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Signal Transduction Model of Magnetic Sensing in Cryptochrome Mediated Photoreception

THESIS

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for the degree of

MASTER OF SCIENCE

in Chemical and Materials Physics

by

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ABSTRACT OF THE THESIS

Signal Transduction Model of Magnetic Sensing
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By

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Master of Science in Chemical and Materials Physics

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Professor Thorsten Ritz, Chair

While migratory birds have long been known to use the Earth's magnetic field for navigation, the precise biophysical mechanism behind this magnetic sense remains unconfirmed. A leading theory of magnetoreception suggests a chemical compass model with a yet undetermined molecular reaction site and unknown magnetically sensitive reactants. The cryptochrome photoreceptor has emerged as a promising candidate site. This investigation numerically models the first order kinetics of cryptochrome mediated photoreception, in order to evaluate its ability to function as a magnetic sensor and transduce orientation information along a neural pathway. A signal-to-noise ratio is defined to quantify the threshold for the functioning of a cryptochrome-based chemical compass. The model suggests that a flavin-superoxide radical pair in cryptochrome functions as the chemical reactants for magnetoreception. Such a cryptochrome-based signal transduction model reasonably predicts the general light intensity and wavelength effects that have been experimentally observed in migratory birds.

INTRODUCTION

Decades of behavioral experiments confirm that numerous animal species have the ability to sense the Earth's magnetic field [21, 4]. Specifically, migratory birds have been shown to use the geomagnetic field as a primary source of orientation information as they navigate thousands of miles along their migratory paths. The geomagnetic field is an ideal source of orientation information, as it is always present unlike sun or star cues, if the sky is overcast. The field may also allow identification of a global location with a unique combination of inclination, polarity, and field strength, which averages 50 ± 10 μT.

However, unlike other sensory systems, the precise biophysical mechanism behind the magnetic sense remains unconfirmed. Specifically, the organ responsible for the sense has yet to be conclusively identified. In addition, the relative weakness of the geomagnetic field makes understanding its detection, with biological materials alone, particularly challenging. Still, an emerging body of recent evidence is lending strong support for a model of magnetoreception based on a chemical compass mechanism.

CHAPTER 1: Chemical Compass Mechanism of Magnetoreception

The chemical compass mechanism of magnetoreception was first introduced by Schulten in 1978 and further detailed by Ritz in 2000 [18, 17]. In this model (figure 1), light that is detected by specialized photoreceptors fixed in the bird's eye, induces a chemical reaction that is sensitive to the receptor's orientation within the geomagnetic field. Changes in orientation result in variations to product yields of the reaction. These fluctuating product concentrations constitute magnetic orientation information that can then be transduced along a neural pathway to produce patterns of signal modulation for different directions. In this way, visual patterns could allow the birds to distinguish their migratory direction from other directions.

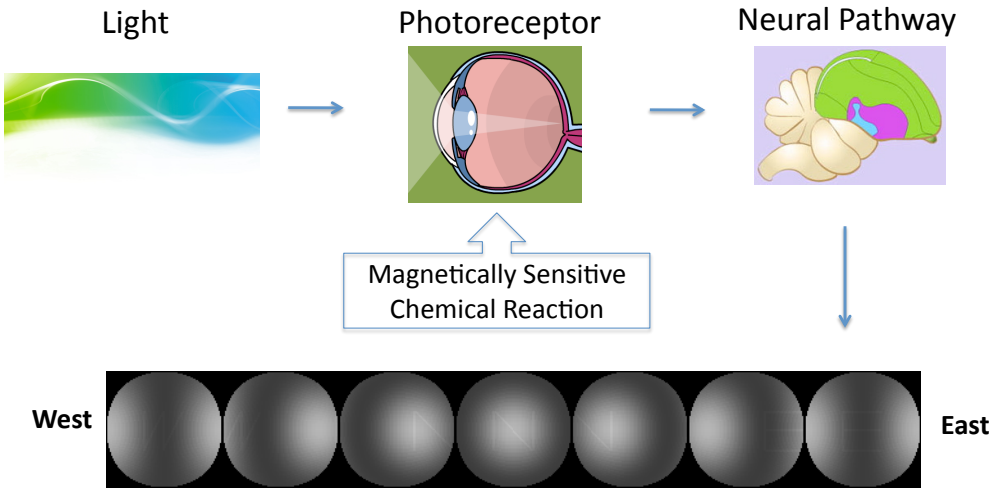

Figure 1. Chemical compass mechanism of magnetoreception. Light, detected by photoreceptors in the bird's eye, triggers a chemical reaction whose products are sensitive to changes in orientation within the magnetic field. Fluctuating product concentrations that correspond to changes in the receptor's orientation within the field constitute magnetic orientation information that is transduced along a neural pathway giving rise to a visual pattern that allows the birds to distinguish their migratory direction.

The body of evidence supporting this chemical compass mechanism of magnetoreception highlights three key aspects of its functioning.

1. The observation that the magnetic compass is light dependent on both wavelength and intensity.
2. The identification of a photoreceptor located within the bird's eye that can be sensitive to external magnetic fields under the appropriate range of light conditions.
3. The development of a biologically feasible mechanism by which magnetic orientation information could be transduced along a neural pathway.

The next four chapters will further detail the research supporting these functional aspects of the chemical compass mechanism.

CHAPTER 2: Light Dependence of the Magnetic Sense

During the migratory season, when strongly motivated to travel in their migratory direction, , individual birds are placed in a funnel cage and exposed to diffuse light of various wavelengths. Under UV, blue or green light, the birds orient correctly toward their migratory direction [21]. However, they become disoriented under yellow & red light (figure 2). This behavior suggests a wavelength threshold for orientation at approximately 570 nm, above which disorientation occurs.

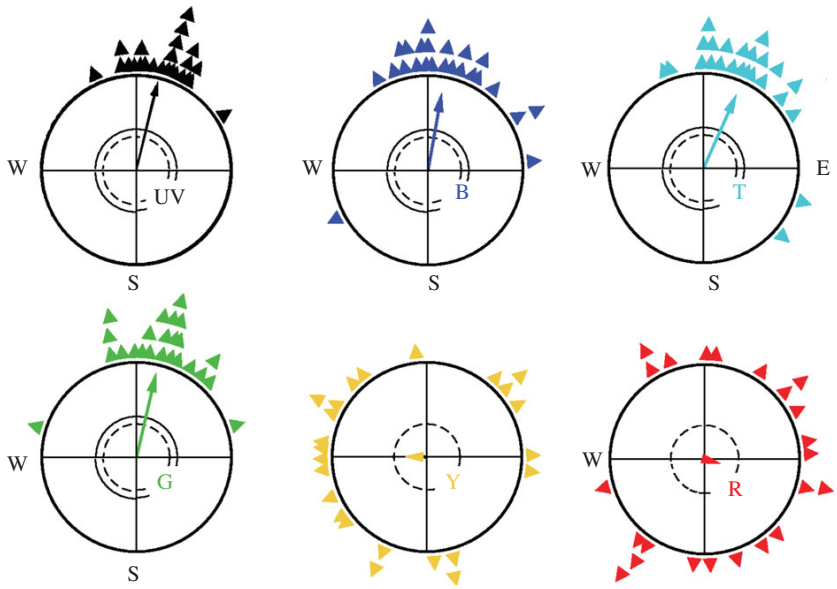

Figure 2. Orientation of European robins during spring migration under monochromatic light of different wavelengths. The light intensity was 7–8 x 10^{15} quanta s^{-1} m^{-2}, except for UV light, which was only 0.8 x 10^{15} quanta s^{-1} m^{-2}. The triangles at the periphery of the circle give mean headings of individual birds based on three recordings each; the arrows represent the grand mean vectors with the length proportional to the radius of the circle. The two inner circles mark the 5% (dotted) and the 1% significance border of the Rayleigh test [2]; arrows exceeding these circles indicate significant orientation. (Reprinted from [21] with permission)

In addition to this wavelength threshold, an intensity cutoff for magnetic orientation also seems to exist. Crepuscular and nocturnal migrants have been found to orient correctly under light of similar intensity to that found at twilight (just before sunrise and just after sunset), when they set off on their migratory path. This falls within an intensity range of approximately 3 - 22 x 10^{11} photons s^{-1} cm^{-2}. However, they lose their ability to orient correctly under stronger light intensities above 26 x 10^{11} photons s^{-1} cm^{-2}, as well as in complete darkness [6].

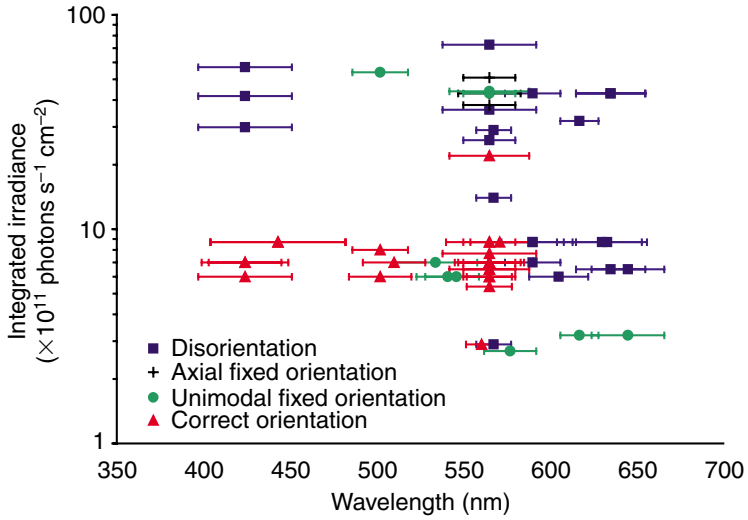

Figure 3. Irradiance spectra of the single-LED conditions under which birds were tested. Values denote central wavelength; error bars denote the range over which intensity is at least half of that at the peak. (Reprinted from [6] with permission)

These patterns of light dependence give rise to specific criteria for evaluating potential photoreceptors that could serve as the molecular site for the proposed magnetically sensitive chemical reaction that is fundamental to the chemical compass mechanism of magnetoreception.

CHAPTER 3: Magnetic Sensitivity of the Cryptochrome Photoreceptor

The exact molecular site for the magnetically sensitive chemical reaction, at the heart of the chemical compass mechanism, has yet to be confirmed. Photoreceptors, including cryptochrome, cone pigments, melanopsin, and rhodpsin, have been considered as potential sites for magnetoreception. However, numerous *in vitro* experiments, attempting to isolate which of these potential receptor systems might be used in magnetoreception, have yielded inconclusive results [6, 14]. Still, cryptochrome remains the only candidate, thus far, that has been shown to meet all of the criteria for a functioning magnetic compass.

Cryptochrome is a photoreceptor that absorbs light in the blue-green spectrum, which corresponds to the wavelength sensitivity range for magnetic orientation. In the same protein family as photolyase, a DNA repair enzyme, cryptochrome's structure conforms to that of other signaling proteins in that it contains two domains – one which allows it to bind to light sensing chromophores (such as flavin), and another that allows it to bind to signaling partners, giving it a means by which to transduce magnetic orientation information along a neural pathway.

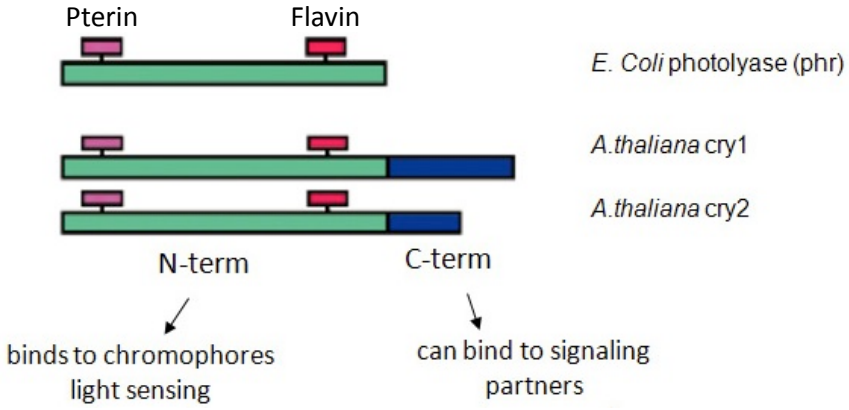

Figure 4. Protein domain structure of photolyase and cryptochrome.

While known for its role in regulating circadian rhythms in various organisms, of specific interest here is recent evidence characterizing the magnetic sensitivity of this photoreceptor. The following research suggests that the functioning of cryptochrome in birds, fruit flies, and plants is sensitive to magnetic fields and light conditions.

Cryptochrome, specifically with the cry1 gene, have been found in ganglion cells located in the retina of the bird eye [12]. A study found that migratory birds exhibited high levels of cry1 gene expression and high neural activity in these cells during magnetic orientation [13]. In contrast, non-migratory birds showed no cry1 gene expression and very low levels of neural activity in the same cells. Thus magnetoreception appears to be correlated with cryptochrome protein expression.

In *Drosophila* fruit flies, cryptochrome has long been known to slow circadian clock rhythms in response to light. Magnetic field exposure was found to enhance this slowing of clock rhythm under blue light, while having no effect under red light [23]. Furthermore, this magnetic field effect was found to be magnified in flies with an over expression of cryptochrome. In contrast, the effect completely disappeared in cryptochrome deficient flies, who were either completely devoid of cryptochrome or simply lacked the protein's flavin light sensing chromophore. Another study found flies correctly sensed and oriented towards or away from an applied magnetic field in full spectrum light and a spectra of wavelengths greater than 400nm [5]. However, the flies lost their magnetic sense under

light spectra limited to long wavelengths of over 420nm. Again, cryptochrome deficient flies showed no magnetic response.

In plants, specifically *Arabidopsis thaliana* which carries the cry1 and cry2 genes, cryptochrome plays a vital role in retarding hypocotyl (seedling stem) growth and in enhancing anthocyanin (pigment) accumulation. A recent study found that increased magnetic field strength resulted in an amplification of cryptochrome's effect in both hypocotyl growth and anthocyanin accumulation, but only under blue light exposure [1]. The effect was not seen on stem growth in darkness, red light, or cryptochrome deficient plants.

All of these studies suggest that cryptochrome's function in biological systems is not only light dependent, but also sensitive to changes in the external magnetic field environment. To understand what gives rise to cryptochrome's magnetic sensitivity, a closer look must be taken at the protein's light sensing chromophore, flavin, and the magnetically sensitive chemical reaction it undergoes upon irradiation.

CHAPTER 4: FAD Formation of Magnetically Sensitive Radical Pairs

Cryptochrome's magnetic sensitivity is a direct consequence of it's light sensing chromophore, flavin adenine dinucleotide (FAD), and its ability to form magnetically sensitive radical pairs. A radical pair is formed when the flavin donates an electron to another molecule, resulting in each molecule having an unpaired valence electron. Flavin has been experimentally shown to form radical pairs with both tryptophan [10] and superoxide [15]. Upon examining both scenarios, the most significant behavior was found with the flavin-superoxide radical pair, which will be the focus of discussion going forward.

In vivo experiments have identified the likely photocycle of flavin (figure 5, table 1), which exists in three distinct states. Upon irradiation, the ground state, FAD, is excited and semi-reduced to the semiquinone state, $FADH^{\bullet}$, which is known to be the signaling state. When the flavin is in this signaling state, cryptochrome can bind to a signaling partner and thereby transfer magnetic orientation information along a neural pathway. The semiquinone state is subsequently fully reduced to $FADH^{-}$, from which a radical pair of semi-reduced flavin and superoxide is formed.

Each of the radical pair molecules contains an unpaired electron whose spins together exist in an initial singlet quantum state. Changes to flavin's orientation within the magnetic field causes the radical pair to oscillate between singlet and triplet states. However, continuation along the photocycle path only occurs from the singlet state, as formation of the diamagnetic products hydrogen peroxide and fully-oxidized FAD is spin forbidden from

the triplet state. Thus, the probability, or time, that any cryptochrome's flavin is in the signaling state is altered by changes to its orientation angle within the magnetic field. This is how changes in magnetic field orientation can alter cryptochrome's signaling along a neural pathway.

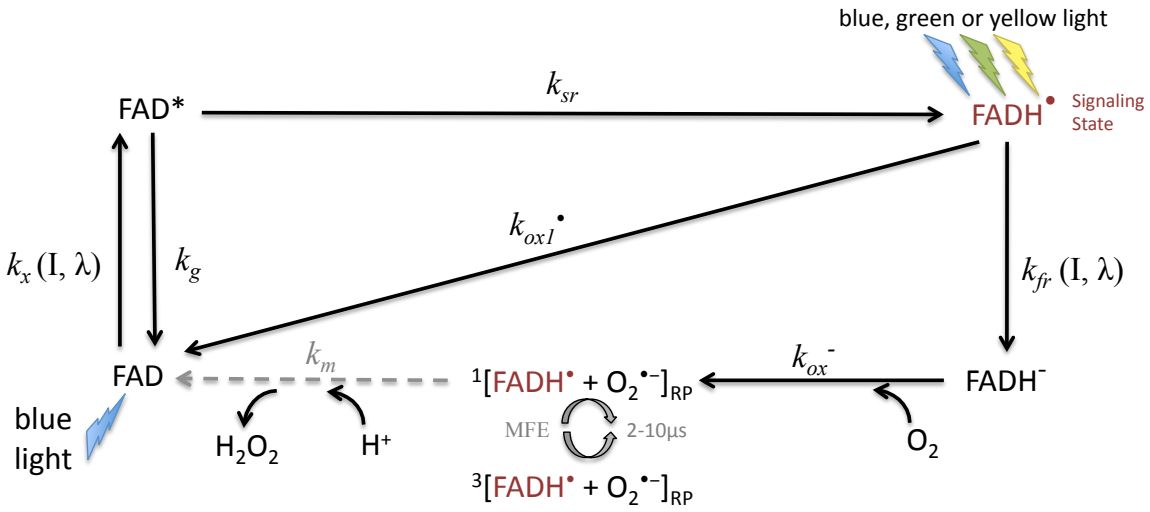

Figure 5. Flavin photocycle forming a radical pair (RP) with superoxide. Superscripts signify a singlet (1) or triplet (3) state.

Table 1. Flavin photocycle reaction rates.

Rate	**(ms^{-})**	
k_x (I,λ)	3.8 x 10^{-5} to 9.2 x 10^{-3} for I = 1 x 10^{-3} photons • ms^{-1}	
k_g	1.94 x 10^6	[7]
k_{sr}	6 x 10^4	[7]
k_{ox1}•	4.6 x 10^{-6}	[15]
k_{fr} (I,λ)	1.1 x 10^{-3} to 5.8 x 10^{-3} for I = 1 x 10^{-3} photons • ms^{-1}	
k_{ox}-	1.1 x 10^{-5}	[15]
k_m	k_m (Φ_s) = k_{ox2}• (1+$\Delta\Phi_s$)	[19]
	k_{ox2}• = 1.000 x 10^{-3} , k_{ox2}• >> k_{ox}- > k_{ox1}•	[15]

In summary,

- a change in direction within the field, $\Delta\theta$, corresponds to
- a change in singlet yield, $\Delta\Phi_s$, or the amount of products formed along the singlet path, which in turn
- changes the concentration of the signaling state, [FADH$^\bullet$], across all cryptochromes, thereby
- changing the amount of cryptochrome able to bind to signaling partners, and the resulting transduction of magnetic orientation information along a neural pathway.

For an ensemble of radical pairs, the fraction of them that will be in the singlet state at any one time depends on the orientation angle of the flavin with respect to the geomagnetic field (figure 6).

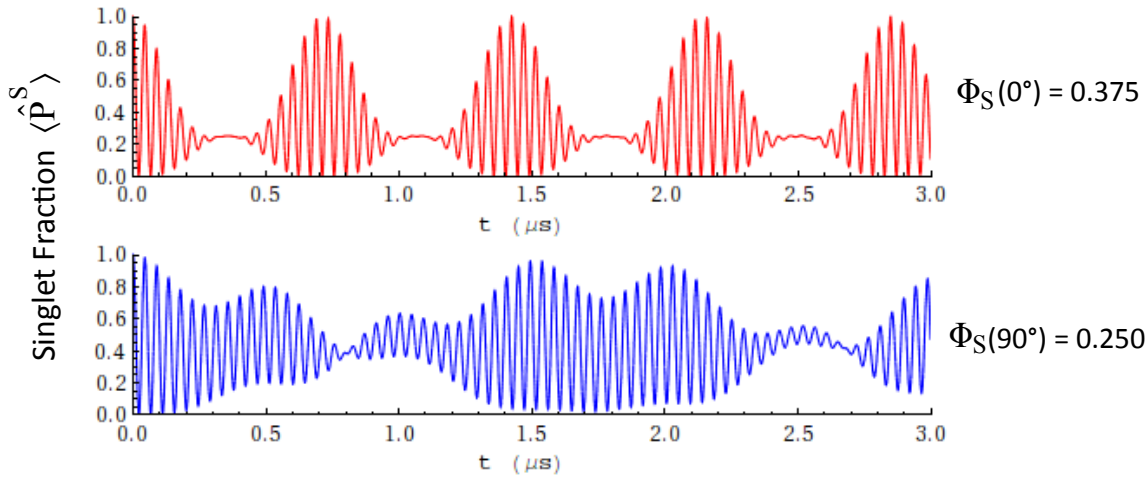

Figure 6. Singlet yield over time. The fraction of an ensemble of radical pairs in the singlet state at time t for a given orientation angle of the flavin with respect to the geomagnetic field. Corresponding singlet yield.

Integrating over the entire lifetime of the radical pair gives the singlet yield,

$$\Phi_S = k \int_0^{\infty} \langle \hat{P}^S \rangle e^{-kt} dt \simeq \frac{5 + \mathrm{Cos}(2\theta)}{16}$$

which undergoes a maximum change of 0.125, as seen in the amplitude of its graph shown in figure 7.

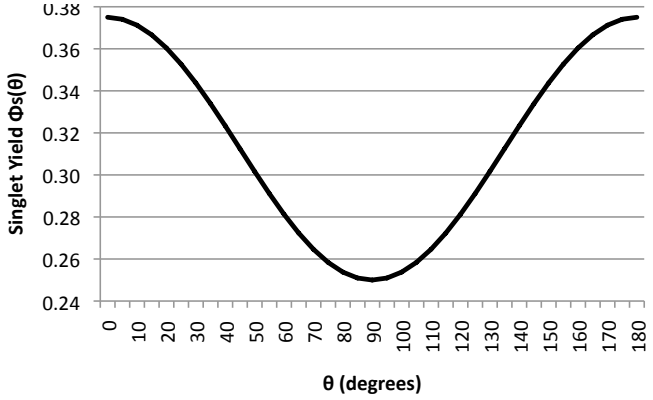

Figure 7. Singlet yield as a function of flavin orientation angle with respect to the geomagnetic field.

A detailed description of the quantum spin dynamics that causes the radical pair's quantum state to fluctuate with changes to orientation within geomagnetic field can be found in Appendices A and B.

Numerous experimental studies have confirmed the aforementioned theory of how radical pairs and their resulting product yields are directionally sensitive to magnetic fields [19]. Most recently, application of an Earth strength magnetic field was found to alter the lifetime, chemical reaction rates and product yields of a photochemically formed radical pair (carotenoid-porphyrin-fullerene) [9]. In addition, directional sensitivity of the radical pair to the magnetic field was also observed. A subsequent study of cryptochrome found

flavin-tryptophan radical yields were suppressed by 10-20% in the presence of a strong magnetic field of 28mT [10].

Cryptochrome's sensitivity to changes in magnetic field strength and orientation results directly from the quantum spin dynamics of flavin radical pairs. The resulting changes in cryptochrome binding to signaling partners directly corresponds to changes within the geomagnetic field. A biologically feasible mechanism for how this signaling information could be transduced along a neural pathway is found in the Weaver model.

CHAPTER 5: Weaver Model of Signal Transduction

Introduced by Weaver in 2000 [20], this model compares a signal, arising from chemical fluctuations due to magnetic field effects, with fundamental stochastic noise and normal physiological temperature fluctuations. The model assumes that cellular production of a ligand molecule *L*, is sensitive to changes in the strength of an external magnetic field. The ligand subsequently binds to free neural receptors R_f as illustrated in figure 8.

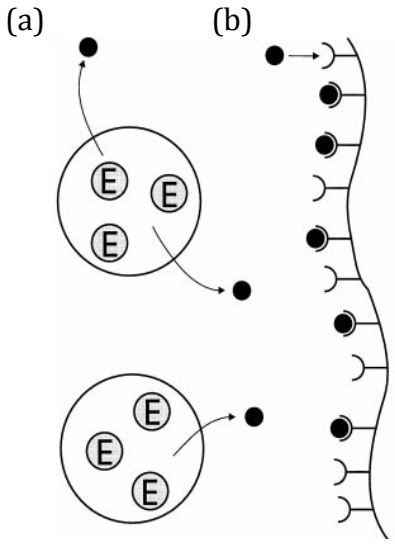

Figure 8. Weaver model of chemical signal transduction. (a) Enzymes within the cell catalyze ligand production. (b) neural receptors bind with ligands. (Reprinted from [20] with permission)

Thus, a dynamic equilibrium exists between bound neural receptors R_b and the available ligands and unbound receptors.

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

Receptor binding is governed by $K_D = k_r/k_f$ the ratio of dissociation and association rates. 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. The field-dependent ligand concentration is then given by

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

where C_s is the substrate concentration. Expansion of k_m(B) about the operating point gives

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

where $k_{m,op} = k_m(\mathrm{B_{Earth}})$ is the operating point of the chemical compass and $\Delta \mathrm{B} << \mathrm{B_{Earth}}$. The parameter $g_1 \approx 100 \mathrm{T}^{-1}$ maximizes the chemical change resulting from the field. The equilibrium average number of bound receptors is a binomial distribution

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

where R_T is the total number of receptors within the local cell volume and $q = K_D/C_s$. Stochastic noise is defined as the variance of this distribution

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

while the signal is the shift in receptor binding from the operating point, due to magnetic field perturbations.

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

The signal-to-noise ratio is then given by

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

where, a signal-to-noise ratio greater than one, characterizes the detection threshold for an operational chemical compass. Weaver also concludes that the molecular changes resulting from field effects will exceed those from temperature fluctuations if the sensory detection system can achieve a small, but biologically feasible temperature coefficient of $\alpha_{det} \leq 5 \times 10^{-5}$ °C.

CHAPTER 6: Cryptochrome Model of Signal Transduction

The aim of this paper is to apply the generalized Weaver model of signal transduction to the specific case of the cryptochrome photoreceptor. In doing so, a determination can be reached as to what extent such a model, based solely on cryptochrome and its flavin photocycle, can actually explain experimentally observed light effects.

In this new model of signal transduction, cryptochromes and neural receptors are fixed within the retinal ganglion cell membrane. The flavin and its principle nuclear spin axis are also fixed within the membrane-bound cryptochrome, providing a reference orientation by which to measure angular changes with respect to the geomagnetic field. A relatively constant pool of free ligands within the local volume, transduces magnetic orientation information between bound cryptochrome and neural receptors. The ligand is assumed to have two conformations, one with an affinity for binding to cryptochrome and the other for neural receptor binding. The mechanism for signal transduction would then progress sequentially as follows, (figure 9):

1. A cryptochrome is unable to bind to free floating ligands in the vicinity, if it has a flavin cofactor that is in a non-signaling state, FAD or $FADH^-$.
2. Upon irradiation, when the flavin enters the signaling state $FADH^\bullet$, the cryptochrome binds with a ligand.
3. The cryptochrome induces a conformational change in the bound ligand that triggers its release and a change in affinity to neural receptor binding.

4. The ligand then binds to a free neural receptor, thereby completing the signal transduction along the neural pathway.
5. Ligand-neural receptor binding and unbinding is governed by an equilibrium dissociation constant $K_D = k_r/k_f = 1 \times 10^{-8}$ M. This rate 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 [8].

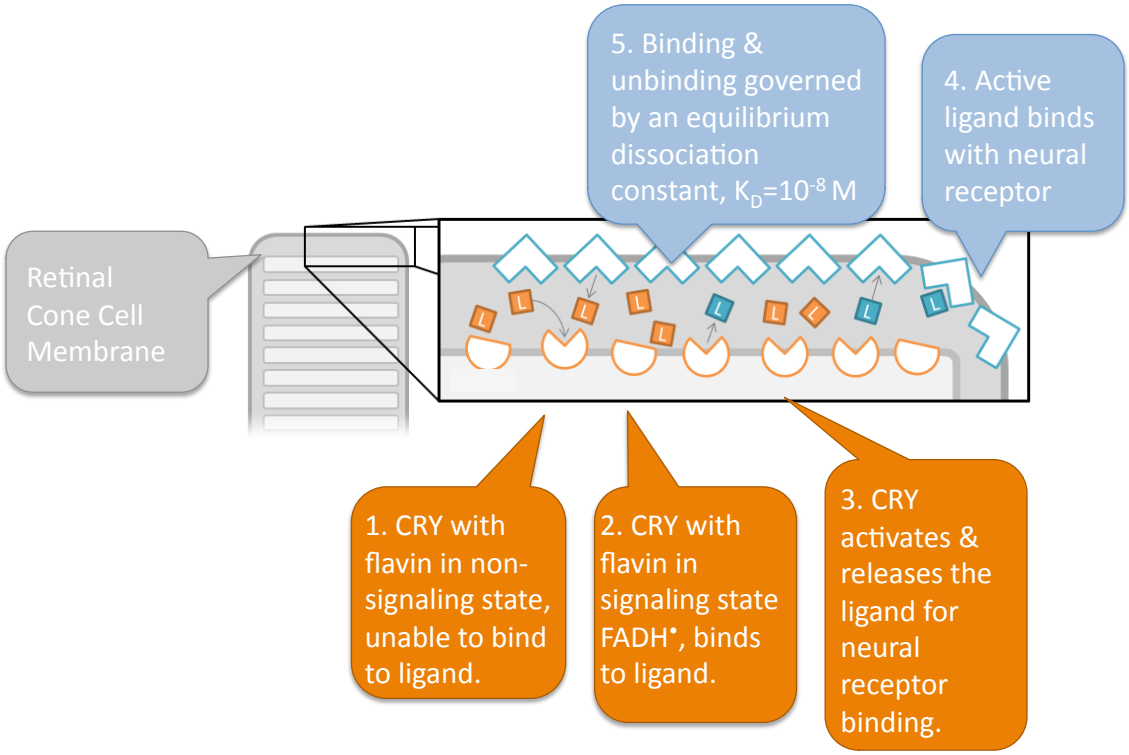

Figure 9. Cryptochrome & Neural Receptors are fixed within the cell membrane. Ligands with cryptochrome binding affinity L and neural receptor binding affinity L transduce magnetic orientation information between them.

Following the Weaver model, the signal from a cryptochrome sensor continues to be defined as the shift in receptor binding from the operating point, due to magnetic field perturbations. Here, however, the operating point is defined as an initial reference orientation within the magnetic field. The perturbation would then be a change to this orientation, $\Delta\theta$. Such a perturbation correlates directly with, and can be defined as, a change in singlet yield, $\Delta\Phi_s(\theta)$. Thus the signal from a cryptochrome compass is the shift in receptor binding due to a magnetic field effect, characterized by a change in singlet yield

$$S \equiv \overline{R}_{b,m} - \overline{R}_b .$$

Stochastic noise continues to be defined as the distribution variance of the equilibrium average number of bound receptors, given by

$$\overline{R}_b = \frac{1}{1+q} R_T \qquad q \equiv \frac{{K_D}^2}{L\,C\,[\mathrm{FADH}^{\bullet}]_{ss}} .$$

However, now the bound receptor average is also a function of the number of cryptochrome in the signaling state, in addition to ligand count, receptor count, and the equilibrium dissociation constant for ligand-receptor binding.

Numerical modeling of the aggregate signal from a single cell, assumes all cryptochromes begin in the FAD ground state, to which all final steady states are then normalized, so that $[\mathrm{FADH}^{\bullet}]_{ss}$ is the percentage of cryptochrome in the signaling state, able to bind to a ligand signaling partner.

The signal-to-noise threshold is then given by

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

where, the change in the concentration of flavin in the signaling state,

$$\delta[\mathrm{FADH^{\cdot}}]_{ss} = \frac{\left|[\mathrm{FADH^{\cdot}}]_{ss} - [\mathrm{FADH^{\cdot}}]_{ss,m}\right|}{[\mathrm{FADH^{\cdot}}]_{ss}}$$

is a magnetic field perturbation defined as the percentage change in $FADH^{\bullet}$ from its reference value, under rate $k_{ox2\bullet}$, to its value under a magnetically perturbed rate,

$$k_m(\Phi_s) \approx k_{ox2\bullet}[1 + \Delta\Phi_s(\theta)]$$

which is a function of the change in singlet yield due to a change in the flavin's orientation angle θ with respect to the magnetic field.

Steady state concentrations of all flavin states, including $[FADH^{\bullet}]_{ss}$, are obtained as the solutions to a system of ordinary differential equations.

$$\frac{d[\mathrm{FAD}]}{dt} = k_g[\mathrm{FAD^*}] + k_m[\mathrm{FADH^{\bullet}}]_{RP} + k_{ox1\bullet}[\mathrm{FADH^{\bullet}}] - k_x[\mathrm{FAD}]$$

$$\frac{d[\mathrm{FAD^*}]}{dt} = k_x[\mathrm{FAD}] - k_g[\mathrm{FAD^*}] - k_{sr}[\mathrm{FAD^*}]$$

$$\frac{d[\mathrm{FADH^{\bullet}}]}{dt} = k_{sr}[\mathrm{FAD^*}] - k_{ox1\bullet}[\mathrm{FADH^{\bullet}}] - k_{fr}[\mathrm{FADH^{\bullet}}]$$

$$\frac{d[\mathrm{FADH^-}]}{dt} = k_{fr}[\mathrm{FADH^{\bullet}}] - k_{ox1-}[\mathrm{FADH^-}]$$

$$\frac{d[\mathrm{FADH^{\cdot}}]_{RP}}{dt} = k_{ox1-}[\mathrm{FADH^-}] - k_m[\mathrm{FADH^{\bullet}}]_{RP}$$

The system is first solved for $\Delta\Phi_s(\theta) = 0$, such that $k_m = k_{ox2\bullet}$, to obtain $[FADH^{\bullet}]_{ss}$. A second solution for the system gives the signaling state concentration under magnetic field perturbation $[FADH^{\bullet}]_{ss,m}$, whereby $0 < \Delta\Phi_s(\theta) \leq 0.125$. All reaction rates have been determined experimentally (Table 1). Two rates

$$k_x(I,\lambda) \propto I \bullet A_{FADox}(\lambda) \text{and}$$

$$k_{fr}(I,\lambda) \propto I \bullet A_{FADsq}(\lambda)$$

are dependent on light intensity and wavelength, being functions of the absorption spectra for the oxidized and semi-reduced states respectively. For better comparison with experimentally observed light effects, these light rates were calculated with a 60 nm half-width Gaussian distribution around each wavelength. Both absorption spectra having been experimentally measured (figure 10).

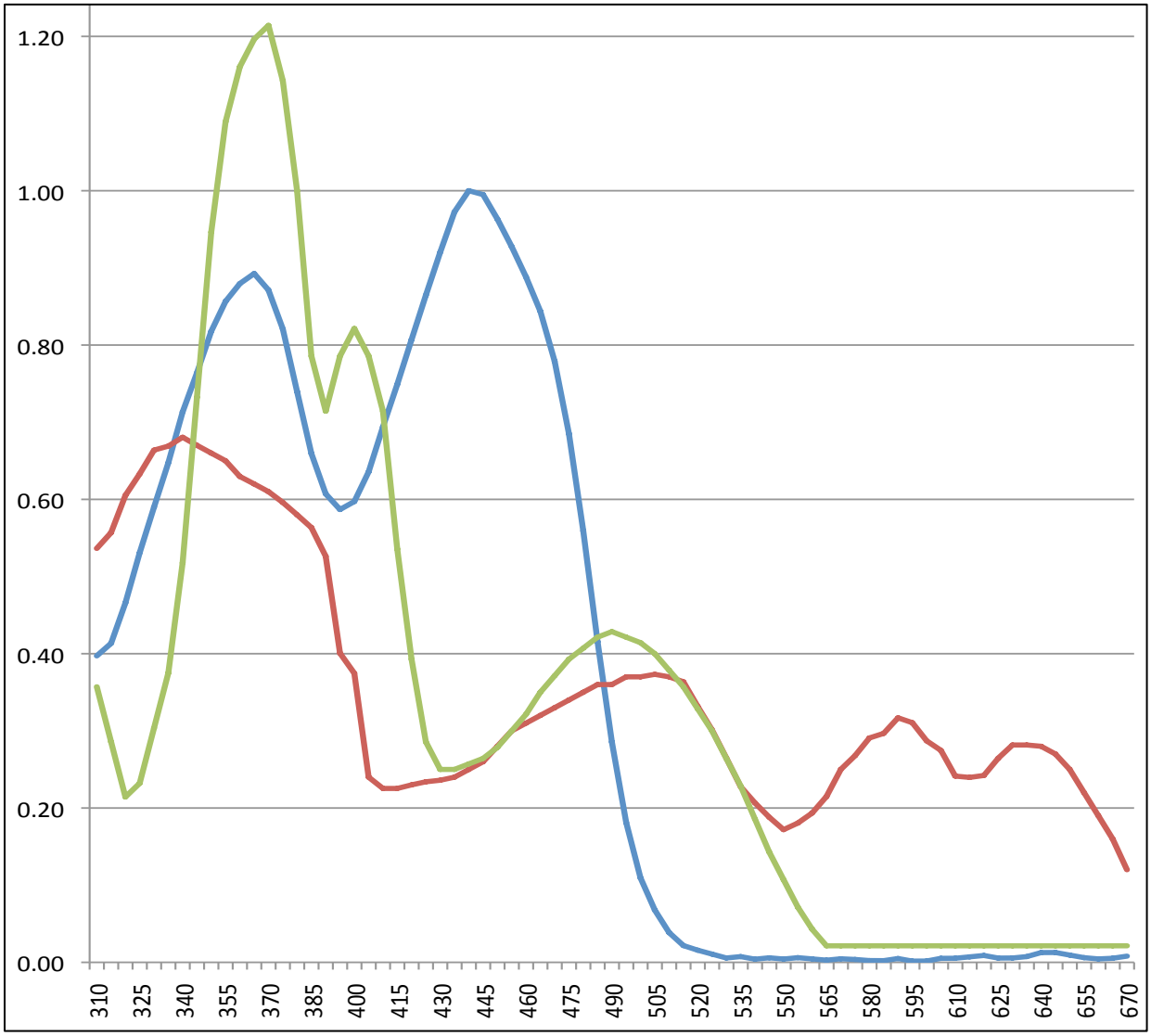

Figure 10. Absorption spectra of fully oxidized flavin FAD_{ox} (blue) and semi-reduced flavin $FADH^{\bullet}$ in neutral (red) and anionic (green) semiquinone form [11].

While it is unclear if the flavin signaling state $FADH^{\bullet}$ exists in the anionic or neutral semiquinone form in birds, no significant computational differences were observed between them, so only the neutral form is considered.

CHAPTER 7: Light Effects of the Cryptochrome Signaling Model

The two principle light effects observed in bird behavioral experiments are a wavelength and an intensity cutoff beyond which orientation no longer occurs. For given parameters, the cryptochrome signaling model predicts this general light behavior extremely well. The cryptochrome model registers a discernable signal above noise for short wavelengths, but not long wavelengths (figure 11).

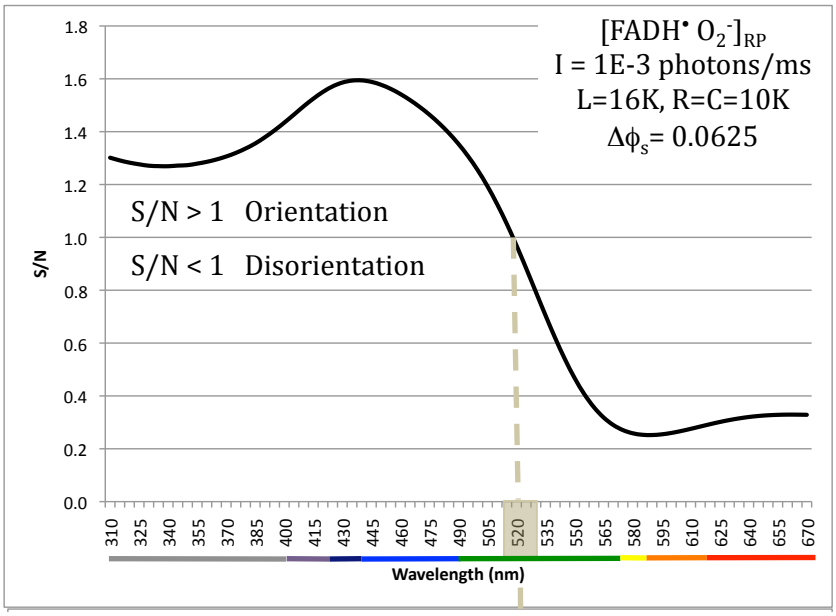

Figure 11. Signal-to-noise versus wavelength for a light intensity of 1 x 10^{-3} photons/ms.

The wavelength cutoff for orientation occurs at 520nm, which is a direct consequence of a loss of absorbance in the absorption spectra of oxidized flavin at this wavelength (figure 10).

Similarly, the loss of orientation at higher light intensities is also well predicted by the model, as the signal approaches the level of noise at 1 x 10^{-1} photons/ms and higher (figure 12).

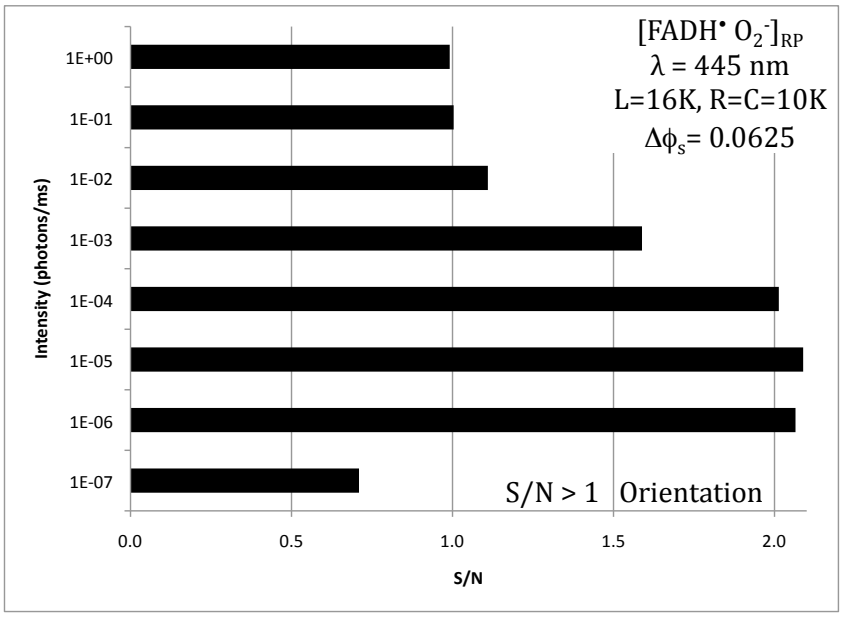

Figure 12. Signal-to-noise versus light intensity for a wavelength of 445 nm.

The given parameters for ligand, cryptochrome and receptor counts are physiologically reasonable and result in a model able to detect changes in singlet yield of 0.0625 or half the maximum potential value. This sensitivity corresponds to angular changes of 30° (from θ_1 = 0°, 45°, 90°) and 45° (from θ_1 = 30°, 60°), which are in line with observed head scanning motions of birds as they perform magnetic orientation. Parameter adjustments, particularly of the ligand count, provides a model with more or less sensitivity to changes in singlet yield (figure 13).

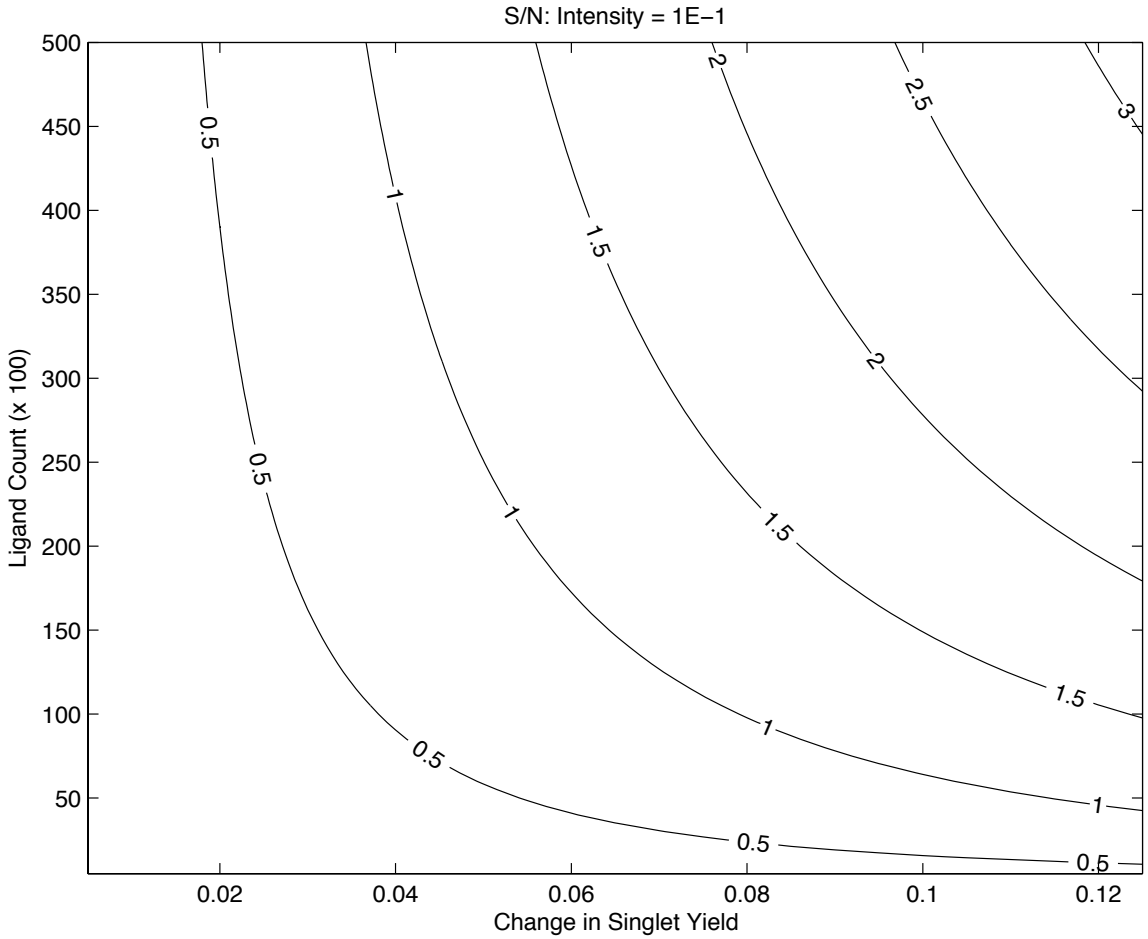

Figure 13. Signal-to-noise values as a function of ligand count and change in singlet yield for a light intensity of 1 x 10^{-1} photons/ms. The cryptochrome signaling model functions in agreement with experimental light observations for S/N = 1 at an intensity of 1 x 10^{-1} photons/ms, permitting all combinations of ligand and singlet yield change along this curve. To detect singlet yield changes of 0.0625, a ligand count of approximately 16,000 is required. To detect maximum singlet yield changes of 0.125, the required ligand count is approximately 43,000. Similar adjustments to cryptochrome and/or receptor counts can also tune the detector sensitivity, though ligands are the only parameter that a biological system can adjust on a time scale of minutes.

Comparison with experimental data reveals that the wavelength threshold predicted from the cryptochrome signaling model is slightly lower than the 570 nm cutoff experimentally observed. However, the general behavior of disorientation under long wavelength light is well preserved. In the future, more accurate measurements of the flavin absorption spectra may bring these values closer in line.

The intensity ranges for orientation and disorientation are well correlated between the model predictions and experimentally observed values. Adjusting for units and the area of the cell membrane, the model for low intensity orientation (1 x 10^{-3} photons/ms), high intensity orientation (1 x 10^{-2} photons/ms), and high intensity disorientation (1 x 10^{-1} photons/ms) correspond to the experimental reported boundaries of 3 x 10^{11} photons s^{-1} cm^2, 22 x 10^{11} photons s^{-1} cm^2, and 26 x 10^{11} photons s^{-1} cm^2 respectively (figure 14).

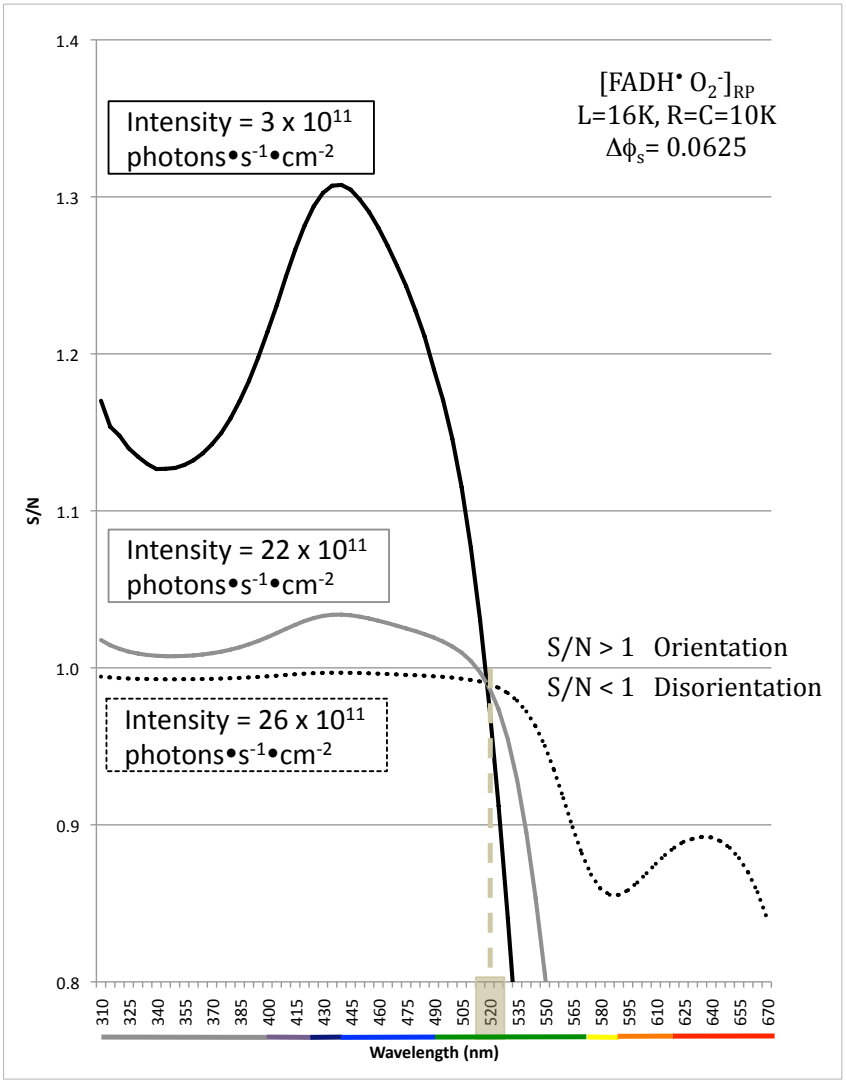

Figure 14. Signal-to-noise versus wavelength for three experimentally observed intensity values: low intensity orientation (3 x 10^{11} photons s^{-1} cm^2), high intensity orientation (22 x 10^{11} photons s^{-1} cm^2), and high intensity disorientation (26 x 10^{11} photons s^{-1} cm^2).

While the preceding results are for a cryptochrome signaling model based on a flavin-superoxide radical pair, flavin has also been found to form radical pairs with tryptophan. A model based on a flavin-tryptophan radical pair achieves the same wavelength cutoff for orientation. However, it fails to agree with the experimentally observed intensity cutoff, simply giving a larger and larger signal for higher and higher intensities. The expected intensity behavior is recovered for a combined photocycle that includes flavin formation of magnetically sensitive radical pairs with both tryptophan and superoxide.

CONCLUSION

Given the range of experimentally observed light effects seen in the magnetic orientation of migratory birds, a signal transduction mechanism based solely on cryptochrome and its flavin photocycle appears to closely model such effects. Still, additional questions and potential applications remain to be explored. Thus far, the model has only been used to examine monochromatic light effects, while dichromatic light effects remain to be investigated. For instance, while birds orient well under blue light, they have been observed to experience disorientation under a mixture of red and blue or yellow and blue light [21].

In addition, growing interest in cryptochrome mutant organisms provides a potential application of the model. In this respect, the model could be used to predict light effects for any bioengineered changes to the flavin and its absorption spectra. Ultimately, the cryptochrome model of signal transduction serves to further elucidate the likely role of cryptochrome and the flavin-superoxide radical pair as the essential elements in magnetic sensing.

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APPENDIX A: Radical Pair Quantum Spin Dynamics

The following pictorial treatment of quantum spin dynamics is adapted from Brocklehurst [3] for the specific case of the flavin-superoxide radical pair. The flavin-superoxide radical pair is formed when the flavin molecule in the fully-reduced state, $FADH^-$, donates an electron to the oxygen molecule O_2, thereby forming the superoxide O_2^-, and semi-reduced flavin $FADH^\bullet$, radical pair. The lone electron of each radical has a spin, which together are correlated with one another in a quantum singlet state (figure A.1a). If these spins were exposed to the same magnetic field, they would precess about the field vector at the same rate, maintaining their relative antiparallel orientation to one another that is 180° out of phase, thereby preserving their singlet state.

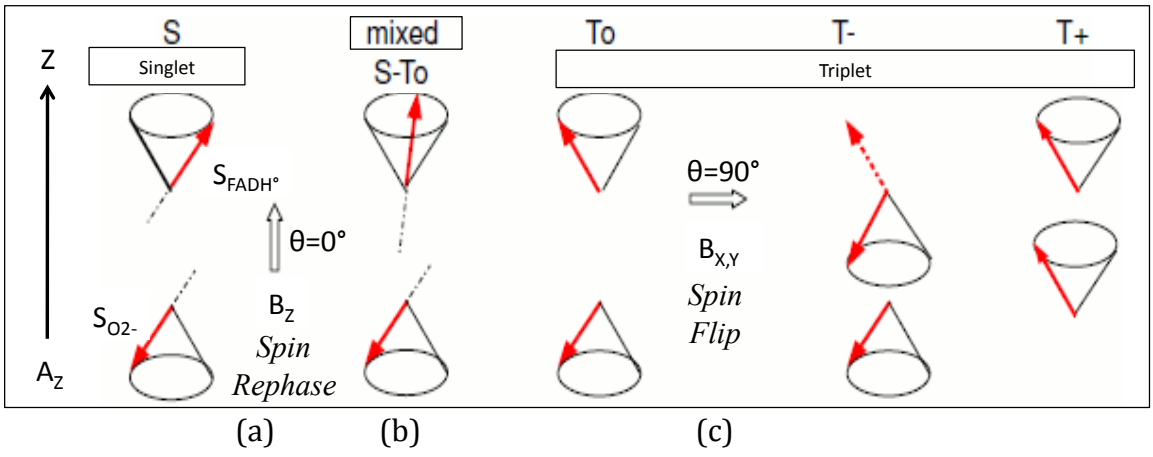

Figure A.1 Model of quantum spin dynamics of the flavin-superoxide radical pair. (a) The lone electron spins of each radical together are created in a singlet quantum state, i.e. their relative orientation is antiparallel and 180° out of phase. (b) Spin rephasing results in mixing between singlet S and T_0 triplet states, which occurs when the spins are exposed to parallel or antiparallel magnetic field vectors of differing strengths. (c) Spin rephasing results in mixing between singlet or T_0 triplet states and T_- or T_+ triplet states, which occurs when the spins are exposed to a non-parallel magnetic field vector.

In actuality, the flavin experiences a local magnetic field produced by its nuclear spin environment, while superoxide does not. Thus the flavin experiences a hyperfine interaction, where the flavin electron spin precesses about the resultant nuclear spin, thereby changing its original orientation with respect to the superoxide electron spin. This spin rephasing causes the radical pair to periodically lose its original singlet state and pass through a triple quantum state where the spins are in phase and antiparallel (figure A.1b). This oscillation between quantum states is referred to as singlet-triplet (S-T) mixing.

In addition, both spins experience Zeeman interactions whereby they each precess about the external geomagnetic field at the Larmor frequency, ω. Zeeman interactions split otherwise degenerate quantum energy levels, thereby opening new channels of oscillation, which enhances S-T mixing (figure A.2).

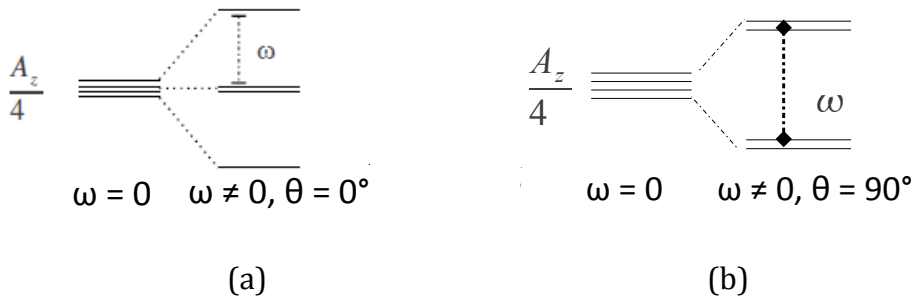

Figure A.2. Energy levels of the Hamiltonian describing the radical pair. Degenerate energy levels under hyperfine interactions alone (ω = 0), are split by the addition of Zeeman interactions (ω ≠ 0), for relative orientation angle between the nuclear spin vector and the geomagnetic field vector of (a) θ = 0° and (b) θ = 90°.

During magnetic orientation, movements inevitably change the orientation angle θ of the geomagnetic field with respect to the flavin nuclear spin. This is because the flavin

orientation is fixed within cryptochrome that is itself bound to the cell membrane within the retina. When the nuclear spin and geomagnetic field vectors are out of alignment with each other, spin flipping results (figure A.1c). This flipping causes the radical pair to pass through additional triplet states, T_- and T_+, which further enhances S-T mixing and further decreasing the fraction of time the pair spends in the singlet state.

APPENDIX B: Mathematical Treatment of Quantum Spin Dynamics

The following mathematical treatment of quantum spin dynamics is adapted from Timmel [18] for the specific case of the flavin-superoxide radical pair.

The Hamiltonian characterizing hyperfine interactions is given by

$$H_{HFI} = \Sigma_j A_j \cdot I_j \cdot S_{FADH^\circ}$$

where S_{FADH° denotes the electron spin of $FADH^\bullet$, I_j is the j^{th} nuclear spin and A_j is the hyperfine coupling tensor characterizing the strength and axiality of the interaction between the electron spin and the j^{th} nuclear spin. The flavin nuclear spin environment has an approximate axial symmetry characterized by $A_z = a + 2a\alpha$, and $A_x = A_y = a - a\alpha$, where "a" is the overall strength of hyperfine interaction, and "α" describes the degree of axiality.

The Hamiltonian describing Zeeman interactions is given by

$$H_{Zeeman} = \omega \cdot (S_{FADH^\circ} + S_{O2^-})$$

where S_{O2^-} denotes the electron spin of superoxide, with the Larmor frequency $\omega = g_e \mu_B B_{Earth}$ and $B_{Earth} \sim 50\mu T << HFI = 1\text{-}10mT$. Thus, the quantum spin dynamics of a radical pair under local and external magnetic fields is summarized by the total Hamiltonian,

$$H = \Sigma_j A_j \cdot I_j \cdot S_1 + \omega(\cos\theta\, S_{1z} + \sin\theta\, S_{1x}) + \omega(\cos\theta\, S_{2z} + \sin\theta\, S_{2x})$$

which includes both hyperfine and Zeeman interactions, and is dependent upon the relative orientation angle θ of the geomagnetic field with respect to a fixed flavin nuclear spin reference A_Z. Here, S_1 is the flavin electron spin vector and S_2 is the superoxide electron spin vector.

The Liouville-von Neumann equation

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

and its density operator solution

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

describe the time evolution of the radical pair. The singlet projection operator, $\hat{P}^S$ acts to isolate the singlet state from the total vector space of possible quantum states (singlet, triplet, and linear combinations of the two).

The expectation value of the singlet projection operator

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

gives the singlet fraction – the fraction of an ensemble of radical pairs in the singlet state at time t, or alternatively the probability of a radical pair to be in the singlet state at time t.

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

Integrating over the lifetime of the radical pair (1/k) gives the singlet yield,

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

whose theta dependence can be approximated in the limit of a radical pair lifetime long enough to allow significant S-T mixing, i.e. $k \ll \omega \ll a$ with $\alpha \neq 0, -2, 1$, as

$$\Phi_S(\theta) \simeq \frac{5 + \mathrm{Cos}(2\theta)}{16}.$$