit in die Bewertung ein, die Entscheidung fiel erst nach dieser. Das Preisgeld beträgt 500 € für den ersten, 300 € für den zweiten und 200 € für den dritten Platz.

Preisträger*innen 2021 sind folgende jungen Wissenschaftler*innen:

1. Preis:
Shani Folschweiller (Albert-Ludwigs-Universität Freiburg, Institut für Physiologie)
Respiration paces prefrontal neuronal activity during intense threat



2. Preis.
Maximilian Hammer (Universität Heidelberg, Institut für Physiologie und Pathophysiologie)
The effects of breathing rate on theta-gamma coupling

3. Preis:
Katrina Deane (Leibniz Institut für Neurobiologie Magdeburg, Systemphysiologie des Lernens)
Optogenetically controlled aggregation of calcium channels in the auditory cortex causes deterministic population dynamics and suppressed impulse responses

Mit diesen nur an Studierende vergebenen Preisen möchte die NWG einen weiteren Beitrag zur Verwirklichung eines ihrer Hauptziele, nämlich die Unterstützung des wissenschaftlichen Nachwuchses, beitragen. Dank einer großzügigen zweckgebundenen Geldzuwendung können die Preise auch auf kommenden Tagungen verliehen werden.

NEU auf dasGehirn.info

Im Mai 2021 konnte in Themenpartnerschaft mit dem Max-Planck-Institut für Hirnforschung das Schwerpunktthema **Konnektom** für dasGehirn.info in zwei Artikeln, einem Interview mit Prof. Moritz Helmstaedter und einem Multimediabeitrag erschlossen werden:

Sind wir unser Konnektom? Weltweit sind Wissenschaftler dabei, einen Schaltplan des Gehirns anzufertigen. Macht die Gesamtheit aller Nervenbahnen unser Ich aus?

Das Neuronengeflecht entwirren: Um Nervenbahnen und Netzwerke im Gehirn zu verfolgen, bedarf es ausgeklügelter Technologien, vor allem aber viel Geduld und Liebe zum Detail

Der dichteste Dschungel: Das Konnektom bezeichnet die Gesamtheit aller Nervenzellen und Verknüpfungen im Gehirn. Mit hochauflösenden Elektronenmikroskopen und künstlicher Intelligenz versuchen Forscher schrittweise, die Hirnarchitektur darzustellen, um mehr über die Funktionsweise des Organs zu lernen.

Multimediabeitrag **Das Connectome:** Wir kennen den großen Schaltplan des Gehirns. Doch was wirklich zählt – Wahrnehmung, psychische Störungen, Bewusstsein – wird zwischen vergleichsweise wenigen Nervenzellen verhandelt. Wie die im Einzelnen verschaltet sind, untersucht die Konnektomik – eine Mammutaufgabe, die noch ganz am Anfang steht. Doch schon jetzt liefert die Forschung erstaunliche Erkenntnisse. Und wunderschöne Bilder.

Im Juni 2021 folgte mit **Schlaganfall** gleich ein weiteres wichtiges Thema, das gemeinsam mit den DFG-Forschergruppen 2795 und 2879 auf dem Portal umfassend vorgestellt wurde. Mit zehn Artikeln und einem Multimediabeitrag konnten die bereits bestehenden Inhalte vertieft und einzelnen Aspekten genauer nachgegangen werden:

Der Schlaganfall: Eckdaten: Kaum ein Notfall lässt dem Arzt so wenig Zeit wie ein Gefäßverschluss im Gehirn – binnen Minuten beginnt das Sterben der Nervenzellen. Was genau geschieht, was kann das Umfeld tun und welche Möglichkeiten hat die Medizin?

Erholung nach dem Hirninfarkt: Während sich Schlaganfallpatienten regenerieren, findet in ihrem Gehirn eine massive Reorganisation statt. Die verbliebenen Nervenzellen verknüpfen sich neu – Regionen übernehmen verloren gegangene Kompetenzen.

Warum reagiert das Gehirn auf einen Schlaganfall so empfindlich? Einige Körpergewebe und Organe können regenerieren. Doch das Gehirn ist im Guten wie im Schlechten ein ganz besonderes Organ – und seine Regeneration nach einem Schlaganfall gestaltet sich eher schwierig.

Steter Tropfen führt zum Schlaganfall: Zwar ist ein Schlaganfall an sich ein plötzliches Ereignis, doch er bildet das dramatische Finale eines langen, schleichenden Prozesses. Und der lässt sich durch die eigene Lebensführung beeinflussen.

Schlaganfall: Die Uhr tickt: Wenn Menschen einen Schlaganfall erleiden, zählt jede Sekunde. Denn das Massensterben von Nervenzellen schreitet in rasendem Tempo voran. Pro Minute sind es rund zwei Millionen Nervenzellen. Hier ein Blick auf die Zeitschiene.

T-Zellen bei Schlaganfall: Nicht schwarz, nicht weiß: Nach einem Schlaganfall wandern T-Zellen in großer Zahl zur Verletzung – und machen anfangs alles nur noch schlimmer.

Die Rolle der Mikroglia beim Schlaganfall: Mikroglia sind die Immunzellen des Nervensystems. Nach Schlaganfällen üben sie ebenso positive, wie negative Einflüsse aus. Genau das macht sie so interessant für Wissenschaft und Medizin.

Sterbende Zellen, bedrohte Netzwerke: Ein Schlaganfall kappt die Sauerstoff- und Energiezufuhr der Hirnzellen. Ionen und Neurotransmitter geraten dann aus dem Lot.

Die Rolle der Astrozyten beim Schlaganfall: Astrozyten sind universelle wichtige Hilfszellen des Nervensystems. Auch an der Pathologie des Schlaganfalls sind sie wesentlich beteiligt – und massiv von ihm betroffen.

Entzündungsprozesse: Nach Schlaganfällen ist „High Life" im Gehirn: Ein Schlaganfall gilt nicht als klassische Entzündungskrankheit. Doch er führt zu einer heftigen Immunreaktion – und zwar im ganzen Körper. Diese Prozesse zu verstehen, liefert Ansatzpunkte für neue Behandlungsmöglichkeiten.

Der Multimediabeitrag **Der Schlaganfall:** Einen Schlaganfall erleiden 250.000 Deutsche jedes Jahr zum ersten Mal. Manchmal ist er so harmlos, dass der Betroffene ihn nicht bemerkt, bei anderen fällt eine ganze Körperhälfte aus. Hier alle wichtigen Aspekte in sechs Minuten.

In dem Format **Frage an das Gehirn** beantworten Experten regelmäßig Fragen unserer Leser. Zuletzt ging es um die folgende Frage: *Wie genau sind Synapsen aufgebaut?* - Eine Synapse ist die Verbindung zweier Nervenzellen – warum lese ich immer wieder von der dreiteiligen Synapse?

In der Rubrik **Neues aus der Wissenschaft** macht dasGehirn.info im Juni 2021 auf die folgenden Pressemeldungen aus den Instituten aufmerksam:
Neuronale Einschlüsse bei Parkinson-Krankheit sehen aus wie Zwiebeln | Zentrum für Proteindiagnostik (PRODI) (15.06.2021)
Wählerische Nervenzellen | Max-Planck-Institut für Neurobiologie (18.06.2021)
Multiple Sklerose beginnt oft lange vor der Diagnose | Technische Universität München, Klinikum Rechts der Isar (21.06.2021)

Möchten Sie eine Pressemeldung an **dasGehirn.info** weitergeben oder Ihr Institut vorstellen, wenden Sie sich bitte an Arvid Leyh (E-mail: a.leyh@dasgehirn.info).

Neueintritte

Folgende Kolleginnen und Kollegen dürfen wir als Mitglieder der Neurowissenschaftlichen Gesellschaft begrüßen:
Pepe Alcami, Dr. (Martinsried)
Julia Büscher (Magdeburg)
Jonas Fisch (Kaiserslautern)
Johanna Habermeyer (Erlangen)
Angelika Harbauer, Prof. Dr. (Martinsried)
Claire Jacob (Mainz)
Matthias Krawutschke (Potsdam)
Tabea Kürten (Mainz)
Christian Reyes Moreno (Oldenburg)
Marta Sanchez Carbonell (Jena)
Johannes Seiler (Mainz)
Lars Wojtecki, PD Dr. (Kempen)

Der Mitgliedsstand zum 21. Juni 2021 beträgt 2.104 Mitglieder.

Ausblick

Davide Raccuglia, Raquel Suàrez-Grimalt
The neural architecture of sleep regulation: insights from Drosophila

Ellen Fritsche
Environmental exposures impact the nervous system in a life stage-specific manner

Frank Winkler
Malignant networks in the brain – a new view on brain tumors

Philipp Rinklin, Bernhard Wolfrum
Recent developments and future perspectives on neuroelectronic devices

Neurowissenschaftliche Gesellschaft e.V. (NWG)

- Beitrittserklärung -

Hiermit erkläre ich meinen Beitritt zur Neurowissenschaftlichen Gesellschaft e.V. (NWG).

Eintrag in das Mitgliederverzeichnis:

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Titel

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Rechte und Pflichten der Mitgliedschaft siehe Satzung (nwg-info.de/de/ueber_uns/satzung). Mit meiner Unterschrift bestätige ich, dass ich die Satzung sowie die Datenschutzrichtlinie (nwg-info.de/de/datenschutz) zur Kenntnis genommen habe und diese anerkenne.

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Ich unterstütze den Antrag auf Beitritt zur NWG e.V.

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Bitte senden Sie Ihren Antrag an die Geschäftsstelle der NWG:

Stefanie Korthals
Neurowissenschaftliche Gesellschaft e.V.
MDC
Robert-Rössle-Str. 10
13092 Berlin
Email: korthals@mdc-berlin.de
Tel.: +49 30 9406 3127

Ich optiere für folgende 2 Sektionen:

- ☐ Computational Neuroscience
- ☐ Entwicklungsneurobiologie/Neurogenetik
- ☐ junge NWG (jNWG)
- ☐ Klinische Neurowisschenschaften
- ☐ Kognitive Neurowissenschaften
- ☐ Molekulare Neurobiologie
- ☐ Neuropharmakologie und -toxikologie
- ☐ Systemneurobiologie
- ☐ Verhaltensneurowissenschaften
- ☐ Zelluläre Neurobiologie

Ich bin Student ☐ ja ☐ nein
(Bescheinigung anbei)
Ich bin ☐ weiblich ☐ männlich ☐ divers

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