Behavioural and psychophysical responses to magnetic and visual stimuli in homing pigeons

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Summary

It has been demonstrated repeatedly, over a period of many years that some animals can perceive magnetic field parameters and use this sensory information to their advantage. The sensory mechanisms involved are, however, still poorly understood. Due to a general lack of understanding of magnetoreception and its properties, experiments to date have been somewhat disjointed with many, often very different, investigative approaches being employed. As a result, direct corroboration of results is uncommon and progress is often slow.

Advancement of this field is important for numerous reasons, such as improving knowledge of factors affecting animal migration in a time of concern over climate change and the possible imminent change in Earth's magnetic field parameters. Perhaps most significantly, such studies might provide a potential keystone in the study of the function of quantum mechanical processes in biological systems. The current study utilises a novel approach, designed to ensure improved reproducibility.

Reported here for the first time, prepulse inhibition of a startle response is used to investigate magnetoreception in the homing pigeon *(Columba livia)*. This powerful, well-characterised paradigm relies on reflexive behaviours and therefore ensures an objective method for demonstrating the perception of a stimulus. Visual psychophysics and polarised light vision are also explored. Proof of concept is provided for the use of prepulse inhibition to investigate magnetoreception. The value of using liquid crystal displays in an investigation of pigeon head movements, in magnetoreception and more generally, is demonstrated. A new, low invasiveness technique for tracking head movements is described. The need to eliminate anthropogenic electromagnetic signals in order to carry out reliable magnetoreception experiments is highlighted.

Suggestions are also made regarding possible future directions of research in this area.

Acknowledgements

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Chapter 1 Introduction

Magnetoreception (the ability of animals to sense and respond to magnetic field stimuli) has become a subject of great interest during the last half century. Many articles have been published, including experimental papers in scientific journals as well as frequent reviews and correspondences in high impact periodicals (e.g. Wiltschko and Wiltschko 2002; Lohmann 2010; Winklhofer 2010). There is also widespread public interest with regular articles on the subject in the national media of numerous countries. One of the current reasons for this is that magnetoreception may be mediated through the visual system and the very recent, exciting idea that quantum effects might facilitate visual magnetoreception (Arndt *et al.* 2009; Ball 2011; Gauger *et al.* 2011). Finding coherent quantum processes functioning in biological systems could have huge implications in a wide range of research areas, from informing a new paradigm in the conservation of ecological goods and services to helping overcome problems in quantum computing. Logically, an improved understanding of how animals navigate also offers great benefit to efforts in areas such as conservation and human navigation.

Despite this widespread interest, surprisingly little is known about this important sensory modality (Johnsen and Lohmann 2005). Advances have been made in understanding how a magnetic sense *might* work, but progress in determining the actual mechanism(s) is limited by several factors (Kirschvink *et al.* 2010). Precisely how many, or indeed which, organisms possess this sense or its relative importance to each species in unknown. The primary role of the work presented in this thesis was to develop a lab-based, easily reproducible assay to investigate aspects of magnetoreception. Then to use these techniques, in the first instance, to demonstrate the dependence (or otherwise) of magnetoreception on the visual system.

Magnetoreception has been identified in a wide range of species, both vertebrate and invertebrate, aquatic and terrestrial (Figure 1.1) (Lohmann 2007; Reppert *et al.* 2010;

Wajnberg *et al.* 2010). It is widely accepted that magnetoreception is utilised by many of these diverse species as an aid to navigation. In fact, this information may be used to navigate both locally and over great distances, such as in seasonal migration, and (sometimes ontogenetically changing) manifestations of the sensory modality are expressed by animals over a range of life stages (Lohmann 2007; Hellinger and Hoffmann 2009; Wajnberg *et al.* 2010; Fuxjager *et al.* 2011; Putman *et al.* 2011).

Recently, it has come to light that magnetoreception may have a role to play in other aspects of an animal's interaction with its surroundings. For example, certain elasmobranch fishes have been shown to alter their feeding behaviour in the presence of magnets and it has been suggested that magnetoreception could be an explanation for the orientation of foxes (*Vulpes vulpes*) prior to striking their rodent prey (O'Connell *et al.* 2010; Červený *et al.* 2011).

Figure 1.1 A wide variety of species use magnetoreception (Lohmann 2010).

Additionally it has been suggested that rodents, such as mice and squirrels, may use the complex, three-dimensional information potentially provided by visual magnetoreception,

to negotiate their immediate environment, quite apart from general, more long distance navigation (Phillips *et al.* 2010). This information could potentially be used for finding food caches in seasonally changing surroundings.

Although this particular field of research is in its relative infancy, thought is beginning to be given to the impact of magnetic field distortions on ecosystem function and potential means of avoiding or mitigating harm (Irwin *et al.* 2004). The European eel (*Anguilla anguilla*), for example, is an enigmatic species, that has received a great deal of research attention recently due to its critically endangered status, which is often attributed to high mortality during migration, although little is currently understood about exactly why this might be (van Ginneken and Maes 2005; Jansen *et al.* 2007; Bonhommeau *et al.* 2009). Magnetic material of a biogenic nature has been identified in the lateral line organ (traditionally considered a mechanosensory organ) of eels (Moore and Riley 2009). The eel may provide an interesting case study on whether the migratory mechanisms of a commercially and ecologically significant species are elucidated in time to avoid their possible untimely extinction.

Aquatic environments provide a number of examples of interesting varieties of magnetoreception. Putman *et al.* (2011) found that loggerhead turtles (*Caretta caretta*) can derive not only longitudinal, but also latitudinal, information using magnetoreception and that they might use this information to operate a bicoordinate map system.

Advancing knowledge of this phenomenon is important for many reasons, among them providing a better understanding of ecological function, such as environmental effects on animal migration, the potential for the development of biomimetic technological devices for navigation, and possible implications for human healthcare.

Numerous mechanisms have been proposed to explain how a magnetic sense could function in animals. The following is a brief overview of the current leading theories:

The map/compass sense

It has been hypothesised that effective navigation requires more than one type of information. A memory map can provide information about where features lie in relation to each other but this information can only be effectively utilised with reference to an orientation, such as a compass bearing. Kramer (1953; 1957) proposed a two stage navigational model (Figure 1.2) which has developed into the commonly accepted "Map" and "Compass" model of avian navigation.

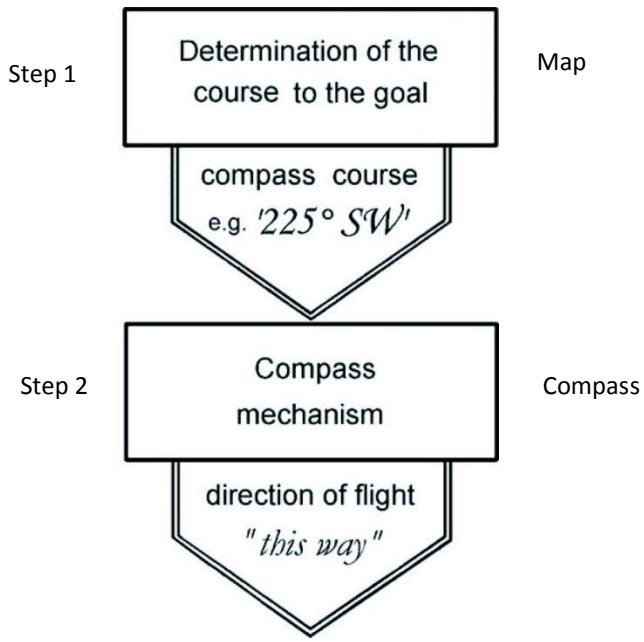

Figure 1.2 Kramer (1953; 1957) 2 stage navigational model. Adapted from Wiltschko and Wiltschko (2009).

In magnetoreceptive birds, the ability to utilise vector navigation appears to be innate (with some species completing their first migration alone), and it is thought that having a hereditary urge to migrate along a certain vector to the magnetic field for a certain distance/ duration may be the basis for this (Wiltschko and Wiltschko 1972; Berthold and Querner 1981). This is commonly referred to as compass sense or vector orientation. In addition to this, displacement experiments have shown that birds can also draw on experience, using environmental cues to recalculate their vector heading based on conditions at their current

location (Wallraff 1974). This implies another 'map-like' sense, and the use of true navigation. Work to date suggests that the compass may be mediated by a visual/cryptochrome system, while the map is based on a magnetite system (Beason *et al.* 1997; Wiltschko and Wiltschko 2007; Zapka *et al.* 2009). It has also been suggested that each of these mechanisms is utilised by the animal for a different purpose (Wiltschko and Wiltschko 2007). The presence of (at least) two separate mechanisms sensitive to magnetic fields highlights the likely evolutionary importance of this sensory modality (W. Wiltschko *et al.* 2007). However, Wallraff (1999) claimed that domestic pigeons (*Columba livia*) are unlikely to commonly use a geomagnetic map sense, as this source of information may be too unreliable at the local scale for accurate navigation.

Candidate mechanisms

The magnetite system

Experiments have indicated that the application of a magnetic pulse impairs magnetoreception in birds, and therefore a magnetic substance must form part of the magnetoreceptor (Wiltschko *et al.* 1994; Beason *et al.* 1995). Subsequent experiments using magnetic pulses indicate the effect may specifically disrupt the map (but not the compass) sense of birds (Beason *et al.* 1997).

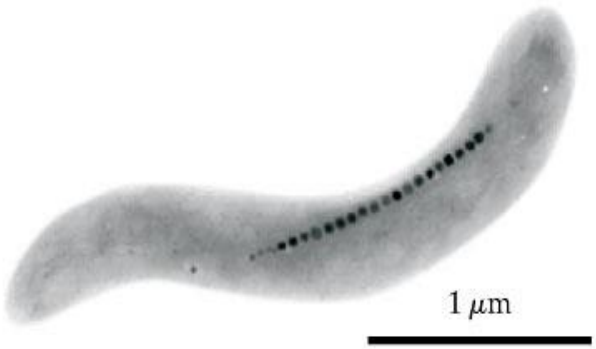

Figure 1.3 Transmission electron micrograph of a bacterium showing magnetosomes (Johnsen and Lohmann 2008).

Two manuscripts written in 1963 (but not published until 2009) describe magnetosensitive bacteria, such as that pictured in Figure 1.3, which were found in fresh water whilst investigating pathogens (Bellini 2009a; Bellini 2009b). In 1975, the crystals responsible for making this magnetosensitivity possible were described and subsequently the biogenic, ferromagnetic material, magnetite, was linked with magnetosensitivity and magnetoreception in a wide variety of organisms (Blakemore 1975; Johnsen and Lohmann 2008). It has been suggested that the magnetite system may involve the ophthalmic branch (V1) of the trigeminal nerve (V) (Beason and Semm 1996). Sectioning of the ophthalmic branch appears to have no discernible effect on the compass sense of birds, supporting theories that the magnetite system may be specifically responsible for the map sense (Beason and Semm 1996; Zapka *et al.* 2009).

Falkenberg *et al.* (2010) found complex neuronal dendritic systems in which iron minerals occur in distinct sub-cellular compartments of the terminals of the ophthalmic nerve, the minerals being predominantly Fe III- oxides. They found similar dendritic systems in the inner dermal lining of the upper beak of several species of migratory and non-migratory bird, suggesting a possible evolutionary significance. Interestingly, trimming of the beaks of chickens (*Gallus gallus*) has been shown to cause magnetic disorientation (Freire *et al.* 2011).

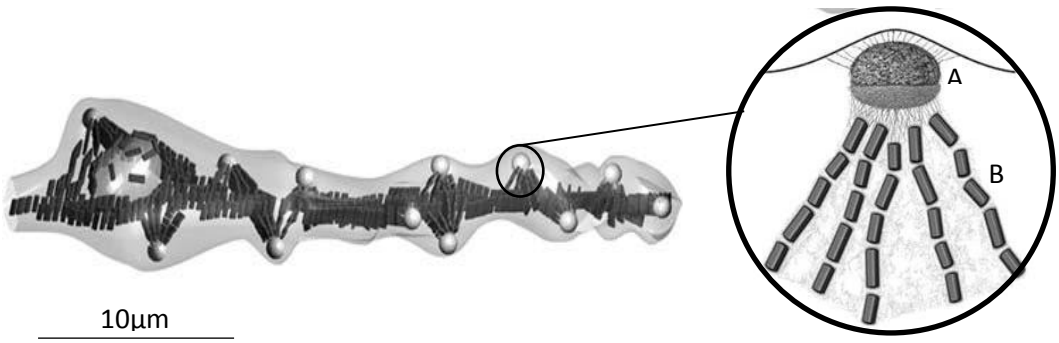

Figure 1.4 Schematic of nerve ending with magnetite spherules, and enlarged view of a magnetite sperule (A) and associated iron platelets (B). Adapted from Fleissner *et al.* (2003).

Hanzlik *et al.* (2000) and later Fleissner *et al.* (2003) used various techniques to investigate the nature, and location of the magnetite receptor in pigeons. They concluded that magnetite is found concentrated in six specific locations in the beak of pigeons and is associated with the necessary nerve endings, which could allow the magnetite to serve as a sensory receptor. These findings were accepted by many investigators as reliable, and numerous more recent theories of magnetite based magnetoreception in birds have been based on the existence of these concentrated areas of magnetite.

Recently, however, Treiber *et al.* (2012) carried out an extensive histological study of some 200 pigeons in order to more accurately describe the distribution of magnetite in the beak. Not only were they unable to find any consistency in the distribution of ferrous substances in the beaks of pigeons, they also discovered that the majority of objects previously identified as magnetite (using Prussian Blue staining, which identifies iron) were actually macrophages. The implication of this finding is that there is now little evidence to suggest that the magnetoreceptor lies within the beak. Although it still remains possible the beak is involved, there have recently been promising developments in investigations that implicate areas of the inner ear and vestibular system in magnetoreception (Wu and Dickman 2012; Ward *et al.* 2015).

The cryptochrome (radical pairs) system

Another magnetoreceptive mechanism has been proposed that depends on changes in the magnetic field inducing different yields in biochemical reactions. Based on quantum mechanics, theories have developed that involve a proposed radical pair process, which may utilise quantum effects, to deliver this change in yield (Schulten 1982). Only recently has this been demonstrated to be feasible, as the forces exerted by the magnetic field were previously suspected to be too low to have a physical effect on biological systems (Rodgers and Hore 2009). However, gradually, this theory has gained favour due to increasing

experimental and theoretical support, and the flavoprotein, Cryptochrome (Cry), found in the visual system of many animals, is a strong contender as the biological molecule responsible for the process (Schulten 1982; Ritz *et al.* 2000; Møller *et al.* 2004; Henrik Mouritsen *et al.* 2004; Ritz *et al.* 2004; Rodgers and Hore 2009; Phillips *et al.* 2010).

There is also a growing body of evidence that the radical pair process is dependent on light, with both wavelength and intensity of ambient light having an effect on magnetic orientation (R. Wiltschko *et al.* 2007a; R. Wiltschko *et al.* 2007b; W. Wiltschko *et al.* 2007; Gegear *et al.* 2008). Gegear *et al.* (2008) used a behavioural assay to demonstrate that cryptochrome from monarch butterflies (*Danaus plexippus*) requires light wavelengths of 420nm and below to function and that, when expressed in transgenic fruit flies (*Drosophila melanogaster*), the flies could use it in detecting magnetic fields.

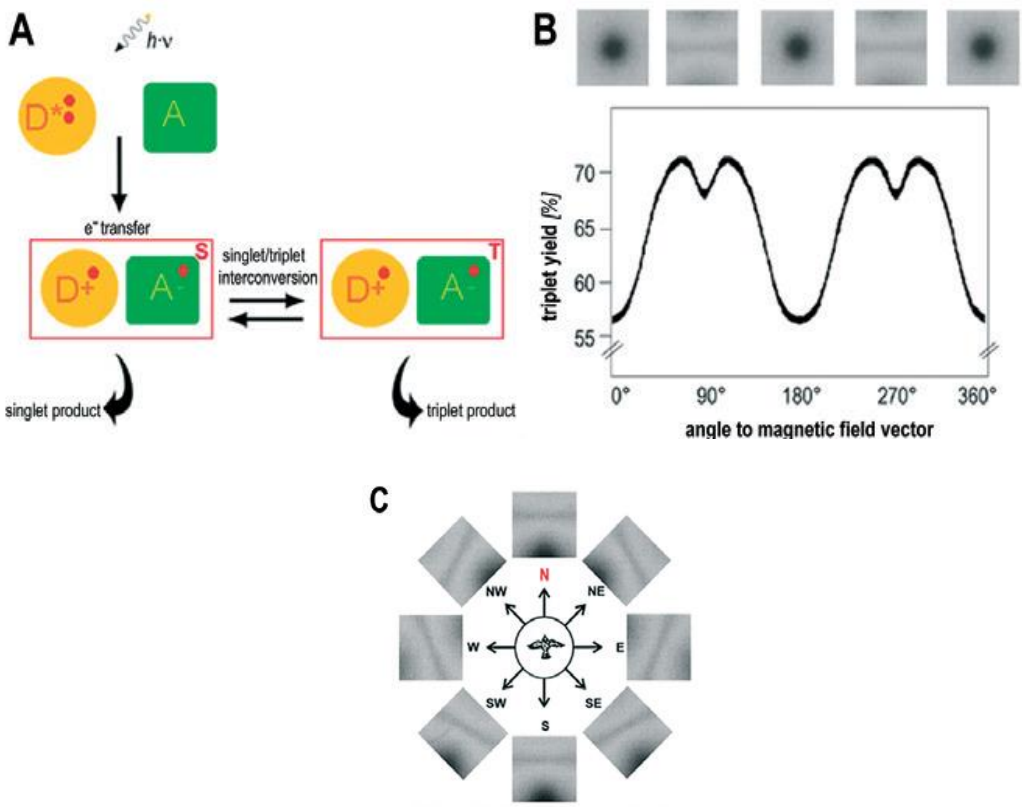

Figure 1.5 A, Photo-excitation results in an electron being moved from a donor molecule (D) to an acceptor molecule (A). The spin of the unpaired electrons can be opposite (singlet state) or parallel (triplet state); B, This in turn affects retinal response (e.g. neural influence of cryptochrome); C, Resultant visual modulation patterns affected by orientation to magnetic field (Liedvogel 2009).

Heyers *et al.* (2007) proposed a visual pathway where retinal neurones propagate the sensory signal via the thalamus to the forebrain region known as "cluster N". In support of this hypothesis, lesioning of cluster N in European robins (*Erithacus rubecula*) resulted in disorientation (Zapka *et al.* 2009).

Manipulated magnetic fields have been used to disrupt the cryptochrome system. Oscillating fields of 1.315 MHz in parallel to the local magnetic field did not appear to change orientation, however, when presented at a sufficient angle (over 24°), these fields disorientated European robins (Thalau *et al*. 2005). Disorientation was also observed in chickens subjected to an oscillating field of 1.566 MHz (W. Wiltschko *et al.* 2007).

Even human cryptochrome has been shown to function as a magnetosensor in transgenic fruit flies, and its function is dependent on the wavelength of ambient light, lending support to the idea that magnetoreception may involve the visual system in some way (Otsuka *et al.* 2001; Foley *et al.* 2011).

Inclination/Declination

There is mounting evidence that many species, including birds, use inclination of magnetic field lines rather than polarity to judge direction (Wiltschko and Wiltschko 1972; Light *et al.* 1993; Wiltschko *et al.* 1993; Vacha *et al.* 2008; Solov'yov and Greiner 2009). The magnetic field lines have a specific inclination (deviation from horizontal) in relation to a given point on the Earth's surface. At the equator, the field lines are parallel to the Earth's surface and have an inclination of 0˚. At the magnetic North Pole, they have an inclination of 90˚ and at the magnetic South Pole -90˚. Thus, latitudinal location can be approximated given the angle of inclination in a given place. This theory does not fully explain how a bird might avoid becoming disorientated in equatorial regions, although complementary information from (for example) olfactory or celestial cues could theoretically solve this problem. It is interesting to consider that this situation is often considered in terms of a standardised

dipole when, in fact, the Earth's magnetic field is asymmetric due to the influence of solar wind (to varying degrees) contracting the extent of the field on the side of Earth nearest the sun and drawing it further away from the planet on the 'leeward' side. There is little if any discussion about the effects that this distortion might have on avian navigation. In experiments described in Chapters 3 and 6, the inclination angle is flipped by inverting the vertical aspect of the ambient magnetic field.

Declination is the deviation of the direction of magnetic North in a given place from the direction of true North (defined by the Earth's axis of rotation) and varies temporally due to irregularities in the movement of the Earth. Due to the inherent variability of magnetic declination, it is considered unlikely to contribute to navigation as meaningfully as inclination, polarity or intensity.

Combined system

The, at times somewhat fierce, debate over whether the magnetite or cryptochrome system really holds the key to understanding magnetoreception has recently developed into a widely held view that *both* systems may well be actively involved (Jensen 2010; Wiltschko, Gehring, *et al.* 2010; Wiltschko *et al.* 2011). Several experiments have demonstrated that, when one system or the other is disrupted, certain aspects of magnetoreception remain functional and that, to some extent, one modality may be able to compensate for underperformance in, or loss of, the other (Wiltschko *et al.* 2005; Jensen 2010; Wiltschko, Stapput, *et al.* 2010; Hein *et al.* 2011).

However, it would appear that each system actually carries out a distinct role and that the two mechanisms complement each other, each making a different contribution rather than one simply being an evolutionary upgrade of, or substitute for, the other (Wiltschko *et al.* 2005; R. Wiltschko *et al.* 2007a; Wiltschko *et al.* 2010). Beason and Semm (1996) found that,

having administered a magnetic pulse and thus rendering birds disorientated with regard to magnetic fields, sectioning of the ophthalmic branch (V1) of the trigeminal nerve alleviated this disorientation, and magnetoreception appeared to return. This suggests that, when one system malfunctions, it may send conflicting neuronal information and confound the effect of the other system, which may otherwise be functioning normally. Another investigator commented that interference with the olfactory system could unwittingly affect the magnetic system giving a misleading indication that olfaction may be involved (Mora *et al.* 2004). This finding highlights the potential complexity of the system underlying magnetoreception and the importance of careful experimental design if a specific question or mechanism is to be successfully answered or determined. Inverting the vertical component of the ambient magnetic field resulted in an axial disorientation where birds tended to orient in the opposite direction as opposed to random directions, an effect which did not appear to vary with the age of animal (Freire *et al.* 2005).

The protein bio-compass

Towards the end of the period of preparation of this thesis, another potential mechanism was proposed, one which provides a concise way in which both magnetite and cryptochrome might function together as a magnetic 'protein bio-compass' (Qin *et al.* 2015). In this proposed mechanism, a protein complex referred to as magR, containing ferromagnetic particles, possibly magnetite, is associated with light dependent cryptochrome (Cry) molecules in such a manner that electron transport is possible between the two. Qin *et al*. (2015) discovered that this association does indeed seem to be in evidence in the retinae of homing pigeons, and describe a possible mechanism for this complex being sensitive to polarity, intensity, and inclination of Earth-strength magnetic fields.

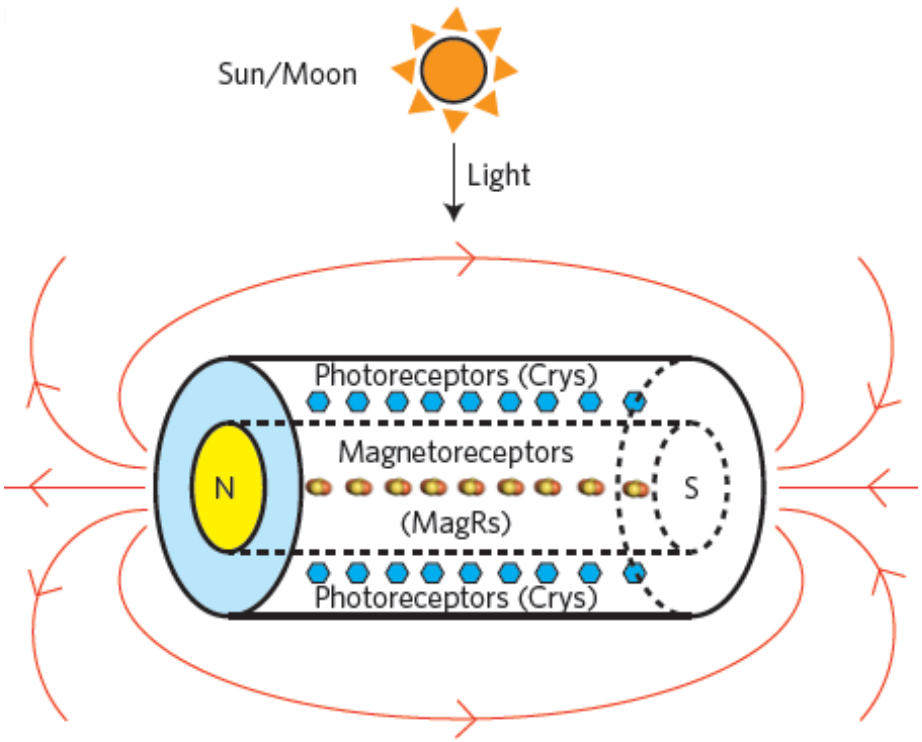

Figure 1.6 Proposed protein bio-compass of Qin *et al*. (2015). Magnetoreceptors orientate spatially to the ambient magnetic field, whilst associated cryptochromes contribute, via a light-mediated response, information about intensity.

This proposed mechanism is however, relatively new in the literature, and robust validation/corroboration is yet to take place. This does however, offer one theory that could explain how the two main proposed mechanisms, may function complimentarily.

Electroreception

As well as magnetite and radical pair mechanisms of magnetoreception, it has been theorised that some highly electroreceptive species, such as elasmobranch fishes, may use electrical induction to detect magnetic field gradients although more experimentation is needed to assess if that is the real mechanism (Johnsen and Lohmann 2008; Molteno and Kennedy 2009). If true, this could have further evolutionary implications as to the origin of magnetoreception.

Polarised light

It is widely accepted that a broad range of species, both vertebrate and invertebrate, can sense and derive spatial information (such as the position of the sun) from polarised light (Von Frisch 1967; Greenwood *et al.* 2003; Johnsen *et al.* 2011). Recent findings suggest some predatory marine species use this information to aid visual acquisition of food items although most work has been centred on understanding how species use the information for orientation (Stephens *et al.* 1953; Von Frisch 1967; Freake 1999; Dacke *et al.* 2003; Johnsen *et al.* 2011). It has been suggested (Able 1982; Moore 1986) that migratory birds use polarised light in migratory orientation, and subsequently many studies have provided evidence to support this proposal with a range of migratory species using experimental manipulations of polarised light (Moore and Phillips 1988; Able 1989; Helbig 1990; Able and Able 1993; Able and Able 1995; Muheim *et al.* 2006; Muheim *et al.* 2007). The mechanisms for detecting polarised light and its specific utilisation by different species remain elusive. Although some progress has been made, most studies have been on insects (Labhart and Meyer 1999). The range of possible mechanisms is fascinating with some means of polarised light sensing not even being reliant on the eye (Phillips *et al.* 2001).

There is a paucity of information regarding the ability of pigeons to sense polarised light (Horváth 2014). Delius *et al.* (1976) found that pigeons could correctly distinguish between different e-vectors (planes of polarisation) in a conditioning experiment, but this was contradicted by subsequent findings (Coemans *et al.* 1990; Vos Hzn *et al.* 1995). Discrimination between a stationary or rotating axis of polarisation appeared to be possible for some birds in a conditioning experiment (Kreithen and Keeton 1974). Furthermore, certain species of bird may use polarised light at certain times of the day to calibrate their magnetic compass sense (Phillips and Waldvogel 1988; Muheim *et al.* 2007).

An advancement of our understanding on whether and how pigeons use polarised light cues would be useful. It has been suggested that certain parallels between magnetic and polarised light sensing could be due to a shared system (Muheim 2011). If pigeons are in fact sensitive to polarised light, then more knowledge about the function of this system may potentially shed light on the mechanism of visual magnetoreception (and vice-versa). Very recently, it has even been proposed that there may be some direct interaction between perception of skylight polarisation, and the ability to discriminate magnetic field information (Muheim *et al.* 2016). Although not a primary focus of the research in this thesis, the involvement of polarised light in possible magnetosensory mechanisms is very intriguing. A novel experiment is carried out to try to identify a reliable response to e-vector stimuli in pigeons, and in Chapter 7, an entirely original theory, of the author's own devising, is proposed.

Experimental design

Magnetoreception is a difficult phenomenon to investigate, partly due to human inability to (at least readily) experience such a perception but even more so because of our limited understanding of the processes involved, such that there are not many logical starting points for investigation. The major difficulty is the lack of reproducible assays that can powerfully test aspects of the mechanisms involved, resulting in too little corroboration (or otherwise) of findings and little scope for pursuing new ideas (Harris *et al.* 2009; Kirschvink *et al.* 2010). In view of the fact that many animals display observable responses to one or more aspects of the magnetic field, it is necessary to develop behavioural experiments that can be used to investigate the different properties and limits of magnetoreception (Rodgers and Hore 2009; Kirschvink *et al.* 2010). Interdisciplinary study is one potential stepping stone to overcoming these problems. The inability of the average behavioural biologist to accurately manipulate magnetic fields (spatially and/or temporally) as well as light conditions constrains the development of new assays in this area. Conversely animal behaviour is not the normal field

of interest or expertise for many engineers. A recent collaboration in these areas here at Cardiff University has led to the development of equipment that is capable of accurately manipulating the temporal and spatial features of an artificially generated Earth-like magnetic field, designed specifically to meet the above mentioned needs (Migalski 2010).

A large body of work has been developed using homing pigeons as test subjects because: they are readily available; have (comparatively) well studied physiology and behaviours; and navigate readily without the need for seasonal migratory restlessness (Zugunruhe). Reproducible experiments have been designed using anuran amphibians and insects (Wajnberg *et al.* 2010; Landler and Gollmann 2011). However, the study of amphibians depends on the animal experiencing migratory restlessness, and knowledge of the mechanisms involved in insect magnetoreception has not advanced as much recently as it has in birds (Mouritsen and Ritz 2005).

Migalski (2010) demonstrated that, using a dynamically controlled configuration of Helmholtz coils, features of a computer generated Earth-like magnetic field that a test bird is exposed to can be accurately manipulated and that this can elicit a qualitative and potentially quantitative behavioural response. Mouritsen *et al*. (2004) observed that birds conduct “head scans” (turning of the head through more than a pre-determined number of degrees from forward facing) prior to taking off.

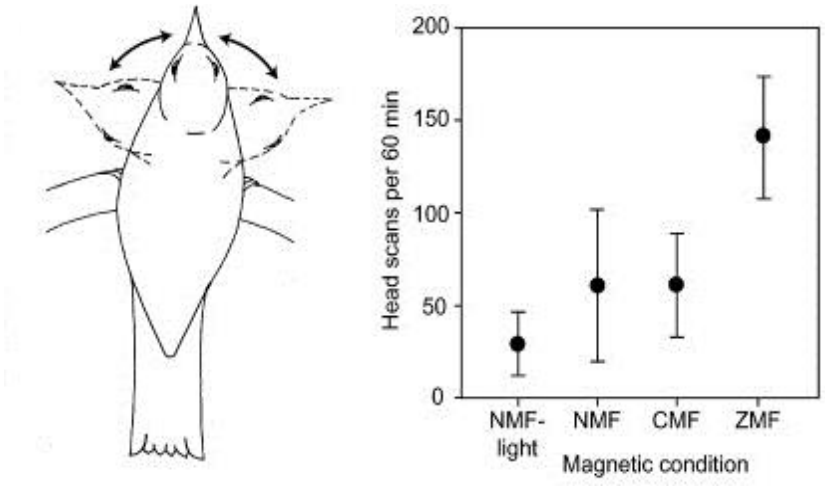

Figure 1.7 Magnetic field affects head scanning behaviour. Head scans per hour under a range of magnetic field conditions. Natural magnetic field daytime (NMF – light), natural magnetic field night time (NMF), changing magnetic field (CMF) and zero magnetic field (ZMF) from Mouritsen *et al.* (2004).

In a zero-magnetic field, the number of such scans increased almost three-fold and the movement direction on take-off changed from consistent (natural migratory direction) to random. This formed the basis for part of a previous study (Migalski 2010), which demonstrated that these head scans can be provoked by transitions between different field conditions produced by the Helmholtz coil apparatus.

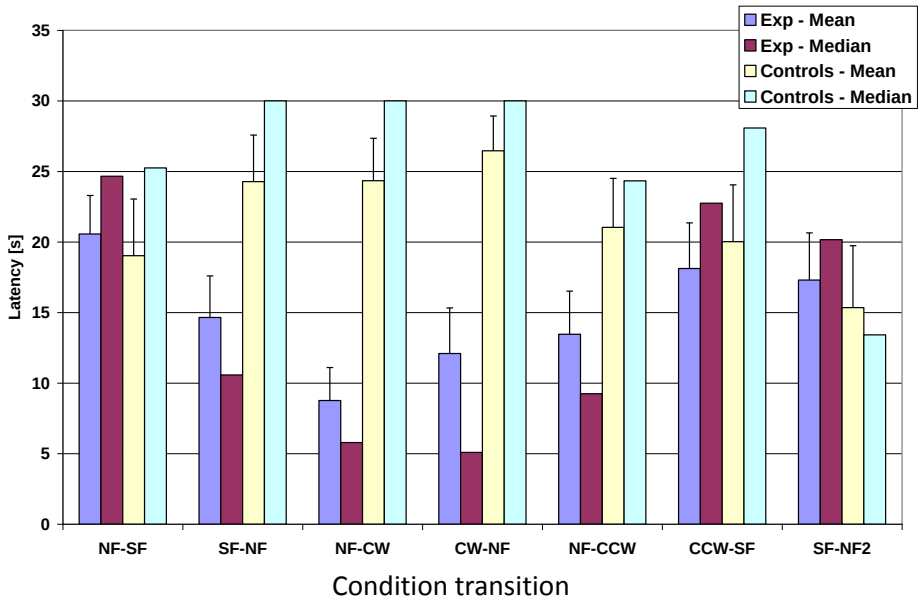

Figure 1.8 Latency of the response (1st head movement > 10°) to field transitions (NF = null Field, SF = Static Field, CW = Clockwise sweeping field, CCW = Counter Clockwise sweeping field, error bars indicate 1 S E). Figure from Migalski (2010).

One objective of the current investigation is to further refine this technique to the point of achieving a consistent, reliable experimental approach, which evokes easily measurable and more reflexive behavioural responses and, if possible, to use this to assess whether (and if so, how) magnetoreception depends on visible light.

Outline of methodology

The following sections give an overview of the approaches used to investigate magnetoreception in this thesis. Detailed methodology is contained in Chapter 2.

Helmholtz coils

In the first instance, an attempt was made to identify spontaneous head movement responses to magnetic manipulations made by Helmholtz coils, after Migalski (2010). This involved simultaneously making various improvements to the equipment, such as increasing the size of the coils, and hence the volume of the experimental arena.

Optocollic reflex

An original experimental setup was also designed to investigate the psychophysics of the optocollic reflex (an involuntary head movement response to whole-field drifting visual stimuli). This would ultimately facilitate investigation into polarisation e-vector vision and visual psychophysics in general, and pave the way for such experiments to be carried out under magnetic manipulation in order to assess the possible impact of the ambient magnetic field on visual perception.

Optokinetic reflex (OKR) is a visuomotor response that involves an involuntary eye and/or head (or occasionally even whole body) movement response to a specific type of moving large visual field stimulus, which occurs only if the stimulus is perceived, making it a good indicator of perception. The optocollic reflex (OCR) is the head movement component of

OKR, with optokinetic nystagmus (OKN) and optomotor response (OMR) being the movements of the eyes and whole body, respectively. OKR has been used extensively to investigate vision in birds and other animals and can be used in pigeons despite the lateral eye position changing some of the standard dynamics observed (Wylie and Crowder 2000; Crowder *et al.* 2003; Crowder and Wylie 2002; Maurice and Gioanni 2004). For example, a type of OKR could be used involving a rotating drum with slits allowing light through it to elicit an OKN response in a pigeon. The light allowed to reach the eye via slits could be at one extreme of the wavelength spectrum that can be seen by pigeons, i.e. 365nm (after Emmerton and Delius (1980)). If a change in magnetic field conditions alters the perception of light wavelength, then a light of wavelength 370nm may change from visible (OCR response) to invisible (no OCR response). This would have to be tested at just above and just below both maxima and minima of wavelength sensitivity with a variety of magnetic field manipulations. However, a proof of concept study could potentially be carried out quickly due to the qualitative nature of the response/no response paradigm and, if successful, could enhance our knowledge about the sensory limits of the visual and/or magnetoreception systems as well as confirming any link between the two.

Startle/Prepulse Inhibition

The acoustic startle response (ASR) is mediated by a simple set of sensory and motor neural circuits, and has been demonstrated to be a good test of perception, as it can be reliably attenuated by a prepulse inhibition (PPI) (if a particular prepulse stimulus is perceived, an altered ASR is elicited) (Koch 1999; Swerdlow *et al.* 2001).

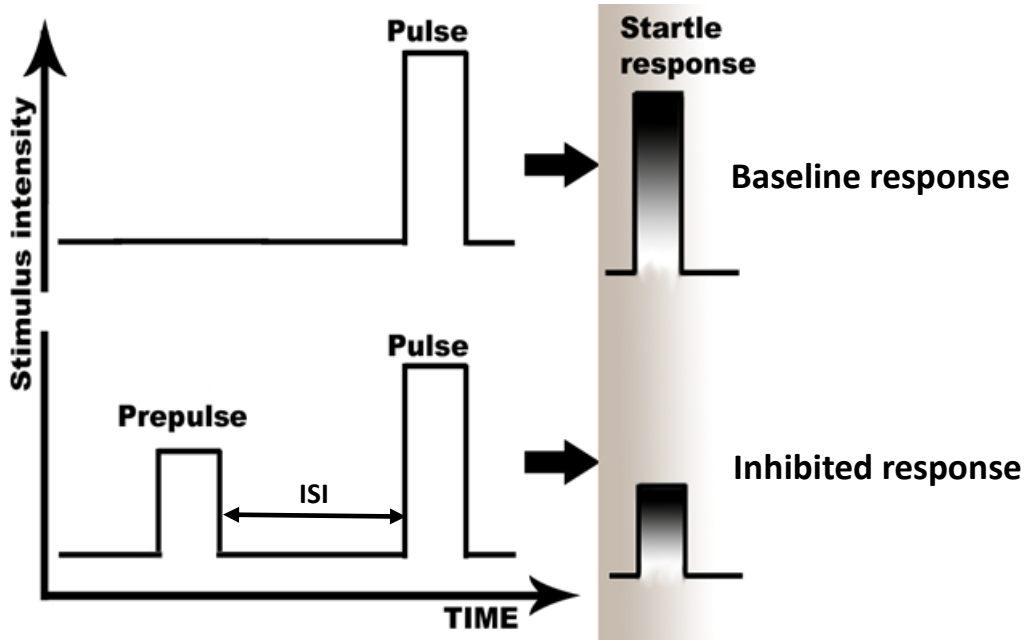

Figure 1.9 Principle of prepulse inhibition (PPI) adapted from (CopperKettle 2007). ISI = inter stimulus interval, Pulse = acoustic startle stimulus, prepulse = sensory stimulus (e.g. light, touch, magnetic field change).

The principle of prepulse inhibition arises from sensory-motor gating whereby the brain can only process and respond to a finite amount of information in a given moment. If a subject is repeatedly startled by a loud noise (ASR) or a flash of light, a response baseline can be built up giving a clear picture of the typical startle response of that subject. Then, if a prepulse stimulus (any stimulus which can be perceived by the subject, but does not cause a startle response) is delivered a short time before the startle stimulus, the sensory systems are effectively overwhelmed and the magnitude of the startle response is reduced (i.e. inhibited). In essence, the perception of a prepulse reduces the amount of perception of the startle stimulus, and hence the amount of response to it. Various aspects of the paradigm can be modified to give the best results in a given circumstance. The prepulse and startle stimuli can be adjusted in terms of magnitude (volume of sound/brightness of light, etc.) and duration (usually in ms). The time between prepulse and startle stimuli, the inter stimulus interval (ISI), also in ms, can also be adjusted, as can the interval between trials or repeats of the experiment, i.e. the inter trial interval (ITI). Usually many trials are carried out to gather

a robust data set, and startle only and prepulse trials are alternated, so that a comparison between the response to each can be made, accounting as much as possible for any order and/or habituation effects.

The duration of the acoustic startle stimulus has an effect on the startle response, and in rats, 8-10 ms has been suggested as optimal because anything less than 8ms reduces ASR amplitude whereas for durations above 10ms, no further increase is observed (Blaszczyk, 2003). Other effects have been found in pigeons with prepulse stimuli 15-2000 ms prior to startle inhibiting the amplitude of response and those presented less than 10 ms before the startle stimulus reducing the latency of response (Stitt *et al.* 1976).

Siqueira, Vieira and Ferrari (2005) used a chamber with sensors in the base to assess the magnitude of startle in pigeons and found especially good responses in the subjective night time, this suggests that such a technique could effectively be used to measure PPI (with change in magnetic field being the prepulse stimulus) in both light and dark conditions.

Atkinson *et al*. (2008) had difficulty using this method to measure PPI in chickens (ISI<500ms) and found that, although it could be done, it only worked well in certain strains of the species. This may indicate that there might also be a potential for variability between individual birds within a species, i.e. during a pigeon study. Background noise, intensity and duration of startle stimulus and prepulse, and the time period between the two, are all important variables in experimental design of PPI paradigms.

As the response measured is reflexive, under properly controlled conditions, PPI is a very reliable measure as no bias is introduced through training of subjects. The principle of PPI works powerfully to indicate whether the prepulse stimulus is perceived, as, by definition, perception of the prepulse will result in some attenuation of the startle response. This gives the opportunity to measure accurately whether subjects can perceive magnetic field

changes, without the need to know where the sensory reception mechanism resides within the body, how it works, or where in the brain the information is processed.

The intention was to use the Helmholtz coils to deliver magnetic field manipulation prepulses in a way that is entirely novel and, if successful, might facilitate many extended experiments involving the titration of the wavelength of ambient lighting, time of day, and almost any other potential variable that becomes relevant in the future.

Operant conditioning

Operant conditioning has been used in many species to assess their ability to perceive magnetic (or other sensory) stimuli (for review see Mora, Davidson and Walker (2009)). Although there has been some success in using operant conditioning in pigeons to investigate magnetoreception, on the whole such assays are often inconclusive (Mora *et al.* 2004; R. Wiltschko *et al.* 2007a). Suggested reasons for this include possible difficulty in associating magnetic stimuli with reinforcement, changes to the magnetic stimulus being significantly different from those that the animal might naturally encounter, and the possibility that animals may at times disregard magnetic field stimuli altogether (R. Wiltschko *et al.* 2007b). The area of operant conditioning, specifically with regard to investigating magnetoreception in pigeons, is another good example of the need for inter-disciplinary study, in this case requiring skills in both biology and psychology (at least) to design and interpret a meaningful assay. It is interesting to note that some of the more successful previous studies involved a more open arena (i.e. with more, if still limited, space to move) rather than a traditional Skinner box approach in which a subject is closely confined and offered buttons or levers to press to indicate a response to a learned cue (Bookman 1977; Mora *et al.* 2004; R. Wiltschko *et al.* 2007a).

An experiment carried out by Mora *et al.* (2004) found that pigeons could discriminate between spatially varying magnetic stimuli in a way that could be refined and adapted to examine the sensory limits of magnetoreception from the point of view of light dependence. That is, the same experiment could be repeated with a range of light wavelengths and intensities to explore functional requirements of visual magnetoreception. Before this could be reliably informative, however, it would have to be clearly established whether the magnetite, cryptochrome or other system is in play, a confounding factor in many magnetoreception studies. Although impairment of one or more systems could be used to isolate that which is under investigation, caution would be advisable to avoid introducing further complication (i.e. suppression of an animal's behaviour in general due to discomfort/disorientation or inadvertently impairing the system under investigation). Having considered these factors, it was decided that the use of conditioning paradigms was beyond the scope of this investigation, and did not offer the potential for robust, reproducible data gathering that the other techniques discussed could provide.

The following chapter gives more detailed information about the construction of apparatus, and design of experiments.

Chapter 2 Materials and development of methods

All work reported in this thesis was done in strict accordance with relevant animal welfare regulations, and carried out under Home Office licence where necessary, in compliance with the Animals (Scientific Procedures) Act 1986, as amended, which encompasses EU Directive 2010/63/EU. Home Office project licence numbers were 30/2724 and 30/3144. Pigeons were obtained from a local homing pigeon breeder, and were housed in mixed sex pairs.

Controlling the ambient magnetic field

Helmholtz coils can be used to accurately control the intensity of the ambient magnetic field with a high level of uniformity. This technique is commonly used in scientific experiments to cancel out aspects of the geomagnetic field. A pair of symmetrical coils placed along a common axis will generate a flux along that axis, which, when properly controlled, can enhance, reverse, or negate the intensity of the ambient magnetic field on that axis.

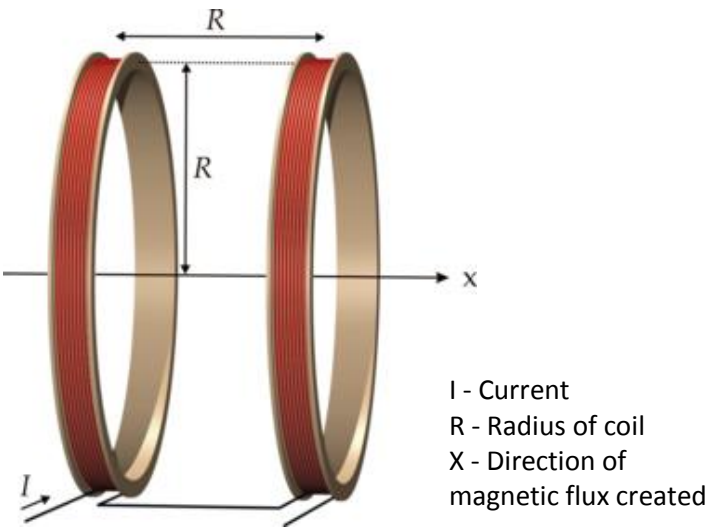

Figure 2.1 A typical Helmholtz coil arrangement (Ansgar Hellwig 2005).

The following formula allows calculation of the intensity of the magnetic field along the common axis of the coils (after Migalski 2010).

$$H = 0.715\frac{NI}{R}$$

Formula 2.1

Where:

- H- magnetic field intensity [$\frac{A}{m}$]
- N – number of turns [-]
- I – current inside the coil [A]
- R – radius [m]

The placement of three pairs of Helmholtz coils, in an orthogonal nested arrangement such that each pair has its common axis along one of the three physical dimensions (i.e. x, y, z axes), allows 3 dimensional control of the ambient magnetic field in such a way as to be able to enhance or negate all aspects of it. This means that the ambient magnetic field can be cancelled out completely (a new approach to behaviour experiments, developed for this research), and a new field effectively superimposed on the space inside the coils. Aspects of

the field that can be altered, which are desirable for the planned experiments, include overall intensity, polarity, and vertical inclination of the field.

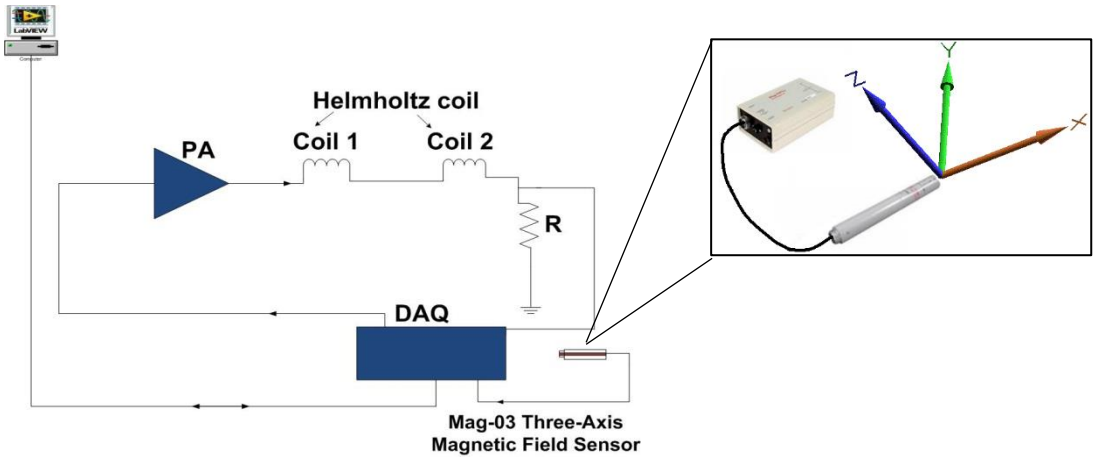

Figure 2.2 Line diagram of one circuit of the Helmholtz coils system (from Aldoumani *et al*. 2013) and expanded view of 3 axis magnetometer (PA = Power Amplifier, DAQ = Data Acquisition Card, R = resistor).

Figure 2.2 shows a single circuit or channel of the coil system. By sampling the intensity of the ambient magnetic field in all three axes, software can, via an external control card, send the appropriate signals to the amplifiers, such that the precise current required in order to generate the desired magnetic field is delivered to the coils. This principle was successfully employed by Migalski (2010) and, for this thesis, was carried out using greatly improved software, developed by Tomasz Kutrowski (Cardiff University), with the technical assistance of Noor Aldoumani (Cardiff University), as well as testing and development feedback carried out by the author.

General rationale for use of Helmholtz coils

Nested Helmholtz coils offer several advantages over other methods of manipulating the geomagnetic field, such as use of permanent magnets, electromagnets, and 1 or 2 axis Helmholtz coils. The main advantages are that the field can be altered very subtly if desired, with the possibility of great precision in producing and adjusting fields (permanent magnets allow no such inherent adaptability), and three dimensional control of the magnetic field,

such that all aspects of a 'natural' field can be emulated, and manipulated temporally, including inclination. Together these advantages facilitate the generation of a field which is theoretically very similar to the normal field an animal might ordinarily experience. Many past experiments in the area of magnetoreception research have employed magnetic manipulations which simply change the ambient field 'a lot', with little control, adaptability or reproducibility between experiments, an issue which has arguably slowed progress in the research field, and which was addressed in the PhD thesis of Migalski (2010).

The coil system of Migalski (2010)

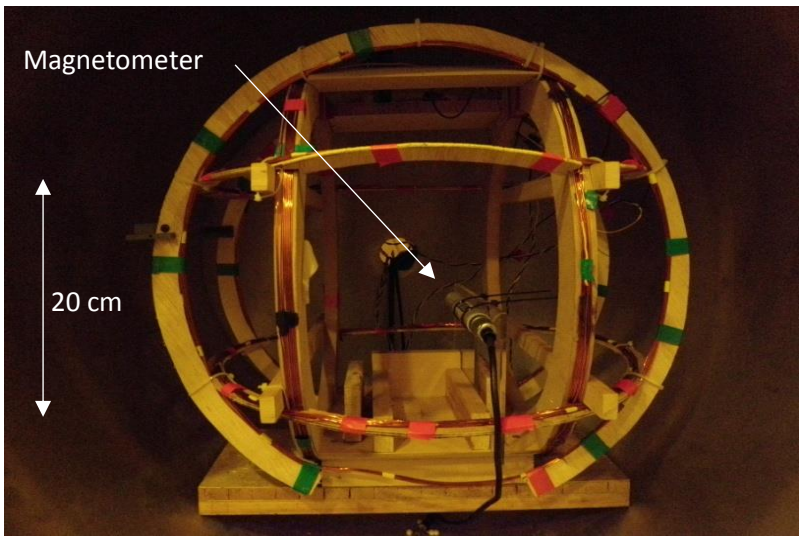

Figure 2.3 The Helmholtz coil system used by Migalski (2010).

Figure 2.3 shows the older coil system used by Migalski (2010). Note the confined experimental area and the three axis magnetometer, which must be removed during experiments (after a sample of the ambient field has been taken).

Whist the experiments of Migalski (2010) did appear to yield some meaningful results, showing that the three axis Helmholtz coil approach is worthy of further use, there were some limitations which the current research sought to overcome. Firstly, the Migalski (2010) experiments depended on physical shielding (5 layers of mumetal) in order to block the

ambient magnetic field, allowing the coils to generate the desired field in its place. The experiments were also carried out inside an insulated room which blocked radio frequency electromagnetic signals. This room was used partly out of convenience, and was not at the time considered essential as deliberate manipulations of the magnetic field were of significantly greater intensity contrast than the oscillations of these signals. Programming was carried out by a summer research student (Dominic Walker) and a post-doctoral research associate (Dr Tomasz Kutrowski) on the software used to operate the coils, which allowed the ambient field to be actively compensated for. That is, the natural field could be actively cancelled out, and the desired field superimposed, without the need for expensive, cumbersome physical shielding. The second desired development was to substantially increase the volume of uniformly controlled magnetic field within the coils from 10x10x10cm to 30x30x30cm, thus allowing a broader range of behavioural experiments, as well as uniformly controlling the field that the whole subject is exposed to, rather than only the head as in Migalski (2010). This required making a new, larger coil system, something difficult to achieve when previously reliant on the available physical shielding.

The new coil system

Early in the design process, thought was given to how visual psychophysics might be investigated whilst simultaneously manipulating the magnetic field. This could potentially provide some means of indirectly measuring an animal's response to magnetic stimuli. Although beyond the scope of the current research, the coils can only be built once within the constraints of the project, and so attempts were made to predict what parameters would be useful.

Figure 2.4 shows an example of one postulated way that visual psychophysics might be explored whilst manipulating the magnetic field. A series of visual stimuli could be presented, for example drifting horizontally, and their effects on the Optocollic Reflex (OCR) response

could be investigated during field manipulation. It is known that pigeons can see a greater range of visual light wavelengths than humans, (Bowmaker 1977), and there are various reasons to suspect some role of visible light in magnetoreception generally (see Chapter 1 Introduction). Therefore, it was considered advantageous to be able to present stimuli consisting of light at a variety of frequencies.

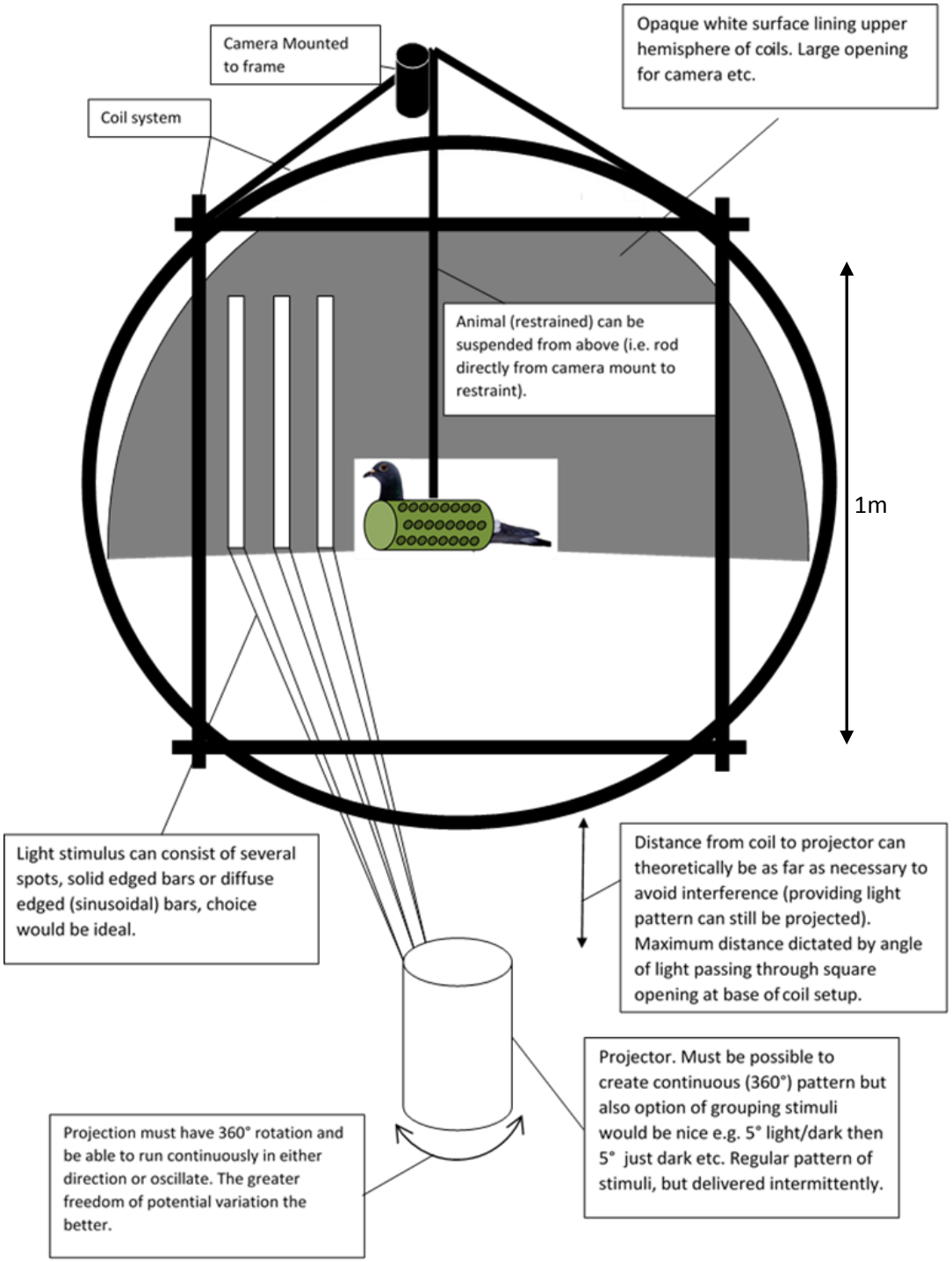

Figure 2.4 An example of an early idea for presenting visual stimuli within the Helmholtz coils without interfering with the magnetic field.

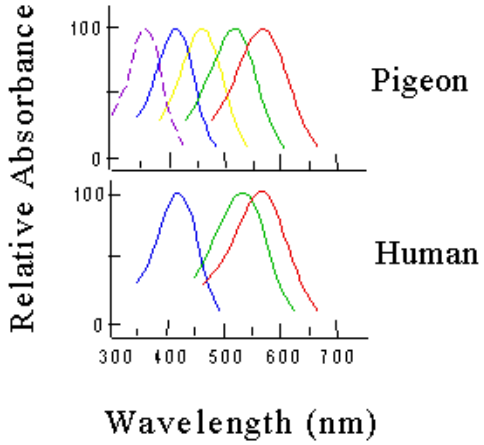

Figure 2.5 Comparison of human and pigeon photopigment sensitivity (Bowmaker, 1991). Each peak represents the maximal activation of a particular photoreceptor cone.

With a view to being able to carry out a range of experiments including presentation of visual stimuli in the future, the larger set of Helmholtz coils was designed and built. Much of the technical work of drawing and designing the coils was carried out by Noor Aldoumani, as part of her PhD research study, being carried out in parallel to that of the author's. The author contributed significantly to the creative process of designing the coils with regard to the envisaged requirements of future experiments. Significant contributions were also made by Dr Tomasz Kutrowski, Dr Turgut Meydan (Cardiff University), and Prof. Jonathan Erichsen (Cardiff University).

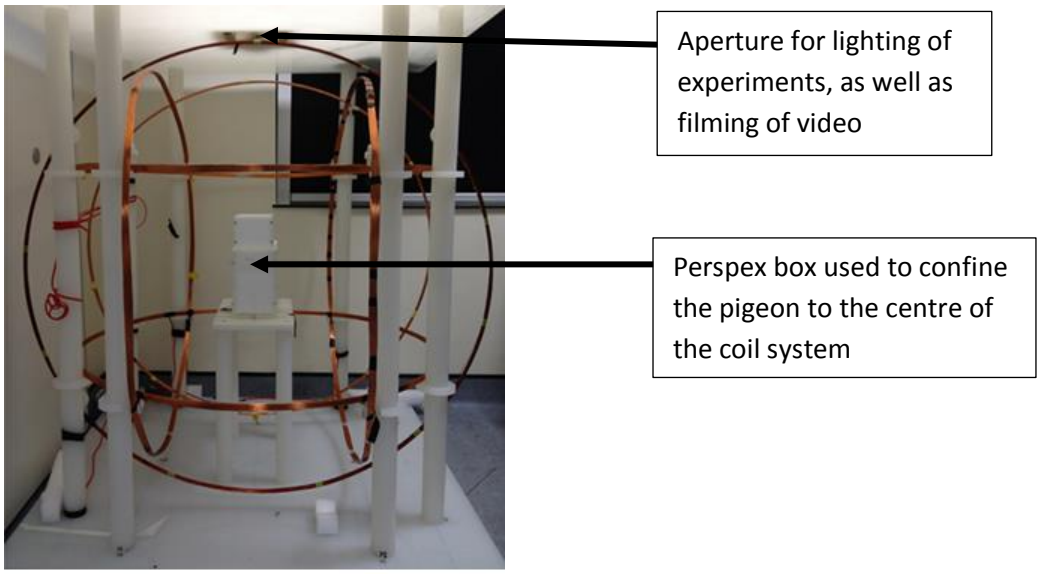

Figure 2.6 the new Helmholtz coil system, *in situ* in the laboratory.

Testing of new coil system

The uniformity of the magnetic field generated by the new coil system was carefully measured by Noor Aldoumani and Dr Tomasz Kutrowski with the assistance of the author and was reported in (Aldoumani *et al.* 2014). The following figures help visualise those measurements.

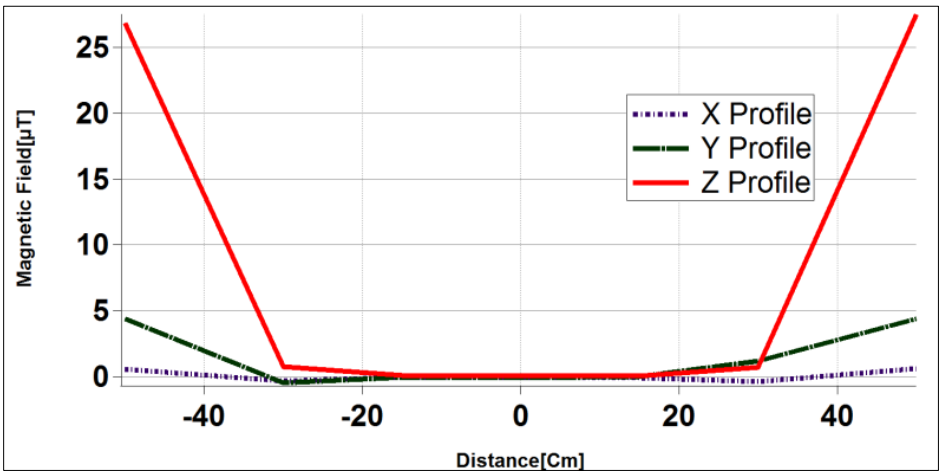

Figure 2.7 Observed intensity of magnetic field in all axes when desired intensity is zero. Note that the expected field condition persists for over the desired ±15cm in all directions (from Aldoumani *et al.* 2014).

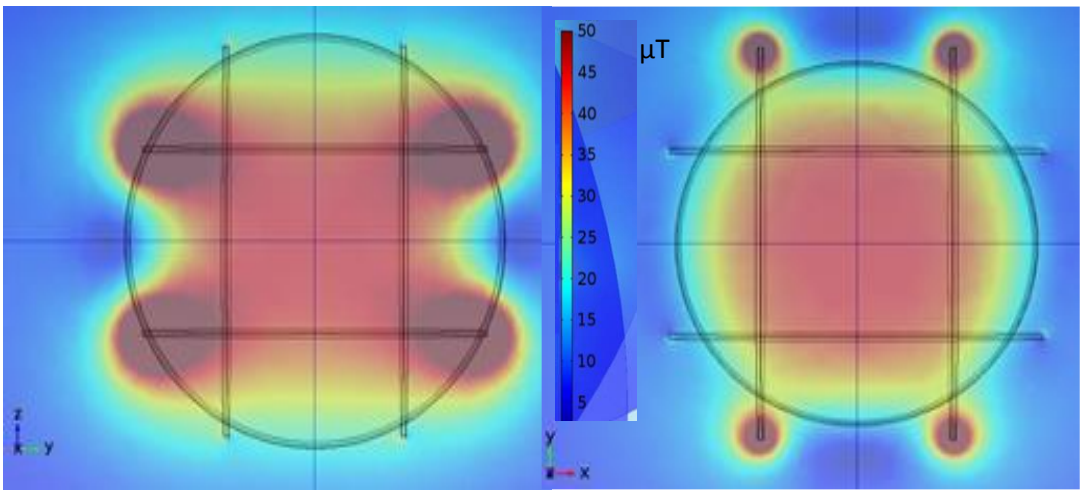

Figure 2.8 COMSOL model showing the uniformity of the generated field as viewed from the side (left panel) and top (right panel) produced by Noor Aldoumani. Consistency of colour implies uniformity of magnetic field intensity (from Aldoumani *et al.* 2014).

Sound and vibration testing

Testing was also carried out by Dr Kutrowski to confirm that no sound or vibration artefacts were produced during magnetic field changes using accurate recording equipment (preamp: Bruel & Kjaer type 2671 serial: 2373743, Mic: Bruel & Kjaer serial: 2407256, Conditioning amp: NEXUS Charge Amplifier serial: 2026082, Accelerometer: KISTLER 8636C5).

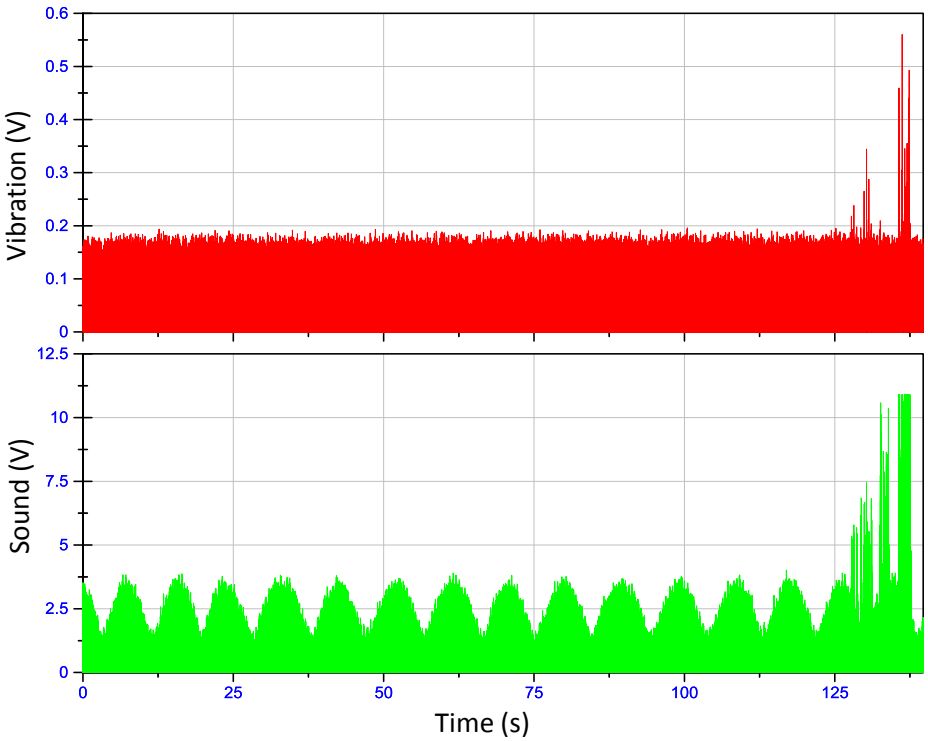

Figure 2.9 Sound and vibration recordings in Volts recorded by Dr Kutrowski. Field manipulations take place in the first 25s, and a light tapping on the apparatus by hand occurs at approx. 127s.

Various field manipulations took place within the first 25s (5s normal field, 5s null field, 10s rotating field with 3 full rotations, 5s null field) and then the signal was terminated meaning the natural ambient field is restored. No changes in sound or vibration recordings was observed when field manipulations took place, but a very pronounced change can be seen as a result of lightly tapping on the structure to which the coils are attached which occurred at approximately 127s (Figure 2.9). The steady fluctuation in sound is a result of the amplifiers and does not change noticeably during field manipulation.

Background field measurements

In order to assess the environmental background 'noise' in the ambient magnetic field, the author used the three axis magnetometer to simply monitor the ambient field over a period of almost 4 days between 23/06/2014 and 27/06/2014. Sampling in three orthogonal directions (i.e. 3 dimensions), at a rate of 1000Hz, a total of 957717 (319239x3) data points were recorded in this period of approximately 88 hours and 40 minutes.

Data were plotted raw, and then values were differentiated to show the amount of change from one point to the next, allowing a clear view of the periods of greater and lesser activity, and an indication of the extent of fluctuation.

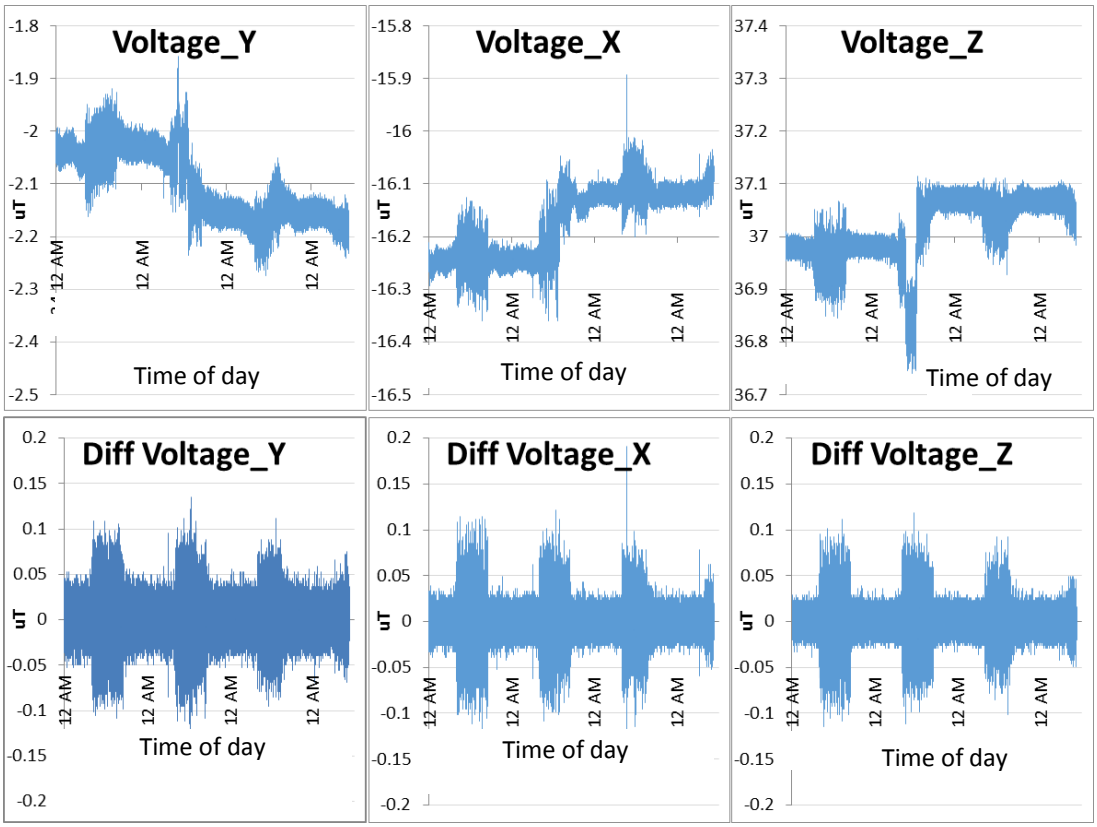

Figure 2.10 Ambient field intensity in all three axes (top row) and the same data differentiated to show spontaneous changes (bottom row).

Figure 2.10 shows the variation in the ambient magnetic field during a time when the lab was unused, and all equipment other than the PC to which the magnetometer recorded data,

was switched off. Note the regular periods of activity, particularly during the daytime. The magnitude of the variation can be seen to be around ±0.05µT during the 'quiet' hours, and around ±0.1µT during the 'working day' (approximately 0.2% and 0.4% the total intensity of the local geomagnetic field respectively). As a result of these findings, it was decided that behavioural experiments would be conducted at night time, in order to minimise any possible disruption arising from this background noise in the magnetic field. It was also assumed that manipulations made during experiments would remain salient, as the changes made would be much greater (as much as 60µT in the case of inclination flipping, during which the vertical aspect of the ambient field is inverted, reversing the angle of inclination) than those occurring in the background field (approx. 0.1 µT).

Experimental design for coil experiments

In order to carry out behaviour experiments using the coil system, a set procedure (developed by the author) was followed as standard.

To minimise perceptual distraction of the subjects, experiments were carried out in a quiet, isolated environment. Carrying out experiments at night had the advantage of minimising the potential influence of background field variation, as previously discussed. Also that there were no other staff on that floor of the building, and only the odd staff member in the building at all. This meant that there were none of the normal daytime noises and vibrations associated with general use (i.e. foot traffic, wheeling trolleys past the lab, or people talking to each other). Additionally experiments were carried out under cover of ambient white noise (65 dB) to further minimise the influence of any unwanted sounds.

All windows to the lab were closed with blackout blinds in place, meaning that the lighting of experiments could be precisely controlled, and that no extraneous stimuli in the form of changing patterns in the ambient lighting would be present. Lighting of experiments was

done using a fibre optic light, presenting white light at 158 lux in emulation of lighting employed in Migalski (2010). Light entered the experimental arena from above the subject, via a fibre optic cable. The light source itself was shielded from the coils using two layers of mumetal shielding. During experiments, the lab was separated into two halves using a secure, light-proof divider which also damped further any unwanted noises. This meant that the subject, within the coil system, was in one half of the lab, with the experimenter, control PC and amplifiers, together in the other half of the lab.

Using the new bespoke software, an experimental trial could be planned and designed (e.g. 1 minute of static field, immediately followed by 1 minute of flipping field, then another minute of static field), prior to bringing subjects to the lab.

All subjects had visited the lab on at least one occasion, and been regularly handled by the author prior to experiments. This was to minimise stress in subjects during experiments which may occur when exposed to unfamiliar people or surroundings. White noise would be playing and amps would be switched on before subjects arrived in the lab. The subjects would then be given a minimum of five minutes to acclimate to the lab conditions prior to experiments. This was again intended to minimise any stress experienced by the subjects.

To carry out an actual trial, the subject would be placed into a Perspex box within the coils and left for 2 minutes to get comfortable in its new surroundings (the initial impulse is to explore and to try to escape, followed quickly by simply standing). The box measured 304x138x209mm and was designed to allow appropriate space to stand comfortably but to prevent the pigeon turning around. Lighting and sound were kept constant (i.e. set up before introducing the subject). On commencing the experiment, the experimenter simply clicks start on the bespoke graphical user interface (GUI) (produced by Dr Tomasz Kutrowski using LabVIEW, a product of National Instruments, Austin, USA) which prompts the initiation of video recording, as well as beginning the manipulation of the magnetic field. From this point

on, the video recording and magnetic field changes are synchronised such that it is possible to tell what was happening in any given frame of the video. Upon completion of a trial, the subject is immediately removed from the arena and placed back into its travel accommodation, either to 'rest' before the next trial (in which case water was provided ad lib), or be returned to the animal housing facility.

The method of measuring and recording pigeon head movements is described in the following section.

Measuring pigeon head movements

In order to automate the otherwise laborious and time consuming process of extracting the head angle of subjects from every frame of recorded videos, Migalski (2010) employed a specially written program created using the LabVIEW programming environment. This was apparently successful and yielded enough data for the analysis desired in that study. The intention was to employ that same program in the current research. Upon testing the software, a serious issue was immediately apparent. The software relied on image recognition algorithms designed to recognise a novel pattern in each frame, and identify its location and rotational orientation. Testing carried out by the author and undergraduate project student, Andrew Moss, revealed that the recognition of the marking used on the heads of pigeons was inconsistent. Running the same data extraction, in exactly the same procedural way, twice, would yield different data each time. A small amount of variation could be tolerated, and indeed expected as a result of slight differences that may arise in the way that the head marking is manually identified in the initial frame before automated analysis begins, however the variation in the data reported was, in some cases, alarmingly large.

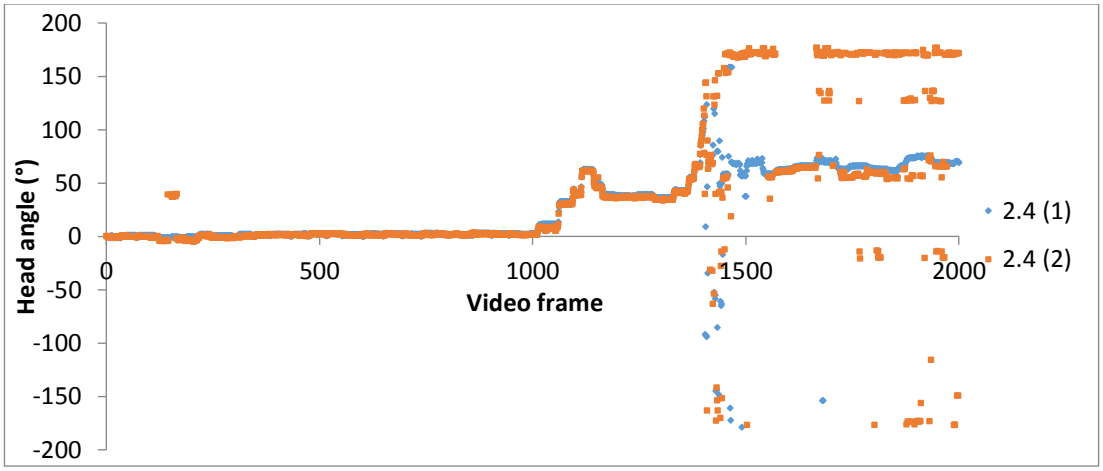

Figure 2.11 Example of inconsistent head angles reported by the old video analysis software on repeat analysis.

The above figure shows the head angle in each frame of a test video, for two separate analyses using the old version of the video analysis software. Note that, when the subject keeps its head relatively still, repeat analyses are fairly consistent. However, as the subject becomes more active (as evidenced by more change in head angle over successive frames) greater inconsistency occurs. Some of the differences between the two data sets are substantial. Many of the frames return a head angle in one analysis that is flipped 180° from that in the other, indicating that the software has failed to tell one end of the template from the other and has somehow rotated it. In other cases, the difference is not 180°, suggesting that it is not a simple case of transposing the ends of the template, but actually recording angles that must be wrong (both cannot be correct if they are different). An investigation was carried out by the author and Dr Tomasz Kutrowski to ascertain whether Migalski (2010) had experienced this issue, and whether it was perhaps overcome with some kind of filtering. As it transpired, it seems that Migalski (2010) had no such issues and detailed investigation into the programming by Dr Kutrowski (assisted by the author) revealed that certain components of the software, specifically a video codec and certain LabVIEW sub-VI's (Virtual Instruments, building blocks which allow pre-defined commands to be employed in scripts)

had been updated since their original use, were no longer fit for purpose, and could not be easily replaced.

This resulted in a situation where there was no option other than to painstakingly sift through the whole program, replacing, updating and re-writing sections as necessary. In doing so, it was decided that the program should simultaneously be improved in certain ways. This was a significant undertaking, requiring several months of intense work, the bulk of which was carried out by the project's lead programmer, Dr Kutrowski, although the author contributed significantly to both the design and extensive testing of the new software. This resulted in a bespoke package which ultimately proved very effective at extracting head angles from video.

Improvements to video analysis

Many ways of improving the method were investigated, including the employment of region of interest (ROI) analysis which limits the search area to a certain distance from the identified position of the head marking in the previous frame, and edge detection, which looked specifically at the shape of the template, rather than simply contrast between groups of pixels. The use of contrast thresholding, such that a high-contrast template could be isolated from its surrounding by adjusting the white-balance of the image, and using a colour template (and camera) to give even greater contrast were also explored. The first three advancements were ultimately implemented, along with a new style of head marking of the author's design (Figure 2.12). This marking further increased contrast, both in luminance (predominantly white, whereas pigeon's head feathers are typically dark grey) and in outline (employed a 3 dimensional, straight edged oblong marker which looked similar in side-on profile to when viewed from above). This allowed the software to recognise it even when the pigeon's head was tilted, something impossible with a 2 dimensional marking, such as the paint streaks used by Migalski (2010).

Figure 2.12 The new style head marking: 3 dimensional, asymmetric, and highly contrasting the colour of the subject's head.

An extensive investigation into the merits of using colour markers was carried out by the author, with the assistance of undergraduate project student, Tim Jerome, which revealed that, although some advantage was offered in the use of coloured markers (in terms of increasing contrast with pigeon feathers), the benefit was not large enough to outweigh the inherent cost of using colour video filming. Specifically, the only available colour camera had a slower frame rate, but created video files at least an order of magnitude larger than those created using the black and white (and lower resolution) camera. This, combined with the fact that only a certain amount of data could be held in the memory of the PC for writing (only a limited amount of data could be recorded in any one continuous run), meant that it was unrealistic to be able to store all of the videos created by an extensive experiment, and videos would have to be shorter than required.

The improvements in image recognition and in the marking used for the head, as well as the employment of some contrast thresholding, did ultimately result in a system which was very reliable. The software development and its validation were summarised in Kutrowski *et al.* (2014).

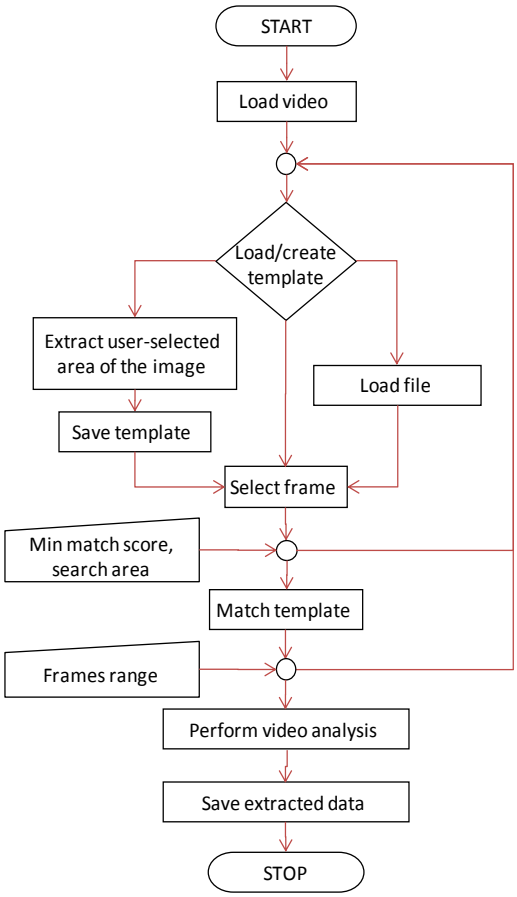

Figure 2.13 Simple flow diagram showing the process of video analysis extracting head angles from each frame (from Kutrowski *et al.* 2014).

Improvements were also made to the GUI used by the experimenter to execute the analysis. Upon opening the GUI and loading the desired video for analysis, the first frame of the video is shown in a window. A scroll bar allows selection of a video frame in which the head marking is seen clearly enough, and in which the subject is facing forwards. The reason for selecting a frame in which the subject is facing forwards is that whatever frame is used for the template, will be assigned zero degrees, and all rotational orientations measured from that set point. An alternative method is to use any frame, and correct the resulting data so that zero equates to straight forwards, this can be achieved using a protractor. For the research presented in this thesis, it was decided that using a frame where the bird was facing approximately straight forwards (±5°) was sufficient, as no specific examination of direction faced was undertaken, only the change in said direction, i.e. movements. Once a suitable

frame is identified, the template is manually drawn around the head marking by the experimenter. This involves clicking the left button of the mouse to create a vertex wherever the cursor is in the window, and then repeatedly clicking around the head so that the marking is encompassed by the resulting box.

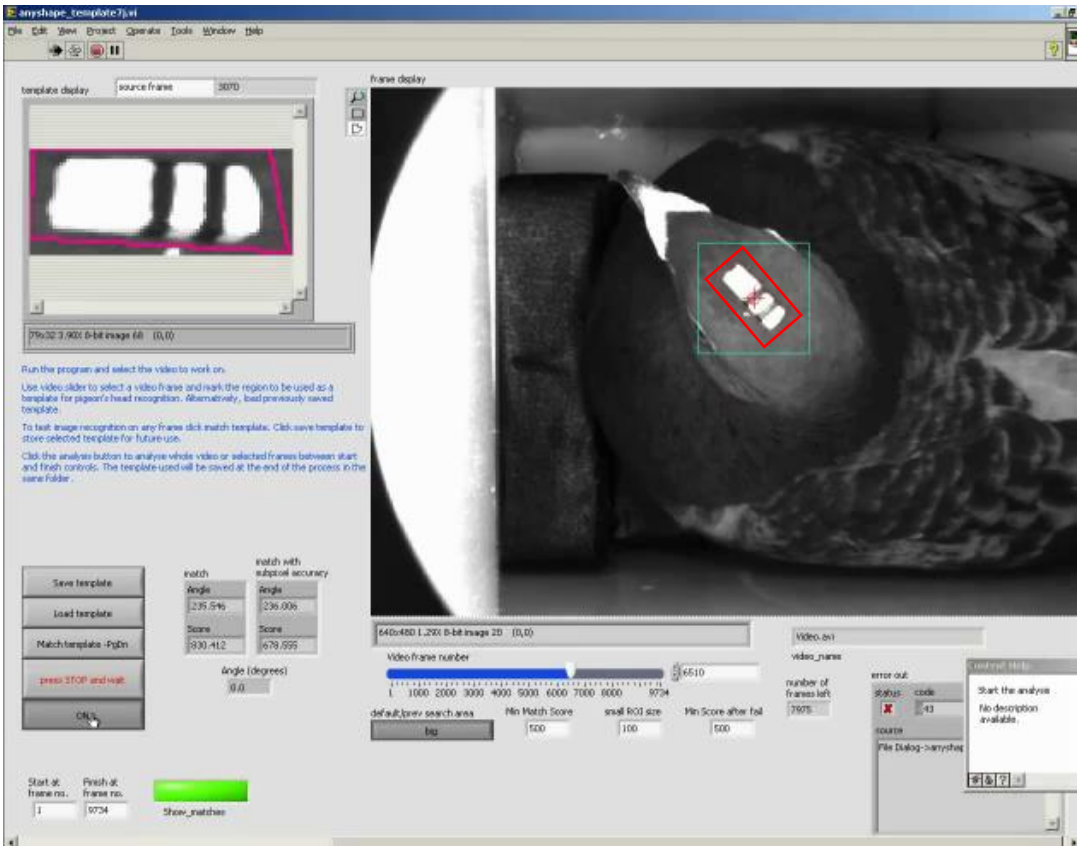

Figure 2.14 A view of the GUI used to control the video analysis software (blue box = region of Interest, red box = marking template).

Drawing the box around the marking, with a small boundary included, means that the template created contains the highly contrasting area where the marker ends, and the pigeon's feathers begin. It is this edge contrast, as well as the overall shape of the marker, for which the software searches in each other frame of the video. Once selected, the template is automatically saved (Figure 2.14) so that it can be used again if necessary. Another crucial advancement in the new software is that a minimum matching score may be assigned. When the software identifies a match to the template in a given video frame, it

calculates how closely the identified pixels match those of the template, 1000 being a perfect match, 0 being no match at all. Due to variation in lighting angle and distance from the camera lenses, the marking does look slightly different in some video frames to others, meaning that searching only for a perfect match results in a huge amount of data being lost. However, setting the matching score too low allows for the including of false matches, where the software has identified entirely the wrong pixels as matching the template (Figure 2.15).

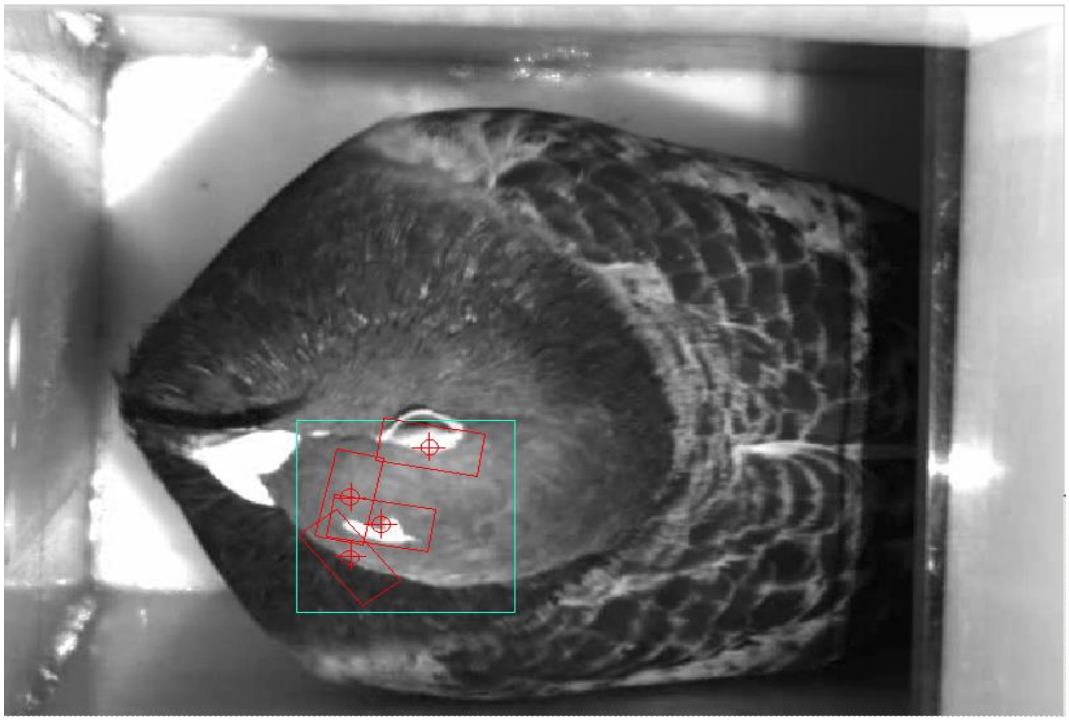

Figure 2.15 False matches where the software has identified the wrong group of pixels typically have a matching score of 225–507, whereas the actual marking has a matching score of 982 in this case (From Kutrowski *et al.* 2013).

After extensive testing, it was decided that setting the matching score no lower than 700 resulted in highly reliable data extraction. This was further assisted by the addition of the new style head marking, meaning that a minimum matching score of 800 could be employed.

The overall result of these improvements was that data were highly consistent and reliable, with no observed instances of transposition (180° rotation) of head angle, and no case of misidentifying the head marking, both of which had been significant issues in the original

software when tested. Any frame for which the software was unable to identify the head marking, was assigned an angle of -999°, i.e. an obviously impossible value which would never occur in the case of a positive match. The process of extracting data from experimental videos was to: open the software; load the desired video; assign a template; test the template on a number of frames using the scroll bar to change frame and the match template button to test the template; then assuming the template is deemed satisfactory press start, upon which the software cycles through all frames of the video in order, assigning each a head angle. The data produced are saved in the form of a comma separated value (.csv) file with one single column of head angles, in the order of the frames in the video. This can then be imported into a range of other software for analysis. Initiation of video recording is done automatically by the software used to operate the coils. This means that each frame is effectively time-stamped to allow users to have confidence about exactly when magnetic manipulations take place.

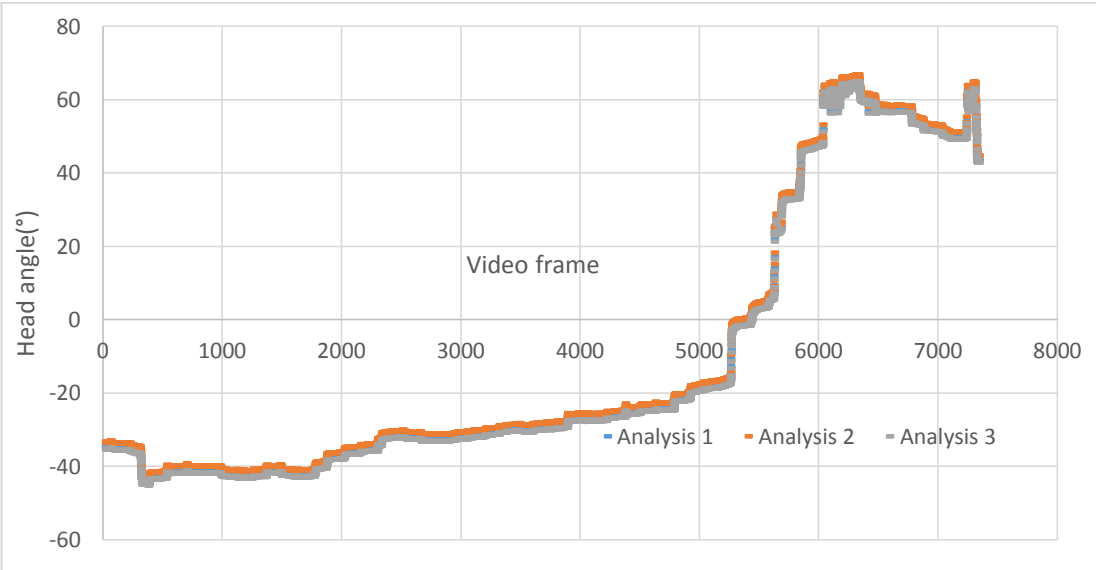

Figure 2.16 Three different extractions of the same video file using the new software.

The above figure shows an example of the data produced by the new software. Three different templates were used. It can be seen that the zero point of each template is slightly different, however the change in position with each frame is highly consistent, meaning

measures of movement over time give identical results. Note that there are no missing data, no 180° transposed values, and no obvious inconsistency as with the old software (Figure 2.11).

Saccade detection

In order to identify saccadic movements in head angle data, MATLAB (MathWorks inc. Natick, Massachusetts, USA.) was used to write a script which carried out several processes. The author produced a script containing many instructions for accessing the correct file, naming output, setting thresholds (if necessary) for any data to exclude, or the possibility of examining discreet portions of the data if desired, and a pre-existing method of identifying saccades was included. A modified script written by Lee McIlreavy (Cardiff University), which identifies saccades based on jerk (the third derivative of position) values, was initially employed with some success. However, tiny, but very fast movements were present in the recorded head angles as noise. This is likely the result of head angles being reported to three decimal places, which may be slightly more accurate than the detection software can reliably achieve, meaning that a small 'jitter' effect is present, where head angles change by a tiny fraction of a degree repeatedly between frames where the head is effectively still. Although small (hence contributing negligibly to any assessment of total movements), and consistent between videos/analyses (meaning comparison between trials is still meaningful), this jitter does affect the use of jerk as a criterion on which to base saccade detection. The result was that many saccades were detected which were clearly not real. The author must at this point give great thanks to Lee McIlreavy for assisting with the extended effort of trying to resolve the issues arising from this jitter. In the end, the most reliable solution was to apply a Gaussian filter to the data (Figure 2.17), as is consistent with eye movement literature (Kolarik *et al.* 2010). This had the effect of smoothing the data significantly, such that the jitter was no longer evident, but the larger movements (over 1° for example) were still clear,

and could then be analysed with the saccade detection algorithm (Figure 2.18). Repeated testing showed that the extended period of development had been successful, as comparison of saccades identified by the software to those manually observed both by watching videos and by examining raw data (head angle), traces showed a high level of consistency.

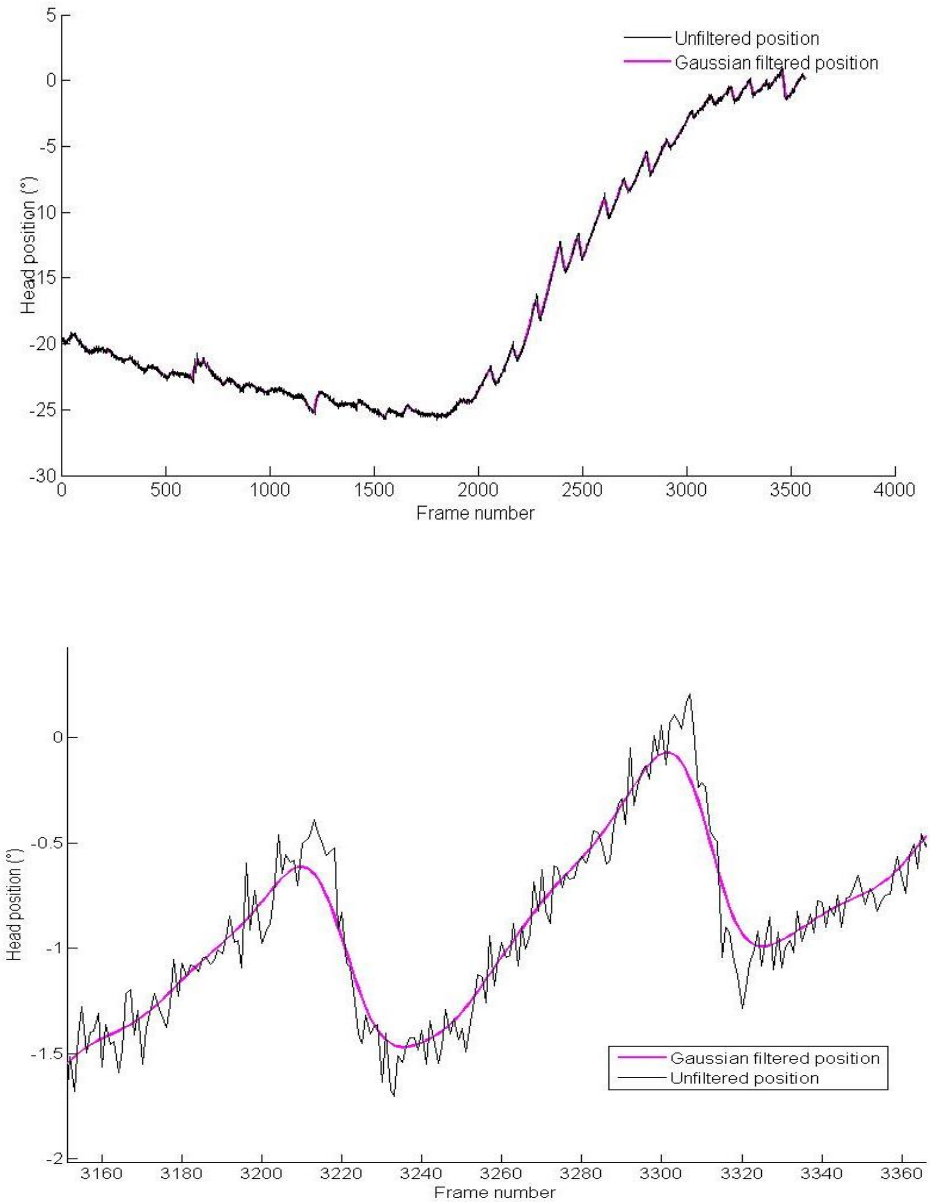

Figure 2.17 Unfiltered vs filtered data plot (top) with zoomed in section to illustrate the effect of filtering (bottom).

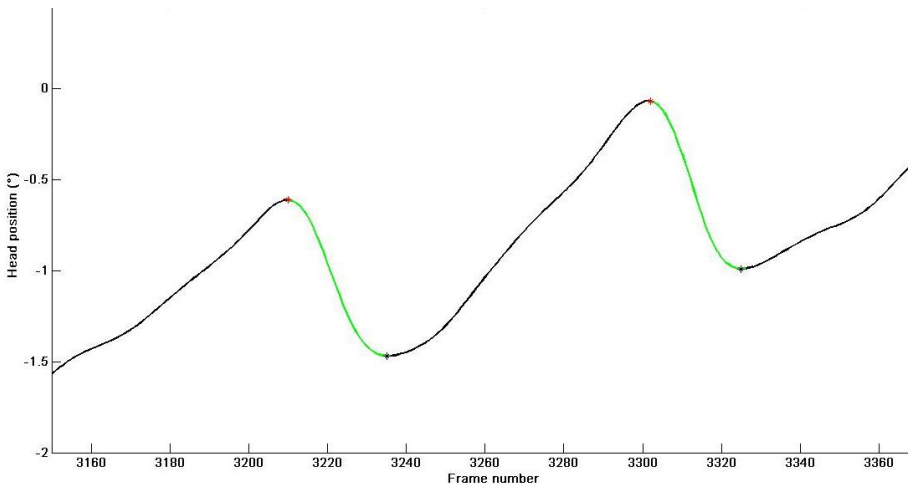

Figure 2.18 Detection of saccades in filtered data is more reliable (green = saccade, black = slow-phase).

The software jointly produced by the author and Lee McIlreavy identifies saccades and slow-phase movements, and writes each to a separate csv file. Data outputs include start frame, end frame, duration (in frames), amplitude (°), mean velocity (°/frame), peak velocity (°/frame), and direction for both slow-phase movements and saccades. Simple additional calculations in Microsoft Excel or similar allow conversion from frames to seconds (duration) and degrees/frame to degrees/second (velocities), as well as gain of slow-phases (velocity of slow-phase/velocity of stimulus). These metrics provide a robust way of comparing head movement responses in pigeons, and are employed in the analyses of data in the following results chapters.

Investigating the Optocollic reflex

Rationale for LCD presentation of regular optokinetic stimuli

Optokinetic nystagmus (OKN) and Optocollic Reflex (OCR) are reflexive movements of the eye and head, respectively, which are primarily involved in gaze stabilisation. The response is highly stylised and can be characterised by a smooth pursuit phase, in which displacement of the visual world about the animal is followed by the eyes and head, and a saccadic reset

where the eyes and head 'flick' back at high velocity to a suitable position to continue smooth pursuit. The movement serves the purpose of stabilising the view of a moving visual environment for the greatest proportion of time possible, thus minimising retinal slip.

OKN is used in a clinical setting for a simple assessment of the function of the visual pathway, as an incomplete pathway (indicative of damage or developmental issues) will not produce the response (Anstice and Thompson 2014). In laboratory settings, OKN and OCR are commonly used in assessment of visual pathway function, often in studies on the effects of contextual, chemical, lesioning, or sectioning treatments on the function of the various neural tissues involved (Conley and Fite 1980; Gioanni and Vidal 2012).

Both OKN and OCR movements have various characteristics that can be quantified and compared, both between species, for example to highlight possible evolutionary trends, and within species to assess effects of treatments or ontogenetic changes, or simply to explore psychophysical limits. Frequency, amplitude, and peak and mean velocity of the reset saccades are commonly used, along with measures of how closely smooth pursuit velocity matches that of the moving stimulus, and the time latency for the onset and ending of smooth pursuit in relation to that of the stimulus. All of these factors vary depending on stimulus parameters such as velocity, luminance contrast, spatial frequency, waveform, or shape of the stimulus, and ontogeny or health of subject.

Each species would be expected to show a range of measurements for each of these parameters, though only for a comparably small number of species have any of these parameters been reported in the literature, and usually only one or two parameters are used, often over a limited range.

Assessment of OKN/OCR is dependent on the ability of the investigator to display a drifting stimulus, often consisting of a square wave grating or a pattern of dots, over as large an area

as possible of the subject's visual field. This is most often achieved by use of a rotating drum, which either rotates the stimulus about an axis in front of the subject, or about an axis vertically in line with the eye, such that the stimulus drifts horizontally (or occasionally vertically or at other angles) across the visual field (Gioanni 1981; Türke *et al.* 1996; Knapp *et al.* 2013).

Stimuli are generally presented using a physical sheet of suitably patterned material. Some more recent studies have employed a rotating dome, containing a light source and penetrated with holes or slots, such that a rotating pattern of light patches is produced on an outer hemisphere which encloses the subject (Gioanni and Vidal 2012). This is limited in terms of exact stimulus presentation (for example, if testing acuity). With both techniques, changing stimulus parameters such as spatial frequency, style (i.e. sinusoidal, square wave, spots etc.), contrast or colour requires a separate trial, as the drum/dome must be stopped, and the subject disturbed, in order that the stimulus be changed. Similarly there can be complications around rotating the drum steadily, but at a variety of accurately controlled speeds and direction changes.

Due to the restrictions mentioned, studies to date have reported limited measurements, representing only the minimum number and range of responses immediately necessary to the study in hand, with very little opportunity for extending/varying the investigation, or utilising the data produced for further studies. There are also issues in some previous studies, particularly in avian subjects, with problematic subject behaviour, resulting in high rejection rates where trials have to be abandoned, and a need for extensive training of subjects to the experimental apparatus (Conley and Fite 1980).

Measuring head and eye movements is problematic in many species. Whilst eye tracking technology has developed significantly in recent years, the accurate assessment of OCR head movements in many species remains a challenge. This results in a situation where

parameters available for a given species often overlap very little between various studies and little or no independent reproduction of such experiments takes place. Attempting to build any kind of extensive range of responses, such as for an evolutionary or detailed psychophysical study, is extremely difficult due to physical constraints on carrying out experiments, and paucity of published data.

In addition to the complications already mentioned, measuring OKN or OCR in species with laterally directed eyes, and hence reduced or non-existent binocular vision, prevents use of single liquid crystal displays (LCDs), as are occasionally used in humans (Anstice and Thompson 2014). Intuitively, only a visual display encompassing the full 360° field of view can be used to maintain a regular OCR or OKN response in subjects of such species (without fixing the head in position in the case of OKN).

The presentation of psychophysical stimuli using LCD or other flat panel electronic displays is not a new one, but its use for OKN/OCR has, until recently, been almost non-existent, due in large part to the technical complications of presenting stimuli that fill enough of the visual field to be suitable for a range of tests. However certain benefits seem clear, with almost any conceivable stimulus parameters being possible, in any combination within a trial.

New LCD method for investigating Optokinetic Reflex

LCD displays offer the possibility of complex, dynamically varying visual stimuli, as well as having the potential for investigating e-vector discrimination. A 360° visual arena was constructed using 6 Philips 190S7FS 48cm (19") SXGA LCD monitors, each having a horizontal viewing area of 375mm, placed in a regular hexagon (Figure 2.19). A wooden frame was built to fit the arena which could support a video camera for filming the experiment from overhead. This was designed such that the camera was positioned vertically directly above the centre of the arena, and hence the pigeon’s head. The wooden frame also served to

support a blackout curtain which entirely covered the arena, ensuring that extraneous visual stimuli were excluded. The camera was positioned within the blackout 'tent', and the cable to operate and record from it passed through a small hole in the fabric. All visible surfaces within the arena were covered with matte black material in an attempt to minimise potential points of visual fixation extraneous to the presented stimuli, as well as to minimise reflection-based artefacts in polarised light experiments.

A platform was placed in the centre of the arena and positioned such that the head of the pigeon, in a normal comfortable position, would be as close as possible to the centre of the arena in terms of being equidistant from each screen, as well as being sufficiently elevated that the head is near the centre of the screens vertically. Between the pigeon and the supporting platform was a contoured piece of soft foam, carefully shaped so that the restrained pigeon would rest in a position such that the angle of its torso to the horizontal plane was as natural as possible. This ensured that the angle of the neck, in holding the head level (eye-beak tip angle approx. 30° decline, Erichsen *et al.* 1989), was as comfortable and unrestricted as possible.

Restraint was in the form of an elasticated tubular bandage, placed around the torso, wings and legs of the bird, so that it was not able to stand, or extend its wings, but, properly supported, would assume a resting posture and sit for 10 minutes or longer without any significant struggle or sign of unease. The bandage used was highly breathable, and to ensure the bird was able to thermoregulate properly, trials were limited to a couple of minutes at a time, interspersed with periods (equal or longer duration than trials) unrestrained and free to move around, and with *ad lib.* access to water.

Because of the blackout curtain enclosing the LCD arena, the small amount of heat generated by the screens could not dissipate as freely as it might normally. This coupled with the fact that the pigeon was restrained and unable to relocate if it felt too warm, meant that it was

important to provide cooling and ventilation to the arena. This is done by having a small fan constantly introducing air through a small aperture at the bottom of the arena, to the rear of the pigeon's resting viewing direction (i.e. bottom-rear of the arena), and another small fan, extracting rising warm air, through a loosely covered opening at the top-rear of the arena. Ambient temperature was checked before and after each experiment, as well as periodically between subjects to ensure it never rose above 24°C in accordance with relevant guidance (Home Office 2014).

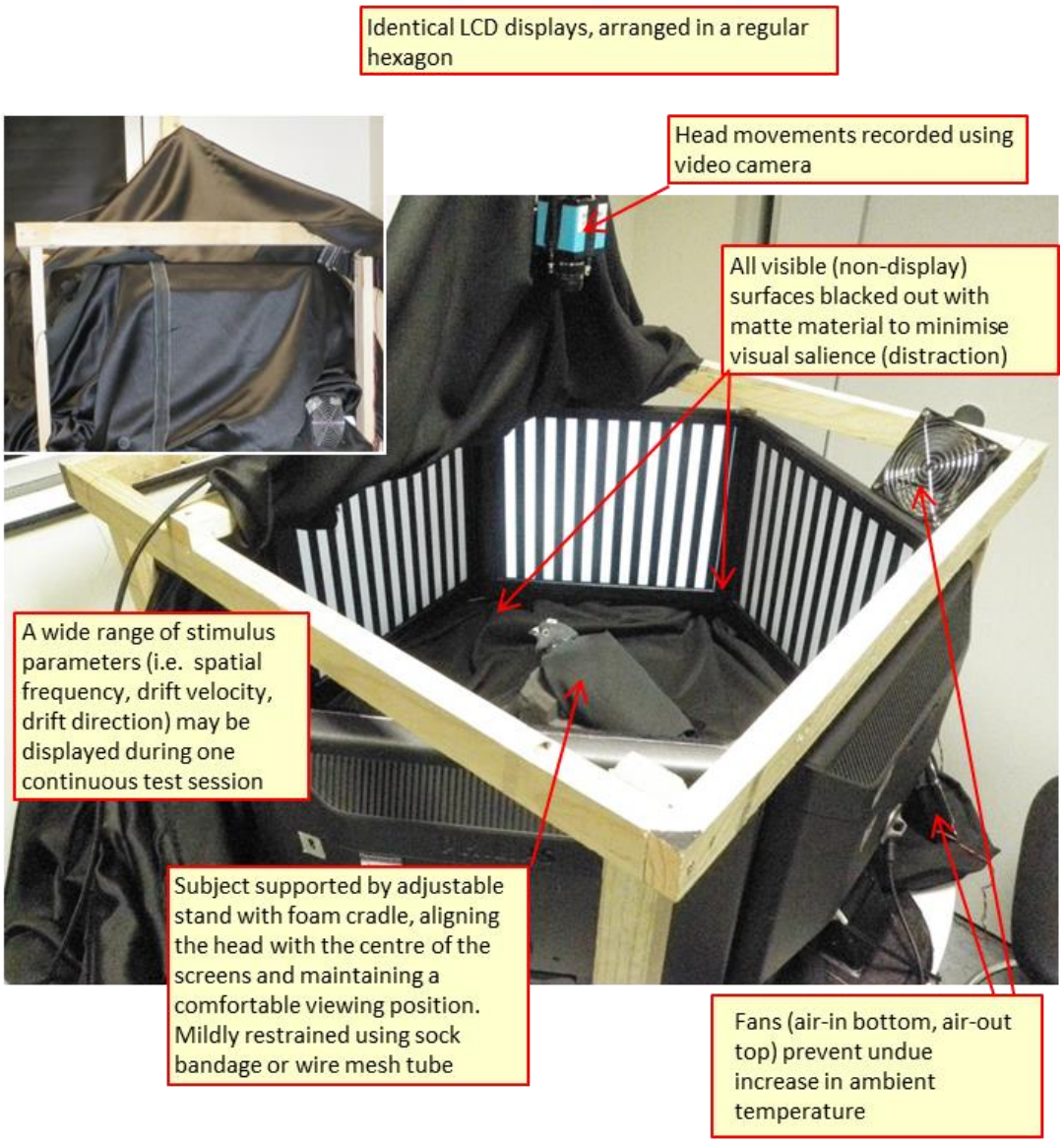

Figure 2.19 Annotated photograph of OCR arena. Inset - the arena when covered by the blackout curtain.

Care was taken to ensure that all screens were functioning properly and had the same on-device settings in all cases. The signal from the PC was split using an 8-way VGA (15 pin) signal splitter. One of the extra ports from the splitter was used to send the signal being displayed on the screens in the arena, to an identical outside test screen. This is helpful for setting up code for experiments/testing stimuli etc., and can simply be turned off as desired during experimental trials, although blackout curtain renders this unnecessary in most cases.

Stimulus presentation

Visual stimuli were created and displayed using MATLAB and the Psychophysics Toolbox extensions (Brainard 1997; Pelli 1997; Kleiner *et al.* 2007). Coding of specific stimuli was done by the author by modifying various parameters of script written originally by Prof. Chris Harris (University of Plymouth) and Dr Matt Dunn (Cardiff University). All stimuli were presented as a square wave grating, with various adjustable parameters available for spatial frequency, luminance contrast, and drift velocity (Figure 2.20).

Figure 2.20 View of grating from within arena.

Camera

Recordings were made using an ImagingSource DMK 21BF04 digital camera connected to the PC via FireWire. This camera can record video at VGA resolution 640x480, at sample rates of 60, 30, 15, 7.5, or 3.75 frames per second (fps).

Recording –LabVIEW

The bespoke video analysis software discussed earlier in this chapter was used to extract head movement data from the videos recorded. This image analysis program is also described in detail in Kutrowski *et al*. (2014).

The soft, removable adhesive foam strip discussed earlier was used as the marker for the video analysis software to identify head orientation in video frames. The size and weight of the strip are such that it has no noticeable effect on the behaviour of the subject, and allows a non-invasive measurement of head angle, whereas previous methods of recording OCR head movements may require the attachment of a mechanical measuring device (which could potentially cause some discomfort or retardation of normal behaviour), magnetic search coils which are inappropriate for subsequent magnetoreception experiments, or complex combinations of individual sensors such as photocells (Fite 1968; Gioanni and Vidal 2012). Homing pigeons, as well as several other bird species, have been shown to be sensitive to ambient magnetic fields, but the location, sensitivity limits, and function of the magnetoreceptive structure remain unknown (Mouritsen and Hore 2012). For this reason, our method is preferable to magnetic search coil techniques, as it reduces the risk of inadvertently adding a potentially distracting stimulus to experiments.

Providing the pigeons are used to being handled, and have experienced the sock restraint method on one or more occasions, (so that they are not unduly agitated by the experience of being put into the arena), they will sit in position quite peacefully and are immediately responsive to the stimulus with no specific conditioning or training required.

LCD technology and polarised light

One of the possible future experiments for the newly developed coil system, would involve the use of visual stimuli, in conjunction with manipulations of the ambient magnetic field. This combined with the fact that polarisation e-vector acuity may be of significance in magnetoreseption and in navigation generally means that an easy way of generating visual stimuli, which ideally can also present stimuli comprising of particular e-vectors of polarised light, was required. At this point, the use of LCD screens for delivering such visual stimuli was considered. LCD screens allow a wide range of visual stimuli to be displayed and have the added advantage of working on a principle of polarised light e-vector manipulation.

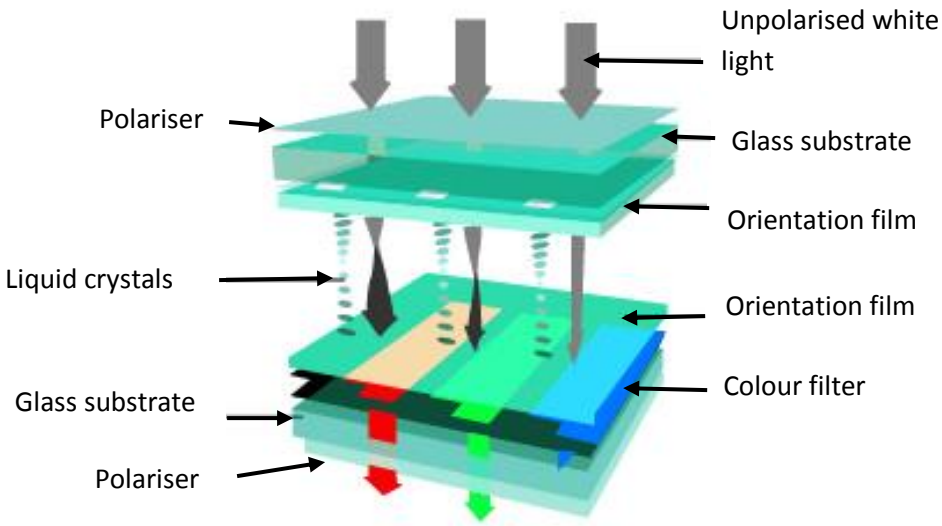

Figure 2.21 Cross section of LCD screen.

In Figure 2.21 it can be seen that several layers make up the screen in a LCD. Initially diffuse, white light is polarised as it enters the rear of the screen. This polarised light passes through an orientation film meaning that e-vectors are lined up appropriately for manipulation by liquid crystals. Depending on the voltage applied to a liquid crystal, it adopts a rotational position relative to the e-vector of incident light, transmitting more light, the closer the match in rotational e-vector. This process is linked with a colour filter, such that light of

various colours, is orentated to different e-vectors. At this point light e-vectors have been twisted to various extents depending on colour desired in each pixel, meaning that if viewed (by a human) together, the screen still appears entirely white. Only the final polarising filter on the outermost face of the screen causes this light to be separated into those parts which complement the e-vector orientation of the screen. This results in the light of each pixel being emitted from the screen and reaching the eye, being the precise RGB scale colour desired.

If only two settings are used for all pixels and component colours (i.e. 0 and 155 in most LCD technology), then pixels will appear either white or black. Which, being dependant directly on e-vector orientation in relation to the outermost filter. Black pixels have all three colours filtered out completely, and white pixels have all three colours transmitted maximally.

If one were to draw black and white bars on the screen in the form of a square wave grating, then the reason the black bars appear black is that the light leaving the liquid crystals is orientated perpendicular to the final filter, whereas the white bars are comprised of light leaving the liquid crystals which is parallel to the final filter. This means that we have a grating of orthoganally orientated e-vecors, such as in Figure 2.22 below.

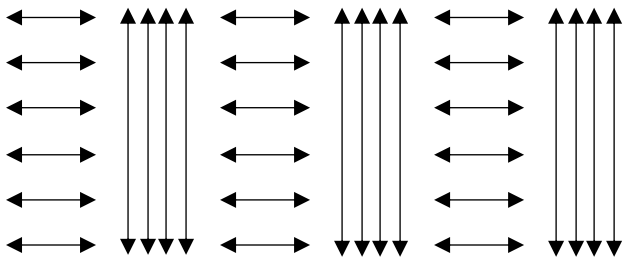

Figure 2.22 The orthogonal arrangement of e-vectors in light arriving at the final filter of an LCD display, when black and white bars are being drawn (in some screens, the e-vectors may all be rotated 45°, however the orthogonal relationship is the same).

With the knowledge that highly contrasting patterns of e-vectors can be produced in this way, it only remains to remove the final filter on the outside of the LCD display, and what is left is a display which looks enirely white (i.e. blank) to humans, and yet to an animal which

can discriminate between different e-vectors has contrasting vertical bars. The level of contrast (i.e. the difference in e-vector) can be adjusted simply by changing the grey-scale values of the bars. This allows the potential for assessing e-vector acuity (the finest difference in e-vector a subject can resolve), although it should be noted, that the rotation of the e-vector does not occur linealy with change in greyscale, due to the sine-wave function of light passing through the filters (i.e. not an all-or-nothing filtration). This technique has been used with some success in cephalapods by Temple *et al*. (2012). For the current research, a simple qualitative assay as to whether pigeons are able to descriminate e-vecter 'at all' is sufficient, meaning that no such calculations are necessary, and fully black and white bars can be used.

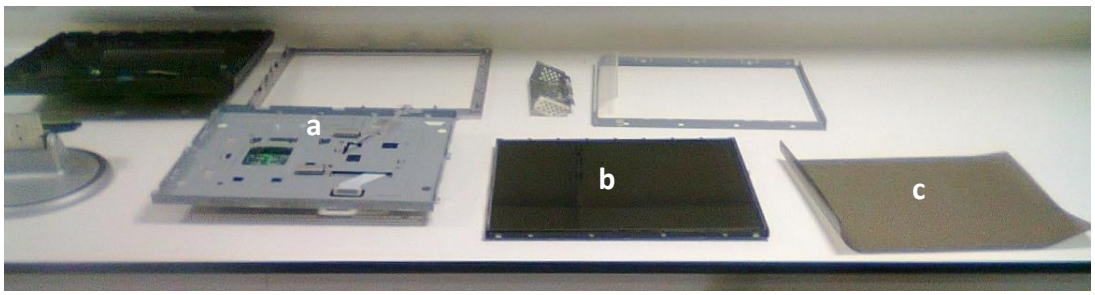

Figure 2.23 Removal of the outer polarising filter from LCD screens requires some dismantling (dependent on screen manufacturer and model); a is the main power and control part of the device, b contains the light source, and materials described in the cross section of an LCD (Figure 2.21), minus the outer polarising filter (c) which lies to the right of the picture. All other items are housing /support.

The process of removing the polarising filter is technically trivial (Figure 2.23), however removing it in such a way as to avoid damaging other screen components requires a good deal of care, and the removal of any residual glue is time consuming. Removing the filter in one piece (useful so that it can be used as a handy removable filter to test screens/stimuli) is also labour intensive, as it is prone to tearing and buckling.

All e-vector only experiments were conducted in the same way as those described in the previous section, but with a separately produced arena of screens with outer polarising filters having been removed as described above.

Prepulse inhibition

As discussed in the Introduction, prepulse inhibition provides a highly controlled and reproducible way to investigate sensory psychophysics. The use of the prepulse inhibition paradigm, to investigate magnetoreception (by using a magnetic field manipulation as a prepulse) is, to the best of the author's knowledge, completely novel. Whilst the adoption of this technique provides a very exciting opportunity to investigate magnetoreception in a more controlled and reproducible (hence arguably more rigorous) way, it also presents some technical challenges. Due to the novelty of the approach, and the scarcity of previous prepulse inhibition experiments involving pigeons, the startle-response measuring equipment, and the methods of delivering prepulse, and startle stimuli had to be developed, mostly from scratch. The system was based around a standard SanDiego Instruments (SDI) (SanDiego, USA) SR-Lab startle response system. Much of the SR-Lab was standard. The software used to operate the SR-Lab, the control box which generates and amplifies signals, the response box which receives incoming data and the data acquisition card (DAQ) (National Instruments) were mostly unmodified. The startle chamber itself however was entirely bespoke, having been built to required specifications to a design primarily conceived by the author and Prof. Jonathan Erichsen (Figure 2.24).

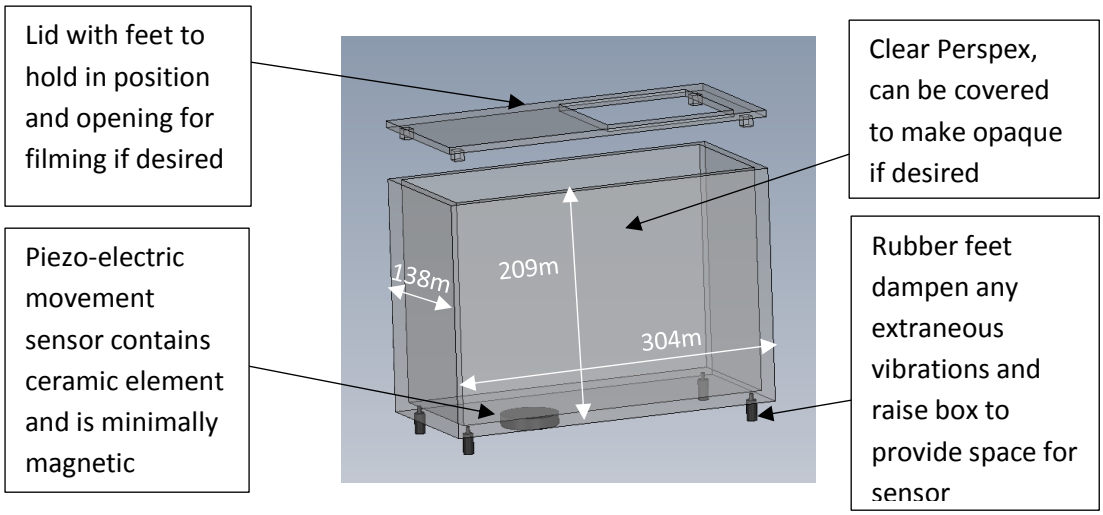

Figure 2.24 Design drawing of the bespoke startle chamber, designed specifically for use in magnetoreception experiments with pigeons.

For the experiments to be carried out as desired, two main differences were necessary from the standard startle chamber. Firstly, the chamber had to be specifically sized such that a pigeon could stand comfortably with a little space facilitating comfort movements and looking around, but restricted enough so that the pigeon could not turn around. For this reason the box was designed to be of identical dimensions (304x138x209mm) to the already existing Perspex box, utilised in the Helmholtz coils, and originally built by Migalski (2010). Secondly the box and all of its connections had to be as non-magnetic as possible. To this end the Perspex sides of the box were bonded together, but not secured with any metal screws or fixings, the feet of the box (which support it slightly off the surface on which it rests meaning the sensor beneath is not under undue mechanical pressure) were made of rubber, and also bonded to the surface of the box. The sensor itself was a piezo-electric disc, highly sensitive in detecting small movements, firmly affixed to the underside of the box, precisely where the pigeon is expected to have its feet (and hence its weight supported) when standing normally, and protected by a large rubber disc surrounding all external surfaces. The cable carrying the startle response signal back to the response box, and ultimately the PC for recording, had a negligible effect on the generated magnetic field, as assessed by Noor Aldoumani and reported in a thesis currently in production.

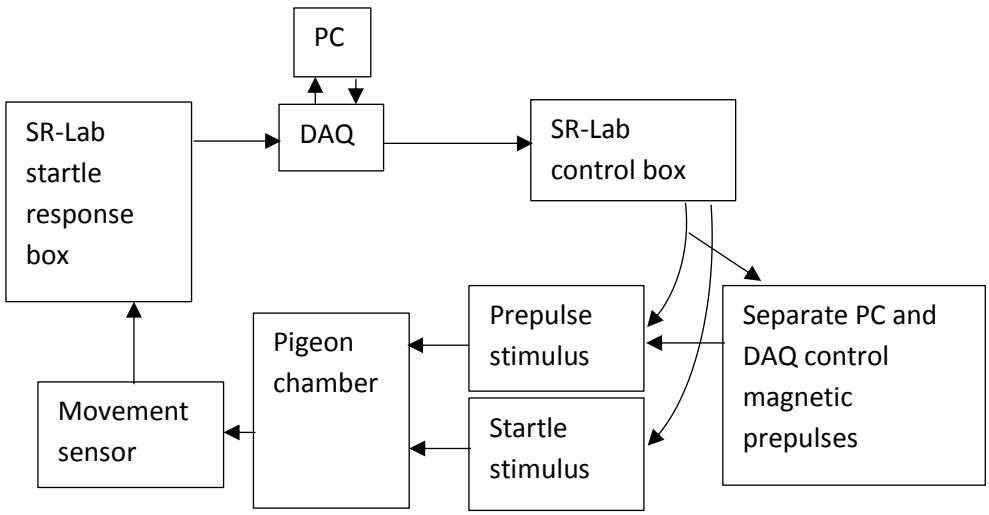

Figure 2.25 Flow diagram of prepulse inhibition apparatus (DAQ = data acquisition card).

Problems with sound stimuli

Initially the hope was that a sound-based stimulus consisting of a square wave click of white noise could be employed for both the prepulse and startle stimuli. One of the few studies in the literature to have explored startle response in pigeons (Stitt *et al.* 1976) discovered that use of a bright flash of light as a startle stimulus was far more effective in eliciting a measurable startle response from pigeons than an acoustic startle stimulus (Figure 2.26), as is normally used in rodents, for example (Koch 1999). The hope was to carry out a similar comparison in the initial stages of the investigation using the new equipment. The intention of carrying out magnetoreception experiments during the night (due to reduced background noise in the ambient magnetic field) was further supported by the finding of Siqueira *et al.* (2005) that startle response is greater, and more consistent in pigeons during the subjective night time.

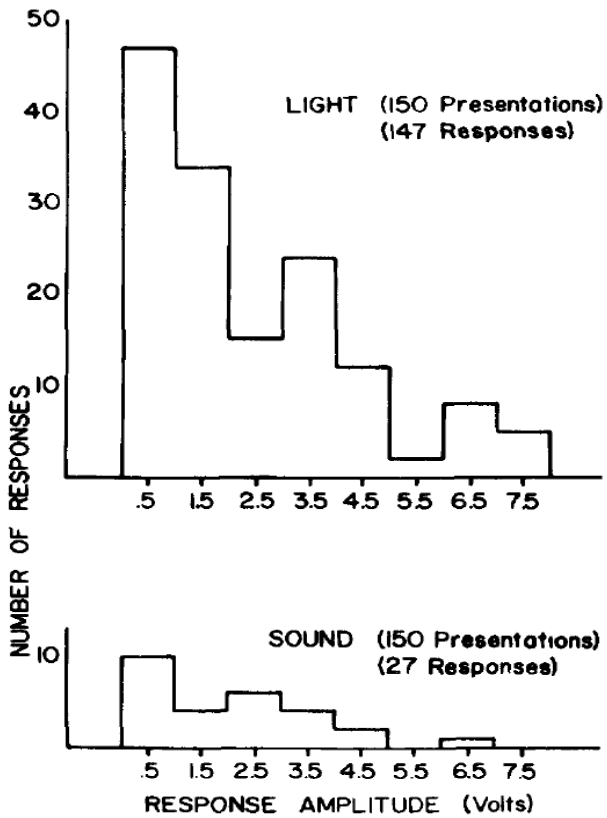

Figure 2.26 Light is far more effective a startle stimulus than sound (Stitt *et al.* 1976).

Upon testing the sound emitted by the startle stimulus set up, it quickly became apparent that the equipment was unable to achieve a loud enough volume to be used for startle (minimum 100dB, but ideally 120dB) during the short time required in order to properly time the inter-stimulus-interval for experiments (Figure 2.27). After much liaison with SDI, it was decided that the control box (containing the small amplifier responsible for sound volume) should be returned to the manufacturer for diagnostics and repair. After several months of not being able to use the equipment, it was declared repaired and returned to Cardiff for use. Upon testing in Cardiff, the issue appeared to persist. It was decided at this point that a light based startle stimulus would be employed from the outset, as the equipment limitations and the literature both suggested this would be the most effective approach.

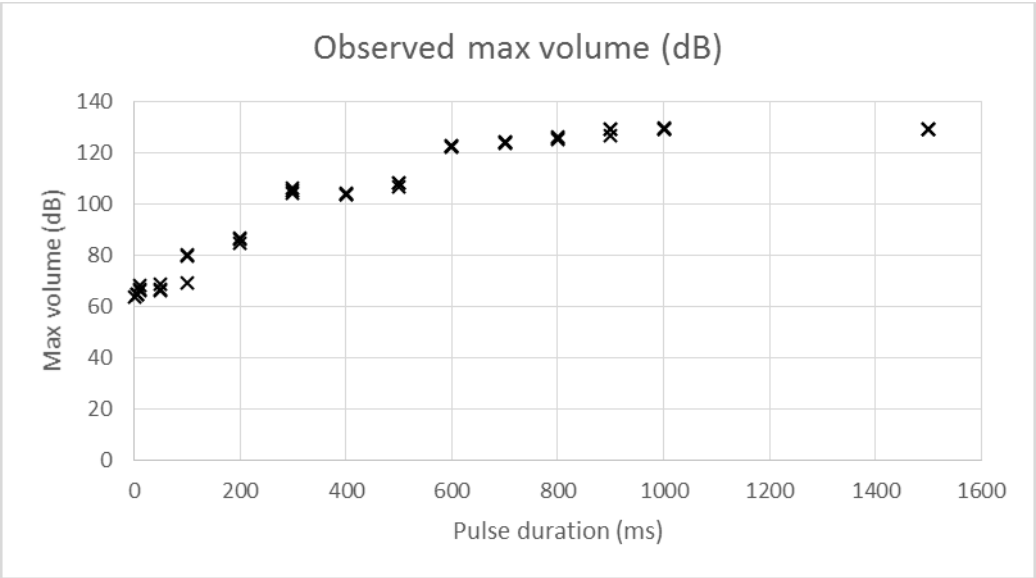

Figure 2.27 Observed stimulus volume. Ideally, for use as a startle stimulus, a volume of 120dB should be achieved in under 10ms. In the above recording, it is clear that this volume is not achieved until approximately 600ms after onset.

Testing the use of sound as a prepulse stimulus (as it does not need to achieve volumes as great as for a startle stimulus, only to be perceived by the subject at all), revealed that the issue with sound amplification went beyond maximal volume. It was also clear that the volume level, even when relatively low, was inconsistent and would waver over time, and eventually build to a high level (completely of its own volition) before dying off into silence.

At this point in the research project, there was not enough time to send the equipment back to the USA for further work, and so technicians in the School of Engineering (Cardiff University) carried out in-house diagnostics and elected to replace the amplifier. After some weeks of delays with parts, etc., the equipment was returned to the lab, only for the same issue to arise within minutes of use. At this point, it was clear that light would be the only option for delivering both prepulse, and startle stimuli.

Using LEDs as a prepulse

It was decided that a strip of LEDs would be used as a control prepulse. Magnetic manipulation would be the experimental prepulse, however a control was needed to verify the parameters of the experiment were suitable and that equipment was functioning as expected. The LEDs were in a single row, at the pigeon's approximate eye level, which extended across the entire front face of the pigeon box and for approximately 10cm along each side, meaning that it was clearly visible to the pigeon even when not looking directly forwards. For experiments conducted in the light, a layer of adhesive tape was placed over the light sources, to reduce the brightness slightly, such that it was clearly visible, but had little chance of causing a startle response in its own right. For experiments conducted in darkness, this was increased to three layers, to account for the greater relative change in luminance. It is imperative to avoid the prepulse causing a startle response, as the timing and recording of the desired startle response is adversely affected. Both LED strips were still clearly visible in their respective lighting conditions, as is evidenced by the successful elicitation of meaningful prepulse inhibition described in Chapter 6. The assistance of Dr Kutrowski in making and attaching a switch to the LEDs such that the 5v TTL (transistor-transistor logic) signal generated by the SR-Lab could be used to turn them on and off at exactly the correct times is greatly appreciated.

Camera flash as startle stimulus

It was decided that a flash of light would be used for the startle stimulus. A Metz Mecablitz 76 MZ-5 digital camera flash was used, which could be activated by means of a 5v TTL signal generated by the SR-Lab equipment. The unit is a 'hammer head' style large photographic flash unit which produces a very bright flash of light. It was not possible to obtain use of a sensor that could measure the brightness of the flash output, as the flash only lasts 6.$\dot{6}$ ms, but as a guide to flash power, the unit was rated with a G/N value of 76.

Effect of flash on sensor

It was noticed very quickly upon initial testing of the setup that the flash discharging had a noticeable effect on the piezo-electric movement sensor attached to the pigeon chamber. This was somewhat surprising as the sensor is meant to respond purely to mechanical signals. Various possibilities have been considered for why this might be. The unit does create a small 'pop' sound upon discharging, it could be that the sound wave itself activates the sensor, particularly if the sound contains low frequencies. Perhaps the discharging of the flash produces enough heat to create a small plume of expanding air emanating from it, or the electromagnetic flux involved could potentially interact with the connecting wire between the sensor and the rest of the SR-Lab equipment, creating an artefact of some sort. The flash was mounted on a tripod, separate from the sensor save for both being in indirect contact with the floor of the lab. The effect of the flash on the sensor was only evident if the sensor was in the path of the light emitted, no effect was noticeable if the sensor was to the side or rear of the device, and the effect was linked with distance. A series of trials were recorded using the SR-Lab to generate flashes, and record the response expected via the sensor, but with no bird present in the experimental box, meaning that any 'response' observed was caused by the flash. This was repeated at a variety of distances.

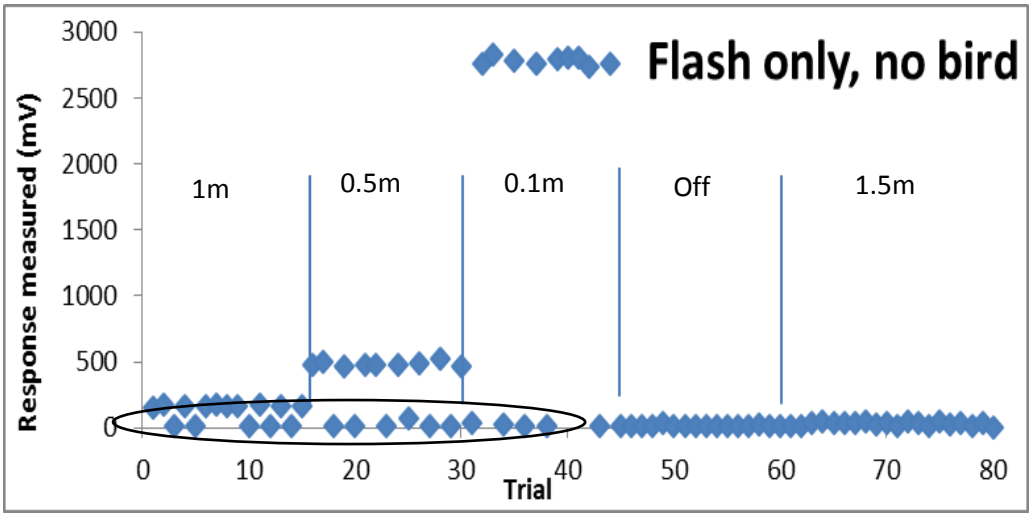

Figure 2.28 The effect of the flash unit discharging on the piezoelectric movement sensor. The flash fails to discharge in some instances (circled). Trials are in the order that different distances were sampled.

It is clear in Figure 2.28 that there is an effect that varies with distance. Evidently the next step of preparation should be to find a distance at which the flash is sufficient to startle the subject, but not to activate the sensor such that the genuine response of the subject becomes swamped and unrecognisable. Examining the data in Figure 2.28 also identified an issue with the flash not discharging on numerous occasions. This was solved by replacing a defective power source, and ensuring a minimum of 25s between discharges, to allow the flash to prime properly each time.

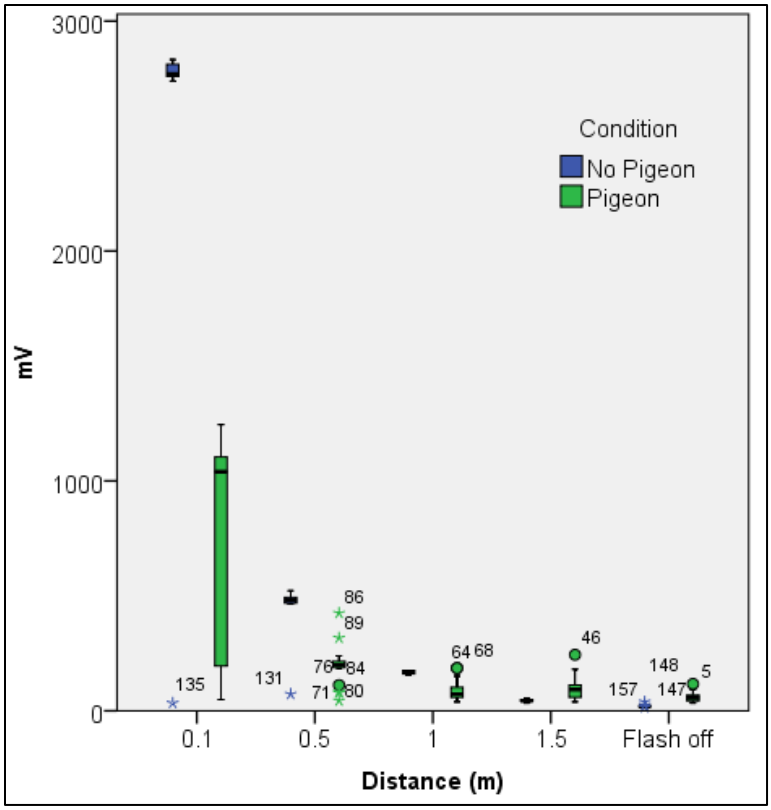

Figure 2.29 Effect on the piezo electric sensor (y-axis, in mV), with the flash at varying distances, compared between two scenarios, one with a subject present, and one without.

Figure 2.29 indicates that in the flash off condition, and flash at 1.5m, the response of the bird is visibly greater than the signal from the flash. At all other distances, the flash signal alone (left side of each pairing) is higher. To make this clearer, the following figure plots only the 1.5m and flash off data.

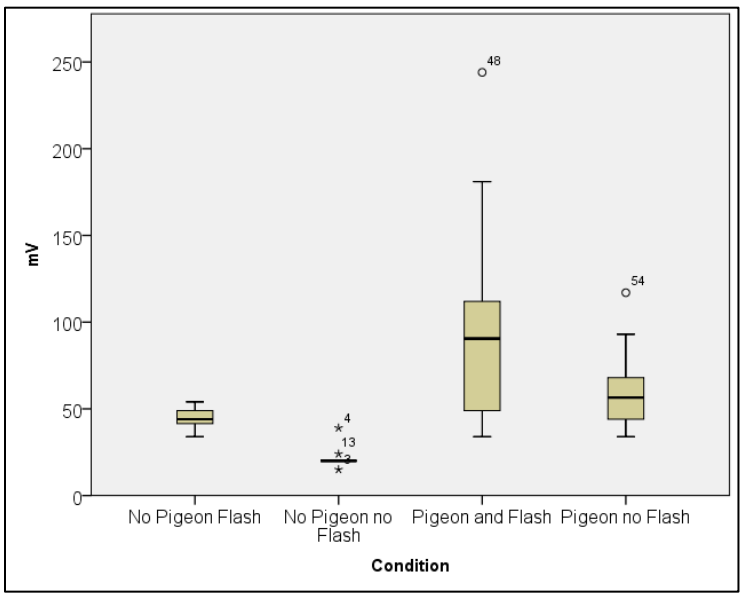

Figure 2.30 Response level with flash unit 1.5m from the subject/sensor (y-axis, in mV). The response with the bird is greater than without, suggesting startle could be meaningfully isolated.

The fact that most of the responses of the bird to the flash, clearly outweigh the flash signal alone, suggests that presenting the flash at 1.5m can give the opportunity to establish a clear startle response ready for PPI experiments.

Due to laboratory space constraints, and the fact that the coils were aligned North/South to assist aspects of potentially desirable magnetic field generation, the only appropriate way to ensure that the flash unit was 1.5m from the subject and sensor was to place the flash unit within the coil system, beneath the table holding the enclosure box and subject. The light from the flash was reflected in a carefully angled mirror so as to direct as much of the light as possible directly into the front of the subject enclosure. Obviously, this positioning has the potential to interfere with the magnetic field generated in the experimental arena, and so two layers of mumetal shielding were employed to cover all areas of the unit except the lens, as well as the small wire which carries the 5vTTL signal from the SR-Lab equipment. Testing with the three axis magnetometer showed no noticeable effect on the field at the position of the subject whilst the flash was re-charging/priming to flash. Some interference was observable when the camera actually discharged, but this was not considered a serious concern, as the crucial time for magnetic field control is during the presentation of the prepulse, before the flash discharges. At this point, it was possible to begin collection of experimental startle data, and investigate the possibility of eliciting a prepulse inhibition in subjects.

Chapter 3 Use of dynamically shielded Helmholtz coils system to investigate behavioural responses to magnetic stimuli

The newly designed Helmholtz coil system was tested in various ways. Initially, during the construction and development phase, there were many tests and assessments of the uniformity and predictability of the field generated, in terms of insuring that the signals sent from the control PC, resulted in the expected manipulation of the magnetic field. This included checking both the 3 dimensional integrity of the field in terms of intensity, as well as the timing of desired changes to the field (more detailed explanation and figures in Chapter 2). It was then necessary to carry out actual experiments using live subjects to assess whether the manipulations of the field would affect their behaviour. Initially, an assessment of the re-analysed data of Migalski (2010) was carried out, followed by a brief experiment using the new coils, and then a subsequent, much more substantial experiment to look for responses.

Re-analysis of Migalski (2010) data

Having identified the shortcoming necessitating the improvements to the video analysis software discussed in Chapter 2, the next obvious step was to re-examine the data presented in Migalski (2010), to ensure that the reported results were reliable and a sound basis for a comparison using newly collected data relying on the new 'active shielding' paradigm. This was also important in confirming the results of Migalski (2010), as, in that study, position was averaged over every 5 frames for ease of subsequent manipulation and analysis, meaning that some of the movement dynamics may have been lost.

Data were reproduced and certain crucial figures from Migalski (2010) were selected for comparison. It should be noted that, although the method of extracting the raw head angle

in each frame of video is expected to be consistent, there may be some slight differences arising from the subsequent procedure of identifying 'head movements' (continuous movements in a given direction, regardless of velocity) in Migalski (2010), as compared to 'saccades' using the newly developed saccade detection software. However, it seems likely that the sudden, large and continuous movements counted by Migalski (2010) were in fact saccadic movements, and so a clear similarity in observed responses should be apparent. With this in mind, a direct comparison was made between the Migalski (2010) figures, and those produced having re-analysed the videos for validation/confirmation of results.

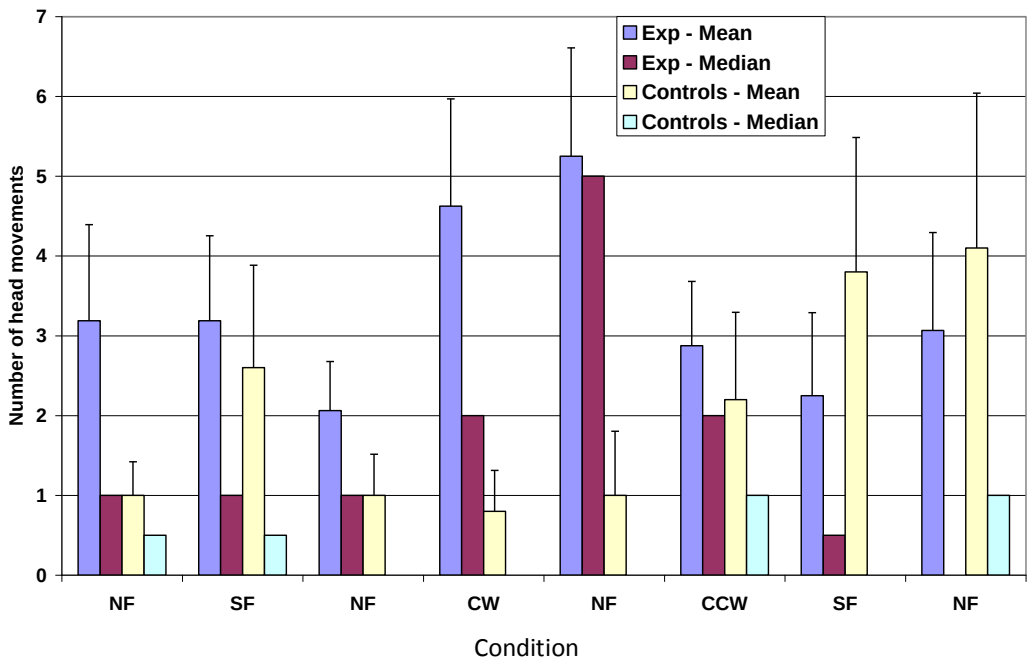

Figure 3.1 Number of head movements in each step in the sequence of experimental and control conditions (from Migalski (2010) Figure 4.2). Error bars represent +/- 1 standard error. (NF- null field, SF – static field, CW – field sweeping clockwise, CCW – field sweeping counter-clockwise).

Figure 3.1 shows the mean and median number of head movements for each condition in the sequence of conditions used, under both control (amp off) and experimental conditions. During the first six stages of the sequence, there are more responses under experimental conditions than there are under control conditions. This gives the distinct impression that the magnetic manipulations are having an effect on head movement frequency.

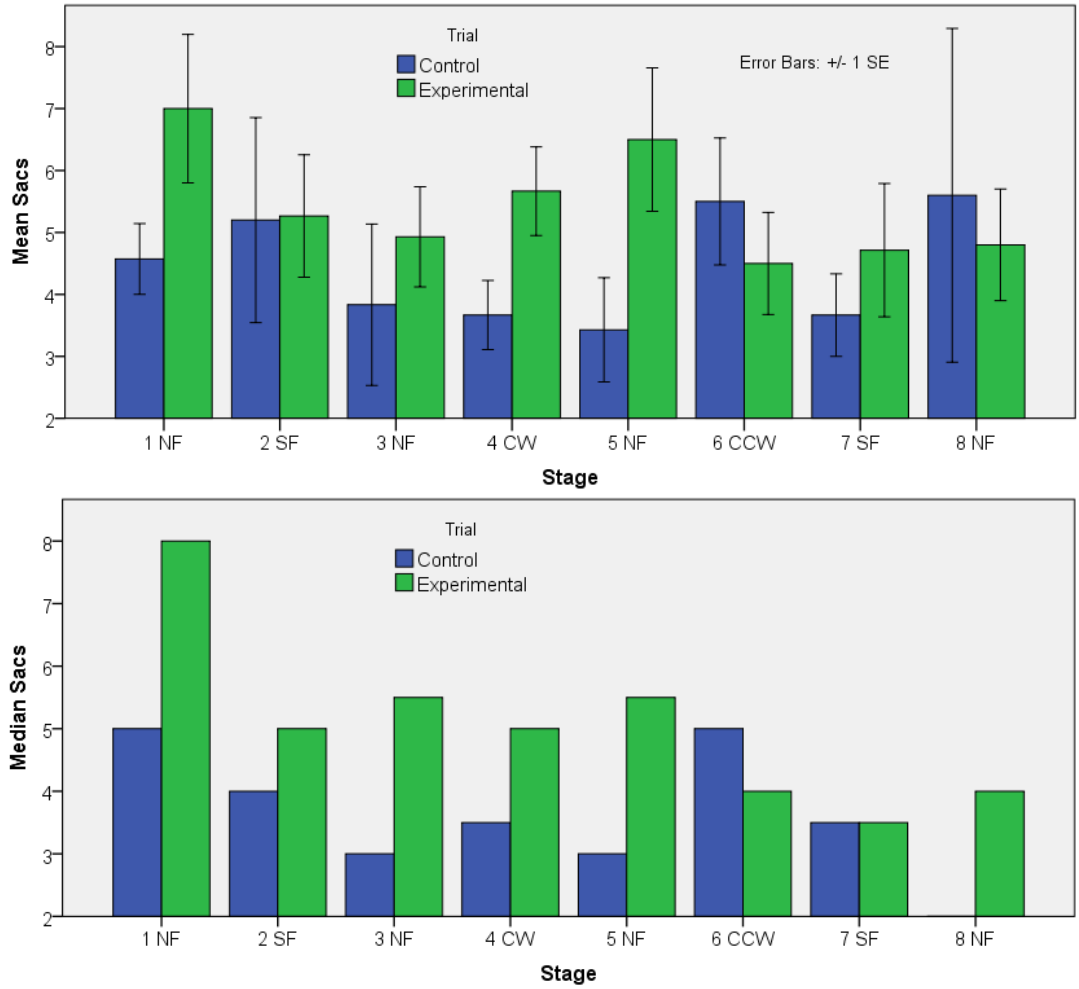

Figure 3.2 Re-analysed version of Migalski data, showing comparison of mean number of saccades (top) and median number of saccades (bottom) between control and experimental trials.

Figure 3.2 shows the re-analysed version of the Migalski data, having been obtained using both the updated video analysis software, and the new saccade detection software. Some differences are evident (likely due to the slightly different methods of identifying relevant head movements) between the original and subsequently re-analysed data (Figures 3.1 and 3.2). However, the overall trend is the same, in that the majority of sequence stages have a greater number of head movement responses under manipulated (labelled experimental) conditions then they do under control conditions.

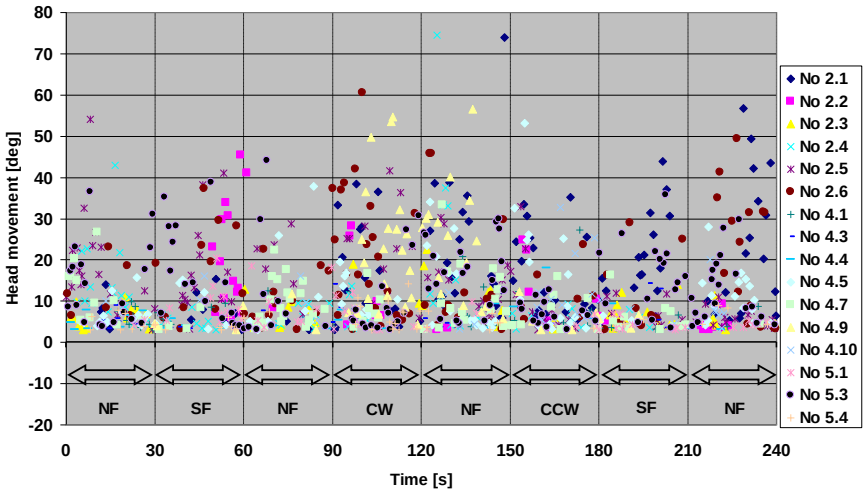

Figure 3.3 Distribution of all head movements of all 16 experimental birds (From Migalski (2010) Figure 4.3). Each point denotes a head movement with its amplitude and the time when it occurred within the experimental sequence (NF- null field, SF – static field, CW – field sweeping clockwise, CCW – field sweeping counter-clockwise).

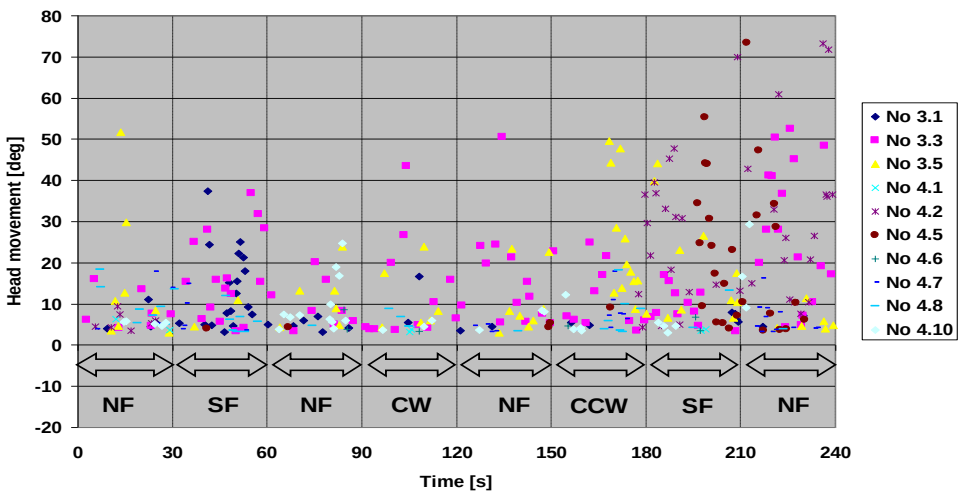

Figure 3.4 Distribution of all head movements of all 10 control birds(From Migalski (2010) Figure 4.4). Each point denotes a head movement with its amplitude and time when it occurred within the experimental sequence (NF- null field, SF – static field, CW – field sweeping clockwise, CCW – field sweeping counter-clockwise).

Comparison of all head movement amplitudes between manipulated (Figure 3.3) and unmanipulated (Figure 3.4) from Migalski (2010) Figures 4.3 and 4.4, respectively, shows a far greater number of responses under the manipulated field condition. This difference is particularly evident when considering only movements of greater than 10° amplitude. It appears that, in several subjects, the Clockwise rotation of the field results in a large increase

in responses compared with the stage before (null field). This marked increase is not evident in Figure 3.4 representing the control sessions.

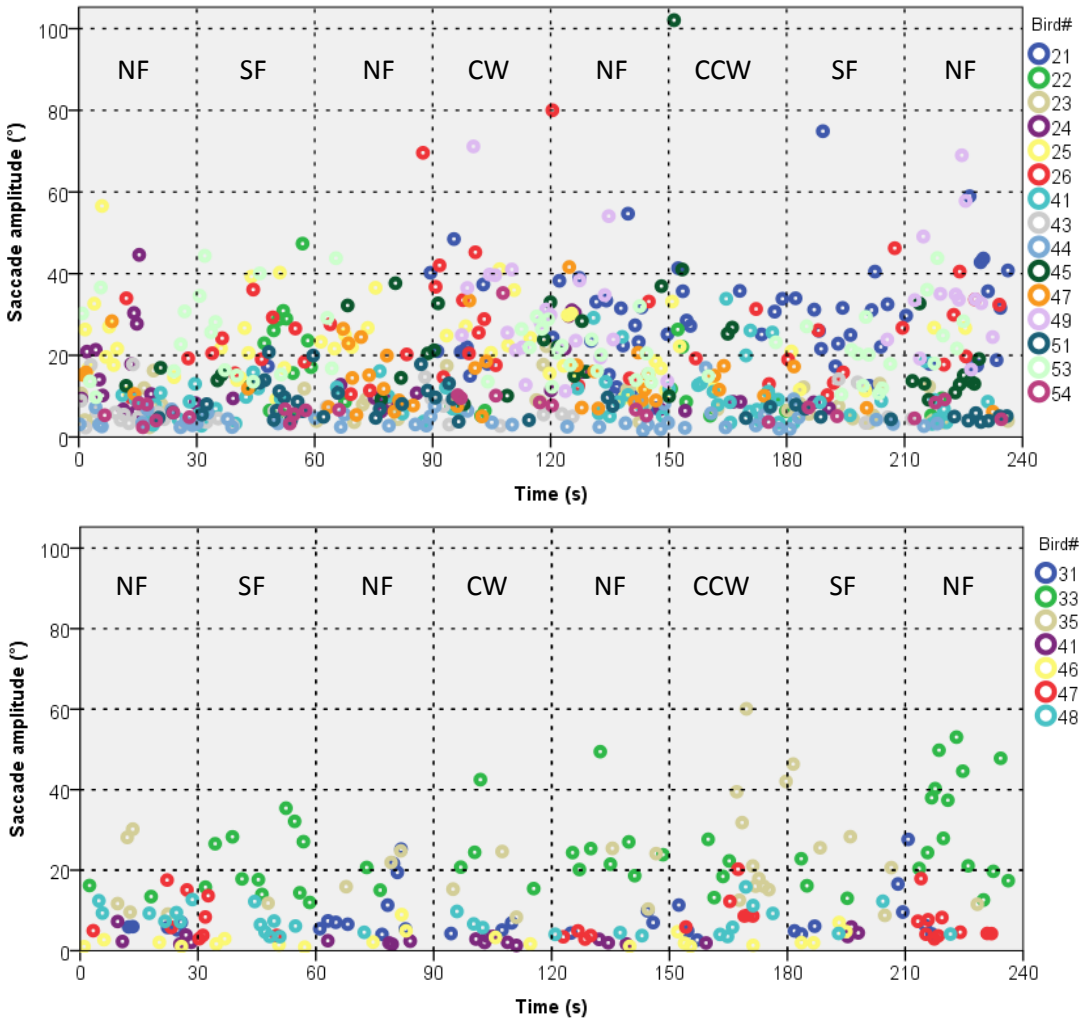

Figure 3.5 Comparison of re-analysed Migalski data showing amplitude of each saccade under experimentally manipulated (top) and control (bottom) conditions.

Comparison of all head movement amplitudes in Figure 3.5 between manipulated (top) and unmanipulated (bottom) trials from Migalski (2010) Figures 4.3 and 4.4, respectively, using the recently re-analysed data show similar trends to those in the previous two figures from the original Migalski (2010) analysis. A much higher frequency of head movements can be seen in the manipulated conditions as compared to the control sequence, and there is also a marked increase in movements from null field to clockwise rotation conditions.

Together these comparisons demonstrate that the process used by Migalski (2010) was apparently reliable in identifying head movements, and that the results reported are accurate. This reasserts the finding of Migalski (2010) that a manipulation of the ambient magnetic field under physically magnetic/electromagnetic shielded conditions will elicit a head movement based behavioural response in pigeons. Having confirmed the reliability of the result of Migalski (2010), it was then logical to carry out a further experiment using the new system to see if any such response could be found. If a response was found, this would show that dynamic shielding is adequate for experiments, whereas should no response be seen, this may be a result of the dynamic shielding being inadequate. If no response was observed, this would suggest that a physical shield blocking high frequency electromagnetic signals is necessary for magnetic manipulations to be salient enough to be recognised over any background 'noise 'in the magnetic environment.

Experiment 1: Identifying behavioural responses to magnetic field inclination flip

After initial setup and testing of the new Helmholtz coil system, an experiment was carried out to look for a clear way to identify head movement responses to magnetic field manipulation. In addition to comparing frequency and size of saccadic head movements, other kinds of head movement, previously identified as possible responses in preliminary set up testing with subjects, were also investigated.

These additional behaviours were defined as:

- Head tilt - Head tilted to the side more than 60°, such that one eye only has a clear view upwards
- Look up - Head tilted backward more than 60°, such that both eyes have a clear view upwards
- Head shake – High velocity oscillatory movement, mostly in the horizontal plane, reminiscent of shaking off water

- Open mouth – Mouth is opened unusually wide, as though yawning
- Eye blinks – Eyes can be seen to close completely.

Eight subjects were used (four male, four female), each being exposed to four separate experimental trials during a single day. Trials consisted of an initial control (amplifiers are switched off), two inclination flip trials (one with a flip every 30s, and one with a flip every 5s), and a final control, for 4 x120s trials. In the flipping trials, the amps were switched on immediately prior to introducing the subject into the experimental arena. The coils did not, however, have any influence over the magnetic field in the arena until the start of the flipping trials (i.e. the amps are on but no signal is sent to the coils until the desired flipping session begins). The flipping trials were ordered differently for different subjects, and all subjects had a break of a minimum 4 minutes between trials. The two sexes of each housed pair were run alternately although they were separated 15 minutes before the experiments began and did not spend any time in the same room until all trials for that pair were completed.

Pair 1			**Pair 2**			**Pair 3**			**Pair 4**		
Trial	Subject	Order	Trial	Subject	Order	Trial	Subject	Order	Trial	Subject	Order
1	33	1	1	38	9	1	32	17	1	35	25
1	34	2	1	37	10	1	31	18	1	36	26
5	33	3	30	38	11	5	32	19	30	35	27
30	34	4	5	37	12	30	31	20	5	36	28
30	33	5	5	38	13	30	32	21	5	35	29
5	34	6	30	37	14	5	31	22	30	36	30
2	33	7	2	38	15	2	32	23	2	35	31
2	34	8	2	37	16	2	31	24	2	36	32

Table 3.1 Order of trials and subjects used in each. 1 = 120s initial control. 30=120s with inclination flip every 30s. 5 = 120s with field flip every 5s, 2 =120s final control.

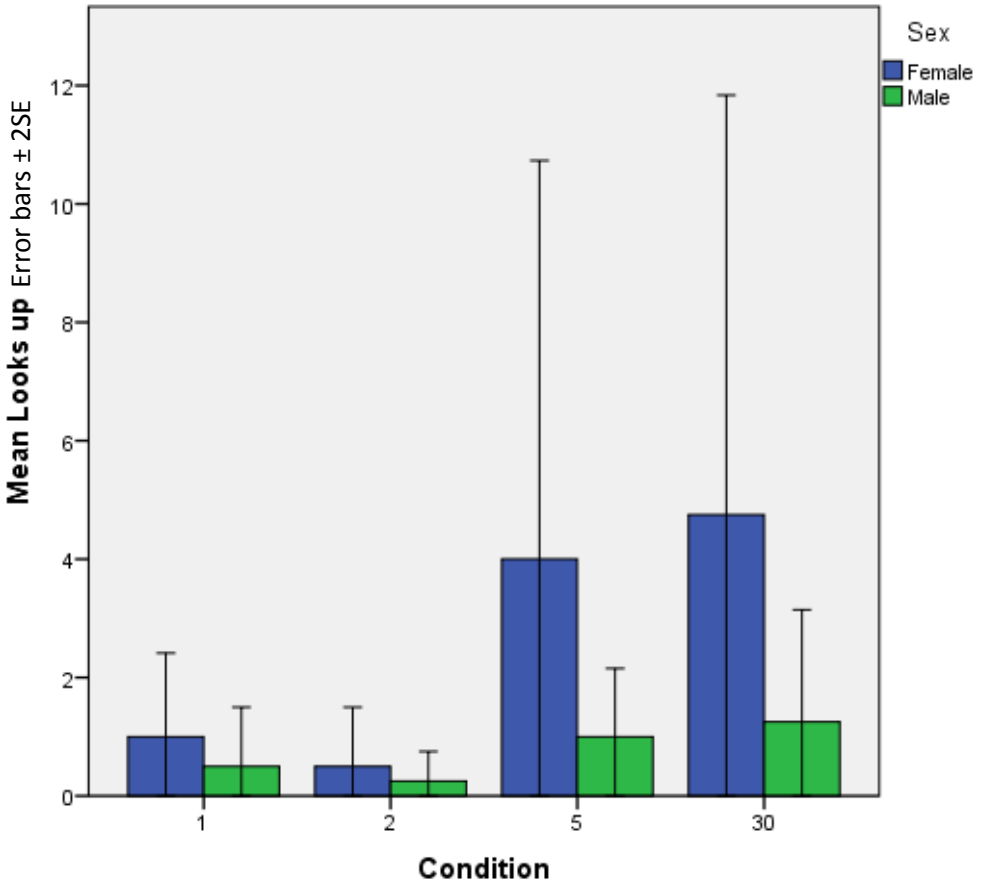

Figure 3.6 Mean number of times a subject looks up under the different experimental conditions (1 = initial control period, 2 = final control period, 5 = inclination flip every 5s, 30 = inclination flip every 30s.

Figure 3.6 presents the mean number of times that the subject looks up during a two minute trial. Conditions 1 and 2 represent the initial and final controls, respectively, 5 and 30 represent the 5s per flip and 30s per flip conditions, respectively. It is immediately clear that there are a greater number of responses during the manipulated field condition trials than during the control trials. Both manipulated field trials have a greater number of responses than both control trials. There additionally appears to be a difference on the basis of sex, with females showing a greater mean number of responses than males in all trials. However, a relatively small number of trials were carried out, and the observed patterns do not reach statistical significance (repeated measures ANOVA), although a disparity on the basis of sex

does appear to come somewhat close ($F_{1,8}$=3.853, P=0.151), and may warrant a future investigation with a greater sample size.

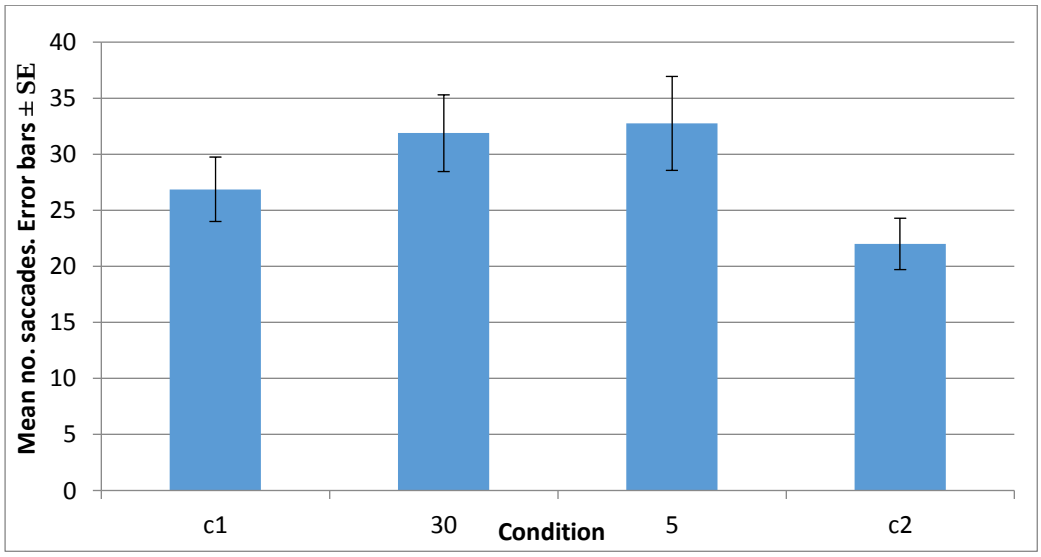

Figure 3.7 Mean number of saccades observed under each different experimental condition (1 = control 1, 2 = control 2, 5 = Inclination flip every 5s, 30 = inclination flip every 30s, Error bars ± SE).

A comparison of the mean frequency of saccadic responses of all subjects combined between the different conditions is shown in Figure 3.7. Error bars represent the standard error of the mean. There appears to be some difference between the number of responses under the 5 second per flip condition and the final control condition, however the final control condition appears to have clearly fewer responses than the initial control also. This might well indicate that the difference between conditions 5 and C2, and 30 and C2 are potentially due to low sampling rate as both approach significance under pairwise comparison (P=0.070, and P=0.115 respectively), whereas repeated measures ANOVA shows a significant difference on the basis of condition ($F_{3,24}$=3.201, P=0.041).

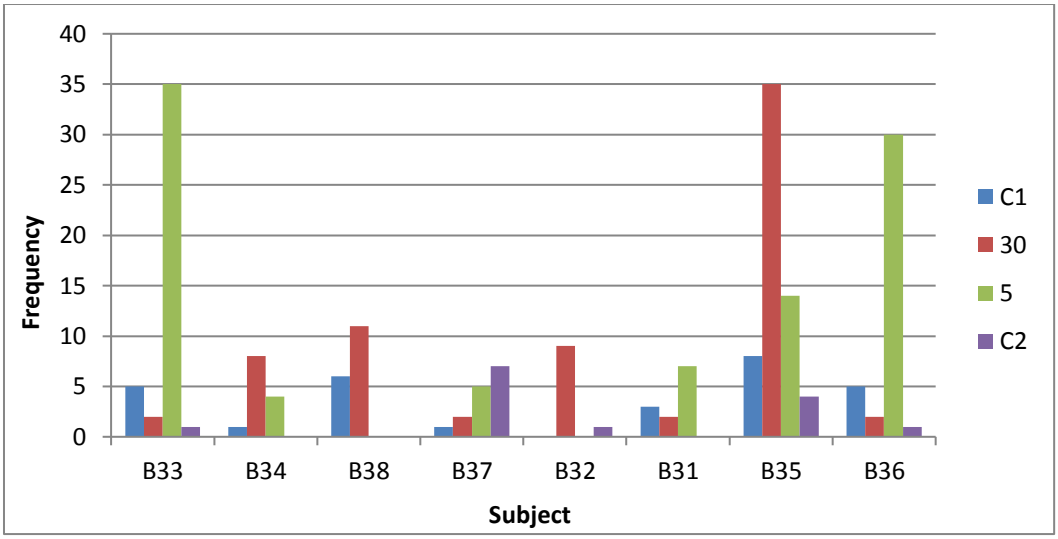

Figure 3.8 Frequency of saccades under each different experimental condition, separated by subject (1 = control 1, 2 = control 2, 5 = Inclination flip every 5s, 30 = inclination flip every 30s).

When considering the number of saccadic movements greater than 20° recorded for each subject, under each different experimental condition (Figure 3.8), it is immediately apparent that, in many cases, the number of saccades is greater under the manipulated field conditions than under control conditions. In all cases except one (subject 37), one or other of the manipulated field conditions shows a greater frequency of saccadic movements than in both control conditions. It is interesting to note that the manipulated field condition that shows the greatest response varies from subject to subject and that in only two out of eight subjects both manipulated conditions show a greater number of saccades than both control conditions.

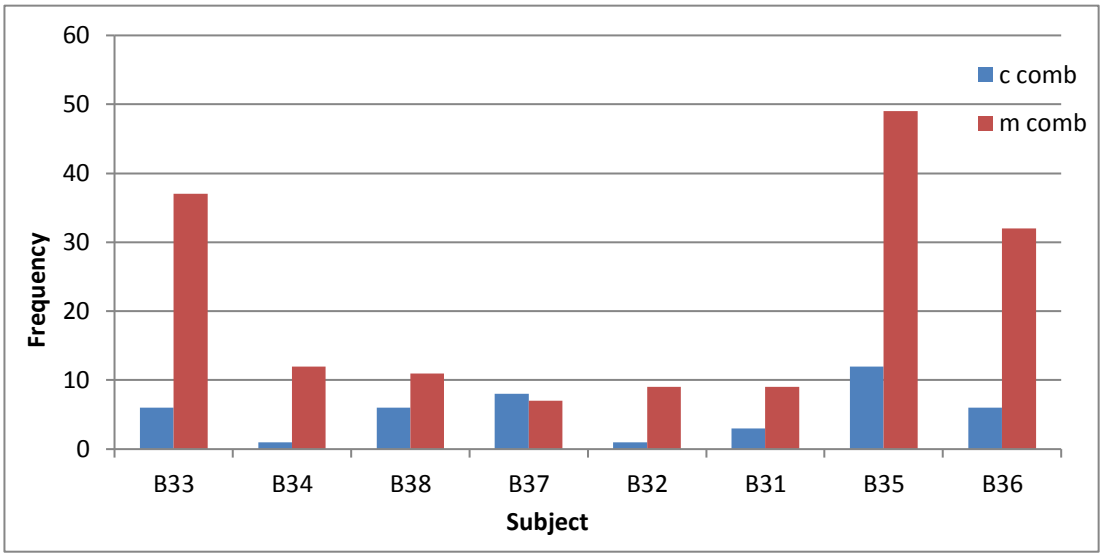

Figure 3.9 Frequency of saccades under combined manipulated condition vs pooled control condition, separated by subject (c comb = both control periods combined, m comb = both manipulated field types combined).

Combining the responses greater than 20° under both manipulated conditions, and comparing with the combined responses under control conditions (Figure 3.9) gives a clearer picture of the apparent overall effect. Only one subject of the eight birds (37) did not show an increase under manipulated conditions. The responses of subject 37 only differ by one saccade between the control and manipulated conditions, with 8 and 7 responses, respectively. All other subjects show a marked increase under manipulated conditions, with 6 of the 8 having more than three times the number of saccadic movements when the magnetic field is manipulated.

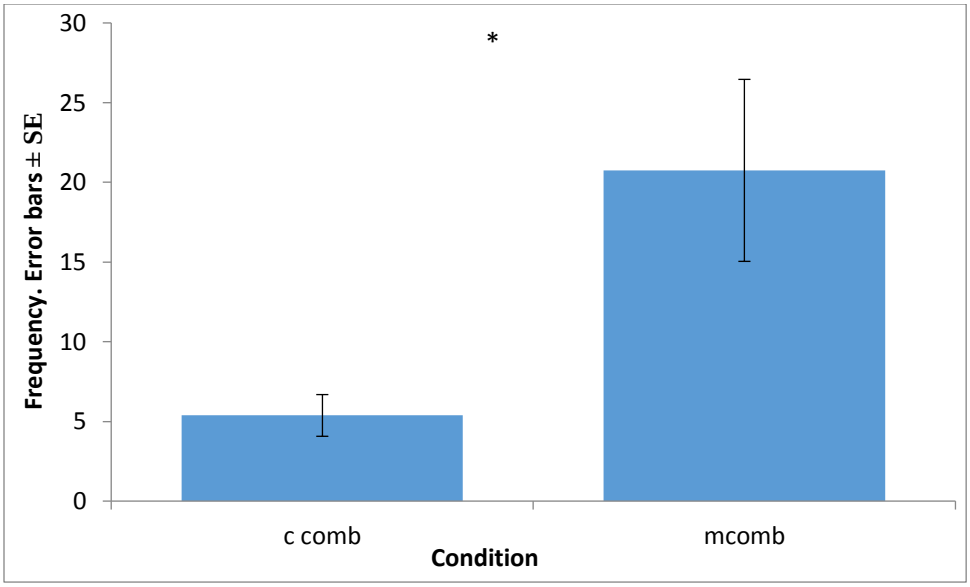

Figure 3.10 Mean number of saccades under manipulated and control field conditions for all subjects (c comb = both control periods combined, m comb = both manipulated field types combined). *=significant (ANOVA P<0.05), Error bars ± 1 SE.

A comparison of the means of all subjects' responses greater than 20° under control and manipulated field conditions (Figure 3.10) shows a significantly greater number of responses under the manipulated conditions than the combined controls. Interestingly, however, a paired samples t-test does not quite reach significance, although it does come very close (P=0.053). Unfortunately, the clearer difference observed in saccades over 20° between manipulated and control conditions is confounded by the trade-off effect of having very much fewer observations overall to compare. For the entire experiment, only 209 responses were recorded, and an average of only 26 responses for each subject over the entire experiment (approx. 6.5 responses per trial) was observed. For this reason, future experiments are expected to be longer in duration, as well as including more subjects and trials, in order to gather a more versatile data set.

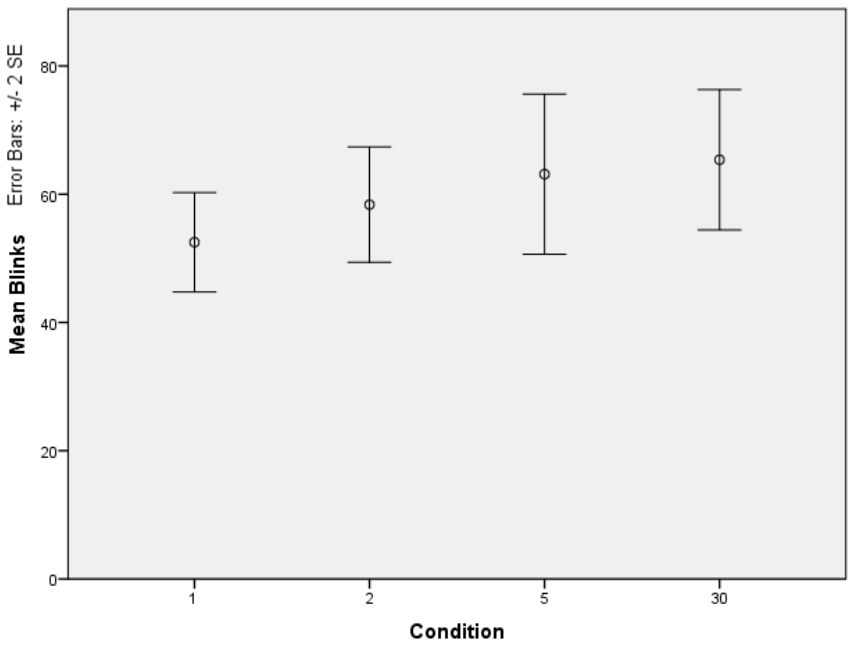

Figure 3.11 Mean number of blinks during experimental trials, comparison between conditions (1 = control 1, 2 = control 2, 5 = Inclination flip every 5s, 30 = inclination flip every 30s).

Past informal observations suggested that there might be a possible correlation between blinking and changing magnetic field conditions. Personal observation, as well as that of Evinger *et al.* (1994), show that pigeons always blink when they carry out a saccadic head movement. However, pigeons are also observed to blink independently of saccadic head movement, meaning that a greater number of responses can be evident. A comparison of the mean number of blinks for all subjects between conditions (Figure 3.11) shows that there is little, if any effect of magnetic field manipulation on frequency of blinks. Whilst there is a slight increase under manipulated conditions, this appears unlikely to be significant with the current data set. Because the process of counting blinks (manually by watching back video recordings) is highly time consuming, and there is no strong suggestion that a real difference might exist, it was decided that this analysis for a much larger data set, which would be needed to investigate saccadic responses effectively, would be outside of the scope and time constraints of the current thesis project.

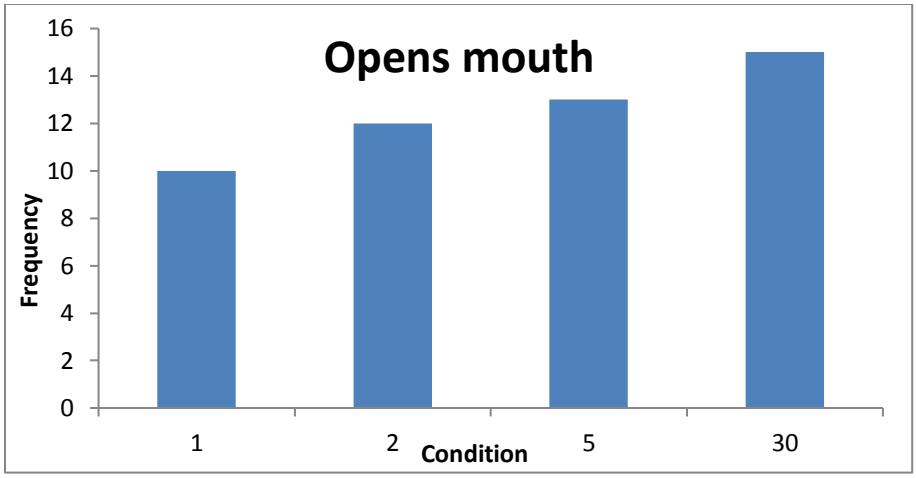

Figure 3.12 Total number of instances of mouth opening behaviour under each different condition (1 = control 1, 2 = control 2, 5 = Inclination flip every 5s, 30 = inclination flip every 30s).

Another interesting behaviour observed in previous trials was a mouth opening, yawn-like movement. Videos were manually watched and these behaviours counted (Figure 3.12). The total number of mouth opening behaviours for all subjects, between conditions, shows a somewhat similar trend to that seen in the eye-blink responses, namely that both experimental conditions show a greater response rate than both control conditions, but only by a very small margin, which, considering the low overall sample number (50 for the entire experiment), explains a lack of significance under repeated measures ANOVA.

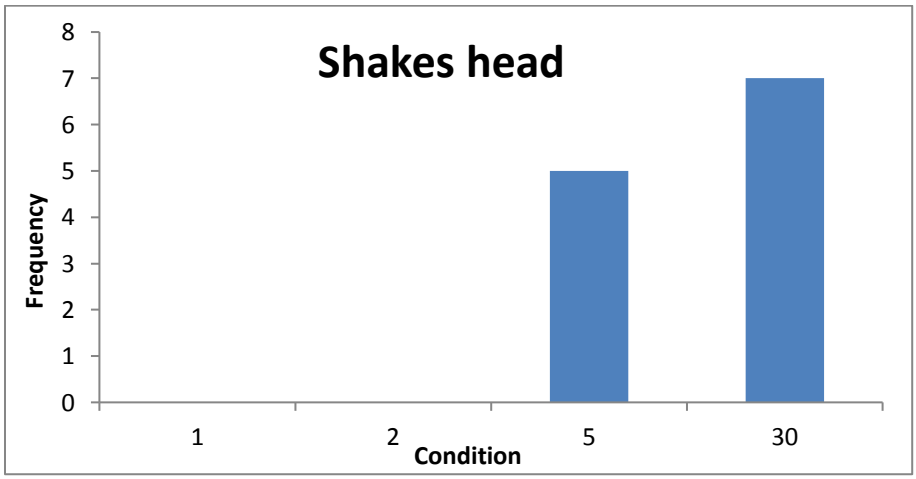

Figure 3.13 Total number of instances of head shaking behaviour under each different condition (1 = control 1, 2 = control 2, 5 = Inclination flip every 5s, 30 = inclination flip every 30s).

The combined number of head shakes for all subjects between conditions (Figure 3.13) shows a similar pattern to the two previous figures in that there are more responses under manipulated conditions. One interesting difference from the previous two figures is that the head shaking response does not occur whatsoever in the control conditions during which the amps are switched off and the magnetic field is unmanipulated. Repeated measures ANOVA shows a significant difference for condition ($F_{3,24}$=3.304, P=0.037).

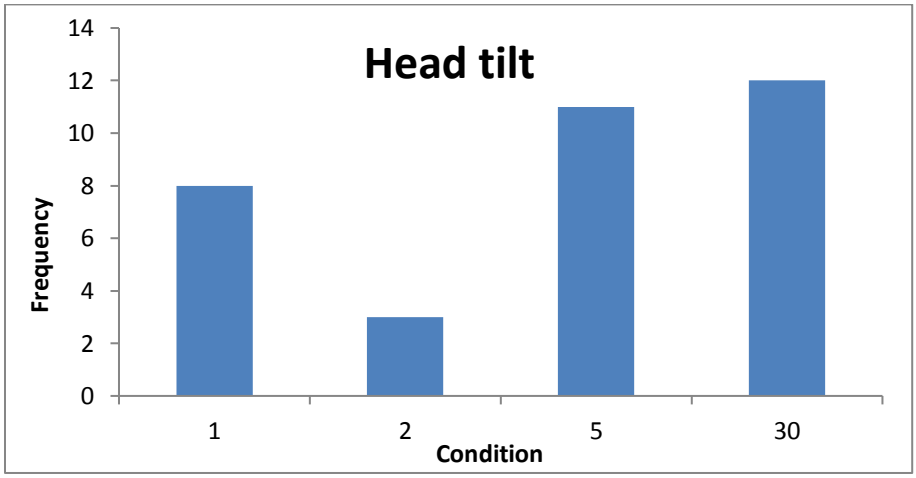

Figure 3.14 Total number of instances of head tilt behaviour under each different condition (1 = control 1, 2 = control 2, 5 = Inclination flip every 5s, 30 = inclination flip every 30s).

Head tilts are also evident in greater numbers under manipulated conditions than under control conditions (Figure 3.14), as are instances of the subject looking up (Figure 3.15).

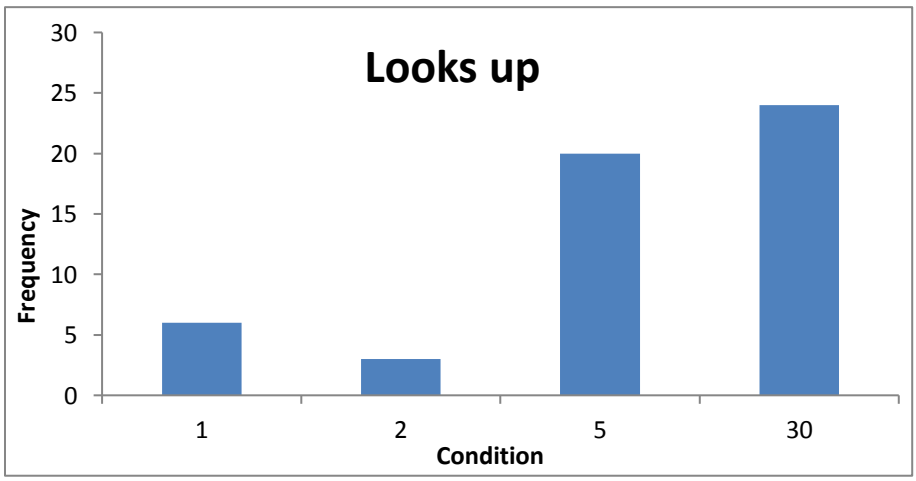

Figure 3.15 Total number of instances of looking up behaviour under each different condition (1 = control 1, 2 = control 2, 5 = Inclination flip every 5s, 30 = inclination flip every 30s).

Repeated measures ANOVA revealed no significant differences between conditions for either looks up, or head tilts ($F_{3,24}$=1.379, P=0.273, and $F_{3,24}$=0.505, P=0.683 respectively).

While the alternative responses investigated show some promise in terms of identifying a novel method of measuring behavioural responses to magnetic stimuli, they are very time-consuming, and in large data sets, this becomes a real issue in terms of carrying out a timely analysis. Some level of automation using similar image-analysis software techniques to those employed in our rotational head angle software would make this type of analysis far more likely to be viable on a larger data set. To this end, initial steps towards developing an inertial measurement unit (IMU) based head tracking sensor were undertaken (described in detail in Chapter 7), which would facilitate the possibility of characterising any type of head movement and automating the analysis of large data sets.

For future experiments within the scope of this thesis, it was decided that analysis would concentrate on the identification and comparison of saccadic rotational head movements, as the characterisation of the behaviour and the automation of video analysis had already been successfully developed.

Conclusions of preliminary experiment

- Alternative behaviours (looking up, head tilt, eye blinks, head shaking and opening mouth) may provide a good indication of a response to a magnetic field stimulus, however a large data set would be required, as each behaviour occurs relatively infrequently, and either automation of video analysis or large amounts of time (to manually count responses) would be required. This is outside of the scope of the current thesis, but may be of merit in future investigations. The development of a 6 degree sensor to measure head movements would remove the need for video analysis, however writing the necessary software to identify these movements accurately from the raw data recorded would be a challenging task.
- Automated saccade detection appears to work well and is suitable for large data sets.

- Larger saccades appear to show a greater difference between manipulated and unmanipulated conditions.
- Frequency and amplitude of saccades are the best option currently available for comparing head movements under manipulated and unmanipulated conditions. Using this measure also allows a direct comparison with the previous results of Migalski (2010).

Experiment 2: Extended investigation into behavioural responses to various magnetic field manipulations

A larger experiment was carried out in order to produce a bigger data set and to investigate a range of different magnetic field stimuli. In addition to the flipping stimulus previously used, clockwise and anti-clockwise rotations (field rotates around a vertical axis), and null field conditions were introduced. Instead of running a sequence of short, varying stimulus types as did Migalski (2010), the experimental design was to present one minute of a generated static (normal) field, followed by one minute of a manipulated field condition, and then another final minute of static field. The main goal was to compare the birds' behaviour during the crucial, second minute of each trial, with controls and the various manipulated conditions being represented in separate trials. If the response is at all comparable to that observed by Migalski (2010), there should be an apparent difference between the response to the control conditions, and that observed when the field is deliberately changed.

In each session, ten pigeons were exposed to two trials per day as follows (SF = static field):

Control trials:

- SF – SF – SF Control 1
- SF – SF – SF (Amps off) Control 2

Experimental trials:

- SF - Null field - SF E 1
- SF - Flipping field (1/3s) – SF E 2
- SF - Flipping field (1/20s) - SF E 3

- SF - CW sweep (20 °/s) - SF E 4
- SF - CCW sweep (20 °/s) - SF E 5
- Control day E 6

Each section of each trial lasts 60s, for total trial length of 120s.

Routine:

Each pigeon received one control trial and one experimental trial each day alternating between control 1 and control 2 and whether experimental condition comes 1st or 2nd, i.e. C2-NF-C2 and C2-C2-C2. The static field (amps off) control was added to compare with the generated static field control, to ensure that it did, in fact, function as a control, and did not elicit an unexpected response.

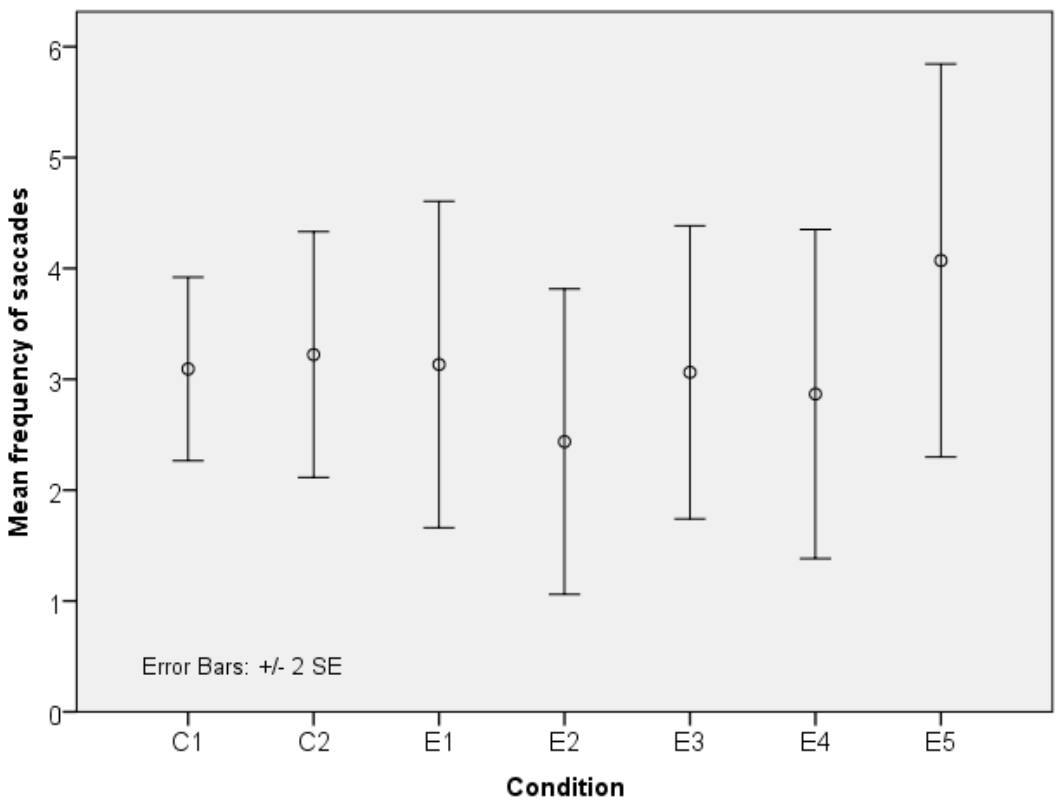

Figure 3.16 Mean number of saccades (over 10°) under each experimental condition. N = 10. C1= Static field control, C2 = amps off control, E1 = null field, E2 = flip every 3s, E3 = flip every 20s, E4 = clockwise rotation, E5 = counter-clockwise rotation (Error bars ± 2 SE).

The chart above represents the data collected in the new experiment. The mean frequency, ±2 standard errors of the mean is presented for the second minute of trials for each condition. All 10 subjects are pooled. Two main observations can be made from this

presentation of the data: Firstly, the data show nothing like the amount of variation between conditions that is apparent in the Migalski (2010) data (see Figure 3.1) with means varying very little, and a very large overlap being in evidence, and no significance found using linear mixed model repeated measures ANOVA. Whereas the Migalski (2010) data show evidence of a large difference between control and experimental conditions, the new data do not. Secondly, the mean number of saccades is between 2 and 4 per minute. This is very much in line with Migalski (2010)'s control condition frequencies, which were typically around 1 per 20s (3 per minute). This gives good confidence that despite the updated method of identifying important movements, the general baseline frequency of movements is consistent between the two experiments, which used different cohorts of pigeons.

Comparing the amplitude of all head movements in the Migalski (2010) data between experimental and control trials (Figure 3.3 and Figure 3.4 respectively), it is clear that more large amplitude movements occur during experimental trials. This is especially evident in the case of the clockwise rotation, which shows no great difference from the preceding null, and static field conditions during the control trials, but a large increase is apparent in the data for the experimental trials. The pattern is particularly evident in movements greater than 10°, the amplitude identified by Migalski as a useful threshold for investigating the response.

Summary of Helmholtz coil experiments

The results presented in this chapter illustrate two important trends in the data:

- The results of Migalski (2010) appear to be confirmed, despite the significantly improved methods of extracting head angles from video frames, and subsequent identification of saccades from head angle data.
- There is no evidence, examining saccades in the data from the newly performed experiment, for a measureable differential response to magnetic field manipulation as compared to control conditions.

Taken together these results would seem to suggest that the experiment of Migalski (2010) was successful in eliciting a measurable behavioural response to magnetic field stimuli, whereas the present experiment was not. Several differences in experimental design were introduced for various reasons, however the changes made were expected to refine and enhance the experiment (more detail in Chapter 2). The most likely explanation for the difference between the results of the experiments was that the experiments of Migalski (2010) were performed inside an electromagnetically shielded room, whereas the recent experiment was executed under active magnetic shielding, with no shielding of high-frequency electromagnetic signals. The field generated was good at replicating the ambient field, and making changes to it, but was potentially susceptible to anthropogenic electromagnetic background noise (see Chapter 2) in the ambient magnetic field.

Taking this result along with the recent findings of Engels *et al.* (2014), who demonstrated that European robins may not be able to orientate using the geomagnetic field when exposed to the general anthropogenic electromagnetic noise of urban or semi-urban environments, suggest that homing pigeons may also experience a reduction in salience of magnetic cues when exposed to such noise. This poses an interesting option for future experimentation, i.e. repeating the recent experiments, in an electromagnetically shielded environment similar to that employed by Migalski (2010). This prospect is discussed in more detail in Chapter 7.

In order to investigate the possible connection between the magnetic sense and vision, the OCR equipment described in Chapter 2 was developed. Ultimately it would be beneficial to be able to investigate visual psychophysics under magnetic manipulation, to explore the possibility that the limits of vision may somehow be altered by changes in the magnetic field. However, producing a more robust response to magnetic field manipulations will be an important first step. The following chapter describes the initial testing, proof of concept, and initial research findings using this newly developed equipment.

Chapter 4 Use of LCD screen technology to investigate polarised light sensitivity in homing pigeons

Introduction

As discussed in the introductory chapter, an understanding of whether pigeons are able to discriminate e-vectors of polarised light would be advantageous, both because it is widely considered an important navigational cue in its own right, and because there may be some potential link between the mechanisms for polarisation sensitivity and magnetoreception (Moore 1986; Moore and Phillips 1988; Muheim *et al*. 2016; Author's own theory Chapter 7).

Having briefly verified that the OCR apparatus could record meaningful and accurate head movement data in relation to the moving stimuli, and having made the necessary modifications for 'e-vector only' stimuli (removal of outer polarising filter, as detailed in Chapter 2), an experiment was carried out to investigate e-vector discrimination in pigeons.

Experimental procedure

Six homing pigeons were used. Each had experience of wearing the sock bandage restraint and had visited the laboratory on at least two occasions, but none had previously been exposed to any e-vector only stimuli.

The stimulus presented consisted of a range of three spatial frequencies of vertical black and white grating, all drifting at 10°/s, repeated in both horizontal directions, giving 6 total stimulus presentations per trial. The three spatial frequencies (0.088, 0.175 & 0.35 cycles/°) used are in the range found during exploratory trials to elicit a following response using normal screens. The rationale for varying this was to look for any noticeable difference in the effect of stimulus spatial frequency between the normal screen paradigm, and the e-vector

only, and 10% contrast paradigms (described below). Direction was alternated, and the order of grating sizes was designed to avoid both presentations of the same grating size appearing successively. All periods of stimulus presentation lasted 60 seconds, and were separated by a 10s period of blank screen (hence no drift).

This produced trials which lasted 430 seconds in total, and each subject was exposed to three separate trials:

- **FF** - Filtered screen (normal LCD with maximal luminance contrast between grating bars)
- **UF** - Unfiltered screen (polariser removed, meaning screen appears all white unless e-vector can be discriminated)
- **10%** - 10% luminance contrast on screen, as opposed to 100% luminance contrast in the other two paradigms (to account for possible artefacts produced by unfiltered screen)

The order of the trials was varied between subjects, and a break of at least 10 minutes was provided for each subject between trials, during which time the bird could move around and had ad lib. access to water.

Results

Slow-phase gain

The first analysis made was to compare the gain of slow-phase movements between the three treatment conditions. Slow-phase gain is calculated as the ratio of head movement velocity to stimulus velocity, with a gain of 1 representing exact following of the stimulus. Figure 4.1 shows a comparison of the frequencies with which different gain values were achieved, including gains for each individual slow-phase movement made.

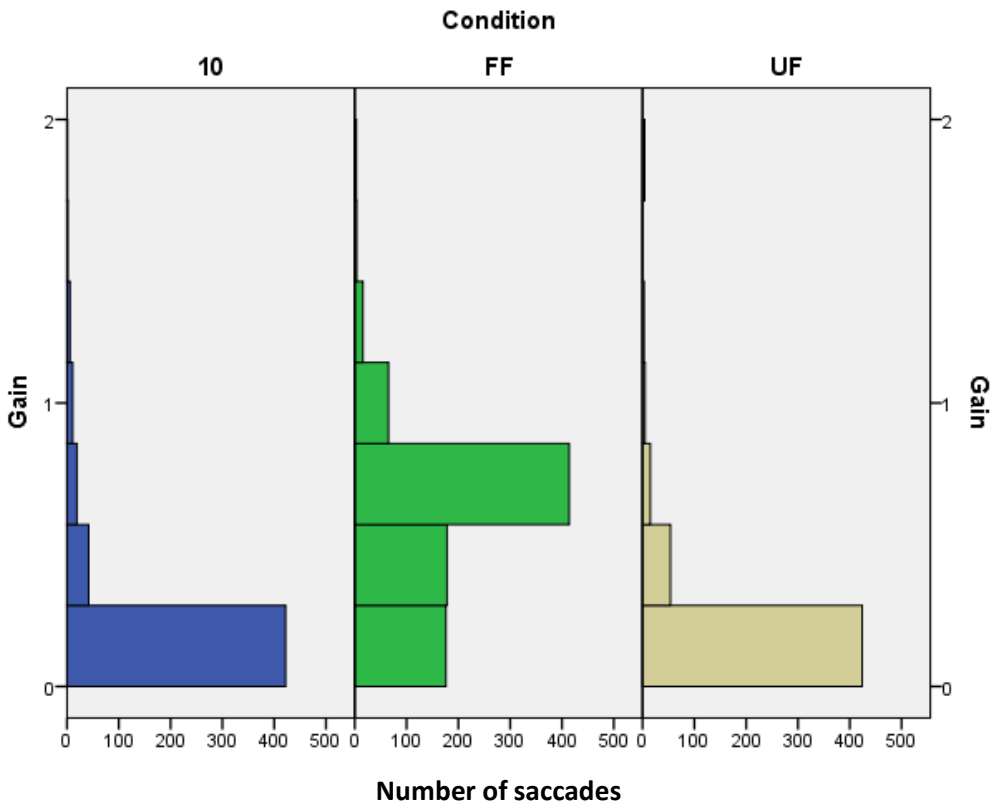

Figure 4.1 Comparison of slow-phase gain of individual slow-phase movements between different treatment conditions (10 = 10% contrast, FF = filtered normal screen, UF = unfiltered e-vector only screen). All 6 subjects combined.

It is clear in Figure 4.1 that the normal 'filtered' screen (FF) was effective at eliciting a strong following response, as evidenced by the high number of movements with a gain close to 1. This is very different to both the 10% contrast (10) and unfiltered (UF) e-vector only screens, each of which elicited very few movements with a gain close to 1. This suggests that only the filtered screen is effective in producing a strong following response. ANOVA revealed that condition has a significant effect on the gain of the birds' responses, with the gain during the FF condition being significantly greater than both 10% and UF conditions ($P<0.001$; Tukey HSD post hoc test).

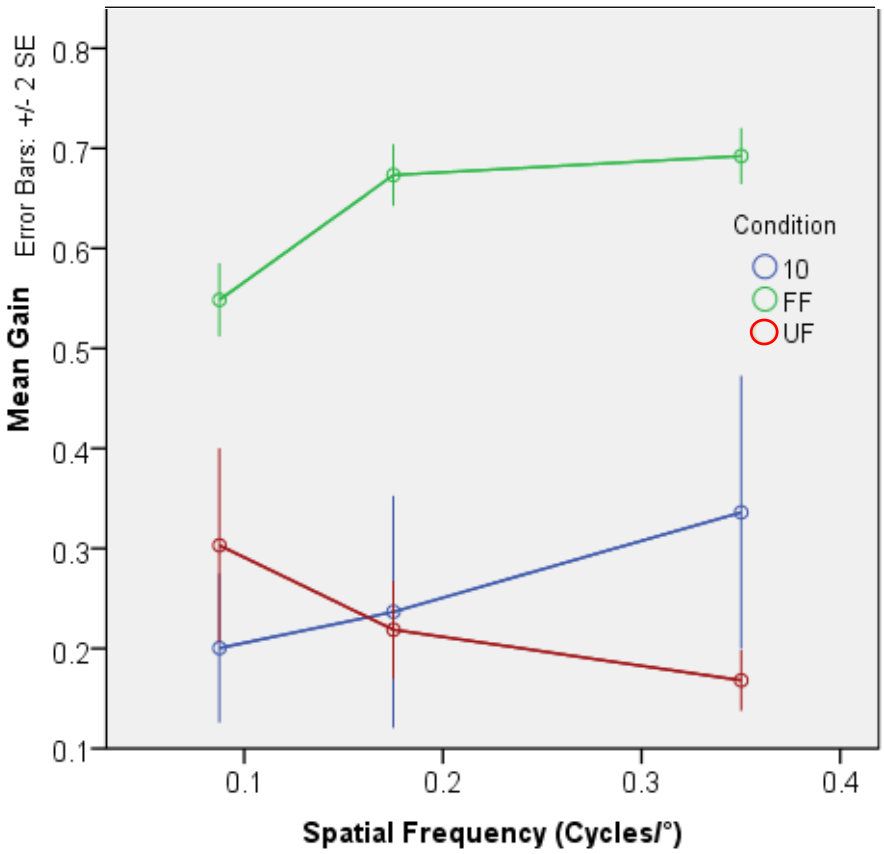

Figure 4.2 Comparison of mean gain across different spatial frequencies, for different treatment conditions (10 = 10% contrast, FF = filtered normal screen, UF = unfiltered e-vector only screen). All 6 subjects combined.

Figure 4.2 illustrates the relationship between mean gain and the different spatial frequencies of grating used. For the filtered screen (FF), an increase in gain can be seen as spatial frequency increases, in accordance with the patterns observed in Chapter 5. However, no such pattern can be seen in the 10% luminance contrast (10) or unfiltered screen (UF) conditions. Although some variation is seen, the large overlap between these treatments (both of which differ from the filtered screen condition), coupled with the generally low gain values in both cases, again suggest that no meaningful following of the drifting pattern occurred at the group level.

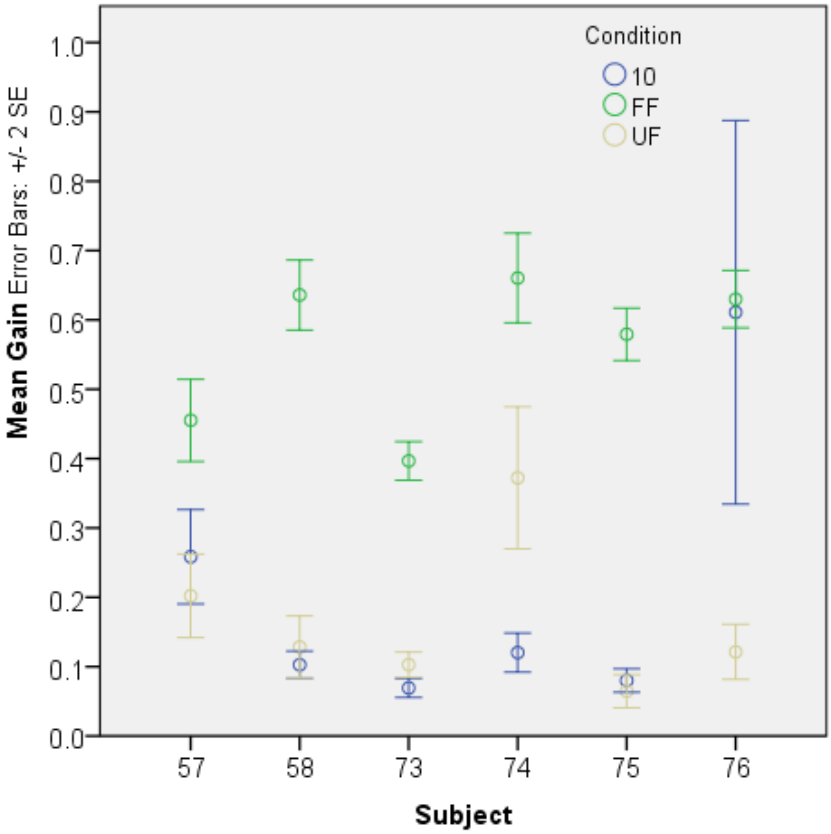

Figure 4.3 Comparison of mean gain across different subjects, separated by treatment condition (10 = 10% contrast, FF = filtered normal screen, UF = unfiltered e-vector only screen). Spatial frequencies combined.

Separating out the results from individual subjects in Figure 4.3 shows a similar pattern to previous figures. The response to the filtered screen (FF) shows a markedly stronger following in general, having in almost all cases, a higher gain value than the 10% contrast (10%) or unfiltered (UF) conditions, which do not seem to differ from one another. Two notable exceptions to this are: subject 76, which had an unusually high mean gain in the 10% contrast condition, although judging by the spread of values, this is likely due to chance, as a large number of slow-phase movements, some of which might have been significantly faster than the stimulus itself, converge on a mean of approximately 0.6; and subject 74, which seemed to respond differently to all three stimuli. In half of the subjects, the mean gain is higher for 10% contrast than for unfiltered conditions, and in the other half, the opposite is true. In all cases, the filtered screen elicits slow-phase movements with higher gain values than in either of the manipulated screen conditions.

Saccade frequency/amplitude

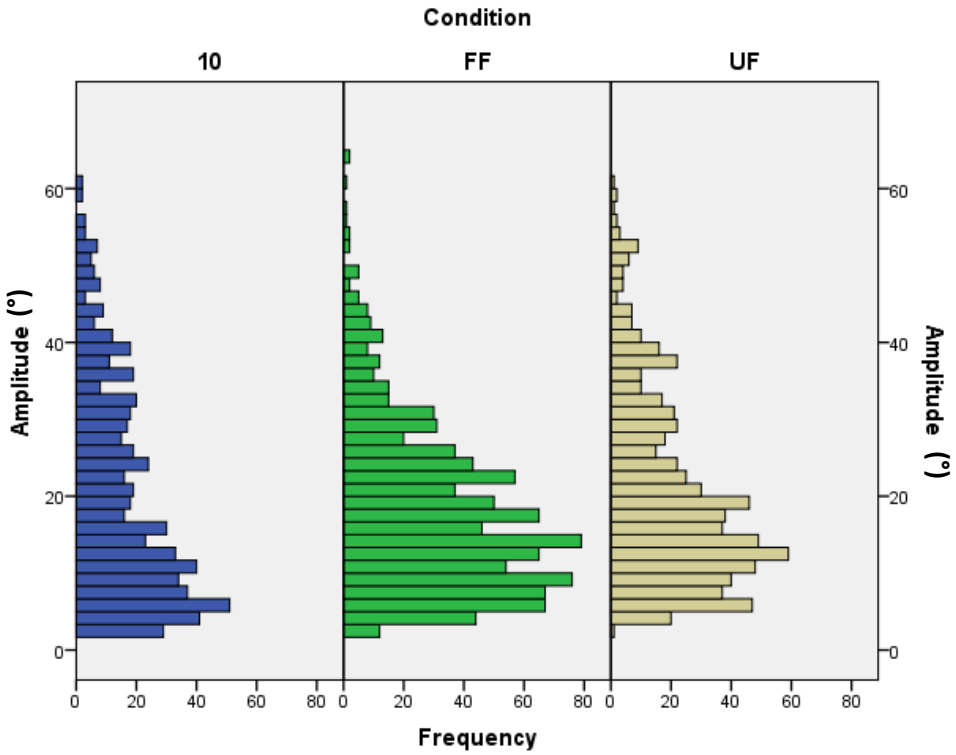

Figure 4.4 Frequency histogram showing the number of saccades of each size (amplitude in degrees), across the three different treatment conditions (10 = 10% contrast, FF = filtered normal screen, UF = unfiltered e-vector only screen). All 6 subjects combined.

Figure 4.4 gives the amplitude (°) of each saccade under each treatment condition, with all of the 6 subjects combined. It is clear that the filtered screen (FF) produces the greatest number of saccades. ANOVA showed that the difference was significant with FF being different from both 10% and UF conditions (P=005 and P=001 respectively, Tukey post hoc test). Interestingly, the 10% contrast (10%), and unfiltered (UF) conditions appear to produce slightly more saccades of over 50°, than does the filtered screen. This may be because the manipulated screen conditions produce some kind of visual stimulus, but not one salient enough to produce the OCR response. The bird is possibly looking around, trying to find a point of fixation, or even exhibiting a mild alarm response, rather than pursuing, as in the filtered condition. Also interesting is that the distribution of saccade frequencies in the unfiltered condition, matches more closely the filtered screen, than the 10% contrast screen, to which it was otherwise more similar in the previous analyses.

Q-ratio

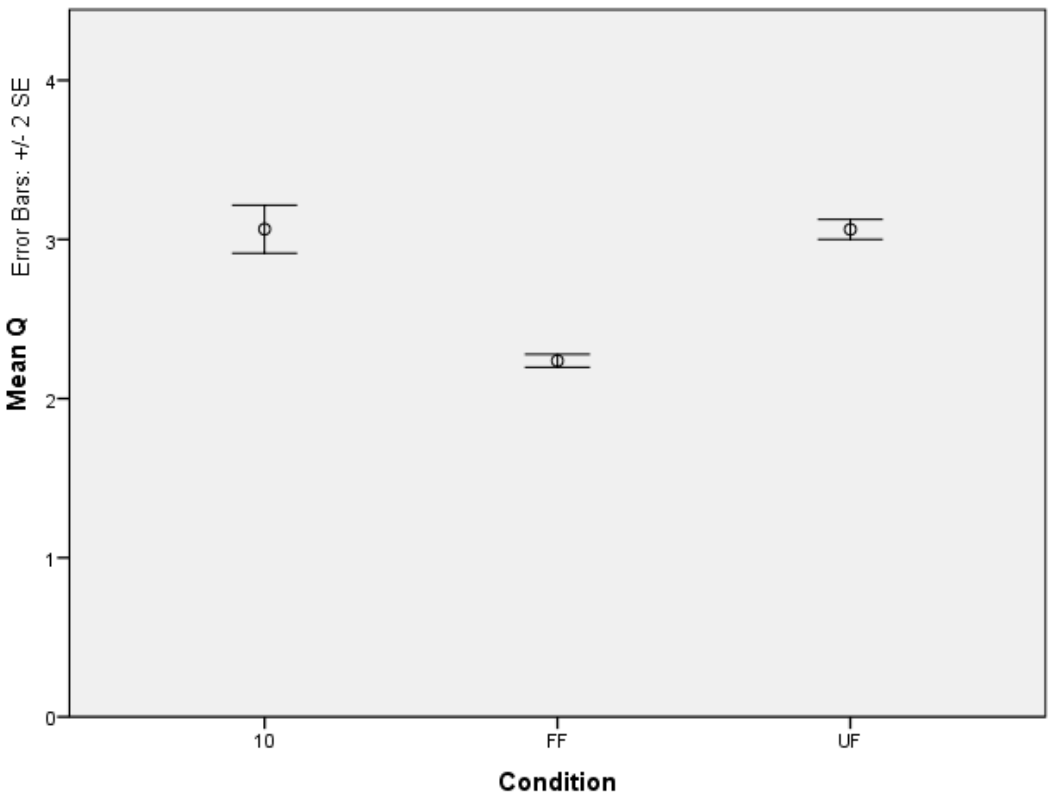

Figure 4.5 Mean Q-ratio over the three different treatment conditions. All subjects combined.

Q ratio represents the slope of the data for individual saccadic movements when peak velocity multiplied by duration is plotted against amplitude $\frac{\mathrm{pV}.Dur}{\mathrm{Amp}}$ (discussed further in Chapter 5). The Q-ratios for saccadic movements in response to filtered (FF) are different from those produced under 10% contrast (10%) and unfiltered (UF) conditions, showing a significant ANOVA result (P<0.001 Tukey post hoc test). This indicates that the saccades observed under unfiltered and 10% contrast conditions are not of the regular, visually guided OKN/OCR type, and may perhaps represent a different behaviour, whose resultant saccades may have different psychophysical or mechanical properties.

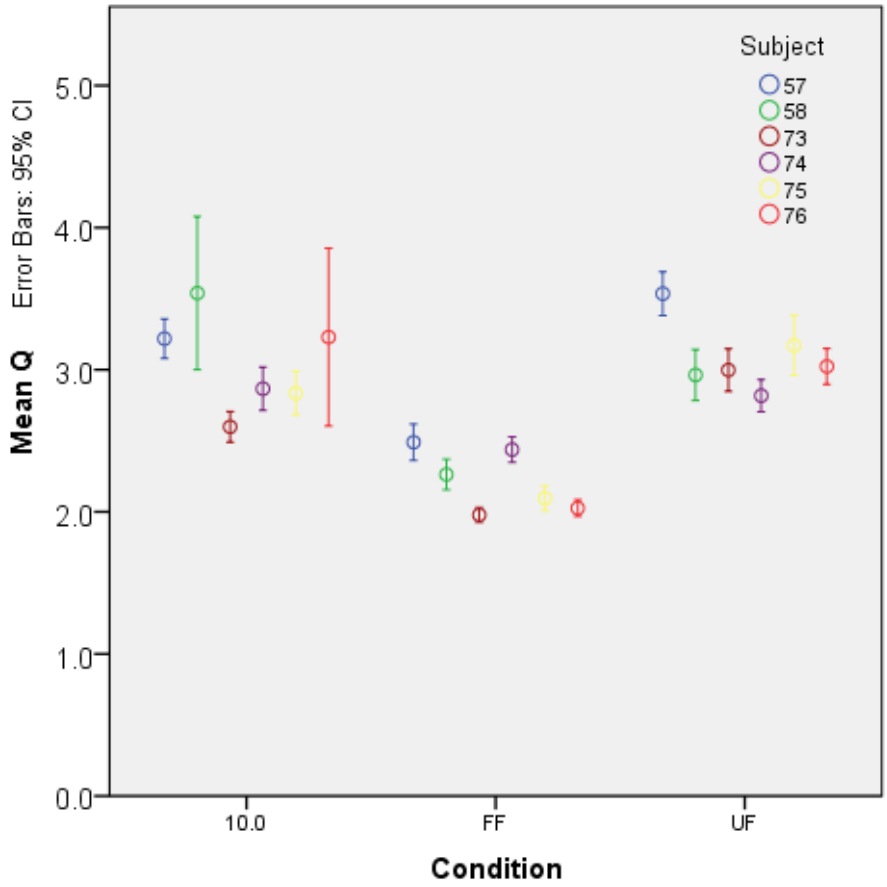

Figure 4.6 Mean Q-ratio across the three treatment conditions, with subjects separated (10 = 10% contrast, FF = filtered normal screen, UF = unfiltered e-vector only screen). All 3 spatial frequencies combined.

Separating out the subjects from one another in Figure 4.6, shows that the patterns observed in Figure 4.5 are not a result of combining data from the various subjects, as the within-subject variation maintains the patterns observed, namely that the Q-ratio in the filtered screen (FF) condition is different from that of the manipulated screen conditions, which tend not to vary as much between one another. It is interesting to note that the spread of within-subject measurements is lower under the filtered screen condition than in the other two. This again suggests some fundamental difference between the presumably visually guided saccades, and those where the stimulus is not meaningfully followed (manipulated screen conditions). Two of the subjects show far greater spread of values under the 10% contrast condition than unfiltered or filtered.

Goldfish control experiment

It is well established that goldfish (*Carassius auratus*) are able to discriminate e-vector orientation and use this information behaviourally (Kleerekoper *et al.* 1973; Pignatelli *et al.* 2011; Roberts and Needham 2007; Hawryshyn 2010). A brief qualitative assessment was carried out to test whether a following response could be induced in goldfish using the same two experimental arenas as used for pigeons. The same drifting grating was presented drifting at 20°/s in a clockwise direction. The goldfish were placed into a 20cm diameter x 5cm deep circular glass bowl, with vertical glass sides of approximately 1mm thickness. The goldfish were able to swim freely in any direction within this container. Therefore, the expected response was optomotor (i.e. the whole body moves) rather than optocollic (head movements). Fortunately, the head movement detection software worked well at identifying the orientation angle of the fish, which confirms its potential usefulness in assessing the responses of other species.

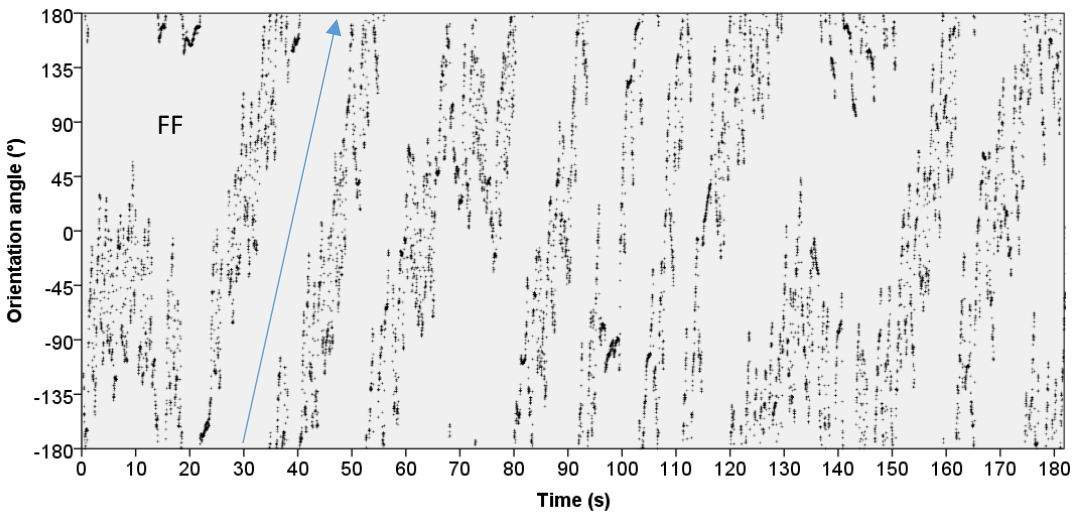

Figure 4.7 Optomotor response of goldfish (1) to filtered grating drifting at 20°/s.

Figure 4.7 shows the response of one goldfish to the drifting grating under the filtered (i.e. clearly visible to humans and, apparently, the pigeons) condition. The behavioural response of the fish consisted of swaying side to side repeatedly, with a strong bias for turning to the right (increase in orientation angle). It can be seen that, although the data appear somewhat

noisy (due to the swaying behaviour), there is a clear trend for the goldfish to rotate around the bowl consistently to the right. The arrow clarifies this steady drift in orientation and its slope represents a drift of 20°/s. It can be seen that the goldfish makes 9 full rotations during the three minute trial. The fact that the goldfish rotates overwhelmingly in the direction of the stimulus drift, and matches the speed of the drift very closely, confirms that it is able to see the stimulus in this condition. Subsequent trials were carried out using the 10% contrast and unfiltered (i.e. e-vector only) conditions.

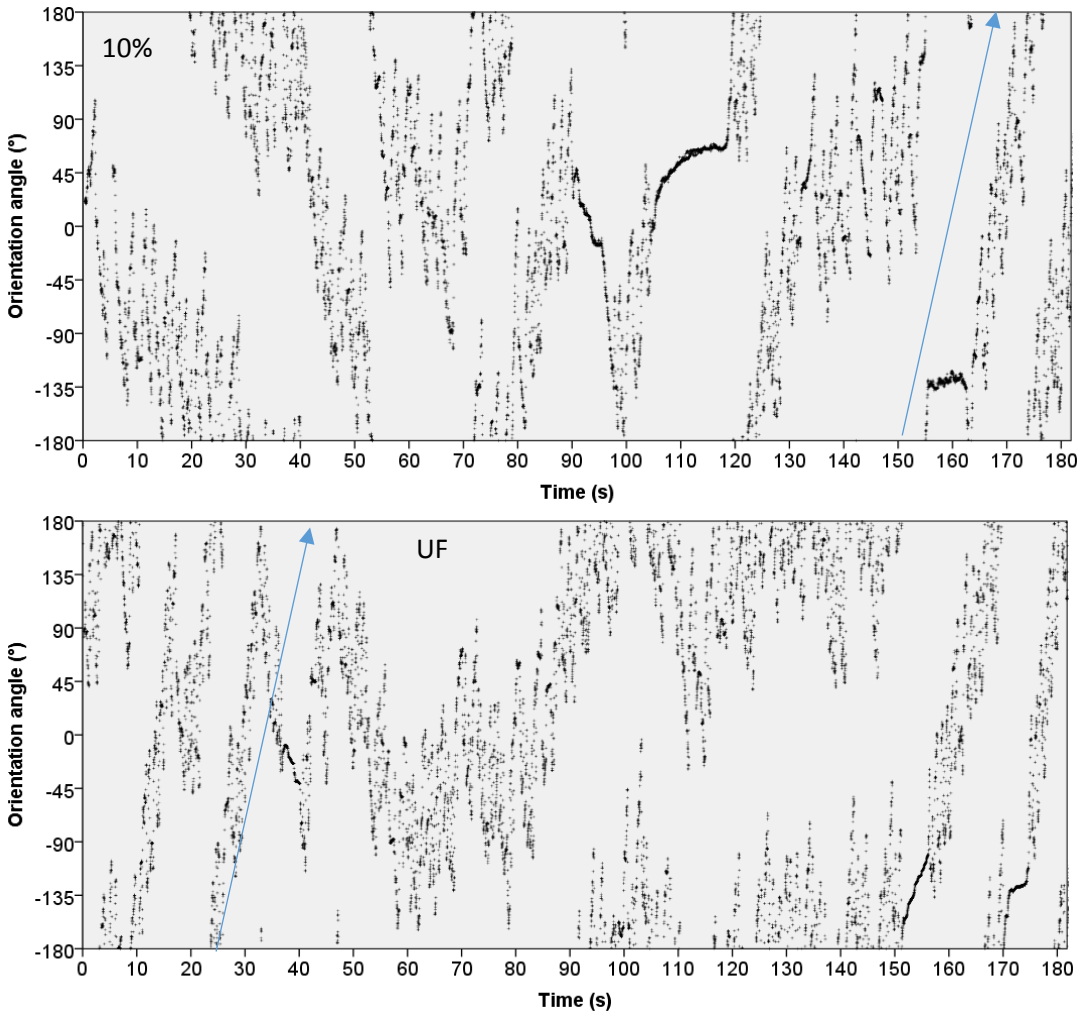

Figure 4.8 Optomotor response of 1 goldfish to a grating drifting at 20°/s with luminance contrast set to 10% (10%-top) and polarising filters removed (UF-bottom).

Figure 4.8 shows a markedly different response from the goldfish to both 10% luminance contrast (10%) and unfiltered, hence e-vector only (UF) conditions, as compared to the filtered (FF) screen condition shown in Figure 4.7. In the 10% contrast condition, there is

some evidence of an overall clockwise drift (i.e. the direction of the drifting pattern) in the second half of the trial. However the rate of rotational drift does not match that of the stimulus nearly as well as in the FF condition, nor does the behaviour display the same clear level of consistency. Under the UF condition an overall trend to drift to the right can be seen, however this response is also far less consistent and well matched with stimulus velocity than the FF condition. This indicates that the fish is not able to follow either the 10% or UF gratings as well as they followed the FF grating. There is a possibility that the clockwise rotation seen under the UF condition could potentially be due to visual artefacts created when the UF stimulus is refracted and reflected by the glass dish in which the fish swims. The lack of a consistent following, however, implies that neither e-vector orientation contrast, nor any visual artefacts, can be clearly seen by the subject. These observations imply that goldfish are unable, or markedly less able, to follow the UF grating and hence, are unable to discriminate the contrasting e-vector orientations in the same experimental setup that failed to demonstrate and OCR response in pigeons. Anecdotally, optomotor responses to rotation gratings of differing e-vector orientation can be induced in goldfish, however that experiment was carried out using daylight-emulating light sources, containing low wavelength and UV light (van der Meer 2011). This is consistent with the fact that the ability of teleost fishes to discriminate e-vector orientation and contrast is associated strongly with availability of UV light (Hawryshyn 2010). Because LCD screens typically do not emit light in the UV wavelengths, a future experiment could be conducted by adding UV light to the display screens. This is discussed in more detail under Future directions in Chapter 7.

Summary of polarised light experiment

The data presented in Chapter 6 provide a clear example of how, using this methodology, meaningful measurements can be obtained for evaluating the head movements of pigeons. However, no evidence can be observed for the existence of e-vector discrimination in the pigeon, the response generally being similar to the response to 10% luminance contrast, both of which differed markedly from that under filtered 'normal' screen conditions in a variety of ways.

The frequency of saccades under unfiltered conditions appeared relatively similar to those under normal filtered screen conditions, noticeably more so than the 10% contrast treatment, which, to the human eye, is easier to see and follow.

The small amount of variation between and within subjects of the Q-ratio, can likely be explained in terms of limitations in the accuracy of recording and analysing head movements (primarily sample rate, see Chapter 5). The greater difference between treatments, and within subjects under the 10% contrast and unfiltered screen treatments appears to be a real effect resulting from the difference in stimulus presentation. This is interesting because a maximal reflexive ballistic movement such as a saccade should have predictable dynamics, particularly within-subject, regardless of stimulus used.

These results suggest that there may be some promise to further investigating this possible sensory modality using the general approach described. If this were to be the case then certain improvements to the technique would be advisable. The original theory of the author combined with the knowledge that pigeons can see into the UV area of the light spectrum (Vos Hzn *et al.* 1994) suggests that it would be worthwhile to display the stimuli used with UV light as is found in regular daylight in a natural environment, or possibly to try the experiment using only the UV range of the spectrum. This was beyond the scope of the

current investigation, but is discussed more in Chapter 7, under Future directions. The results of testing with goldfish show a similar trend, with the FF condition eliciting a strong following response, which was not present under 10% or UF conditions. This further supports the implication that UV light might be necessary for a following response to be obtained using this experimental approach.

Nevertheless the methodology does seem effective in obtaining a rapid, and highly adaptable means of investigating visual psychophysics in pigeons and the optomotor response in fish. The next chapter will investigate more closely the way that this method can be applied to studying vison in pigeons.

Chapter 5 Using the LCD arena to explore the optocollic reflex more generally

Having designed and built a system capable of psychophysical testing using pigeon head movements, consideration was given to how this may be used more generally. In the long term (see 'Future directions' in Chapter 7), it would be very interesting to use this approach to establish psychophysical limits of vision, for example spatial acuity and/ or contrast sensitivity, and then assess whether these are affected by manipulating the ambient magnetic field. To test acuity, screens of higher resolution than those available would be required and the time needed for such an investigation was beyond the scope of this thesis. However, it was possible to test whether such techniques could work in principle, even if the theoretical limits of spatial vision cannot be reached with the current setup. To this end, a series of investigations were carried out to establish the reliability of the method in eliciting a consistent reflexive response, and to begin investigating how the methodology might be employed in future investigations of visual psychophysics. The following experiments were conducted during the prolonged period during which debugging and repair of the prepulse inhibition equipment was in progress.

Effect of stimulus velocity on saccadic head movements

In order to verify the reliability of the measurements of head movement responses using the experimental approach, a comparison was made with data available in the literature, obtained using more traditional methods (i.e. physical rotating drum stimulus).

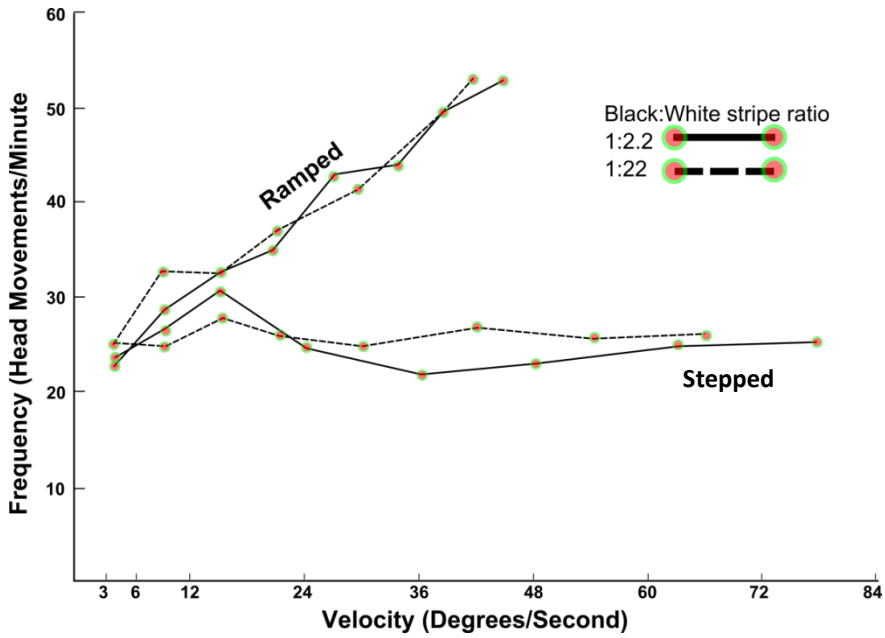

Figure 5.1 Effect of stimulus velocity on frequency of saccades, from Fite (1968).

Figure 5.1 shows data recorded by Fite (1968) illustrating the relationship between frequency of saccadic head movements and stimulus velocity, for two different paradigms. In the ramped trials, velocity was increased in regular equal increments, from one velocity to the next, meaning any one change in velocity was relatively small. In the stepped paradigm, each velocity was presented with an abrupt step from 0°/s, presumably incurring a greater increase in retinal slip for higher stimulus velocities, as the new target begins travelling much faster than the one currently being pursued. Consequently, it can be seen that in the paradigm where the increase in stimulus velocity is gradual, head movement frequencies increase steadily up to stimulus velocities of almost 50°/s. Whereas those changes in velocity which are more abrupt, show only minor, if indeed any, real changes in saccadic head movement frequency.

An attempt was made to replicate the above findings using the newly developed apparatus, but this was limited by certain factors. In order to present a smoothly drifting visual stimulus using Psychtoolbox, it is important that the spatial frequency of the stimulus be a factor of screen width, each being measured in pixels. This also has a bearing on the precise stimulus

velocities which can be reliably employed. Despite this, a wide range of stimulus velocities can be presented, meaning a comparison of differing paradigms for increasing velocity can be made. The only outstanding difference in the stimulus presentation, is that using the current method, contrasting bands are delivered in the form of a regular square wave grating, with a 'Black to White stripe ratio' of 1:1, as opposed to the 1:2.2 and 1:22 ratios used by Fite (1968). Consequently, direct comparison of saccade frequencies with Fite (1968) is not possible, however similar variation in the trends shown under abruptly changing vs gradually changing velocities should be apparent. A trial was carried out using 5 birds, with 2 presentations of each stimulus velocity (one in each rotational direction), utilising 2 differing paradigms;

- Ramped velocities changed in regular equal increments
- Randomised velocities involved a random order of velocities, with a 10s pause between each presentation, meaning that, each time a stimulus began to move, it was changing instantaneously from a velocity of zero to the presentation velocity.

Subjects were 5 homing pigeons, 2 male, 3 female, all of which had been handled regularly and had experienced having the sock-bandage restraint put on them at least once. Two continuous trials were run for each bird, such that each stimulus velocity was presented in both rotational directions. In the ramped trial, velocity increased in 10°/s steps over the course of 4 minutes 40 seconds, giving 20 seconds of continuous stimulus motion at each chosen velocity. This meant that each subject experienced two 280s trials, and a total of about ten minutes of mild restraint for the entire experiment. A break of several minutes was given between trials, during which time the restraint was removed and the pigeon was free to move around and extend its wings (but not fly), as well as having ad lib. access to water. Intervals of 20s for each stimulus were chosen as preliminary trials showed this to be adequate time to produce a response, whilst minimising trial time, and hence potential stress to the subject. In the randomised trials, each velocity was separated by a period of 10s of

stationary stimulus, and the direction of trials was alternated. This was done in order to minimise any effect of lag effects from one stimulus presentation carrying over to affect the subject's ability to pursue the next. For subsequent analysis of saccades and slow-phase gains, data for the two different directions was combined. Because of the self-imposed time-limits for restraining the pigeons, and the introduction of stationary phases, a slightly different number of stimulus velocities were employed. There are, therefore, some important differences between the paradigms employed here and those of Fite (1968):

- The spacing of black and white bands.
- The specific stimulus velocities employed.
- The duration of each stimulus presentation (and overall trial), was shorter than in Fite (1968).
- The randomised trial was more broken up than the stepped paradigm of Fite (1968), with changes in stimulus velocity being greater in some cases.

Despite these considerations, it was still possible to present a wide range of stimulus velocities, extending to include previously published limits, and to explore the effect of the motion elicited by one stimulus on the ability to pursue the next.

Consequently, based on Fite's (1968) results, one would expect to observe a much steeper curve in the case of the incrementally ramped velocity trial, than in the trial involving abrupt (and in some cases large) increases in velocity from zero (Randomised).

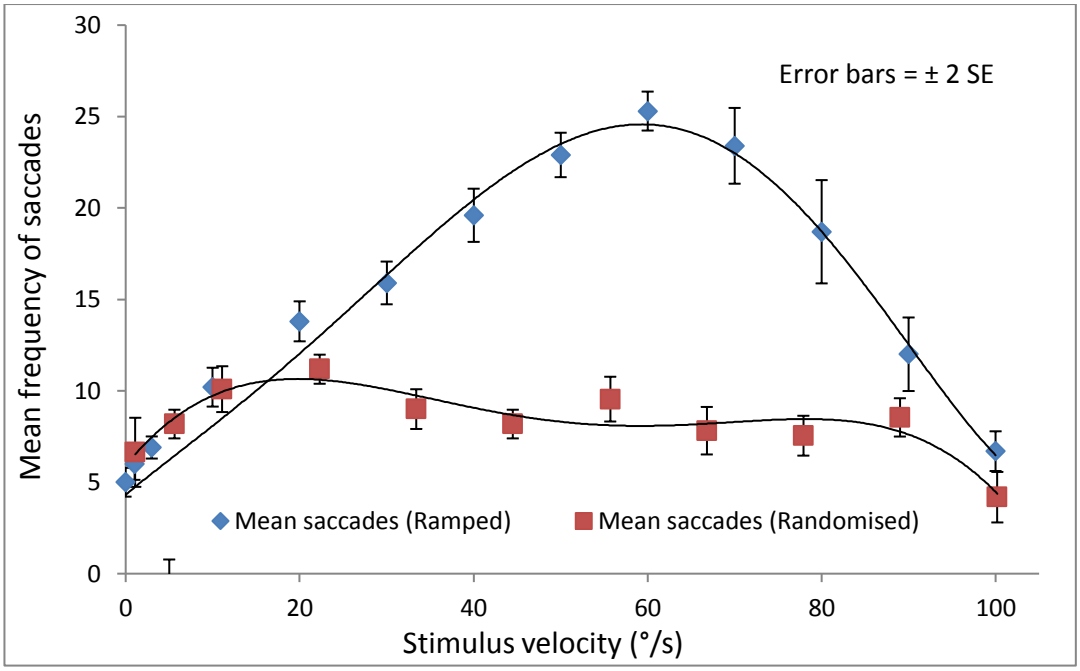

Figure 5.2 Effect of stimulus velocity on frequency of saccades (Error bars ± 2 SE) N = 5.

In Figure 5.2, a clear distinction can be seen between frequencies of saccades under the two paradigms. A gradual increase in velocity clearly results in a steady increase of saccadic frequency up to approximately 60°/s, whereas in the randomised paradigm, the abrupt changes in stimulus velocity result in only small variations in a relatively constant saccade frequency, which do not appear to be related to stimulus velocity. The general patterns observed between saccade frequency and stimulus velocity, and the effect of stimulus change from one velocity to the next, appear to match well with the previous findings of Fite (1968).

Relationship between amplitude and peak velocity of saccades

The nature of a saccadic head movement is such that there is a very close relationship between the amplitude of a movement, and the peak velocity achieved during that movement. This is due to the reflexive, maximal nature of the movement in question, meaning that over greater movement amplitudes, greater maximum velocities are achieved. Head movements were analysed using the MATLAB code developed by Lee McIlreavy described in Chapter 2. The following figures show that a clear relationship between head movement amplitude and condition is evident in the data.

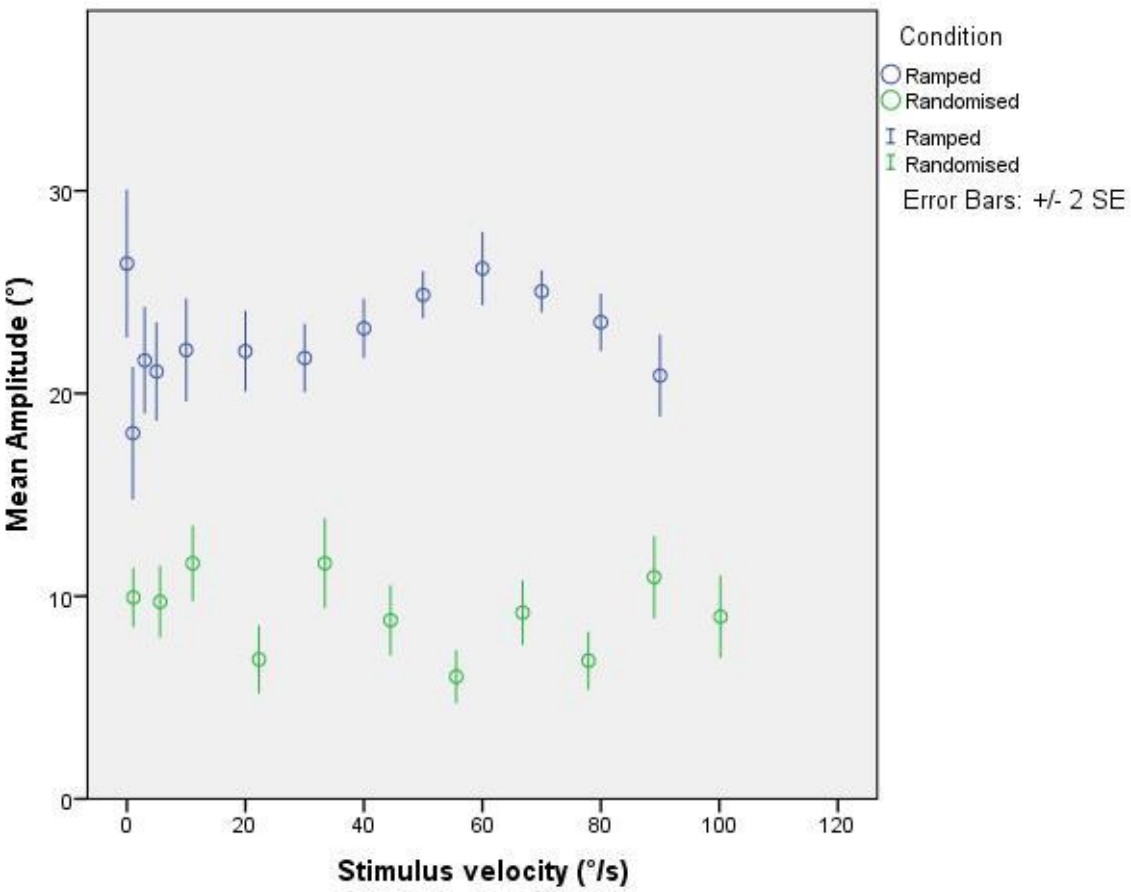

Figure 5.3 Effect of stimulus velocity on mean amplitude of saccades (all 5 subjects combined).

Figure 5.3 suggests there is a gradual (albeit small) increase in the mean amplitude of saccades between velocities of approximately 30 to 40°/s, which then falls gradually back to

starting level beyond 60°/s. In the randomised data, no such relationship with stimulus velocity is apparent.

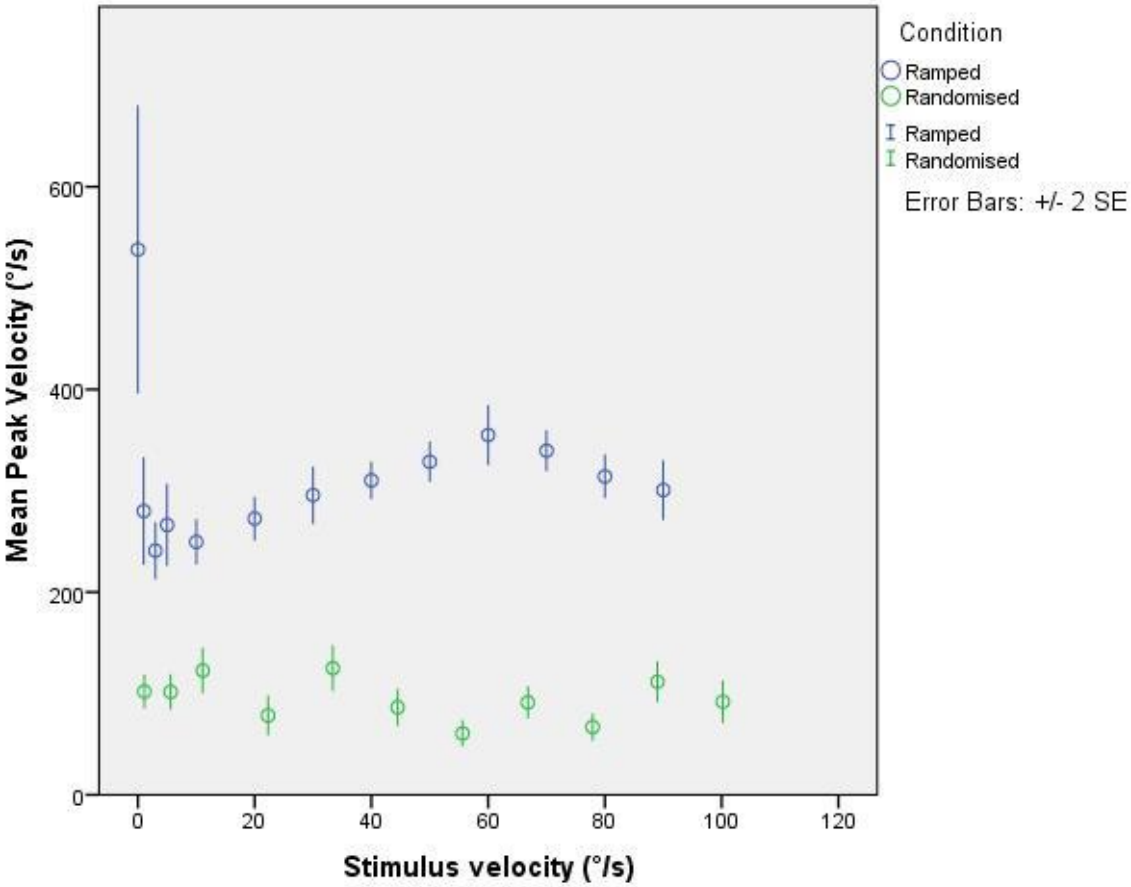

Figure 5.4 Effect of stimulus velocity on mean peak velocity of saccades (average of all 5 subjects).

Figure 5.4 shows an almost identical relationship between mean peak velocity of head movements and stimulus velocity as between mean amplitude and stimulus velocity in Figure 5.4. This clearly demonstrates the known relationship between amplitude and peak velocity of saccadic movements, and provides supporting evidence that the apparatus and paradigms used are effective in eliciting the Optocollic Reflex, whose dynamics are affected by experimental condition.

Further investigation into the main sequence of saccades under each paradigm may be possible and will be discussed further in Chapter 7.

Effect of stimulus velocity on slow-phase gain

As well as demonstrating that the apparatus is capable of producing results in accordance with previous studies, the above findings also pose an interesting question about how moving targets are seen by pigeons: how does the relative change in stimulus velocity affect their ability to pursue the target?

In order to shed light on this issue, a comparison of slow-phase gains was made between the two paradigms. Gain, in terms of visual pursuit, is an effective measure of the ability of an observer to accurately pursue a moving visual stimulus. Expressed as a ratio, visual pursuit gain is the mean slow-phase rotational velocity of the head divided by the velocity of the target. Such that a target moving at 30°/s that is eliciting a following response at 27°/s would be expressed as a gain of 0.9. Pursuing a visual target with eye/head velocity perfectly matching that of the stimulus would be expressed as a gain of 1. Generally head movement pursuits would rarely be expected to be exactly 1, as some contribution is made by movements of the eyes, which was not measured here.

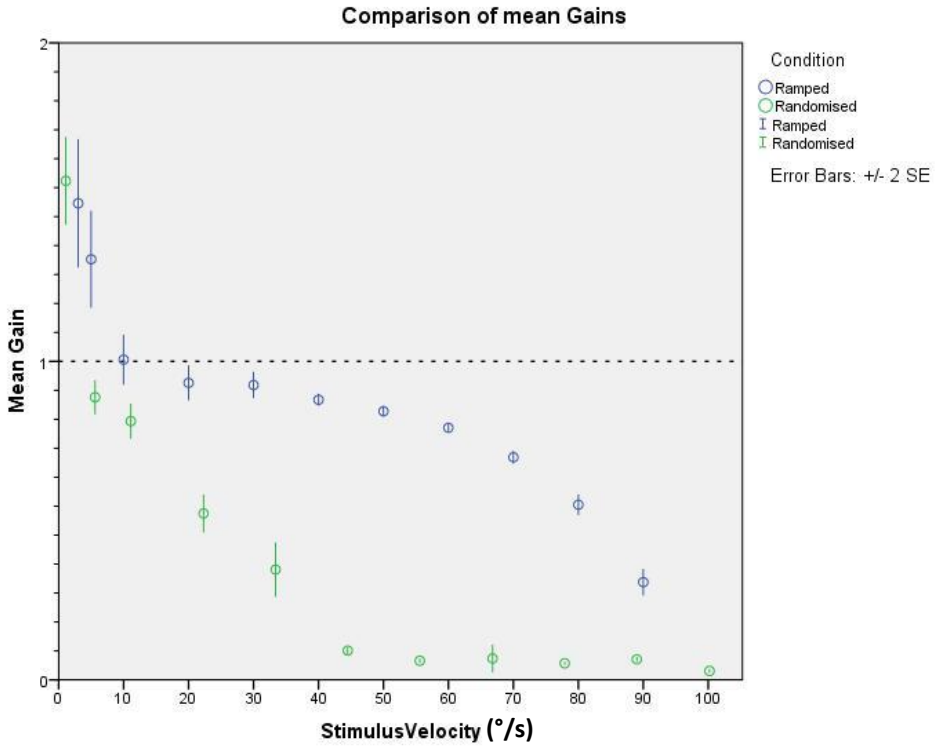

Figure 5.5 Effect of stimulus velocity on mean gain of slow-phase movements under two different conditions (all 5 subjects combined).

It can be seen from Figure 5.5 that the relative change in stimulus velocity has a clear effect on the ability of the pigeon to accurately pursue the stimulus, with abrupt increases in stimulus velocity (randomised) resulting in a much steeper drop in gain than gradual increases (ramped).

At very low velocities, both paradigms show gains of greater than 1. This occurs when the stimulus is not being effectively pursued, and is moving at a lower velocity than the typical resting head movements of the pigeon. The fact that these very slow velocities are not being pursued is suggestive of a minimum low-velocity threshold for activating the Optocollic reflex in pigeons. The data presented in Figure 5.5 show that this threshold is likely to be in the region of 10°/s. At greater velocities than 10°/s, gain begins to reduce. This reduction in gain is steep and apparently somewhat linear in the randomised paradigm, with little or no pursuit occurring at velocities above 40°/s. In the ramped paradigm, gain falls away more

gradually with the slope of the curve steepening with increased velocity. It appears that the pigeons may be pursuing the target to some extent, right up to 90°/s, which was the highest velocity used in that paradigm, although by that point the gain value has dropped considerably, and whether they can be described as meaningfully pursuing the target is a simple case of applying an arbitrary threshold for gain.

'Build up' and 'Break down' effects

The observation that the amount of retinal slip may be affecting the ability of the bird to pursue, begs the question of whether the bird requires a period of exposure to the moving stimulus ('building up') in order to reach pursuit velocities, i.e. gains closest to 1.

The figure below shows the same ramped data as plotted in Figure 5.5 with the notable difference that each period of a given stimulus velocity has been divided into two, allowing comparison of mean gain during the first and second half of each velocity presentation.

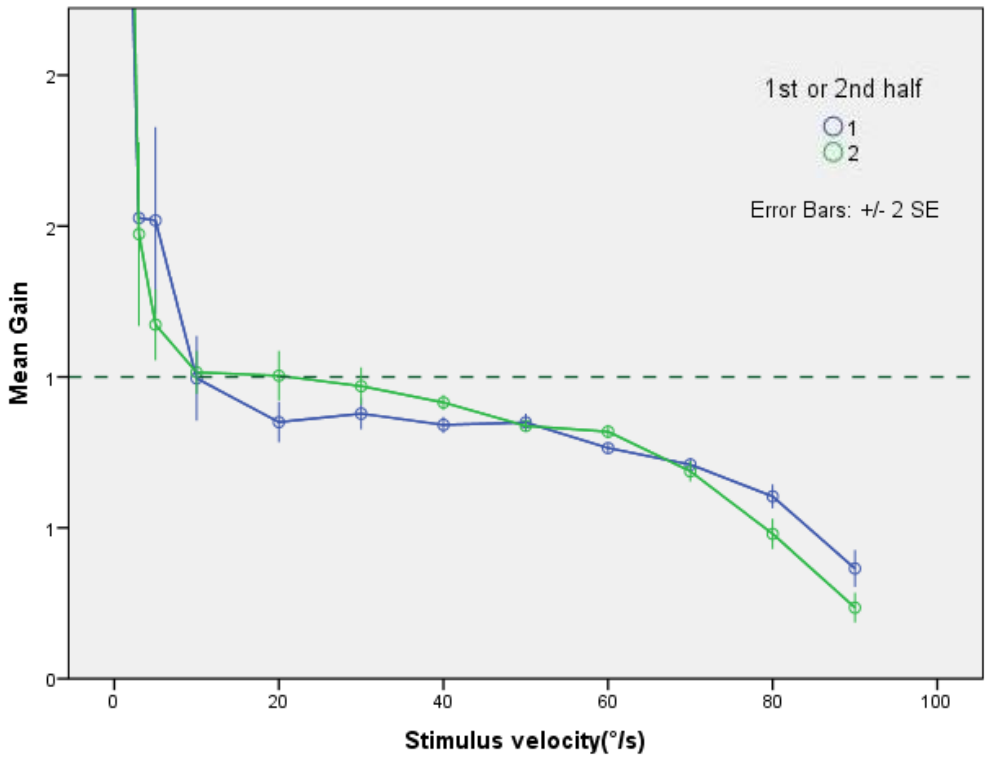

Figure 5.6 Effect of stimulus velocity on mean gain of slow-phase movements, 1st and 2nd half of each velocity presentation separated (all 5 subjects combined).

The fact that the first and second half of each stimulus presentation shows differences in gain at certain velocities is potentially very interesting (Figure 5.6). When the green (2nd) gain value is higher, this suggests that the bird is 'building up' to the stimulus velocity, rather than achieving a gain of close to 1 in under 50ms, as would be expected in adult humans (Crane *et al.* 2007). When the green marker shows lower gain than the blue, the bird is finding it increasingly difficult to follow the stimulus, and hence gain (for a given stimulus velocity) 'breaks down'.

In order to investigate this effect further, a comparison was made between two different stimulus velocities, arising spontaneously from a stationary stimulus (i.e. from the randomised recordings above). Hence, in the figure below, 22°/s stimulus is twice the velocity of 11°/s.

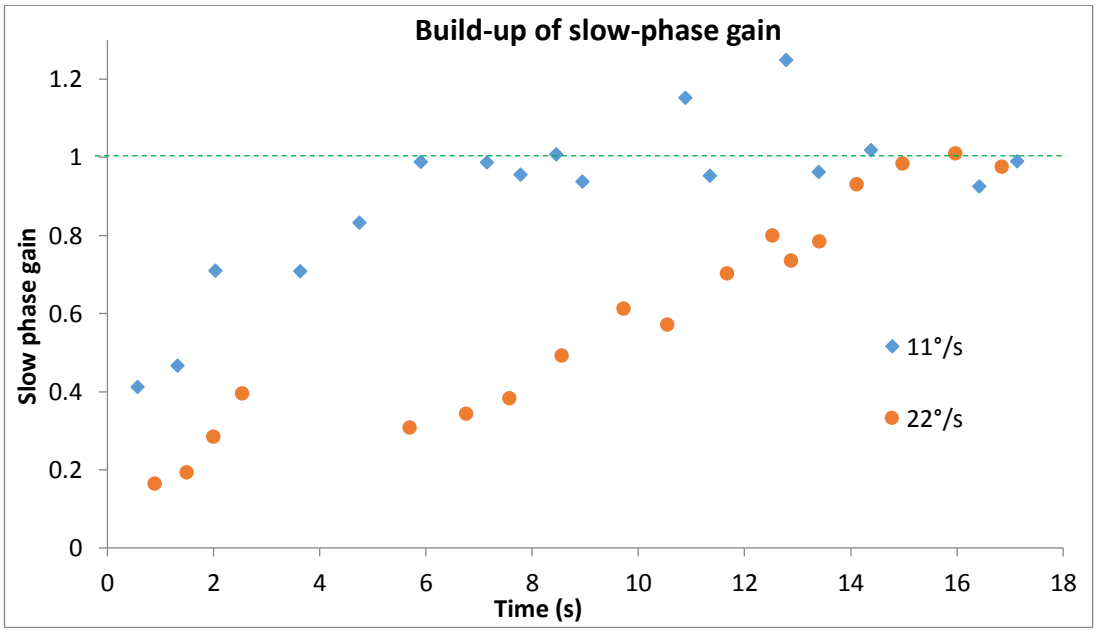

Figure 5.7 Build-up of slow-phase gain (1 subject, ID B1).

Figure 5.7 shows that, rather than instantaneously reaching pursuit velocity, the head movements of the pigeon actually build gradually towards a gain of 1. Not only is this build

up effect clearly present, but it also appears to be a function of stimulus velocity (and/or change therein).

The above chart implies that the duration of stimulus presentation (in this case 20s) is too short to explore the full effect. This provides an opportunity for future investigations, utilising greater durations of stimulus presentation, combined ideally with a greater sampling rate, in order to better characterise this build up effect. This also presents the possibility of comparing directly with humans (which, in adulthood, are not thought to express such a build-up effect in pursuit eye movements (Crane *et al.* 2007)), and other species, in order to investigate evolutionary relationships in visual pursuit behaviour

Acuity

A common property of vision that may be assessed using OKN/OCR techniques is visual acuity. This denotes the finest distinction in spatial resolution that can be made by the eye, and can be measured as a function of spatial frequency (width of bars in this approach). Luminance contrast can also be tested by varying the brightness contrast between bars. A combination of the two, using the greatest point at which both measures elicit a response can be plotted to produce a contrast-sensitivity curve. In some case 1/contrast threshold is employed as a contrast sensitivity function (CSF), thus, low contrast thresholds of vision represent high CSF values.

An attempt was made to measure the effect of spatial frequency on slow-phase gain. A simple, exploratory trial was conducted which involved only one subject, and one presentation of each stimulus. For a detailed investigation more subjects/trials would clearly be required.

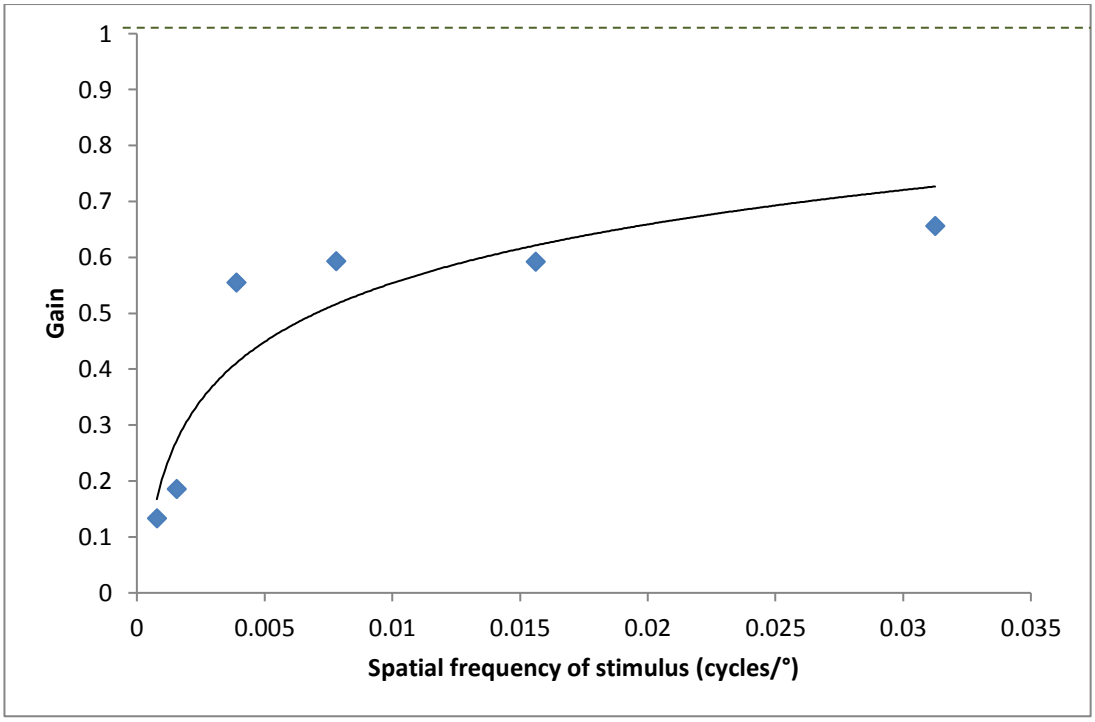

Figure 5.8 Effect of spatial frequency on slow-phase gain (1 subject ID B1).

As can clearly be seen in Figure 5.8, some stimuli are too wide to follow (low number of cycles per degree). This is due to the fact that edges (areas of visible contrast) are only visible for a small proportion of the time, the bars being so wide that they show no visible change during large periods of the presentation, meaning that movement cannot be observed, and the optocollic reflex is not stimulated. As spatial frequency increases, so does gain, as the bars presumably become small enough for the subject to track the edges. This trend should reach a peak and come back down, as the bars now become so narrow they become harder and harder to discriminate from one another, to determine the narrowest bar the pigeon can see. The spatial frequency of this narrowest bar (at maximum contrast) is known as the spatial acuity of the subject. This experiment is however limited by screen resolution. Due to the size of pixels in the Philips displays used, and the specific number of pixels that a stripe must contain in order to be redrawn smoothly enough to appear as a steady drift, the greatest spatial frequency possible was slightly over 0.03 cycles per degree. No comparison of luminance contrast was performed in this trial.

Pigeon acuity has been measured in various ways, with values between 3 and 12 cycles per degree having been previously reported (Bloch and Martinoya 1982; Gunturkun and Hahmann 1994). Future experiments may be conducted using much higher resolution screens, allowing more of the spatial contrast sensitivity curve to be assessed. The numerous screens used were selected simply on the basis of their availability, having been kindly offered for use in this research by Cardiff University. Screens with resolutions that can theoretically produce gratings suitable for a full investigation into spatial acuity, have recently become commercially available, and are discussed in more detail in Chapter 7.

Main sequence of saccadic movement

Saccadic eye and head movements have a stereotypical relationship between the amplitude of a given saccade, and its peak velocity. Similarly, peak velocity and mean velocity are closely correlated. This stereotypy is referred to as the saccadic main sequence, and is useful in various ways (Bahill *et al.* 1975). It is possible to compare features of the main sequence (such as the slope/ratio of peak velocity multiplied by duration and mean amplitude $\frac{\mathrm{pV}.Dur}{\mathrm{Amp}}$ of saccadic movements known as Q), between subjects/species. This can be useful to investigate differences and similarities in the musculature employed in facilitating the movements, as well as the underlying neural substrate responsible for their control. Comparisons within subject may be useful in comparing before and after treatments, such as lesioning or sectioning certain pathways.

In the case of this investigation, the main sequence is useful in validating the experimental approach, as any problems in recording or calculating saccades may potentially be visible as a deviation from the stereotypical shape of standard plots. The plots commonly employed are: peak velocity vs amplitude, duration vs amplitude, and peak velocity x duration vs

amplitude (the gradient of the slope created by this chart also gives Q-ratio). An example of these plots can be seen below.

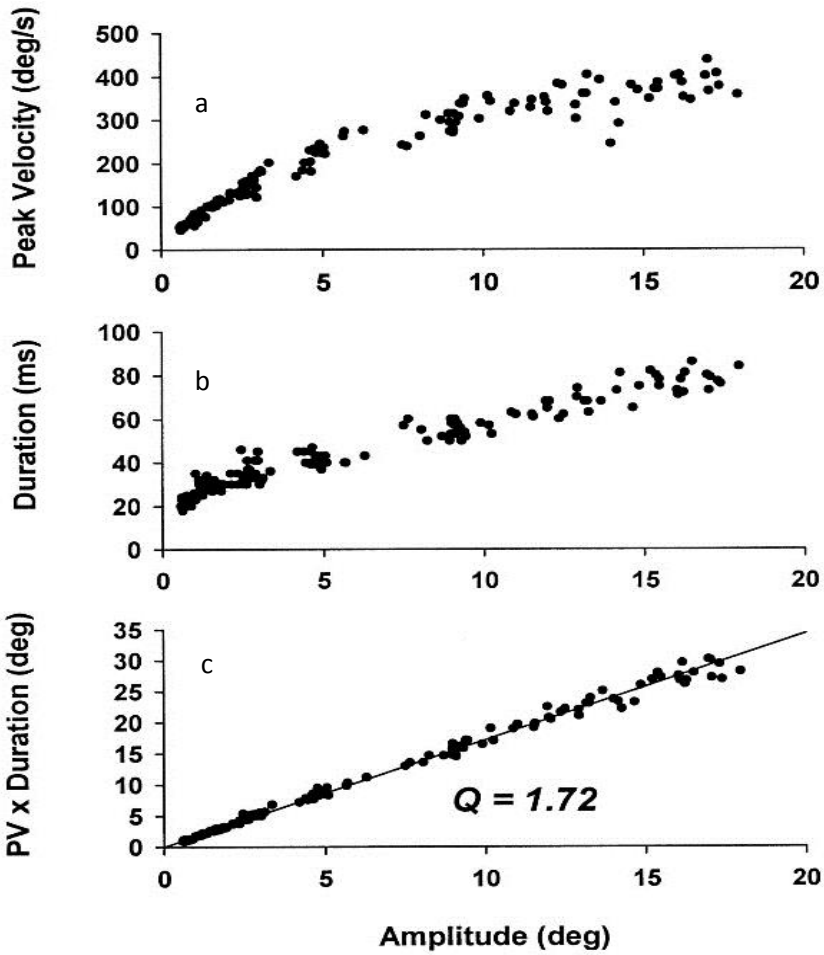

Figure 5.9 Stereotypical aspects of the saccadic main sequence, in human eye movements from Harwood *et al.* (1999).Showing: a) peak velocity vs amplitude, b) duration vs amplitude c) peak velocity x duration vs amplitude.

These figures (5.9 a-c) were reproduced using the data recorded in the LCD arena. Figure 5.10 (a-c) contains combined data from all birds/trials, hence shows a more 'noisy' signal than in the above example, which only employs one single subject. However, the stereotypical trends should still be apparent.

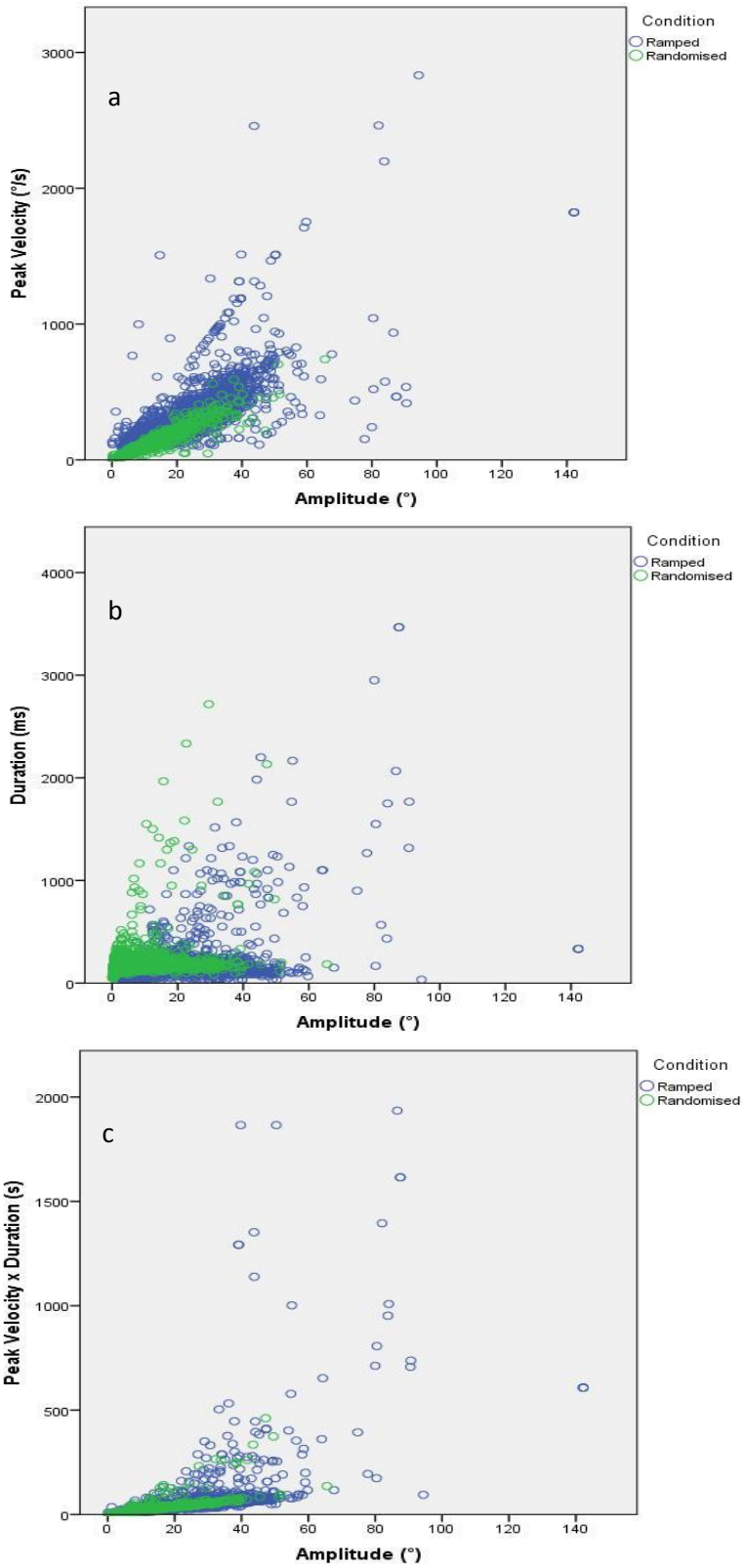

Figure 5.10 Reproduction of Figure 5.9, using the data from the 5 bird experiment recorded to compare saccade frequency with Fite (1968). Showing; a) Amplitude vs Peak Velocity, b) Amplitude vs Duration, and c) Amplitude vs Peak Velocity x Duration.

As can be seen in Figure 5.10, the plot of peak velocity vs. amplitude (a) shows the stereotypical trend expected. The data is predictably noisy due to variation between subjects and individual saccadic movements recorded. The actual values achieved are somewhat different from those of Harwood *et al*. (1999), due to differences in eye and head movement saccades, and the small amount of noise or jitter present in the raw data (discussed in Chapter 2). However, the expected trend is clearly visible. The trend in plot b, on the other hand, is markedly different from that expected based on the results of Harwood *et al*. (1999). Further investigation is needed to find the cause of this anomalous finding. However, the most likely reason is that the sampling rate of the camera used for these experiments was too low (60 Hz vs. 1000 Hz used by Harwood *et al*. (1999)). This would severely bias the measurements of saccade duration. This suggests that, for detailed analysis of saccadic main sequence features, a camera with a greater sampling rate and/or an improved filtering method (to remove more noise from the recorded data) may be necessary. Plot c shows again the stereotypical trend pattern that might be expected, but due to the issue with measuring duration, the values are likely to be somewhat inaccurate, meaning that this approach requires refinement before measures of Q-ratio can be derived using it that will be comparable with existing published data.

Vertical OKR

As well as the optocollic reflex, various other types of visually induced head movement can potentially be investigated using this apparatus. The optokinetic reflex may manifest as extending and shortening of the neck, allowing the head to remain stable vertically when the rest of the animal moves, as well as the classic head bobbing movement seen in mobile birds, which fixates the image of the world for the maximum possible time, with fast bobs equivalent to OCR saccades in allowing the following to continue after the animal reaches its

physical limit (i.e. maximum head rotation in OCR or neck extension in head bobbing) (Frost 1978).

In order to assess the suitability of the apparatus for measuring head bobbing, an exploratory trial was carried out using vertically drifting stimuli. In order to fill as much of the vertical visual field as possible, the subject was placed nearer to the front of the arena, allowing the front screens to subtend a larger vertical angle. The stimulus drifted upwards at 20°/s for three seconds, before reversing (i.e. downwards) for 3 seconds. This was repeated such that the stimulus was presented twice in each direction. For these trials, the pigeon was videoed from the side, and positions were extracted manually, by measuring the distance travelled on the viewing screen.

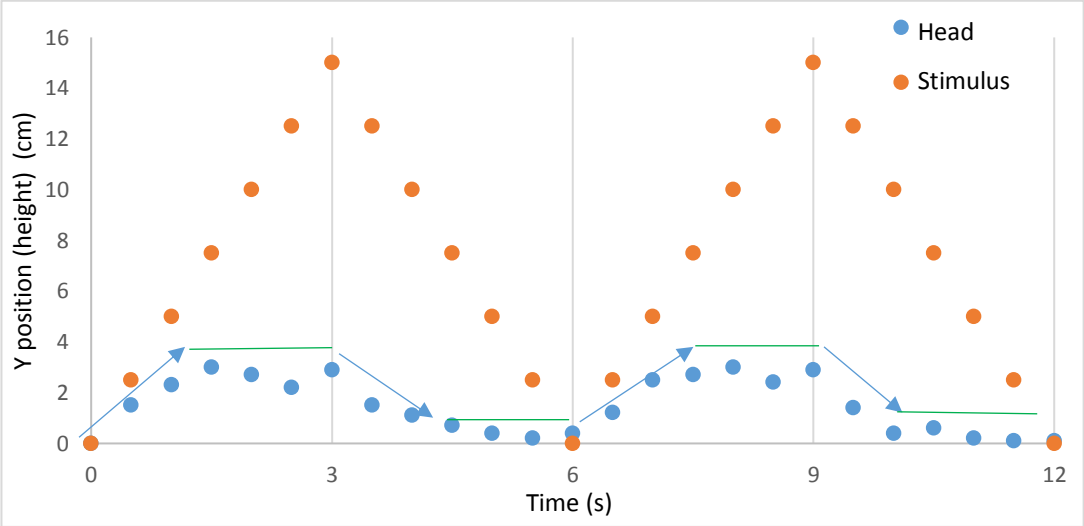

Figure 5.11 Pigeon response to vertically drifting stimulus (1 subject).

Figure 5.11 shows the relationship between stimulus motion and vertical head movements (neck extension). In this trial, the range of head movement in this subject was approximately 2cm. Typically the pigeon achieved a full range movement in approximately 1 second, beyond which the head position was relatively stable. At full extension, there were signs of regular small amplitude bobbing/nystagmus where only minor movements were made in

order to fixate the stimulus, however these were observed visually, and higher precision recording of position is required to characterise this more accurately.

During the initial 1s of movement, the head follows the stimulus with a mean gain of 0.56. With greater sampling rates it should be possible to develop an accurate picture of head bobbing dynamics in both the horizontal and vertical direction.

Direction of saccades

An investigation was made into the effect of stimulus velocity on saccade frequency, when separated by direction. Below is a chart showing all saccades recorded during counter clockwise (CCW) stimulus drift, under the 'Ramped' paradigm already described.

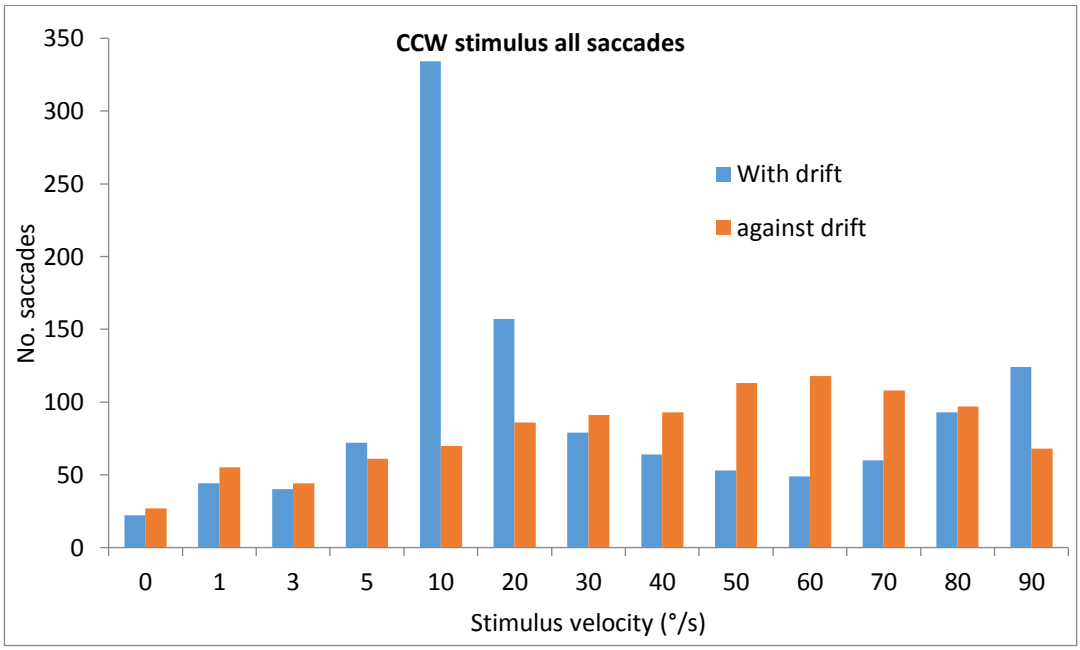

Figure 5.12 Frequency of saccades, separated by direction (all 5 subjects combined).

Figure 5.12 shows an interesting relationship between the different directions of saccade. At low velocities, there is no obvious difference in the frequency of each. At 10- 20 °/s there is a large increase in saccades moving with the drection of drift (these are variously referred to as 'catch-up saccades, or mis-directed saccades). Between 10 and 90°/s stimulus velocity,

there appears to be an inverse relationship between the two directions. The saccades 'against' stimulus drift follow the now familiar pattern of building to a peak around 60°/s before falling away, whereas the catch-up saccades follow what appears to be the opposite pattern, being greatest at 20°/s and 80°/s, and dipping in between.

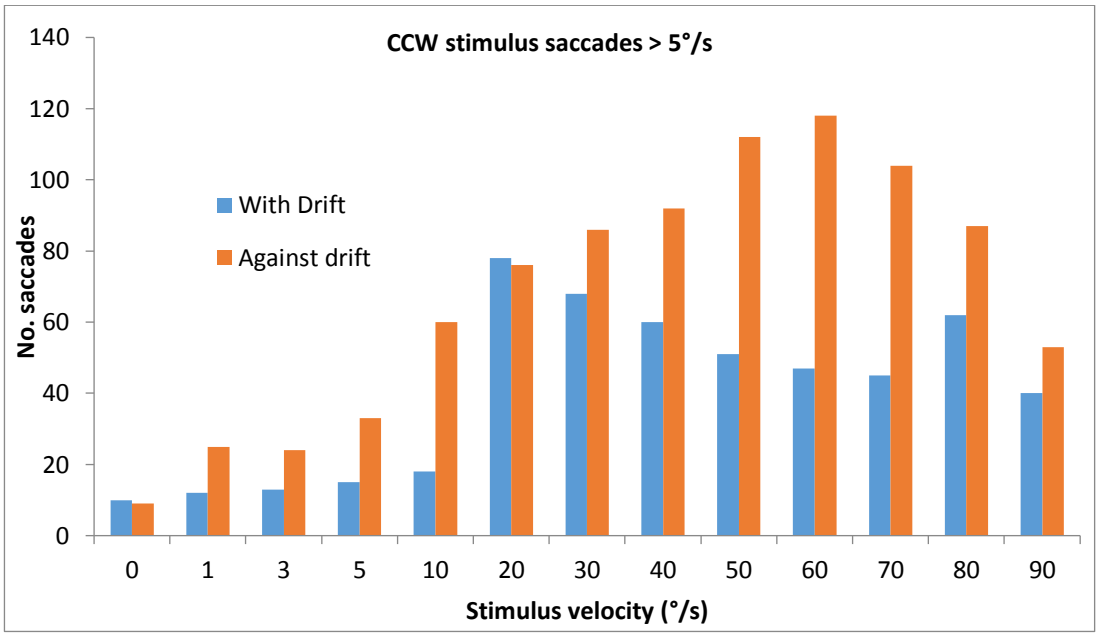

Figure 5.13 Frequency of saccades, separated by direction, and with saccades of less than 5° amplitude removed.

At this stage it was observed that a large number of catch-up saccades at the 10°/s velocity were less than 5° amplitude. An arbitrary limit of minimum 5° amplitude was imposed and the same chart plotted to examine the trend more closely (Figure 5.13).

The inverse relationship described above is still in evidence (although the gradual increase in catch-up saccades towards the greatest velocities may be eliminated), and poses two interesting questions:

- What is the relationship of catch-up saccades to the velocity of the stimulus?
- Why are so many of these catch-up saccades less than 5° in amplitude?

Future work will concentrate on answering these questions and utilising the resulting knowledge to further improve the method of measuring saccades. This will be discussed in more detail in Chapter 7.

Summary of OCR experiments

The relationship between stimulus velocity and frequency of saccades produces a 'velocity profile' which, as with other measurements of OCR, is highly stylised for a given species of subject. The data presented show this stylised response, with a small amount of saccadic movement during presentation of a stationary stimulus (baseline movement), which then builds gradually as the stimulus velocity increases, up to a point where the stimulus moves too quickly for the subject to effectively track the drifting pattern. Saccades continue to occur, however their frequency drops off significantly, once the smooth pursuit (i.e. the slow-phase head movements of the subject) can no longer keep up with the stimulus velocity.

This characteristic profile reflects that already published, but has been accomplished by the use of a much lower number of birds. The established accuracy of the approach, coupled with its increased efficiency in use of subjects, suggests that this technique will be of considerable use to those wishing to assess the dynamics and other properties of OKN/OCR in the future.

We have concentrated here on head movements (OCR), but with the addition of eye-tracking equipment (available commercially) the eye movements of OKN might be assessed just as easily, and with the same range of stimulus variants available.

Previous studies on OCR in pigeons have reported up to 50% rejection of trials (Conley and Fite 1980) and a requirement for extensive training. The new method described produces a far lower rejection rate, with 11 out of 12 trials being included for final analysis. Video analysis does not return an automated head angle measurement if the bird looks directly

upwards or downwards as the head marking is out of view. A threshold is set for how many head angles it is acceptable to miss. For this validation, any video from which 5% or more of the frames did not return a head angle was simply rejected. With an additional analysis and/or manual correction for missing frames, 100% of all head angles can be obtained. Here, no extra analysis or manual correction of video's was done, carrying out only a straight single run of the automated analysis, which allows acceptance (>95% frames detected) in over 90% of trials. The technique requires no training of the subject beyond familiarisation with handling by humans, and a previous experience of wearing the sock bandage restraint. All subjects can be tested without difficulty in the present case, without the need to reject any prior to automated analysis of the data.

This method also gives the opportunity to use reasonably short trial times, as the stimulus can be presented in the absence of other visual stimulation and the subjects remain alert but unstressed, resulting in a clear, consistent OCR response. This facilitates testing of many different parameters, in a short session, allowing larger amounts of useful data to be obtained, with little or no impact on the subjects.

The ability to present a huge range of possible variations in stimulus parameters while using an accurate (and crucially non-invasive) method for recording head movements, as well as the possibility of short quick-fire trials, makes it possible to collect a far greater amount of useful quantitative measurements, over a much greater range of stimulus parameters, in a shorter time, and critically, with the use of fewer subjects.

The use, in previous chapters, of Helmholtz coils and LCD screen technology as well as our newly developed video analysis and saccade identification software, has demonstrated that meaningful experiments can be carried out using these approaches. In the future, the optocollic response/LCD screen approach may be combined with the Helmholtz coil system

in order to examine the psychophysical limits of vision under different ambient magnetic fields (for detailed discussion see Chapter 7 – Future directions).

This work was useful and informative in its own right, but also served the purpose of allowing meaningful investigations to continue, despite unexpected delays in making ready the equipment for other experiments. For the next chapter of this thesis, another highly novel and experimental approach to investigating magnetoreception will be employed, relying on the principle of prepulse inhibition.

Chapter 6 Using prepulse inhibition to investigate magnetoreception

The field of magnetoreception research is beset by a very serious lack of reproducibility in results between research groups, due, in large part, to the necessity of having to create experiments, and the necessary bespoke equipment supporting them (often involving biologists who are unlikely to be trained or experienced in electrical engineering). A fundamental tenet of the approaches in this thesis is to use novel, but reproducible, equipment and techniques, to examine known, reliably reproducible responses, such that with the necessary resources, the experiments can be adequately reproduced. Prepulse inhibition is a well-established method for investigating psychophysical aspects of animal perception. The use of large numbers of trials, coupled with the relatively well understood nature of the reflexive startle response in vertebrates, makes for a highly reliable and, crucially, reproducible method of experimentation. More details on prepulse inhibition, and the approach to the methodology are in Chapter 2.

An unexpectedly large amount of time and effort had to be invested in the design and implementation of the equipment used for prepulse inhibition (PPI) experiments, due to a series of unfortunate problems with the hardware. However, it was eventually possible to carry out useful experiments with a flash (visual) startle stimulus, allowing for proof of concept, and indeed original investigative experiments to be carried out.

Verifying prepulse timing

A short experiment was undertaken to verify the timing of stimulus presentation. Two things were to be established, 1, that the timing of the prepulse in relation to the startle stimulus (inter stimulus interval or ISI) was as intended, and 2, that the magnetic field changes could be presented with suitably accurate timing to be used as a prepulse. The first question can

be answered simply using exploratory trials with actual subjects because if a significant PPI can be elicited, then the ISI is by definition, an effective one. Evidence for this efficacy of delivered ISI is presented in Figure 6.1. Additionally, there is no reason to doubt the accuracy of the SDI equipment sending the signals at the planned intervals. In order to elucidate the other potential concerns mentioned, data were plotted for both magnetic field variation times, and expected session times, calculated by examining the inter trial intervals (ITIs) and an additional 1.067s that the SDI software takes to begin executing each trial. If these intervals match with no signs of drift, it can be taken that both computers involved in the experiment are synchronised, i.e. the signal from the SDI software computer is reaching the computer that runs the coils, and the act of changing the magnetic field conditions takes place at the intended time.

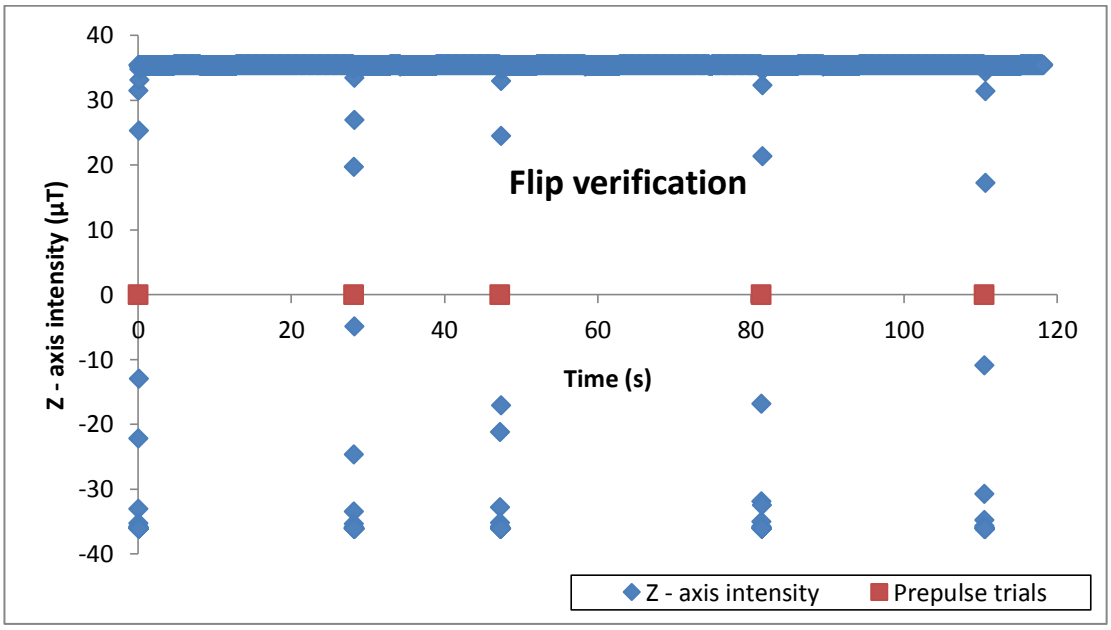

Figure 6.1 Verifying prepulse timing. Red squares represent expected time of trial.

Figure 6.1 shows periodic change in z axis intensity of magnetic field, as measured with a magnetometer, which represent inclination flip prepulses. The red squares represent the expected time of each prepulse trial. No signs of time offsets or drift are apparent. This figure contains only 5 magnetic field flips for illustration. However, when similar charts are plotted

containing an entire session of 40 prepulse trials, there is still no sign of any drifting being present, and all prepulses align well with the expected prepulse trials. This figure cannot be displayed here due to the very large size necessary to visibly discern important details.

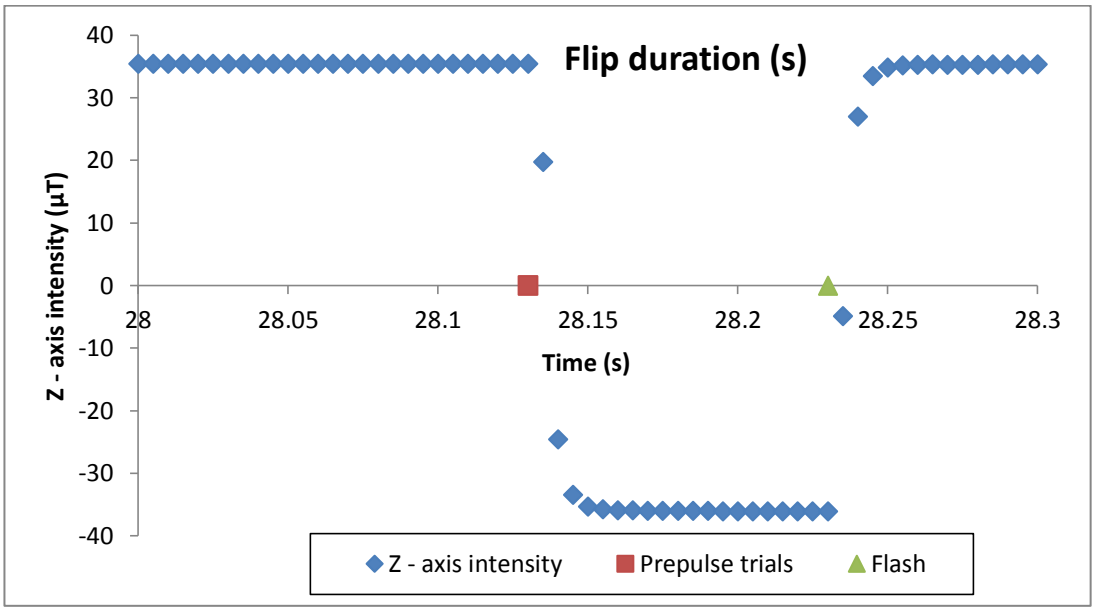

Figure 6.2 A closer look at the timing of inclination flip prepulse. Red square represent start of trial, green triangle represents end of trial.

Figure 6.2 shows an even more magnified view of the same data. One prepulse trial has been selected to look in more detail at timing of the all-important prepulse. It is clear that very shortly after the expected start time of the trial (red square) the field inclination flips, and that it remains flipped until the startle stimulus is presented (camera flash) at which point it duly returns to the normal/unmanipulated condition. This is exactly what one would hope to see and confirms that the physical aspects of the experiment are being timed, and crucially, executed as intended. The period over which the inclination is inverted is exactly 100ms as programmed and ends immediately after the startle stimulus is presented.

PPI experiment – Initial exploration

Upon realising that the use of a sound based startle pulse or prepulse was unlikely to be achieved in the time available for the project (see Chapter 2), the use of a LED prepulse and bright light flash startle was implemented. Various exploratory trials were carried out in order to refine the technique and then some data were formally recorded to test its efficacy.

Four birds were used, in experiments that were carried out over six days. Initially, a trial was recorded using one bird, in which the flash was unplugged. This was to ascertain that the prepulse does not itself cause a startle, and to give a baseline against which to measure the parameters of the startle response.

Then a brief comparison of inter-stimulus intervals (ISI) was carried out, to explore whether the standard (see Chapter 2) delay of 100ms would be suitable.

Following this, trials were carried out using a LED prepulse, which appeared 100ms before the flash startle pulse and was left on, such that the LEDs were illuminated for the full 100ms preceding the startle stimulus. In magnetic field treatment trials, the LED prepulse was replaced by a sudden flip of the inclination (vertical) aspect of the ambient magnetic field. This flip began 100ms prior to startle stimulus, maintaining the same inter-stimulus interval as in the LED control. As shown in Figure 6.2, the flip takes only a few milliseconds to occur, and the inclination remained inverted for the remainder of the 100ms ISI.

In a given session, each bird was exposed to 2 trials of 80 startle stimuli each. In one trial there would be 40 startle only presentations and 40 with LED prepulse presentations, and in the other trial, there would be 40 startle only and 40 magnetic prepulse presentations. Two sessions were carried out on each bird, one in darkness and one in white light (158lux). Due to equipment and animal licencing constraints, dark and light trials had to be conducted on different days, though the order was varied between subjects and a mixture of light and dark

sessions carried out each day on different birds to minimise effects resulting from testing on different days.

Four birds were used initially, however problems with the flash (battery failure) resulted in data being compromised for subject 52 in the initial 'dark' trial, so 52 was not used in the following 'light' trials, during which there was also an equipment based problem with the data for bird 51, hence dark experiments have data for 3 birds, and light experiments, only 2. Two important measures are returned by the SDI software, these are:

- Vmax = Maximal voltage (mV) of the response, the 'peak' amount of movement.
- Tmax = Time (ms) between startle stimulus presentation and Vmax, the 'latency' of the response.

Baseline (no startle stimulus) 1 subject

A session was carried out with the flash turned off, meaning all aspects of the experiment remain unchanged, but no flash stimulus was presented, hence no startle response should be observed. The purpose of this was to assess the likely magnitude of movements involved in regular behaviour with no startle stimulus, and whether they would be large enough to be mistaken for startle responses as identified by the SDI software.

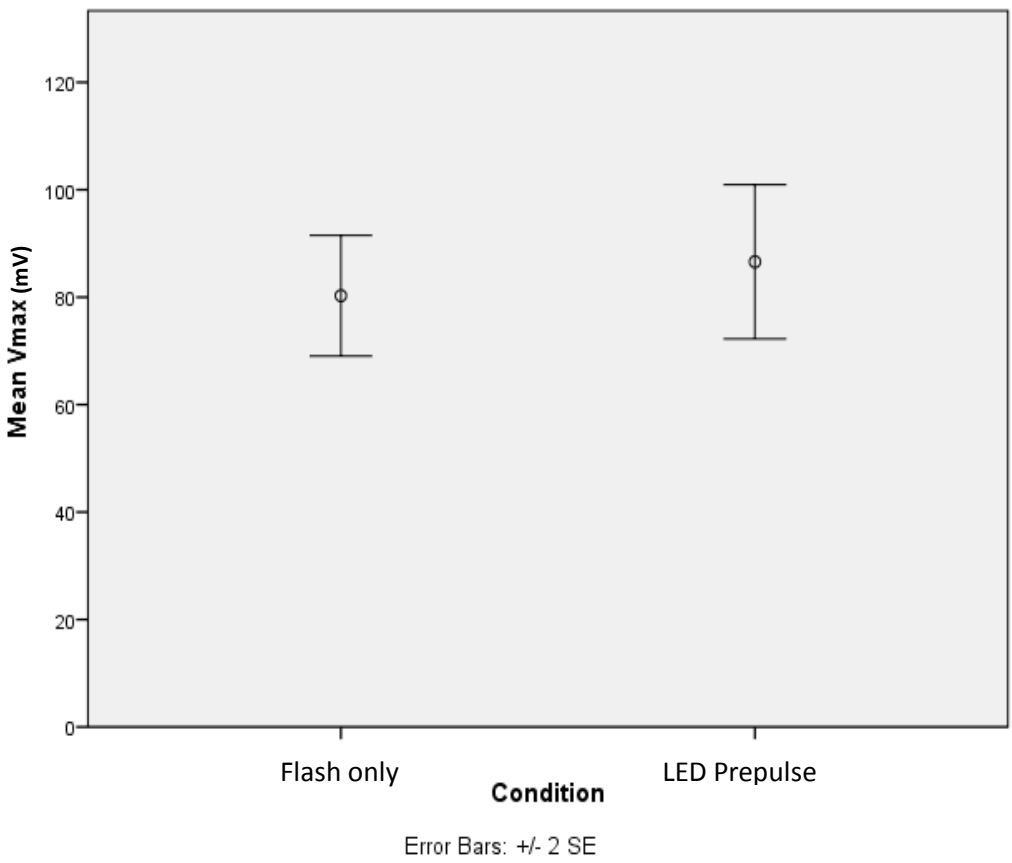

Figure 6.3 Comparing mean amplitude of response between Flash only (startle) and LED prepulse sessions, when the flash startle stimulus is turned off.

As seen in Figure 6.3, there is no obvious difference in Vmax arising from PPI, indicating that no startle is occurring

1 bird ISI comparison

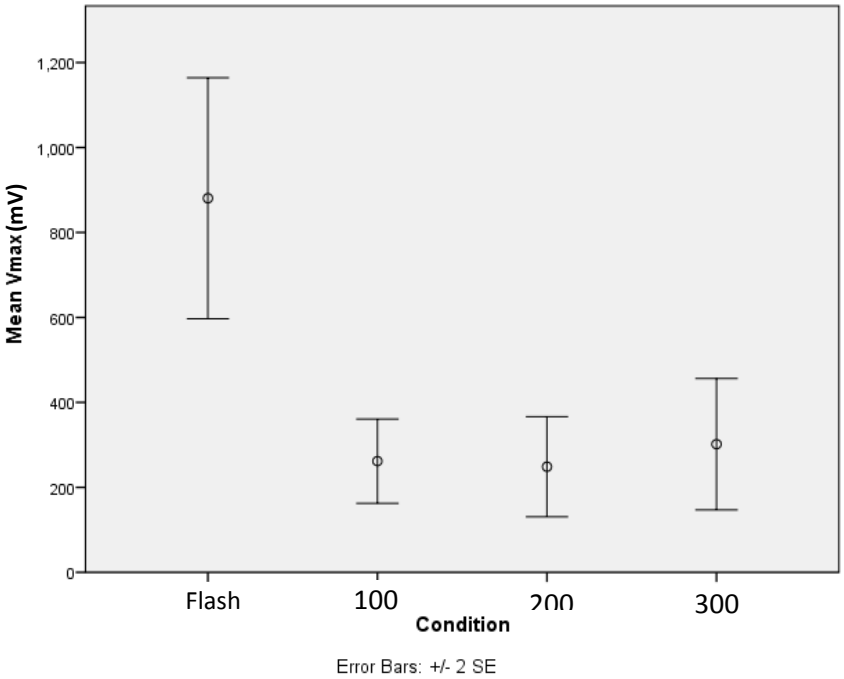

Figure 6.4 Comparison of inter trial intervals. Prepulse inhibition is apparent, and ISI of 100-300ms are effective (1 subject).

The ISI comparison in Figure 6.4 illustrates three important points. Firstly, there is a good clear sign that PPI is elicited utilising the LED prepulse, with mean amplitude of response being reduced from approximately 900 on the startle only condition, to around 300 in all prepulse conditions. Secondly the PPI is consistent at 100 ms ISI and even with longer (200-300ms) ISI times, which suggests that the planned 100ms ISI is effective at inhibiting the startle response. The third useful observation is that the amplitude of response when the startle is used, both with and without a prepulse, is noticeably greater than those in Figure 6.3, representing the activity when no startle stimulus is presented (mean amplitude of response is around 300 mV in response to startle with a prepulse, as compared with a mean of around 80-90mV with no startle).

Darkness LED prepulse

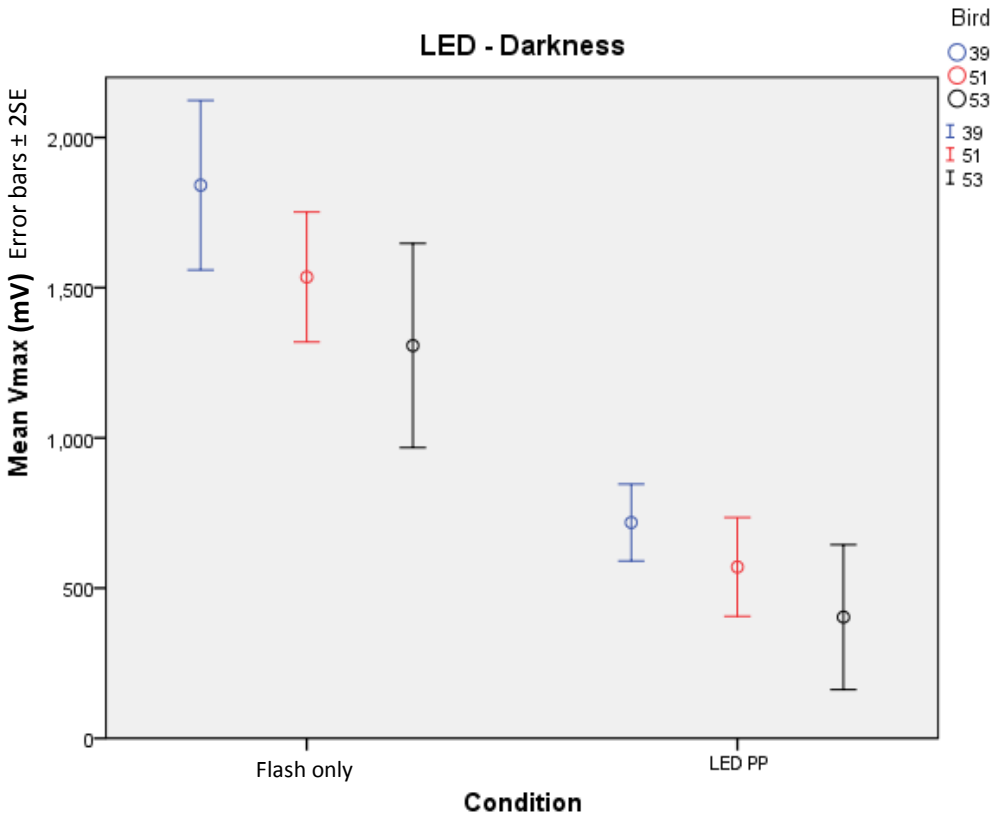

Figure 6.5 Comparison of mean startle amplitude between Flash only (startle) and LED PP (LED prepulse) sessions, for 3 subjects.

Figure 6.5 shows that a clear decrease in startle response is apparent when preceded by the LED prepulse under dark conditions. All three subjects show a marked decrease in the mean amplitude of response when the prepulse is used, and the reduction in amplitude seems remarkably constant across individuals, despite there being clear variation between individuals receiving the same treatment i.e. there is a variation in responsivity of individuals under the startle only condition, and under the prepulse condition, but the decrease in response between startle only and prepulse conditions appears constant for all individuals with reductions in response amplitude, being on the order of approximately 1000mV.

Darkness magnetic prepulse

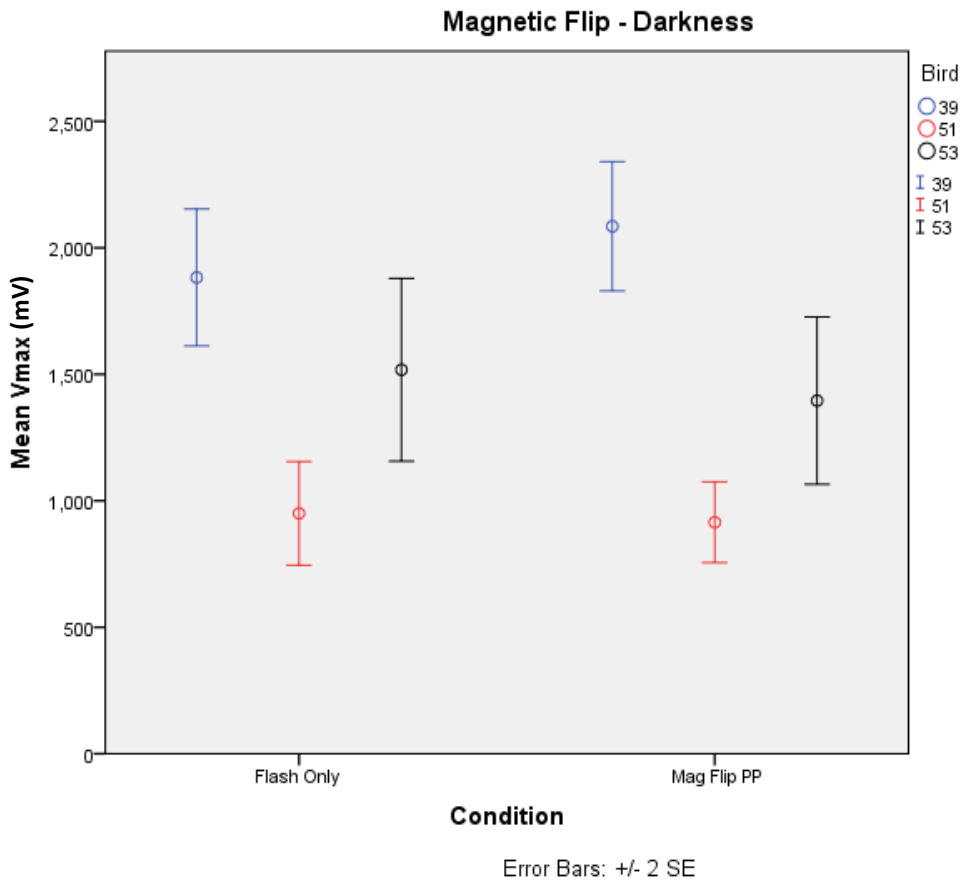

Figure 6.6 Comparison between mean startle amplitude between Flash only (startle) and Mag Flip PP (magnetic prepulse) conditions in the dark for 3 subjects.

A very different pattern is observed in Figure 6.6 (comparing mean amplitude of response when using a magnetic inclination flip prepulse) to the preceding figure of LED prepulse under dark conditions. The variability between subjects receiving the same treatment is still present, however in this case all signs of PPI are absent. For each subject the mean amplitude of response is approximately the same under both startle only, and magnetic inclination flip prepulse conditions, with error bars overlapping almost completely. Subject 53 shows signs of a very slight decrease in mean response amplitude under the prepulse condition, but subject 39 shows an increase. In both cases this trend is unlikely to be of any meaningful

significance. Thus, there is no evidence of prepulse inhibition occurring when using a magnetic inclination flip prepulse under dark conditions.

Light LED prepulse

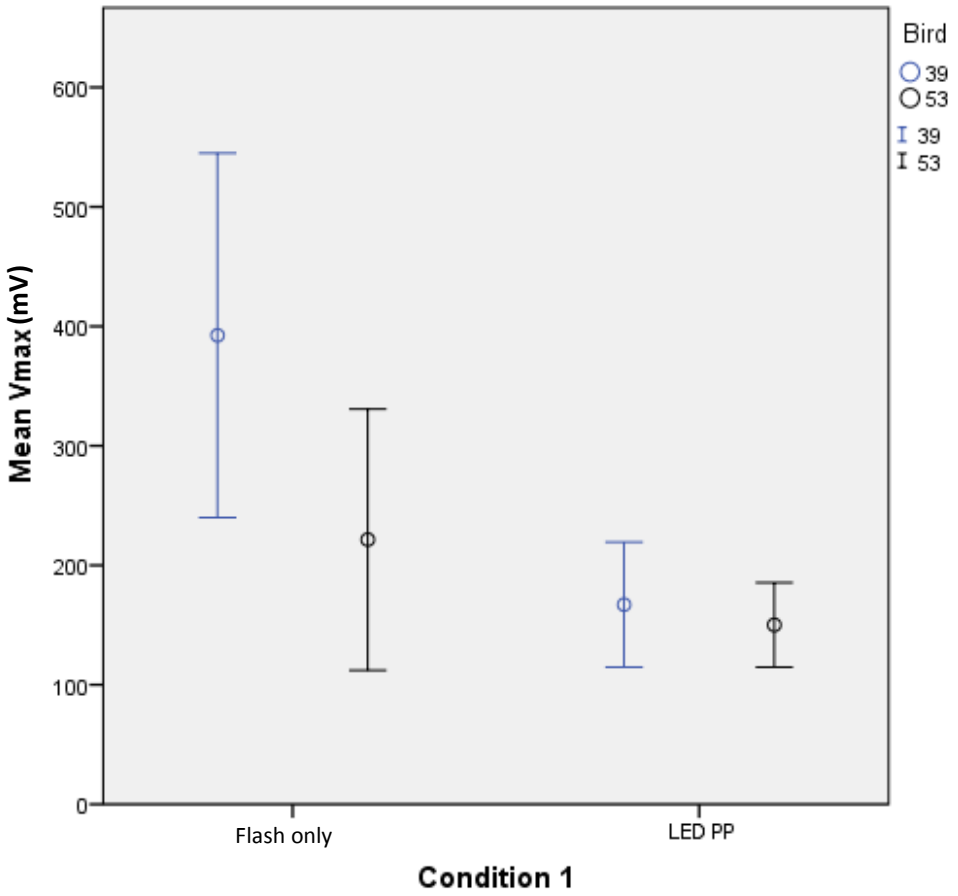

Figure 6.7 Comparison between mean startle amplitude between Flash only (startle) and LED PP (LED prepulse) conditions in the light for 2 subjects. Error bars +/- 2SE.

Figure 6.7 shows clear signs of a reduction in the startle response under LED prepulse lit conditions compared to the startle only condition in one subject (39), and some suggestion that PPI is occurring in both subjects, although the reduction is not as large in subject 53. This, however, may be the result of low responsivity to the startle, which makes it difficult to discern the reduction caused by PPI. This suggestion is supported by the fact that, in both subjects, the mean amplitude of response to the LED prepulse condition is approximately similar, and thus the reason for a less significant reduction resulting from PPI is simply that

the subject responded less intensely to the startle stimulus alone. This, taken together with the observed variation between subjects in previous figures, suggests that some subjects will perhaps give far more clear/useful results than others, something which should be considered in experimental design of future experiments.

Light magnetic prepulse

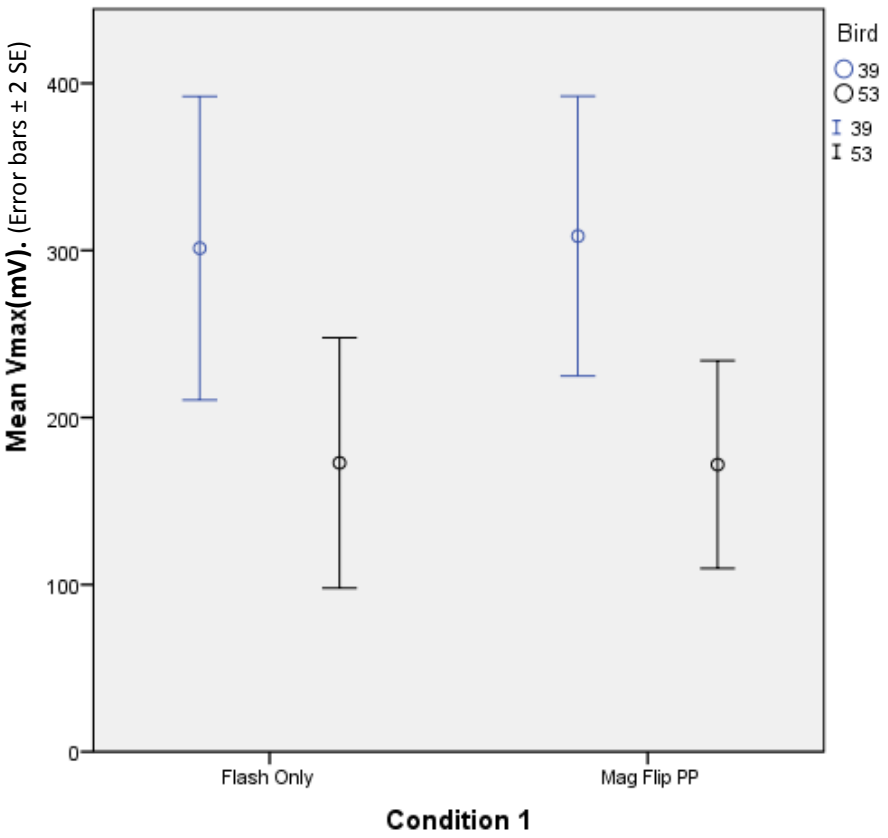

Figure 6.8 Comparison between mean startle amplitude between Flash only (startle) and mag flip PP (magnetic prepulse) conditions for 2 subjects (Error bars ± 2 SE).

When using a magnetic inclination flip prepulse under lit conditions, the mean amplitudes of response, as in Figure 6.8 show no signs of a reduction under the magnetic flip prepulse condition compared with the startle only condition. Subject 39 responds more strongly to

the startle stimulus in both cases than subject 53, reflecting the pattern seen in previous figures, but no evidence for PPI is present.

Conclusions

From this exploratory investigation it appears that a reliable PPI in response to a light prepulse stimulus can be elicited in darkness, and potentially in light, although startle response amplitudes are so much lower in lit conditions (200-400mV as compared to >800mV in darkness) that meaningful comparison between LED and magnetic prepulse in light may be problematic. The overall outlook is that there does not seem to be a PPI effect from the magnetic prepulse, however a more detailed study with a greater number of subjects is necessary. This is the basis of the following experiment.

Main PPI experiment

The previous exploratory experiment demonstrated a measurable PPI in 3 birds in darkness, using a control prepulse of an LED switching on 100ms before the startle stimulus (flash of light) was delivered, and appeared to show some effect in 1 of 2 individuals tested in lit (158 lux) conditions.

No noticeable effect of a magnetic inclination (z-axis) flip could be found, however due the small number of subjects in this preliminary experiment no clear conclusions could be drawn. Extra batteries were obtained for the startle flash unit, meaning more subjects could be tested in a session than in the previous exploratory trial.

In the current study 5 homing pigeons were subjected to various paradigms over 4 days.

Details

Five birds were used, labelled here: A, D, F, G & H. Prepulses (presented before the flash startle stimulus) used were:

- LED switches on 100ms prior to startle (stays on until startle)
- Flip of inclination (z-axis only) 100ms prior to startle (stays flipped for the 100ms)
- Null field (geomagnetic field cancelled) for 100ms prior to startle

Each different prepulse was tested on a different night:

- Night 1. Flash startle vs LED prepulse. Lights on only – to identify responsive subjects
- Night 2. Flash startle vs Flip prepulse. Duplicated with lights on and off
- Night 3. Flash startle vs Null Field prepulse. Duplicated with lights on and off
- Night 4. Flash startle vs LED prepulse. Duplicated with lights on and off

On nights 2-4, there were two sessions for each subject, one in light (158 lux) and one in darkness. Light and dark sessions comprised 80 trials, sub-divided into 40 startle only trials

and 40 with a prepulse. Between nights 1 and 2, a batch of new batteries became available which also allowed longer sessions Therefore, night 1 has only 'light on' sessions.

LED lights on test

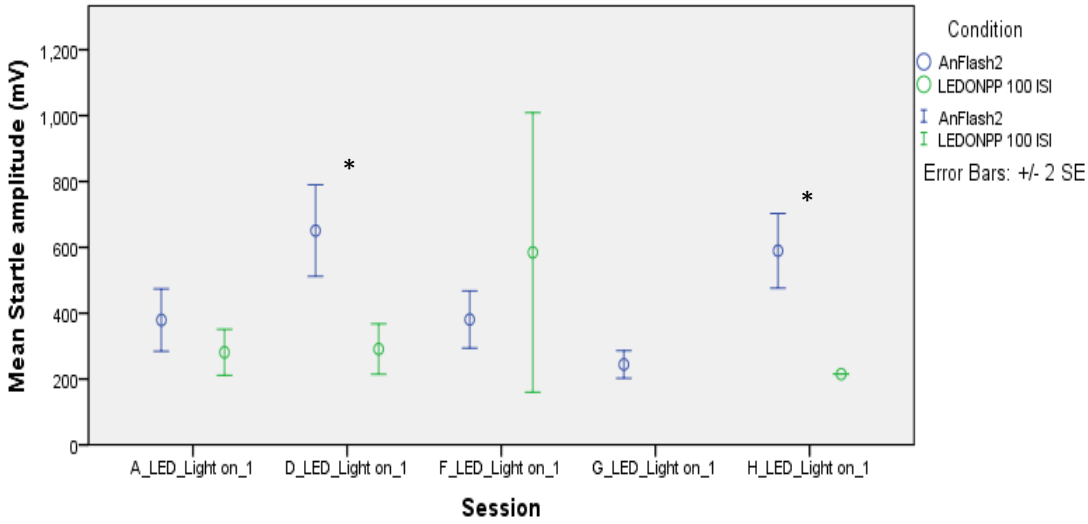

Figure 6.9 Comparison between mean startle amplitude between Flash only (startle) and LED prepulse conditions for 5 subjects (*=significant p<0.05, Repeated measures ANOVA). The capital letter prefixing each session denotes the subject. Blue markers (left hand marker in each session pair) are startle only trials, green markers represent prepulse trials. AnFLash2 (Blue) = Startle only trials, LEDONPP 100 ISI (Green) = LED prepulse trials.

Figure 6.9 depicts an all subjects comparison of mean peak amplitude of startle response in startle only and LED prepulse trials. Lights are on in all sessions. This was to identify any subjects which would display evidence of PPI when using a flash of light as a startle stimulus, in light conditions.

Initially, a trial was carried out to assess whether an LED prepulse was effective in eliciting a prepulse inhibition when used under lit conditions. Figure 6.9 shows a comparison of the mean startle amplitude in millivolts, between startle only and LED prepulse conditions, for each subject separately (error bars represent 2 standard errors of the mean). It can be seen that subjects D and H show a significantly different response to the startle only, and LED prepulse conditions, both having an inhibited response under the LED prepulse condition, hence demonstrating PPI. Subject G also has a clear difference between the two conditions,

with no measurable startles occurring under the LED prepulse condition. However, the low mean startle amplitude for the startle only condition suggest that low responsivity in general may be a confounding factor in this particular instance. Subjects A and F do not show clear PPI, however there is some difference between the mean startle amplitude under the different testing conditions for each subject, suggesting that the LED prepulse might be having some kind of effect. Taken together, these results indicate that, in at least some of the subjects, the LED prepulse is perceived and hence does result in prepulse inhibition of the startle response.

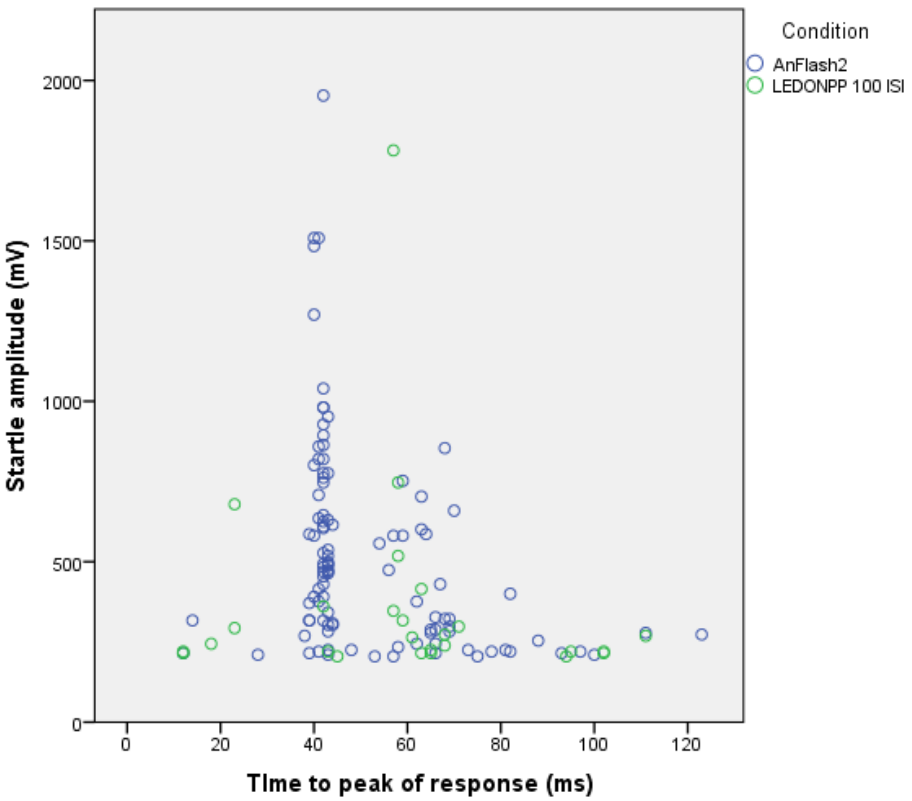

Figure 6.10 Time to peak of startle response for startle only vs LED prepulse in the light (5 birds).

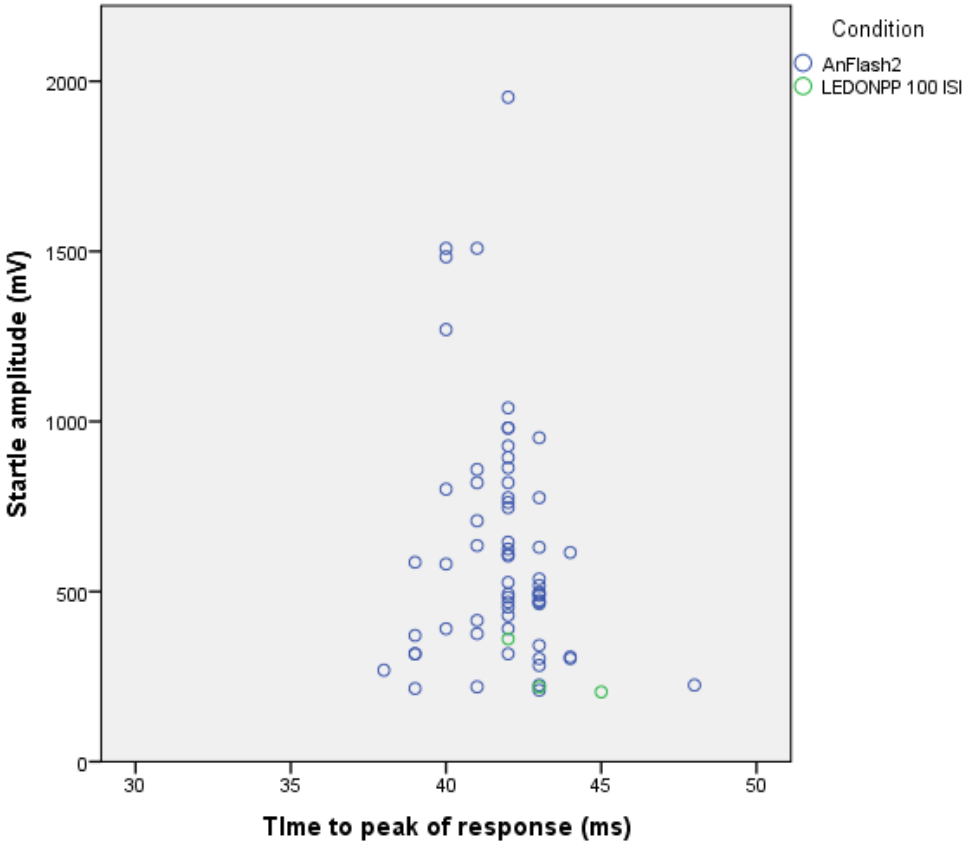

Figure 6.11 Closer look at peak response timings for startle only vs LED prepulse in the light.

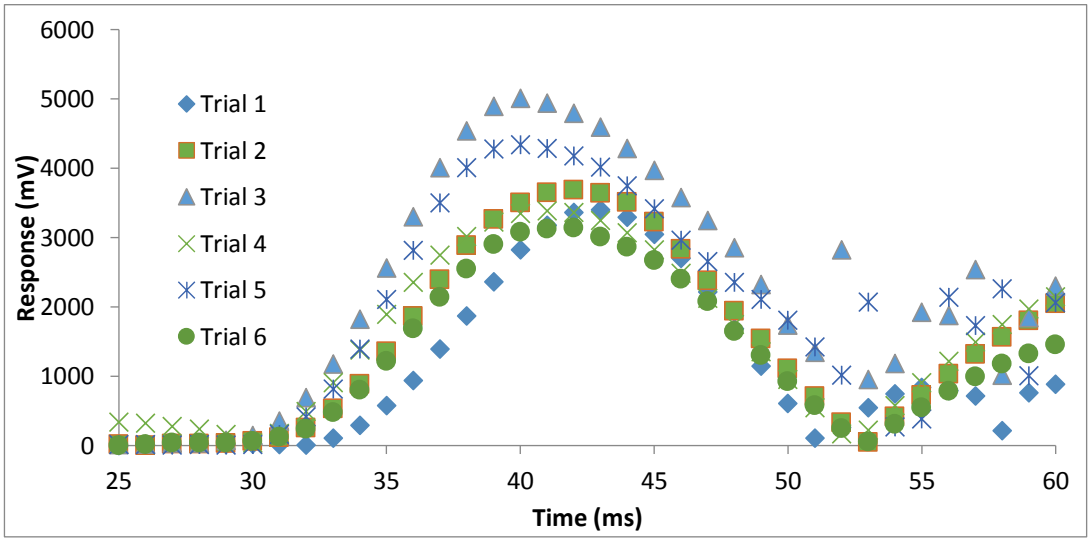

Figure 6.12 Latency of startle response. The peak of the startle response (in mV) can be seen to occur approximately 40-43 ms from the time of startle stimulus onset (t=0). No prepulse used.

Figure 6.10 shows the time to *peak* of the startle response (latency of response) for all birds combined under the LED light on paradigm, with a magnified view in Figure 6.11. A clear trend can be seen in the timing of the response, with the majority of large amplitude responses clustering around 42 ms after the startle stimulus is presented. Stitt *et al*. (1976) reported mean startle latencies of approximately 32ms, measuring from stimulus presentation to the *start* of the startle response. They also reported that PPI does not affect latency, except when extremely small (<10ms) inter stimulus intervals are used. The measurement of time to peak of response used in the current study represents one measure of latency (automatically reported by SDI software), however this obviously differs from the measures used previously in the literature (i.e. time to start of the response). Figure 6.12 shows a sample trace of raw data (1 bird) showing the startle response to 3 startle only (blue), and 3 LED prepulse trials (green). It can be seen from these data that the approximate time of the start of the startle response is 30-33ms after the startle stimulus onset, and that the time from start of the response, to the peak of response is approximately 10ms. Therefore the current measures of latency of around 42ms to peak of the response, agree very closely with the previously reported latencies of approx. 32ms to start of response. This result is encouraging as it demonstrates again that the equipment is working as expected, that timings of stimulus presentation and response measurement appear to be accurate, and that the parameters of the response itself are in keeping with those previously reported for pigeons.

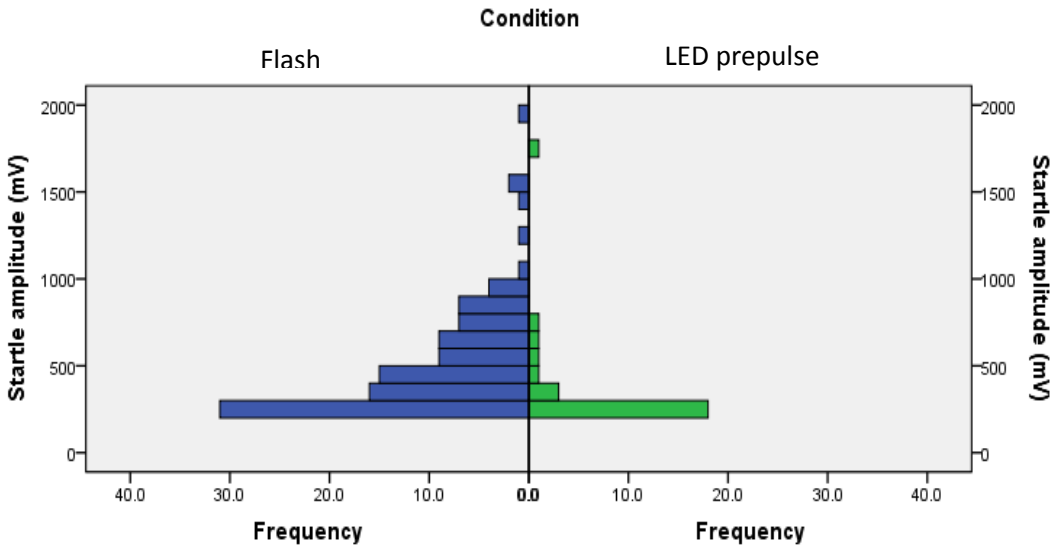

Figure 6.13 Frequency histogram comparing Startle amplitude frequencies between startle only (Flash) and LED prepulse trials.

Figure 6.13 displays a frequency histogram, representing a simple count of all startle responses in the LED lights on test session (subjects combined), binned by startle amplitude. Two clear patterns are immediately evident: 1, the overall number of startle responses is far greater in the startle only condition than in the LED prepulse condition, and 2, many more large amplitude startles occur in the startle only condition. Whilst the general low amplitude trend in the prepulse responses is to be expected (signifies that PPI is indeed occurring), it is clear that the prepulse also has an effect on the number of startles occurring. This is likely to be because the startle response is inhibited to such an extent, that the movement is no longer recognised as a startle response by the SDI software. This presents the interesting possibility that frequency of startle responses may offer some useful measure of PPI, along with the expected limitation of response amplitude.

Taken together these results clearly indicate that an effective prepulse inhibition is elicited at least in some subjects.

Main experimental sessions

Comparison of mean response amplitude

Inclination flip prepulse

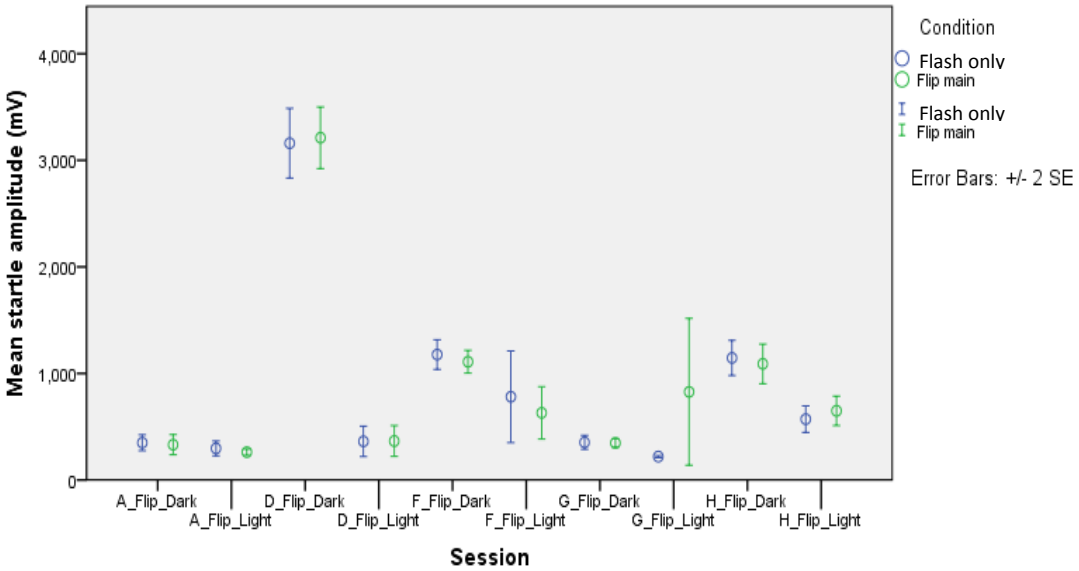

Figure 6.14 All subjects/sessions comparison of mean peak amplitude of startle response in startle only and Inclination flip prepulse. The capital letter prefixing each session denotes subject. Blue markers (left hand marker in each session pair) are startle only trials, green markers represent prepulse trials. Flash only (Blue) = Startle only trials, flip main (Green) = Inclination flip prepulse trials.

Comparing the mean amplitude of startle response under the different magnetic field conditions/sessions shows a number of relevant patterns. There are no obvious instances of PPI occurring in either the flip (Figure 6.14) or null (Figure 6.15) sessions, under either lit or unlit conditions. In the flip session (Figure 6.14) all subjects can be seen to have responded with measurable startle responses under each treatment condition. Subject D is clearly more responsive than the others under unlit (dark) conditions, in this and in other sessions, yet despite having a very clear and pronounced startle response, no evidence of PPI is apparent in the comparison of means of startle amplitude. Only subject G, under lit conditions shows any sign of a difference between startle and prepulse conditions, and in this case the large range of standard errors, overlapping the values for startle only, suggest this difference is

not significant. It may be that very few startle responses were recorded under these conditions. This will be investigated later in this chapter.

Null

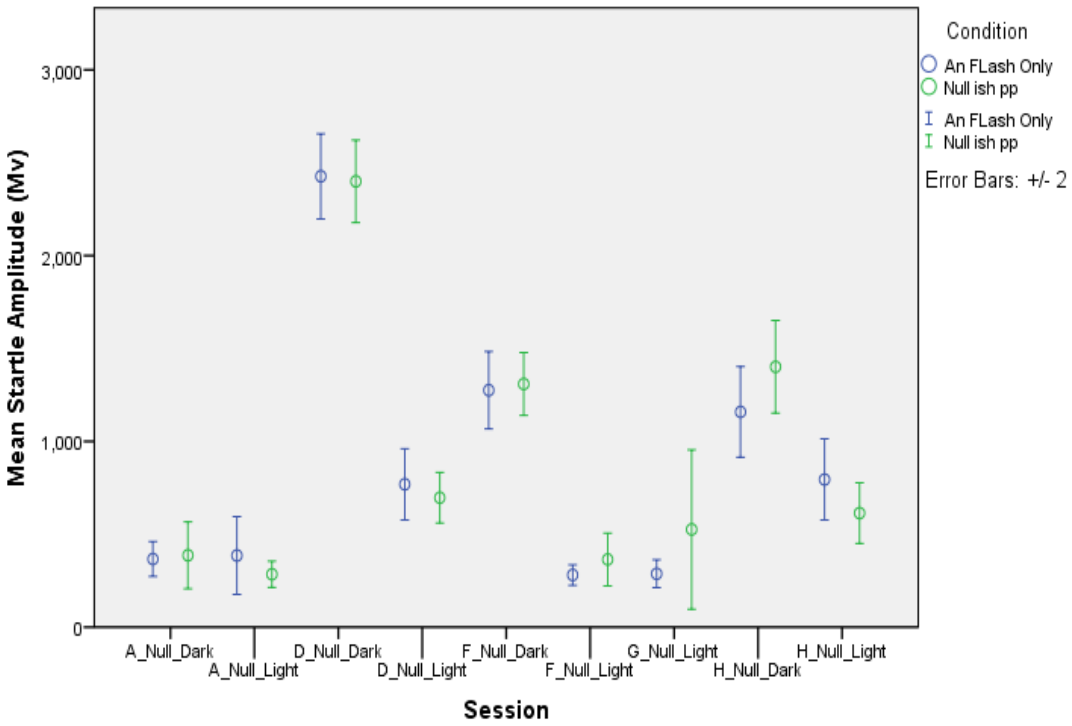

Figure 6.15 All subjects/sessions comparison of mean peak amplitude of startle response in startle only and null field prepulse. The capital letter prefixing each session denotes subject. Blue markers (left hand marker in each session pair) are startle only trials, green markers represent prepulse trials. An Flash Only (Blue) = Startle only trials, null PP (Green) = null field prepulse trials.

A comparison of the mean amplitude of startle response for the null field prepulse session showing some similar trends to those observed under the flip prepulse session can be found in Figure 6.15. Subject D is again very responsive under the unlit condition. Subjects F and H also respond clearly in unlit conditions, having startle amplitudes with a mean above 1000mV (similar to those shown in the previous figure representing the flip prepulse), and other subjects have mean responses between 300-800mV. Again, no subject shows any clear sign of prepulse inhibition occurring under lit or unlit conditions. In 2 subjects (A and H) under lit conditions, the startle response in the prepulse condition is lower than in the startle only condition, however the overlap of error bars, combined with the fact that the opposite is true in some other cases (F and G lit, H unlit) suggest this is not a significant difference arising

from meaningful PPI. As with the previous figure, looking in more detail at the number and size of responses from individual subjects may shed light on this, and is carried out later in this chapter.

LED prepulse

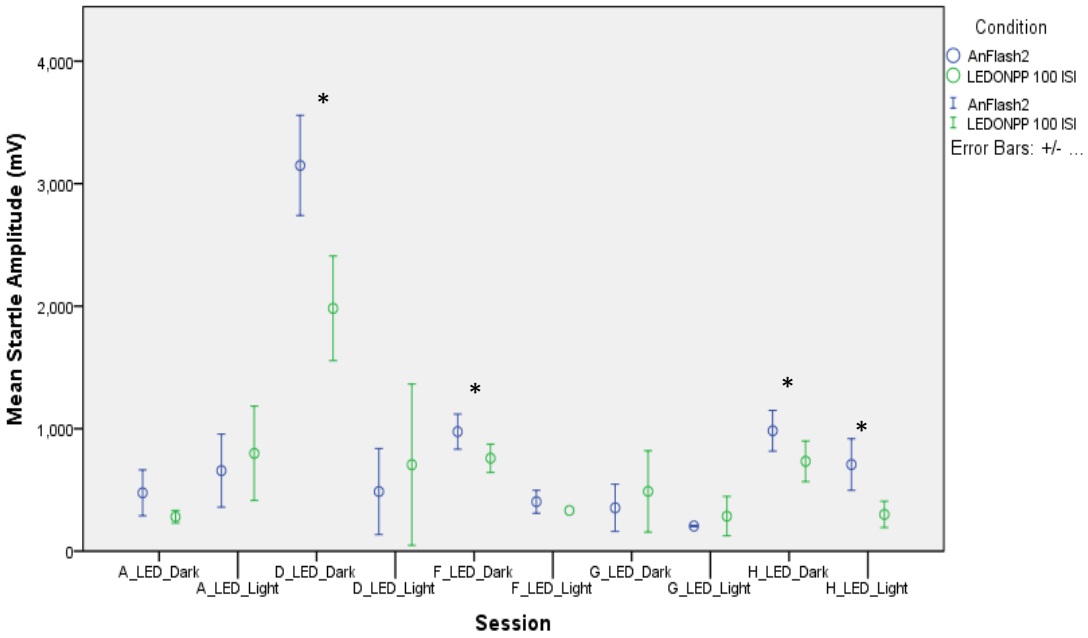

Figure 6.16 All subjects/sessions comparison between Startle only and LED prepulse, trials repeated in darkness and lit room conditions. The capital letter prefixing each session denotes subject. Blue markers (left hand marker in each session pair) are startle only trials, green markers represent prepulse trials (*=significant: D_LED_Dark F=17.251 df=1 P<0.001; F_LED_Dark F=5.319 df=1 P=0.024; H_LED_Dark F=4.063 df=1 P=0.047; H_LED_Light F=14.021 df=1 P<0.001).

Figure 6.16 displays the mean amplitude of the startle response for all subjects under lit and unlit conditions both with, and without, an LED prepulse. Subject D responds more vigorously than all others under unlit conditions, matching its trend in previous sessions, however in this case there is a clear indication that PPI is occurring, as the mean is over 100mV less in the prepulse condition, additionally, the 2 x standard error bars do not overlap, suggesting that this may be a significant difference in the means. Subject H under lit conditions also shows a difference between means with error bars that do not overlap, and in some other subjects, a reduction in mean response is evident, although error bars do overlap (A-lit, F-dark, F-lit, H-dark). With a prepulse of LED light, over half of the sessions show some sign of

PPI occurring, this pattern is very different from the previous 2 figures (representing the same data for null and flip magnetic prepulse conditions), in which no subjects/sessions show any clear sign of PPI.

Timing of response (startle latency)

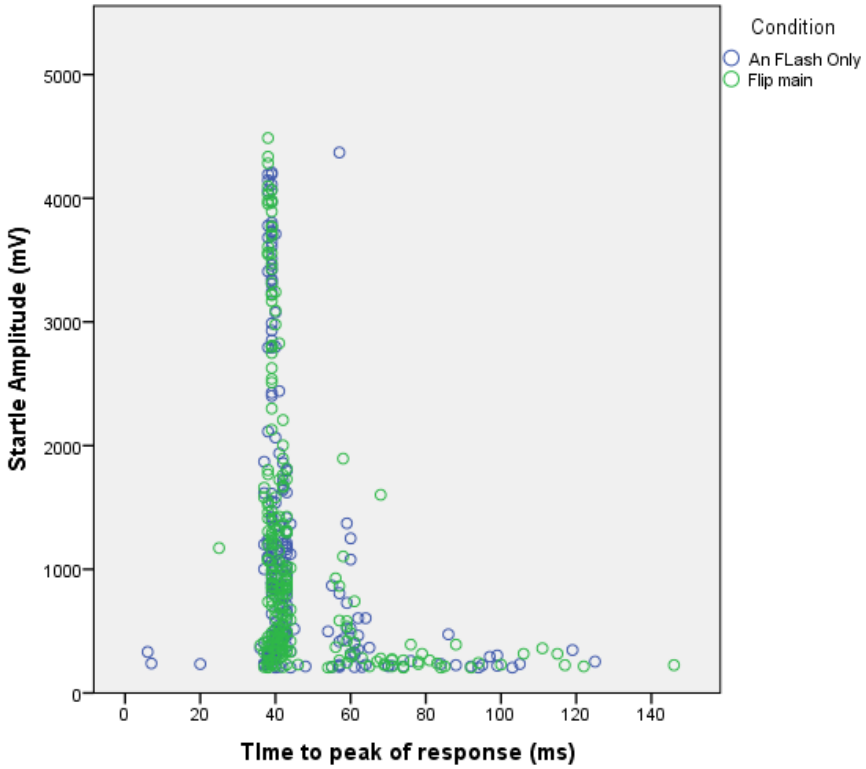

Figure 6.17 Time to peak of startle response for inclination flip prepulse and startle only.

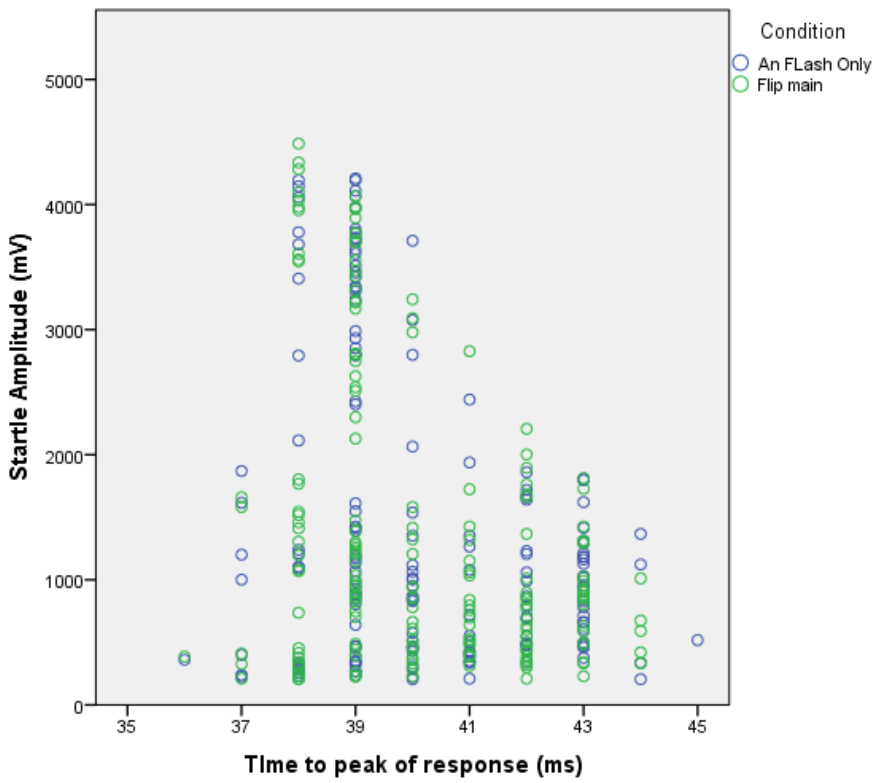

Figure 6.18 Closer look at peak response timings for Inclination flip sessions and startle only.

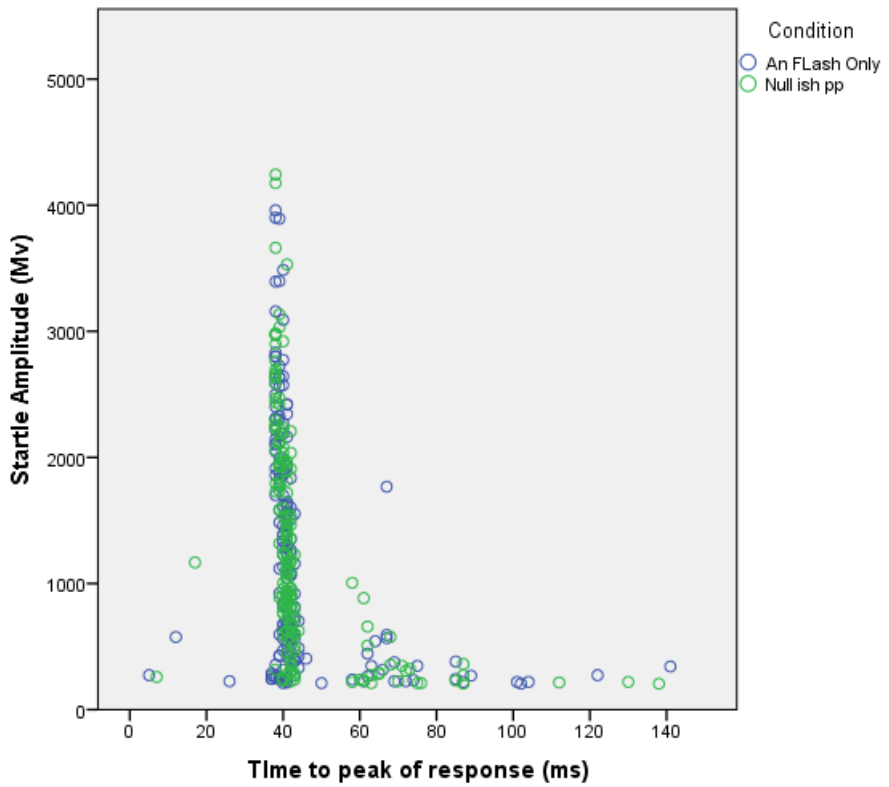

Figure 6.19 Time to peak of startle response for null field prepulse and startle only.

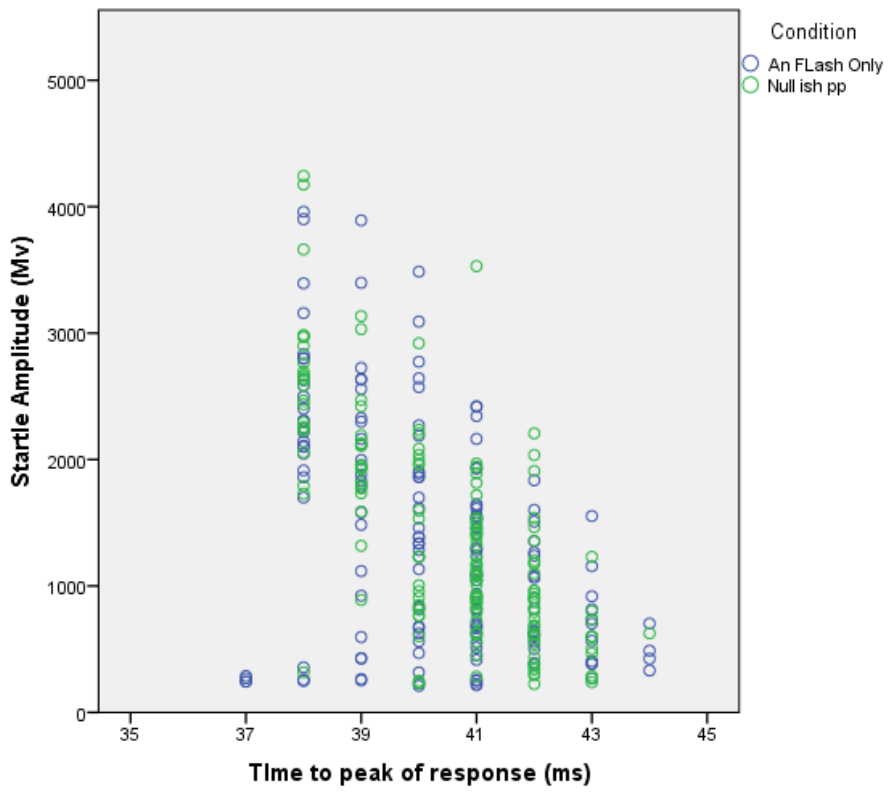

Figure 6.20 Closer look at peak response timings for null field prepulse and startle only.

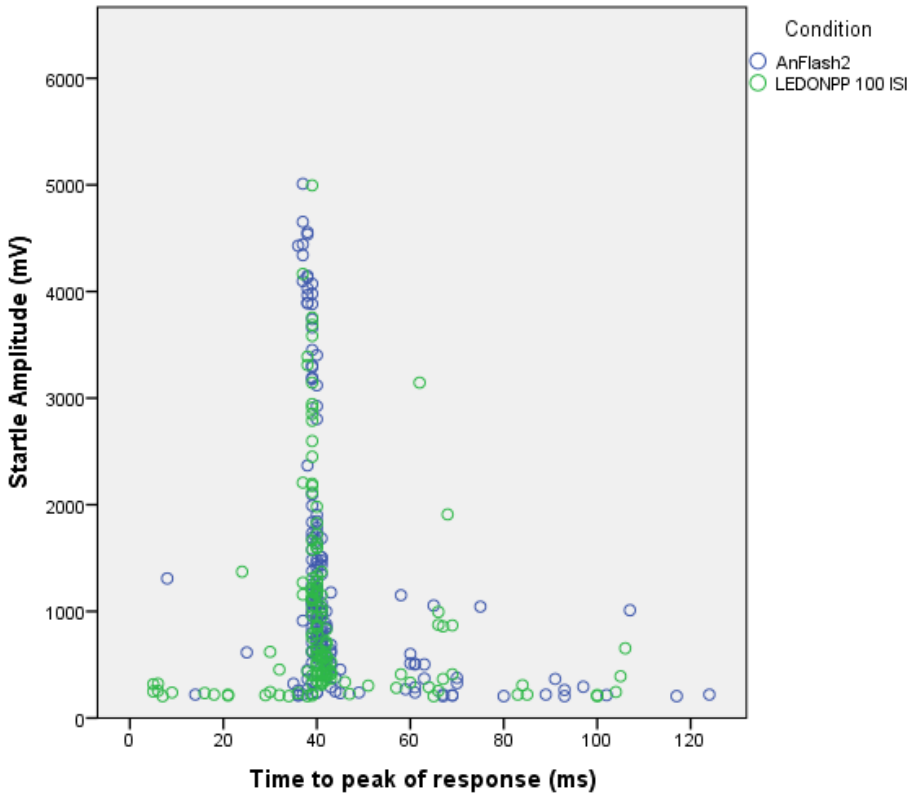

Figure 6.21 Time to peak of startle response for LED prepulse and startle only.

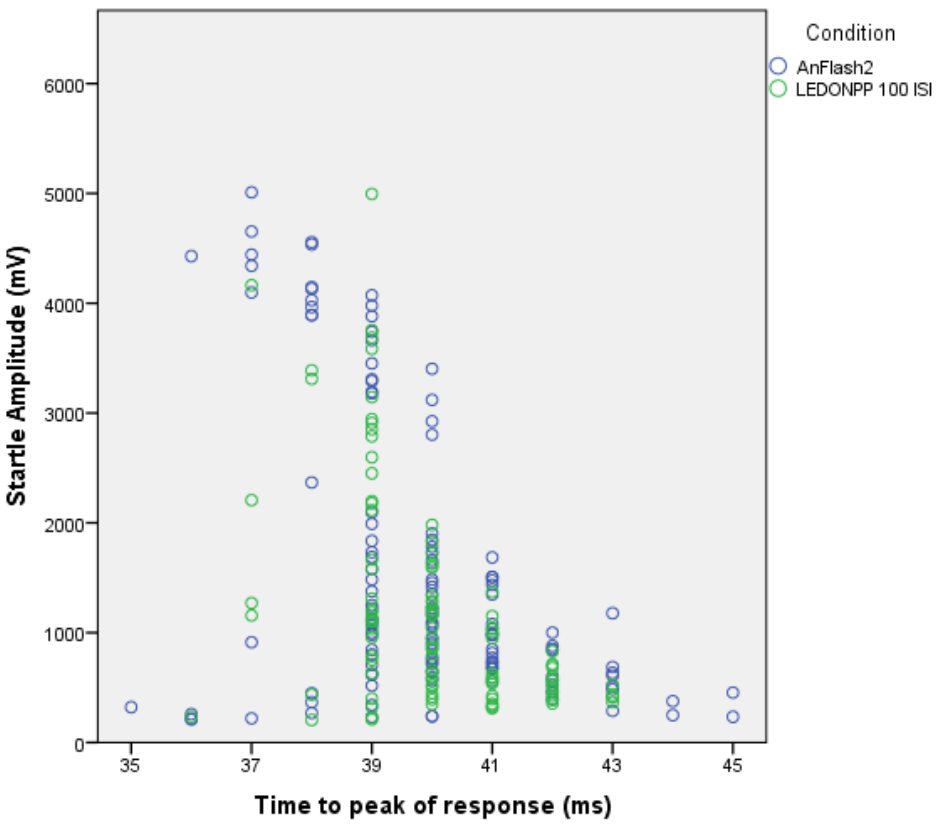

Figure 6.22 Closer look at peak response timings for LED prepulse and startle only.

Figures 6.17 - 6.22 show the relationship between peak amplitude of response in mV, and time to peak of response in milliseconds. Comparing the figures makes it clear that the timing of the response latency is very similar under all treatment conditions. Separating the latencies between startle only, and prepulse conditions shows no clear difference arising in timings as a result of introducing a prepulse 100ms before the startle stimulus. This is in keeping with observations reported by Stitt *et al*. (1976). There is also no clear difference resulting from the type of prepulse, with the majority of startle responses reaching their peak amplitude between 39 and 42 ms after startle stimulus presentation, under LED prepulse, null prepulse, flip prepulse and startle only conditions. As with Figure 6.1 this provides some reassurance that the equipment is functioning and that genuine startle responses are being observed. It is interesting to note that presence or absence of a prepulse, and even PPI, has no discernible effect on startle latency.

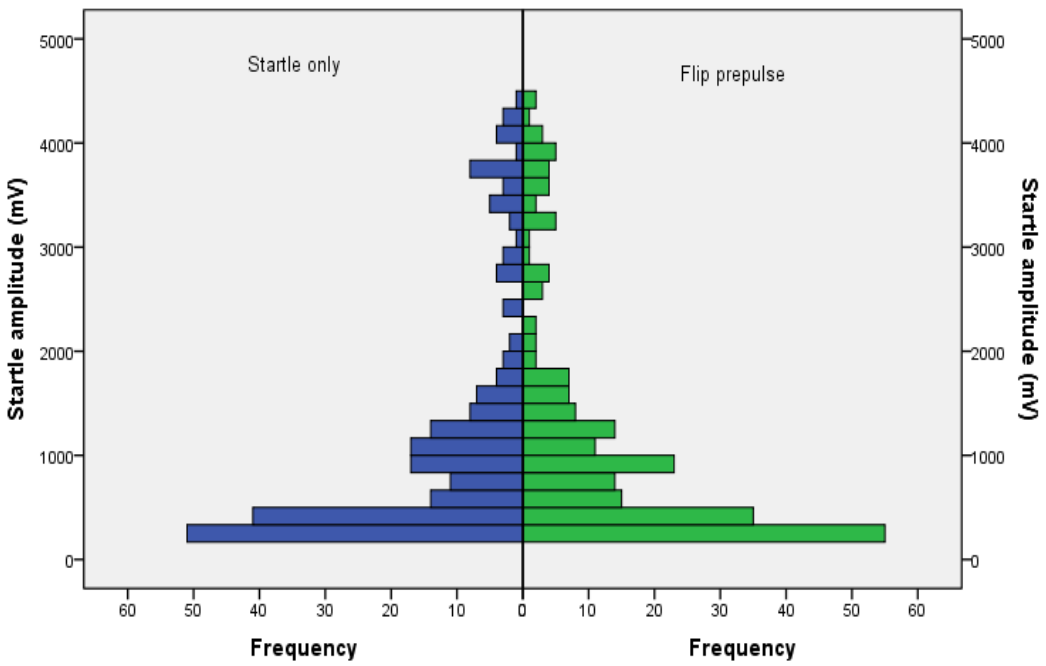

Figure 6.23 Frequency histogram comparing Startle amplitude frequencies between startle only and Inclination flip prepulse trials.

Figure 6.23 shows the frequency of startle responses, binned by amplitude and separated by condition (startle only vs prepulse) for the magnetic flip prepulse treatment session. A similar pattern arises in both cases. The overwhelming majority of responses have an amplitude between 200 and 1000 mV, with numbers tapering off towards 2000mV. A number of responses do occur with amplitudes between 2000 and 5000mV, and while the patterns are not perfectly symmetrical, approximately the same number appear to occur under each condition. The smaller number of responses in this range, combined with the variability between subjects observed in the earlier figures might explain this lack of symmetry.

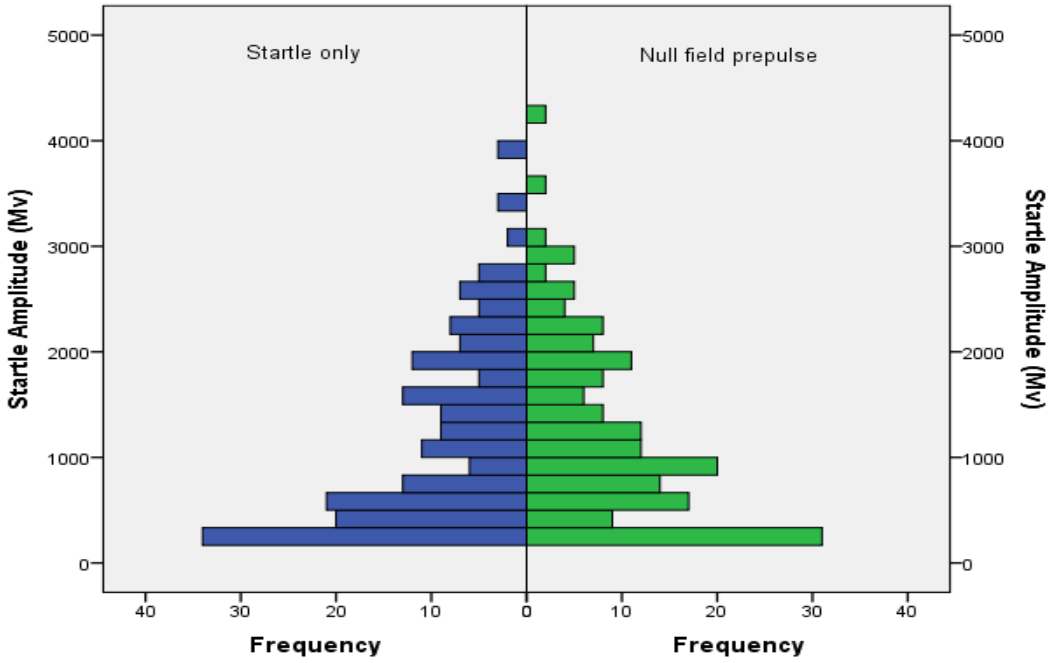

Figure 6.24 Frequency histogram comparing Startle amplitude frequencies between startle only and null field prepulse trial.

Figure 6.24 shows the number of startle responses, binned by response amplitude and separated by condition for the null field prepulse sessions. Some similarities are present to the previous figure representing the flip prepulse. Firstly, the majority of responses are clustered towards the lower amplitude range, with numbers tapering off as amplitude increases. However in this case the responses seem to taper quite smoothly towards 4000-5000mV. This is the case for both startle only and prepulse conditions, suggesting that any difference from the flip prepulse histogram is unlikely to be the result of experimental differences (the startle only treatment is exactly the same in all regards in all sessions). This apparent difference may simply be a result of chance, and a greater sample may potentially result in histograms which are much more similar. Importantly, there again seems to be no obvious difference in the number, or amplitude of responses between the startle only and null prepulse conditions.

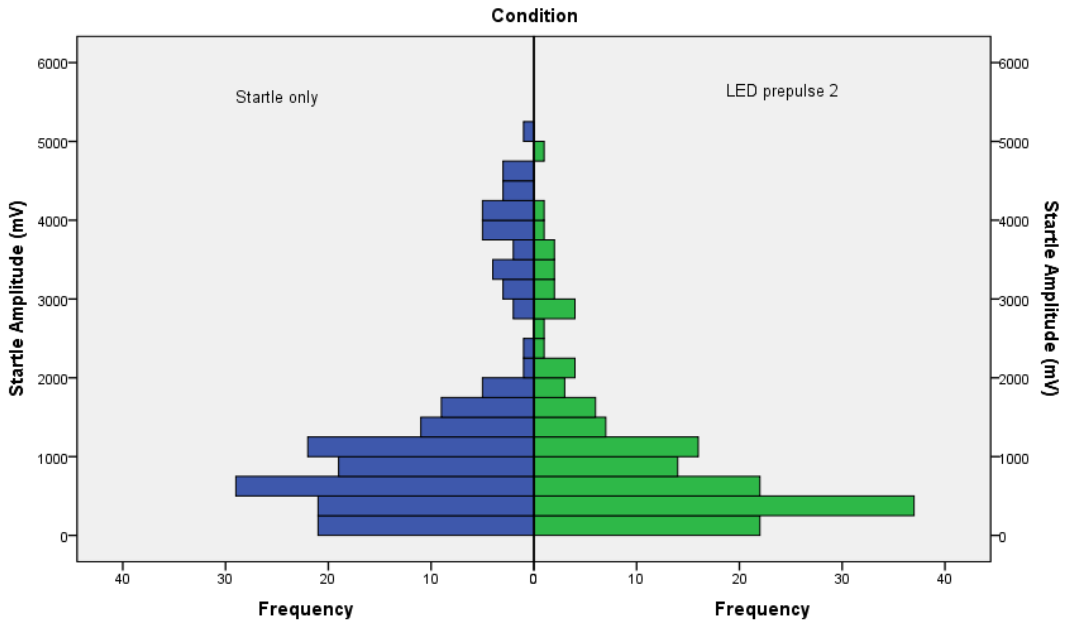

Figure 6.25 Frequency histogram comparing startle only trials with LED prepulse trials (sessions combined).

Figure 6.25 Shows the frequency of startle responses in the LED prepulse sessions, binned by amplitude, and separated by condition (i.e. startle only vs LED prepulse). In this figure some differences can be observed resulting from the different treatments. As opposed to the previous two figures, representing the flip and null field prepulse sessions, which showed a high level of symmetry and very little difference between treatments, this figure shows a more apparent effect of the introduction of a prepulse. Specifically the responses in the startle only condition have a distribution which includes fewer low amplitude responses, and more high amplitude responses than does the prepulse condition. This is indicative of prepulse inhibition, because the startle responses under the prepulse condition, have a generally lower amplitude.

Together these results appear to support the implications of Figures 6.9, 6.14, 6.15 & 6.16, representing the mean amplitudes of response, namely that no PPI is in evidence under the null, or flip prepulse conditions, but some evidence for PPI is apparent under LED prepulse conditions. Repeated measures ANOVA comparing startle only responses with prepulse

responses for each treatment shows that this pattern is statistically significant. Separating the light and dark LED conditions shows that a significant PPI occurred under dark LED conditions (F=9.827, df=1, P=0.002) but not lit LED conditions. This contrasts with the initial LED test session in which only lit conditions were used to test the LED prepulse, and which does show a statistically significant difference in means representing effective PPI (F=6.001, df=1, P=0.016).

These results suggest that a deeper understanding of effects may be gained by: 1, dividing frequency histograms by subject, to isolate those which responded well from the confounding effects of those which did not; and 2, understanding whether there is some possibility that a decrease in responsiveness over subsequent sessions may be occurring, particularly under lit conditions, as this may explain the apparent loss over time of PPI in the LED lit condition. The following charts will seek to elucidate these trends and hopefully answer some of the questions raised.

Change in responses over time (session days)

Because the response to the LED prepulse paradigm under lit conditions varies substantially between the first and last day of recording, a simple comparison was made to assess whether the subjects startled less often, or with a lower Vmax, over subsequent sessions.

Dark

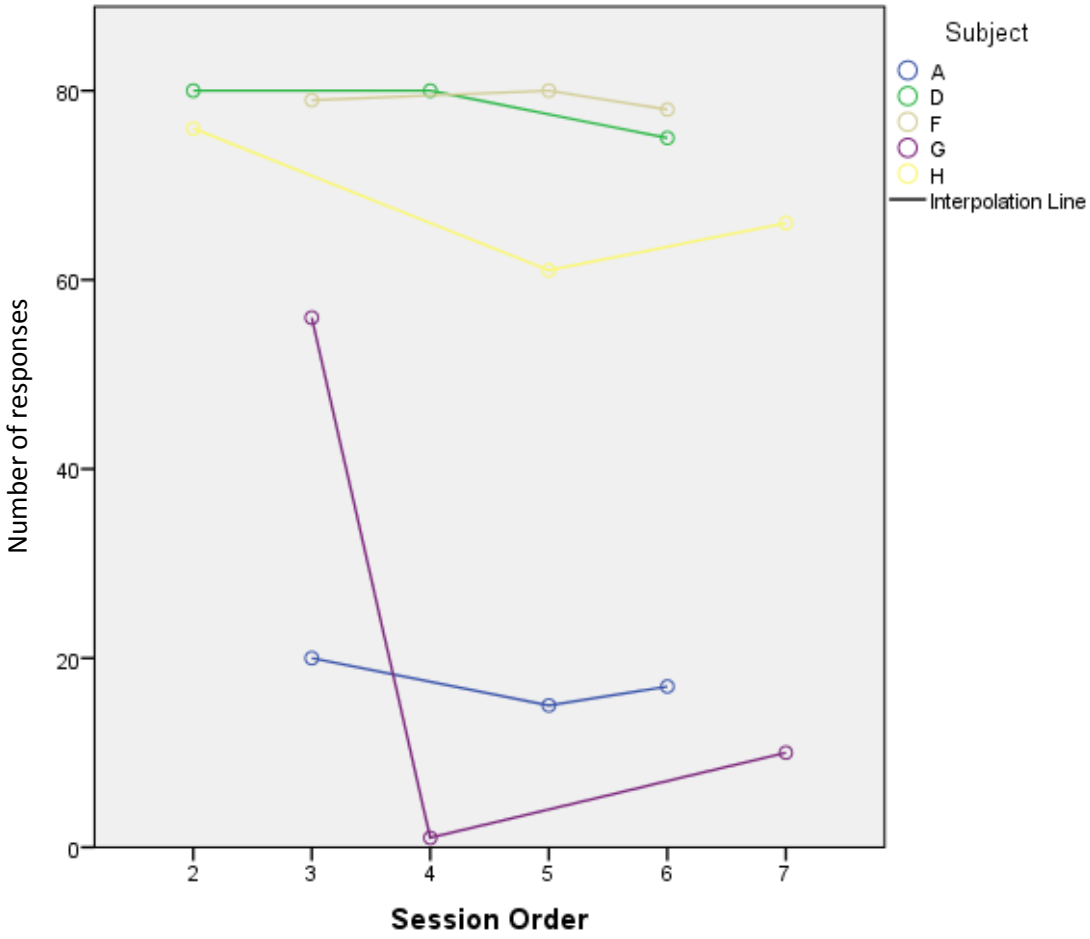

Figure 6.26 Number of responses for each subject, during each experimental session in darkness.

Figure 6.26 shows the number of startle responses for each subject, in each session under dark conditions. All subjects show a decrease in the number of valid startle responses measured over the course of the experiment. Subjects A, D, and F show only a modest decrease as compared to subjects H, and G, with G showing by far the largest reduction. This

reduction in responsivity over successive sessions may indicate the necessity of a period of acclimatisation to successive startle sessions, so that a baseline may be reached, giving more stable results. Due simply to time constraints, no such period was possible during this experiment, as is discussed in more detail in Chapter 2. The data in Figure 6.34 also make it abundantly clear how variable responsivity can be between subjects, indicating another way to improve a future experiment, i.e. to have some selection criteria for subjects, perhaps assessed during the acclimation period. Again, this was not possible because of the limited number of subjects available. With the exception of subject G, the overall trajectory of reduction in startle frequency is not steep. Accordingly there is no reason to assume that the reduction represents a crossing of any important threshold in terms of measuring meaningful startle responses.

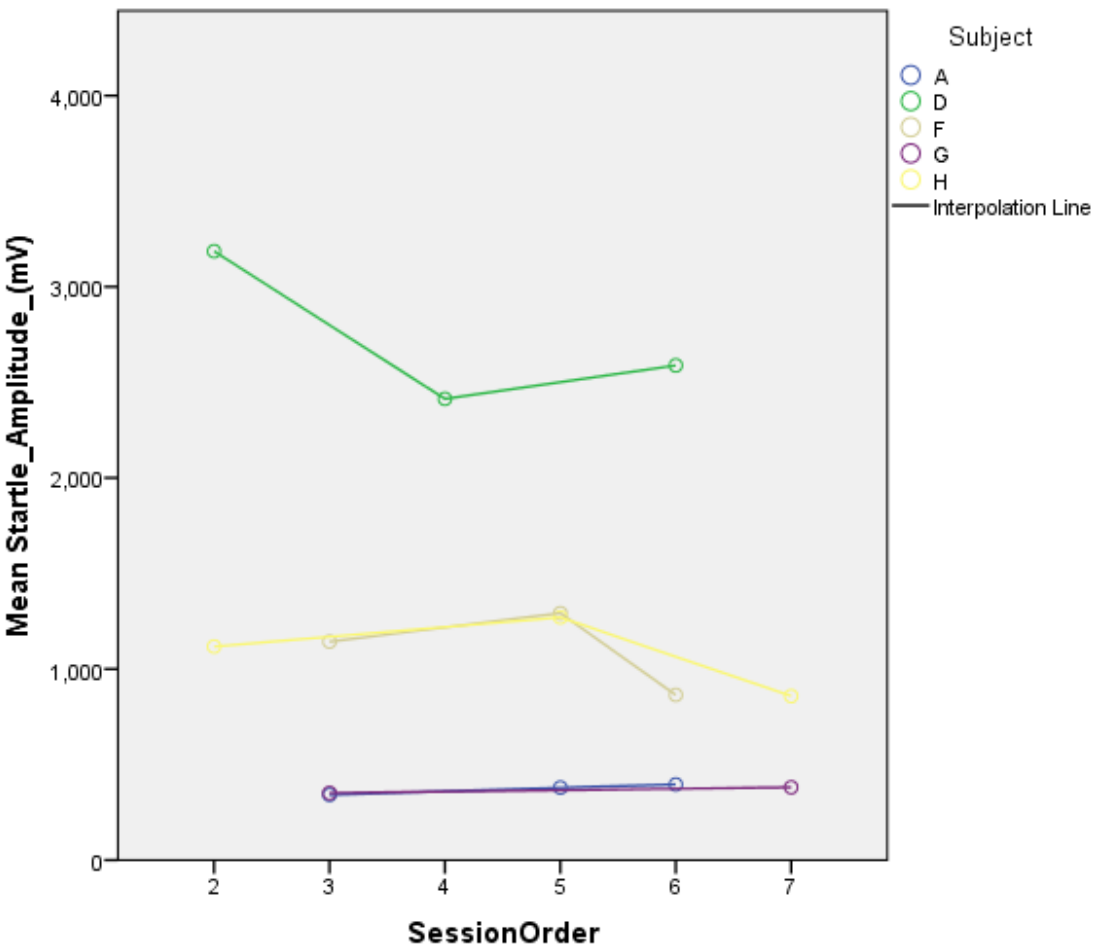

Figure 6.27 Mean amplitude of response for each subject during each session in darkness.

Figure 6.27 shows the mean amplitude of startle responses over successive sessions, separated by subject. A slight downward trend is apparent in subjects D, H, and F, whereas the response amplitude of subjects A, and G remain stable. Subjects A and G do however exhibit generally very low numbers of startle responses, and hence could arguably be considered under-responsive. Overall the general trajectory of reduction does not appear to be pronounced in terms of the ability to measure meaningful responses over successive sessions. One interesting possibility is that the decrease in PPI between the first and last LED sessions might be due, at least in part, to some sort of acclimation to the LED prepulse, rather than to the startle stimulus itself.

Light

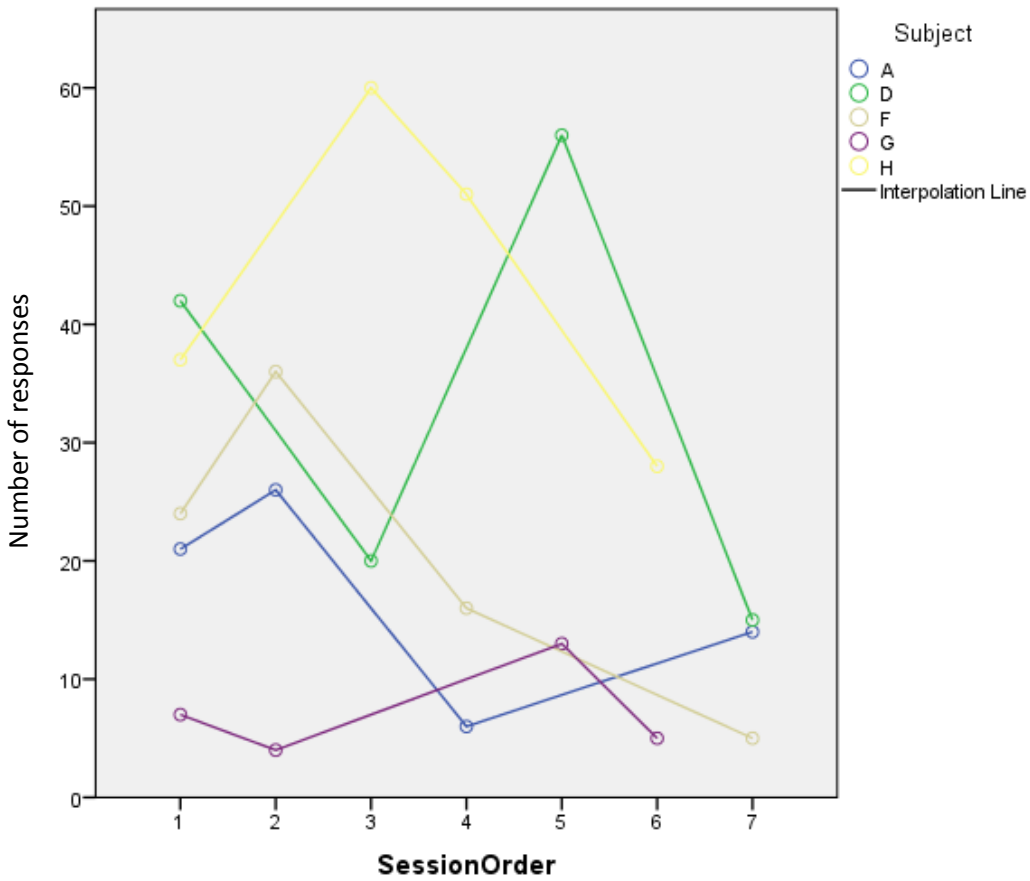

Figure 6.28 Number of responses for each subject, during each experimental session in the light.

Figure 6.28 shows the number of valid startle responses measured over successive sessions under lit conditions, separated by subject. A great deal of variability can be seen. All subjects

have a lower number of startle responses on the last session than they do the first, however frequencies do not follow a steady downward trajectory, but vary widely from session to session. All subjects show responses greater in one or more sessions than they do in the first, and interestingly, the session order in which these peaks and troughs in responsiveness occur do not coincide between subjects, suggesting that it is not a case of the specific day, or session of the experiment dictating changes, but perhaps variability in the arousal of each subject between different days. That said, with the low numbers of responses measured, it is also possible that the data are simply noisier and thus more samples are required for a clear signal to emerge. This also supports the notion that a period of acclimation and observation/subject selection would be beneficial, as variability of specific subjects over successive sessions could then be better taken into account.

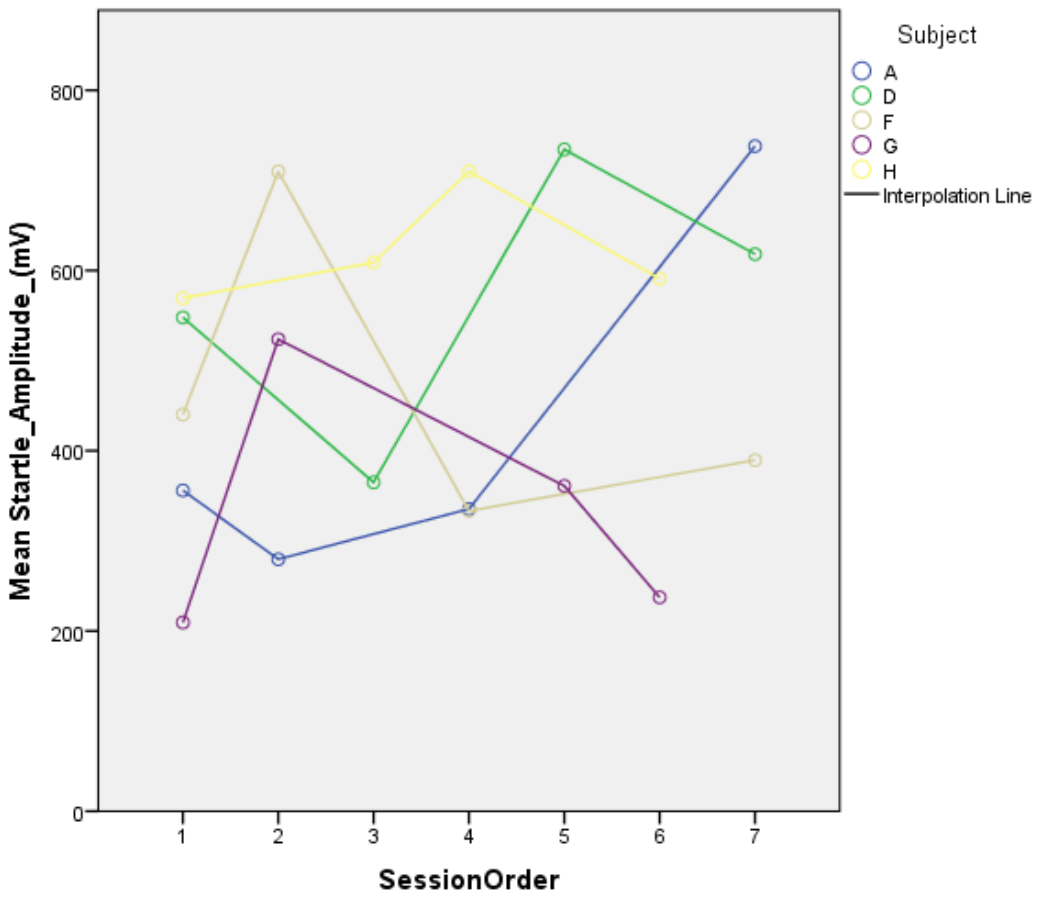

Figure 6.29 Mean amplitude of response for each subject during each session in the light.

Figure 6.29 shows the mean startle amplitude, for each successive session, under lit conditions, separated by subject. In this case, no clear downward trend is apparent. Indeed in some cases there is rather an interesting upturn in responsivity. This might perhaps be due to the generally low amplitude responses not representing 'good clear startles'. There is some evidence in Figures 6.17 – 6.22 that low amplitude startles do not fit the latency values expected and hence might not represent very reliable measures of the startle response.

Summary of results

- The startle equipment and experiment appear to function as expected, with startle only and prepulse trials differing significantly in Vmax ($F_{1,1961.740}$=24.432, P<0.001).
- No clear PPI effect of magnetic prepulse can be seen, but a PPI can be elicited using an LED prepulse, the two being significantly different ($F_{3,27.289}$=46.382, P<0.001).
- Startle is elicited under all conditions in most subjects however PPI is significantly more effective at changing Vmax in darkness than in light ($F_{1,1947.037}$=184.175, P<0.001).
- PPI is evident when using the control (LED) prepulse.
- There is some evidence that an acclimation period would be beneficial.
- It may also be worthwhile to include some selection criteria for both the responsivity (in frequency and amplitude of responses), and in terms of consistency of responsivity over successive sessions.
- PPI is present in some subjects in both first and last sessions, suggesting that had a meaningful PPI been possible during the magnetic prepulse sessions, the effects of changes over successive sessions are unlikely to have affected the results.

Summary of prepulse inhibition experiments

Several lessons can be learned from the results in this chapter. The first is that there has been a demonstrable level of success in eliciting startle responses, and prepulse inhibition, when using an LED prepulse. This shows that the experiment works in terms of equipment specifications, and experimental parameters employed, such as the timing of the relevant stimulus presentations. It seems clear that there is, however, no sign of PPI occurring when

a magnetic field manipulation is employed as a prepulse. Whether that manipulation takes the form of a null field prepulse, where the field in all three physical dimensions is negated for 100ms prior to startle, or if the vertical element and hence inclination of the field is inverted, no evidence is present to suggest that the prepulse is perceived by the subject.

This suggests very strongly that under the experimental conditions used, pigeons are unable to perceive these supposedly salient changes in the ambient magnetic field. In light of the positive results obtained by Migalski (2010) (see Chapter 3), this may well be due to interference swamping the sensitivity of the magnetoreceptor, a concept which is discussed in more detail in Chapter 7.

Other significant lessons learned in terms of carrying out these experiments pertain to the large amount of variability between subjects when carrying out PPI experiments, regardless of the type of prepulse stimulus being used. The susceptibility/sensitivity of birds to the startle stimulus is highly variable, and this is borne out in the large differences observed in responsivity, both in number of startles, and the amplitude of individual startles.

Future experiments should seriously consider establishing rejection criteria, and select only responsive subjects before moving on to real PPI experiments, as the effect of unresponsive subjects is confounding in terms of grouped data. This step was not taken in the current study due to constraints of both time and the limited number of subjects available. Another recommendation for future PPI experiments in pigeons would be to expose all subjects to a standardised period of acclimation, during which numerous sessions of startle only stimulus presentation should be employed. This would provide two clear benefits: 1, subjects should hopefully attain a baseline level of response, alleviating the effects observed in Figure 6.29 of the responsivity varying significantly from session to session, independently of stimuli used; and 2, this in turn would provide an ideal assessment period in which to exercise whatever rejection criteria are to be employed in subject selection.

To facilitate this, a larger initial cohort of subjects should be employed, in order to cover any shortfall in subjects that may arise from rejections if a smaller cohort were used. Some consideration would also have to be given to session timings, if larger numbers of subjects were to be used. It would be important to avoid unwanted confounding effects resulting from the subjects being predisposed to different behaviours at different times of the day (for example courtship rituals such as the bow-coo towards the end of daylight hours).

Chapter 7 Conclusions and future directions

Magnetoreception experiments

No clear evidence was found in the experiments detailed in Chapters 3 and 6 to support pigeons being able to detect magnetic field stimuli. However, referring back to the primary literature (see Chapter 1 – Introduction), there is clearly some evidence that pigeons in fact can sense changes in the ambient magnetic field. Moreover, the re-evaluation of the data of Migalski (2010) identified signs of a significant response to changes in the magnetic field (see Chapter 3). This suggests that there was a crucial difference in the experimental design and/or execution of the present experiments as compared to those of Migalski (2010).

All experiments were carried out in a highly controlled and experimentally rigorous way, with great care being taken over the planning and execution of all approaches. Additionally, a good deal of effort was made both by the author and by various colleagues to ensure that all aspects of the equipment set-up functioned as intended before relying on them in the experiments. Thus, although not impossible, it seems unlikely that both the coil-only head movement experiments and the prepulse inhibition (PPI) experiments would produce negative results erroneously. Therefore, the lack of a positive result may have its origin in one or more of the known ways in which experiments were performed` differently to those of Migalski (2010).

Many changes were made to the procedures used by Migalski (2010). All were made with the best of intentions, hoping to improve the way such experiments were carried out. It seems evident that changing the head markings and software used to investigate the head movements of pigeons was a success, with greater accuracy (and much less lost data) being achieved in the video recordings. The handling and care of subjects was improved slightly in that, as opposed to Migalski (2010) who had to move the subjects across campus to the

laboratory (a somewhat disruptive procedure involving going outside and travelling in motorised vehicles), the current study involved simply moving them to a different room on the same floor of the building in which they were housed. Additionally, subjects were exposed to this local travelling experience a number of times before experiments took place, meaning that they were calmer and more relaxed during experiments. Whilst this reduction in stress is arguably a great benefit to the subjects in terms of welfare, and in terms of trying to observe changes in 'normal' behaviour of pigeons, it cannot be ignored that possibly some element of heightened arousal is important to experimentally eliciting a behavioural response to magnetic manipulations, and that the transfer of subjects in Migalski (2010) helped to achieve this.

Carrying out experiments at night time was also planned as an improvement, minimising environmental noise and disruption, both magnetically and in general. However, given the necessary time, it would seem advantageous in future experiments to change the lighting regime of the subjects' housing, such that subjects could be used at night time, but for some at least, it could be during their own, subjective daytime, in case there is any link between magnetoreceptive abilities and time of day. This could even be extended to carrying out (brief) experiments during the subjects' own subjective twilight hours. If it were possible to house subjects or perhaps pairs of subjects separately, lighting regimes could be adjusted such that all animals are tested during the same subjective time of day. Personal observations showed that even a slight disruption to daylight hours could induce seasonal mating and /or moulting behaviour in the subjects, which would take several weeks to abate. Hence, any changes to the daylight schedule of the subjects' housing must be planned carefully, and executed early in the preparations for experiments.

The most important change between the work of Migalski (2010) and the current research was the use of physical shields (magnetic/electromagnetic) by Migalski (2010), as opposed

to the active/dynamic shielding of only the magnetic field used in the current research. The intention was that, by dynamically cancelling out the ambient magnetic field, far more flexibility would be afforded in the design of experiments. Dynamically shielded coils, if effective, could potentially be used in a wide range of sizes and locations, even potentially being usable on a mobile platform, for various field-based experiments, perhaps on wild and/or migrating subjects. Towards the end of the current research it became clear that dynamically shielding only steady magnetic fields might be insufficient. The recent work of Engels *et al*. (2014) strongly suggests that a method of shielding from electromagnetic signals may in fact be necessary, however this was simply not known at the time of building the apparatus and planning the experiments reported here. It was supposed by the author and colleagues that, because deliberate manipulations of the field would be significantly larger than variations resulting from background noise, they would still be salient to the subjects. However, the implication of the work of Migalski (2010) and Engels *et al*. (2014), taken together with the findings presented in this thesis, is that such background noise can, in fact, disrupt the effect of magnetic manipulation. Perhaps it is the case that, particularly in today's modern era of high-technology, electromagnetic noise is so common that it can interfere with birds' ability to respond to magnetic field manipulations. Presumably there is a certain amount of magnetic field variation, beyond which all stimuli are filtered out such as to prevent potentially harmful distractions occurring. The lack of results in the PPI experiments suggests very strongly that, if such filtering does indeed occur, it must do so before subjects become aware of such signals. That is to say, that signals are not consciously ignored, but subconsciously filtered before the subject becomes conscious of them, as, by definition, if the subject became conscious of the magnetic prepulse, prepulse inhibition should have been observed. Therefore, the main, overarching recommendation for work continuing from that reported in this thesis, would be to reproduce the experiments reported here, inside high-frequency electromagnetic shielding similar to that used by Migalski (2010).

Main findings of magnetoreception experiments

- Head movement analysis (software and markings) has been greatly improved, and offers a non-invasive way to measure head movements, even at high sampling rates, which is very useful for a range of behavioural assays.
- The use of prepulse inhibition to investigate magnetoreception is feasible. Although the present results were negative for magnetic field stimuli, the experiment was a success using light stimuli. This provides at the very least, strong proof of concept that PPI is an effective means of investigating magnetoreception. This represents an entirely novel approach, and a potentially very powerful one.
- Time of day might potentially have some influence on magnetoreceptive abilities, and should be controlled for in future experiments.
- Anthropogenic electromagnetic background noise may swamp the sensory abilities of pigeons in such a way as dynamic magnetic shielding is insufficient. Therefore, physical shielding of high frequency electromagnetic noise should be employed.

Future directions

The logical next step for this type of investigation is to repeat the experiments reported in this thesis, using physical shielding as did Migalski (2010), i.e. a high frequency shielded room removing unwanted ac signals. It would be the prediction of the author, based on the findings reported, that such experiments would be likely to prove very effective, especially properly shielded PPI experiments. If it should transpire that PPI experiments in the physical shielding do not elicit a response, but experiments involving longer lasting manipulations do, this would indicate that the time taken for field manipulations to be perceived by pigeons is greater than the 100ms used in the PPI experiments. Such a result would potentially shed light on the function of the neural processes and/or sensory mechanisms involved.

Additionally, once a reliable response can be identified, many other experiments would be possible. Repeating the experiments reported here, under electromagnetic shielding, could

be complemented by experiments controlling for time of day. Then, responses could be assessed under different wavelengths of monochromatic light and even involving other visual stimuli. One example of the latter, would be to elicit an OCR response using monochrome coloured bars, and test the limit of the response in terms of acuity, contrast and/or colour. To achieve this, a response could be obtained as in the OCR experiments described in Chapter 5, and the bars incrementally reduced in width (i.e. increased spatial frequency) to test the subjects acuity and/or the wavelength of the colour could be changed gradually to test the limits of the subjects colour vision range under these circumstances (i.e. what the bird is able to visually perceive and thus pursue when in motion). This could be repeated under normal magnetic conditions, and under a series of manipulated field conditions, to investigate the link between magnetoreception and vision, and whether the identified psychophysical limits change at all as a result of magnetic manipulations. The author would strongly recommend that a reliable PPI response to magnetic field stimuli be established, using the high-frequency shielding now deemed necessary. Then a titration of ambient lighting wavelength could be carried out to assess the contribution of different parts of the visual spectrum. Together these experiments may offer some insight into the contribution of vision to magnetoreception. As well as performing the experiments mentioned in the high-frequency electromagnetically shielded environment, there is also the possibility of carrying out experiments in some locations without the need for high frequency shielding. In the future it may still be possible to employ dynamically shielded coils in experiments in areas of low ambient electromagnetic noise. However a detailed investigation into proposed areas would need to be carried out in advance to establish the presence or absence of electromagnetic noise, and suitable areas are likely to be in remote locations.

OCR experiments

The combination of the new head movement detection software and LCD screens for presenting 360° stimuli was, at the time of conception, highly novel. Due to the flexibility and range of different stimulus patterns available, and the ability to change them dynamically if required, it is likely that this type of approach will be widely used in the future. The work reported here has provided a number of beneficial insights. The OCR response can be investigated in an extremely time-efficient, and minimally invasive way, which provides highly accurate data. Potential uses include studying the effect of stimulus velocity and spatial frequency on frequency and amplitude of saccadic movements as well as slow-phase gain. The LCD setup was also briefly used as proof of concept that vertical head movements can also be elicited and recorded. This gives the opportunity for future studies of the visual system that produces head bobbing behaviours.

The research presented here also uncovered a number of interesting behavioural effects which merit further investigation. There appears to be a delayed effect whereby pigeons do not pursue moving stimuli with maximal gain immediately, but rather their ability to pursue 'builds up' over time, and only reaches maximal pursuit ability (the highest gain the subject can achieve for a given velocity) after several seconds. This may give some insight into evolutionary similarities and differences in brain structure and function between birds and other animals, such as humans, which exhibit no such build-up effect.

Additionally, the presence of a break down effect, where pursuit does not simply stop when maximal velocity is exceeded but exhibits a gradual decrease in gains towards zero and the evident increase in the prevalence of saccades in the same direction as stimulus drift under certain conditions seem interesting and potentially worthy of further research.

OCR future directions

The OCR experiments reported here open up a potentially rich vein of possible future areas of research. Studies of the build-up/break-down effects may prove of interest to neuroscientists and behavioural ecologists, as may the cause of saccades in the same direction as stimulus drift. Improvements to the screens used (i.e. greater resolution and high contrast ratios) would facilitate the investigation of the contrast sensitivity curve in pigeons and other birds. This involves identifying the smallest and largest spatial frequency at which the subject can perceive the movement of the drifting bars, and combining these data with those for the lowest level of luminance contrast that can be perceived by the subject. This luminance threshold varies with spatial frequency such that a curve can be produced illustrating the contrast sensitivity of the subjects' visual system across a range of spatial frequencies. Thus the lowest luminance contrast that can be seen is plotted on the y-axis, often as a contrast sensitivity function (1/contrast threshold), for each different spatial frequency that can be seen (on the x-axis).

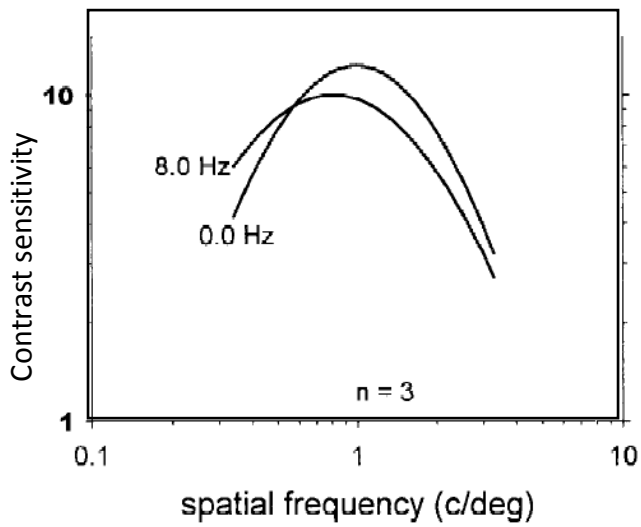

Figure 7.1 Example of a contrast sensitivity curve for pigeons, recorded using operant conditioning techniques (Hodos *et al*. 2002). 0Hz = stationary grating, 8Hz = drifting grating 8 cycles/s.

The methodology employed by Hodos *et al.* in Figure 7.1 involved substantial training of subjects to recognise gratings, for the purpose of later comparison with more rapid, but

highly invasive electrophysiological recordings. The approach reported in this thesis is minimally invasive, with no harm to the subject, and requires no training. At the time of writing, a full set of screens with 4k resolution (3840 × 2160 pixels) has been procured, which can not only present a grating of high enough spatial frequency to test pigeon acuity, but also is able to display the moving lines smoothly enough that the grating drifts steadily across the visual scene. Plans are currently being made for using these screens to reproduce the contrast sensitivity curve in Figure 7.1 (Hodos *et al.* 2002), by which demonstrating the proof of concept that this much more time-efficient, and minimally invasive approach can be used in this way. A grant application has been made to Cardiff University for an undergraduate summer studentship to carry out these experiments in 2016. This will then allow the combining of tests of acuity and contrast sensitivity with magnetic manipulations to investigate the relationship between magnetoreception and vision as previously discussed. Additionally, the same approach, with only very minor modification, could be used to investigate similar psychophysical properties in almost any bird species and, with a little modification of the restraint method, a potentially huge range of different species of animal.

Polarisation experiment conclusions

No evidence was found here for the ability of homing pigeons to discriminate different e-vector arrangements. There are two main possibilities for why this might be. Firstly, the ability to see e-vector may not function in such a way that drifting whole field movements are salient, and secondly that the equipment used was for some reason unable to provide a salient stimulus. Because the mechanism by which birds detect e-vector orientation remains unknown, and the fact that there is a considerable amount of contention over whether pigeons can see polarisation patterns of light at all (see Chapter 1), it is difficult to be sure that the stimulus presented contained any visually salient information. If pigeons cannot in fact discriminate e-vector, or if only one orientation can be detected at a time (for instance

if a tiny portion of the visual field is dedicated to e-vector discrimination), then no positive result would be expected in these experiments. However, the paradigm is easy to carry out, and the LCD arenas have such a diverse range of applications, that is would be a shame to abandon this avenue of research completely. One technological advancement that could potentially be worth investigating would be to add shorter wavelength light to the emissions of the screens. As discussed in Chapter 1, there is some suggestion in existing literature that wavelengths of light at the UV to blue end of the visible spectrum might be important in magnetoreception, and the original theory of the author presented later in this chapter describes how low-wavelength light may be important for discrimination of e-vector (and indirectly detecting magnetic field intensity). Unfortunately, this low-wavelength light, due to its high-energy interaction with matter, is often deliberately removed from displays to safeguard human health. Ultraviolet light, for example, is absorbed by body tissues over only a shallow penetration, meaning that a great deal of energetic interaction occurs in the outer layers of tissue. Consequently, excessive UV light has been linked with various maladies, including skin cancer and eye damage in humans (Willmann 2015; Holm 2015; van Norren and Vos 2015). For this reason, most electronic display screen equipment (DSE) has no UV light component, either in its light source or, due to filtering, in its emitted light. In order to investigate whether this low-wavelength light has a special role in any of the visual phenomena discussed in this thesis, it would be advantageous to be able to engineer LCD displays that emit full daylight spectrum light, including UV. This could potentially require some reverse engineering of screen components, such as the light source, and also some aspects of the screen itself may have to be changed. For example, most types of glass filter out a high proportion of UV light. Consequently, an investigation into appropriate materials would be needed, as well as due consideration of health and safety and any potential risks involved, followed by the actual engineering task of assembling such a device. The author would recommend that it would be beneficial to assess the feasibility of creating such a

display. Should the work seem feasible and the necessary time and funding be available, then it would be very interesting to be able to carry out OCR and OMR experiments using full spectrum daylight, both from the point of view of investigating polarisation vision and facilitating the investigation of visual psychophysics under magnetic field manipulation, as it would appear that full spectrum daylight might also be important for this in its own right.

IMU head tracking

During the course of this research, the author was highly involved in the conception, design, and early stage testing of a device for tracking avian head movements based on an inertial measurement unit (IMU) (Figure 7.2). The IMU can measure six degrees of motion, that is, translocation in the x, y, and z axial planes, as well as pitch, roll and yaw (rotation about the three axes). Thus, all kinds of movement of the head can be accurately recorded in three dimensions, rather than the two-dimensional movements in the horizontal plane extracted from video frames. The prototype was built with an on-board battery and Bluetooth wireless connectivity device for transfer of data, attached to a small backpack worn by the pigeon. The IMU itself was attached to a hood, specially designed for subduing falcons before and after flights by comfortably covering their eyes. Holes were simply cut into the hood so that vision was unobstructed. Validation of the accuracy of the device in two dimensions was carried out by the author by recording a simple OCR session using both the now well established video extraction method and the IMU at the same time. The work on analysing the IMU data for comparison to the video extraction data provided by the author, and the design and building of the device itself, was carried out by Noor Aldoumani and is to be reported in her PhD thesis, which is being prepared in parallel to this one.

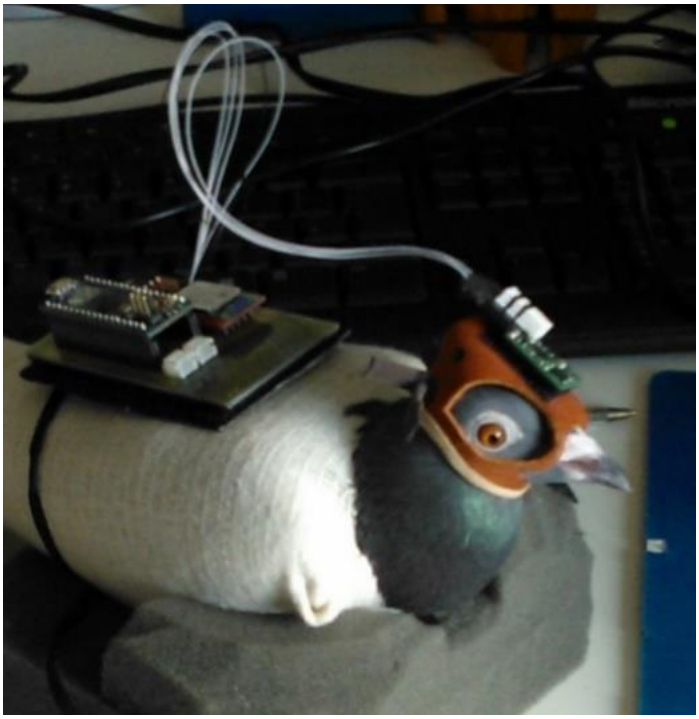

Figure 7.2 Sensor and head marking in place for validation of IMU device.

The clear advantages of the IMU approach to pigeon head tracking are that movements in all directions are measured, allowing for much more complex analyses of head movements, and also allowing for analysis of head different movements such as bobbing or monocular foveation of visual targets, in a freely moving pigeon (as the subject no longer needs to be confined to the area a camera can see). More testing would be required, however, before the device could be used in magnetoreception experiments, to ensure that Bluetooth signals sent to the recording PC do not interfere with magnetoreception. If this is the case, a hard-wired connection or on-board data storage may be more suitable.

Magnetic field effects in humans

A growing body of anecdotal evidence suggests that humans may be able to detect very strong magnetic fields due to their effects of causing mild dizziness, nausea, or slight loss of balance. Although these phenomena have only very recently come to the attention of primary researchers, the growing exposure of humans to anthropogenic electromagnetic fields, and the increased vigilance being adopted in terms of health and safety, means that

there is growing interest in this area. By analysing the posture of walking human subjects, Theysohn *et al.* (2014) demonstrated that there may be some vestibular effect resulting from exposure to strong magnetic fields (effects were observed at 7T, but not at 1.5T or 0T). Even more relevant to the research presented in this thesis is a study by Ward *et al.* (2015) in which human subjects are reported to experience a strong horizontal nystagmus (eye movement) effect when entering and leaving the bore of a 7T MRI machine, and it is proposed that this is the result of a Lorentz force interacting with the vestibular system. An alternative approach to studying magnetoreception in humans and/or other species could be to begin with very strong magnetic fields, and try to investigate whether the effects of strong and weak fields bear any common causes or mechanisms. As described in Chapter 1, there is a growing body of evidence suggesting the involvement of the vestibular system in magnetoreception. Perhaps the vestibular effects of very strong fields have a similar mechanism, meaning that use of strong fields may be helpful in describing the physiological processes involved. One specific experiment which the author has considered is to use eye-tracking equipment (cameras + software) to measure nystagmus in humans exposed to a strong magnetic field in MRI machines. This would be similar to the study described in Ward *et al.* (2015), but more comprehensive in scope, building a robust data set that can then be compared across different field strengths. This could then be expanded to include various other species that are reported to be able to detect Earth strength magnetic fields to look for any physiological or psychophysical correlates to field strength changes. The fundamental questions are: do all species respond the same way or differently, and does a response at weaker field strengths depend on sensitivity variation between species, or are there completely different mechanisms involved? Eye tracking equipment suitable for use in MRI scanners is already available.

In addition, the prepulse inhibition experiment can easily be adapted for use in humans, and the author, with some assistance from Dr Matt Dunn, has begun to design and test the

equipment and experimental protocols necessary for this. Specifically the main adaptations involve adjustments to the piezo electric movement sensor used to measure the startle response, and the addition of a large, non-metallic bed. Use of a bed means that the head of the human subject can be comfortably supported in the 'sweet spot', where the uniformity of the generated field is well characterised, and where the controlled magnetic manipulation prepulses occur. This adapted setup facilitates testing in both waking and sleeping human subjects, as well as allowing longer exposure (non PPI-based) experiments, such as examining the effect of manipulated magnetic field conditions on sleep patterns.

A proposed mechanism for the polarimetric detection of geomagnetic field properties by animals

The following is a theory initially conceived by the author in early 2012, and gradually refined over time. To the best of the author's knowledge, this proposed mechanism of navigation in animals is entirely novel. This theory proposes that the interesting coincidence of magnetoreception and polarised light vision might be because the two mechanisms are fundamentally linked. The following pages describe how well known physical effects of magnetic fields on polarisation e-vector of light, could allow animals to derive magnetic field information using e-vector acuity alone.

It is known that many animals are able to discriminate between different e-vectors of linearly polarised light. The precise mechanisms for this are somewhat poorly understood and are considered to vary between different species (Horváth 2014). There is also a growing, and increasingly informative, literature demonstrating that many animals are able to in some way sense features of Earth's magnetic field. The mechanism by which this is achieved is still unknown. However, of the two leading theories, the more popular, and arguably better supported, relies on the availability of light of particular wavelengths, suggesting a visual component. (Wiltschko *et al.* 2005; Phillips *et al.* 2010). Whilst many of the species identified

as having the ability to discriminate e-vector, are also sensitive to Earth's magnetic field, there has hitherto been very little consideration of whether, or how, these sensory modalities might interact or support one another in some way.

What follows is a summary of a potential way in which these two seemingly separate sensory modalities may in fact be one and the same in certain cases. A theoretical analysis of how the mechanism could work is presented together with some suggestions for how this might be investigated experimentally.

Polarised light

Light propagates as an electromagnetic wave, the electric and magnetic components of which oscillate orthogonally to one another and to the direction of propagation (Figure 7.3).

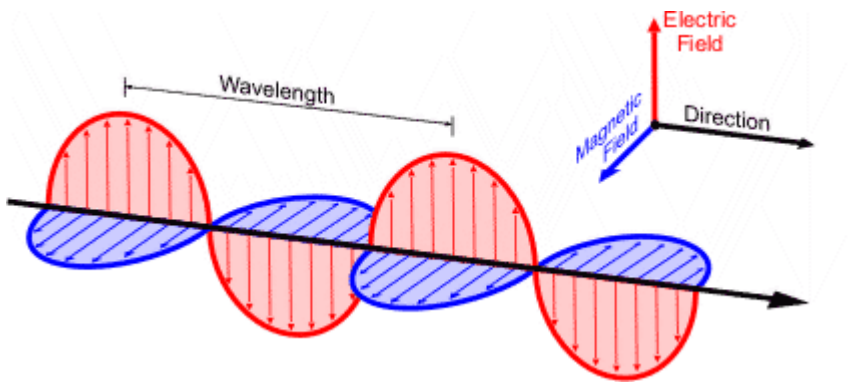

Figure 7.3 the relationship of electric and magnetic wave propagation in light (NOAA 2012).

Diffuse light, such as from the sun or many man-made sources, is comprised of light with the electric field (and hence, inextricably the magnetic field) oscillating in all directions perpendicular to the direction of propagation. However, when reflected off the surface of an object or passed through a filter that only allows one direction of oscillation, the light becomes (predominantly) linearly polarised. This property is used to great effect in LCD display screens (see Chapter 2 for description) as well as many other applications. In natural settings, the polarisation of light is somewhat more chaotic due to the vast number of unpredictable interactions a propagating photon may encounter on its journey. However,

there are many situations where the amount of light that is polarised can be significant to animal vision. For example anglers commonly benefit by applying a polarised filter to their view when looking into water and aiming to avoid surface reflection, and photographers regularly employ methods of filtering certain e-vectors from a visual scene, which can have a very striking visual effect. Many animals are known to be able to discriminate different e-vectors of light and numerous experiments have linked this sensory capability with communication with co-specifics, prey detection, and navigation (Muheim *et al.* 2006; Temple *et al.* 2012; How *et al.* 2012). One main theory for how polarised light may be used in navigation is by viewing the sky at dawn and dusk. Because light reflects off water and other molecules in the atmosphere, and light reflecting orthogonally is maximally polarised, there is a strongly polarised band of light right across the sky at 90° to the sun (Figure 7.4). When looking straight upwards, which gives a view orthogonal to the sun's direction (i.e. at dawn and dusk), a dark band can be observed running approximately North-South through the zenith. Humans usually require a (linearly) polarising filter to see this dark band, but many animals are suspected to be able to see it readily, and to use it in navigation (Muheim 2011). It has been suggested that there is a link between this ability and the magnetic compass, and that perhaps the polarisation pattern is used to calibrate the compass sense in animals before a day of travelling/migrating (Muheim *et al.* 2006).

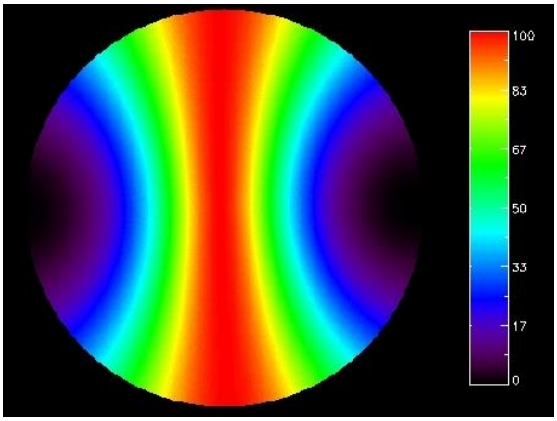

Figure 7.4 False colour image showing the degree of polarisation of light (%) at various points in the sky at twilight (Hallsw 2010). In this scenario the sun is positioned on the horizon at 90° to the band of maximal polarisation.

Earth's magnetic field

The generation of the geomagnetic field, and the variations with location, are complex but generally obey certain rules. The field can be expressed as a sum-vector, comprising the intensity (usually expressed in units of micro Tesla, µT) of the magnetic flux, in each of the three dimensions, all added together, to give an intensity in a particular direction. At the poles, the vertical component is the biggest contributor, and at the equator the N-S horizontal component is strongest. The overall intensity is greater at the poles (up to 60uT) than at the equator (as little as 25uT).

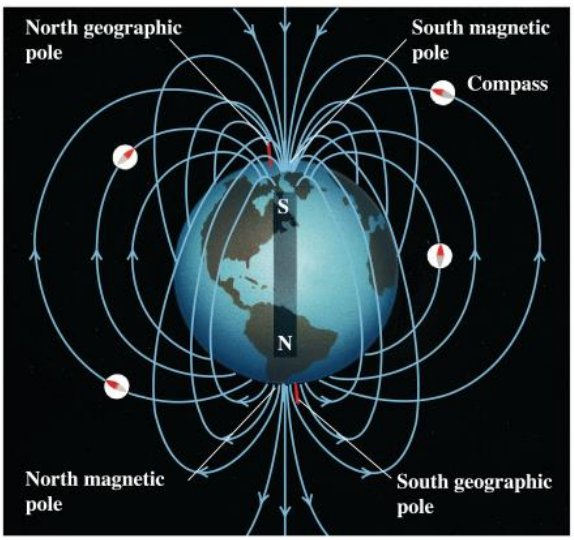

Figure 7.5 Field lines showing the sum vector intensity of Earth's magnetic field (Becker 2002).

Thus, at the poles the field 'points' almost directly away from Earth's surface, whereas at the equator the field lines lie parallel to Earth's surface (Figure 7.5).

Interaction between light and magnetic field– The Faraday Effect

Michael Faraday observed that linearly polarised light will experience a twist in e-vector orientation as it travels through a magnetic field. This can be characterised as an amount of rotation, for a given distance at a certain intensity of magnetic field as illustrated below (Figure 7.6). The Verdet constant is a function of the light scattering properties of the medium (e.g. air/water etc.). This effect is exploited in modern science with great accuracy. For example, in satellite polarimetry, to assess the intensity of Earth's magnetic field from orbiting satellites, by measuring the faraday rotation of e-vectors in linearly polarised light reflected off the planet's surface in predictable patterns.

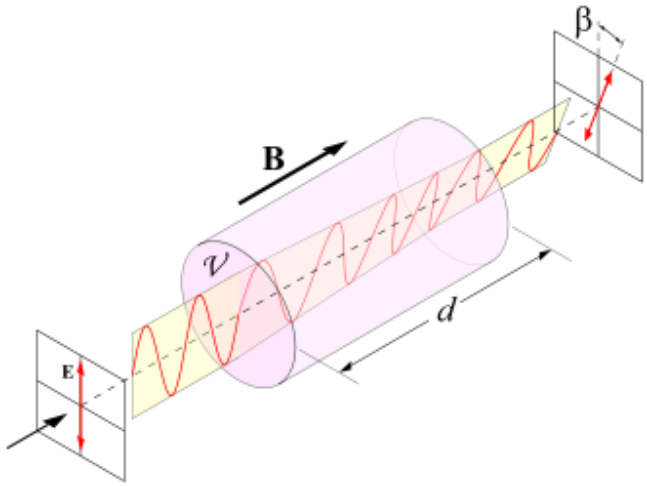

Figure 7.6 Interaction of light and magnetic field intensity (DrBob 2007).

The amount of e-vector rotation can be calculated using the equation:

$$\beta = \mathcal{V}Bd$$

Equation 7.1

Where:

β = angle of rotation (Radians)

B = Magnetic flux intensity in the direction of light propagation (Teslas)

d = Distance over which the light is influenced by the magnetic field (Meters)

$\mathcal{V}$ = Verdet constant, a proportionality constant defining the relationship between magnetic flux, Faraday rotation and distance, which arises from the influence of different material compositions, and is affected by temperature and wavelength of light.

Assessment of Verdet constant in atmospheric air

The relationship above is well understood and commonly applied in lab-based settings under highly controlled conditions, often being used to assess the optical qualities of different types of glass for example. Measurements of Verdet constant in atmospheric air are much rarer, and should be treated with greater caution in terms of their variability, because of the changing nature of the medium over large distances. However, recent advances in satellite

polarimetry have resulted in some reliable measures of Verdet in the atmosphere in terms of the average effect over known distances, and assuming relatively consistent conditions.

Verdet is dependent on light wavelength, being greatest for shorter wavelengths. The shorter wavelengths of the visual spectrum show the greatest Faraday rotation, and hence might be crucial to e-vector discrimination of magnetic field intensity over practical distances.

Due to the limited availability of Verdet constants at various wavelengths in atmospheric air, a table had to be produced of known values, and then regression (exponential) applied in order to find the desired values for Verdet at shorter wavelengths.

Wavelength (nm)	Verdet	Units	Expressed as Rad/T/m
650*	5.884×10^{-5}	°/mT.cm	0.001710
635*	6.221×10^{-5}	°/mT.cm	0.001810
532*	6.448×10^{-5}	°/mT.cm	0.001880
450**	10^{-4}	°/mT.cm	0.002910
1064†	3.91×10^{-4}	Rad/T/m	0.000391
634.8‡	1.39×10^{-9}	Rad/G/cm	0.001390

Table 7.1 known Verdet constant measurements from published literature (sources - *Li and Li (2012); ‡Zhang *et al.* (2007); **Li *et al.* (2008); †Wu *et al.* (2003)).

Taking the known values from Table 7.1 and performing an exponential regression provides the projected values in the table below.

λ (nm)	Verdet	λ (nm)	Verdet
250	0.005343	700	0.001279
300	0.004558	750	0.001091
350	0.003889	800	0.000931
400	0.003317	850	0.000794
450	0.00283	900	0.000677
500	0.002414	950	0.000578
550	0.00206	1000	0.000493
600	0.001757	1050	0.000421
650	0.001499	1100	0.000359

Table 7.2 Projected values of Verdet constant in atmospheric air.

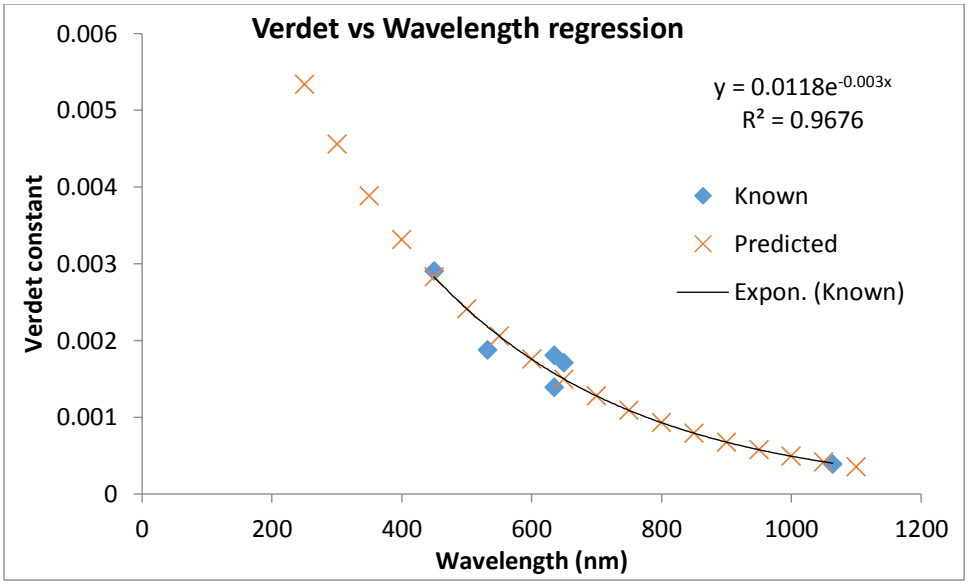

Figure 7.7 The known values of Verdet in air, and those predicted by the regression analysis.

Using the values obtained and equation 7.1, it was possible to estimate the approximate distances light of different wavelengths must travel in order to be rotated by a measurable amount in Earth-strength magnetic fields. These values are plotted in Figure 7.7. The commonly used approximate distance to the edge of the atmosphere (100km) was used as a baseline for the distance light will travel through the atmosphere if travelling vertically, i.e. the shortest possible path to the surface of the planet (Figure 7.8).

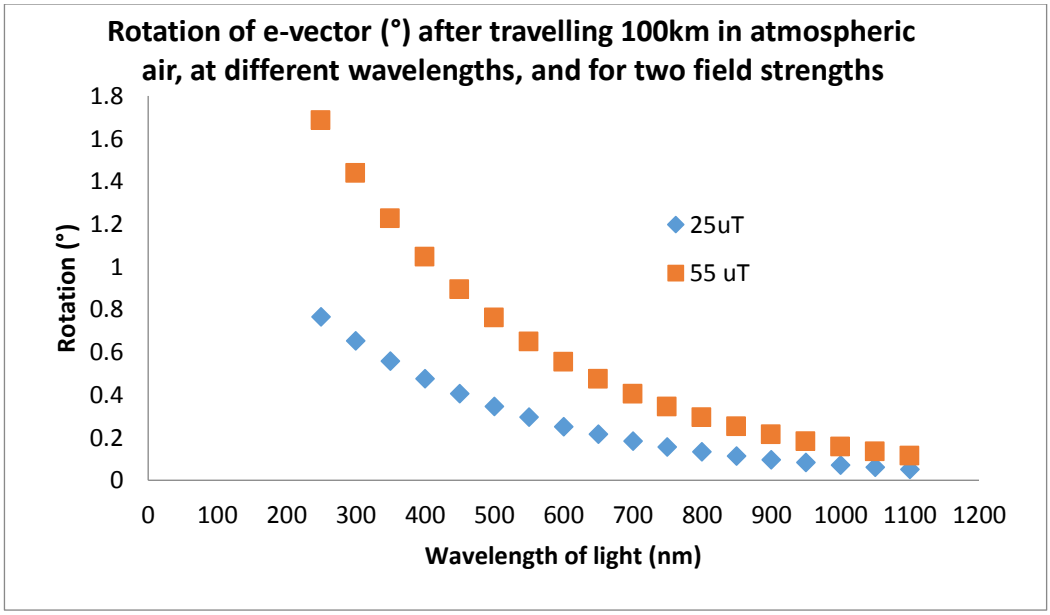

Figure 7.8 Rotation of light of various wavelengths after travelling 100km in atmospheric air.

Then 1° of rotation was arbitrarily assigned as a hypothetical minimum rotation that may be discernible (Figure 7.9). In reality, e-vector resolution of various species may be greater or less than this, but it is at least in the range of potential discrimination (Temple *et al.* 2012). Unfortunately, in most terrestrial animals such as birds, polarisation vision has been tested only using rotations of 45° or more, however there is some evidence that the nature of the effect on electrophysiological recordings in fruit flies and locusts (*Schistocerca gregaria*) (Bech *et al.* 2014; Velez *et al.* 2014) is such that the response varies in a continuous fashion with changing e-vector, meaning that e-vector acuity of a couple of degrees or less is at least theoretically possible.

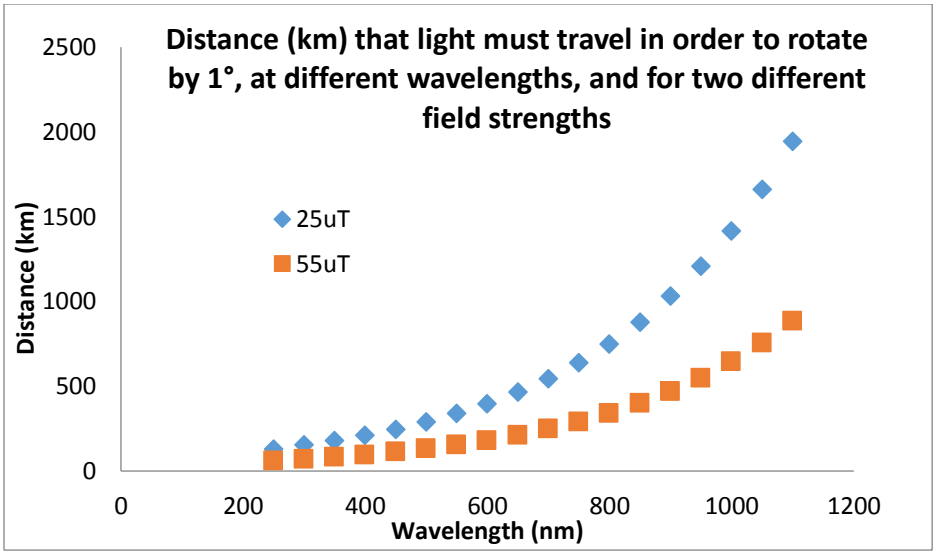

Figure 7.9 Distance light of various wavelengths must travel in air to rotate by 1°.

Realistic distances of light paths

If we estimate the distance to horizon for an observer at 100km elevation, we find ~1134km. If reversed this is effectively the distance that light will have travelled from the 100km altitude (edge of atmosphere) point to an observer on the ground who is looking just above the horizon (and extending into the atmosphere). Thus, an observer, looking at a point just above the horizon will (not accounting for interference/refraction of planet surface) be seeing light that has travelled approximately 1134km in the atmosphere. This is true in both (opposite) directions.

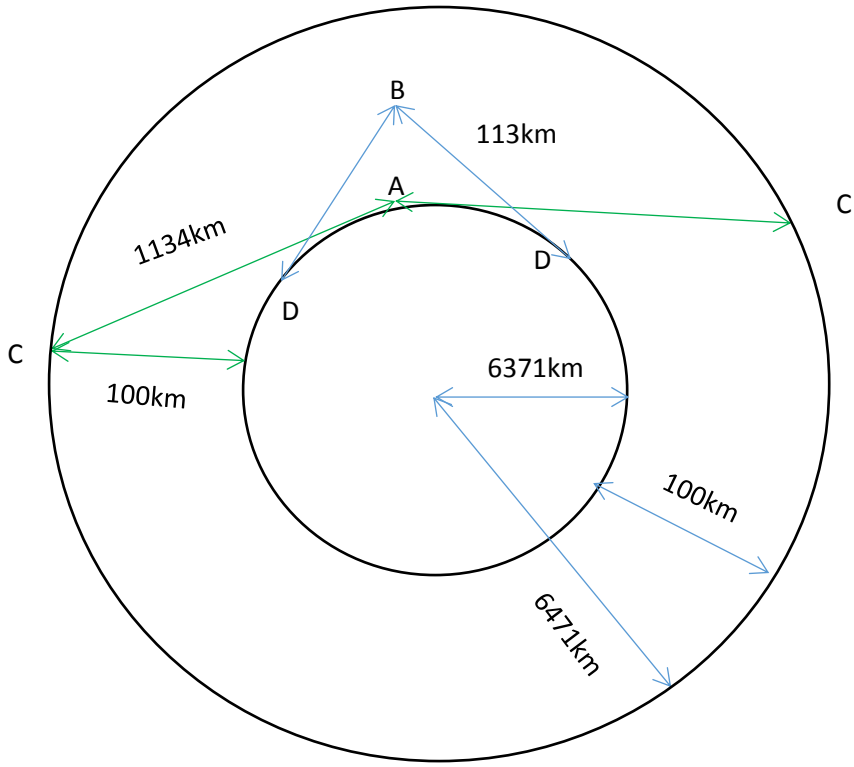

Figure 7.10 Diagram showing estimated distances of maximal light path in the atmosphere. The above is not to scale, and measurements are approximate. This illustrates the possible distances light may travel through the atmosphere before arriving at the eye. Inner circle=Earth's surface. Outer circle= edge of atmosphere (approximate transition between mesosphere and thermosphere), a point commonly cited as the point at which particles become dense enough to have a significant interaction with incident light (see Figure 7.11).

- A=Observer on Earth's surface
- B=Observer at 1km altitude
- C=Edge of atmosphere in line with horizon from point A
- D=Horizon when viewed from B

Figures 7.8-7.10 show that, when viewing the sky just above the horizon, light reaching the eye has travelled on the order of 1000km through Earth's atmosphere. However light of certain wavelengths only needs to have travelled approximately 100km for a difference in e-vector to potentially be discerned. If we conservatively say that light at each end of the North-Zenith-South plane has travelled at least 500km through the atmosphere upon reaching the eye, it is perfectly possible that an animal with e-vector resolution of a couple

of degrees or better, would be able to discern poleward from equatorward, using the effect that the magnetic field has on the e-vector of light.

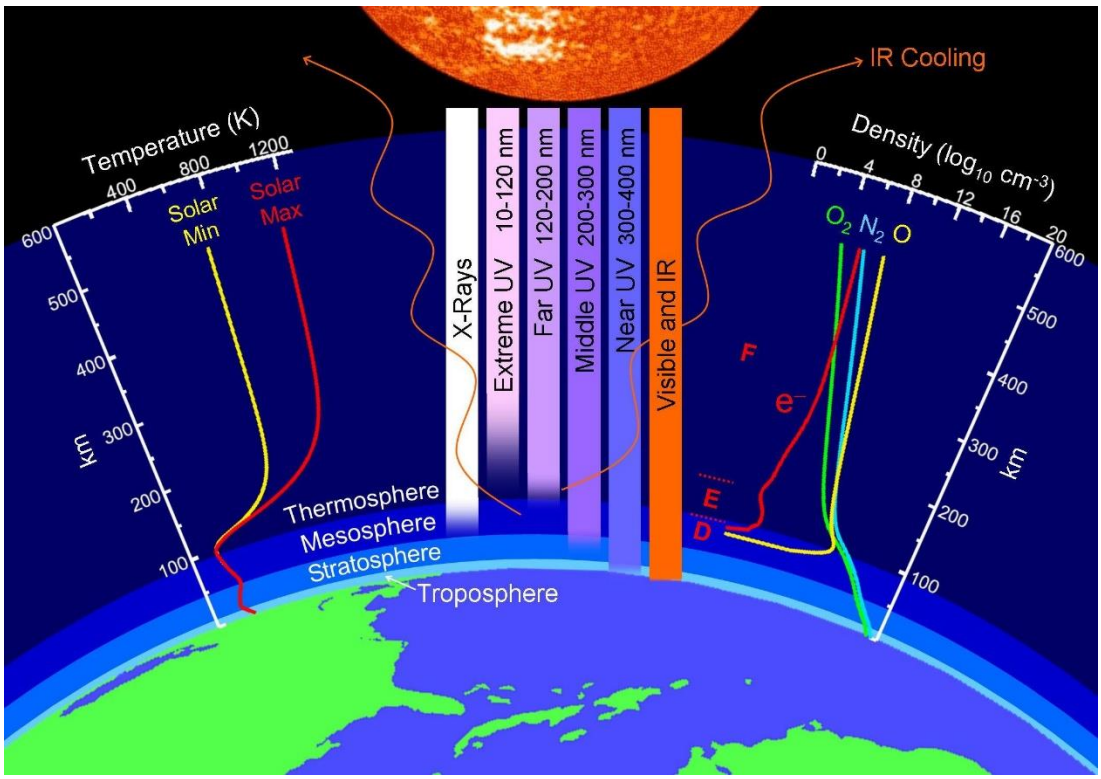

Figure 7.11 Layers of Earth's atmosphere and their effect on incident light (NASA 2015).

This implies that any animal that can discriminate a difference of 5 degrees in e-vector for a given (probably short e.g. <500nm) wavelength can tell magnetic poleward from equatorward, at dawn and dusk, with no other mechanism required.

Because the optical properties of air are affected by the density of particles, and hence altitude, the Verdet constants given must be assumed to be averaged for the entire path from 100km altitude to Earth's surface (whether the path is vertical or otherwise). However, they have been shown experimentally to be a good approximation in satellite polarimetry (Wu *et al.* 2003; Zhang *et al.* 2007; Li *et al.* 2008; Li and Li 2012).

Accounting for change in magnetic field intensity

The intensity of the Earth's magnetic field also changes along the path of the light, and with altitude, therefore some approximation must be made as to the net effect of the various field intensities along the light path. It is reasonable to assume that if the intensity changes steadily with location (true on a global scale but not locally), we may estimate the average intensity of the field that the light passes through. This is a simple case of taking the median value for field intensity over the course of the light path. For example, if the light enters the atmosphere where the field intensity is 50uT, and arrives at the observer in a region of 30uT, then the median intensity along the path, and an approximation of the intensity over the entire path, could be 40uT. However, this makes the assumption that the field is 50uT at 100km altitude, which is unlikely to be accurate as magnetic field strength tends to degrade exponentially with distance from source. Using the NOAA magnetic field calculator (NOAA 2013), it can be seen that 100km altitude reduces intensity by only 2-3uT, approximately 5-10%, and so making suitably conservative estimates should account for this.

So, if we shorten the path of the light by, say, 113km to account for having to look slightly above the horizon and if we take a median value for intensity looking equatorward from a mid-latitude as, say 35uT and the same median value looking poleward as, say, 47uT, we can again, plot the expected amount of rotation, for a range of light wavelengths.

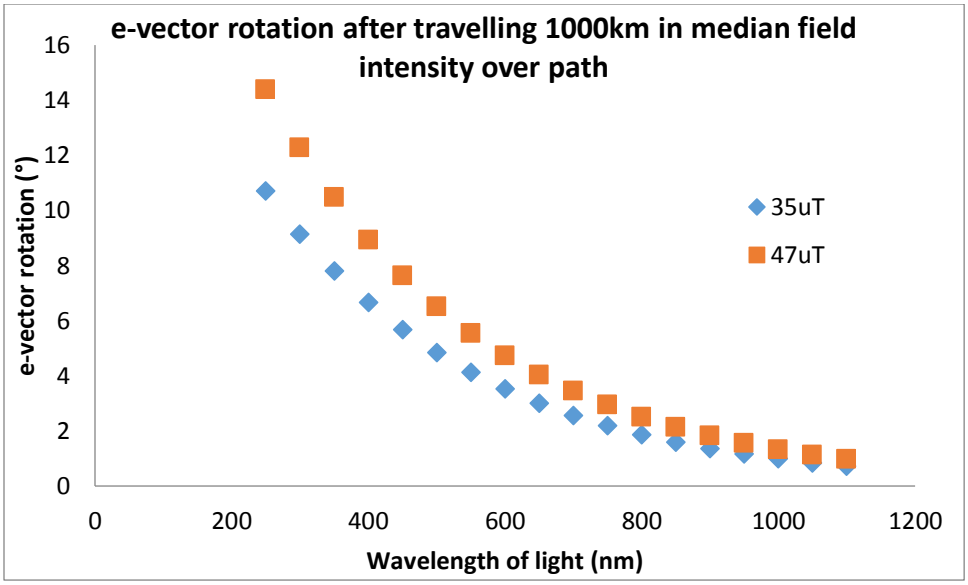

Figure 7.12 E-vector rotation after 1000km travelled at median field intensity for different wavelengths.

It can be seen in Figure 7.12 that as much as a 14° rotation can be seen at lower wavelengths. Dependant on abilities of specific species, this difference could also be discerned using higher wavelengths.

There are, however, two more important factors that must be taken into account in order to understand whether this phenomenon will exist in a real situation. Both relate to the fact that not only does the intensity of the magnetic field have an important effect, but so does 'direction' of the summed field vector.

Accounting for 'direction' of magnetic field vector

The first of these to account for is that the field 'points' toward the geographic North Pole (technically South magnetic pole). This means that light travelling from the equator toward the North is travelling 'with' the field direction, whereas that travelling South from the pole toward the equator (assuming we are in the geographic Northern hemisphere) will be travelling 'against' the magnetic field. This, in the simplest sense, means that a negative value of field intensity should be used for the calculation of Faraday rotation when light travels against the field (i.e. geographic N-S).

Making this adjustment gives the following values for e-vector rotation.

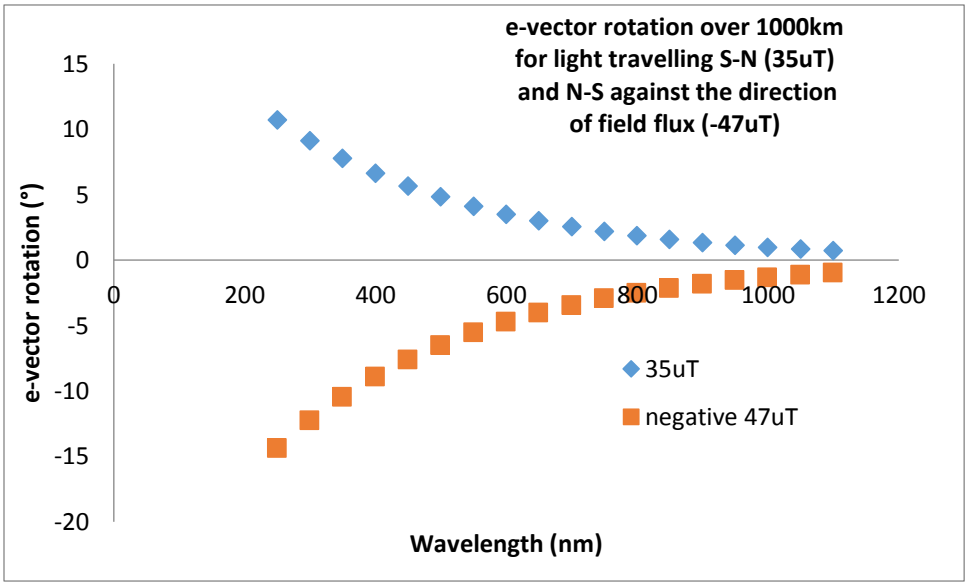

Figure 7.13 Rotation of e-vector having taken into account direction of travel in relation to Earth's magnetic field.

This adjustment, far from confounding the effect, exaggerates it, meaning that an even greater divergence in e-vector is theoretically observed (Figure 7.13), making it significantly more likely for a wide range of species to be able to discern the difference. It is reassuring to note that a rotation of over (or even close to) 180° has not been observed. Such positions are given arbitrary notation by humans to distinguish say 181° from -179°, but this distinction may not be possible for animals. Hence, it is reassuring that the values do not diverge enough to 'overlap'.

The remaining correction that must be made pertains to the fact that the sum intensity of the field does not point along the path of the light exactly, hence an approximation is required of the proportion of the field intensity, which does align (positively or negatively) with the path of the light. This must account for the fact that, near the pole, the vertical component is by far the strongest component, whereas at the equator it is the horizontal component that has most influence. This calculation, and the setting of definite distance

limits, together with a thorough breakdown of the appropriate mathematics, will be carried out prior to publication.

This theory provides what appears to be a possible mechanism of using the Earth's magnetic field to tell poleward from equatorward, and even to navigate in other directions in areas of high magnetic field variability, by means of polarisation e-vector acuity alone. Very recently (February 2016), a paper was published by Muheim *et al.* in which the key finding was that zebra finches (*Taeniopygia guttata)* orientated normally when the axis of maximal polarisation of skylight was parallel to the magnetic field, and became disorientated when the skylight polarisation and magnetic field were perpendicular. This was interpreted by Muheim *et al.* (2016) as suggestive that the polarisation level of skylight is an integral factor in the functioning of cryptochrome-modulated visual magnetoreception. However, it seems plausible that the above theory of the authors may provide an alternative. The implication being that it is actually the e-vector being seen by the bird, and the magnetic field intensity/direction is being derived from this information.

The mathematical and engineering aspects of this theory are well established physical principles, often utilised in industry (e.g. satellite polarimetry). The crucial question, it seems, is whether any animal considered to be magnetoreceptive, also has the ability to visually resolve e-vector rotation of a couple of degrees. To try to examine this principle in the homing pigeon, the extension of the experiment described in Chapters 2 and 4, recommended in Chapter 7 would be necessary.

Further explanation

One nice way to imagine how an animal might perceive the difference in magnetic intensity via e-vector is to imagine they have a filter, like one might use in photography. The dark band at sunrise/sunset runs approximately N-S, but this is not to say that a linear polariser must be orientated N-S to see it. The pattern is the region of maximally polarised light in relation

to the sun, at the point where it scatters in the atmosphere, but does not necessarily represent the orientation of the e-vector of the light arriving at the eye. That is to say, light in this polarised band has struck a particle in the atmosphere and been reflected at right angles, such that it is now linearly polarised and heading directly towards the observer. At the point of reflection, it is polarised predominantly N-S, but on arrival at the eye, the above described Faraday effects have taken place, and the field strength becomes apparent through e-vector rotation.

Imagine that you look at the poleward end of the dark band and have to turn your filter to, say, 5° to get the darkest possible stripe. Then if you look to the equatorward end of the dark band, you now find that you must turn your filter to -10° in order to see the dark band most clearly. The difference in the way you need to orientate the filter to best see the dark band is a function of the intensity of the magnetic field along the path the light has taken (Figure 7.14). The smaller the rotation you are able to discern, the more accurate a measure of the difference in magnetic field intensity you can achieve.

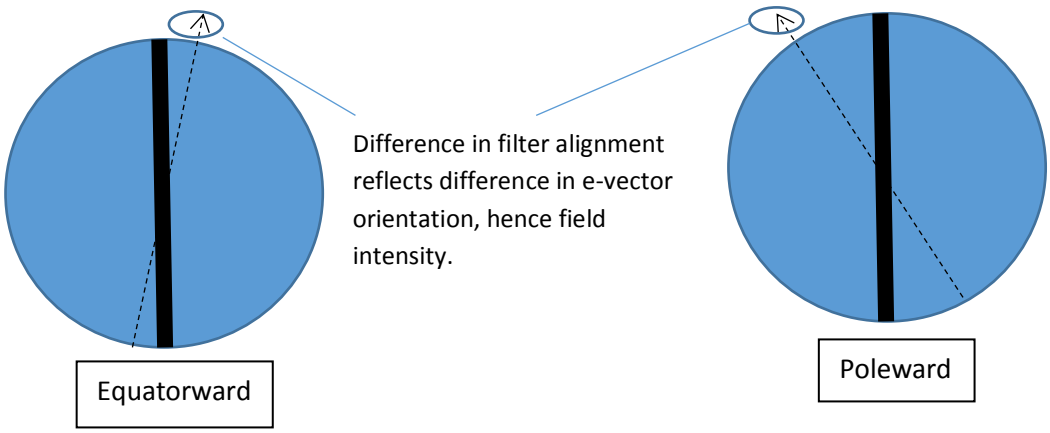

Figure 7.14 Hypothetical example of deriving magnetic field intensity from e-vector acuity.

Although very much beyond the scope of this research study, an obvious experiment to carry out would be to use a man-made polarimeter (or simply a light intensity sensor with polarising filters which can be very accurately rotated) to assess the amount of Faraday rotation actually observed when viewing the two different ends of the plane of maximal

polarisation. Such devices are beginning to be used for solving navigational questions, however the author is unaware of any studies directly measuring Earth's magnetic field intensity by means of ground-based polarimetry.

It may also be possible that information about Earth's magnetic field intensity could be derived by simply looking straight up at the sky, in different locations at the same time of day, possibly tying in with the notion that migratory birds use polarised light to calibrate their magnetic compass (Muheim *et al.* 2007). Additionally, it might be feasible for a travelling bird to use this method with polarised light reflecting off the surface of the Earth, such as a water body. However, in order for the light to travel far enough, it would seem that the bird would have to be flying at considerable altitude (>1km).

It must not be overlooked that many different animal classes appear to use polarised light cues for navigating, some perhaps to a greater extent than birds (Horvath 2014), to which this theory is not limited.

Closing statement

The work presented in this thesis has demonstrated that interdisciplinary collaboration can offer new directions, and improved reproducibility in research into magnetoreception and other sensory modalities. The novel use of prepulse inhibition and other techniques has demonstrated the importance of electromagnetic shielding in magnetoreception experiments. The use of LCD arenas for investigating head movements in pigeons and the optomotor response in fish was successful. These findings, along with the knowledge and experience gained in the process of obtaining them, offer great insight into how future experiments should be conducted. Whilst interdisciplinary research and design of novel experimental approaches are by no means without their challenges, they offer the potential for significantly improved ways of investigating enigmatic sensory mechanisms.

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