Cluster N: The Bird Geomagnetic Compass

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April 2nd, 2025

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Introduction

Many birds can migrate far distances with high accuracy. Birds can sense the earth's magnetic field and use this magnetic field information to guide migration orientation over long distances (Mouritsen and Ritz, 2005; Mouritsen et al., 2016). In other words, birds have a "geomagnetic compass" that both transduces magnetic signals and projects this information to the brain to coordinate behavior (Mouritsen et al., 2016). The transduction and integration of avian magnetoreception is a question for both the physical and natural sciences: while much has been learnt since birds were first observed to exhibit geomagnetic orientation in 1965 (Mouritsen et al., 2016), many questions of the geomagnetic compass and itsf putative brain region, Cluster N, are left unanswered. Magnetoreception in birds is thought to be sensed by cryptochromes in the retina that is known to share structural connectivity to Cluster N, within the Visual Wulst. Cluster N is thought to be responsible for representation and, perhaps, integration of geomagnetic information and/or night vision (Bingman et al., 2021; Langebrake et al., 2024; Zapka et al., 2010). Structural molecular mapping experiments of Cluster N aim to better characterize its morphology using the gene expression patterns of nearby brain regions. Cluster N shares connectivity with regions of the hyperpallium, mesopallium and hippocampal formation. Additionally, structural and functional variation of Cluster N within and between species remains poorly characterized. Functional mapping experiments aim to understand the behaviors controlled by Cluster N by mapping immediate early genes that signal brain activity, such as rapid depolarizations, in the brains of behaving birds. At first, Cluster N was thought to be an area responsible for night vision in dim-light conditions. Subsequent experimental data suggested that this region may be implicated in the control and/or "tuning" of a geomagnetic compass at times where other orientation-relevant cues are unavailable or irrelevant. To better understand the mechanisms of how birds can integrate geomagnetic information, future research directions should integrate methods from the behavioral, ecological and biochemical sciences. In this review, I will synthesize what researchers do, and do not understand about each part of geomagnetic orientation in birds. I will start by describing magnetoreception and its' related brain regions. Following the discussion of neuroanatomy, I will characterize the behaviours associated with different brain regions involved in migratory behaviour. I will finally suggest future research directions for each topic discussed.

Magnetoreception

Magnetoreception in birds is likely to act as a magnetic inclination compass through the cryptochrome proteins in the bird retina (Mouritsen and Ritz, 2005; Mouritsen et al., 2016). In the past, proof-of-concept experiments have determined that radical pair reactions can be sensitive to geomagnetic fluctuations, indicating that a biological geomagnetic compass is physically viable (Schluten et al., 1978). Only recently have these proof-of-concept ideas been validated in birds. In-vitro proteins, particularly cryptochrome 4 (CRY4) and cryptochrome 1a are sensitive to earth-like magnetic field variation through stable radical pair signalling states between adjacent tryptophan or tyrosine side chains and flavin cofactors (Liedvogel et al., 2007; Xu et al., 2021). Proteins of the cryptochrome family have been found in both migratory and nonmigratory species of birds ranging from passerine european robins (*Erithacus rubecula*) to chickens (*Gallus gallus*; Xu et al., 2021, Günther et al., 2018, Bolte et al., 2021). Xu et al. (2021) demonstrated that in-vitro sensitivity of european robin CRY4, a migratory species, to magnetic fluctuations is higher than that of the nonmigratory chicken and pigeon CRY4, suggesting evolutionary pressure on the structure of these proteins related to migration. In-vitro characterization does not alone prove that these proteins are capable of transducing geomagnetic information into neural signals: further research should include manipulations of cryptochromes in-vivo (Xu et al., 2021). In-vivo, magnetic field-dependent, radical pair reactions within the cryptochrome(s) would suggest the ability of birds to transduce magnetic field information into a biological signal to the brain, thus responsible for migratory behavior. While such an experiment is yet to inform the mechanisms magnetoreception, structural mapping experiments may allow us to make inferences regarding the transduction of geomagnetic information.

Neuroanatomy and Functional Connectivity

The avian pallium, or forebrain, refers to the area dorsal to the striatum in the cortex. Within the avian pallium is the hyperpallium, the most dorsal region of the avian pallium. The visual wulst corresponds to the ultimately dorsal and medial region of the hyperpallium and stretches four inter-hyperpallium regions, the mesopallium as well as the anterior hippocampal formation (Heyers et al., 2022; Jarvis et al., 2013). The lateral and posterior area of the Visual Wulst contains a brain region named Cluster N that spans the same dorsal-ventral layers but is shorter in the anterior-posterior axis compared to the Visual Wulst (Jarvis et al., 2013). Cluster N lacks region-specific gene expression, meaning that its borders are determined by relative expression patterns of nearby brain regions (Heyers et al. 2022). Immunohistochemical tracing experiments in garden warblers (*Sylvia borin*) have illustrated that these Cluster N subdivisions share neuronal projects with each other (Heyers et al., 2022). Because these subdivisions are responsible for different behaviors and respond to different stimuli (Bingman et al., 2021, Heyers et al., 2022), it is possible that biological information is shared and integrated within Cluster N to produce downstream behavior (Heyers et al., 2022).

The biological information shared between these regions is likely geomagnetic information: Heyers et al. (2007) demonstrated that the garden warbler retina projects to cluster N via the hypothalamus using axonal projection tracing and immunohistochemistry. Because the retina is known to contain geomagnetically sensitive proteins, it follows that this projection scheme is a well-accepted physiological mechanism for neural transduction of geomagnetic compass in a wide range of migratory species. The structural relationships between cryptochromes and Cluster N is then a functional one. If the goal is to better characterize the structure and function of the cryptochrome-Cluster N circuit, future research should aim to better understand the phylogeny and evolution of the structures that govern geomagnetic compass orientation.

Studies of the geomagnetic compass and its transduction are limited to a narrow range of European model species (Gulson-Castillo, 2024). A recent functional mapping study has illustrated that magnetoreception-related Cluster N immediate early gene (IEG) expression is conserved across all major Passeriform radiations, but not all species within these radiations (Gulson-Castillo, 2024). Additionally, a genome analysis of passerines suggested that the evolution of the CRY4 gene is characteristic of a sensory protein. CRY4 was also found to be deleted in night-migratory species, indicating alternative migration mechanisms (Langebrake et al., 2024). Evidently, recent investigation into the evolution of magnetoreception has revealed new information about the function and evolution of magnetoreception.

Cluster N Function and Functional Mapping Experiments

Neuroanatomical tracing and protein marker mapping experiments provide spatial locations in the brain that can be used to expand our understanding of behavior. Following the discovery of a shared circuit that contains the two regions, Zapka et al. (2009) demonstrated that Cluster N lesions disrupt the geomagnetic orientation ability of european robins. Either Cluster N or its host region, the visual Wulst, is required for geomagnetic orientation in these night-migratory birds in the absence of other visual cues (eg. inside of Emlen Funnels). Furthermore, Cluster N-lesioned, and Sham-lesioned european robins orientated in the same direction when confronted with sunny and celestial cues, suggesting that sun and star orientation are still intact. As a result, Cluster N may not be necessary for the use of sun and star orientation cues.

Functional mapping experiments utilize immunostaining of immediate early genes and in-situ hybridization of the ZENK protein and RNA, respectively, to determine regional brain activity patterns during behaviours or environmental conditions (Nordmann et al., 2020; Zapka et al., 2010). ZENK is a transcription factor and immediate early gene that is rapidly induced in neurons following synaptic stimulation, linking it to activity-dependent gene regulation (Nordmann et al., 2020). In experiments of migratory behaviour, birds are typically euthanized between 5-45 minutes after exhibiting a behaviour of interest (Brodbeck et al., 2023; Zapka et al., 2010). Brain activity induced ZENK mRNA is expressed rapidly, while peak protein expression is usually seen at 1-2 hours after stimulus onset (Nordmann et al., 2020). As a result, levels of ZENK mRNA and protein expression are often used synonymously to brain activity. Cluster N activity increases at night in night-migratory european robins and garden warblers but not in nonmigratory zebra finches and canaries, independent of circadian and circannual rhythms that typically govern migratory behavior (Mouritsen et al., 2005, 2016). Subsequent work suggests lateralization towards the right hemisphere of cluster N and provides further evidence for night-time activation (Liedvogel et al., 2007). The idea that cluster N is lateralized has not held up to further experimentation in a wider range of species (Rudolf et al., 2024). In absence of behavioural data, it is difficult to distinguish ZENK activation as a result of night-time conditions versus the “tuning” of a geomagnetic compass in response to the motivation to migrate. Consequently, behavioural paradigms that allow researchers to better determine whether cluster N is a visual region that shows increased to night vision in dim light conditions or is responsive the tuning of a geomagnetic compass are crucial to better understand the regulation of cluster N. One way to test for the motivation to migrate is by using a behavioural measure called migratory restlessness.

Migratory Restlessness

Migratory restlessness is an indicator of the time to migrate in the wild. Birds that show higher migratory restlessness in captivity tend to migrate sooner than birds who show lower levels of migratory restlessness (Eikenaar et al., 2014). Consequently, behavioural measures of migratory restlessness are often used synonymously with the motivation to migrate. The finding that cluster N activity increases with migratory restlessness is supported by the work of Rudolf et al. (2024). The authors found that night-active Swainson's thrushes displayed higher cluster N activity than day-active birds. Additionally, Zapka et al. (2010) demonstrated that cluster N activity is higher in night active than day active in the meadow pipits, a bird that migrates both during the day and night. The Zapka et al. (2010) experiment suggests that these birds are differentially regulating the activity of their geomagnetic compass; however, the reason for this regulation is unclear. It is possible that day and night migratory birds preferably use geomagnetic orientation cues in dim light conditions because other orientation cues (eg. Solar) are unavailable. This conclusion is reasonable because, when migrating during the cloudy skies during the day, meadow pipits are either unable to correctly orient themselves because relevant cues are unavailable or not motivated to migrate (Zapka et al., 2010). A limitation with these two experiments is the lack of non-active nighttime controls. In any case, these experiments in combination with the finding that cluster N lesioned birds are unable to correctly orient themselves (Zapka et al., 2009), provides evidence that cluster N is a region responsible for geomagnetic orientation, and can be regulated by migration-relevant cues. A recent experiment demonstrated that Cluster N shows more activity in white-throated sparrows that were exhibiting nighttime migratory restlessness compared to daytime restlessness and nighttime resting sparrows (Brodbeck et al., 2023). This finding eliminates some ambiguity surrounding the lack of a night-time resting group. Overall, these behavioural mapping studies suggest that the geomagnetic compass has differential regulation regardless of how migratory cues are weighed.

The novel understanding that Cluster N is regulated by the “motivation to migrate” implies that this brain region can regulate or be regulated by other areas in the brain in response to changing conditions. Presumably, the ability to regulate migratory orientation tools should result in an evolutionary advantage particularly in situations where individuals are able to migrate during unexpected bouts of a good migratory environment by avoiding the mortality costs of migrating during poor conditions (Alerstam et al., 2003). Further research should measure the degree to which Cluster N is regulated by different migration-related stimulus and follow up by understanding the neural mechanisms that are involved in regulation that responds to environmental conditions.

Hippocampal Formation

The avian Hippocampal Formation (HF) is the brain region in birds that is involved in spatial memory and cognition (Mouritsen et al., 2016). HF is an elegant illustration of how natural selection may act on behavioural and neurophysiological traits: HF size varies between species of birds that have different food-caching behaviour, and within species that contain migratory and nonmigratory populations (Mouritsen et al., 2016). These variations, presumably because of natural selection, reflect the idea that the avian’s neural circuits represent specialized behaviours. HF-lesioned homing pigeons show an impaired ability of orienting around a memorized, “home” area but have intact long-range orientation (Mouritsen et al., 2016). The HF seems to be involved in migration, however the specific role that it plays is still unclear.

Because cluster N includes regions of the hippocampus (Heyers et al., 2022), it is possible that these different regions support different functions within the geomagnetic compass. A discrimination training paradigm experiment in Homing pigeons has shown that hippocampal lesions resulted in impaired intensity discrimination but not inclination discrimination, while the opposite was found in birds with lesions to the visual Wulst (Bingman et al., 2021). These data suggests that migratory behaviour pertaining to magnetic signals is integrated by other regions of the brain. The HF seems to both have increased neuronal activity in response to magnetic field intensity changes while not being necessary for geomagnetic compass orientation (Bingman & MacDougall-Shackleton, 2017). These two points illustrate an argument that the HF may be implicated in the function of a geomagnetic “map”: in other words, the HF fosters a system of memory that uses multiple sensory systems to help remember familiar locations in a scale different than that of the nearby landscape (Bingman & MacDougall-Shackleton, 2017).

If the goal is to better characterize the neural signature of the HF as it pertains to migratory behaviour and orientation, researchers should identify and make use of behavioural paradigms that distinguish between orientation at a large (migratory) and small (landscape scale).

Future Directions

To confirm or reject our own ideas about magnetoreception, future experiments should consider what is known and unknown about cluster N and its related proteins and behaviours. If the goal is to better characterize the role of geomagnetic orientation from an ultimate perspective, future investigations of cryptochromes should utilize a broad range of species to better understand how magnetoreception has evolved across different migratory strategies. Comparative analyses between migratory and nonmigratory cryptochrome proteins can reveal how differences in cryptochrome structure and function are shaped by ecological or evolutionary pressures. Expanding research beyond European model species will provide insight into the diversity and conservation of geomagnetic transduction mechanisms. These approaches should use modern molecular biology techniques including cheap and accessible genome wide sequencing and statistically driven protein structure prediction models. By better understanding the origin of magnetoreception, those studying all aspects of geomagnetic orientation behaviour will benefit from having a better developed idea of the evolutionary benefits and costs of a geomagnetic compass protein.

If the goal is to understand the proximate mechanisms of geomagnetic compass control, behavioural scientists will need to expand their behavioural paradigm “toolbox” in addition to utilizing immunostaining. One way to better characterize cluster N’s role in the output of migratory restlessness is by utilizing the birds’ ability to choose whether to migrate or not. The behavioural paradigm should 1) hold all birds in a constant environment, followed by 2) a sharp change in environmental conditions and 3) measure both ZENK and migratory behaviour in the same instance. The latter step proves to benefit from the biochemical regulation of ZENK protein expression. Because peak ZENK expression typically takes 1-2 hours after the onset of a stimulus (Nordmann et al., 2020), a clever experiment should make the change in environmental conditions and thus the point of decision within this “window”. The results from experiments that utilize this paradigm should be able to parse whether the geomagnetic compass (cluster N) is up- or down-regulated in response to fluctuations environmental conditions. If regulation is found, follow-up experiments should aim to find particular brain regions responsible for selective migratory restlessness.

To better understand the observed communication between cluster N and the HF, broad experiments that utilize natural variation in migration distance and ability may be useful. By exploiting the within and between species variation of migratory ability, behavioural scientists will be presented with an artificial experimental group of birds that dedicate more resources to orienting themselves on a larger scale. The studies that utilize this variation should examine both HF activity in response to changes in geomagnetic inclination, similar to the data discussed in Bingman & MacDougall-Shackleton (2017). By better understanding patterns in HF to cluster N communication across species, researchers may be able to make inferences regarding the function of this communication.

Conclusion

Scientists are only beginning to better understand the mystery of magnetoreception. It is likely that the magnetoreception-sensitive pigments are in the retina and are cryptochromes. These cryptochromes transduce geomagnetic inclination information, and project it to the visual Wulst via the thalamus. Once in the Visual Wulst, neurons in Cluster N are activated. It is still unclear whether Cluster N is sensitive to geomagnetic signals at night or night vision in general. Cluster N's functional interactions with other regions of the brain are also unclear. To better characterize Cluster N and its' function, researchers should continue to examine a wider range of migratory and nonmigratory species. Research questions should then incorporate functional mapping experiments in combination with sophisticated behavioral paradigms across a wider range of both migratory and nonmigratory species.

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