Title
A Photochemical Signal Transduction Model of Magnetoreception in Cryptochrome

Author
Todd, Phillise Tiffeny

Publication Date
2016

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA,
IRVINE

A Photochemical Signal Transduction Model of Magnetoreception in Cryptochrome

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Chemical and Materials Physics

by

Phillise Tiffeny Todd

Thesis Committee:
Professor Thorsten Ritz, Chair
Professor Zuzanna Siwy
Professor Jun Allard

2016

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGMENTS

It is with great appreciation that I recognize the committee chair, Professor Thorsten Ritz, for his mentorship in all aspects of my research and academic career.

I would also like to express my gratitude to committee members, Professor Zuzanna Siwy and Professor Jun Allard, for their thoughtful insights and careful scrutiny of my work.

Financial support for this research was received from the University of California, Irvine, AGEP, LifeChips, and Eugene Cota-Robles fellowships.

CURRICULUM VITAE

Phillise Tiffeny Todd

Education

6/10-9/16	UNIVERSITY OF CALIFORNIA *Master of Chemical & Materials Physics – Physics, 2014* *Ph.D. in Chemical & Materials Physics – Physics, 2016*	IRVINE, CA
9/08-6/10	CALIFORNIA STATE UNIVERSITY *Bachelor of Science degree in Physics*	LOS ANGELES, CA
6/06-5/08	PACE UNIVERSITY SCHOOL OF EDUCATION *Master of Science for Teachers – Adolescent Mathematics Education*	NEW YORK, NY
9/98-6/00	BABSON COLLEGE *Master of Business Administration*	WELLESLEY, MA
9/91-6/95	NORTHWESTERN UNIVERSITY *Bachelor of Arts degree in Economics*	EVANSTON, IL

Experience

6/15-8/15	UNIVERSITY OF CALIFORNIA *Teaching Assistant*	IRVINE, CA
8/06-8/08	MILLENNIUM HIGH SCHOOL *Mathematics Teacher*	NEW YORK, NY
11/03-11/04	SANOFI-SYNTHELABO *Senior Sales Professional*	NEW YORK, NY
2/02-11/03	ABBOTT LABORATORIES *Cardiovascular Representative*	NEW YORK, NY
7/97-8/98	JP MORGAN *Trade Support Specialist, Proprietary Trading Middle Office*	NEW YORK, NY
9/96-6/97	*Temporary Trade Support, Foreign Exchange Middle Office*	

FIELD OF STUDY

Biological Magnetic Sensing

PUBLICATIONS

"A Photochemical Signal Transduction Model of Magnetoreception in Cryptochrome." Dissertation, University of California. Irvine: ProQuest/UMI, 2016.

ABSTRACT OF THE DISSERTATION

A Photochemical Signal Transduction Model
of Magnetoreception in Cryptochrome

By

Phillise Tiffeny Todd

Doctor of Philosophy in Chemical and Materials Physics

University of California, Irvine, 2016

Professor Thorsten Ritz, Chair

For decades, scientists have documented the ability of numerous animal species to sense the Earth's magnetic field. Migratory birds detect and use this magnetic information to navigate their migratory paths. The precise mechanism underlying this apparent biological compass is unkown and remains an active area of ongoing research. A bio-compass based on the chemistry of magnetically sensitive radical pairs is one of the leading theories under investigation. Such chemistry is known to form under illumination in the cryptochrome photoreceptor, which has been found within the retina of migratory birds. This work seeks to create a model that details how a cryptochome-based bio-compass could signal magnetic information. The model developed here considers the physical mechanism by which signal transduction could occur given the unique biology of the cryptochrome protein structure. Further, a quantitative description of cryptochrome magnetic sensitivity and signaling is devised based on well documented, as well as approximated, aspects of the receptor's photocycle. The functioning threshold for a biologically feasible chemical compass is thus proposed based on a signal-to-noise ratio. Flavin adenine dinucleotide (FAD), the light

sensing chromophore of cryptochrome, is widely viewed as a likely site of radical pair chemistry. Using the Runda-Kuta method to solve the first order kinetics of the cryptochrome photocycle provides a baseline for analyzing the effects of different magnetic field orientations on the steady state concentrations of flavin redox states. These concentration fluctuations constitute magnetic information that can be signaled along a neural pathway. Flavin has been found to form radical pairs with tryptophan or superoxide, both potentially magnetically sensitive. Modeling both radical pair formations suggests that a flavin-tryptophan radical pair strongly aligns with light effects observed in behavioral experiments, while a flavin- superoxide does far less so. Still, the model suggests a flavin-superoxide radical pair may have a role in explaining magnetic sensing under dark conditions. The model also suggests further experiments that may elucidate which radical-pair, flavin-tryptophan or flavin-superoxide, and which flavin signaling state, semi-reduced or fully-reduced, contributes most strongly to the magnetic sensitivity of the biological compass.

INTRODUCTION

Behavioral experiments have long strongly supported the existence of a magnetic sense among numerous animal species[1,2]. Much of this attention has focused on migratory birds, which appear to use an internal biological compass to align themselves the geomagnetic field for the purpose of navigating during migration periods. While birds can and do use other cues, such as the Sun, Moon and stars, these markers are not always present particularly under overcast conditions. The geomagnetic field in contrast is ubiquitous and possesses location specific information in a unique combination of polarity, inclination, and field strength, which averages 50 ± 10 μT.

Contrary to other sensory systems, the biophysical mechanism underlying magnetoreception is still being sought by multiple lines of experimental and theoretical investigations. Numerous experimental observations that the biological compass is disabled under certain monochromatic light conditions, has led to extensive research into a chemical compass mechanism. This work seeds to further detail a theoretical analysis for how such a chemical compass could function under biologically feasible conditions. To understand why focus on such a mechanism is warranted, a general overview of magnetic sensing provides a useful context.

CHAPTER 1: Magnetic Sensing – Evidence of Quantum Biology

Though once criticized as physically impossible, numerous animal species are now known to perceive the Earth's magnetic field and use it as a primary source of navigational information. However, despite the discovery of magnetic orientation in migratory birds over 40 years ago, the biophysical mechanism behind the magnetic sense has remained an open question. Of the leading theories proposed, those based purely on classical electromagnetic and mechanical forces, have failed to explain key aspects observed in avian magnetoreception. Alternatively, new evidence is now pointing towards a solution found in quantum biology; a quantum-based mechanism that involves radical pair reactions.

Evidence for Magnetoreception

The first confirmation of magnetic orientation was found in night-migrating European robins[3]. Without access to celestial cues, these birds appropriately adjusted their migratory orientation in response to shifts in the direction of the ambient magnetic field[4]. Further behavioral and electrophysiological studies have demonstrated a magnetic sense in other passerine migrant birds, several vertebrate animals, and even certain molluscs, crustaceans and insects[5].

Electromagnetic Induction

One hypothesis for how magnetoreception might occur in elasmobranch fish, like sharks and rays, is through a process known as electromagnetic induction. These fish have electroreceptors called ampullae of Lorenzini, which as they swim, detect voltage drops

across conductive canals within skin pores. Like a conducting rod moving non-parallel to a magnetic field, a potential difference forms across these jelly-filled canals due to the Lorentz force. The surrounding sea water acts as a stationary conducting medium and completes a circuit with the canals, through which an induced current flows[6]. While electromagnetic induction may eventually be shown to give rise to magnetoreception in these fish, it cannot explain magnetic sensing in land dwelling animals where air is not a conducting medium. Furthermore, most species like birds, have not been found to possess sensitive electroreceptors like ampullae of Lorenzini.

Magnetite: Magnetotaxis vs. Magnetoreception

A second theory of magnetoreception suggests that magnetic fields exert mechanical forces on magnetic crystals found in a wide number of magnetically sensitive animals, including birds. One confirmed example of this is seen in aquatic bacteria who are physically rotated into alignment with the geomagnetic field by magnetite (Fe_3O_4) crystals, whose dipoles align in the field[7]. Consequently, the subsequent movements of these bacteria are involuntarily directed downward toward the anaerobic environment, without the need to process or evaluate magnetic information.

For more complex animals that must interpret magnetic stimuli to navigate, magnetite mechanisms have been proposed based on single-domain magnetite particles[8], fixed super-paramagnetic magnetite particles[9], and magnetite-containing liquid crystals[10]. Both single-domain and super-paramagnetic magnetite crystals have been found within the ethmoid region and upper beak of migratory birds and homing pigeons[11,12]. Experiments to test the

magnetite hypothesis suggest that while the beak receptor does process certain components of magnetic information, it falls short of enabling complete orientation behavior.

Map and Compass

To understand these findings, a distinction must be made between two types of information that can be garnered from the Earth's magnetic field. Directional information is used as a 'magnetic compass,' which allows animals to maintain a consistent heading, like north or south during migration. In migrating birds the magnetic compass is sensitive to the inclination or axis of the geomagnetic field, but to the field's polarity[13]. A change in inclination through inversion of either the horizontal or vertical field component, changes the bird's orientation. On the other hand, a change in polarity from reversing both components has no effect on behavior[14]. The second type of information inherent within the geomagnetic field is positional, which allows certain animals to determine their geographical location. This 'magnetic map' is sensitive to the polarity and intensity of the geomagnetic field[15].

Magnetite Receptor Provides Positional (Map) Information

A magnetite-based receptor in the upper beak appears to detect magnetic intensity and polarity, and thus could provide the source for a magnetic map. Electrophysiological recordings of the trigeminal nerve, which connects the magnetite regions of the upper beak to the brain, detected responses to fluctuations in the intensity of the ambient magnetic field[16,17,18]. In addition, conditioning experiments showed that homing pigeons lost their

ability to detect magnetic anomalies when their upper beaks were impaired. Attaching magnets to the cere, anesthetizing the upper beak, or bilateral section of the ophthalmic branch of the trigeminal nerve, all resulted in a lost ability to distinguish magnetic intensity variations[19].

Magnetite Receptor Fails to Provide Compass Orientation

However, while the detection of magnetic field intensity may be mediated by a magnetite receptor in the beak, this mechanism does not appear to provide the directional information that is the source of compass orientation. Following bilateral section of the ophthalmic branch of the trigeminal nerve, migrating birds maintained their ability to orient using their magnetic compass[20]. Other studies have subjected migratory birds to a strong magnetic pulse, in order to disrupt the magnetic orientation of single-domain magnetite particles and observe the subsequent affect on behavior. Again, magnetic compass orientation was unaffected by the pulse treatment[21,22,23].

Furthermore, these pulse treatments were only found to cause course deflections in experienced, but not inexperienced migrants who have not yet learned the magnetic map of their migratory path[24,25,26,27]. Similarly, experienced homing pigeons deviated from correct headings after pulse treatments only when they were at distant sites, but not when they were close to home where intensity variations may not be as significant[28]. These findings lend further support to the hypothesis that magnetite receptors provide intensity and positional information for a magnetic map.

A 2nd System Provides Compass Orientation & is Light Dependent

Since many animals, such as migrating and homing birds, rely on both magnetic map and compass information for successful navigation, a second magnetoreception system is thought to exist. Some researchers expect to find that this second compass system is again magnetite-based, perhaps with super-paramagnetic particles. In an attempt to ascertain the type of particles involved in the magnetite mechanism, pulse treatment experiments applied a second identical pulse several days after the first. Similar behavior was observed after each pulse; initial course deflection followed by a period of disorientation then course correction to the proper migratory heading. This could suggest the existence of super-paramagnetic particles rather than single-domain particles, which would not have experienced further magnetization following the first pulse[23].

Alternatively, mounting evidence suggests that this second system is based on a completely different mechanism from magnetite; one that is light dependent. In an analysis of 62 light-dependent magnetoreception experiments, correct migratory orientation only occurred under low light intensities close to those found at twilight when night migrants set off on their migratory paths. When exposed to light intensities higher than those found at sunset, disorientation and fixed-direction responses were observed[29]. Fixed responses are non-seasonal orientations in one direction, or alternatively along a single axis, i.e. with some birds heading north and the others south. This fixed directional choice does not change with season and thus is not reflective of normal migratory orientation. Unlike normal compass orientation, which is an inclination magnetic response, fixed directions were found to be polar magnetic responses[30,31]. This could indicate that the inclination compass

is disabled under high intensity light, in which case the birds are only left with polarity information from a magnetic map mechanism.

While a change in motivation towards daytime activities could also explain the disruption of compass orientation at higher light intensities, it would not explain a similar response observed under complete darkness. Behavioral experiments conducted in total darkness also showed fixed-direction responses that were independent of the inclination compass. Furthermore, local anesthesia of the upper beak caused the migratory birds to become completely disoriented[32]. This again implies that the fixed-directional response results from magnetic information mediated by magnetite in the upper beak, while the inclination compass is absent in the absence of light.

Quantum-Based Radical Pair Mechanism is Light Dependent

As such, attention has shifted to, a quantum-based radical pair process, which has been proposed to be the light-dependent mechanism behind the avian magnetic compass[33,34]. In this model, light absorbed by photoreceptors in the bird's eye, induces an electron transfer from one molecule to another. In this way, the molecules are transformed into radicals that each have an unpaired electron. This radical pair can exist in either a singlet or triplet spin state, which corresponds to the relative orientations of their electron spins. The electron spins precess around the net magnetic field established by the Earth and nuclear spin environment. This coupling causes the radical pair to oscillate between singlet and triplet quantum states.

It is likely that one radical experiences a stronger coupling with surrounding nuclear spins than the other radical does. Thus the geomagnetic field exerts an additional perturbation, particularly on the precession rate of the electron spin that is weakly coupled to its nuclear environment. In this way, the orientation of the geomagnetic field, relative to fixed nuclear spins, affects the interconversion dynamics between quantum states. Being highly reactive, the radical pair eventually recombines into reaction products that reflect the final spin state that it was in. Consequently, by altering the oscillation between quantum spin states, the orientation of the geomagnetic field affects the reaction rates and product yields of radical pairs. Fluctuations in product yields are in tern signaled to the brain along a visual transduction pathway. In this way, the bird experiences different visual modulation patterns as it looks in different directions relative to the ambient field.

Evidence Supporting the Radical Pair Mechanism (RPM)

Three key findings strongly implicate the radical pair mechanism in magnetoreception. The first is that the avian magnetic compass is not only sensitive to light intensity, but also to light wavelength. Migrant birds are able to orient normally under monochromatic blue and green light, but experience complete disorientation when exposed to yellow or red light[35,36].

This sensitivity of the magnetic compass to blue light has direct implications for the second discovery of blue-light photoreceptor proteins, known as cryptochromes, fixed within the retina of the bird's eye[37,38]. Cryptochromes, which have flavin adenine dinucleotide (FAD) as a photoactive chromophore, are the only molecules in birds known to form radical

pairs[39,40]. Furthermore, cryptochrome in plants has been shown to mediate magnetic field processes. These plants experienced retarded growth in fields ten times the geomagnetic field strength, if they were raised under blue light. This effect was not observed, in red light or darkness, nor in plants that did not possess cryptochrome[41]. Thus, cryptochrome is suspected to be the molecular site of magnetically sensitive radical-pair reactions in the quantum model of magnetoreception.

The third finding reveals a visual transduction pathway by which magnetic signaling effects in the retina may be transmitted to the brain for neural processing. The 'cluster N' brain region was found to be specifically active in the night vision of night-migrating songbirds, but not in non-migrating birds. Located in the hypothalamus at the dorsal surface of the brain, cluster N is connected to the bird's eye via a known visual pathway. Consequently, neuronal activity in cluster N ceased when the birds had both eyes covered[42]. Later experiments found that following bilateral lesions of the cluster N region, night migrants lost their compass orientating ability, but could still navigate using star and sun cues[43].

RF Diagnostic Tool Further Supports the RPM

Thus, the fundamental components of a quantum-based chemical compass are largely supported by experimental observations. However, to further distinguish this model from a magnetite system, as the basis for the magnetic compass, several experiments have employed radio frequencies as an indirect diagnostic tool. The radical pair model predicts that the avian magnetic compass would be disabled in an environment of oscillating magnetic fields superimposed with the static geomagnetic field[34,44]. At the low radio

frequency range, an oscillating field in resonance with the splitting between spin states would drive singlet-triplet interconversions and disrupt a radical-pair mechanism[45]. In addition, the alignment of the oscillating field with respect to the static field would also affect the frequencies at which the radical-pair reaction is perturbed[46]. Conversely, magnetite particles should not experience alignment disruptions in such weak oscillating fields, thus leaving a magnetite-based system unaffected[47].

The results of these experiments showed that migratory birds exhibited normal orientation in oscillating fields of 7 MHz and 1.315 MHz that were parallel to the geomagnetic field. However, as predicted by the radical pair model, these birds were disoriented when these resonant oscillating fields were presented at an angle of 24° with respect to the static field[46,48]. The strong resonance at the Larmor frequency of 1.315 MHz, the frequency at which the electron spin precesses, suggests one radical experiences negligible electron-nuclear spin interactions. Normal orientation was therefore restored for oscillating fields at lower, non-resonant frequencies of 0.01 MHz and 0.03 MHz, presented at 24° angles to the ambient field. Increasing to 0.1 MHz and 0.5 MHz, a weak fixed axial response was observed, suggesting the magnetic compass began to reach the limits of its operational range as resonance was approached.

Also, as predicted by the radical pair model, doubling the static field strength resulted in a shift in the resonance frequency response to twice its original value, i.e. from 1.315 MHz to 2.63 MHz. The observed resonance effects point to FAD and superoxide as the most likely radical pair formed in cryptochrome, since superoxide experiences no electron-nuclear

spin interactions. The strong agreement between these experimental observations and the resonance effects predicted by the radical pair model are quite compelling. Radio frequency tests may not exclude the possibility of a super-paramagnetic system that re-magnetizes quickly. However, taken together with the light-dependent nature of the magnetic compass, these results strongly point towards a quantum-based radical pair mechanism in migrant bird orientation.

It is clear that attempts to explain the magnetic sense through purely classical processes have fallen short of providing a comprehensive description of the avian magnetic compass. At the same time a growing body of experimental and theoretical evidence suggests that investigations in quantum biology may hold answers to the mystery of magnetoreception. If proven correct, the quantum-based radical pair model could illuminate our understanding of how biological systems have evolved to optimize magnetic sensing through the control of quantum spin states. This question has broad implications to other scientific fields that are seeking to control quantum coherence in physical and computational systems.

CHAPTER 2: The Chemical Compass Mechanism

The proposal of a chemical compass by Schulten and Ritz[49,50] is predicated on the observation that certain photoreceptors undergo magnetically sensitive chemical reactions upon illumination. That is to say that the products of these reactions fluctuate corresponding to the receptor's alignment within an external magnetic field. Neural receptors would then interpret these fluctuating concentrations as magnetic information, which could then be transduced along a neural pathway allowing a visual image to arise. Three important experimental and theoretical results led support to the chemical compass model. The finding that the biological compass in migratory birds is disabled under certain light conditions, gives support to a photo-induced chemical compass mechanism. The discovery of a magnetically sensitive photoreceptor within the bird's eye further strengthens this support. And finally, a generalized theory of signal transduction for the chemical compass. The following sections will explore the research developments in each of these aspects of the chemical compass theory.

Biological Compass Light Dependency

Numerous behavioral experiments on migratory birds have been conducted under various light conditions[51]. The birds are tested individually in a funnel cage lined with scratch paper during the migratory season, when they are strongly motivated to fly in the direction

of their migratory path. The cage is then lit under varying conditions. Experiments under monochromatic light reveal the biological compass is disabled under long wavelength light. Specifically, the birds are able to identify their correct migratory direction under UV, blue, or green light, showing a strong preference for flight in that direction. However, under yellow or red light, their flight directions are random and directionless, suggesting a loss of magnetic orientation capability. In short, birds do not appear to orient at wavelengths above 570 nm[52,53]. Such a phenomenon, could be explained by a chemical compass based on a photoreceptor that absorbs within this short wavelength spectrum, such as cryptochrome.

A result that has hitherto been less easily explained is that migratory birds also become disoriented in light intensities much stronger than that under which they typically orient. The majority of behavior experiments have been performed on night migrants who set out on their migratory paths at twilight. Intensities of this magnitude range from around 3 - 22 x 10^{11} photons s^{-1} cm^{-2} as seen in figure 1. These same birds become disoriented in somewhat brighter monochromatic light of 26 x 10^{11} photons s^{-1} cm^{-2} and above.

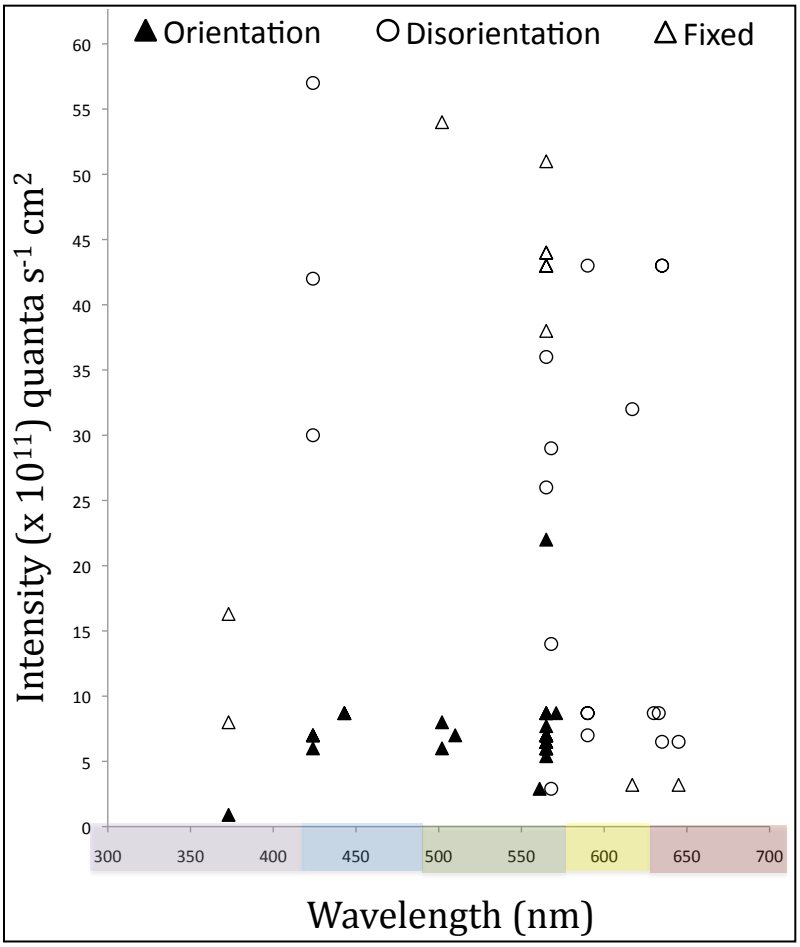

Figure 1. Experimental Light Effects. Migratory bird orientation behavior under monochromatic light conditions of varying wavelengths and intensities. Solid triangles indicate proper orientation under the specified light conditions. Open triangles and circles indicate improper orientation (fixed response and disorientation respectively)[54].

Photoreceptors do not generally function within such a narrow intensity band. The prevailing logic assumed stronger light would simply yield a stronger signal from photo-induced chemical reactions. This work elucidates a possible origin of such intensity dependence as arising form the specific photocycle and signaling state of cryptochrome, thus making a significant contribution to the ongoing debate about these aspects of the theory.

With the goal of identifying the biological site of the chemical compass, modeling the predicted signaling of candidate photoreceptors under different light conditions and comparing those results to light effects seen in behavioral experiments, becomes a useful tool. In this work, we find that the expected signaling from a cryptochrome photoreceptor aligns well with experimental light effects only under certain photocycle and signaling state conditions that are currently being debated.

Cryptochrome Functioning under Magnetic and Light Effects

As researchers continue their search for the biological site that could underlie the chemical compass, significant attention has been given to the cryptochrome photoreceptor protein. Of all photoreceptors considered, including rhodpsin, melanospsin, and cone pigments[55], cryptochrome is the only one to possess all the criteria necessary for a functioning biochemical compass – fixed orientation, blue-green light sensitivity, formation of magnetically sensitive radical pairs under illumination, ability to bind to signaling molecules.

The cryptochrome photoreceptor absorbs light primarily within the blue-green region of the electromagnetic spectrum, as is seen in behavioral experiments as the wavelengths at which orientation occurs. Similar to other signaling proteins, cryptochrome's two domain structure provides one site for binding to light sensing chromophores, like FAD, and another for binding to signaling molecules.

Cryptochrome regulates various biological functions in numerous biological species. Numerous in vivo experiments suggest that the functioning of cryptochrome in these organisms is not just light dependent, but also dependent on external magnetic conditions.

Identified within retinal ganglion cells within the bird eye[56], the cry 1 gene of cryptochrome was found to have high expression and neural activity during magnetic orientation[57]. Cellular activity in non-migratory birds, by contrast exhibited no cry 1 gene expression with insignificant neural activity. These results suggest a correlation between magnetic sensing and cryptochrome gene expression.

Cryptochrome also regulates circadian clock rhythms, in numerous organisms. In daylight, cryptochrome slows circadian clock rhythms in *Drosophila* fruit flies. In the presence of an externally applied magnetic field, this slowing was intensified by blue light exposure, but unaffected by red light[58]. These magnetic effects were stronger in mutant flies with over expressed cryptochrome, while absent in cryptochrome or flavin deficient flies.

Other fruit fly experiments observed proper orientation within an externally applied magnetic field under short wavelength, but not long wavelength light[59].
Similarly, cryptochrome deficient flies were disoriented under all light conditions suggesting cryptochromes mediation of light dependent magnetic sensing.

In *Arabidopsis thaliana* plants, the cry1 and cry2 genes of retards hypocotyl (seedling stem) growth and intensifies anthocyanin (pigment) accumulation. Both functions were found to

be enhanced with increased magnetic field strength under blue light, but not in darkness, red light, or cry gene deficient mutants[60]. The effect was not seen on stem growth in darkness, red light, or cryptochrome deficient plants.

These *in vivo* results in birds, insects, and plants suggests the light induced chemistry of cryptochrome in some way mediates magnetic sensitivity within the protein. Further isolation of these effects between flavin abundant versus deficient groups, suggests the photochemistry on flavin is possible source of this magnetic sensitivity.

Magnetic Sensitivity of Flavin Adenine Dinucleotide

Magnetic sensitivity in cryptochrome likely arises from the flavin adenine dinucleotide (FAD) light sensing chromophore. FAD has been observed to form magnetically sensitive radical pairs. A radical is a molecule that has a lone unpaired electron. Numerous *in vitro* experiments have documented flavin radical pair formation with a tryptophan radical. Magnetic sensitivity of the flavin-tryptophan radical pair was observed under a 28 mT applied magnetic field, which resulted in a 10-20% suppression of reaction products[61].

Other investigations have documented flavin radical pair formation with superoxide[62]. While magnetic sensitivity in a flavin-superoxide radical pair is theoretically possible, such sensitivity has yet to be observed experimentally. Directional magnetic sensitivity of radical pairs has also been experimentally demonstrated, though not for flavin pairs[63]. And while the afore mentioned studies observed magnetic field effects at field strengths far

greater that that of the geomagnetic field (50 μT), one recent study found directional magnetic sensitivity in a photochemically created radical pair, carotenoid-porphyrin-fullerene[64].

The fluctuating reaction products of radical pairs sensitive to magnetic field orientations, constitute magnetic information that can be communication through fluctuating binding activity with signaling partners and neural receptors. The Weaver model, subsequently discussed, provides a general framework for how such signal transduction processes might occur.

Biochemical Compass Signal Transduction Model

Proposed in 2000, the Weaver model of signal transduction defines signaling as a function of the chemical fluctuations that result for magnitude changes in an external magnetic field[65]. While his treatment considers magnetic field affects attributable to changes in field strength, he also illustrates that the mathematical formulism is transferrable to directional changes, without loss of generality. The model aims to discern if such a signal can overcome molecular shot noise to be discerned. In this model, the magnetically sensitive reaction product takes the form of a ligand molecule L, that is produced by a cell which functions as the chemical compass. When the ligand binds to a free neural receptor, R_f, the subsequent percentage of bound receptors, R_b, constitutes the transference of magnetic information along a neural pathway.

A dynamic equilibrium is established among these three factors,

$$L + R_f \underset{k_r}{\overset{k_f}{\rightleftharpoons}} R_b$$

governed by a forward, k_f, and backward, k_r, reaction rate, which can be defined by a dissociation constant $K_D = k_r/k_f$ that determines receptor binding.

Ligand production is magnetically sensitive with a production rate of $k_m(B)$. Ligands are removed from the region to a downstream resevoir at a rate k_p.

The magnetically sensitive rate of ligand production is given by $k_m(B)$ while k_p is the rate of ligand removal via a downstream passive permeability that acts as a degradative sink. At any given time, ligand concentration is also a function of the number of productive cell, the substrate concentration, C_s.

$$L = \frac{k_m(B)}{k_p} C_s$$

The production rate under a magnetic field effect $\Delta B << B_{Earth}$ is given my a first order expansion of $k_m(B)$ about an initial operating point field strength, $k_{m,op} = k_m(B_{Earth})$ as

$$k_m(B) \approx k_{m,op}(1 + g_1 \Delta B) .$$

where $k_{m,op} = k_m(B_{Earth})$ is the operating point of the chemical compass and $\Delta B << B_{Earth}$. The parameter g_1 measures the chemical change expected from a given field strength, and is maximized at $g_1 \approx 100 T^{-1}$.

The signal arising from a chemical compass is then a function of the number of neural receptors bound to ligands. In equilibrium this average number of bound receptors is given by a binomial distribution

$$\overline{R_b} = \frac{R_T}{1+q}$$

where R_T is the total number of free and bound receptors occupying a local cellular volume, where q = K_D/C_s. The variance of the binomial distribution represents the molecular shot noise as

$$\mathrm{N} \equiv \delta R_{b,\mathrm{op}} \approx \sqrt{\frac{q_{\mathrm{op}}}{\left(1+q_{\mathrm{op}}\right)^2} R_T}\ .$$

The signal that must overcome this noise for a functioning compass, is simply the difference between the number of bound receptors at the operating point versus after some perturbative magnetic field effect.

$$\mathrm{S} \equiv \overline{R_b} - \overline{R_{b,\mathrm{op}}} \approx g_1 \Delta \mathrm{B}\ \frac{q_{\mathrm{op}}}{\left(1+q_{\mathrm{op}}\right)^2} R_T$$

A signal-to-noise ratio can be simplified to be expressed as

$$\frac{S}{N} = g_1 \Delta \mathrm{B} \sqrt{\frac{q_{\mathrm{op}}}{\left(1+q_{\mathrm{op}}\right)^2} R_T}$$

where, a value of one represents the threshold for a functioning chemical compass. Light conditions and magnetic field effects that produce a signal-to-noise ratio greater than one represent parameters under which proper orientation by the chemical compass would occur.

CHAPTER 6: Model of a Cryptochrome-Based Compass

The goal of the work presented in this dissertation has been three-fold:

1. Quanlify a biologically feasible mechanism by which cryptochrome could signal magnetic information along a neural pathway.
2. Quantify the threshold for the functioning of a cryptochrome-based chemical compass under reasonable physiological conditions.
3. Elucidate which magnetically sensitive reactions and signaling state might most contribute to observed light effects?

The signal transduction model developed here, is an application of the generalized Weaver model adapted to the specific biology of the cryptochrome. In agreement with the Weaver model, it is assumed that the orientation of a cryptochrome-based biochemical compass should be fixed within a cellular membrane, in order to provide a reference direction with respect to the geomagnetic field. Indeed, fixed cryptochrome proteins have been found to permeate the membranes of cone cells within the retina of the bird eye[66,67].

As depicted in figure 2, Cryptochrome has a two domain structure. The N-terminal domain binds to light sensing chromophores, such as flavin adenine dinucleotide (FAD), which absorbs blue-green light. The C-terminal domain binds to signaling partners. The photochemistry of flavin has been well documented and is known to primarily exist in one of three redox states: fully oxidized, FAD, one-electron semireduced semiquinone, $FADH^{\bullet}$, and two-electron fully reduced hydroquinone, $FADH^{-}$. The flavin rests in the FAD state

under darkness, but then transitions to the other states upon illumination. The FADH• state induces a conformational change in the C-terminal of cryptochrome, which allows binding with a signaling partner[68,69]. In accordance with the Weaver model, this binding is assumed to be with a yet unknown ligand. Binding is assumed to activate the ligand, inducing a conformational change or electro-chemical change, which gives it an affinity for binding with a neural receptor. The ligand activation rate replaces the production rate in the Weaver treatment. This activation rate is given a 1-to-1 correspondence with the number of cryptochromes in the semi-reduced flavin signaling state.

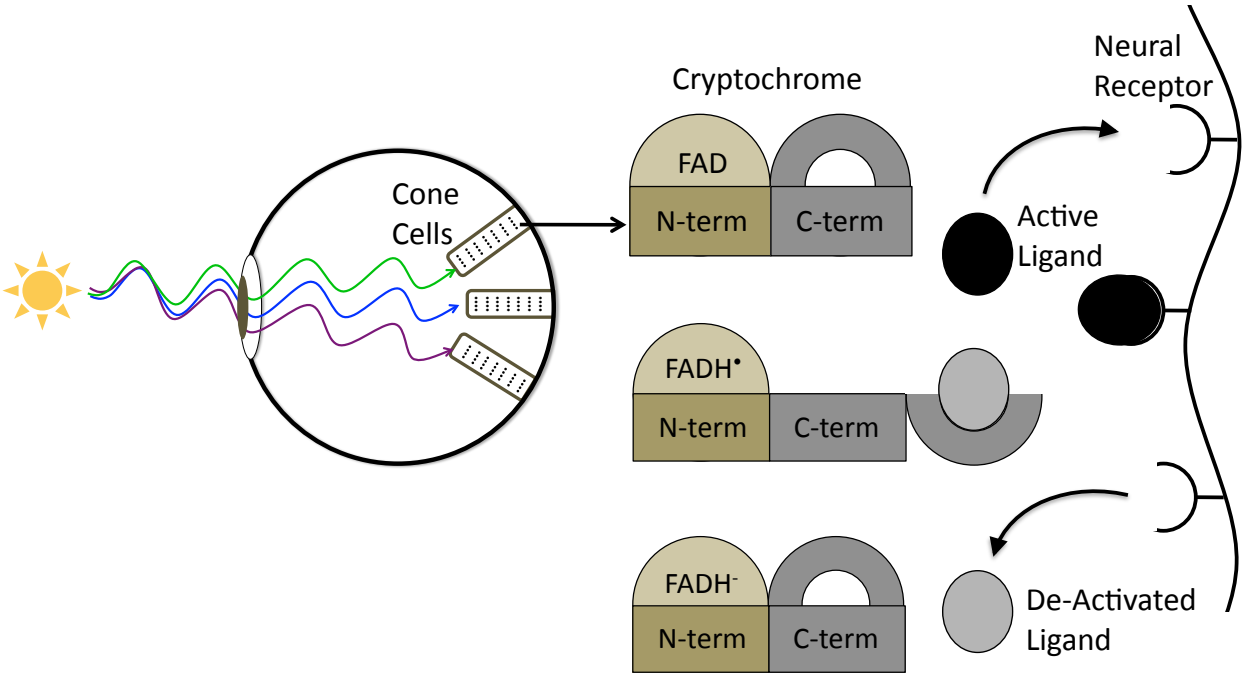

Figure 2. Cryptochrome Model of Signal Transduction. Cylindrical cone cells line the retina of the bird eye. Cryptochromes are fixed within the cell membrane depicted as black dots. In the dark, cryptochrome flavin exists in the FAD redox state, but upon illumination can transitions to FADH• and FADH- redox states. Only the signaling state, FADH, creates a conformational change in the cryptochrome C-terminal that allows it to bind and activate a ligand. Subsequently binding with a neural receptor completes the signal transduction process whereby the number of bound receptors corresponds to the signaling state concentration, which is sensitive to magnetic effects.

The flavin ground state FAD absorbs primarily in the blue region of the electromagnetic spectrum (figure 3). Upon illumination flavin is reduced to FADH•, which itself also absorbs strongly in the green region of the electromagnetic spectrum[70,]. Thus the wavelength and intensity of light determines the rates at which FAD is converted to the other states.

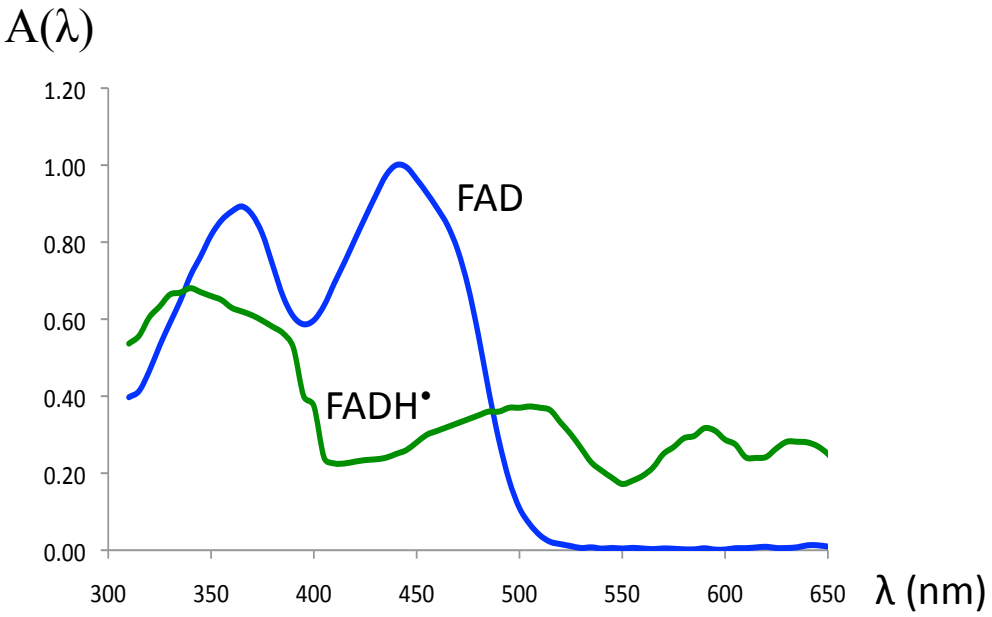

Figure 3. Two State Absorption Spectra. Fully oxidized flavin FAD (blue) and semi-reduced flavin FADH• in neutral form(green)[70,71].

Estimation of Light Dependent Kinetic Rates

In the model described here the rates at which fully oxidized flavin is semi- and then fully-reduced are estimated as follows,

$$FAD_{ox} \longrightarrow FADH^{\bullet} \qquad k_x(I,\lambda) \propto I \bullet A_{FADox}(\lambda)$$

$$FADH^{\bullet} \longrightarrow FADH^{-} \qquad k_{fr}(I,\lambda) \propto I \bullet A_{FADsq}(\lambda)$$

where A_{FADox} and A_{FADsq} are the absorption spectra of the semi- and fully-reduced states respectively. This treatment is only an approximation of the rates using available data. More accurate rates are calculated as $k(\lambda) = I' \sigma(\lambda)$, the light intensity multiplied by the

photoconversion cross-section $\sigma(\lambda) = 2.3\ \varepsilon(\lambda)\ \phi(\lambda)$. The extinction coefficient, $\varepsilon(\lambda)$, is given by the Beer-Lambert Law of absorption $A(\lambda) = d\ \varepsilon(\lambda)\ c$, where c is the concentration of cryptochrome and d the path length light travels through the cell. The quantum yield, $\phi(\lambda)$, is the ratio of the number of cryptochrome undergoing redox state conversion to the number of photons absorbed by the photoreactive substance.

Only recently have *in vitro* and *in vivo* measurements of flavin state quantum yields been obtained for λ=450 blue and λ=560 green light[72]. However, the estimates vary widely between three orders of magnitude from 2.13 x 101 to 1.4 x 10-2 for φ(λ=450). We assume a quantum yield of 1.2×10^{-3} $mol_{FAD}/mol_{photons}$ at 450 nm wavelength. To align our approximated rates to the intensities observed in behavioral experiments the following conversion was utilized: $I\ A(\lambda) = I'\ \sigma(\lambda) = I'\ (2.3\ \varepsilon\ \varphi)$; $I = 2.3\varphi/[C]d$, where $[C] = 2.6 \times 10^{-5}$ (mol/m^3) is the cryptochrome concentration and $d = 4 \times 10^{-6}$ the cell diameter.

Estimation of Magnetically Dependent Kinetic Rates

While the forward reduction rates are dependent on light conditions, two backward re-oxidation rates are potentially magnetically sensitive (figure 4). This magnetic sensitivity arises from flavin's ability to form radical pairs whose quantum spin dynamics are dependent upon orientation angle within an external field.

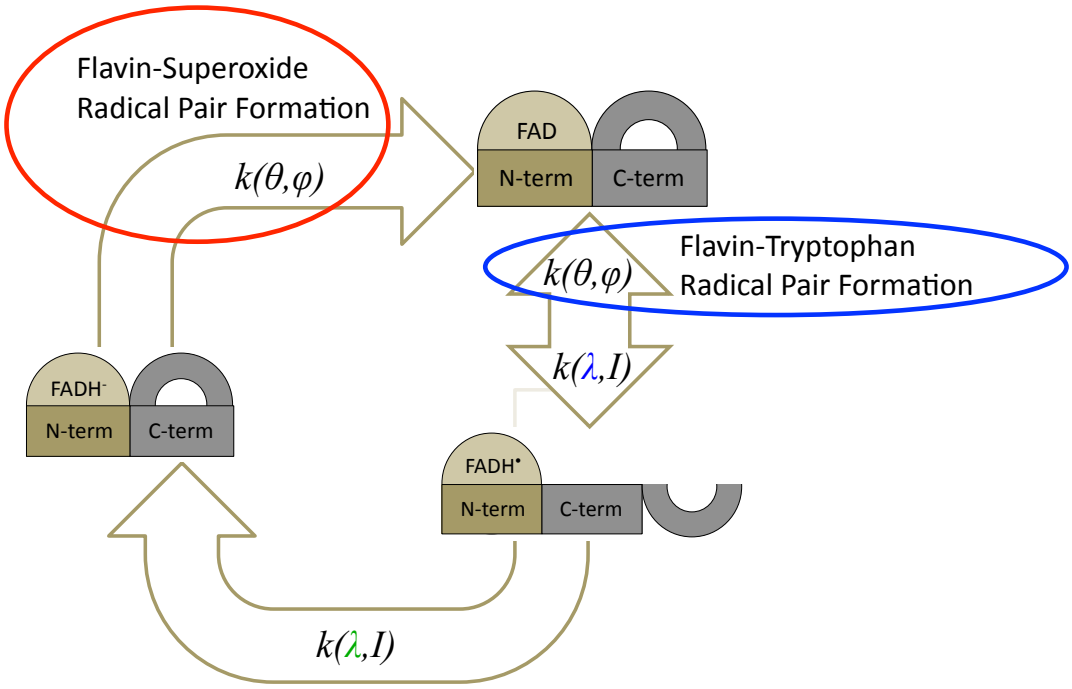

Figure 4. Three State Photocycle of Flavin Adenine Dinucleotide. From the FAD ground states, forward reduction rates are light dependent, while backward re-oxidation rates are potentially magnetic field dependent.

A radical is a molecule that has one lone, unpaired electron. This electron will precess about a magnetic field vector that is the resultant of the geomagnetic field vector and the internal field of nuclear spins (except for superoxide which has no nuclear spin environment). When a radical pair is formed, the electron spins of each radical are created in a spin-correlated quantum state of either singlet or triplet. In the singlet state, the electron spin of one radical precesses 180° out of phase with the other radical. When a radical pair is formed in a triplet state, the two electron spins are in phase. However, since each radical precesses about a different resultant magnetic field, due to differing nuclear spin environments, they quickly lose their initial quantum state and oscillate between states that are singlet, triplet, and linear combinations of the two according to the Hamiltonian[73]

$$H = \Sigma_j A_{1j} \cdot I_{1j} \cdot S_1 + \omega(\cos\theta\, S_{1z} + \sin\theta\, S_{1x}) + \Sigma_j A_{2j} \cdot I_{2j} \cdot S_2 + \omega(\cos\theta\, S_{2z} + \sin\theta\, S_{2x})$$

where A represents the strength of the hyperfine interactions between nuclear spins, I, and electron spins, S for radical 1 and 2. The Larmor frequency $\omega = g_e \mu_B$ and $B_{Earth} \sim 50\mu T <<$ HFI = 1-10mT. The Liouville-von Neumann equation

$$\frac{\partial \hat{\rho}}{\partial t} = \frac{-i}{\hbar}\left[\hat{H},\, \hat{\sigma}\right]$$

has the following density operator solution

$$\hat{\sigma}(t) = e^{-i\hat{H}t}\, \hat{\sigma}(t_0)\, e^{i\hat{H}t}, \qquad \hat{\sigma}(0) = \frac{1}{M}\hat{P}^S$$

which characterizes the radical pair's time evolution. From the total vector space of quantum states, the singlet character is isolates via the singlet projection operator, $\hat{P}^S$. This operator has an expectation value

$$\langle \hat{P}^S \rangle = \mathrm{Tr}\left[\hat{P}^S\, \hat{\sigma}(t)\right] = \frac{1}{M}\sum_{m=1}^{4M}\sum_{n=1}^{4M} \left| P^S_{mn} \right|^2 \cos(\omega_{mn}\, t)$$

that gives the probability of a radical pair to be in the singlet state at any given time, or alternatively the percentage of radical pairs in an ensemble that are in the singlet state at any given time. Integration over the radical pair lifetime (1/k) gives the singlet yield,

$$\Phi_S = k \int_0^\infty \langle \hat{P}^S \rangle\, e^{-kt}\, dt = \frac{1}{M}\sum_{m=1}^{4M}\sum_{n=1}^{4M} \left| P^S_{mn} \right|^2 \left(\frac{k^2}{k^2 + \omega_{mn}{}^2} \right)$$

where $\omega_m = \langle m \,|\, \hat{H} \,|\, m \rangle$, such that $\omega_{mn} = \omega_m - \omega_n$ are the eigenvalues of the Hamiltonian.

Flavin has been experimentally found to form radical pairs with tryptophan and superoxide. With tryptophan, flavin forms a radical pair with electron spins in a singlet

state[74]. With superoxide, flavin forms a radical pair with electron spins in a triplet state[75]. However, for both radical pair formations, re-oxidation back to ground state FAD only occurs for radical pairs that are in the singlet state at the end of the radical pair lifetime. Modeling the quantum spin dynamics of both radical pairs using principle hyperfine axes[76], an angular change from 0-90 results in an approximately 3% change in singlet yield for a flavin-tryptophan radical pair and an approximately 20% change in yield for a flavin-superoxide radical pair (figure 5). The orientation dependent rates in the flavin photocycle, k(θ,ϕ), are assumed in our model to change by the same percentage as the yields.

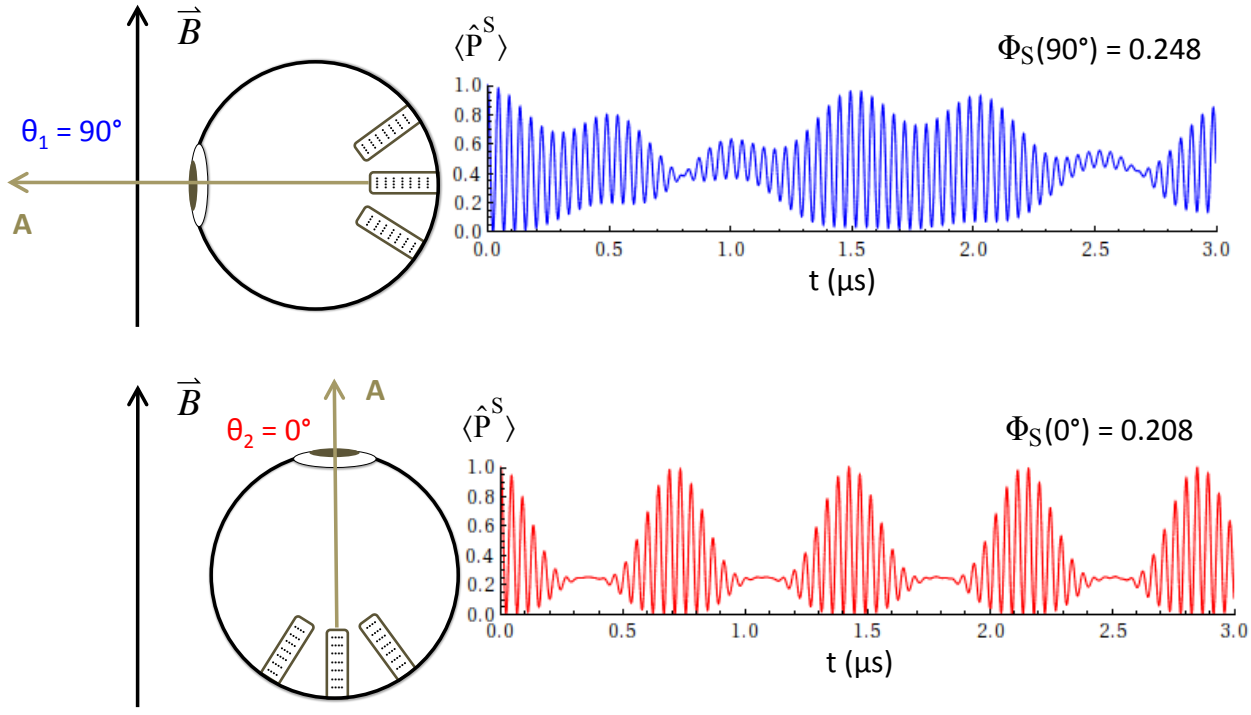

Figure 5. Singlet Fraction and Yields. Singlet fraction over time and subsequent yields of a flavin-superoxide radical pair at 0 and 90 degrees. The angle represents that between flavin net nuclear spin and external geomagnetic field vectors.

Calculation of Signal-to-Noise

The model developed here considers a photocycle in which flavin forms radical pairs with both tryptophan and superoxide (figure 6). The contribution of each radical pair to magnetic sensitivity of the cryptochrome compass is evaluated seperately.

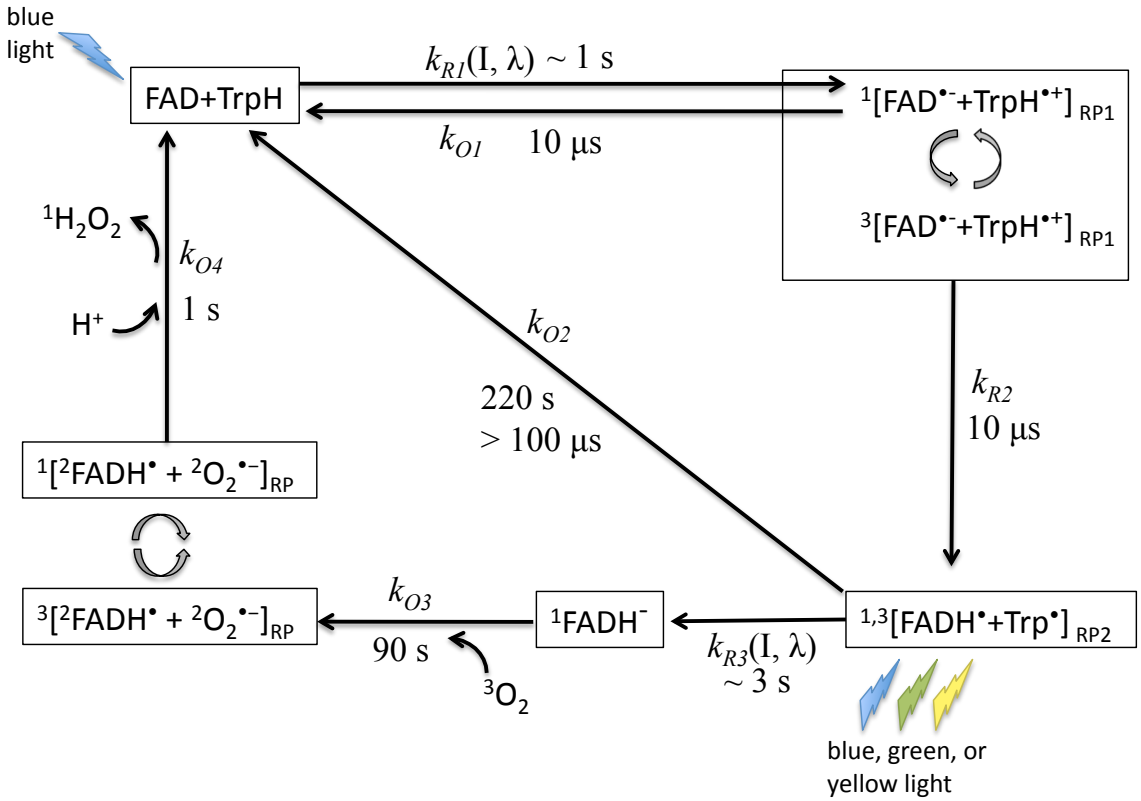

Figure 6. Cryptochrome Photocycle with Kinetic Rates. Radical pairs (RP) undergoing electron spin singlet-triplet interconversions are indicated by circular arrows, the quantum state indicated by a superscript.

Fixed rates ($k_{R2} = k_{O1} = 1\times10^5$ s^{-1})[77,78] and ($k_{O2} = 4.6\times10^{-3}$ s^{-1}, $k_{O3} = 1.1\times10^{-2}$ s^{-1})[79] have been measured experimentally. Solving the first order reaction kinetics in MatLab using the Runge-Kutta method, the relative steady state concentrations of each redox state are found for a given light condition and magnetic field orientation using the following equations:

$$\frac{d[\mathrm{FAD}]}{dt} = -k_{R1}[\mathrm{FAD}] + k_{O1}[\mathrm{RP1}] + k_{O2}[\mathrm{FADH^{\bullet}}] + k_{O4}[\mathrm{RP3}]$$

$$\frac{d[\mathrm{RP1}]}{dt} = k_{R1}[\mathrm{FAD}] - k_{O1}[\mathrm{RP1}] - k_{R2}[\mathrm{RP1}]$$

$$\frac{d[\mathrm{FADH^{\bullet}}]}{dt} = k_{R2}[\mathrm{RP1}] - k_{O2}[\mathrm{FADH^{\bullet}}] - k_{R3}[\mathrm{FADH^{\bullet}}]$$

$$\frac{d[\mathrm{FADH^{-}}]}{dt} = k_{R3}[\mathrm{FADH^{\bullet}}] - k_{O3}[\mathrm{FADH^{-}}]$$

$$\frac{d[\mathrm{RP3}]}{dt} = k_{O3}[\mathrm{FADH^{-}}] - k_{O4}[\mathrm{RP3}]$$

The system is first solved to steady state at the fixed operating rates, with the exception of the light dependent rates that are calculated for each light intensity and wavelength (with a 60 nm half width Gaussian spread about the peak wavelength). This solution gives a baseline concentration of the signaling state, $[\mathrm{FADH^{\bullet}}]_{SS}$. A second solution allows the photocycle to evolve over 5 seconds with a 5% and 20% change in either k_{O1} or k_{O4} respectively depending on which radical pair a magnetic field effect is being investigated. This secondary solution gives $[\mathrm{FADH^{\bullet}}]_{SS,m}$, the signaling state concentration after a change in orientation within the external magnetic field. In this way the signal-to-noise ratio is calculated in a similar way to the Weaver treatment as

$$\frac{S}{N} = \delta\,[\mathrm{FADH^{\cdot}}]_{ss}\sqrt{\frac{q}{(1+q)^2}\,R_T}$$

$$\delta[\mathrm{FADH^{\cdot}}]_{ss} = \frac{\left|[\mathrm{FADH^{\cdot}}]_{ss} - [\mathrm{FADH^{\cdot}}]_{ss,m}\right|}{[\mathrm{FADH^{\cdot}}]_{ss}} \qquad q \equiv \frac{{K_D}^2}{L\,C\,[\mathrm{FADH^{\bullet}}]_{ss}}$$

with $K_D = k_r/k_f = 1 \times 10^{-8}$ M over the cone cell volume of 6.3×10^{-13} L. This magnitude of K_D allows for a sufficiently rapid response time for navigational sensing, requiring only 3.6 seconds to reach 95% equilibrium receptor binding if no bound receptors are initially present[80]. The number of neural receptor is calculated from the neural density[81] $\rho_R \sim 10^4$

μm^{-2} giving an area occupied per receptor of $A_R \sim 10^{-16}$ m^2, which applied to a cellular surface area with radius 2 x 10^{-6} m and length 5 x 10^{-5} m, gives $R_T \sim 1 \times 10^7$.

CHAPTER 6: Signal-to-Noise Predictions

Flavin-Tryptophan Radical Pair Magnetic Sensitivity

The model of cryptochrome signaling with a magnetic field effect only on the flavin-tryptophan radical pair shows strong correlation with the general behavior seen in behavioral experiments. Generally, the model predicts a signal above noise under low frequency light conditions and within a narrow intensity range (figure 7).

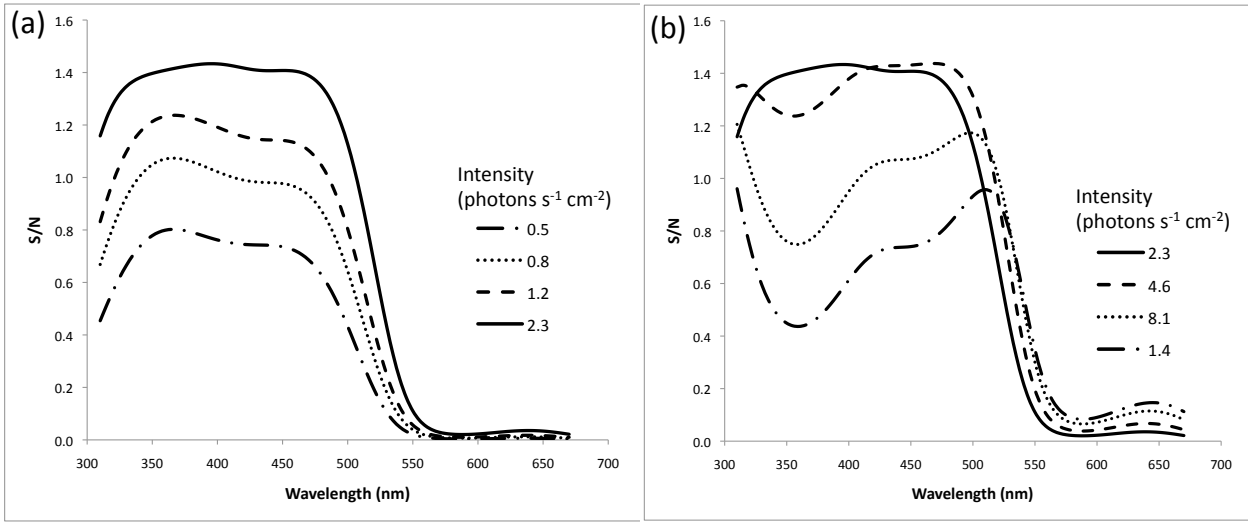

Figure 7. Signal-to-Noise of Flavin-Tryptophan Radical Pair. Shows (a) a rising signal for increasing intensities followed by (b) a falling signal for further increases in intensity. Parameters include a -5% change in spin selective rate k_{01}, 10 thousand ligands and cryptochromes with 13 million receptors.

More specifically, the light intensity range in which orientation is predicted to occur by the model closely matches that observed in behavioral experiments. Hitherto, no quantitative explanation has been offered for this behavior. However, analysis of steady state concentrations at different wavelengths reveals a region of intensities at which the signaling state concentration is maximized. It is within this window of intensities that orientation occurs, presumably because the signal is a function of a change in this state's concentration. At lower intensities the kinetics are not driven significantly and most of the cryptochrome concentration remains in the ground state, FAD. At high intensities, the kinetics drive most of the concentration to full reduction in the $FADH^-$ state. The semi-reduced state peaks at intermediate intensities (figure 8).

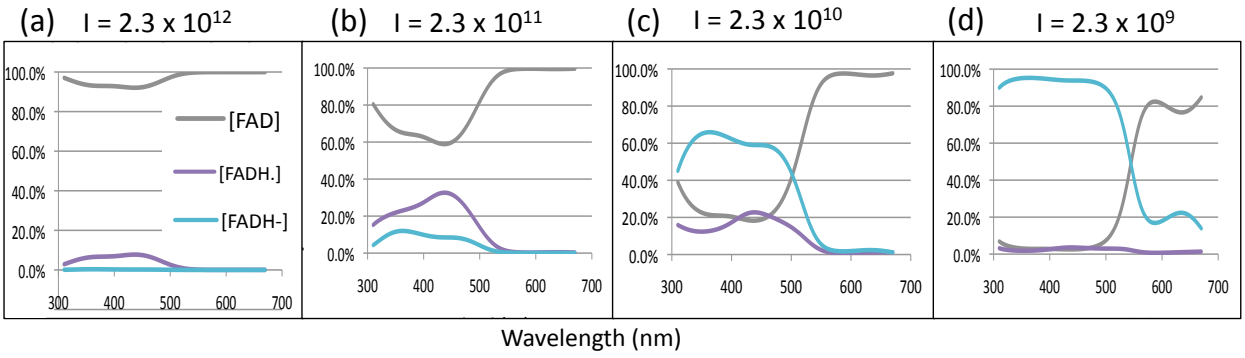

Figure 8. Steady State Concentrations. Depicted over increasing intensities (from a to d) in photons s^{-1} cm^2.

The wavelength cutoff correlation between the model and experiments is less precise. While behavioral experiments observe orientation up to 560 nm, within the region of green light, the model falls short of this, predicting a wavelength cutoff at around 530 nm (figure 9).

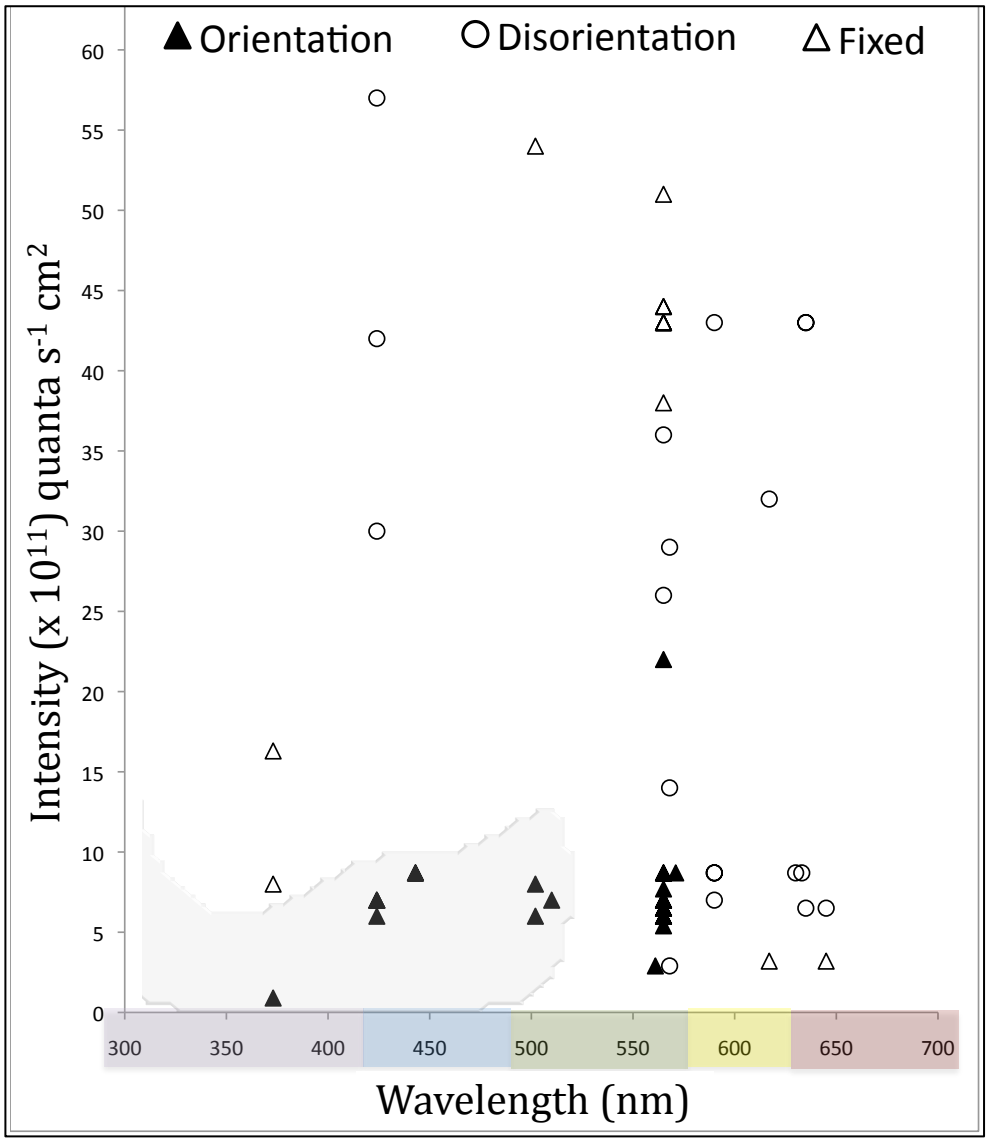

Figure 9. Signal-to-Noise vs. Experimental Light Effects. Migratory bird orientation behavior under monochromatic light conditions of varying wavelengths and intensities. Solid triangles indicate proper orientation under the specified light conditions. Open triangles and circles indicate improper orientation (fixed response and disorientation respectively)[82]. The shaded region represents light conditions under which a signal to noise greater than one is predicted by the cryptochrome signal transduction model.

The model wavelength cutoff is a direct consequence of the FAD absorption spectrum. If the actual *in vivo* absorption spectra in birds ended at 560 nm instead of 520 nm, then the model would correlate with behavioral experiment observations. Another possible

explanation is that only absorption of the neutral semiquinone FADH• has been modeled. If absorption of the anionic semiquinone state FAD•- is also at work, then more green light absorption may increase signaling in that region of the spectrum (figure 10).

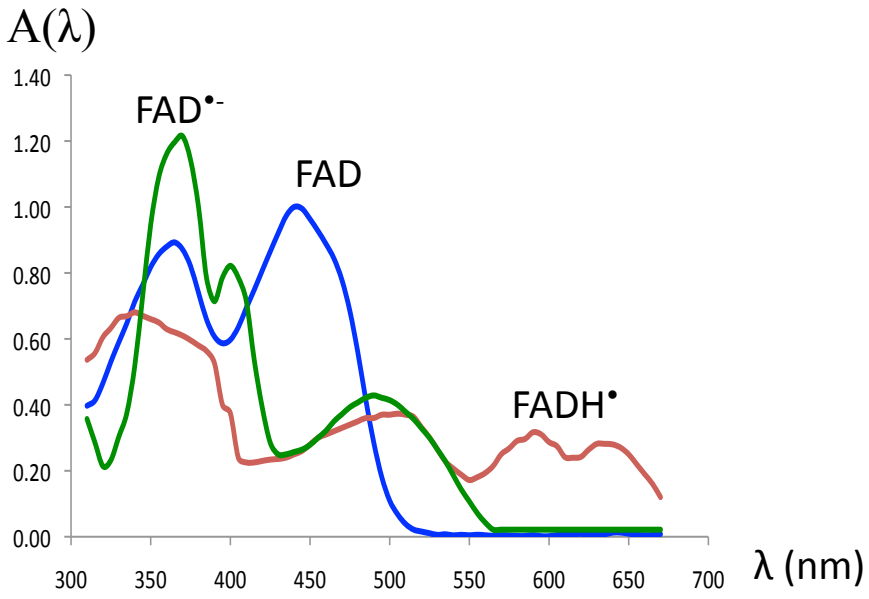

Figure 10. Three State Absorption Spectra. Fully oxidized flavin FAD (blue) and semi-reduced flavin FADH• in neutral form(green) and FAD•- anionic form (red)[83].

Flavin-Superoxide Radical Pair Magnetic Sensitivity

Modeling the signal-to-noise with only the flavin-superoxide radical pair exhibiting magnetic sensitivity appears to correlate less with behavioral experiment observations (figure 11). This is particularly true in the ultraviolet region of the spectrum where orientation only occurs at light intensities less than what is seen under blue light.

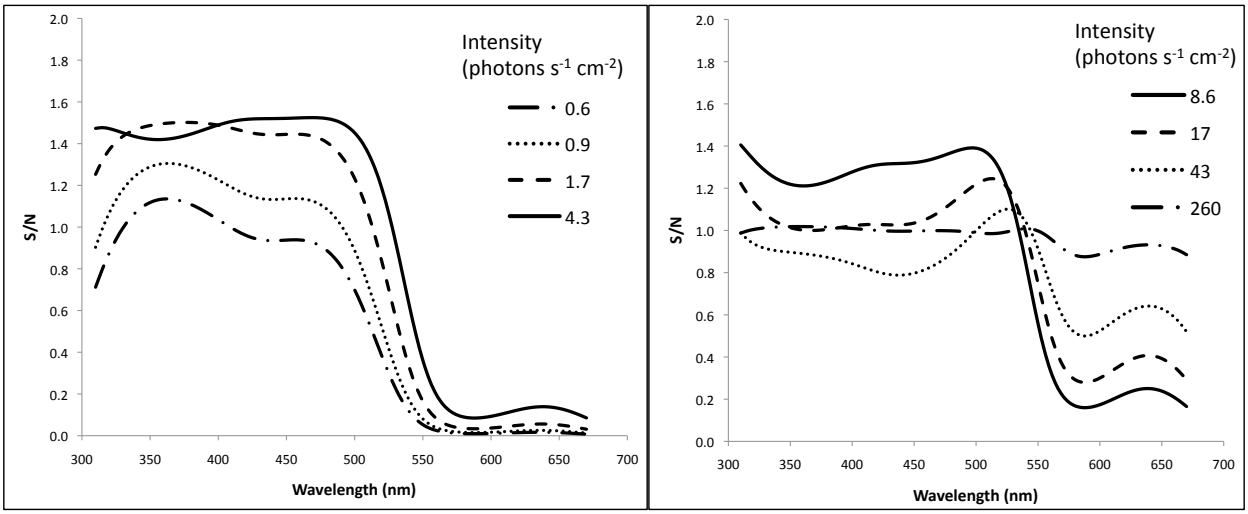

Figure 11. Signal-to-Noise of Flavin-Superoxide Radical Pair. Shows (a) a rising signal for increasing intensities followed by (b) a falling signal for further increases in intensity. Parameters include a +20% change in spin selective rate k_{04}, 10 thousand ligands and cryptochromes with 10 million receptors.

CONCLUSION

In an attempt to explain experimentally observed light effects of the biological compass of migratory birds, the cryptochrome model of signal transduction offers the first quantitative descriptions of how such affects may simply be attributable to photocycle kinetics. The simplest signal transduction model conceivable, based solely on cryptochrome's photochemistry, can reasonable explain short wavelength (UV/Blue) orientation selectivity of the biological compass. While this had always been qualitatively understood given the absorption spectrum of cryptochrome, the close agreement in the UV and blue region is telling. Particularly the prediction that UV light orientation requires light intensities less intense than those at which blue light orientation occurs, a recently observed light effect in behavioral experiments.

The origins of disorientation at high light intensities is elucidated by the model as arising from a concentration rising and falling of the flavin signaling state, between which maximum concentration occurs and maximum signaling occurs.

As researchers continue to isolate the radical pair at the heart of a cryptochrome compass, the model developed here gleans important insights. Particularly that a flavin-tryptophan radical pair most closely resembles intensity ranges and short wavelength orientation observed experimentally. However, a flavin-superoxide radical pair would also give orientation with a reasonably similar set of light conditions. Thus, continued investigations into both radical pair formations is warranted.

REFERENCES

[1] Wiltschko, W., Wiltschko, R. Magnetic compass of european robins. *Science*, 176(4030):62-64, Apr 1972.
[2] Emlen, S. T., Wiltschko, W., Demong, N. J., Wiltschko, R., Bergman, S. Magnetic direction finging: Evidence for its use in migratory indigo buntings. *Science*, 193(4252):505-508, Aug 1976
[3] Wiltschko, W. and Merkel, F. W. Orientierung zugunruhiger Rotkehlchen im statischen Magnetfeld. Verh. Dtsch. Zool. Ges. 59, 362-367, 1966.
[4] Wiltschko, W. Über den Einfluß statischer Magnetfelder auf die Zugorientierung der Rotkehlchen (Erithacus rubecula). Z. Tierpsychol. 25, 536-558, 1968.
[5] Wiltschko, W., Wiltschko, R. Magnetic Orientation in Animals, *Zoophysiology* Springer-Verlag, 1995.
[6] Kalmijn, A. J. The Detection of Electric Fields from animate and Animate Sources Other Than Electric Organs. In: Handbook of Sensory Physiology., A. Fessard, (ed). Springer-Verlag, New York, 1974.
[7] Blakemore, R. Mgneto Tactic Bacteria. *Science* 190 (4212): 377-379, 1975.
[8] Kirschvink, J., and Gould, J. Biogenic magnetite as a basis for magnetic field detection in animals. *BioSystems*. 13:181–201, 1981.
[9] Kirschvink, J., M. Walker, S. Chang, A. Dizon, and K. Peterson. Chains of single-domain magnetite particles in chinook salmon, On- corhynchus tschawytscha. J. Comp. Physiol. A. 157:375–381, 1985.
[10] Edmonds, D. A sensitive optically detected magnetic compass for animals. *Proc. R. Soc. B.* 263:295–298, 1996
[11] Beason, R. C., Nichols, J. E. Magnetic Orientation and Magnetically Sensitive Material in a Trans Equatorial Migratorial Bird. *Nature* 309(5964): 151-153, 1984.
[12] Walcott, C., Gould, J. L., Kirschvink, J. L. Pigeons Have Magnets. *Science* 205(4410):1027-1029, 1979.
[13] Wiltschko, W., and R. Wiltschko. Magnetic orientation in birds. J. Exp. Biol. 199:29–38, 1996.
[14] Wiltschko, W., and R. Wiltschko. Magnetic compass of European robins. Science. 176:62–64, 1972.
[15] Lohmann, K. J., Lohmann, C. M. F., Putman, N. F. Magnetic maps in animals: nature's GPS. *The Journal of Experimental Biology* 210:3697- 3705, 2007.
[16] Beason, R. C., Semm, P. Magnetic Responses of the Trigeminal Nerve Sstem of the Bobolink Dolichonyx-Oryzivorus. *Neuroscience Letters* 80(2):229-234, 1987.
[17] Beason, R. C., Semm, P. Does the Avian ophthalmic nerve carry magnetic navigational information? *Journal of Experimental Biology* 199(5):1241-1244, 1996.
[18] Semm P, Beason, R. C. Responses to small magnetic varia- tions by the trigeminal system of the bobolink. *Brain Res Bull* 25:735–740, 1990.
[19] Mora, C. V., Davison, M., J. Martin Wild, M., Walker, M. M. Magnetoreception and its trigeminal mediation in the homing pigeon, Letters to Nature, pg. 508-511, 2004.

[20] Zapka, M., Heyers, D., Hein, C. M., Engels, S., Schneider, N. L., Hans, J.., Weiler, S., Dreyer, D., Kishkinev, D., Wild, J. M., Mouritsen, H. Visual but not trigeminal mediation of magnetic compass information in a migratory bird. *Nature* 461(29) October 2009.
[21] Munro, U., Munro, J.A., Phillips, J.B., Wiltschko, R. Wiltschko, W. 1997 Evidence for a magnetite-based navigational 'map' in birds. *Naturwissenschaften* 84:26–28, 1997.
[22] Wiltschko W, Munro U, Ford H, Wiltschko R. Bird navigation: what type of information does the magnetite-based receptor provide? *Proc R Soc B* 273:2815–2820, 2006.
[23] Wolfgang Wiltschko, Hugh Ford, Ursula Munro, Michael Winklhofer, Roswitha Wiltschko. Magnetite-based magnetoreception: the eVect of repeated pulsing on the orientation of migratory birds. *J Comp Physiol A* 193:515–522, 2007.
[24] Wiltschko W, Munro U, Beason RC, Ford H, Wiltschko R (1994) A magnetic pulse leads to a temporary deXection in the ori- entation of migratory birds. *Experientia* 50:697–700.
[25] Wiltschko W, Munro U, Ford H, Wiltschko R. EVect of a magnetic pulse on the orientation of Silvereyes, Zosterops l. lateralis, during spring migration. *J Exp Biol* 201:3257–3261, 1998.
[26] Beason RC, Dussourd N, Deutschlander M. Behavioural evidence for the use of magnetic material in magnetorecep- tion by a migratory bird. *J Exp Biol* 198:141–146, 1995.
[27] Munro U, Munro JA, Phillips JB, Wiltschko R, Wiltschko W. Evidence for a magnetite-based navigational 'map' in birds. *Naturwissenschaften* 84:26–28, 1997.
[28] Beason RC, Wiltschko R, Wiltschko W. Pigeon homing: eVects of magnetic pulse on initial orientation. *Auk* 114:405– 415, 1997.
[29] Sönke Johnsen, Erin Mattern and Thorsten Ritz. Light-dependent magnetoreception: quantum catches and opponency mechanisms of possible photosensitive molecules. *The Journal of Experimental Biology* 210:3171-3178, 2007.
[30] Wiltschko W, Wiltschko R. Magnetic orientation and mag- netoreception in birds and other animals. *J Comp Physiol A* 191:675–693, 2005.
[31] Roswitha Wiltschko, Katrin Stapput, Peter Thalau and Wolfgang Wiltschko. Directional orientation of birds by the magnetic field under different light conditions. *J. R. Soc. Interface* 7: S163–S177, 2010.
[32] Katrin Stapput, Peter Thalau, Roswitha Wiltschko, and Wolfgang Wiltschko. Orientation of Birds in Total Darkness. *Current Biology* 18:602–606, 2008.
[33] Schulten, K. Magnetic field effects in chemistry and biology. *Festko ̈rperprobleme*. 22:61– 83, 1982.
[34] Ritz, T., Adem, S. & Schulten, K. A model for photo- receptor-based magnetoreception in birds. *Biophys. J.* 78, 707–718, 2000.
[35] Wiltschko, W., U. Munro, H. Ford, and R. Wiltschko. Red light disrupts magnetic orientation of migratory birds. *Nature*. 364:525–527, 1993.
[36] Wiltschko, W., and R. Wiltschko. The effect of yellow and blue light on magnetic compass orientation in European robins, Erithacus rube- cula. *J. Comp. Physiol. A.* 184:295–299, 1999.
[37] Mo ̈ller, A., W. Sagasser, W. Wiltschko, and B. Schierwater. Retinal cryptochrome in a migratory passerine bird: a possible transducer for the avian magnetic compass. *Naturwissenschaften*. 91:585–588, 2004.
[38] Mouritsen, H., U. Janssen-Bienhold, M. Liedvogel, G. Feenders, J. Stalleicken, et al. Cryptochromes and neuronal-activity markers colocalize in the retina of migratory birds during magnetic orientation. *Proc. Natl. Acad. Sci*. 101:14294–14299, 2004.

[39] Liedvogel, M., M. Maeda, K. Henbest, E. Schleicher, T. Simon, et al. Chemical magnetoreception: bird cryptochrome 1a is excited by blue light and forms long-lived radical-pairs. *PLoS ONE*. 2(10):e1106, 2007.
[40] Giovani,B.,M.Byrdin,M.Ahmad,andK.Brettel. Light-induced electron transfer in a cryptochrome blue-light photoreceptor. *Nat. Struct. Biol.* 6:489–490, 2003.
[41] Ahmad,M.,P.Galland,T.Ritz,R.Wiltschko,andW.Wiltschko. Magnetic intensity affects cryptochrome-dependent responses in Arabi- dopsis thaliana. *Planta.* 225:615–624, 2007.
[42] Mouritsen, H., Feenders, G., Liedvogel, M., Wada, K. &Jarvis, E. D. Night-vision brain area in migratory songbirds. *Proc. Natl. Acad. Sci.* 102:8339 – 8344, 2005.
[43] Zapka, M. et al. Visual but not trigeminal mediation of magnetic compass information in a migratory bird. Nature 461, 1274–1277, 2009.
[44] Henbest,K.B.,P.Kukura,C.T.Rodgers,P.J.Hore,andC.R.Timmel. Radiofrequency magnetic field effects on a radical recombination reaction: a diagnostic test for the radical pair mechanism. *J. Am. Chem. Soc.* 126:8102–8103, 2004.
[45] Cintolesi, F., Ritz, T., Kay, C., Timmel, C. & Hore, P. Anisotropic recombination of an immobilized photoinduced radical pair in a 50 mT magnetic field: a model avian photomagnetoreceptor. *Chem. Phys.* 294, 384–399, 2003.
[46] Ritz, T., Thalau, P., Phillips, J. B., Wiltschko, R. & Wiltschko, W. Resonance effects indicate a radical- pair mechanism for avian magnetic compass. *Nature* 429,177–180, 2004.
[47] Kirschvink, J. et al. in Sensory Transduction (eds Corey, D. & Roper, S.) 225–240 Society of General Physiologists 45th Annu. Symp., Rockefeller Univ. Press, New York, 1992.
[48] Thalau, P., Ritz, T., Stapput, K., Wiltschko, R. & Wiltschko, W. Magnetic compass orientation of migratory birds in the presence of a 1.315 MHz oscillating field. *Naturwissenschaften* 92, 86–90, 2005.
[49] Schulten, K. Magnetic field effects in chemistry and biology. *In Festko ̈rperprobleme*. J. Treusch, editor. Vieweg, Braunschweig. 22:61– 83, 1982.
[50] Ritz, T., Adem, S. & Schulten, K. A model for photo- receptor-based magnetoreception in birds. *Biophys. J.* 78, 707–718, 2000.
[51] Roswitha Wiltschko, Katrin Stapput, Peter Thalau and Wolfgang Wiltschko. Directional orientation of birds by the magnetic field under different light conditions. *Journal of the Royal Society Interface,* 7(2): S163–S177, Apr 2009.
[52] Sönke Johnsen, Erin Mattern, and Thorsten Ritz. Light-dependent magnetoreception: quantum catches and opponency mechanisms of possible photosensitive molecules. *The Journal of Experimental Biology*, 210:3171-3178, Jul 2007.
[53] Roswitha Wiltschko, Ursula Munro, Hugh Ford, Katrin Stapput, Peter Thalau, Wolfgang Wiltschko. Orientation of migratory birds under ultraviolet light. *Journal of Comparitive Physiology A*, 200(5):399-407, May 2014.
[54] Sönke Johnsen, Erin Mattern, and Thorsten Ritz. Light-dependent magnetoreception: quantum catches and opponency mechanisms of possible photosensitive molecules. *The Journal of Experimental Biology*, 210:3171-3178, Jul 2007.
[55] Sönke Johnsen, Erin Mattern, and Thorsten Ritz. Light-dependent magnetoreception: quantum catches and opponency mechanisms of possible photosensitive molecules. *The Journal of Experimental Biology*, 210:3171-3178, Jul 2007.

[56] Andrea Möller, Sven Sagasser, Wolfgang Wiltschko, and Bernd Schierwater. Retinal cryptochrome in a migratory passerine bird: a possible transducer for the avian magnetic compass. *Naturwissenschaften*. 91:585–588, 2004.
[57] Henrik Mouritsen, Ulrike Janssen-Bienhold, Miriam Liedvogel, Gesa Feenders, Julia Stalleicken, Petra Dirks, and Reto Weiler. Cryptochromes and neuronal-activity markers colocalize in the retina of migratory birds during magnetic orientation. *Proceedings of the National Academy of Sciences of the United States of America*, 101:14294–14299, 2004.
[58] Taishi Yoshii, Margaret Ahmad, and Charlotte Helfrich-Förster. Cryptochrome Mediates Light-Dependent Magnetosensitivity of Drosophila's Circadian Clock. *PLOS Biology*, 7(4):0813-0819, Apr 2009.
[59] Robert J. Gegear, Amy Casselman, Scott Waddell and Steven M. Reppert. Cryptochrome mediates light-dependent magnetosensitivity in Drosophila. *Nature*, 454:1014-1018, Aug 2008.
[60] Margaret Ahmad, Paul Galland, Thorsten Ritz, Roswitha Wiltschko, and Wolfgang Wiltschko. Magnetic intensity affects cryptochrome-dependent responses in Arabidopsis thaliana. *Planta*, 225:615–624, 2007.
[61] Kiminori Maeda, Alexander J. Robinsona, Kevin B. Henbesta, Hannah J. Hogbenb, Till Biskupb, Margaret Ahmadc, Erik Schleichere, Stefan Webere, Christiane R. Timmela, and P. J. Hore. Magnetically sensitive light-induced reactions in cryptochrome are consistent with its proposed role as a magnetoreceptor. *Proceedings of the National Academy of Sciences of the United States of America*, Mar 2012.
[62] Pavel Müller and Margaret Ahmad. Light-activated Cryptochrome Reacts with Molecular Oxygen to Form a Flavin–Superoxide Radical Pair Consistent with Magnetoreception. *The Journal of Biological Chemistry*, 286(24): 21033–21040, Jun 2011.
[63] James C. Weaver, Timothy E. Vaughan, and R. Dean Astumian. Biological sensing of small field differences by magnetically sensitive chemical reactions. *Nature*, 405:707-709, Jun 2000.
[64] Kiminori Maeda, Kevin B. Henbest, Filippo Cintolesi, Ilya Kuprov, Christopher T. Rodgers, Paul A. Liddell, Devens Gust, Christiane R. Timmel and P. J. Hore. Chemical compass model of avian magnetoreception. *Nature*, 453(7193): 387-390, May 2008.
[65] James C. Weaver, Timothy E. Vaughan, and R. Dean Astumian. Biological sensing of small field differences by magnetically sensitive chemical reactions. *Nature*, 405:707-709, Jun 2000.
[66] Nießner C, Denzau S, Gross JC, Peichl L, Bischof H-J, et al. (2011) Avian Ultraviolet/Violet Cones Identified as Probable Magnetoreceptors. *PLoS ONE* 6(5): e20091.
[67] Bolte P, Bleibaum F, Einwich A, Günther A, Liedvogel M, Heyers D, et al. Localisation of the Putative Magnetoreceptive Protein Cryptochrome 1b in the Retinae of Migratory Birds and Homing Pigeons. *PLoS ONE* 11(3): e0147819, 2016.
[68] Banerjee R, et al. The signaling state of Arabidopsis cryptochrome 2 containsflavin semiquinone. *J Biol Chem* 282:14916–14922, 2007.
[69] Bouly JP, et al. Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. *J Biol Chem* 282:9383–9391, 2007.
[70] Vincent Massey. The chemical and biological versatility of riboflavin. *Biochemical Society Transactions*, 28(4): 283 -96, Aug 2000.
[71] Evans et al. 2013 Interface Focus.
[72] Maria Procopio, Justin Link, Dorothy Engle, Jacques Witczak, Thorsten Ritz, Margaret Ahmad. Kinetic modeling of the Arabidopsis cryptochrome photocycle: FADHo accumulation correlates with biological activity. *Frontiers* (submitted for publication 2016).

[73] Christiane R. Timmel, U. Till, Brian Brocklehurst, Keith A. Mclauchlan & Peter J. Hore. Effects of weak magnetic fields on free radical recombination reactions. *Molecular Physics*, 95(1): 71-89, 1998.
[74] Kiminori Maeda, Alexander J. Robinsona, Kevin B. Henbesta, Hannah J. Hogbenb, Till Biskupb, Margaret Ahmadc, Erik Schleichere, Stefan Webere, Christiane R. Timmela, and P. J. Hore. Magnetically sensitive light-induced reactions in cryptochrome are consistent with its proposed role as a magnetoreceptor. *Proceedings of the National Academy of Sciences of the United States of America*, Mar 2012.
[75] Pavel Müller and Margaret Ahmad. Light-activated Cryptochrome Reacts with Molecular Oxygen to Form a Flavin–Superoxide Radical Pair Consistent with Magnetoreception. *The Journal of Biological Chemistry*, 286(24): 21033–21040, Jun 2011.
[76] F. Cintolesi a, T. Ritz b,1, C.W.M. Kay c, C.R. Timmel a, P.J. Hore. Anisotropic Recombination of an Immobilized Photoinduced Radical Pair in a 50-μT Magnetic Field: a Model Avian Photomagnetoreceptor. *Chemical Physics* 294 (2003) 385–399.
[77] Kiminori Maeda, Alexander J. Robinsona, Kevin B. Henbesta, Hannah J. Hogbenb, Till Biskupb, Margaret Ahmadc, Erik Schleichere, Stefan Webere, Christiane R. Timmela, and P. J. Hore. Magnetically sensitive light-induced reactions in cryptochrome are consistent with its proposed role as a magnetoreceptor. *Proceedings of the National Academy of Sciences of the United States of America*, Mar 2012.
[78] Ya-Ting Kao, Chuang Tan, Sang-Hun Song, Nuri O¨ ztürk, Jiang Li, Lijuan Wang, Aziz Sancar, Dongping Zhong, Ultrafast Dynamics and Anionic Active States of the Flavin Cofactor in Cryptochrome and Photolyase, *JACS* 130:7695–7701, 2008.
[79] Pavel Müller and Margaret Ahmad. Light-activated Cryptochrome Reacts with Molecular Oxygen to Form a Flavin–Superoxide Radical Pair Consistent with Magnetoreception. *The Journal of Biological Chemistry*, 286(24): 21033–21040, Jun 2011.
[80] Douglas A. Lauffenburger and Jennifer L. Linderman. Receptors: Models for binding, trafficking, and signaling. Oxford University Press, 1993.
[81] Kandel 1991 *Principles of Neural Science*
[82] Sönke Johnsen, Erin Mattern, and Thorsten Ritz. Light-dependent magnetoreception: quantum catches and opponency mechanisms of possible photosensitive molecules. *The Journal of Experimental Biology*, 210:3171-3178, Jul 2007.
[83] Vincent Massey. The chemical and biological versatility of riboflavin. *Biochemical Society Transactions*, 28(4): 283 -96, Aug 2000.