**Project Title – Is Cryptochrome 4 a magnetic sensor?**

**Supervisor – Professor David Keays, LMU.**

**Background.** The ability to detect magnetic fields is a sensory faculty exploited by a wide array of animals on the planet to aid navigation. This sense is not limited to species that undertake lengthy annual migrations, but has also been reported in animals that undertake localised journeys such as bees, bats, mole rats, lobsters, and pigeons. How these species detect magnetic information and transduce it into a neuronal impulse remains an unresolved question in modern biology. One hypothesis that provides an intellectual framework for tackling the problem is known as the radical pair mechanism (RPM). It predicts that the spin state of light-induced radical pairs are influenced by local magnetic fields, altering the photochemical properties of a receptor protein. This hypothesis is supported by evidence showing that the magnetic orientation is dependent on the presence of blue/green light in migratory birds. The cryptochrome (Cry) molecules are widely considered to be the best candidates for a RPM based magnetoreceptor as they are blue light sensitive photosensors.

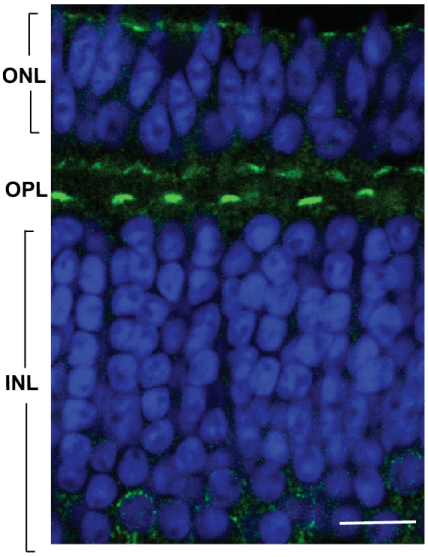


**Fig. 1 Cry4 staining in the retina.** Immunostaining showing the localisation of Cry4 in the pigeon retina (green) and nuclei (violet). Cry4 is enriched post synaptically in horizontal cells within the the outer plexiform layer (OPL). ONL indicates outer nuclear layer, and INL the inner nuclear layer.

**Preliminary Data.** We have cloned 5 cryptochromes in the pigeon (Cry1a, Cry1b, Cry2a, Cry2b, and Cry4) and investigated their biophysical properties. This has revealed that only Cry4, binds the co-factor FAD and absorbs UV and blue light, whereas Cry1 and Cry2 do not. Exploiting spectroscopic methods we have further shown that Cry4 forms long lived radical pairs, a pre-requisite for it to function as a magnetoreceptor (Hochstoeger et al, in revision, *Science Advances*). We have generated pigeon specific Cry4 antibodies, which has revealed that the molecule is enriched post synaptically in horizontal cells within the retina (See Fig. 1). A proteomic survey for retinal specific clCRY4 interactors identified molecules that are involved in receptor signalling, including glutamate receptor interacting protein 2 (GRIP2) which co-localises with clCRY4.

**Hypothesis:** We predict that magnetic stimuli influences the efficiency of glutamatergic synaptic transmission in the outer plexiform layer of the pigeon retina through a clCRY4-GRIP1/2 signalling pathway in the presence of light.

**Proposed experiments**. To test this hypothesis the student will employ molecular and cellular methods to interrogate how GRIP1 and GRIP2 interacts with Cry4. Specifically he/she will ask whether light and/or magnetically induced structural changes in Cry4 alters its interaction with GRIP1/GRIP2. This in turn will lead to an assessment of glutamatergic signalling and neuronal activity in the outer plexiform layer employing calcium indicators. For this experiment the student will exploit our unique "magnetoscope" that permits live cell imaging in a temperature controlled chamber while exposing explant retinas to light and/or magnetic fields. Finally, the project will employ CRISPR based methods to undertake a loss of function experiment targetting Cry4 and/or GRIP1/2.

**Methods:** Cell culture, molecular cloning, immunoprecipitations, calcium imaging, CRISPR-Cas9 genome editing.

**Website:** Keayslab.org