INSTRUMENTATIONS TO INVESTIGATE MAGNETORECEPTION IN HOMING PIGEONS (COLUMBA LIVIA)

A thesis Submitted to Cardiff University in candidature for the degree of

Doctor of Philosophy

By

Noor Shuaib Aldoumani, BEng.

Wolfson Centre for Magnetics

School of Engineering

Cardiff University

United Kingdom

January 2017

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ACKNOWLEDGEMENTS

This thesis is dedicated to the memory of my father, Shuaib Aldoumani. I would like to thank him for his love and support, as well as plenty of encouragement. I wish only that he could have been here to see his daughter complete her PhD.

I would like to take this opportunity to express my deepest appreciation and gratitude to my supervisors Dr. Turgut Meydan and Professor Jonathan Erichsen for their supervision and guidance, without which this work would not have been possible.

I would like to express my special thanks to Dr. Christopher Dillingham for his encouragement and invaluable guidance and his advice throughout my experimental investigations.

I'd like to thank Dr. Anoor Aldoumani for his excellent guidance, encouragement, caring, and providing me with an excellent advises during this project. I appreciate his vast knowledge and skill in many areas, and his advice during my study, in writing papers.

I would also like to thank the other members of Wolfson centre, Dr. Fatih Anayi and Dr. Tomasz Kutrowski for their advice and comments at all levels of my research.

I would like to acknowledge Leverhulme Trust for their financial support during my study at Cardiff University.

I would also like to acknowledge the technicians in the mechanical workshop in the School of Engineering for their help and cooperation.

I am grateful to my mother for her unconditional love, support and encouragement throughout my life.

Finally I extend my deepest thanks to my sisters and my brother in law for standing beside me during happy and sad moments.

ABSTRACT

Seasonal migration of birds from one place to another is a very complex phenomenon investigated by researchers for many years. Birds move from one area to other often covering thousands of kilometres, and some species even fly only at night. In order to find the proper direction and their way to specific areas, birds use many cues, which can vary, for example objects that can be seen, smell or the perception of other environmental features. The type of cue depends upon whether the birds have to travel a short distance or if they have to travel to distant locations. The cues for birds, which migrate at day time, are rather different from those who travel at night. The first chapter of this thesis covers details of the strategies that pigeons use when homing and the interplay of the various navigation cues used.

Various experiments have been carried out on pigeons but the results are quite complex. However, previous experiments have demonstrated that pigeons have the ability to use geomagnetism as a reference in order to find their way. The inclination and intensity of the field vector are both used as references.

In this PhD research, I mainly focused on getting an answer whether homing pigeons are able to sense the Earth's magnetic field, which could then be used as a navigational cue in order to navigate and migrate. I have designed a 3D Helmholtz coil setup to a create variety of artificial magnetic fields and tried to evaluate the bird's horizontal head movements recorded by a camera located above the coils. The effect of different fields has been examined, such as sweeping, null, steady and flipping fields.

Evidence was found that homing pigeons are able to distinguish a flipping field from a steady field, and this can be observed by changes in their head movement. Homing pigeons have also been shown to distinguish different frequencies of flipping inclination field conditions. Data analysis has revealed that the pigeons respond more obviously to a field rotating in both directions, clockwise and counter clockwise. This response has been seen whether compared to a natural baseline or artificial baseline. However, the pigeons' response to the other magnetic field conditions varied significantly depending on the baseline type.

Also, I focused on developing a head tracking system in order to extract pigeon's head saccades more accurately while the pigeons are experiencing the various field conditions. The main aim of the new tracking system is to investigate a pigeon's response to the Earth's magnetic field, which requires 3D monitoring of its motor responses to various stimuli. Conventional video analysis (VTA) involves tracking a 2D image, and the resulting data can be noisy and limited to a single field of view (one rotational angle).

LIST OF PUBLICATIONS

First-author papers

1. Aldoumani N, Kutrowski T, Barnes J, Meydan T, Erichsen JT, ,"Instrumentation for monitoring animal movements,"IEEE SENSORS 2014, 2014. pp. 1295-1299.

2. Aldoumani N, Meydan T, Dillingham C, Erichsen J, "Enhanced Tracking System Based on Micro Inertial Measurements Unit to Measure Sensorimotor Responses in Pigeons," IEEE Sensors Journal 2016. (16): pp. 8847-8853.

3. Aldoumani N, Meydan T, Dillingham CM, Erichsen JT, "Enhanced tracking system based on micro inertial measurements unit to track sensorimotor responses in pigeons," IEEE SENSORS 2015, 2015. pp. 1-4.

Co-Author papers

1. Kutrowski TM, Meydan T, Barnes J, Aldoumani N, Erichsen JT, "Instrumentation for monitoring animal movements," IEEE SENSORS 2014, 2014. pp. 1295-1299.

2. Erichsen JT, Dillingham CM, Barnes JA, Meydan T, Kutrowski T, Aldoumani N, "Saccadic and optocollic head movements in homing pigeon (Columba livia)," Investigative Ophthalmology & Visual Science, 2015. (7): p. 2909.

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ABBREVIATIONS

AC	Alternating Current
AHRS	Attitude and Heading Reference System
CCW	Counter clockwise
CW	Clockwise
DAQ	Data Acquisition
DC	Direct Current
DCM	Direction Cosine Matrix
DLL	Dynamic-LInk Library
DSP	Digital Signal Processing
FEM	Finite Element Method
FF	Flipping Magnetic Field Condition
GPS	Global Positioning System
IMU	Inertial Measurements Unit
IR	Infrared Light
LCD	liquid crystal display
MEMS	Microelectromechanical systems
MF	Microelectromechanical systems
NF	Null Magnetic Field Condition
NI	National Instrument
OCR	Oculocardiac Reflex
OKN	Optokinetic Nystagmus
PC	Personal Computer

PCB	Printed circuit board
PPI	Pre-Pulse Inhibition
SF	Static Magnetic Field Condition
VI	Virtual Instrument
VOR	Vestibulo–Ocular Reflex
VTA	Video Tracking Analysis
2-D	Two Dimensional
3-D	Three Dimensional

Nomenclatures

A	Cross Section area
B	Magnetic Flux Density
D	Diameter
f	Frequency
H	Magnetic field strength
h	Height
H_e	External magnetic field
H_i	Internal magnetic field
I	Current
L	Distance between coils
N	Number of turns
n	Sample size
R	Radius
S	Shielding factor
S_{diff}	Standard deviation of the difference
$S_{\bar{x}}$	Estimate standard error of the mean
t	Time
w	Width
$\bar{x}_{diff}$	Sample mean of the difference
Ω	Angular frequency
μ_1	Sample mean of the difference

μ_2	Standard deviation of the difference

CHAPTER 1

INTRODUCTION TO THE ORIENTATION AND NAVIGATION IN HOMING PIGEON'S

1.1 INTRODUCTION

Homing pigeons (*Columba livia*) have the ability to fly for extensive journeys and investigators have been trying to unravel the secret of how this is accomplished.

As a matter of fact, although not used any longer, pigeons were the most ancient technique of sending messages in the world. In spite of a lengthy history and large body of knowledge about homing pigeon coming back home from distance locations. This mechanism is still not obvious. No doubt scientific research of this practice started early in the twentieth century but it became more deeply investigated over the last four decades, when many theories and assumptions were created about animal migration and/or homing. Pigeons, being comparatively easy to study, became the most closely inspected or observed model system [1].

The remarkable ability of animals to carry a message over a long distance is no doubt very fascinating. Although it has been studied, it is still not understood. The performance of birds, used for transferring messages, is the result of some animal instincts like moving in search of food, coming back to their nest or migrating when the weather changes [1]. Homing pigeons apply this homing ability to fly back to their nest area even if they are not familiar with their current location. This capability can be compared to humans while using a GPS device (Global Positioning System) in which signals are sent through satellites in company with a compass in order to give an estimation of the required location coordinates. It is still a question how magnetic field can be used as navigation cue for the birds or animals [2].

Over the last many decades, researchers have discovered many approaches to unravelling the mystery. Many of high impact publications have been published in this field[3].

Some evidence has been found to suggest that a change in conductivity in the part of the bird's brain giving rise to a nerve stimulus may influence the visual system to achieve magnetoreception. In this regard, it is essential to better understand the reason for animal and birds migration and the cues that might affect the migration such as:

1. Weather conditions changing continuously.

2. Anthropogenic magnetic pollution.

3. Magnetic reversal of the Earth's magnetic field. If the navigation technique, which is used by the animal, can be revealed and explored, then it will be very helpful for the human design of new navigation systems means [4].

During the past decades, many experiments have been carried out in order to investigate the process of the pigeon navigation accurately (regardless of the cues that the pigeons use during the navigation). However, it is not a single process but many which vary in complexity depending on the group of animals concerned, each of which may possess its own strategy in navigation. Three properties necessary for all navigation have been utilized by animals, whatever the mechanisms involved, i.e. compass, map, and internal clock. These three fundamental components are essential in any navigational strategy. Various compass systems have been found to be utilized by the Homing Pigeons, such as the magnetic (e.g. celestial [5], Sun [6]; and stars [7]).

Several types of maps which may depend on various cues, including olfaction or magnetic field. Indeed, the map could also depend on the spatial distribution of a cue such as gradient or mosaic maps. The internal clock provides important temporal information, for example, for appropriate interpretation of the Sun or star position in the sky.

In this thesis, each possible navigation cue that might be used by the Homing pigeon is discussed, such as the celestial cue (the Sun and the star compass), land marks, and polarized light. More attention is paid to the magnetic compass because a significant part of my thesis was focused on the sense (magnetoreception) underlying this compass. In (Figure 1-1), the various environmental cues believed to play a role in navigation.

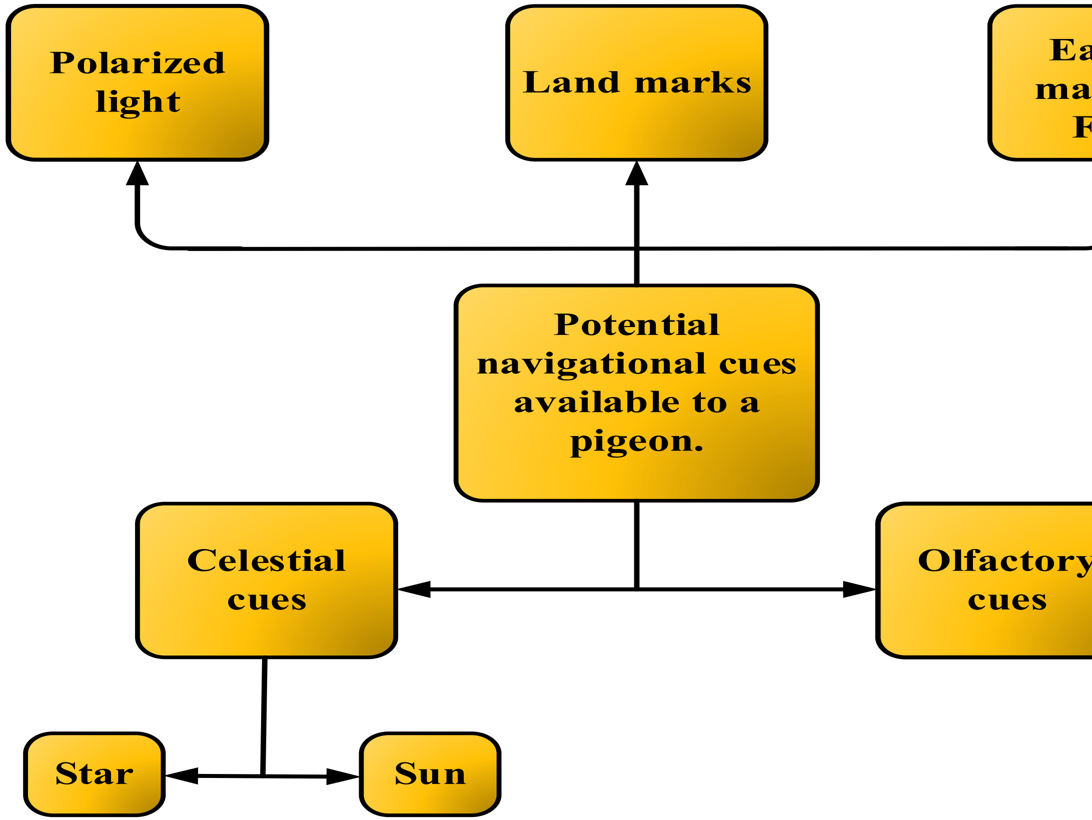


Figure 1-1 Possible Cues Used by Homing Pigeons

1.1.1 CELESTIAL CUES:

There are various kinds of navigational sources that the pigeons use while homing which play a significant role in some cases and are considered as the primary sources of their navigation. These include the Sun, stars, polarized light, and odours. Similarly, homing pigeons show instinctive behaviour and can remember the visual landscape features to create a map. By using those memories, they can find their way and reach their home destination [1].

For homing pigeons, the light of the Sun during the day time, skylight polarization, the light of stars at night are found out to be other references for finding their way [1].

The Sun or stars if observed from a certain position of Earth, at a specific time, seem to have a fixed position in the sky. However, Earth position is not fixed due to the rotation that it performs around its own axis as well as around the Sun. Therefore, the celestial bodies also show movement with respect to given location on Earth. The axis or the path along which celestial bodies move changes depending on the season in a regular manner. Hence, we can say that by analyzing the position of any star or Sun at a specific time, it can provide the necessary information for navigation. Similarly, pigeons use these celestial bodies in order to find their way but this ability of pigeon is a hereditary characteristic (called also the internal “clock”). The movements of celestial bodies are strictly related to time and follow a regular schedule. Different experiments were carried out to understand the navigation at phenomenon shown by migrating animals and homing pigeons. For example, both artificial celestial bodies and artificial sky have been employed to investigate pigeon reaction to different positions of celestial cues [1].

Sun and stars

In 1950, an experiment or study conducted on European Starlings [7], provided the very first proof or the birds' dependency on using the position or movements of celestial objects like stars and the Sun. When the food was placed in a cage and the position of the Sun (sunlight) was managed or changed by mirrors, the birds showed an ability to locate their food by using the Sun's position.

Later experiments were performed in order to prove the pigeons' ability to orient using the Sun compass. This was achieved by using “clock-shifted” pigeons, whose –night internal clock phases were artificially modified in lab [8]. Then their behaviour towards Sun orientation was observed. It was concluded that the artificially modified pigeons displayed a predictable diversion with respect to the movement of the Sun.

Then Stephen Emlen[9], carried out more experiments but on different birds and animals like Indigo Buntings, *Passerina cyanea*, in a planetarium [10] to record the bearing vectors as the position of the stars were artificially shifted.

At that time when initial experiments were being carried out on the navigational behaviour of birds, Gustav Kramer was the person who stated that the navigational behaviour of birds using celestial bodies' position is dependent upon time, which later become known as the hypothesis of time-compensated celestial compass orientation [11].

Various experiments were also achieved under a clear sky as well as on overcast day. These experiments also showed that celestial body’s position is used by the birds to perform their navigation. It was also observed that the pigeons, which have not been trained to migrate from a specific location, have experienced a disruption in finding their way under cloudy conditions or in the dark, whereas these same pigeons have oriented and navigated easily in day time or in the presence of sunlight 12]. Hence it can be stated that birds always can use sunlight for finding their ways (navigation). In contrast, pigeons which have learned their way to their nests can travel under an overcast sky as well as at day time. The pigeons which have learned the way back to their home are not only dependent upon the Sun but they can use any other reference like Earth's magnetic field, or features of the land or odours.

Different studies have been carried out in order to study the effect of sunlight on birds finding their way [13]. Such experiments can be performed by changing the “internal clock of birds”. The word “clock” in case of this study of navigation of the birds means the ability of the bird to imagine or perceive the position of the Sun with reference to the time of the day. In order to perform experiments to verify birds' ability and prove the hypothesis of time-dependent Sun compass, the birds were “clock shifted” for many hours and then the direction of movement of birds was observed. The “Clockshifting” procedure is performed inside a room in which the timing of darkness or brightness has been set differently as compared to the outside natural environment for several hours and birds (pigeons) were allowed to stay inside that ran for several hours. After this, the birds were released or tested in funnels to observe their orientation. It was observed that the birds move in a direction in which they would be expected to move in the light of Sun when they are in open air before staying in room. A deflection and scattering often occurs in some cases when the directions of the birds' bearings do not coincide accurately to the values indicated. This could happen due to the birds' confusion when two compass inputs are not coinciding such as their magnetic compass and the now "clock-shifted" Sun compass. Similarly, if wild birds which generally fly all the time in forests are kept inside, they became confused and therefore they respond differently [14]. This effect of clock shifting has been observed only in some specific species of birds. The study also indicates that birds first use to manage their internal ability to perceive the position of Sun (internal clock) and the Sun's actual position, then by comparing both they found out in which direction they have to fly to reach their homes. This is also notable that their speed of travelling directly relates to the speed of Sun movement as it changes its position or its angular position with reference to Earth throughout the day known. At noon, the rate of movement or its speed is greater as compared to morning and evening.

Another study [15] shows that there is a considerable interrelationship between the heading of the pigeons which have experienced a clock-shifting and the amount of the clock-shifts. This suggests that the Sun plays an important role as a navigation source for the pigeons. A deviation in the pigeon's migration way is noticed which is equivalent to the anticipated angle that can be calculated theoretically based on the clock shifts. Pigeon's familiarity with the local areas has not been taken in account as it also could be influence the pigeon Sun compass orientation [16].

Some other experiments were carried out in which the birds were partially exposed to the Sun but not completely [17]. For this type of research, pigeons were grown in an enclosure or in an artificial environment where they have never seen Sun. Then they were divided into two groups: test and control groups. The control group is allowed to see the Sun for whole day whereas the tested group is allowed to see the Sun only after noon to sunset. Both groups were prepared to discover their food at a particular bearing in an open air enclosure so that they have to visit through sunlight but they are not allowed to see the surroundings.

After some time, both groups were released in the morning time but no change in their behaviour was notable. Which means that the second group, which has never been exposed to the morning sunlight, was capable of using the Sun's movements in order to achieve a compensation to their own compass orientation. Moreover, both groups oriented randomly when they were released under completely overcast sky.

The researches have suggested that the celestial compass is considered to be a learned mechanism in pigeon.

Other experiments [18] have been performed where the pigeons have been divided into two groups: The first group were young pigeons (under the age of 2 months) which have never seen the Sun. But when this group has released, they were able to return home without using the Sun compass for orientation, which suggests that the younger pigeons rely on the geomagnetic field for navigation because the Sun, landmarks and odours have to be learned in advance. The second group (at the age of approximately 3 months) had learnt the ability to use the Sun compass; this became obvious when the clock-shifted pigeons shifted their orientation at release sites [19].

Stars in the sky are also used as a reference for navigation (especially for birds which migrate at night time)[9]. Experiments on birds using stars as a reference have been carried out in outdoor open air (with no moon) or in a planetarium (with the absence of planets in the artificial sky but not stars). These tests indicated that birds only use stars as a reference. These studies also suggested that birds possess a time-independent stellar compass. Also, the North could be deduced by pigeons from the geometrical pattern of the stars, without taking into account the rotation of stars. (For more detailed description of these hypotheses see [10]).

How birds can use these references for migration is still debatable, because of the shortage of studies conducted on this topic. Nevertheless, the results obtained from some of these studies that support specific hypotheses can be considered for further discussion.

Experiments on buntings [8] regarding stellar cues shows that birds do not change their direction of motion regarding the position of stars in the sky. When changed, they did not change their direction of motion accordingly. Stephen Emlen deduced that birds do not use any time-compensated stellar compass, but the birds can use the geometrical distribution of stars in the sky as a reference. However, afterwards when specific constellations were displayed to the birds (even with absence of the Polaris), the results failed to support the hypothesis.

Saucer had stated [8] that birds have genetically inherited the ability to use stars as a reference in order to find their way and the map of stars in sky has already been imprinted on the birds' mind.

Stephen Emlen has disproved the presence of the star map in the pigeon's mind. He suggested instead that the ability of the pigeon to use the stars can be learned and they use rotation of stars as a reference instead of their position as a map. So, the rotation of celestial objects could help the birds in learning the importance of certain stars which they then use as compass.

Stephen Emlen's results [10] have demonstrated that the axis of rotation of the night star pattern could be perceived by the birds and the least rotation will remember by them (it coincides with the position of the North Star in the case of the natural sky). Then, this will be used in order to locate the geographic North, which means a stellar compass is available to the birds during the migration.

Further tests using pied flycatchers and blackcaps for stellar orientation [20] showed the impact of changing the position the Polaris (the North Star) which will cause a deviation in the birds' migration routes which coincides that the imposed orientation shift.

Further tests have been carried out (for more information about analysis, see also Stephen Emlen [21] but all show that birds do not use time compensation for the stellar compass as they do in case of Sun compass. Similarly, similar experiments been designed for mallards, Anas platyrhynchos, by using artificially sky patterns, but the birds again do not show the orientation change regarding or with respect to time [22].

Liepa had done more experiments [23] that concluded that birds always use a reference star in any environment. In a planetarium, they use Betelgeuse, and in open sky outdoors, they use the North Star as a reference. Both skies are different in many features such as the spectrum of light. Birds can memorize the pattern of each sky and respond differently based on the pattern that has been memorized in order to solve this problem. Clearly, more research needs to be carried out.

1.1.2 POLARIZED LIGHT:

Sunlight coming from the Sun is electromagnetic waves. When this light enters the Earth's atmosphere, it will be polarized, a phenomenon known as polarization. Electromagnetic waves (light), comprise an electric field vector E and a magnetic field H which are perpendicular to each other. Most of ambient light is not polarized, so at any given moment the unaided eye will experience a set of pairs of waves from different direction (i.e. perpendicular waves). Therefore, polarizing filters are used to allow waves at certain angles to pass through. The angle of light polarization is the angle of the vector of the electric field. Humans usually have to use devices for observing such phenomenon but many animals can view this phenomenon by their natural eyes [1].

Light when it enter into the atmosphere and under the water shows a specific and regular polarization pattern dependent upon the position of Sun on the sky. For that reason, it been suggested that these patterns of polarization may provide additional navigational information for animals. Hence, we can say that, indirectly, the polarization of light is used as a reference for birds in order to find their way. Polarization of light is a very useful phenomenon for birds in navigation and orientation, e.g. finding the right direction to find food and for defence purposes [24],etc.

It has been difficult for researchers to carry out definitive experiments on birds hence it has been considered disputable. One group of experiments shows that the birds use the polarization of light for finding their way during their seasonal migration. Passerines often move or travel at night times and keep migrating for a short period after the sunrise. This helps them to use the polarization of light to re-correct their direction after they deviate from the right route during the night time [25].

Some birds of North America known as warblers have also been tested but it appears that these birds more efficiently find their location using polarized light in early morning as compared to noon and overcast skies [26]. Several researchers have found that the different patterns of birds orientation could be caused based on the axis of polarization [26]. For that reason, some experiments have also used artificially polarized light in order to find out its effect on the bird's orientation. It is a challenge to use artificial polarized light in this kind of research. But the study on blackcaps has proved the bird's capability to respond to the artificially polarized light is different in the presence of natural polarized light (i.e. sensitivity of birds towards both type of light is different) [27].

During some experiments the birds have not responded to the polarization of light but the researchers state that this was due to condition of the environment. Birds' sensitivity to polarized light depends on how the e-vector direction is going to be recognized. Typically, this process occurs in the bird's cones in the retina of their eyes. Birds can sense the polarization of light because they possess double cones. Another research has proved that insects and cephalopods have photoreceptors that are sensitive to polarized light, but this has not been seen in pigeons [28].

Other work [29] has shown that Savannah sparrows have recalibrated their magnetic compass when exposed to the natural external environment (above the horizon) so they have used the information of the sky light polarization pattern over the horizon and compared it with their magnetic compass. The results became clearer when the birds that are exposed to a magnetic field while seeing the natural sky are compared to those who have not been exposed to natural sky.

From all of the above, we can conclude that birds can use polarized light as a reference in their orientation. It is also clear that birds always use polarization of light as compared to other animals for finding their direct orientation. However, not all birds possess the ability to sense polarized light. For example, pigeons cannot sense the polarized light, as suggested by one study [30]. An experiment [31] has been carried out in order to prove that the pigeons do not perceive polarized light. Pigeons were divided into two groups. The first group has exposed to under polarized light and the second group was exposed to different spectra (including white and UV) of polarized light flashes. The response of the second group was recorded using Electroretinograph. This has revealed that both groups showed no response.

European starlings and Japanese quails, Coturnix japonica, were trained to use the polarized light patterns for foraging but they also failed to show any sensitivity to the polarized light [32].

Other researchers have used conditioning paradigms to investigate pigeon's sensitivity to polarized light. An experiment was carried out by conditioning pigeons using a Skinner box in order to discriminate directions of polarised light and found that they were able to discriminate the direction, but they were not capable of using the information to orient spatially by themselves [33].

1.1.3 OLFACTORY CUE:

Using olfaction as a reference by birds and animals in order to find out their orientation remains controversial. Many studies have supported this hypothesis out of which some are experiments carried out on the animals by depriving them of their sense of smell. Birds can also be made senseless regarding smell by using various methods [1]:

1) Cutting their nerve responsible for smell.

2) Chemically anaesthetising their sense of smell.

3) Using wax to block their nostrils.

It is not simply a matter of whether birds might be using their olfactory organs as a reference. There is also evidence that supports the hypothesis of using the olfactory organs for magnetic navigation to provide a putative magnetic "map" sense. Specifically, the bird's ability to deduce the putative magnetic field is disabled when olfaction is lost or impaired, i.e. when cottons plug has been inserted in birds' nostrils [1].

Some researchers have proved that using the olfaction sense in homing pigeon is crucial during the short distance and middle distance navigation.

Others have concluded that it is not common to use olfactory organs for navigation in birds, including pigeons [34]. When using olfaction in navigation, the air and direction of wind are also two important factors which have to be considered.

Flow pigeons are found to be the most studied vertebrate as most of the experiments for testing olfactory organs as a reference have been carried out in pigeons. A hypothesis of using olfactory navigational map has also been formulated.

Papi and his team [35] suggested and carried out an experiment by dissecting the anosmic nerve of pigeon. Two groups of pigeons were compared. The first group was left with a normal olfactory nerve and the second group experienced a dissection of the anosmic nerve, thus impairing the olfactory sense.

In order to find out whether by treating the pigeons' anosmia will affect on the pigeon's orientation or it will further harm them. Birds have been divided into 3 groups [36]:

1. Control birds which have not been treated.
2. Control birds, in which the olfaction sense of birds was disabled by zinc sulphate application and nasal plug on the same side.
3. Experimental birds, with unilateral $ZnSO_4$ and a nostril plug on the opposite side. The pigeons' ability to use olfactory organs for navigation has been tested after these 3 groups of pigeons were released into the open air distant that exceeds the range of the pigeon's familiar area, which is 55-79km. The results showed that the birds of the third group experienced a disturbance in their navigation route that was clearly worse than the first and second groups. The two control groups were not significantly different in their ability, but the group of pigeons having blocked olfactory organs were became directly affected and lost their ability to come back home or have the right orientation. However, blockage of their olfactory organs had no effect on their brain at all.

Therefore, we can say that the sense of smell can be a very crucial reference used by the birds for their navigation. So, the olfactory hypothesis assumes that the different trace substances, which are available in the atmosphere, varies in the stability of the spatial gradients [37]. The low and high concentration of gases in atmosphere would lead to the mapping of a region or particular area by birds, and these maps could then be deduced based on the gradients. The fundamental basis of this hypothesis is questionable because researchers have argued about the stability of the gradients [38]. Moreover, the composition of air is gradually changing over time.

It has been suggested that the pigeons utilize olfactory cues in order to deduce a mosaic map (a map with a small components irregularly arranged together). Pigeons use this map during short distance migration, whereas other birds' species might use it as a primary cue of navigational information in the final phase of long-distance migration.

It would be logical and reasonable to conclude that the birds use the olfaction for navigation in short distance migration. Although birds use other cues in long distance migration, but they could still use odour and landmarks in the final stage of their long distance migration.

More researches are required in this field in order to settle the role of the olfactory cue in birds' navigation.

1.1.4 LANDMARKS:

Landmarks can also be used for navigation while coming back to home over short distances [64]. The landmark term was renamed by the word "piloting" (using features of nearby areas to find their way to home) by Klaus Schmidt-Koenig [39]. Piloting can be defined in many ways. Definitions of piloting relate to the extent to which landmark features have been used by birds and other animals in order to come back to their homes and over what distances it is valid, but more studies and research on this topic are required. The most meaningful definition of piloting refers to the distribution of familiar features on a map of a terrain or surrounding areas which the birds can see, remember and then use to find their direct way to their homes [40]. This map can also be referred to as "familiar area map".

Up until now, it has not been very clear which reference is being used by the birds most of the time, but according to some researchers, piloting can also be used as a sure or certain reference by birds for short navigation.

The navigation provided by their ability to use Sun or magnetic field as a reference was indirect, but piloting can provide a direct reference. It has also been suggested by researchers that birds memorise the landmark features of their surrounding areas for 5 minutes before leaving their homes [41]. But when they have to travel to unknown or unfamiliar sites, then this type of reference did not work anymore [42]. For birds, it is very easy to see the landscape features of the surrounding areas from where they have been released many times to come back to their homes, and they can easily remember their routes home but to preview the features or way or areas which had been visited by them a long time ago is very different and will certainly take more time.

It can be concluded that a previous view of an area helps the birds to recognize from their prior visits the way back home and they do not need to waste their time by thinking about that way. Thus, they already know their direction and their goal if they have already visited that area [43]. Furthermore, other than the landmark features, the other aspects of the way or path cannot be recognized by the birds. Studies of the routes, terrains or paths of birds if measured by using GPS technology, suggest that the birds can remember routes only up to 1 km from the site from which they have been released. Hence, the ability of birds to remember their previously visited terrains is more for straight line paths. When released birds often start in order to discover their way. Landmarks help them determine if they have been let go from there before or not.

Similarly, distances of more than 1km from the familiar release site are difficult to recognize [44]. Data also suggested that the map of already visited areas provide birds with the orientation or direction to fly before being released from a certain site. Pigeons always use complex routes to fly as compared to other birds. Hence, more experiments are needed to obtain clearer results. Using GPS technology has also helped in research. Small transmitters have been attached to pigeons and then their flight is observed by the cameras and interpreted as well. It has also been observed that a pigeon's complex way of travelling is due to the fact that they first go in the wrong direction or orientation, but then after recognizing their way, they come back again and fly in the right direction [45]. Further experiments and research are needed to determine the importance of landmark features in pigeon homing. Features of landmark will certainly provide information that can be used along with other cues to navigation [46].

Landmark features being used as a reference for navigation depends upon many factors, such as the ability to remember and the distance to be covered. In order to cover short distances, the birds usually use landmark features as a reference instead of compass (either of Sun or magnetic). But when travelling at night or travelling over longer distances references other than landmarks are required, although some of features like city or street light can be used to some extent.

1.1.5 Earth's Magnetic Field:

The Earth's magnetic field provides navigational information for birds, which can be either directional and/or positional. The directional information (bird's compass sense) could be deduced from a bird's ability to sense the compass direction of the Earth's magnetic field. Also the bird's ability to use geomagnetic cues could provide the positional information (map sense), i.e. birds are capable of determining their approximate position depending on Earth's magnetic field information. Earth's magnetic field for birds is not only used for orientation and navigation but also for many other purposes, such as a reference for changing the bird's behaviour, such as motivation for travelling.

Birds' physiological mechanisms(s) for perceiving Earth's magnetic field is still unknown. Biologists have shed the light on the bird's ability to perceive such a weak magnetic field (Earth's magnetic field).

Theoretically, there are two fundamental phenomena that could be used in animals in order to achieve navigation using Earth's magnetic field:

- Mineral-based magnetoreception mechanism
- Radical-pair-based magnetoreception mechanism

In birds, there are some brain areas including plausible primary sensory molecules that are candidates for processing magnetic information of the above types.

In spite of the presence of two possible types of magnetoreception mechanisms, it has not clearly understood its nature. Up until now, no evidences that birds use one or both of these mechanisms in navigation. This remains a challenging field for future researches.

1.2 MAGNETIC FIELDS

A charged particle moving without acceleration produces an electric as well as a magnetic field i.e. the electric charges are usually electrons. Taking the microscopic scale into account will show that the magnetic field can be produced from spinning the electron and nucleus. Also, it will show that the magnetic field could be generated around a wire when a current goes through a wire. The strength B of the generated magnetic field at a specific location can be described as a three-dimensional (3D) vector. Magnetic flux density is measured by Tesla [T]

$$\mathbf{1T = \frac{1V\times s}{m^2} = \frac{1\,N\times s}{C\times m} = 10,000\ G} \qquad \text{Equation 1-1}$$

(V=Volt, s=second, m=meter, N=Newton, C=Coulomb, G=Gauss)

The right hand rule is used to find out the direction of magnetic field. Taking or holding the wire in the right hand, the thumb will be pointing in the direction that the current will pass. The direction of the magnetic field will be of holder remaining fingers circling around the wire. Magnetic field is inversely related to the distance; as distance increases, the magnetic field decreases. If we are using a coil of wire instead of a single straight piece of wire, the magnetic field created will be stronger inside the coil as compared to outside. Hence, where strong and artificial magnetic fields are required, coils are usually used. Magnetic field is a 3D vector quantity. The resultant of adding an artificial field to the Earth's magnetic field could be performed by doing vector addition of the two fields. Ferrimgatic material is material magnetized by an external magnetic field. If the magnetized field is removed, the magnetization will remain. Magnetitite (Fe_3O_4), an iron oxider, is a well-known example of a ferrimagnetic mineral [47].

1.3 THE EARTH'S MAGNETIC FIELD

The electric currents in the fluids or liquids which present in the outer core of the Earth create a magnetic field that is known as the "dynamo effect" or Earth's magnetic field. The natural magnetic field of Earth could be imagined to be similar to the field that could be obtained from placing a large dipole magnet in the centre of the Earth (Figure 1-2).

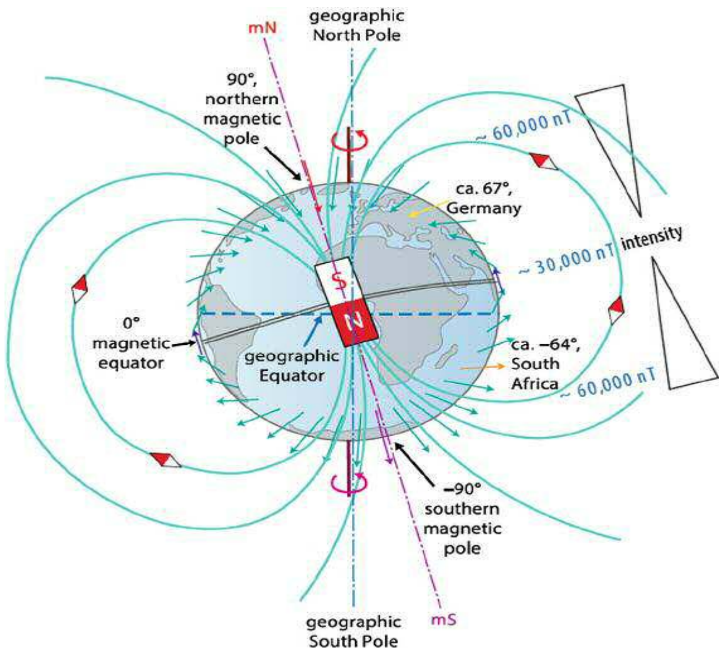


Figure 1-2 Earth's Magnetic Field [169]

It demonstrates that the magnetic poles (Southern and Northern) and the geographical poles (Southern and Northern) do not coincide. Also, the magnetic equator does not coincide with geographical equator.

(Figure 1-2).also showing the inclination angle, which is the angle at which magnetic field lines intersect the Earth's surface depending on the magnetic latitude. Magnetic inclination is +90° at the Magnetic North Pole (red vector), +67° at the latitude of Germany (yellow vector), 0° at the magnetic equator (dark blue vectors), −64° at the latitude of South Africa (orange vector), and −90° at the Magnetic South Pole (magenta vector). Also, it can be seen from (Figure 1-2).that the magnetic field intensity varies from 60 μT near the magnetic poles to 30 μT along the magnetic equator.

Magnetic field of Earth possesses two poles, North pole and South pole. The South pole of the magnetic field is located at the geographical North pole of Earth, and hence used as magnetic North of the Earth in biology. Throughout this thesis the magnetic field near geographical South pole of Earth will be stated as magnetic South (that is not actually the South pole magnetically); similarly the magnetic pole near geographic North pole of Earth is stated as “magnetic North” (that is not actually the North pole magnetically) [47].

Lines of magnetic fields emitted from the magnetic South Pole enter into the magnetic North pole. The polarity of the magnetic field lines always points toward Magnetic North. And hence can be used as a strong reference everywhere on the Earth by birds except at the poles where the case is different. At the extreme poles, the magnetic fields lines point directly towards the sky or toward Earth. At magnetic South Pole, lines point directly towards the sky, and at the magnetic North poles, the lines point directly towards Earth. Whereas at the centre (equator), the magnetic field lines become parallel to the Earth’s surface.

The magnetic inclination hence keeps on changing. At extreme magnetic South pole, it will be -90 degrees and then gradually it becomes 0 degree at equator and then rises again until it reaches extreme magnetic North pole where it becomes +90 degrees. The intensity of magnetic field ranges from c.30 μT near to the equator and 60 μT near the magnetic poles.

The intensity of Earth magnetic field can be measured by a three dimensional fluxgate magnetometer.

The angular deviation between the magnetic and geographic North Pole is known as declination. Intensity of magnetic field and angle of inclination changes or ranges between magnetic South and North Pole. These vary on a daily basis and hence affects the accuracy of the magnetic map as a reference.

The total circumference of Earth is supposed to be 40,000 km, and the total change in magnetic inclination is supposed to be 0.0009 degree per Km along the South to North axis whereas the total change in magnetic intensity is supposed to be 3nT/Km. These numbers should be considered alongside the fact that natural variations occur in the geomagnetic field somewhat stochastically, in the order of 30-100nT and in more or less random directions. During magnetic storms generated primarily by the Sun, the variability can reach 1 μT. It is then clear why any map sensing system based on magnetic fields is unlikely to have a precision better than a few kilometres. Both magnetic intensity and magnetic inclination can theoretically be used on a larger scale for locating one's position on the Earth.

On earth the magnetic intensity and inclination varies continuously from North to South, but there is not much variation in the field parameter from East to West. Because the declination is negligible at low latitudes and increases to become substantial at the poles which can cause a serious problem in the bird's navigation unless they find another method for compensation. So the declination can be used for navigation is still in theory solely, as the researchers think it can help to give more accurate information about their position or location on any part of Earth can be found out by the assessment of East- West variations.

1.4 Birds Use Information from The Earth's Magnetic Field for Orientation and Navigation

All migratory birds use orientation and navigation skills in order to find their way. Young birds migrating for first time face a difficulty in finding an unfamiliar wintering area that thousands of kilometres away [48]. In day time, adult birds migrate in a group, so the young birds might follow the adult birds due to their experience of knowing the migration routes. However, small songbirds migrate during the night and travel alone without following their parent's routes. So their navigation ability will depend only on inherited sensory capabilities and strategies [48].

Thus, strategies for the orientation of solitary, first-year migrants cannot involve cues requiring previous experience of the goal. The number of possible orientation cues is thereby limited to a few classes of globally or regionally consistent cues [48]:

1. Celestial cues, such as the stars, the Sun, and perhaps the sky's polarised light pattern
2. Geomagnetic cues

Moreover, other chemical cues, including odours,[49], infrasound (sound with frequency below 20 Hz; [50], and/or Coriolis forces might also be used for orientation and navigation. Coriolis force is the phenomenon of deflection in the direction of moving liquids and moving air, which is to the left in the Southern Hemisphere and to the right in the Northern Hemisphere because of the Earth's rotation [51].

However, studies on birds have shown no physiological structure that would give them the ability to detect the Coriolis Effect with a sensible signal-to-noise ratio [52].

Similarly, it seems difficult for young birds with no prior experience of travelling and migrating to know the infrasound or odour "landscapes" through their migratory path. Also, it is difficult for the infrasound and/or odour landscapes as a primary map cue to be utilised by inexperienced birds over thousands of kilometres [48].

Moreover, it is difficult for the birds to recognize the sources and the direction of infrasound because the width of the wavelength of infrasound is much bigger than the width of the bird's head, which is 2 cm wide [48]. Thus, the young inexperienced migrating birds are using celestial and magnetic cues as a primary orientation system in their first autumn migration. Also these young birds (in their first-time autumn migration) are not capable of correcting the displacements that occur in their migration routes [53]. Hence, they choose a migration route that is parallel to their normal route as an alternative route which means that they do not possess a map sense (see Figure 1-3).

The primary travelling program of birds (young birds) can be named as "clock and compass", "calendar and compass", and "vector navigation strategy" [54], according to which the birds fly for the first time in a specific direction for a period of time without taking into account their present location. Because the system showed independency for the location, so the migration of the young birds is mathematically considered to be as a random walk or flight because every evening the birds decided their direction or orientation of flight randomly and did not depend upon the previous experiments [54]. This strategy explains that the statistical distribution of first flight of a bird should be parabolic, and it has been reported in the first flight of many birds of different species in Western Europe [54].

Researchers have proved that the orientation of young migrants (flying for the first time on long routes) and direction of adult migrating birds (which have been migrating for many years) in spring will be different from their orientation in autumn [54].

Adult migrants and young migrants on their first spring migration use local (map) information that they gained through their migration routes. This map information is then utilized to migrate back toward a region with which they have had experienced previously.

Birds that have the ability to use all their senses or all types of cues (e.g., magnetic sense, olfaction, vision, and hearing) during their migration can improve their navigation and can fly more directly as compared to these which can use or which have the ability to use only one cue or sense. Therefore, the flights and migration of adult birds (which can use many cues and can have prior experiences and have learned maps) are more accurate [54]. Indeed, in contrast to first-time migrants, the birds that are experienced in migration are capable of correcting displacements in their routes [55], and for that reason, the learned map is involved in their navigation program (see Figure 1-3). This map is also functional in correcting the birds' direction and orientation when they are displaced to faraway locations where have never been before [55]. Therefore, displacement experiment has been achieved in order to provide details of the spatiotemporal orientation strategies of migratory birds, so demonstration of Perdeck’s classical experiments has been shown in (Figure 1-4 A) experiments in which more than 10,000 starlings have displaced during autumn migration from holland s/sse (S/SSE (→) to Switzerland. It has showed that young starlings (•) on their first autumn migration were unable to correct for the displacement. A parallel displaced migration pattern has been performed by the young birds which is relative to the wintering area of non displaced controls (dashed area) while adult starlings (Δ) corrected their direction instantly back to the normal population specific wintering area of non displaced controls.See Figure 1-3 (A). Emlen funnels was used to test the displacement experiments of Eurasian reed warblers (Acrocephalus Scirpaceus) (before and after displacement), which demonstrated the young birds already able to correct for 1000-km Eastward displacements to a location in the first spring migration where they have certainly never been before see Figure 1-3 (B).

Orientation of birds at the capture site (Rybachy) and in their orientation after the 1000 km Eastward translocation to Zvenigorod have been monitored which can be seen see (Figure 1-3 a) and Figure 1-3 b). Also the map of the displacement region has shown below in Figure 1-3 b, which shows a comparison. Also birds that undergone to cut of ophthalmic branch of the trigeminal nerve could no longer compensate for the displacement see Figure 1-3 d-f)

If the map learned by the bird or imprinted on the mind of the bird is based on prior experience or on landmarks which have been visited by the bird before, then it should not have been helpful in unknown locations. Up until now, the actual application of imprinted map is not clearly understood [56], It is most likely based on several cues, and is likely to involve the detection of gradients at larger scales. This can then be extrapolated, thereby enabling birds to navigate home from unfamiliar locations.

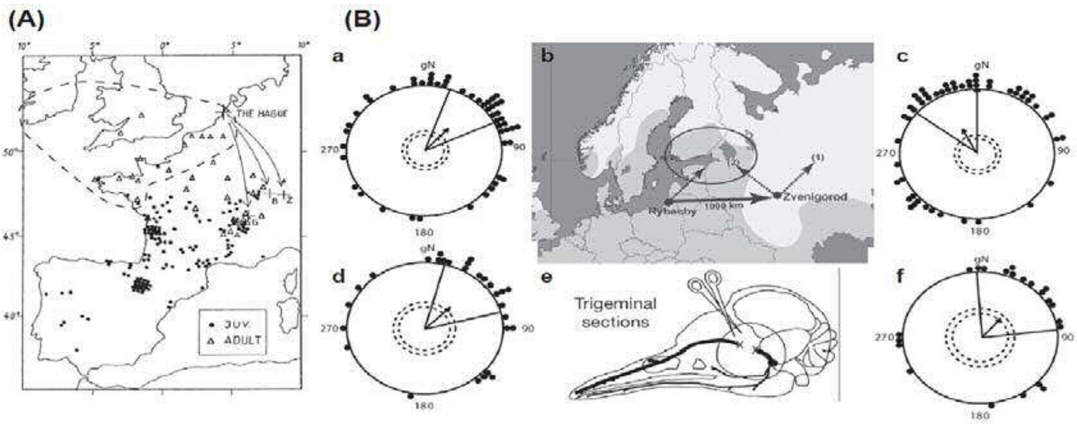


Figure 1-3 Displacement experiments (A) Perdeck's classical experiments, (B) comparison of birds displacement. [56]

1.5 THE MAGNETIC COMPASS OF BIRDS

In mid the 1960's the scientists Friedrich W. Merkel and Walfgang Wiltschko discovered that birds possess a magnetic compass[57]. They took advantage of the fact that birds show migratory restlessness when they have been left in rounded cages over night (Zugunruhe in German [58]). The birds first jump in their migratory direction, but when the direction of the magnetic field is changed horizontally in the absence of (Sun and stars) cues, the birds turn their direction with the applied magnetic field (see Figure 1-4). Hence, this demonstrated that birds can use a magnetic compass for their orientation and can turn their direction along with the magnetic field [59].

The ability to respond to the magnetic compass has also been found in many other migratory bird species [59]; consequently, the assumption could be made that all migratory birds possess a magnetic compass. Two magnetic field properties contribute input to a magnetic compass:

1. A magnetic polarity compass (e.g., the human ship compass) of which only horizontal lines out of all field lines have been used; anywhere on Earth, it points towards Magnetic North except at the magnetic poles.
2. A magnetic inclination compass which detects only the angle which is between magnetic field lines and the Earth's surface or gravity—i.e. not depending on the polarity of the field lines. The smallest angle between the field lines and Earth surface indicates that the direction is towards the magnetic equator on Earth. Whereas the greatest angle represents that the direction is towards one or other of the magnetic poles. The inclination angle for the Northern and Southern hemispheres are opposite. Therefore, the latter includes both. All bird species on which experiments have been performed possess a magnetic inclination compass [60] see Figure 1-4.

   Experiments in order to investigate the inclination compass see Figure 1-4 has been performed using Emlen funnel because the Emlen funnel is the most ordinarily used orientation cage. scratch-sensitive paper lining the inclined wall of the funnel is used to record the mean jumping direction of the bird . Indications that birds possess an inclination compass, which means that the birds are capable to estimate the angle between the magnetic field lines and the Earth's surface or gravity. Consequently, the birds are capable to separate between pole ward and equator ward. which means it is not like a polarity compass i.e. birds are not capable to separate between North and South (Birds should have oriented in the direction indicated by the red end of the inserted technical compass if they use a polarity compass). Similar what is occurring at the magnetic equator, birds are made to lose their sense of direction in a horizontal magnetic field. The direction of the flight of the inserted bird indicates the springtime mean direction chosen by all bird species tested so far in the given magnetic field.

Hence, the birds which travel at night possess a magnetic compass that cannot differentiate between North and South direction just like a human ship compass but can differentiate between "toward the magnetic equator" and "toward the magnetic pole" (the Magnetic North Pole and the Magnetic South Pole, which are in the Northern Hemisphere and in the Southern Hemisphere, respectively). Further, the magnetic compass that birds possess appears to be limited by a narrow functional intensity window. However, it seems possible to extend this window to new intensities within a few hours by allowing adaptation to an unfamiliar magnetic field [56].

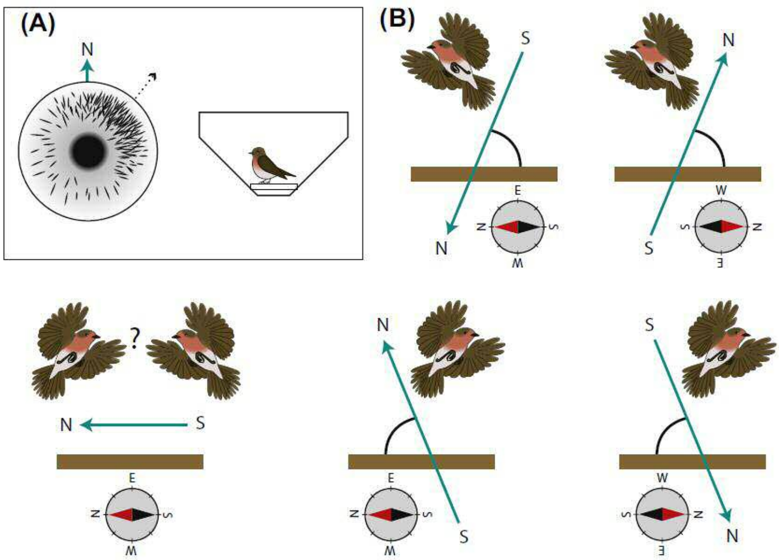


Figure 1-4 Investigation of inclination compass using emlen funnel, (a) The emlen funnel, (b) Indications that birds possess an inclination compass. The direction of the magnetic field lines has been represented in red arrows. Brown bar indicates to the Earth's surface, N indicates to geographic North, S indicates to geographic South. [56]

1.6 Do Birds Possess a Magnetic Map

Studies show that the magnetic field is used as a reference by birds in order to determine their position, i.e. birds have a magnetic map. But the actual basis of this magnetic map is still confusing. Researchers' views have ranged from a magnetic map with an accuracy of a few kilometres being an established fact [59] to the sense of magnetic mapping being thought of as an evergreen phantom [53]. One thing can be said for sure, the natural ability of bird to use the natural map is dependent upon many factors, such as olfactory organs [53], sense of vision [61], and sometimes on sensing of magnetic fields [62]. Sense of hearing (infrasound) can also be a factor but see [63]. The studies indicate that pigeons fitted with opaque lenses, which cannot see the landmark features, are able to travel up to 5 Km before they become lost [64]. Hence, without using landmarks as a reference, birds can cover or travel a short distance only. However, there may be some other references being used by the pigeons, not having the ability to use landmark features as a reference to come back home from 5 Km area, has raised a question for the researchers concerning which reference it would be. At present, the studies have been carried out on the dependency on olfactory organs and magnetic map, but still controversaries remain [34].

Researchers have found evidence suggesting that chemical cues (odours) play an important role in the absence of the landmark-based part of pigeons' map sense which is much more convincing than the evidence supporting the use of magnetic cues in the pigeons' map (e.g., [59]).

But at the end of all the discussion, both cues proved to be effective. The map sense is essential for the survival of bird and birds can use all of their senses in deducing the maps. Whatever the case, it remains very difficult to understand how a bird manages to find its location in less than 10 Km using the magnetic field as a reference. The problems being faced by the birds using or wanting to use the magnetic field as a reference is that the intensity of magnetic field changes from North to South axis about 3nT/Km, whereas the change in geomagnetic field is 30 μT from magnetic poles to magnetic equator, a distance of about 10,000 Km. The magnetic inclination only changes up to 0.009 degrees/ Km along the North to South axis (a 90° change over 10,000 Km). Whereas change in the intensity and inclination of the magnetic field along the East-West axis much smaller. Hence, birds using the magnetic field as a reference should have a strong sense of gravity as well as a magnetic sensory system.

However, even if birds do possess such navigation systems, natural stochastic variations in the geomagnetic field occur daily in the order of 0.03–0.1 μT. This happens in more or less random directions, meaning it is unlikely for a magnetic-field-based map sense to have a finer precision than 10–30 Km. Indeed, during magnetic storms primarily generated by Sun activity, the variability in the geomagnetic field can reach 1 μT [65].

Hence, magnetic parameters are utilized to determine position where the expected differences in the daily magnetic variations are consistently less than the magnetic field parameters [49]. Birds' navigation for short distances probably depends more on odours and familiar landmarks as their significant map. So, it has been suggested that the magnetic map is utilized on a much larger spatial scale. Also, magnetic cues have been used as an approximate geographic “signpost” by songbirds, which, for instance, give the birds information when they have to increase their fat reserves before crossing the Sahara Desert [66] or when they change their migratory direction [67]. Consequently, the magnetic parameters are referred to as a magnetic signpost when they trigger a change in behaviour [49].

1.7 Interactions with Other Cues

The primary strategy that pigeons use to orient during their homeward flights became more complicated due to the availability of a large number of potential cues suggested to be used by homing pigeons. The ability to sense and to respond to the magnetic field also interacts with other references. For example, the birds travelling at night use their magnetic compass along with the Sun and star celestial bodies' compass [67]. These birds can use all three compasses or any one out of these to find their way [68].

Sometimes the use of all the three compasses can create confusion and hence, birds can choose any one, which one is used depends upon various conditions, like the surrounding area conditions and the phenomena and variations occurring in nature. So Figure 1-5, shows an experiments that is used to investigate the birds capability to use the celestial cues in the time of the sunset in order to calibrate their magnetic compass.

Tracks of free-flying gray-cheeked thrushes (a) and Swanson’s Thrushes (b) released from Champaign, Illinois The migratory flights of not handled individuals have been demonstrated in black arrows. The migratory flights which experienced a magnetic field turned to 80° East before flyoff (experimental birds) are represented in red arrows , and yellow arrows represents the migratory flights of the experimental birds during following nights. the birds which did not migrate on the night of magnetic treatment have shown a migratory flight paths which are represented in white arrows. Arrows connection show flights of the same individual during subsequent nights. Because the experimental and control birds are different individuals for grey- cheeked thrushes, the data are showed differently in (a) and (b). Whereas in Swainson's Thrushes, the same experimental individuals were followed for at least two subsequent nocturnal migrations (because of the large spread in natural headings). When birds were lost during tracking at the site, this was indicated by broken lines. When the birds exposed to a magnetic field turned 80° to the East during sunset and that were released after all light from the Sun had disappeared. Then they migrated toward the West when they started migration on the same night. on subsequent nights, they migrated in a direction of northerly spring migratory. these results mean that the birds has used the Sun compass in order to calibrate their magnetic compass before take off and that this calibration occurs daily. The reasons are illustrated in (C–F): (C) for control bird all cues gives the same information. (D) if experimental birds calibrate based on magnetic compass from sunset-related cues, so the supposed to calibrate their magnetic compass so that 80° counter-clockwise to the magnetic field lines will be their "North" for the subsequent night. (E) After release, the experimental birds exposed to the natural field lines. Because all light from the Sun has disappeared, no new calibration is taken a place at time of release and their wrongly sunset-calibrated magnetic compass makes them fly towards the West for the rest of the first night, which is 80° counterclockwise with respect to the natural field lines? (F) On then followed night after release, Sun and magnetic cues are in agreement and the birds will restore the flight direction into their intended migratory direction. the horizontal direction of the magnetic field lines that exposed to the birds have been represented by the four thin parallel arrows (from C to F). The thick arrow indicates the expected orientation of the birds. The setting Sun and the three lines with double arrowheads indicate whether Sun and polarized light cues were obtainable as a calibration cue for the bird [56].

The experiment that used truly freeflying birds showed that two species of North-American songbirds, while they are travelling in spring, use the magnetic compass as their primary compass, but they also use celestial bodies' reference at Sunset in order to calibrate the magnetic compass. However, this is not true for all bird species [69].

Other birds might be using polarization of light in order to calibrate this compass [33]; but the detection mechanism for polarized light in birds is not yet clear. Pigeons show more complex interactions among different cues for navigation and orientation. As mentioned previously, homing pigeons have been proven to use olfactory cues [53]. Also, the landmark features have been used as a reference for homing pigeons [54], as well as prior travel experience (reviews in [59] and sometimes magnetic cues. All the above cues have been used by the birds when they travel along unknown paths after being released in order to find out their direction and orientation.

Many researchers have carried out experiments on the use of these cues as a reference in orientation of birds, but sometimes the results or outcome of the research are found to be contradictory. The reason behind this may depend on location. For example, one reference or one type of reference can be used for one location but the same reference cannot be used for another location. Therefore, birds and other animals have to use different references for different locations. One experiment performed in order to address the above mentioned debate was cutting the olfactory nerves or cutting of the ophthalmic branch of the trigeminal nerve in pigeons. The results showed that some pigeons, mostly in areas of Italy, use the sense of smell as a reference but do not use Earth magnetic field as a reference (see next topic for more details) [53]. Also, Procellariiform seabirds seem to need the olfactory nerves more than “magnetic” nerves [54].

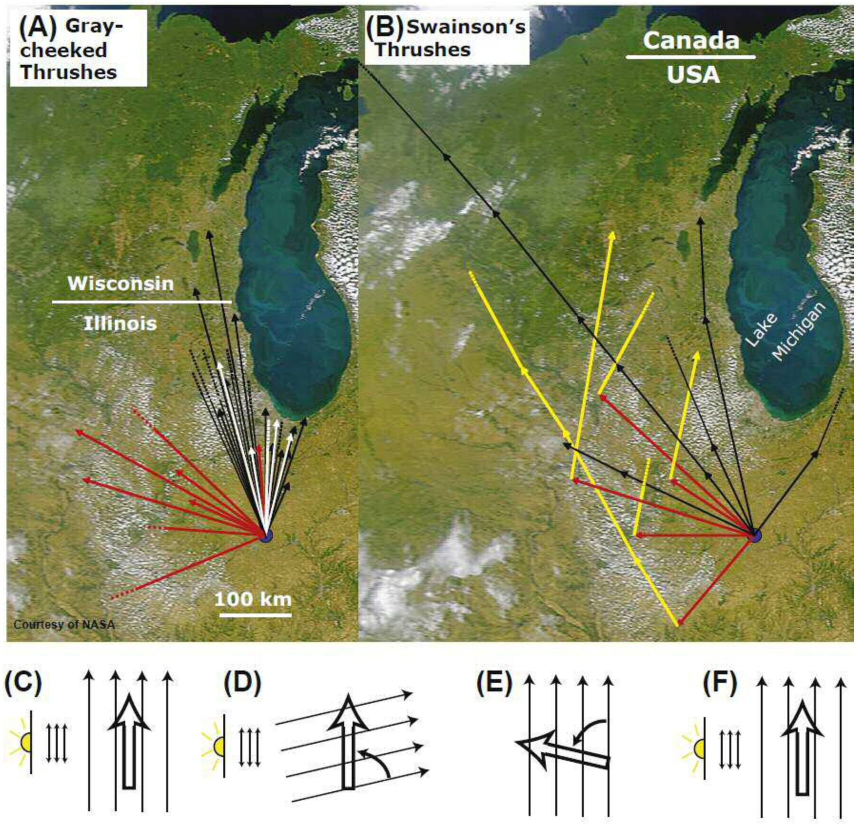


Figure 1-5 Using the celestial cues in the time of the sunset in order to calibrate their magnetic compass. (A) Release of free-flying gray-cheeked thrushes (B) Release of Swanson's Thrushes. (C to F) the process of birds' utilization of the Sun to calibrate magnetic comps. [56].

1.8 How Do Birds Sense the Earth's Magnetic Field?

It is highly complex to detect the weak Earth's magnetic field using biological materials. Taking into account the constraints of the bird's anatomy and physiology, careful models of sensory mechanisms often find it is difficult to explain how a 50 µT magnetic field can be used as a reliable cue for navigation in the presence of noise sources such as heat fluctuations, measured in (kT). In fact, various biological phenomena that can, in general, allow measurements of 50 µT fields have been considered [70]. There are three basic mechanisms currently approved by researchers:

(1) Induction in highly sensitive electric sensors,

(2) Iron-mineral-based magneto reception, and

(3) Radical-pair based magneto reception.

1.8.1 The Induction Hypothesis

When the conductor is placed in a constantly moving magnetic field, or when the conducting material itself moves or rotates within a constant stationary magnetic field, a current flows through the conductor material. Current is thus induced in the conducting material due to the magnetic field. This phenomenon is known as electromagnetic induction [71]. This principle can be demonstrated by holding a wire in one's hand and moving or rotating it within a magnetic field. A current would be induced in the wire. If the shape of the wire is coil shaped, then the sensitivity regarding direction can be obtained [72]. The tissue cells within biological bodies contain ring shaped, liquid filled structures. These structures can produce electric signals which can be detected by the receptor cells. Saltwater fish possess specialised cells known as Lorenzini ampullae, which can sense electric currents. This suggests that electricity can pass through saltwater and subsequently be detected by aquatic animals [49]. In the case of land animals or birds, it is also necessary for them to possess specialised cells for sensing electric signals [49].

1.8.2 The Iron-Mineral-Based Hypothesis:

The geomagnetic field has been used in order to find direction and to orient human beings; a magnetic compass is used for that purpose. This compass contains a needle made up of magnetized iron or compound of magnetic iron that moves in the horizontal plane.

Therefore, when discussing the bird's ability to use the magnetic field as a reference, the first thing comes to mind is that the bird must possess a magnetic compass needle in their heads, which can sense or move in response after detecting the magnetic field signals. This is the first hypothesis presented by scientists, i.e. that birds possess some kind of magnetic compass in their bodies [73]. It has also been thought that the magnetotactic bacteria that can sense magnetic fields also possess the compass needles inside them. If the magnetotactic bacteria are placed in magnetic field, then these bacteria can show alignment behaviour along the magnetic field lines. So the magnetosomes (magnetic organelles) have main contribution to lead to the alignment because these magnetosomes are usually placed inside lipid membrane and they contain iron oxide magnetite, Fe_3O_4, or the iron sulfide greigite, Fe_3S_4.

The magnetosomes have a magnetic moment in order to align the whole bacteria with the Earth's magnetic field lines so they will be presented as a compass needle because these magnetosomes are connected to a cytoskeletal filamentous structure and their magnetic moment along the axis of filament. so scientists have confirmed the magnetic field lines that are around the bacteria can help them to move oxic-anoxic transition zone magnetiv field lines and the gradient of oxygen (which loss it concentration with increasing the depth) are anti-parallel in the direction of the Northern hemisphere, and parallel in the direction Southern hemisphere [74].

Magnetotactic bacteria navigate via magnetic nanoparticles which contain magnetite; these particles are considered as ferrimagnetic. So with linear extension 30-10 nm they can be considered to generate large dipole moments. So these bacteria demonstrate a permanent magnetic dipole moment depending on single magnetic domain. It can be noticed the dipole-dipole interaction in a chain of magnetosome particles [75]. Also this interaction has an energy that can be calculated as shown in Equation 1-2:

$$E_{12} = -\frac{\mu_0}{4\pi}\frac{1}{r_{12}^3} \times \left(3\frac{(r_{12}.m_1)(r_{12}.m_2)}{r_{12}^2} - m_1 m_2\right) \qquad \text{Equation 1-2}$$

Where

$m_{1,2}$ are the magnetic moments of the two particles and r_{12} is the vector connecting them.)

Ferrimagnetic materials are the materials in which magnetic moments are anti-parallel (Figure 1-6) but the values do not compensate completely. This happens because this material are consist of different materials or ions (such as Fe^{2+} and Fe^{3+}) [76].

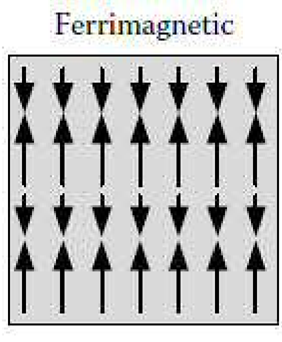


Figure 1-6: Ferrimagnetic ordering [76].

Ferrimagnetism is represented by ferrites and magnetic garnets. So the magnetite which is considered as the oldest loadstone to be used as a compass magnetic substance of the ferrimagnet materials are magnetite (iron(II,III) oxide; Fe_3O_4) [76].

The structure of this magnetic material consisted of two magnetic sublattices, which is usually defined as A and B which are separated by oxygen. Oxygen anions have the main job to achieve the exchange interactions which is called superexchange interactions. So the antiparallel alignment of spins between the A and B is happening due to the strong superexchange interactions. This could explain the reason behind the remaining of spontaneous magnetization which is due to the inequality in the magnetic moments of the A and B sublattices. Ferrimagnetism is therefore similar to ferromagnetism [76].

So Magnetite, Fe_3O_4 become in a crystal form with the spinal structure. The oxygen ions which are considered to be large ions are arranged in cubic format and the Fe ions which are considered to be small ions are filling the rest gaps.

These gaps can be classified into the following:

1.Tetrahedral site: Four oxygen are surrounding Fe ion.

2. Octahedral site: Six oxygen are surrounding Fe ion.

The two magnetic sublattices, A and B of the magnetite are shaped up by the tetrahedral and octahedral sites (See Figure 1-7) [76].

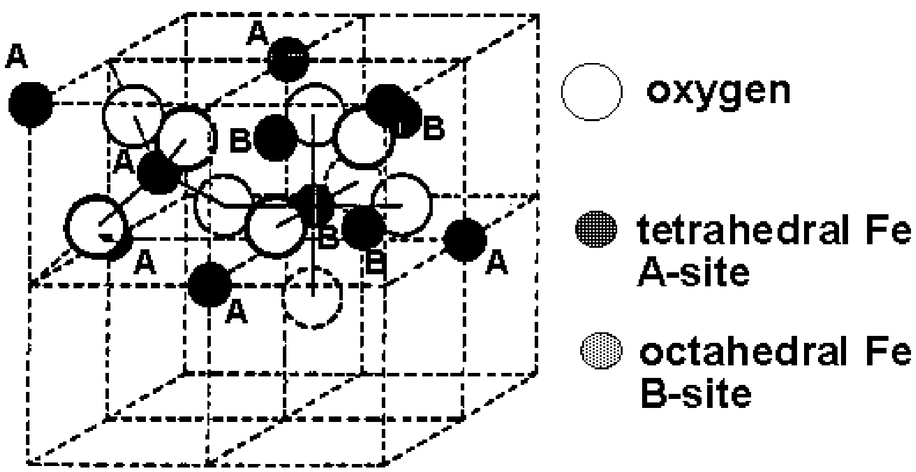


Figure 1-7: The structure of magnetite [76].

An experiment has been conducted in order to investigate the relative proportions of single domain and multiple domain particles in sample of material is depending on two the detection through magnetic measurements of the relative proportions of SPM, SD, and MD particles in a sample is based on two basic facts. (1) The high saturation remanence of the single domain particles without taking in account the grain size, and the low saturation remanence of multiple domain particles. (2) The temperature has an effect on superparamagnetic SPM threshold. So in this specific experiment, the researcher has used four magnetite powders 3mm in diameter, so the saturation hysteresis has been measured with room temperature and low temperature. Vibrating sample magnetometer (VSM) was used to measure the Magentization Curve at 77^{o} and 300^{o} K. Also, large cylindrical clay samples was used in order to measure the High-temperature hysteresis. It was concluded that stable single domain ranges are presented in large grain magnetite. So Ti-rich natural titanomagnetites are should not be single phase, also d_o (The critical size at which domain structure develops in equidimensionagl grains of magnetite) and d_s are the relevant threshold parameters [77].

So these particles would not exhibit a flux closure configuration and hence the interactions between the particles could result in the saturation of individual grains hence forming chains leading to a segmented object that would behave like a compass needle particularly in a liquid environment which is the case in Magnetotactic bacteria [77].

Compass is extremely simple device which consists of a small light weight magnet; this magnet is generally called "needle". One end of the needle is marked N for North or coloured in some way to indicate that it points toward North direction. So compass works by looking at the anatomy of the Earth. Its centre of the Earth is considered as a core, then the mantle and the main surface which is known as the crust. The Earth's core consists of molten iron and the pressure inside is so great which makes that iron to crystallize into solid convection caused by heat radiating from the core along with the rotation of the Earth. This will cause the liquid iron to move into rotational patterns. It is believed that these rotational forces in the liquid iron layer lead to weak magnetic forces around the axis of spin. This form a magnet like bar inside the surface of the earth the South pole of the magnet faces the North Pole and the North Pole of the magnet faces South pole. As it knows that North and South attract each other and by this way the

South part of the needle minds in that specific direction and that is how compass helps in various expeditions around the globe [77].

It has been reported that birds possess a chain of single-domain magnetite crystals that can detect the magnetic field or iron oxide may be present in order to detect the magnetic field. Thus, the iron-mineral crystals arrangement in the bird's body could be employed as a magnetic field detector [78]. The crystals of iron-mineral are expected to transduce magnetic signals via the opening or closing of pressure-sensitive ion channels [74].

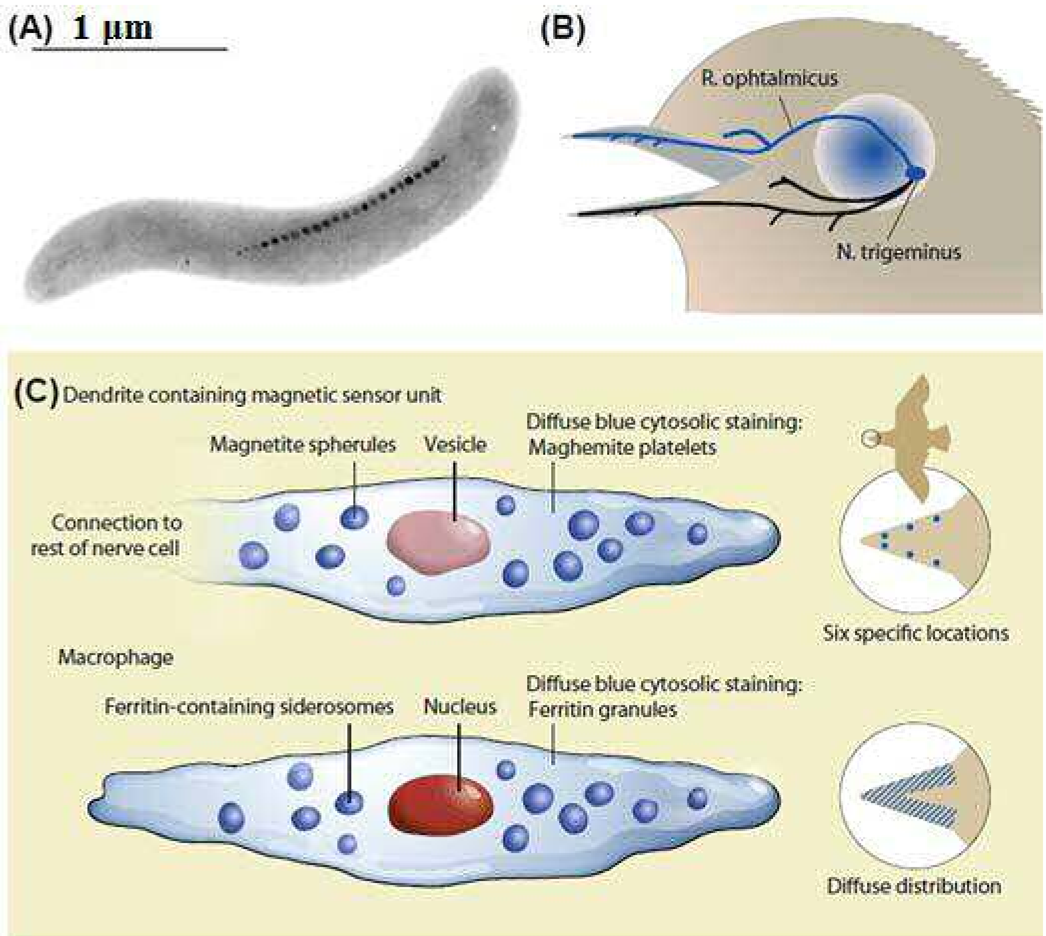


Figure 1-8 Iron-mineral construction in birds. (A) Chains of intracellular iron in a magnetotactic bacterium (B) Schematic drawing of the bird head to illustrate the anatomical location of the three branches of the trigeminal nerve. (C) iron mineral-containing structures in birds' upper beak illustrated schematically which indicates the disagreement interpretations of [56].

Studies have indicated that many or almost all animals possess magnetite or some other form of iron mineral crystals, when scientists started looking for or search for such iron mineral crystals in animals (e.g., in *Caenorhabditis elegans*, mollusks, insects, crustaceans, and various vertebrates) [56].

However, the presence of iron mineral crystals in any form does not necessity mean that they are playing an important role in the detection of magnetic field or that they affect their ability to detect the magnetic field in any way [56].

By nature, iron is the one of the most important compounds found in biological bodies, as it is involved in the organisms functions. The presence of iron must therefore be balanced in the biological bodies. Any iron excess has damaging effects as too little, and so, iron mineral crystal deposits are a way to avoid excess iron in bodies [56].

Iron mineral structures only qualify as serious magnetosensory candidates if they are found at specific, consistent locations associated with the nervous system [56]. In bacteria, the magnetic crystals are present in the form of chains which detect magnetic field and help the bacteria to orient in the direction of magnetic field. Therefore, such organisms are called magnetotactic bacteria [78]. Also, it has been thought that living cells in biological bodies can make or produce magnetite, which can then be oriented with respect to the magnetic field.

But the presence of magnetite crystals in the bodies of bacteria is not considered to be an additional sense, it just leads to a passive alignment with the field [56]. The most promising phenomenon, but certainly not proven, concerns structures in the olfactory epithelium of fish, which could be good candidates for iron mineral-based magnetoreceptors [79].

Researchers have reported that iron-mineral-based structures are employed as magnetoreceptors and are found in the upper beak of the birds [56]. Recent findings, however, suggest that these structures actually constitute macrophages that are involved in iron homeostasis [56]. Therefore, there are currently no iron-mineral-based magnetoreceptive candidate structures in birds that have been convincingly documented (see Figure 1-8 (C); [80]).

Conditioning of birds towards a magnetic field has also proven to be very complex, and independent replication is rare. It has been observed that pigeons, *Colomba livia*, homing over long distances might show a response towards magnetic field reference [81].

The ophthalmic branch of the trigeminal nerve might give pigeons the ability to detect strong magnetic field changes [79]. Therefore, if the animals would use the geomagnetic field information as a map for navigation they must be sensitive to minor changes (i.e. the magnitude must be 3-5 orders smaller than the normal magnitude that has been used in successful experiments). A similar condition adapted for European robins and using weaker fields has been used for pigeons, which resulted in a failure to provide a conditioned response to geomagnetic effect but the same birds were satisfactorily conditioned to auditory stimuli [82].

The trigeminal nerve's ophthalmic branch (Figure 1-8 (B)) terminates at the principal (PrV) and spinal (SpV) tract nuclei of the trigeminal brainstem complex [83] (see Figure 1-9. Studies have proved that the sub-branches of neurons in PrV and SpV in European robins (Erithacus rubecula), a night-time travelling songbird, can be activated by variations in the external magnetic field effect but not by a null magnetic field [83]. In addition, cutting the ophthalmic branch of the trigeminal nerve results in the disappearance of the activation in the changing magnetic field.

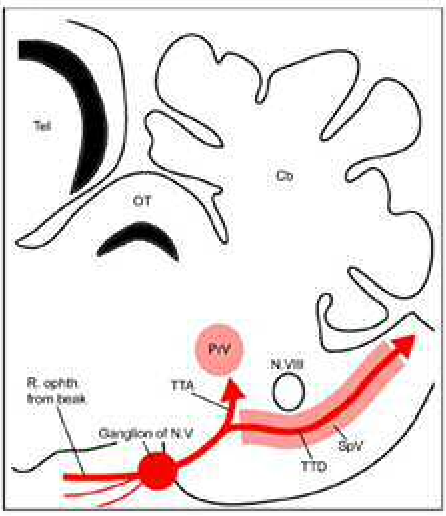


Figure 1-9 Termination of trigeminal nerve in the hind brain of birds [56].

These results show that the ophthalmic branches of the trigeminal nerves are responsible for transferring the magnetic field related information birds [83]. Nevertheless, the iron-mineral-based and the biological importance of the trigeminal mediated magnetic information are still not well understood at present [56]; see Figure 1-8 (C).

Night-time migrating songbirds do not require any information from the ophthalmic branch of the trigeminal nerve for their magnetic compass navigation [56] , (see

Figure 1-8). In Pisa, Italy, pigeons have homed with intact olfactory nerves but not just with intact trigeminal nerves [56].

Large-scale changes in the strength of the magnetic field and/or magnetic inclination have been detected by using the trigeminal nerve to determine the average position.

Researchers have proved that Eurasian Reed Warblers, *Acrocephalus scirpaceus*, are capable of correcting the deviation in their migration routes (approximately 1000 Km Eastward displacement) [56], but their ability to correct disappears when the ophthalmic branch of the trigeminal nerve is severed ([56], see Figure 1-3). Other experiments exposed night-migratory songbirds to strong magnetic pulses. The pulses are thought to disturb the magnetite-based magnetic sense for a period from days to weeks but with no effect on light-dependent magneto-reception mechanisms. The studies support the assertion that the magnetic map or signpost sense is an iron mineral-based phenomenon [84].

The avian's lagena which is a part of the birds' vestibular system, has recently been reported to be involved in providing information to achieve magnetoreception)[85]. It has not yet been proven whether the lagena gives the primary gravity information or magnetic information, but the electrophysiological data are very satisfactory [85] and can be independently replicated. Consequently, this indicates that the lagena play a significant role in magnetoreception, and the vestibular brainstem nuclei would be a very important point for utilizing the magnetic field information.

The majority of light dependent experiments were performed on birds such as the European robin, Erithacus rubecula see (Figure 1-8). Birds detect a reference direction from the Earth's magnetic field. Proteins named cryptochrome are the most likely molecules to be light dependent. After absorbing light, long-lived flavin-tryptophan radical pairs are generated within crypto chromes in the retina. The yield of the reaction is determined by the orientation of the molecule relating to the geomagnetic field vector. If cryptochromes exist along the membrane disks of the photoreceptors' outer segments, a structure is formed in a very orderly manner. To provide a visual impression of compass bearing, the various yields of reactions present in different parts of the retina can be compared (see Figure 1-9). Light-dependent magnetic compass information is transmitted from the retina, through the optic nerve, to the visual thalamus. Then it passes through the thalamofugal visual pathway to move towards cluster n in the forebrain (Figure 1-11). If cluster n has been destroyed, a European robin's magnetic compass can no longer be used for orientation (Figure 1-12).

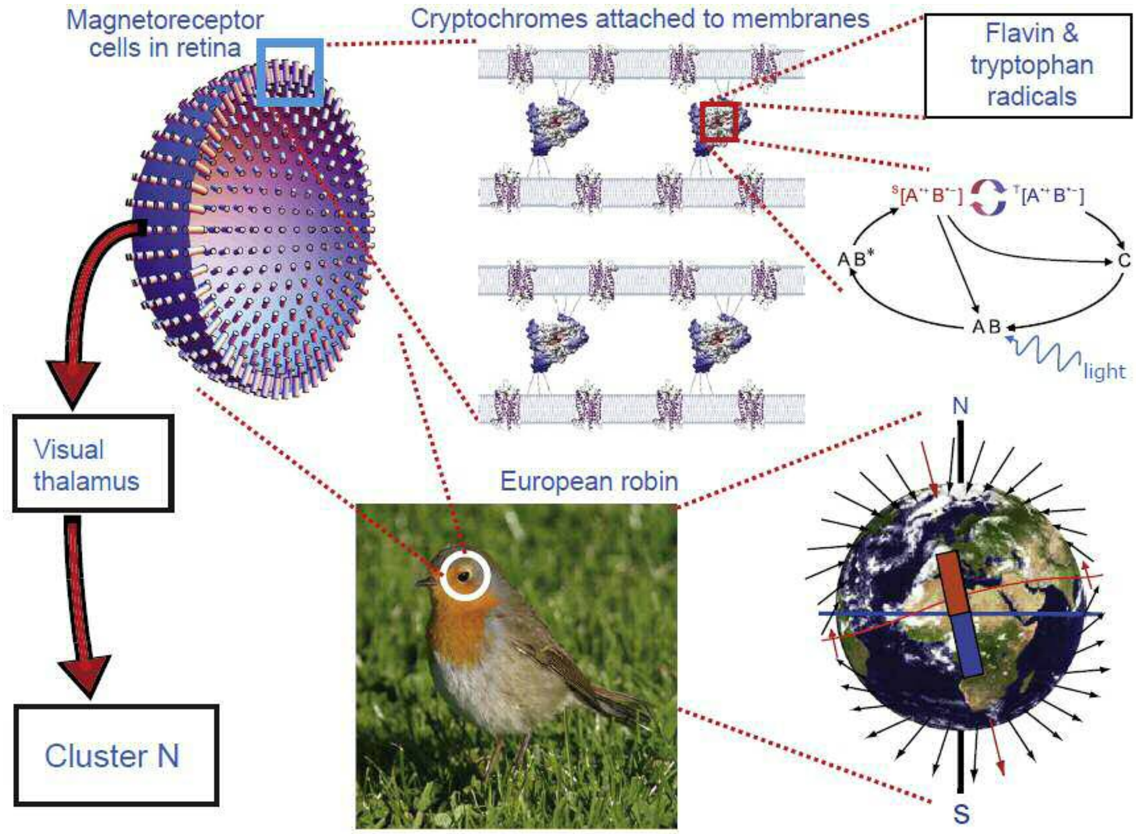


Figure 1-10 A Summary of the hypothesis for magnetic compass sensing in birds, which is believed to be light dependent. [56]

1.8.3 The Light-Dependent Hypothesis:

The wavelengths of light available during experimental behavioral tests has an important role in affecting the magnetic compass behaviour of newts [86] and birds [87]. Klaus Schulten has suggested a hypothesis, in the 1970s, which stated that the magnetic compass sense is based upon the chemical reactions in photosensitive molecules [88]. Figure 1-10 demonstrates the concept of the light-dependent magnetic sensing mechanism. The radical pair could be produced when the light-sensitive molecule (D) absorbs light, and then the electron will be transferred to an acceptor (A) by utilizing the energy of the absorbed light. If this radical pair remains for long time (>1 μs), then it can be shown in different states:

1. A singlet state (spins antiparallel)
2. A triplet state (spins parallel)

The above states could be determined based on the spin of the electrons. Because chemical properties of these states are different, so they will produce different chemical products.

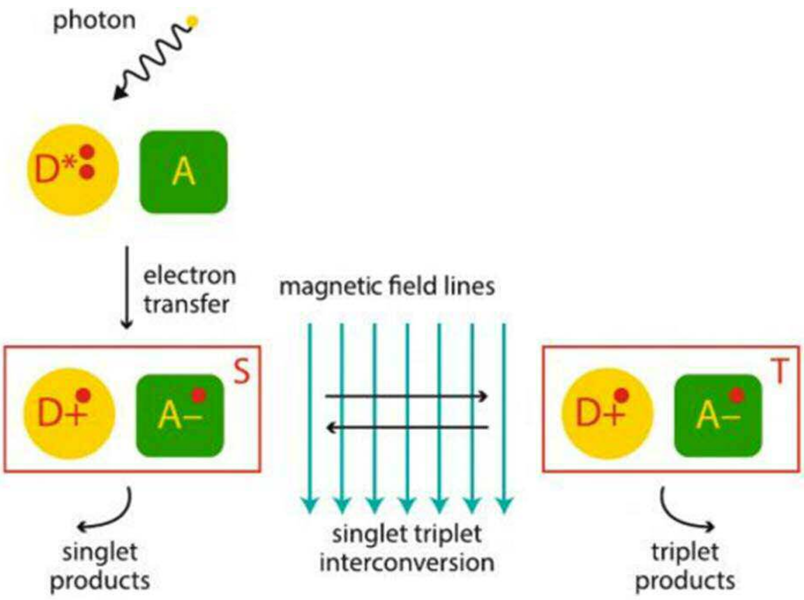
In theory, Earth-strength magnetic fields can affect the statistical equilibrium of these states, and an unknown biochemical pathway can thereby be modulated [89]. Researchers have suggested that the birds are using the radical-pair mechanism in order to detect the magnetic field. They have suggested that a resultant visual image might enable the birds to realize the direction of the magnetic field lines (e.g. [90]).

If a simple assumption is made, that the sensory molecules are oriented in a direction perpendicular to the eyeball (see Figure 1-11), then molecules oriented in all axial directions would occur due to the half ball shape of the retina [89].

When a bird looks in a direction which is nearby aligned with the magnetic field lines direction, then this would lead to a light pixel because the magnetic field lines would be parallel to the retinal molecules. Darker pixels would be produced when the molecules are perpendicular to the magnetic field at the edge of the eye. In between, different shades of gray pixels could be observed when the molecules are oriented at various angles relative to the magnetic field. Altogether, a virtual image would be produced which is shown on the right side of Figure 1-11 [89].

This pattern is only for explanatory purposes consistent with the hypothesis. The patterns will be more understandable when the bird moves its head in a scanning motion. Consequently, the head scanning will allow the patterns to move across the retina [91]. If the radical-pair mechanism is indeed responsible for magnetoreception, then it must be based on a quantum mechanical effect [92]. Furthermore, it might be the only biological sensory mechanism that is inherently quantum based in nature. However, those opposing the concept of the radical-pair mechanism have identified that the interaction energy between a radical and a geomagnetic field is generally several orders of magnitude lower than the background thermal energy, k_BT [53].

a)



b)

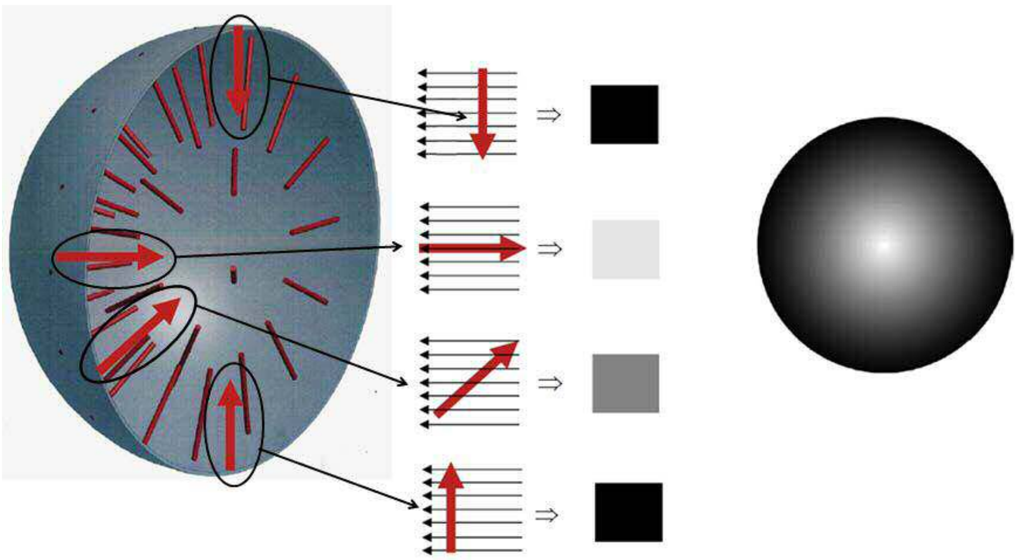


Figure 1-11 depiction of how the light-dependent magnetic sensing mechanism[56].

a) A simplified version of the primary reaction [56].

b) The radical pair-based mechanism explaining how birds could convert a magnetic stimulus into a putative visual image [56].

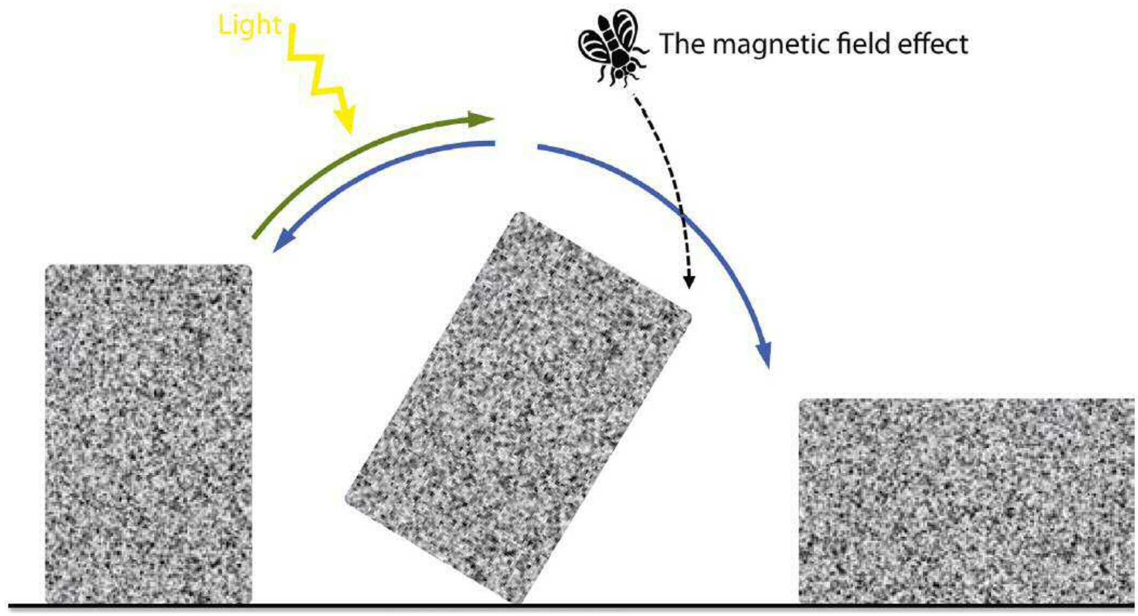


Figure 1-12 A granite block analogy can enable an understanding of how a radical pair-based mechanism may theoretically be used to sense Earth-strength magnetic fields [56].

It is not a fundamental problem that the spins are not in equilibrium. Radical pair reactions could be affected by interaction with the very weak magnetic fields for three general reasons [93]: 1. The electron spins in the radicals control the whole radical pair chemistry; 2. Spinning of the electron are not at thermal equilibrium; 3. The spin of the electrons behaves in a quantum mechanical manner. The spins of electrons are affected by the weak magnetic fields according to the theory of quantum mechanics in which k_BT has played no role. It is better to do a compression between the period of time that is required for the magnetic field to achieve an effect (T1) with the period of time that is required for the system to reach thermal equilibrium (T2). This comparison is much better than comparing interaction energies to k_BT [93]. Thus, the magnetic field can have an effect if T1 is shorter than T2 [93]. For more explanation, it is worth imagining a rectangular granite block and a fly in the air. The fly cannot flip the granite box if the box is placed on one of its side (conventional physics, equilibrium). In fact, quite a lot of energy is needed to move the granite block onto one of its corners, but the fly has a chance to flip the granite box on the side if the box was placed on one of its corners (quantum mechanics, nonequilibrium), i.e. a very small amount of energy can influence which way the granite block falls. This example helps to understand how the radical pair-based mechanism can work theoretically and how it might be used to sense Earth-strength magnetic fields, although the energy exerted by the magnetic field on the radical is much lower than thermal energy, k_BT. In the light-dependent hypothesis of radical pair-based magnetoreception, the magnetic field has much less energy than the light. Then, if the light is absorbed, then in a photoreceptor molecule (a cryptochrome) is brought into an exited state (moves the granite block onto its corner). Placing the granite box on its corner will make it very sensitive to even very small magnetic field effects (the fly landing onto the granite block in the analogy) (Figure 1-12) [93]. Such a system would require a steady bias field which is come from the continuous Earth's magnetic field.

Because opsins utilize light energy to change a chemical bond, not to transfer an electron, then it was not taken into account as a molecule that could be responsible for light-dependent magnetoreception. Cryptochromes are the only photoreceptor molecules found in vertebrates that utilize light energy to form long-lived radical pairs [94].

Cryptochromes are mainly found within photoreceptor cells and ganglion cells in the eyes of birds [95], and cryptochromes are the only candidate molecules for radical-pair-based magnetoreception in birds [96]. For example, the retina of migratory birds has magnetosensitive cryptochrome molecules [95].

It has also been suggested by Klaus Sculten that the colour (wavelength) of the light that is used in the experimental room has an effect on the compass orientation behaviour of night-migrating songbirds [56]. In birds, the magnetic orientation compass needs the photoreceptor molecules in the pineal organ [181] but not the pineal organ as it is not essential for magnetic compass orientation [96].

Thorsten Ritz and colleagues reported that even very weak oscillating magnetic fields (0.015 μT (0.03% of the geomagnetic field strength), in the low MHZ range, can disturb the orientation capabilities of birds' magnetic compasses. These effects are difficult to understand theoretically, but once independently confirmed, they have the potential to be used as a diagnostic tool for the radical pair mechanism in magnetic compass sensing [97].

Taking neuroanatomy into account, Cluster N is a region in the forebrain of the night-migrating birds, which shows an increase in its activity in birds that migrate using the magnetic compass orientation (Figure 1-13). However, the activation of this region disappears when the bird's eyes are completely covered. [98]. The hyperpallium and the dorsal mesopallium are the two parts that form Cluster N [99], so in European robins the lateral-most part of the visual Wulst in because the neuronal input is delivered to the Cluster N via their eyes (i.e. via the thalamofugal visual pathway) [100]. Other experiments have been performed by Archi Rastogi [101] using Black-Headed Bunting (Emberiza melanocephala) (another kind of songbird) in order to approve the presence and activation of Cluster N.

Researchers have confirmed that Cluster N is the centre where the light-dependent magnetic compass information is processed. This has been proved when the bird's orientation using the magnetic compass has been distributed when Cluster N lesioned. ([56], see Figure 1-14). In contrast, sham Cluster N lesions or bilateral sections of the ophthalmic branch of the trigeminal nerves did not influence the robins' ability to use their magnetic compass for orientation ([56], see Figure 1-14). Cluster N lesions only affect the magnetic compass because Cluster N lesioned robins can still orient well using their Sun and star compasses ([56], see Figure 1-14).

It can be concluded from the above data that:

(1) This species is capable of orienting and navigating using Cluster N in order to perform magnetic compass orientation.

(2) It has also been proved that Cluster N is the centre for processing magnetic compass information.

(3) It has been suggested that migratory songbirds depend on vision in order to achieve a magnetic compass orientation.

(4) It has been suggested that, in robins, the lagena's input is not sufficient for magnetic compass orientation.

(5) In European robins, the magnetic information that is transmitted from the trigeminal nerve to the brain is not sufficient for magnetic compass orientation [56].

Mouritsen [56] has discussed the exact role of Cluster N within the magnetic compass, and it has been suggested that birds are capable of seeing the magnetic compass information via this small part of the visual system.

Cluster N hypothesis does rule out other hypotheses that have been presented in the literature, such as iron-mineral- based, trigeminally mediated and lagenamediated magnetoreception.

For example, night-migratory songbirds are not using trigeminally and lagena mediated magnetoreception as a primary mechanism for the magnetic [56], but it could still work as a primary mechanism for magnetic map information [56].

In fact, it is likely that light-mediated, radical pair-based magnetoreception and iron-mineral-based magnetoreception mechanisms are both available in the animals in order to provide them with various types of magnetic information [102]. It is common in biology to support two hypotheses if both of them have proved their validity. In addition, non-essential redundancy is common in biology, because some multi-function organisms are more capable of adapting to different conditions and thus be preferred by the evolution [56].

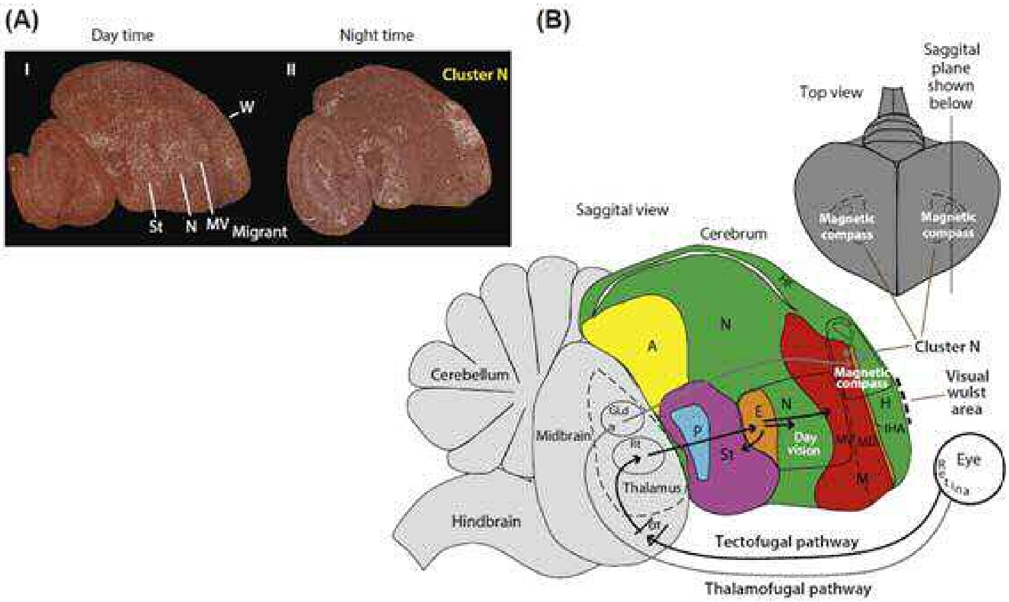


Figure 1-13 (a) Cluster N is the most active area of brain in migratory birds performing magnetic sensing or compass orientation at night (b) A top view of the brain, indicating the medial-lateral and the frontal-caudal extent of cluster N. [56]

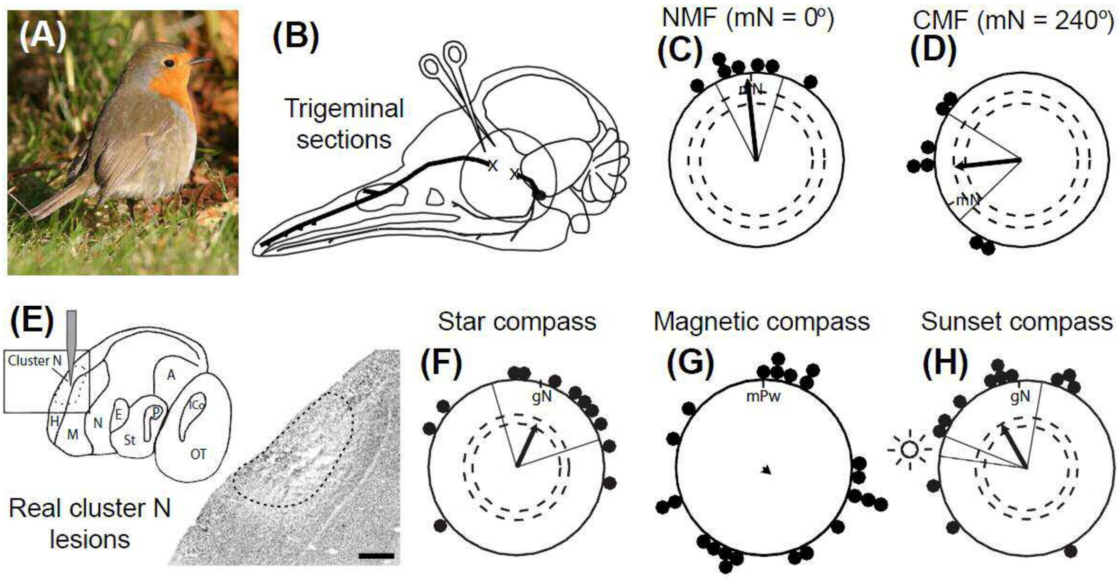


Figure 1-14 Cluster N is necessary in birds to enable orientation with respect to their magnetic compass (A) The European robin. (B–D) bilateral sections of the ophthalmic branch of the trigeminal nerve. (E) Cluster N destroyed the magnetic compass capabilities of the birds. (F) Cluster N contribution to star compass (G) Cluster N contribution to magnetic compass (H) Cluster N contribution to sunset compass [56].

1.9 Methods to Study and Quantify Orientation and Navigation

Magnetorecption investigation involves studying different aspects, such as physiology, behaviour, and anatomy. Various techniques have been employed to do the investigations and each technique has its own advantages and drawbacks.

1.9.1 Vanishing Bearings:

Bird's magnetoreception has only been investigated theoretically until the 1950s, because of the lack of methods to be used in experimental labs.

Moreover, some approaches were unreliable and impractical such as following the birds from a plane. Consequently, a new method was introduced for the first time by Geoffrey Vernon Townsend Matthews [103] which is called "vanishing bearings method"

The concept of this technique depends on measuring the last seen direction of the flying pigeon when it is released from its home loft or release site.

Advantage:

This method aims to reduce the harm to the animal (non-invasive method), so it cannot affect the animal's behaviour.

Disadvantage:

It cannot reveal which system, magnetite or cryptochrome, is involved.

It is also considered to be an imprecise method as only the last seen direction is considered as it may be different from their average direction back to the loft [104].

1.9.2 Nervous System and Sectioning Lesions

This is an invasive method, used by researchers to test animal behaviours. The advanced and disadvantage can be concluded as the following:

Disadvantage:

The lesions within the nervous system can be imprecise and the impact of damaged to adjacent areas could prove to be harmful. To ensure the absence of any kind of harmful occurrence within the concerned brain area can be affected.

Advantage:

When it is done properly, it provides sufficient information regarding the identification of areas which are responsible to detect the magnetic field information and their pathway [105].

1.9.3 Stephen Emlen's Emlen Funnels

Stephen Emlen used the Emlen funnel in order to record the bearing vectors of experimental animals and to investigate the bird, (indigo buntings, Passerina cyanea), stellar navigation and orientation by using the Sun and star cues in a planetarium [56]. It has been called by this name based on the inventors (Emlen and Emlen 1966) [56] The principle of this method is simple.

During a bird's migratory restlessness, it is kept in a funnel-shaped cage. The bottom of the funnel is coated with ink or the touch-sensitive walls are connected to a computer. This will help to demonstrate the intended migratory direction when the birds jump against the walls of the funnel, as birds will leave in marks on the walls (or contact one region of the wall) corresponding to the direction they choose.

Circular statistics have been used in order to calculate their preferred direction. A validation for this method was achieved by comparing the funnel bearings of redstarts, *Phoenicurus phoenicurus*, with the vanishing bearings of released birds [56]. Results of this test showed that the case method was adequate.

Nevertheless, this method should be used with care, since any results obtained from Emlen funnels will be much stronger if any corroboration can be obtained using other methods, especially telemetric data which provides the advantage of analyzing the whole track of an animal or at least a major part of it.

Using Emlen funnels in the last few decades' has contributed to providing evidence that vertebrates are migrating depending on celestials cues, from expedients carried out during bird's migratory restlessness (also called Zugunruhe, see Figure 1-15).

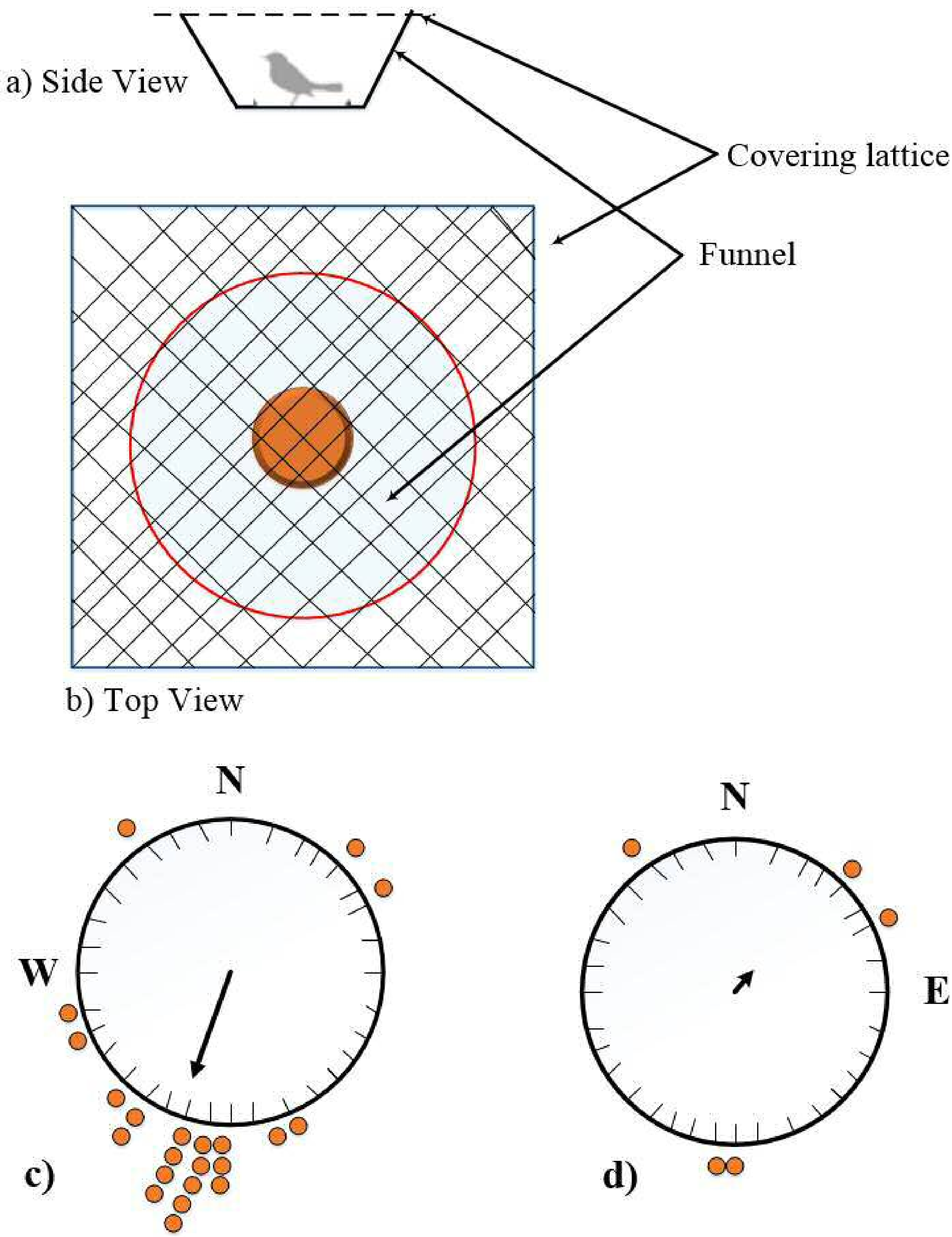


Figure 1-15 the principal of the Emlen funnel. Birds leave marks on the wall of the funnel as they jump from one direction to another. These marks were recorded (orange dots on c and d). Based on the directions shown on the readings chart, their direction was analysed using circular statistics. Adapted from [56]

The disadvantages/ shortcomings/drawbacks of the Emlen Funnel methods are:

1. Noise in orientation data:

Although birds will leave marks on the funnel's wall, not every mark is related to orientation behaviour. Because some scratches are related to escape behaviour which will result in showing a direction that are not related to the orientation behavioural experiments.

It has been recommended to test a large number of birds during migratory restlessness and to obtain enough tests with each individual bird, to increase the signal to noise ratio in the orientation behaviour data gained by using this method.

2. No temporal resolution:

As has been mentioned previously, the marks that the birds make on the funnel wall depict all the bird's movements during their orientation in migratory restlessness, and each test lasts for 40 to 60 min. The researchers have therefore found it difficult to determine the exact occurrence time of each scratch. Consequently, online automatic registration have been achieved using touch sensitive wall (e.g., [56]). But these attempts can still produce unreliable data, especially for the outdoor experiments.

3. Applicable mostly to nocturnal migrants:

Those migratory birds that perform their migratory restlessness during the night time show specific locomotor activity which is related to their migration and can be distinguished from the locomotor activity which is related to their feeding and exploring activities. Hence, researchers who use the Emlen funnel method should perform their experimental tests on nocturnal, not diurnal.

Advantages:

Non-invasive: the results can be obtained without affecting the animal behaviour.

1.9.4 Operant Conditioning:

It includes providing training to birds and teaching them how to respond in certain specific conditions.

Disadvantages:

It is very difficult to control all the variables during training, and it is time consuming. In spite of all the efforts, there is always the risk of affecting the animal's behaviour by habituating it to do what is needed for the results [106].

Advantages:

Can be used to detect animal behaviour at specific experimental condition without affecting other organs.

1.10 Navigation Based on Inclination Compass:

There is a great deal of evidence that pigeons are not sensitive to the polarity of magnetic field lines, which means they depend on inclination (vertical component). For example, birds were turning their heads according to the change in the resultant field vector only after the horizontal component of the field was flipped. Pigeons have not shown any changes in their headings when both components (i.e. horizontal and vertical) of the field vector were altered [107].

1.11 Magneto Reception Investigation Based on The Nature of the Head Movements

Homing pigeons can distinguish between different magnetic field condition, and this can be observed by the changes in their head movement. However, some studies have proved that pigeons make head sweeps to orient themselves before taking off [56]. Also, a disorientation in pigeon's migration routes can be observed when a magnet has been attached to their head, which supports the idea that the pigeon's head is the location for a magnetoreceptor [56]. Research has been carried out by Henrik Mouritsen [56] has further supported this. This research has focused on studying a bird's initial behaviour before it decides its direction of orientation, which is happens directly after their release. It was revealed that migratory garden warblers used head scans to find a reference direction from the Earth's magnetic field. These head scans were well suited to the symmetrical pattern of the axis of field lines running between the poles. The birds tend to turn their heads in various directions and fix it in each direction for a short period of time. So, the researchers have suggested that this is the way that birds estimate the direction of the geomagnetic field lines which in turn will assist the bird to orient towards their correct migratory direction.

However, the birds were unable to orient when the magnetic field was eliminated to zero, because the birds made triple the number of head scans and their migrating directions became random.

It is noteworthy that pigeon's head movements are a sufficient way to measure and analyses the characteristics of the pigeon's response to various conditions of magnetic field. Therefore, these previous studies have led the way for us to design a novel and accurate methodology to be developed and tested in this thesis.

1.12 Where do We Go from Here?

Many researchers have supported the magnetoreception hypotheses (iron-mineral-based and the light-dependent), because they have proven their involvements in the magnetic senses. However, there is still a mystery related to magnetoreception. For example, although the iron-mineral-based has proven its involvement in magnetoreception, it is not considered as an active sensory system [56]. Similarly, there is a need to understand the mechanism that enables the receptors of radical-pair to sense the geomagnetic fields at physiological temperatures. Manmade radical- pair reactions have been achieved but did not give a full answer [108]. In addition, experiments have proved that cryptochromes involved in formatting long-lived radical pairs [56], but the effect of the magnetic field on the cryptochrome is still unknown.

Based on the neuroanatomical investigations, the mechanisms that the brain use to process the magnetic information have been explored, but it has not been proven how the birds transform magnetic information to a directional choice, which is thought to be made depending on integration of information from multiple sensory systems.

Magnetoreception is an important phenomenon of life for birds and for a various species of other animals. New research is required to answer all of the questions related to this field.

1.13 Novelties of This Research

A new method of research has been developed to increase the researchers' opportunity to obtain accurate data that could be collected during the year-around instead of relying on a bird's migration periods. Therefore, working in such a laboratory environment will assist the researchers to avoid outdoors experiments that might consume time and efforts to achieve the arrangements necessary for a single experiment.

The number of a bird's head scans and their orientation are influenced by magnetic field alterations. Therefore, controlling the external magnetic field conditions are likely to affect the bird's behaviour. Hence, this research has built based on this approach. Previous research has limited their magnetic field manipulations to either exposing the birds to Null field (eliminating the external magnetic field) or changing the direction of the field vector. It was noticed that birds respond to these simple field manipulations but they were insufficient to study the bird's choice.

The approach of the present research is to expose birds to more intense spatiotemporal alterations in the magnetic field and then investigate the bird's response to the field changes and how these changes in magnetic field can directly change the bird’s behaviour.

In this case, the subjects were exposed to a sequence of different magnetic field conditions, or steps, consisting of six scenarios/conditions : normal static field, Fast Flipping Field (i.e. inclination); One flip every 3 seconds), Slow Flipping Field (i.e. inclination) One flip every 20 seconds) , Sweeping Field Clockwise direction (Rotation 20 deg/s) around a bird’s head, Sweeping Field Counter-clockwise (Rotation 20 deg/s) around a bird’s head, Null field (absence of the field) and Control field. Each condition was preceded and succeeded by a static field. Each scenario (sequence) lasted 180s except the sweeping which lasted for 300s. And each scenario was preceded by 180s of static magnetic field as a baseline period to habituate the pigeon for the experimental location. The sequence could be constructed from a different number of these magnetic steps, and the order of the steps could be varied as well as the period of time that each step is present within the sequence and even the speed of the flipping and sweeping field could be altered. This study aims to use novel methods to increase reliability and repeatability, while at the same time reducing invasiveness and the training time needed to investigate magnetoreception. Creating and manipulating the Earth magnetic field was fully automated by using an active shielding system, which was constructed especially for this purpose. This has added numerous benefits to this research that the passive shielding system could not provide (see chapter 2). In this research, the intensity and inclination values of the geomagnetic field have been replicated,(see Figure 1-16), in order to generate an artificial Earth-like magnetic field.

Latitude	Longitude	Altitude	Date
51.486	-3.179	0.00	04.09.2015

Comp	D	I	X	Y	H	Z	F
MF	-1.836	66.344	19507	-625	19517	44554	48642
SV	9.1	-0.5	16.0	51.3	14.4	16.8	21.1

	D= Inclination	I= Inclination	X=North Intensity	Y=East Intensity	H= Horizontal Intensity	Z= Vertical Intensity	F= Total Intensity
MF=Main Field	Degrees east	Degrees down	nT	nT	nT	nT down	nT
SV=Secular Variation	Arcmin/year	Arcmin/year	nT/year	nT/year	nT/year	nT/year	nT/year

Figure 1-16 Local Earth magnetic field condition in Cardiff. [109]

The dependence of orientation on the biochemical compass shows that the eyes are the place where the oxidation reduction reaction is occurring [56]. The eyes have been used conveniently as a model throughout the experiment because large numbers of experiments have been performed on light-dependent magnetoreception and to test the compatibility of the results of these tests is rather easy.

For illustration purposes, Ritz has imagined a bird's eye as an ideal sphere. On the front side, a pinhole opening has been shown and the scattered circle is representing the retina which is uniformly placed along the internal wall of the eye as shown in Figure 1-17.

The axis of the photoreceptors are thought to all be pointing towards the centre of the eye (c).

Furthermore, if a theoretical eye is placed in the centre of the head (and in front of it), a line can be thought of which connects the infinitesimal opening of the eye and its centre. This axis reflects the orientation of the head, [56] and depicts the bird's simplified zone of vision. It appeared as a totally grey and even pattern of increased colour density within a black circle in the centre of the vision zone. Figure 1-15 shows a schematic illustration of this concept.

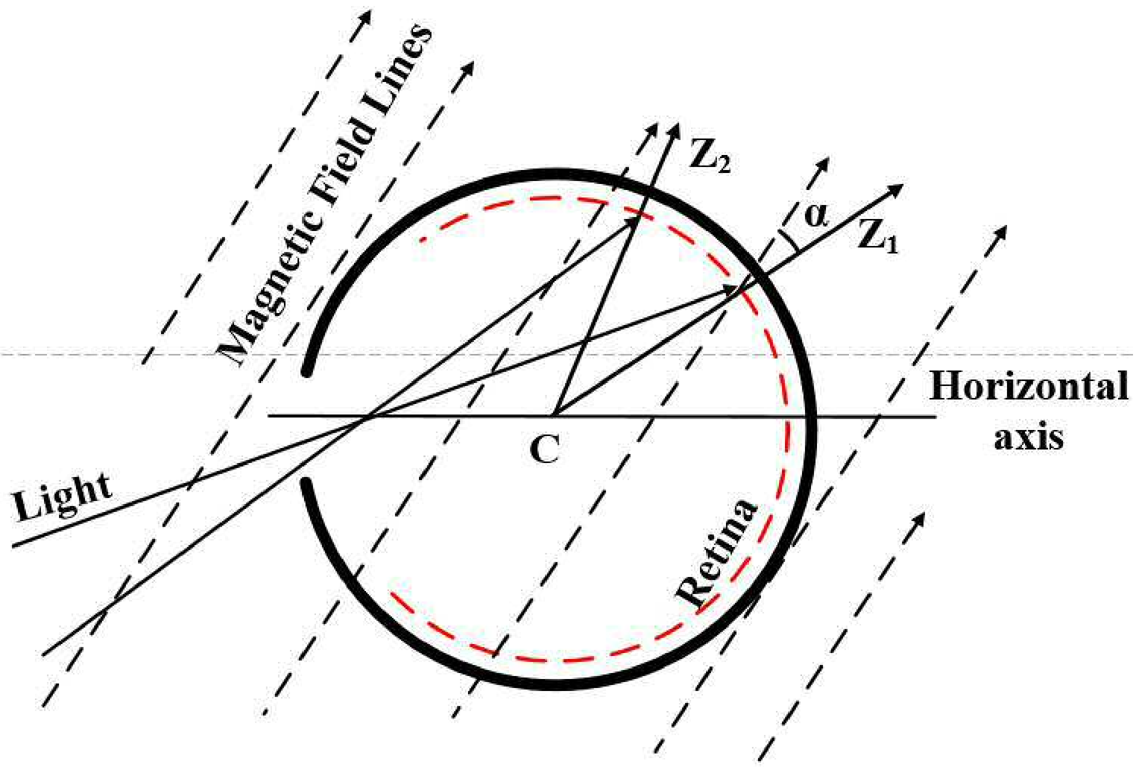


Figure 1-17 Bird's eye diagram used for illustrating visual pattern modulation, as it depends on the radical pair mechanism. The Z1 and Z2 arrows represent the axes positioned towards the retina. C represents the centre of the sphere. . Adapted from [56]

The presence or absence or any change in the magnetic field would be responsible for changing the frequency of the bird's head movements [107]. In novel approach used in this thesis, the head movements have been chosen as an indication for the bird's ability to detect Earth's magnetic field. This research has involved recording a pigeon's head angles during the manipulation of the magnetic field conditions; this is considered a non-invasive method. So, investigating the pigeon's head movements in the laboratory environment is contributing to obtaining more accurate data because more variables can be controlled. Thus, any magnetoreception experiment could be performed year round with no need to do any training for the birds. Also, modifications to the experiment setup and for the system could be achieved easily. Using the same 3D Helmholtz coil and camera tracking setup in other labs could help to replicate the paradigm independently. This could contribute to providing reproducibility for the results, especially in viewing the lack of consistent results in magnetoreception research area.

Moreover, this approach has focused on eliminating other navigational cues (Sun, star and etc.). So it helps guarantee that the magnetic field is the only available cue for the birds during the experiment. This was easy to perform because the experiments of this research were conducted in a lab environment.

Homing Pigeons, *Columba livia*, are relatively convenient study object which have become one of the most extensively examined model systems, they have been chosen for this research for the following reasons:

1. Agreat deal or previous massive of old research has approved the pigeon's ability to use Earth's magnetic field as a navigation cue. In the middle of the nineteenth century when it was thought that birds might use Earth’s magnetic field for orientation, von Middendorf [110] suggested the term "animal magnetic compass". In 1882, Viguier proposed the idea the homing pigeons, *Columba livia*, used magnetic field intensity and inclination of the geomagnetic field to find out their navigation direction. However, the laboratory and experimental attempts to support these hypotheses did not start until the mid- twentieth century, when Wiltschko and Merkel [[111] demonstrated that the shifts in the direction of the artificial magnetic field cause a change in the pigeon's orientation.

2. Pigeons are common species for experimental investigations, so they are easily available.

3. On the neuroanatomical level, many studies of the pigeon brain have been conducted, and much is known about their visual system [112].

These have been a long history of the practical application of homing pigeon's magnetoreception during the twentieth century. Later, many hypotheses have been produced, especially in the second half of the twentieth century, from the experimental analysis but the magnetoreception mechanisms remain unclear even today. Thus, it is clear that new experimental paradigms are required [113].

The main focus of this research is on investigating pigeon's behavioural reactions, manifested as head movements, which can be monitored in the presence of the magnetic field alterations. Hence, the birds' orientation and/or navigation abilities are not taken into account in this research.

The second important thing about this research is the improved tracking system that is used in measuring a pigeon's head movements in order to study its reaction to the external stimuli properly. So, investigating a pigeon's ability to perceive the Earth magnetic field can require 3D monitoring of its motor responses to various changes. Video analysis (VTA) involves tracking a 2D image, relying on software, but the resulting data can be noisy and limited to a single field of view (one rotational angle). Our research demonstrates that the validity of a research, low-cost system based upon motion-detection using micro Inertial Measurement Unit (IMU) technology. This new technology will pave the way for researchers to study the pigeon's behaviour accurately and without any limitations.

Tracking head movements is crucial in this research, and only limited number of techniques are available for the analysis of often complex behavioural responses. Methods used to track the movements of animals, such as birds, have varied depending on the degree of accuracy required. Most conventional approaches involve the use of a camera for recording and then measuring an animal's head movements in response to a variety of external stimuli, such as changes in magnetic fields. However, video tracking analysis (VTA) will generally only provide a 2D tracking of head angle. Moreover, such a video analysis can only provide information about movements when the head is in the view of the camera. In order to overcome these limitations, the novel invention reported here utilises a lightweight (<10g) IMU unit (Inertial Measurement Unit), positioned on the head of a homing pigeon, which contains a sensor with tri-axial orthogonal accelerometers, gyroscopes, and magnetometers. This highly compact (20.3×12.7×3 mm) system, can be programmed and calibrated to provide measurements of the three rotational angles (roll, pitch and yaw) simultaneously, eliminating any drift, i.e. the movement of the pigeon's head is determined by detecting and estimating the directions of motion at all angles (even those outside the defined areas of tracking). Using an existing VTA approach as a baseline for comparison, IMU (Inertial Measurement Unit) technology can track a pigeon's normal head movements with more precision and in all 3 axes.

A third task has been suggested to be tackled by my biologist (second part of magnetorection group): using PPI (pre-pulse inhibition). This method is based on the idea that a brief pre-stimulus will contribute to reducing the startle response's amplitude if the pre stimulus (i.e. magnetic field change) is detected. The startle response occurs when the animal flinches when exposed to a strong set stimulus such as a sudden onset loud sound or bright flash of light making the animal surprised. The PPI experiments have been carried out in the lab using the 3D Helmholtz coils to generate the pre-pulse for the startle paradigm. PPI can be reproduced and replicated independently.

1.14 Hypotheses and Aims

Before carrying out this research some questions to be answered have already been discussed. These hypotheses include:

1. Pigeon's head movements are a goal indicator of the perception of the magnetic field, which is determined by the presence or absence of a reaction.
2. Pigeons can easily and rapidly detect the stimuli or changes in magnetic field.
3. Using a tracking method to measure their head movements will provide insight into the nature of the birds' responses to changes in the magnetic field.

The main purpose of this study is to validate two systems that can be used for magnetoreception experiments. This experiments have been performed using the new coil system (with active shielding), and this research will validate on new tracking system (IMU).

1.15 Thesis Outline

Immediately following this literature review, the next chapter (i.e. **Chapter 2**) includes an overview as well as details of the system which was developed and built in the laboratory for investigating the magnetoreception of homing pigeons. Subsequently, in the **Chapter 3**, the verification of the produced artificial magnetic field conditions is discussed; also the uniformity of the field inside the 3D Helmholtz coil is presented in detail and compared with simulation results.

Chapter 4 explains the experimental design and the preparation for the magnetoreception experiment. **Chapter 5** described the magnetic field sequence that the pigeons have experienced in order to record their reaction to the changes in the magnetic field. Analysis of the pigeon's head movements has been performed by comparing the pigeon's activity in different field conditions.

A new tracking system based on an Inertial Measurements Unit is detailed in **Chapter 6**, including the validation of the new system. It has then been used to measure sensorimotor responses in pigeons, and its accuracy has been compared with the old tracking system (VTA) that has been used in the experiments presented in **Chapter 5**.

The general conclusion of this research and suggested further work are given in **Chapter 7.**

CHAPTER 2

SYSTEM OVERVIEW

Behavioural experiments require a system that is designed to generate the magnetic field, which is controlled by software. In this chapter video recording system that is recording the birds' behaviour will be demonstrated. Also the details of designing the coils and system construction will be presented. System overview will be discussed as well as the various stages of the system design will be demonstrated in details.

2.1 SETUP FOR GENERATING AND CONTROLLING A MAGNETIC FIELD

It is usually essential to have a uniform magnetic field source in order to provide a field of known uniformity. To obtain the uniform magnetic field and manipulate its temporal and spatial properties and enable the recording of the bird's behaviour while it is experiencing the magnetic field, a special instrumentation setup has been constructed. This instrumentation consists of the following parts:

- LabView software (for controlling and manipulating the magnetic field)
- Power amplifier (to inject the coils with appropriate current (A))
- Active magnetic shielding (three-dimensional Helmholtz coils).
- 3-axis magnetometer (Mag-03 Three-Axis magnetic field sensor).
- High resolution camera (for recording the bird's behaviour)
- Illumination

2.1.1 Controlling and Manipulating the Magnetic Field Using LabView Software:

The different properties of the magnetic field, such as amplitude and spatial and temporal settings, have been controlled by LabView. Also, the head movements tracking and related analysis have been accomplished by using LabView (See Figure 2-1) which shows the different magnetic field conditions that has set by the user.

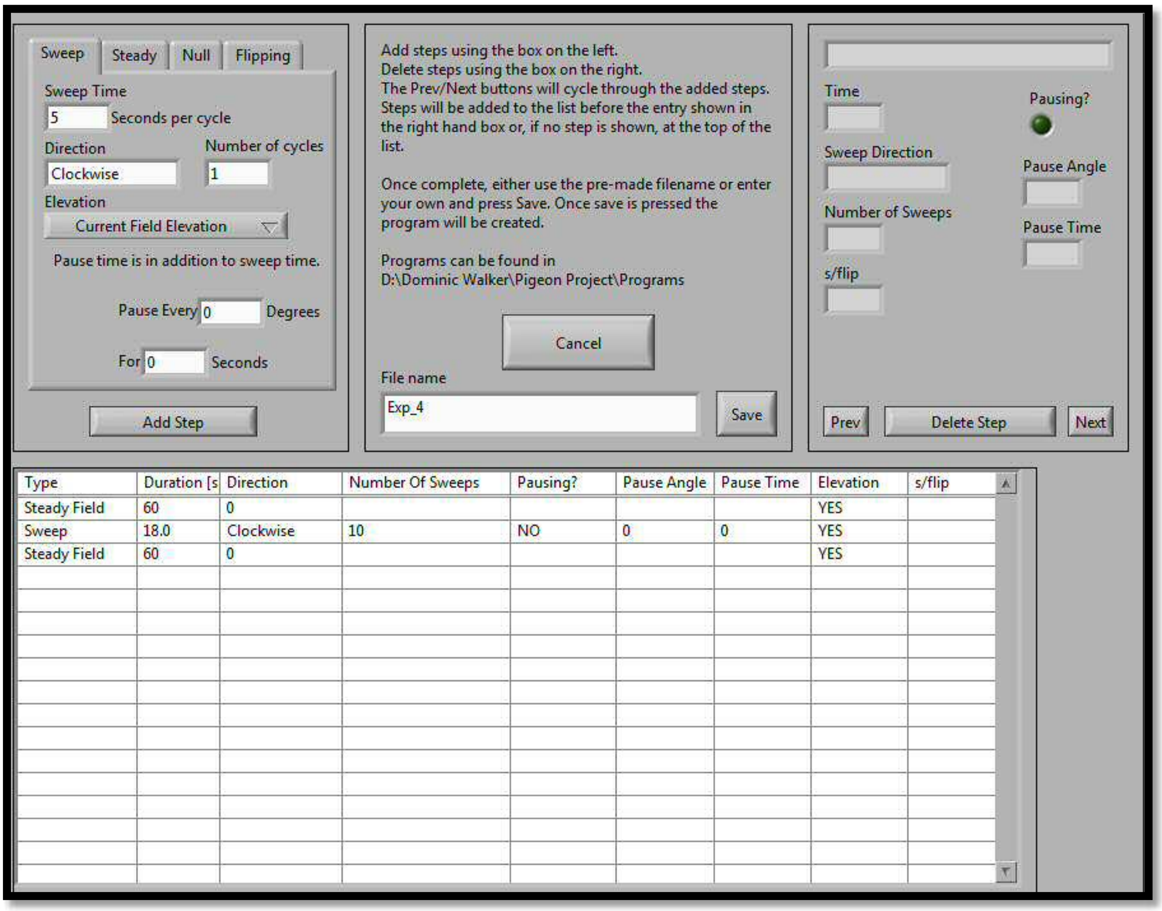


Figure 2-1 Front panel of the LabView programme.

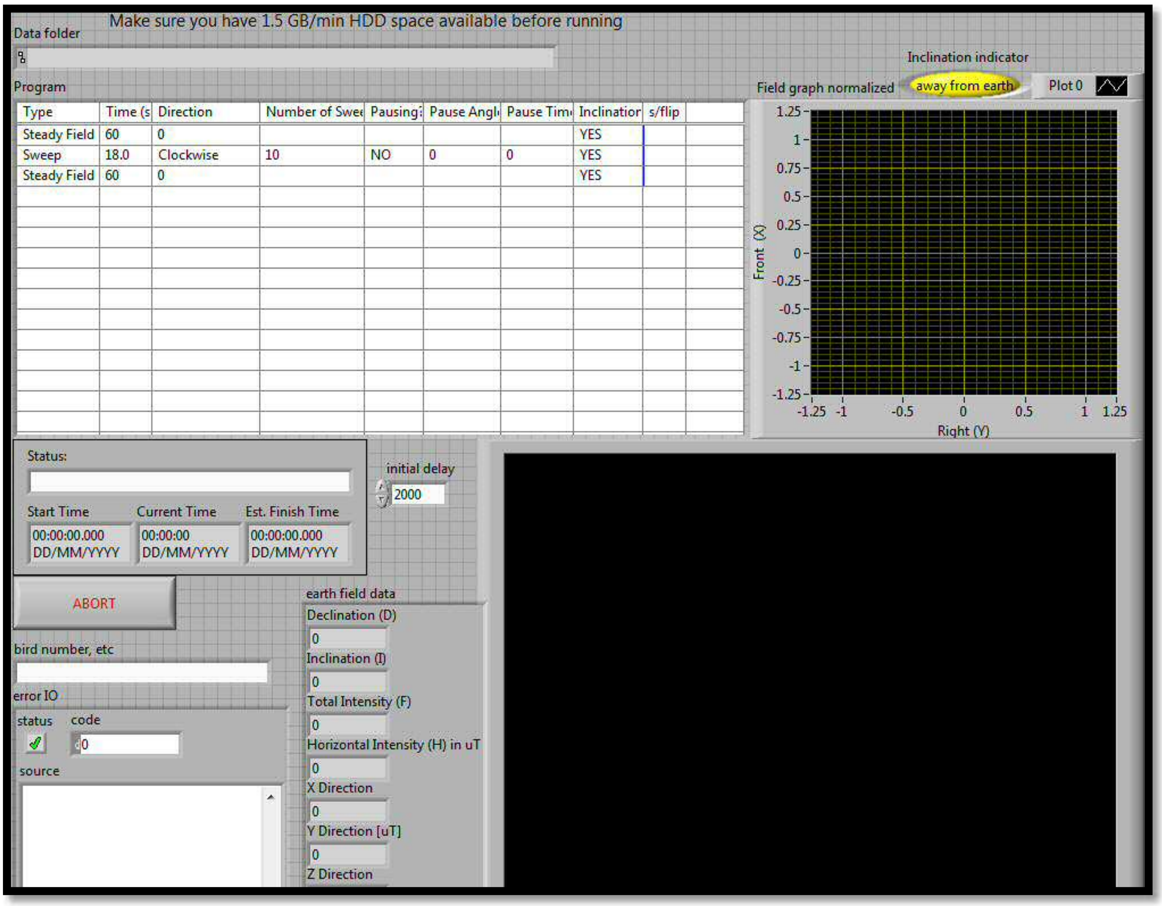


Figure 2-2 Front panel of the sub-program.

LabView (short for Laboratory Virtual Instrument Engineering Workbench) is a system-design platform and development environment for a visual programming language from National Instruments. It is a highly productive development environment for creating custom applications that interact with real-world data or signals in fields such as science and engineering. This language is distinguished from other languages as this one is based on the graphical language named "G", which results in:

- Intuitive, using flowchart-like dataflow programming model.
- Shorter learning curve than traditional text-based programming.
- Naturally represented data-driven applications with timing and parallelism.

Normally, any program written in LabView should consist of: the front panel window and block diagram widow. The first is the user interface, see Figure 2-2 , which shows a sub-VI (Virtual Instrument) as each one can be designed for a specific experiment. Every LabView block diagram has an associated front panel, which is the user interface of specific application. On the front panel, the user can place generic controls and indicators such as strings, numbers, and buttons or technical controls as well as indicators such as graphs, charts, tables, thermometers, dials, and scales.

LabView supports for thousands of hardware devices, including: scientific instruments, data acquisition devices, sensors, cameras, motors and actuators. It is a common programming model for all hardware devices and its portable code can support several deployment targets. LabView has freely available drivers for thousands of NI (Nationsl Instruments) and third-party hardware. In the rare case that a LabView driver does not already exist, LabView offers good tools to create our own, reuse a DLL (Dynamic-LInk Library). The feature that distinguishes this language from other languages is not requiring any written code, which makes it more user friendly. Alternatively, commands are embedded in various blocks in a library. An example of the block diagram in LabView programming is shown in Figure 2-3 where the programmer can write the functions, constants, structures, and wires of the programming.

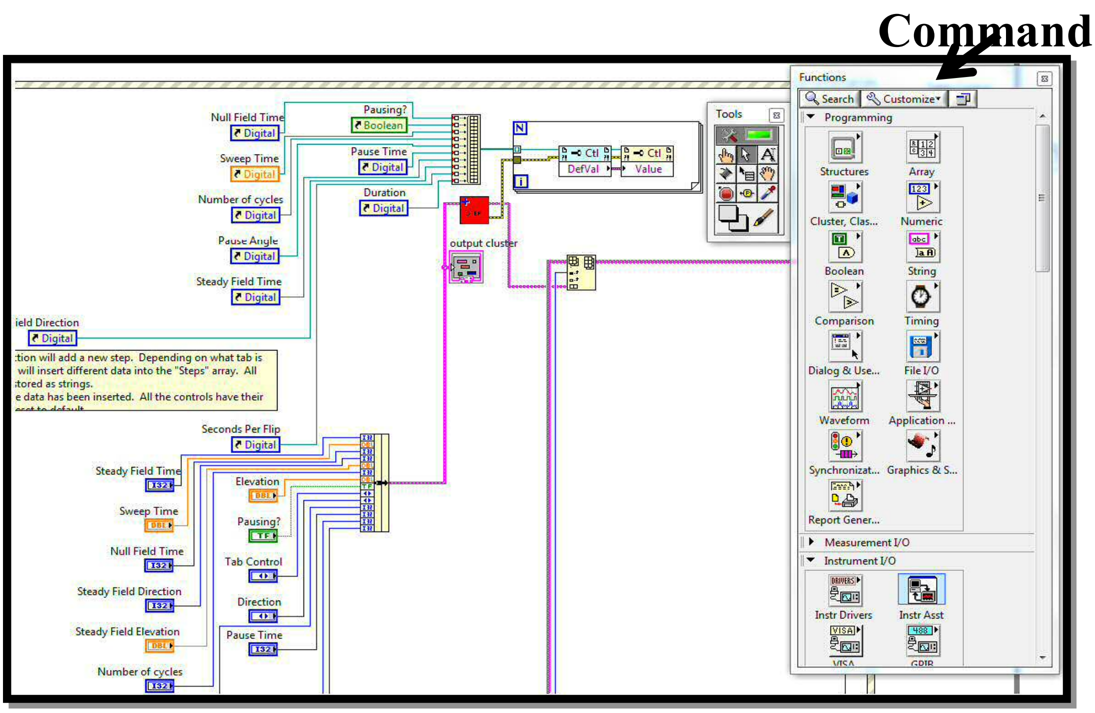


Figure 2-3 the block diagram in LabView programming

Programming in the LabView environment has been utilized to control the magnetic field conditions, tracking and recoding, and analysis of the results. The software has been developed and designed during the course of this PhD research.

The program, named "Create_Program.vi", was developed by Dominic Walker and Tomasz Kutrowski to achieve the experiment requirements, so the program was capable of giving the user the ability to determine and control the following:

- Condition type of the magnetic field
- Number of scenarios to be included in one session
- The duration of each of magnetic field condition.
- Elevation to be decided by the user to be ON or OFF.
- Field direction in the case of the steady field.
- Speed of the sweep in the case of the sweeping field in a clockwise (CW) or counterclockwise direction (CCW).
- A pause every specific degree for certain of seconds in the case of the sweeping field
- The speed of the flipping in the case of the flipping field (Z coils are included).
- When preparing the complete scenario, which is made up of a sequence of magnetic field conditions, the user can easily amend the sequence at any point by removing steps or inserting steps in the middle of the sequence.

So the user can make a record of different sessions with various scenarios, these sessions are saved as individual experiments (Sub-programme) under any name that the user prefers. This feature helps to save time as the user might re-use any of these saved sessions in the future. The front panel (Figure 2-1) of the software has been developed in a way to be convenient for the user to access the above features.

The new session (sub-program) appears as a front panel, which is user-friendly as well. Furthermore, it offers some features such as:

- After creating the sub-program with specific sequences of magnetic field condition, the camera will be turned ON automatically before exposing the pigeon to a magnetic field. This period is called a preparation period which gives the user the ability to adjust the position of the object (Pigeon) and monitor live the box during the experiment. The user can exclude any recording video in any circumstances.

- Compare the head angle to North, i.e. correlate the head angle with the position of the North.
- Magnetic field values are displayed for the user.
- Start time, current time and predicted finish time are displayed for the user.
- The program stores all the details about the experiments such as: the sequence of the magnetic field conditions, speed of the sweeping, number of sweeps, speed of flipping, the angle of the steady field, the presence or not of the inclination factor, the time, date, magnetometer's measured data, and expected artificial magnetic field. All these data are automatically saved a. tdms and excel file, in a given directory, with a date and time in the file name which is presented in the saved file box in Figure 2-2. As the measurements have been repeated in this research, so saving such details for each experiment will be helpful to distinguish each experiment from the others to re-use them anytime for experimental purposes.

The user has five options to set up the sequence of magnetic field conditions:

- Static field (SF)
- Sweeping field (with a speed of 20°/s) in clockwise rotation (CW).
- Sweeping field (with a speed of 20°/s) in counter-clockwise rotation (CCW).
- Fast flipping field (adding inclination factor).
- Slow flipping field (adding inclination factor).
- Null field.

Although the speed of the sweeping and flipping can be adjust at any time by the user, in most experiments the sweeping speed was left at 20°/s, unless stated otherwise. Previous work, which has been carried out by Szymon Migalski [114], had a limitation is that all of the steps of the magnetic field conditions in a given sequence needed to be the same duration. Whereas in this research, the program was further developed to give the user flexibility to specify the various lengths of each of magnetic field condition.

Two NI DAQs (Data Acquisition cards) were used to acquire and generate a signal. The first DAQ was used to acquire the signal, which has been obtained from the magnetometer. Using A/D (convertor data acquisition card), the signal will pass to the computer in digital format to compensate and cancel the actual earth magnetic field and to generate the desired value of the field. The generation of the signal can be achieved by connecting another DAQ which converts Digital to analogue and it will generate desired value (DC generated signal) which will then be amplified and injected in the coils with the specific current value to get the desired magnetic field that the pigeon will be exposed to. These DAQ's are manufactured by the same company that produces LabView and consequently are fully compatible with this software.

2.1.2 Amplifying the Signal:

Another piece of equipment is the power amplifier. There is a power amplifier for each axis (Analogue Kepco and Amcron amplifiers). Due to the age of these amplifiers; each one has its own specific characteristics. The amplifiers have been tested at DC and low frequencies. Separate measurements were performed where an input was desired with field value in microtesla and the output field was measured with a magnetometer. The obtained data allowed fitting of a linear calibration curve, as shown in Figure 2-4**.** The calculated parameters of the fit were then fed back to software (Figure 2-5) so as to correct for the gain of the amplifiers. This program takes an input in Teslas, and outputs a voltage. This voltage will vary depending on which Amplifier is selected (Top and Bottom are used to identify which amplifier is used.) If a voltage is input into the relevant amplifier, the current producing the requested field strength via the coils is output from the amplifier. This amplifier has a variable gain, which should be set to half way (exactly up on the dial) in order to get the expected characteristics shown in the data file. In the event amplifiers need to be re-arranged, or a new amplifier is used, code would need to be changed. The calibration program has been written by Dominic Walker and Tomasz Kutrowski (private communication).

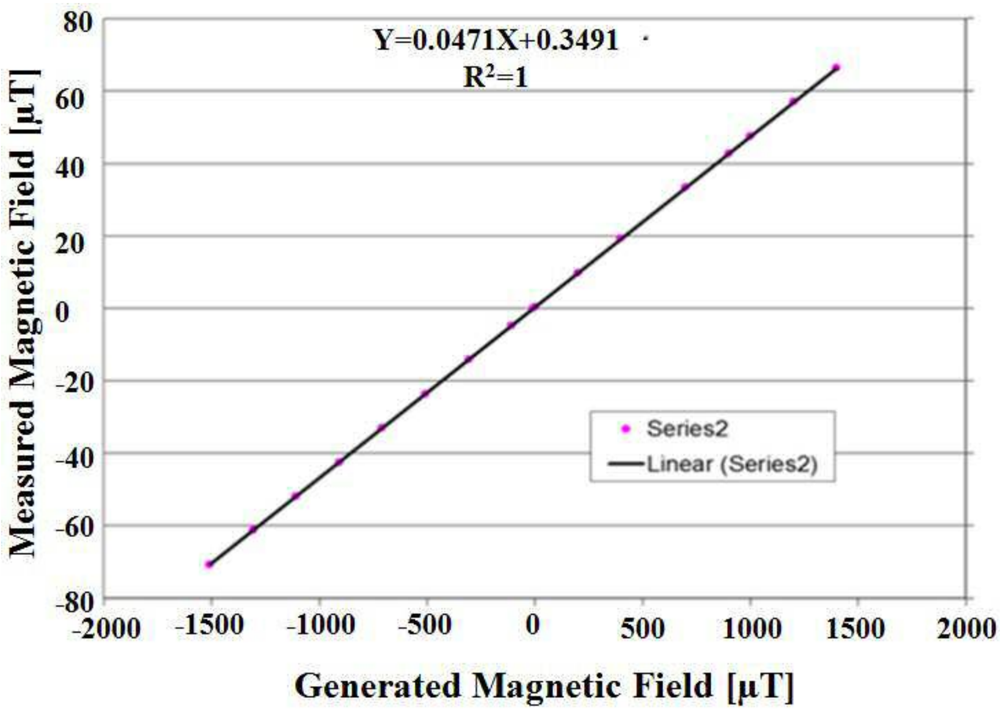


Figure 2-4 an example of the amplifier calibration characteristic

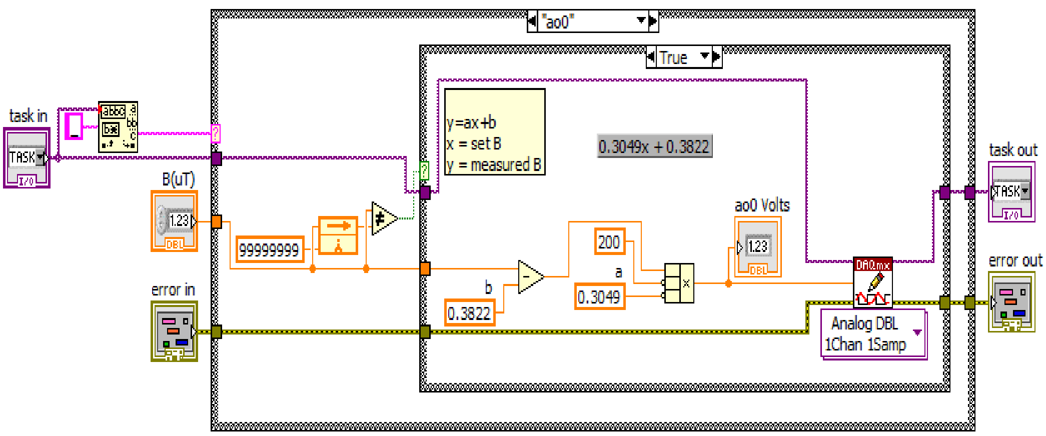
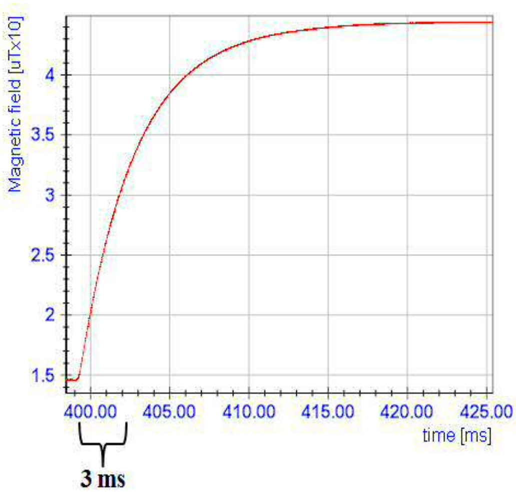


Figure 2-5 Gain_select.vi subroutine created to account for amplifiers' gain characteristics.

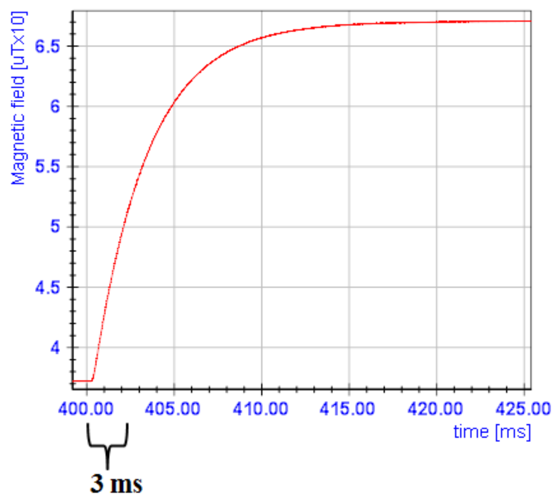
2.1.3 RISE TIME AND FALL TIME

It was important to determine the amplifiers' rise and fall times (defined in electronics as the time required for the signal to reach from 63% of a specified value (rise time) or 37% in (fall time) in the system for confirmation of the equipment suitability. The field inside the new coils was being recorded with a three-Axis Magnetic Field Sensor, (MAG-03MC of Bartington Instruments [115]) , one axis at a time, while the field was switched from 0 to 30 μT (rise time) and 30 μT to 0 (fall time); X, Y direction – Amcron amplifier[116], Z – Kepco amplifier [117].

The rise and fall times were found to be very similar for both amplifiers used, close to 3ms, and regarded as sufficiently low for the proposed investigation of magnetoreception in pigeons (See Figures 2-6 and 2-7).

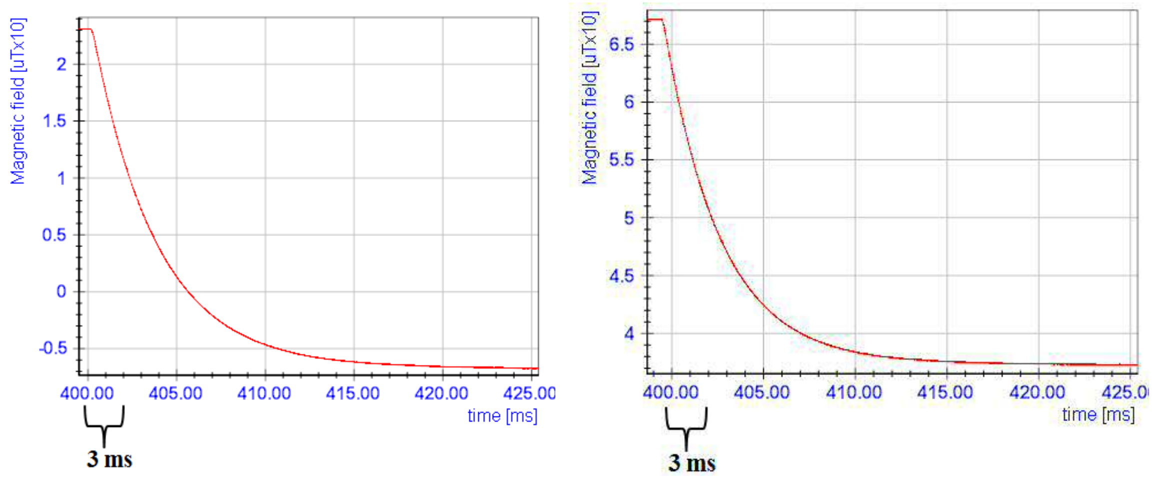


One of the alike characteristics recorded on both channels of the Amcron amplifier (rise time about 3ms).



Kepco amplifier (rise time about 3ms).

Figure 2-6 Rise times



Falling edge for Amcron (about 3ms) Falling edge for Kepco (about 3ms)

Figure 2-7 Fall times

2.1.4 Instrumentation for Magnetic Field Generation:

It is usually essential to have a uniform magnetic field source in order to provide a field of known uniformity, and a Helmholtz coil is the most commonly used source of a uniform magnetic field (sometimes also known as a standard of magnetic field).

2.1.4.1 Helmholtz Coil Background

Helmholtz coils are the most commonly used source of a homogeneous magnetic field (sometimes also as a standard of magnetic field). A pair of Helmholtz coils (named in honor of the German physicist Hermann von Helmholtz) are constructed of two identical circular coils placed symmetrically one on each side of the experimental area along a common axis. Both coils must have the same number of turns n, same diameter, and equal currents flowing in the same direction. Each coil is carrying a current I. Coils are separated with a distance L, which is equal to the radius of the circular loops r (Figure 2-8). That uniform magnetic field will be generated in the region which is between the two circular coils. The magnetic field's intensity increases proportionally with the number of turns wound in the coil and the current pass thorough the coil.

The magnetic field generated by the circular coils according to Equation 2-1 can be determined as:

$$\boldsymbol{H_1} = \frac{\boldsymbol{nIr^2}}{\boldsymbol{2}}\left[\boldsymbol{r^2} + \left(\frac{\boldsymbol{L}}{\boldsymbol{2}} + \boldsymbol{x}\right)^{\boldsymbol{2}}\right]^{-\boldsymbol{3/2}}$$ Equation 2-1

And

$$\boldsymbol{H_2} = \frac{\boldsymbol{nIr^2}}{\boldsymbol{2}}\left[\boldsymbol{r^2} + \left(\frac{\boldsymbol{L}}{\boldsymbol{2}} - \boldsymbol{x}\right)^{\boldsymbol{2}}\right]^{-\boldsymbol{3/2}}$$ Equation 2-2

The magnetic field between those two coils is therefore:

$$\boldsymbol{H} = \boldsymbol{H_1} + \boldsymbol{H_2} = \frac{\boldsymbol{nIr^2}}{\boldsymbol{2}}\left\{\left[\boldsymbol{r^2} + \left(\frac{\boldsymbol{L}}{\boldsymbol{2}} + \boldsymbol{x}\right)^{\boldsymbol{2}}\right]^{-\boldsymbol{3/2}} + \left[\boldsymbol{r^2} + \left(\frac{\boldsymbol{L}}{\boldsymbol{2}} - \boldsymbol{x}\right)^{\boldsymbol{2}}\right]^{-\boldsymbol{3/2}}\right\}$$ Equation 2-3

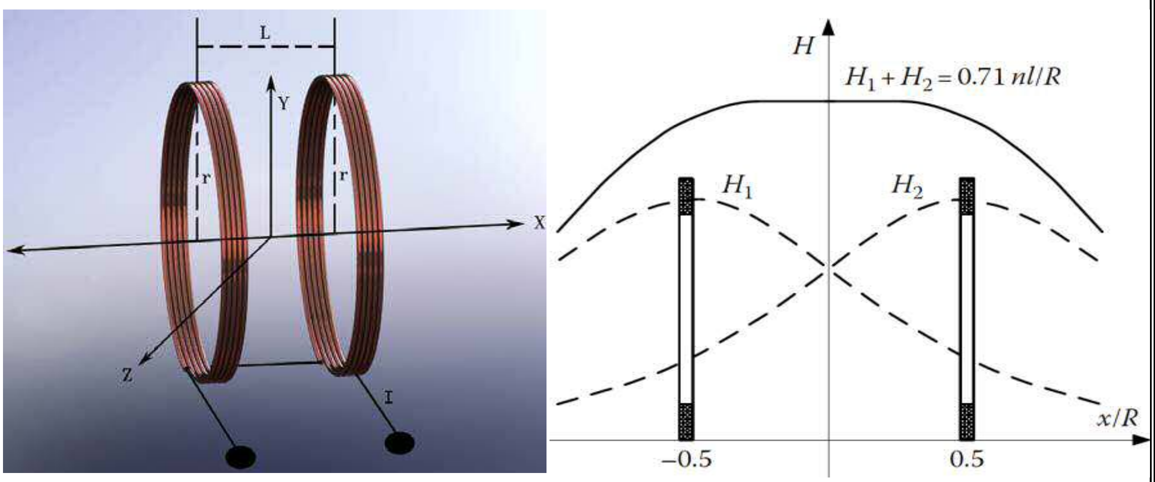


Figure 2-8 the Helmholtz coils and the magnetic field distribution inside the Helmholtz coils system. Adapted from [118].

This relation is presented in above Figure 2-8 The Helmholtz coils and the magnetic field distribution inside the Helmholtz coils system and we can see that in the central part between these two coils, the magnetic field is uniform. It can be proved that if *r*=L this uniformity is optimal, and for *x*=0, *r* = L. A uniform magnetic field will be generated in a region between the two circular coils. The magnetic field's intensity increases proportionally with the number of turns wound on the coil and. The current passed through the coils generates a magnetic field at the geometrical centre, and its strength in the middle region of the Helmholtz coils can be calculated using the formula:

$$\boldsymbol{H_o = nIr^2(r^2 + 0.25r^2)^{-3/2} = 0.7155\frac{nI}{r}}$$ Equation 2-4

Where: H- magnetic field intensity [$\frac{A}{m}$]

n – Number of turns

I – Current inside the coil [A]

r – Radius [m]

At a horizontal distance 1/4 L from the centre, the magnetic field value drops by less than 0.42% as compared with the centre. Perpendicular to the coils axis at the same distance of 1/4 L, this difference is not larger than 0.75%.

The region of uniform magnetic field varies with the relationship of the coil's radius and the separation distance of the Helmholtz coils. The uniformity region can not be achieved when L< r and L > r, hence L = r was implemented in this research to obtain a volume that has homogeneous magnetic field.

The field uniformity of the Helmholtz coil system can be significantly improved by using another two pairs of coils connected in series. In this research, three pairs were connected in series without causing any field influence as they are oriented 90^o on each other.

2.2 Magnetic Shielding:

A long time ago, (della Porta) [119] defined the shielding from the fact that the magnetic field does not pass the ferromagnetic layer. First calculations and the theory of a spherical shield were presented by Rücker [120].

2.2.1 MAGNETIC SHIELDING PRINCIPLE

Magnetic shielding does not allow the magnetic field that causes the induction from reach the low-level circuit. The shield is surrounding the complete circuit. The principle of operation of the magnetic shield is demonstrated in Figure 2-9, the principle of shielding by a ferromagnetic cylinder [121]. If the shield is made out of high permeability material, the magnetic field is concentrated in the shield and prefers passing into the shield, bypassing the interior part of the shield around the circuit to be shielded and out at the other side. The effectiveness of shielding is described by the shielding factor, S, as the relationship between external $\boldsymbol{H_e}$ and internal $\boldsymbol{H_i}$ magnetic field:

$$\boldsymbol{S} = \frac{\boldsymbol{H_e}}{\boldsymbol{H_i}} \qquad \text{Equation 2-5}$$

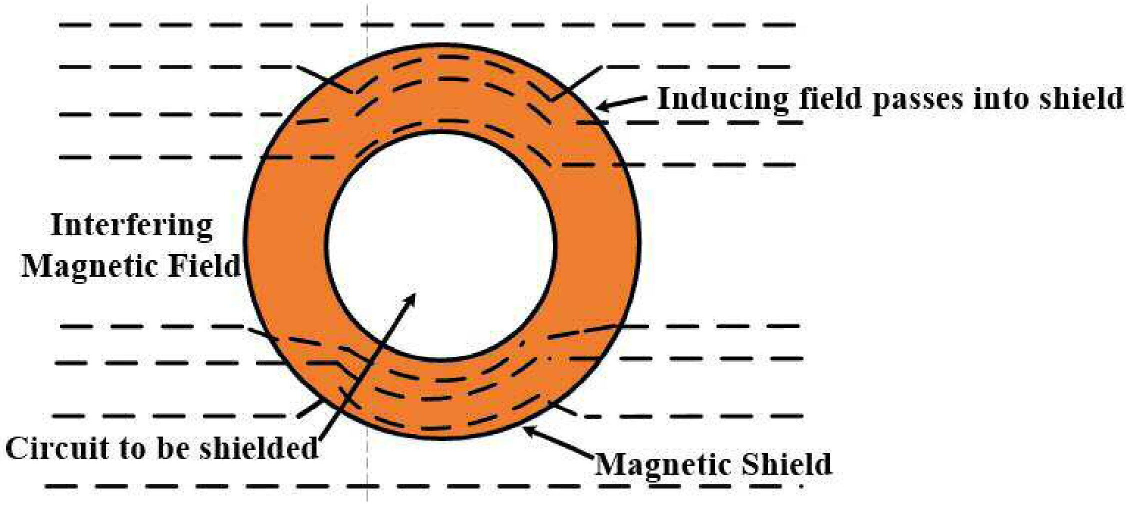


Figure 2-9 The principle of shielding by a ferromagnetic cylinder. Adapted from [121]

There are different shapes that can be used to achieve the shielding (sphere, cylinder, or cube), so the shielding factor of a DC magnetic field has been calculated via approximate formulas [122-124].

For a cube with length, a, and wall thickness, *t,*

$$S = 1 + \frac{4\,\mu\,t}{5\,a}$$ Equation 2-6

While for a long cylinder with diameter *D*

$$S = 1 + \frac{\mu t}{D}$$ Equation 2-7

We can, therefore, see that the factors that influence the shielding effect are:

- Relative permeability, μ, of the material. That is why usually high-permeability materials as NiFe (*mumetal*) with μ=50,000–100,000 or amorphous materials reaching even permeability as high as 800,000 are used.
- Wall thickness.
- It was proved that, instead of increasing the wall thickness, multiple shells [125-127]. For example, for a cylinder consisting of two layers with diameters are more effective *D*1 and *D*2 and shielding factors *S*1 and *S*2, the resultant shielding factor is:

$$S = 1 + S_1 + S_2 + S_1 S_2 \left[1 - \left(\frac{D_2}{D_1}\right)^2\right]$$ Equation 2-8

2.2.2 Passive Magnetic Shielding

Passive shielding is produced by a closed container surrounding the shielded space, this structure consisting of relatively large pieces of ferromagnetic materials such as mu-metal. Also, the high permeability of mu-metal provides a low reluctance path for magnetic flux, leading to its use in magnetic shields against static or slowly varying magnetic fields, i.e. it is a more attractive path for the magnetic field than the air. Consequently, mu-metal shields are often made of several enclosures one inside the other, each of which successively reduces the field inside it. Because mu-metal saturates at such low fields, sometimes the outer layer in such multilayer shields is made of ordinary steel. Its higher saturation value allows it to handle stronger magnetic fields, reducing them to a lower level that can then be shielded effectively by the inner mu-metal layers [128].

The previous research performed by (Szymon Migalski)[114], employed passive magnetic shielding (see Figure 2-10).

Figure 2-10 Chamber passive shielding.

The system was a shielding chamber, which consisted of five layers of mu-metal and , five superimposable lids. This structure was used to eliminate all external magnetic fields, such as Earth's magnetic field. Consequently, it was useful to get an artificial magnetic field without any external disturbance. In Figure 2-10 , the chamber shielding with different lids is pictured.

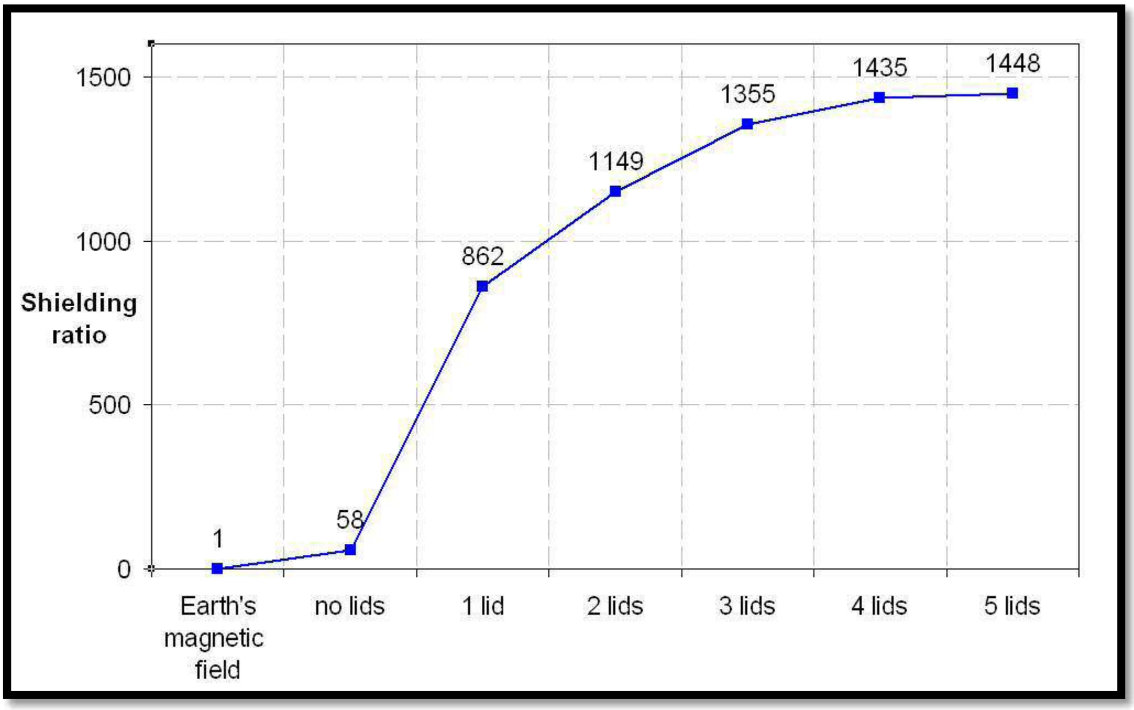


Figure 2-11 Plot showing shielding factor versus number of lids of the shielding chamber [114].

It was sufficient to use only one lid; see Figure 2-11, as using all five lids every time would have produced unwanted noise and stress for the pigeon, which in turn could affect the pigeon's behaviour.

The limitations of this technique with respect to our research are:

- The bird is always isolated from the ambient magnetic field by the enclosing shielding chamber so the pigeon will experience a null field straight away when it goes in the chamber, which is sometimes not desirable as some experiments require a magnetic field condition other than null field.
- Space limitation, as the system was designed to be small so as to fit inside the chamber, which limits the user's flexibility.
- Using a small system required increased value of the currents value that flow in the 10 turns of the coils to get the desired magnetic field, which in turn was causing heating up the coils and heating up the space. Increased temperature in the space might affect the pigeon's behaviour, which in turn may limit the experimental period.

2.2.3 Active Magnetic Shielding

Because of the above limitations of passive shielding, an alternative used with static or low-frequency fields is active shielding, which ensures appropriate accuracy in measuring and emulating the Earth’s magnetic field. For this reason, it was necessary to design and set up a system dependant on an active magnetic shielding concept. Theoretically, this involves building a second magnetic system on the outside of the main magnetic system with a field of the opposite sign, thereby eliminating the unwanted external magnetic fields. It is also possible to cancel the magnetic field by applying a feedback magnetic field—the method known as active shielding or magnetic field compensation. Because even the Earth’s magnetic field is not stable, the compensating magnetic field should be controlled. The volume under test is usually surrounded by three-axis Helmholtz coils, and the magnetic field generated by these coils is proportional to the field detected by three-axis magnetic field sensors. The active shielding technique can substitute for conventional ferromagnetic shields, but more often it supports the conventional shield technique [118]. It was essential to ensure a high level of magnetic shielding when working with such low magnetic fields. Therefore, a specially designed active shielding unit (3D Helmholtz coil) as shown in

Figure 2-19, which has a shielding factor of 40, was used for the generation of a new field, see Figure 2-12.

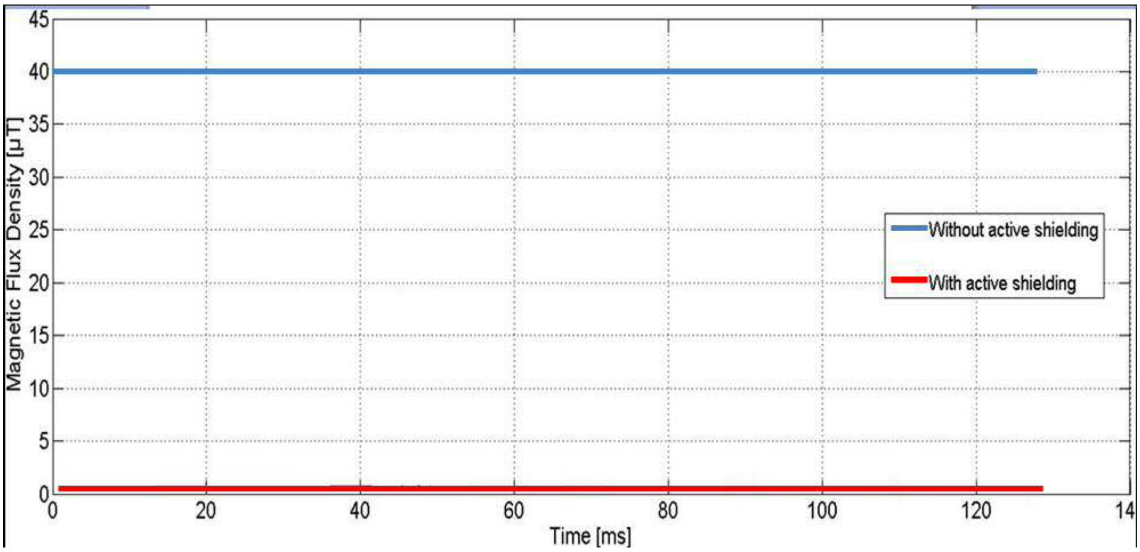


Figure 2-12 Shielding factor field reduction.

2.3 Three Dimensional Helmholtz Coil Setup as a Source for Magnetic Field

In this research, a special instrumentation setup of three-dimensional Helmholtz coils was used to cancel out the Earth's magnetic field and then generate a field within the range of the Earth's magnetic field, controlled by a specific current source. Active magnetic shielding is used in an attempt to accurately eliminate and then re-create the magnetic fields to be experienced by an animal. The details of the designed and constructed 3-D Helmholtz coil system are described here.

2.3.1 System Setup

The main thrust of this 3-D Helmholtz coil design is to generate a uniform magnetic field in a region convenient for positioning the animal, as well as for including external stimuli in the investigation. SolidWorks is solid modelling CAD [130] (computer-aided design) software that runs on Microsoft Windows, and utilizes a parametric feature-based approach to create models and assemblies. The software is written on Parasolid-kernel. It provides a suite of product development tools for mechanical design, design verification, data management, and communication tools.

In order to achieve precise results in this research, SolidWorks software was used to design the 3-D system with accurate dimensions. The details of the software design will be demonstrated below. Finite Element Analysis techniques were employed to determine the coil configuration needed for producing the ideal uniform magnetic field used in this investigation as well as to identify non-uniform magnetic field regions. The details of the simulation will be explained in detail in the next chapter.

2.3.1.1 Software Design

This system (Figure 2-13) is designed in way to remove all of the limitations that the previous system had, with larger dimensions to create convenient space for the user and for the subject to proceed with smooth experiment. Also, bigger coils mean more turns which will reduce the value of the current (Ampere) that flows in the coils. Thus, neither the coils nor the middle region of coils will not heat up. The system encompasses various parts:

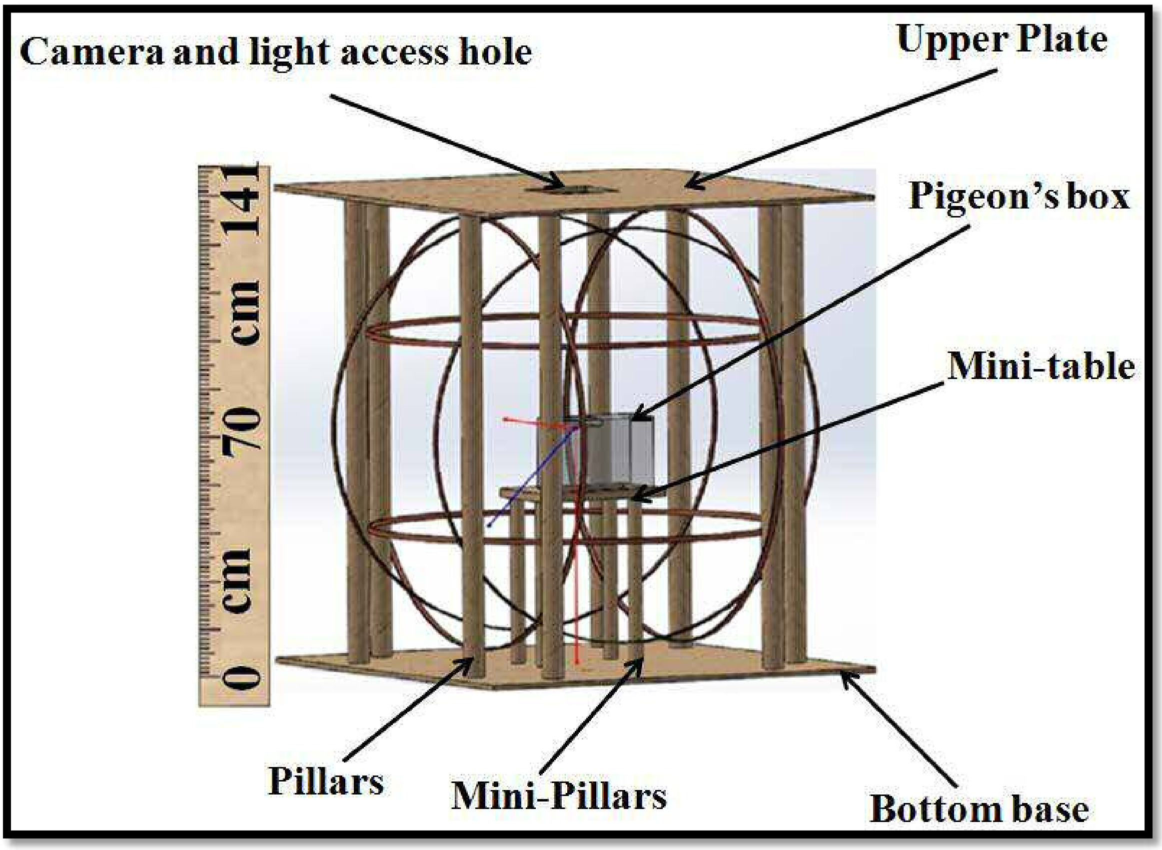


Figure 2-13 a picture of the 3-d Helmholtz coil system.

1. Three pairs of Helmholtz coils each with different dimensions and a different number of turns have been designed. Table 2-1 illustrates the specifications of

the coils. These specifications have been used after investigating them by using finite element technique to guarantee producing the equivalent Earth's magnetic field with such specification.

Table 2-1: Specifications of three dimensional Helmholtz coil system.

Parameter	X Pair	Y Pair	Z Pair
Number of turns	144	138	132
Inner diameter [Cm]	127	122	117
Outer diameter [Cm]	128	123	118
Resistance [Ω]	25.2	23.7	20.7

2. Two acrylic plates have been attached to the top and bottom of the system (129 x 124 cm). The top plate has a 20x20 cm hole, providing access for a camera and/or light source.
3. Eight large pillars: Eight acrylic pillars (height 138.6cm) were constructed to support the coils from all directions and maintain their circular shape. The physical locations of the pillars were distributed in the ring so that easy access to the animal box was maintained, as can be seen in (Figure 2-14)

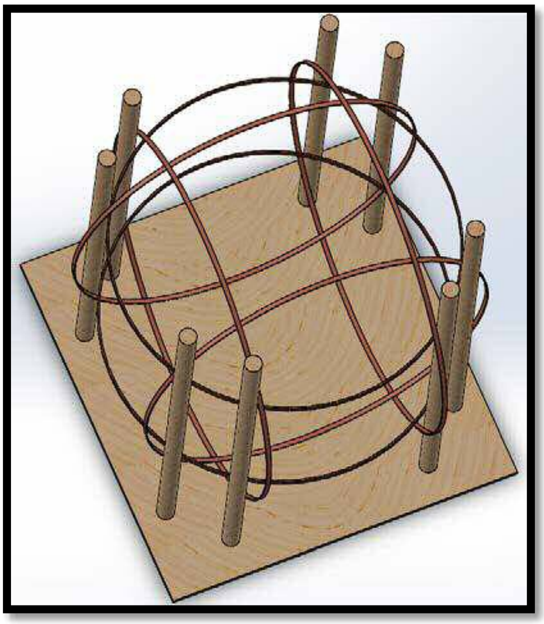


Figure 2-14 Top view of the 3d holmholtz coil.

4. Mini-table (60 x60 x 2.5cm): Because the animal should be located entirely within the uniform magnetic field created (Central region), an acrylic mini-table was designed as a base to support the pigeon box in which the pigeon experiences the magnetic field). It is also important as it works as a means for ensuring that every time the experimental box, in which the bird is placed for the time of an experiment (see in figure how box is positioned with help of the limiters), was positioned so that bird's head would be situated within the area of the uniform magnetic field.

5. Mini-pillars: acrylic pillars (height 49 cm) were constructed to support the mini-table to hold the pigeon's box while the experiment is running. Also, these pillars have a role to position the pigeon's head (eyes) in the cantered of the system. Thus, the pigeon's eyes will experience the desired magnetic field.

6. Coils Supporters:-Two kinds of supporters are added to the system:
   A. Two of supporters are added to each pillar to aid in keeping the circular shape of the coils in the X and Y directions. The windings of the coils will pass through each supporter, which will thus support the coils (see Figure 2-15).

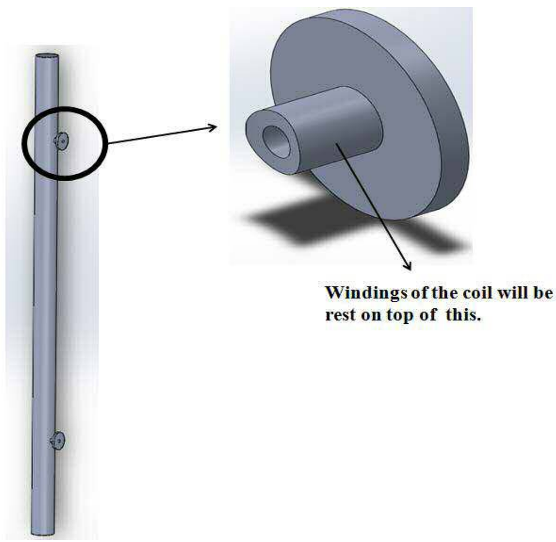


Figure 2-15 Pillars and supporters.

B. Two supporters are added to each pillar to aid in keeping the circular shape of the coils in Z directions. The windings of the coils will rest on top of each supporter (see Figure 2-16).

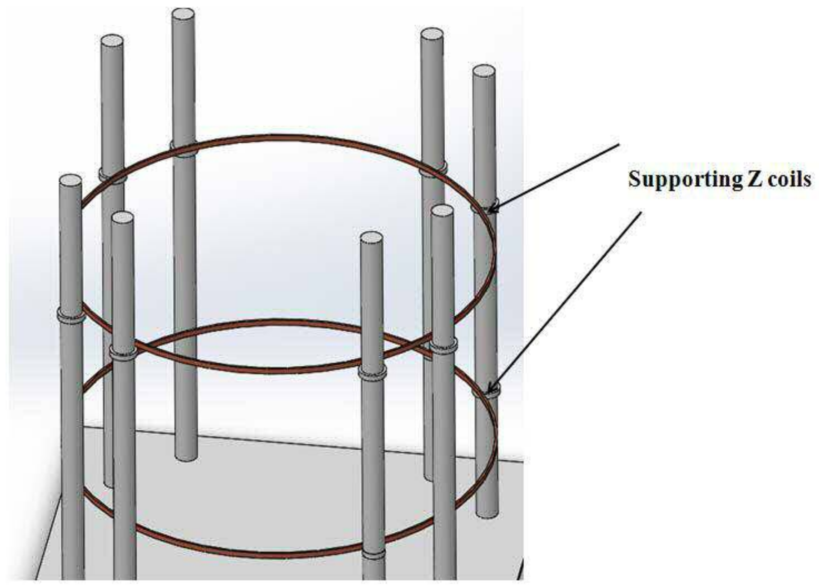


Figure 2-16 Side view of the system that is supported by the pillars

7. Sensor holder: 3-axis magnetometer (Mag-03 Three-Axis magnetic field sensor) is fitted onto the mini-table close to the centre of the system. This is to keep the sensor in place near the centre in order to send the field reading which is corresponds to the centre of the coil. Consequently, this will help perfect the accuracy of the field compensation (see Figure 2-17).

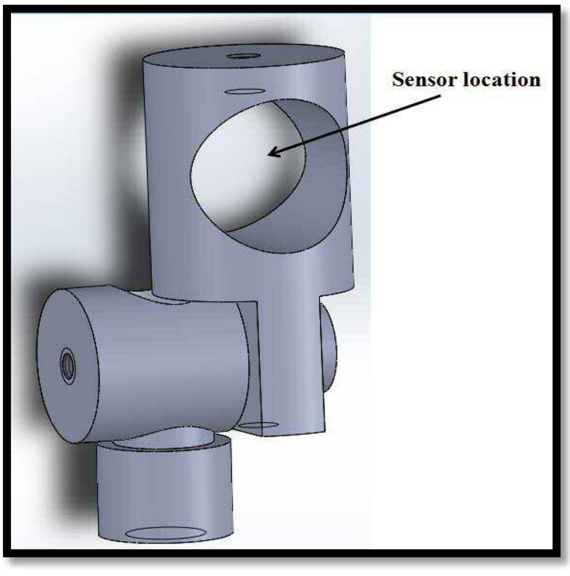


Figure 2-17 Sensor holder

8. Pigeon box: During this research, two different kinds of boxes for placing the pigeon inside were tried. Both boxes have exactly the same design, but the material was different.

1) White non-translucent Perspex experimental box with non-transparent lid.
2) Translucent Perspex experimental box with transparent lid.

Although the pigeon could sit or stand comfortably in the box, it was designed to prevent the bird from turning around inside. However, head movements were not restricted in any way; the bird could move its head freely in any direction. An additional Prespex chimney (either translucent or non- translucent) was placed around the hole in the Prespex lid so that particularly active birds were restricted from looking out of the box or even escaping from the box. Also, it played a beneficial role in aiding ventilation while the experiment was run (see Figure 2-18).

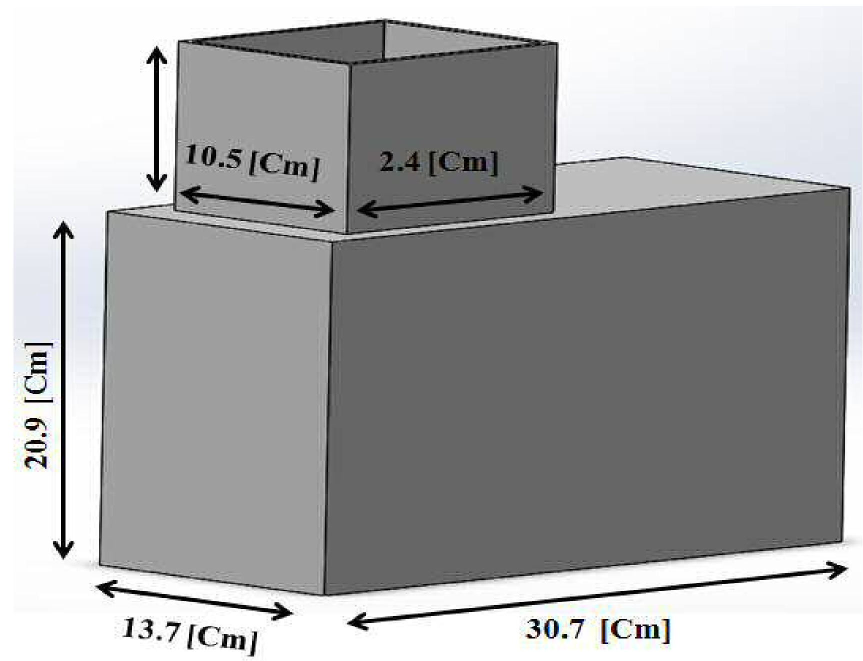


Figure 2-18 Pigeon's box

2.3.1.2 ACTUAL DESIGN OF 3 PAIRS OF HELMHOLTZ COILS:

Three pairs of coils with different diameters and dimensions were produced. The system on Figure 2-19 has been constructed with help from the Mechanical Workshop in the School of Engineering at Cardiff University.

Figure 2-19 A picture of the constructed 3-D Helmholtz coil system

This job has been achieved by cutting three wooden wheels with different dimensions (large, medium, and small), see Figure 2-20. Each wooden wheel has been used as a former to produce a symmetrical pair of coils by winding the coils on top of these formers. Thus, a small (wooden wheel) former has been used to produce pair of small coils, medium (wooden wheel) former has been used to produce a pair of medium coils, and large (wooden wheel) former has been used to produce a pair of large coils. Auto-bonded coil, with a diameter of 0.71mm, has been used in the research. Grey PVC open slot trunking (19x19mm) has been fixed around the wooden wheel, which was provided as a tunnel to help the windings of the coils to settle around the wooden wheels, and subsequently, the windings of the coils will take the rounded shape.

In order to get stiff and solid rounded coils in the absence of the formers' support (wooden wheels), DC current 4 amperes and 130 volt was applied to the windings to heat up the coils due to that current. The internal tunnel of the trunking has been covered by masking tape to protect it from the heat that we used to provide the stiffness to the coils.

The diameter of the these three wooden wheels are : 1276 mm, 1222 mm and 1168 mm taking into account the dimension of the trunking.

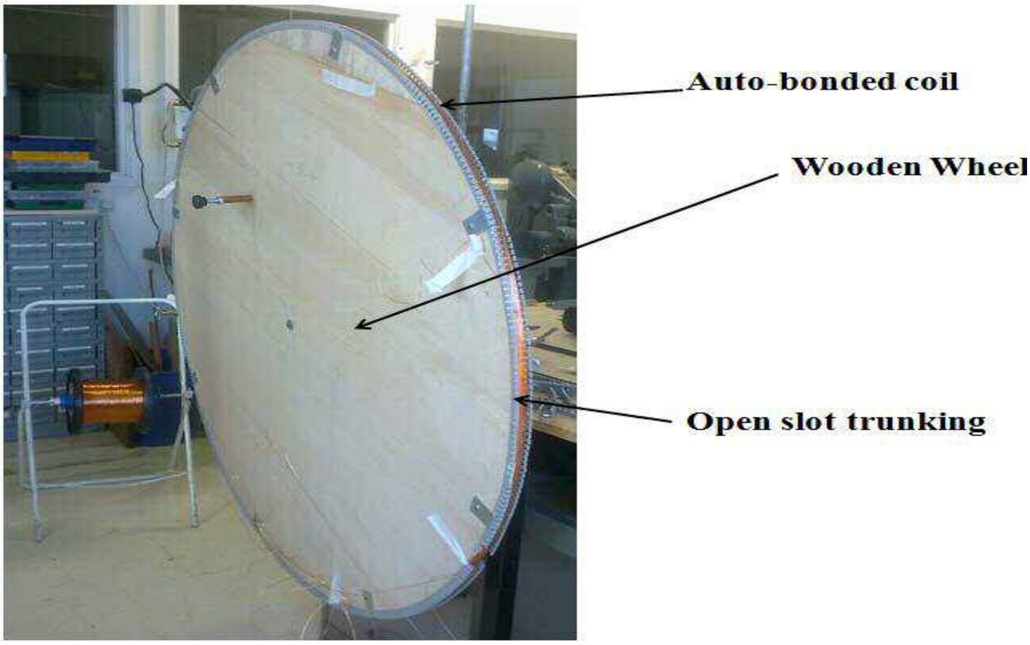


Figure 2-20 Winding one of the Helmholtz coil in Cardiff university workshop

2.3.1.3 Initial Trials and Modifications for The Design

In order to design a convenient measurements system, Solid Works software has been used to do many trials. The first four trials had some limitations but such trials were useful as they led us to design a perfect system that is convenient for the pigeons as well as the user.

Stage 1: Initially, the system has been designed with only 4 pillars (see Figure 2-21).

Limitation: When the PVC open slot trunking was removed from the coils, four pillars could not offer enough support for the 3 pairs of coils to remain in rounded shape.

Solution: Then, the number of the pillars was doubled to support the coils and give them more stiffness.

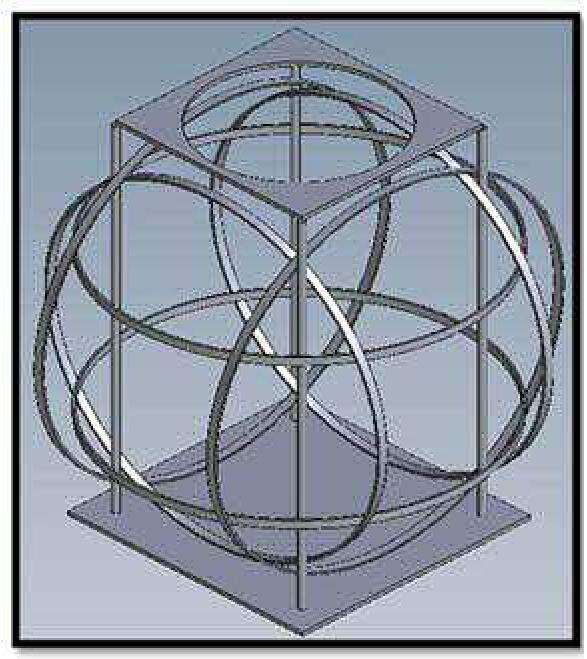


Figure 2-21 First stage of the design, 4 pillars only

Stage 2: In this stage, 8 pillars have been added. In addition, in order to ensure a dark environment for the pigeon, the laboratory's windows were painted black and a black curtain used. To ensure darkness inside the design, external curtains were added around the design (these curtains can be added and removed as they will be attached to the top plates by hooks). The length of these curtains is 1386mm. For darkness, a small cylinder can be attached to the top hole to keep the space for the camera to record the pigeon behaviour. By doing that, we will ensure that 100% darkness inside the system (see Figure 2-22).

Limitation: is the lack of ventilation which might stress the pigeon.

Solution: Some ventilation facilities have been added to the design as shown in the next stage.

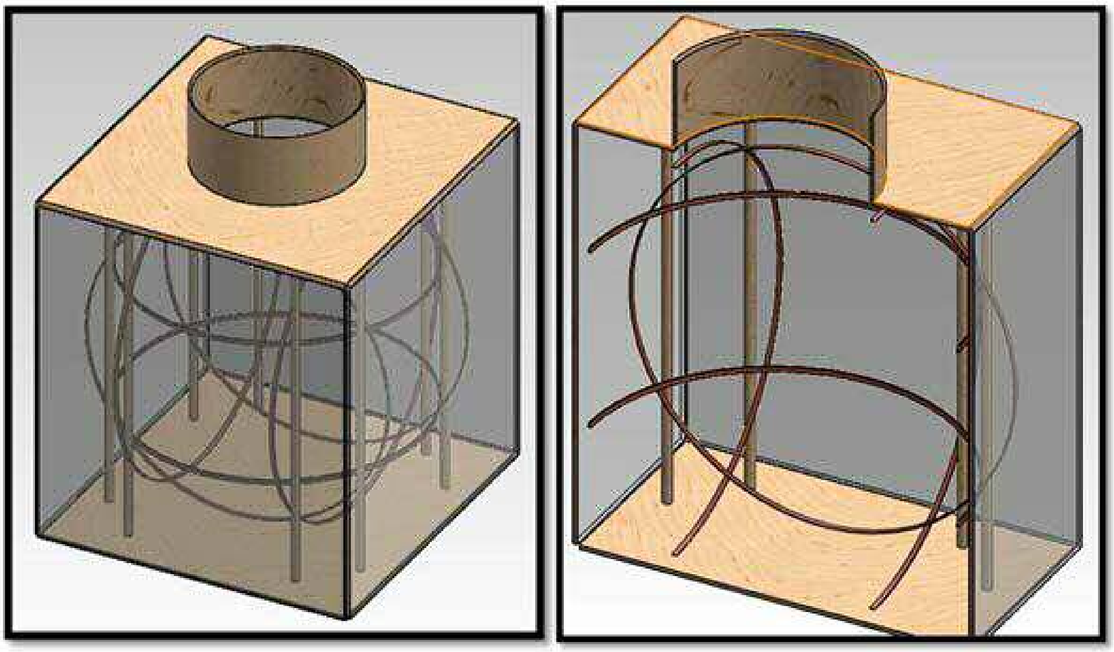


Figure 2-22 Second stage

Stage 3:

The below design has offers a ventilation via a ventilation tube, and the darkness has been ensured by the top cylinder (see Figure 2-23).

Limitation: This design is not convenient for mounting the camera or the fibre-optic light.

Solution: Remove the curtain completely and consider turning off the light and using infrared source excitation in the darkness experiments.

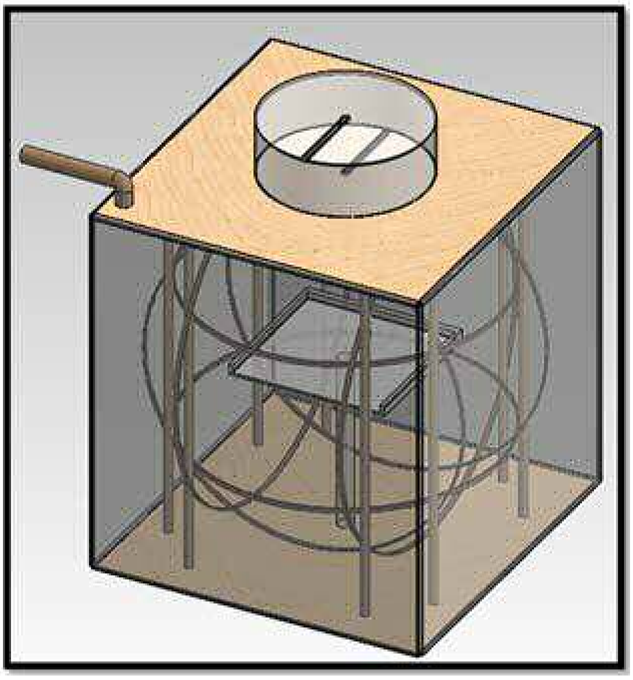


Figure 2-23 Third stage

Stage 4:

In this stage, the upper plate has been removed. Alternatively, two bars have been crossed to each other to give stability to the eight pillars.

In order to lift the pigeon to the middle point of the system, a cylinder has been added to the middle of the system. A glass base has been attached to the top of the cylinder to support the pigeon box. A small hole has been added to the bottom of the cylinder. Light can be inserted via this hole which will pass through the glass and then will be reflected via the top mirror. This reflected light will illuminate the pigeon box (see Figure 2-24).

Limitation: Camera and light positions are not stable. Also, it is not convenient for the user.

Solution: The configuration explained in (section 2.3.1.2) was used as the final design.

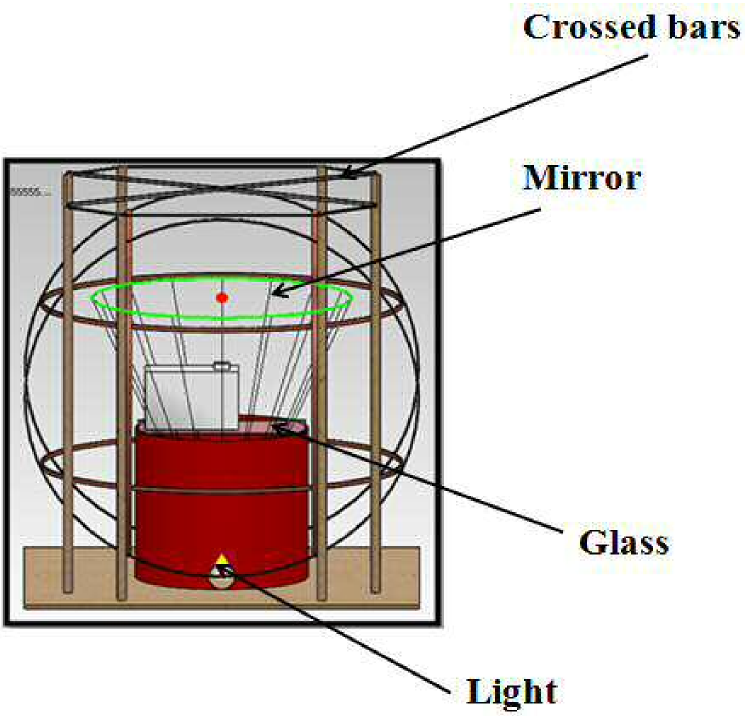


Figure 2-24 Forth stage

2.3.2 Electrical Setup

Each of the windings of the small, medium and large Helmholtz coils is connected in series to improve significantly the field uniformity and also to compensate for the Earth's magnetic field in every direction and location (see Figure 2-25).

The three components of the Earth's field can be compensated for by supplying accurate values of currents from the power amplifier, which is controlled by LabView software. The exact compensation values required were detected by a Three-Axis Magnetic Field Sensor (MAG-03MC of Bartington Instruments), which was placed in the centre of the system where the animal was to be located. The computer software controls three output channels on the control card (National Instruments NI) connected to three amplifiers, which in turn, send a signal to the coils. An artificial magnetic field, equivalent to that of the Earth, was generated with the help of the set of three Helmholtz coils perpendicular to each other, which are capable of producing a static field in any direction as well as a field rotating clockwise or anticlockwise or, by suitable dynamic screening, even the absence of any field, i.e. a null field. The static field, field rotating clockwise or anticlockwise, and null field are referred to hereafter as SF, CW, ACW, and NF, respectively.

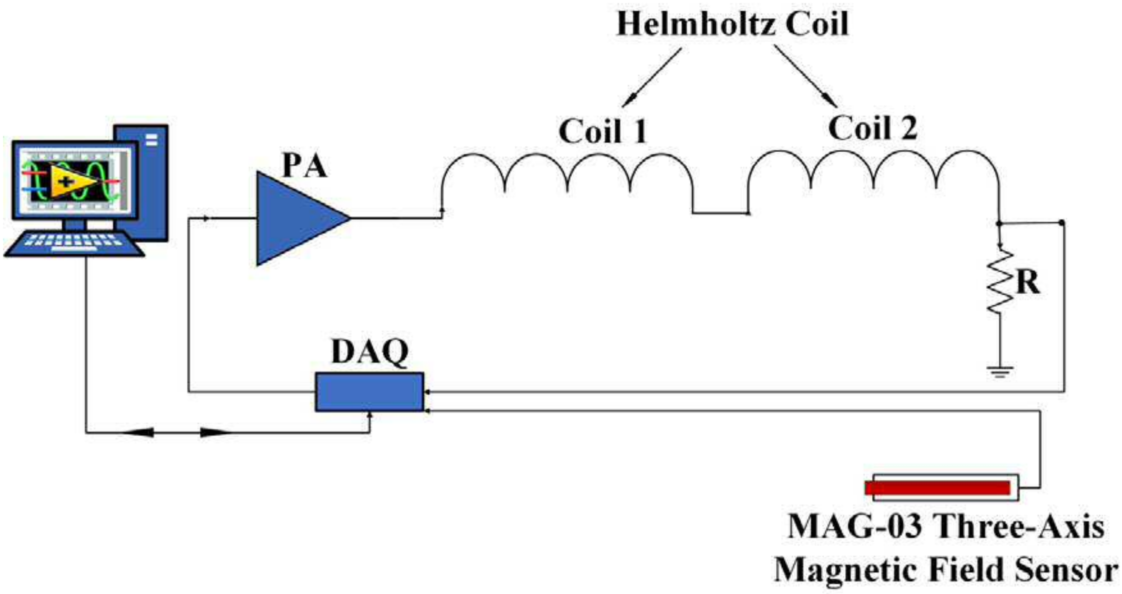


Figure 2-25 Electrical connection of one channel of the overall system.

Figure 2-26 illustrated the generation of the magnetic field in three dimensions – (XYZ) and the resultant field. So the concept of producing the magnetic field based on the following principle: Coils producing field in the X and Y directions were used to produce the static and sweeping fields in the XY plane. Both signals are sinusoidal waveforms, but the Y's phase is shifted with respect to X by 90^0. Furthermore, both X and Y signals were changing in the case of a sweeping field, but in a manner that the sum of both vectors was always equal to the constant value of the horizontal value of the field. That value was set to be 19,5μT in order to meet the conditions of the local magnetic field in Cardiff. Such a signal, when combined on the XY plane, results horizontal resultant which will be represented as a blue circle in Figure 2-26. The radius of the blue circle is 19,5μT (blue vector Figure 2-26). The resultant field was only the horizontal component of the field. The system also allows the inclination angle of the artificially generated Earth field to be altered via the coil producing a constant field in the Z direction, to give an inclination factor equal to 44,4μT (red vector Figure 2-26). Therefore, the resulting field in three dimensions (XYZ) was equal to the local Earth magnetic field in Cardiff (i.e. 48,5μT) – and its inclination of 66.342^o downwards. All three coils together, i.e. the horizontal component created by the X and Y coils and the vertical component of the Z coil, formed a resultant field –i.e. an artificially created reproduction of the local magnetic field in Cardiff (green circle in Figure 2-26). This field has been used for all of the experiments except the situation of switching off the field (null field). The generation of the field was fully automated using LabView.

Amongst other features, this software controlled the sequence and the duration of different magnetic field conditions to which the animal (in this case, a pigeon) was exposed as well as enabling the moving magnetic fields to rotate at different velocities. The static field and rotating fields were a reproduction of the local magnetic field in Cardiff, UK (Latitude: 51.487° North, Longitude: 3.181° West) with intensity equal to 48.48 μT and inclination angle of 66.342° downwards.

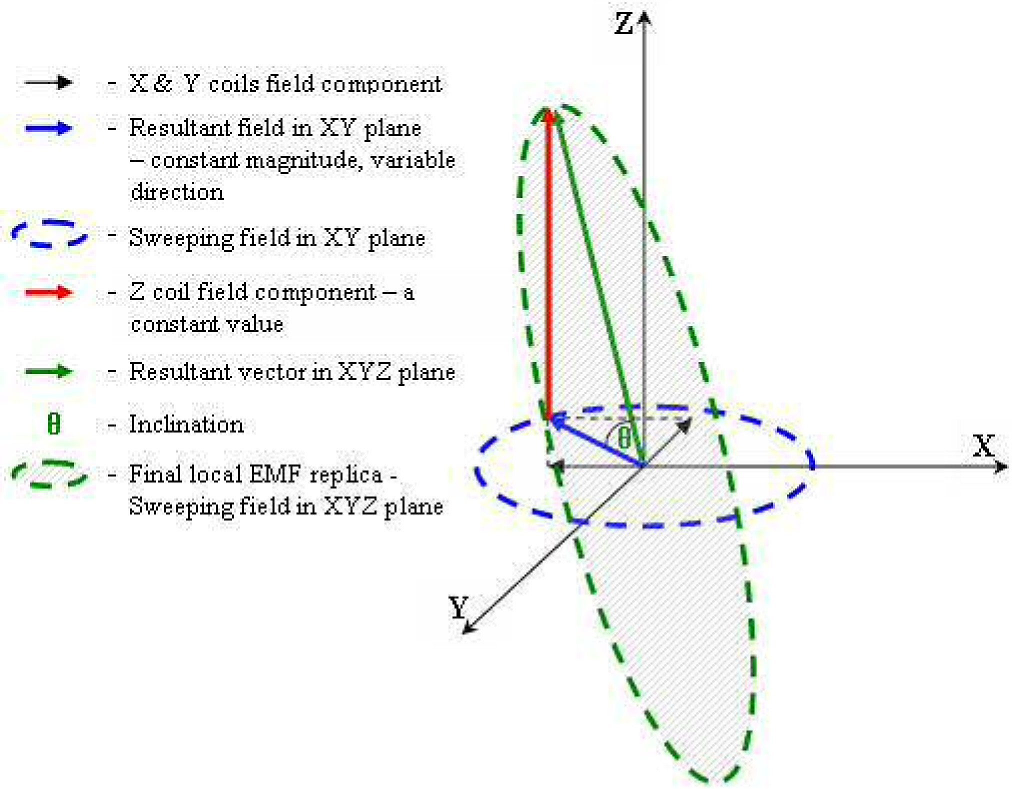


Figure 2-26 The generation of the magnetic field in three dimensions – xyz and the resultant field [114]

As mentioned above, the program saves all the experiments details in .tdms and .xls files. This makes the recording of measurements convenient, as files are named by the date and time of the experiment, and they automatically record all of the information about the magnetic field steps, etc. used. This makes the data very accessible, facilitating and ensuring the accuracy of all subsequent analysis.

The correctness of the generated magnetic field within Helmholtz coils was checked with a Mag-03MC Three-Axis Magnetic Field Sensor. As the sensor has been fixed in the holder (middle of the system) to keep measuring the magnetic field which is inside the coils while the experiment is running to ensure that the magnetic field that the pigeon is experiencing is the desired magnetic field.

2.3.3 Camera Recording System and Illumination

The aim of the project is to concentrate on the pigeon's behavioural and reaction after perception of magnetic field. Thus the head movements were recorded by high quality, monochrome, firewire video camera (Imaging source DMK 21BF04) with a Sony Diagonal 4.5mm (Type 1/4) Progressive Scan CCD Image Sensor [129]. This camera has been preferred as the image sensor has progressive scan feature, which confirms of recognition of all video's frames.

As mentioned previously, the software incorporated a system that activated an overhead camera for recording the bird's behaviour. (LabView software, Create_program.VI). This is based on synchronisation of the camera operation and the generation of magnetic field.

The software is capable of starting to record the head movements along with the start of the magnetic field condition. Also, the software gives the opportunity to the user to visualize the recording area while the user is adjusting the box position; this adjustment period is not included in the recorded video. So, after running the experiment, a fixed time video will be obtained to analyses the bird's responses. These analyses will correlate the specific magnetic field condition at any time during the running of the experiment with the pigeon's response.

The frame rate utilized by this camera was 60 frames per second were found to be accurate. Thus, the orientation of the pigeon's head position could be specified precisely 60 frames per second.

A fibre optic ring light was positioned near the camera to illuminate the bird sufficiently for the head to be seen clearly. The light consists of all colours of spectrum, being a white light. The light condition, luminance, in the box where a pigeon's located during an experiment was approximately 220lux as measured with an ISO-TECH ILM350 luxmeter. It is worth mentioning that the light does not produce any heat in the surrounded area..

2.3.4 Camera Setup and Illumination for Dark Experiments

Some experiments in this research have been achieved in the darkness environment. To achieve total darkens and to allow the camera to record in complete darkness, the infrared range of light in spectrum has been used. As shown in the figure below, the infrared light spectrum is not visible to either the human or bird eye, thus allowing the camera to record a video in this range of bandwidth. In order to check the light sensitivity of the camera, its operational frequency range has been checked, and it was found that its light sensitivity extended to near infrared (IR), i.e. up to 1000nm. Thus, to give the camera the ability to do head tracking in the near IR, an infrared source light has been utilized. Previous research shows that the pigeons are not able to detect the light with a wavelength higher than 700nm [131]. Consequently, an infrared LED was mounted next to the camera and the fibre optic light on the top of the system. The fibre optic light was switched off in the case of darkness experiments in the dark. (See Figure 2-27).

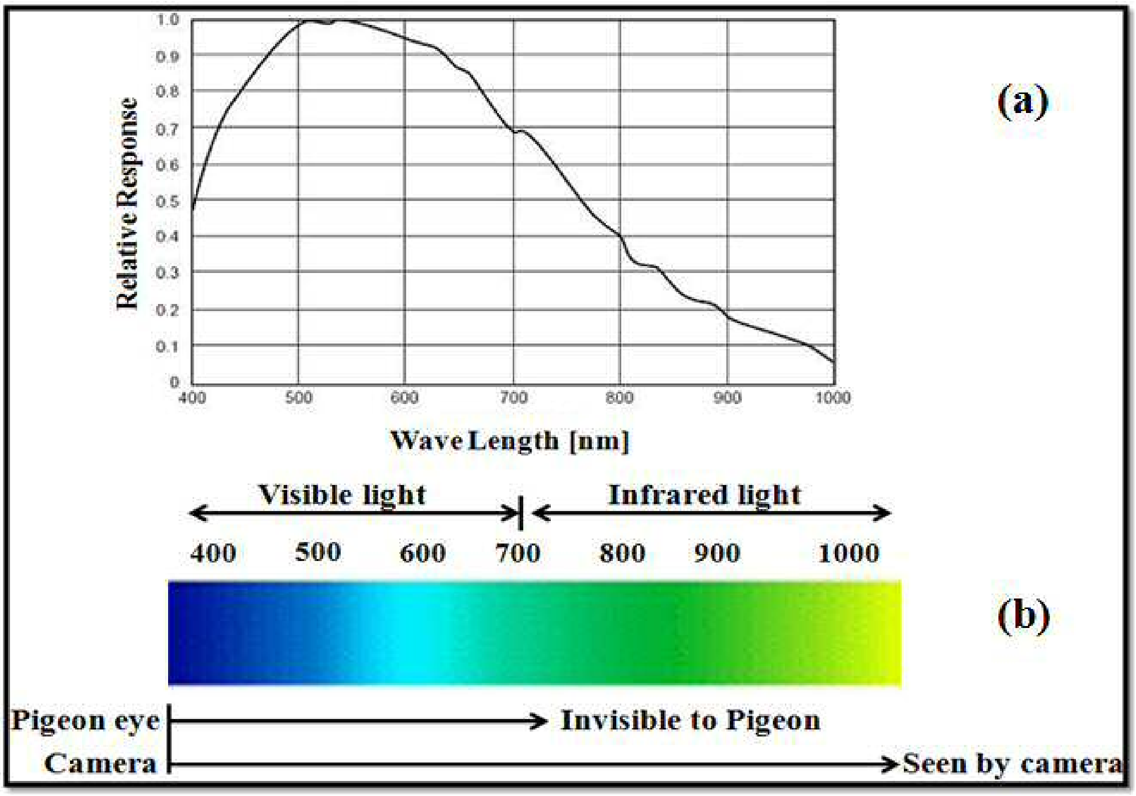


Figure 2-27 (a) Spectral sensitivity characteristics of the Sony image sensor used in the digital camera [129], (b) pigeon sensitivity to the light.

2.3.5 Adaptations in The System for Darkness Experiments

As the camera possesses a near IR operational bandwidth, it has been used to record experiments in the darkness condition. As long as the excitation of an IR light source is available, the camera can record in full darkness. Achieving darkness experiment in this research is more convenient than the previous research which is conducted by Szymon Migalski as the procedure can be done simply by switching off the room light and turning on the IR LED while the experiment is running, which means that the user has access to the IR torch at any time of the experiment. In contrast, in Szymon Migalski there is no remote access to the torch and thus the IR light cannot be switched off unless the lid is taken off. Normal light experiments can be achieved in the presence of room light/day light as well as the IR light as the latter allows recording the video tracking in the darkness without affecting the light condition in any way. Also, as mentioned before the laboratory in this research has blackout installed to the doors and windows.

CHAPTER 3

MAGNETIC FIELD VERIFICATION

3.1 GENERATION OF ARTIFICIAL MAGNETIC FIELD

An artificial 3-D magnetic field, equivalent to the Earth's field, was generated by the 3-D Helmholtz coils using active shielding. The coils are capable of producing static or rotating magnetic fields (rotating in space) as well as the absence of any field (zero magnetic field condition). The artificial magnetic field that is produced in the experiment is the sum of the fields produced by the three coils in X, Y and Z directions, respectively. Coils producing fields in the X and Y directions can generate static or rotating fields in the X-Y plane. The system also allows the inclination angle of the artificially generated Earth field to be altered, giving full control of all of the Earth's field variations that animals may experience (see Figure 3-1).

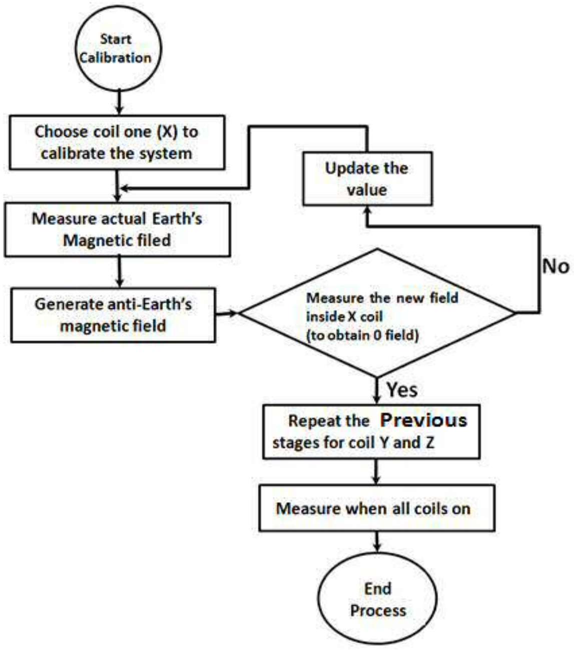


Figure 3-1 Process of producing artificial magnetic field in one of the pairs of this helmholtz coil

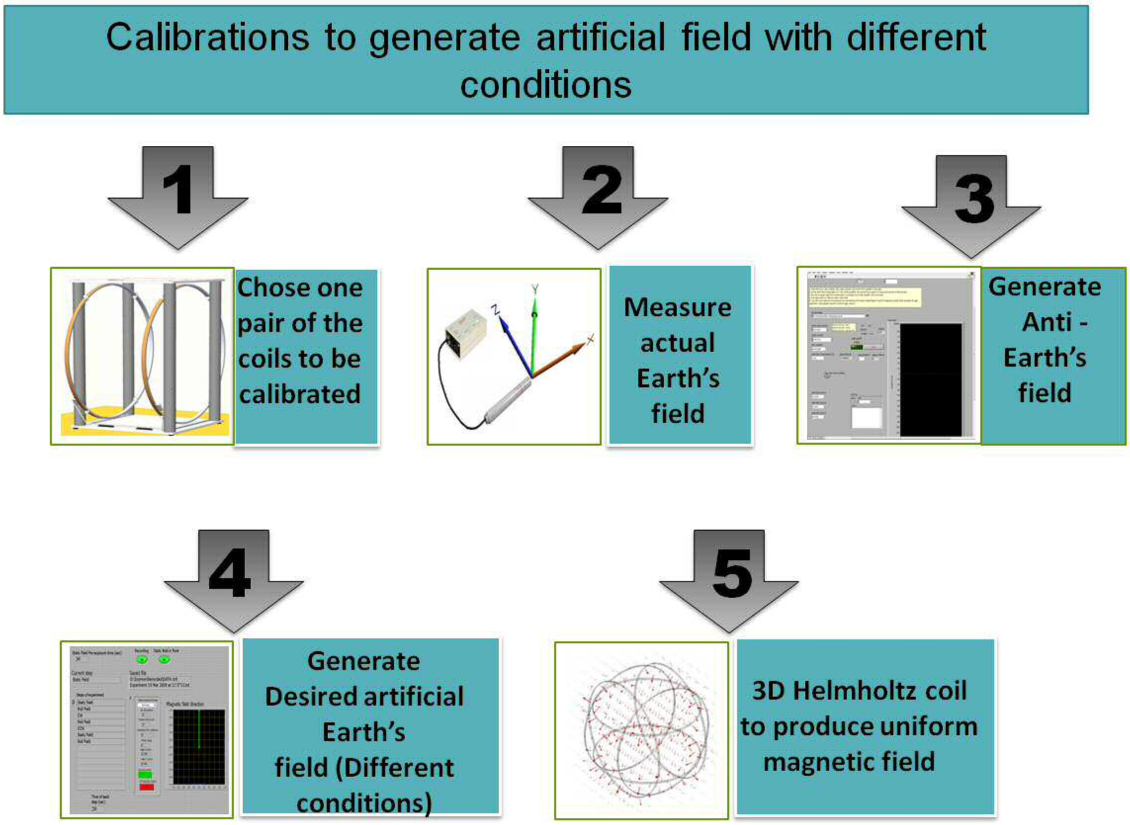


Figure 3-2 Calibration to generate artificial magnetic field

Hence, National Instruments LabView software was used to measure and cancel out the Earth's magnetic field as well as other ambient fields generated by the light source equipment, the camera system, and other field generating sources, see Figure 3-2. This degree of control would not have been possible using passive shielding systems. Figure 3-3 shows some of the simulated Earth field conditions that can be generated, indicating that the system can emulate the magnetic fields in any geographical location. The system can also produce many different magnetic field conditions, including both spatial and temporal control, to facilitate investigations of an animal's responses. These conditions are programmed within LabView for any specific paradigm being tested. The sensor was placed as close to the centre of the Helmholtz coils as possible to verify the precision of the created field's uniformity.

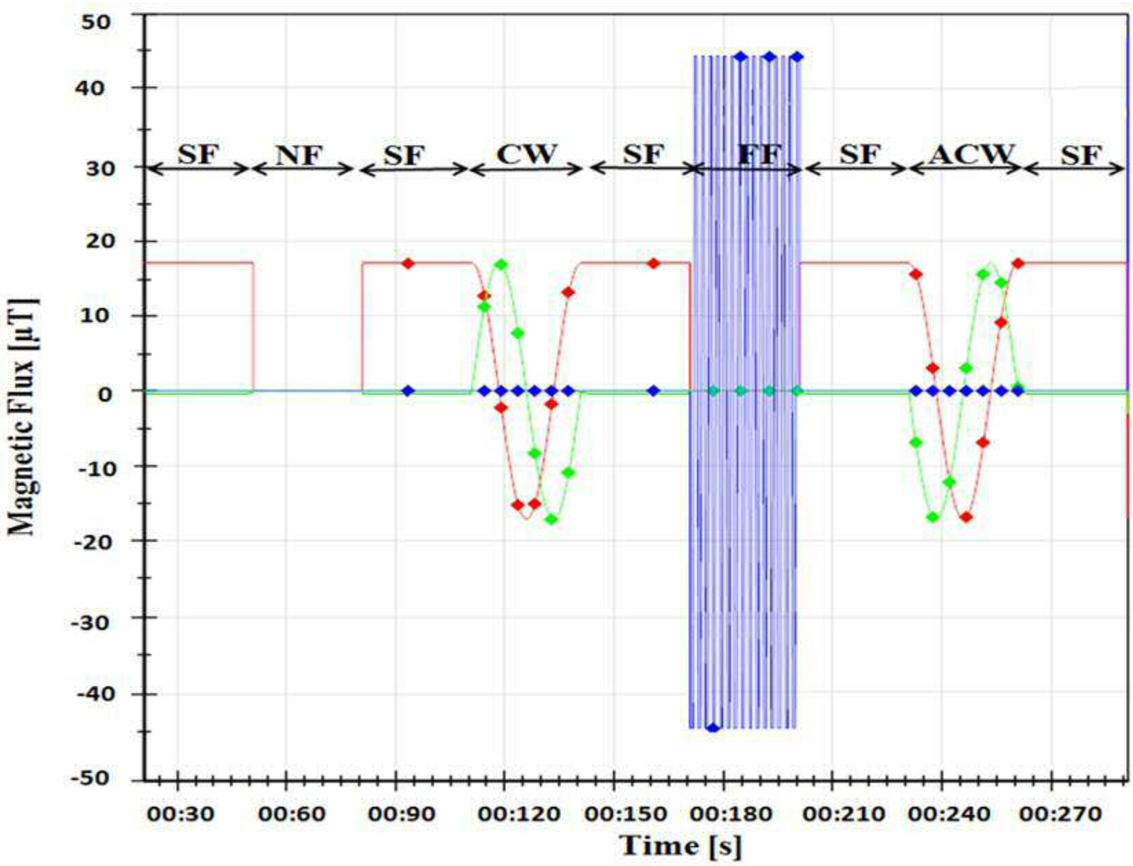


Figure 3-3 Different magnetic field conditions [SF=static field; NF=null field; CW=clockwise rotation; ACW= anticlockwise rotation].

3.1.1 Null Field Condition:

The Earth's magnetic field was completely eliminated by operating a 3-D Helmholtz coils system. The zero field profile was tested by scanning a Mag-03 Three-Axis Magnetic Field Sensor through the coils every 10 cm to provide the field profile in a ±15cm cube. This step is just to verify that the system is capable of cancelling the Earth's field so we ensure that the pigeon can experience different field conditions without any external influences. The zero field was fully achieved in ±15cm, but there is a tiny of field leakage after ±15cm. However, this is negligible for two reasons. First, the pigeon box (region of interest) can be placed within a ±15cm cube where it will certainly be included in the Null field. Second, the field outside ±15cm is very small. As shown below in Figure 3-4 the system can maintain accurate zero fields in a ±15cm cube, which is sufficient to encompass the volume of the animal's box.

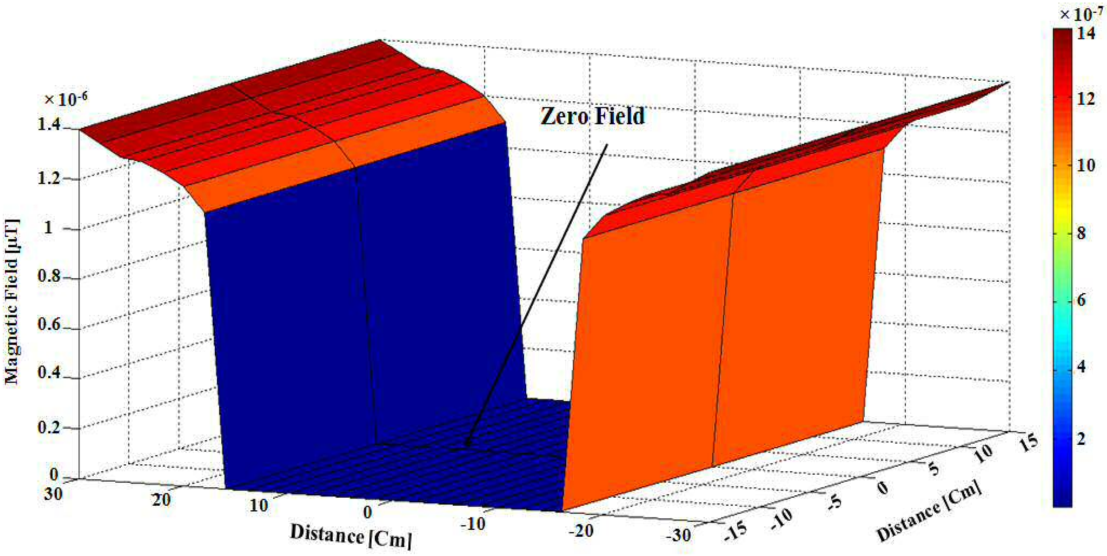


Figure 3-4 Contours of zero magnetic field at the centre of the constructed system

3.1.2 SWEEPING FIELD CONDITION:

This condition is used to rotate the field around the pigeon's head. The generation of the rotation field is based on the following principle: One coil is fed with a signal from the X output and the other from the Y output. Both signals are sinusoidal waveforms, but the Y phase is shifted with respect to X by 90^0. Which means that both X and Y signals were changing in the rotation, but in a manner that the sum of both vectors was always equal to the constant value of the horizontal value of the field 19,5μT.

The system can produce CW and CCW rotation field based on the below equations:

CW:

$$B_{X\,CW} = B_X\,.\,sin\,(\omega.t) \quad \text{Equation 3-1}$$

$$B_{Y\,CW} = B_Y\,.\,cos\,(\omega.t) \quad \text{Equation 3-2}$$

CCW:

$$B_{X\,ACW} = B_X.\,sin\,(\omega.t) \quad \text{Equation 3-3}$$

$$B_{Y\,ACW} = -B_Y.\,cos\,(\omega.t) \quad \text{Equation 3-4}$$

Where: $B_X\,(t)$, $B_Y\,(t)$ - orthogonal components of B; B_X, B_Y - peak value of B orthogonal components, [T]; $\omega = 2 \cdot \pi \cdot f$ - angular frequency, [rad/s]; t - time, [s].

Due to the oblique (inclination factor), the flux density vector trajectory becomes elliptical in shape.

Clockwise and counter clockwise sweeping fields are shown in Figure 3-3 where each one has lasted for 30 seconds for 1 cycle without any elevation.

3.1.3 Constant Field (Static Field):

This condition represents the horizontal intensity of the field (X and Y plane) without taking into account the inclination factor. Five static field conditions are shown in Figure 3-3 above each lasting for 30 seconds. Note that this static field is the control field when the pigeon is expected to behave normally. This field can be presented before any other condition to provide a baseline for any change in the pigeon's behaviour when the field is altered. The axis of the constant field can be controlled to be aligned with any angle required. This can be achieved based on trigonometric functions.

The results indicate that the system is capable of producing and pre-defining a magnetic North in any direction. The shift angles were chosen to verify the reproducibility of any condition.

Two conditions of static field are shown below Figure 3-5, each lasting for two minutes.

i. Constant Field (North Pole 30° shifted):

The system was tested by shielding the natural Earth field and then re-creating a static magnetic field with the North Pole moved by 30°.

ii. Constant Field (North Pole 310° shifted):

The system's capability was further tested by re-creating a constant magnetic field with the North Pole shifted by 310°. The results indicate that the system is capable of producing and pre-defining magnetic North in any direction. The shift angles were chosen to verify the re-reducibility of any condition.

The field values in both cases (30° and 310°) were as expected based on trigonometric functions. For example, the horizontal intensity in Cardiff (X=19.5µT ,Y=-0.73µT) is 19.5µT. When it is shifted by 30°, the individual horizontal intensity components will be changed (X=16.89µT, Y=9.75µT), but the total horizontal intensity will remain the same 19.5μT. A shift of 310° again changes the individual components but the field strength remains constant.

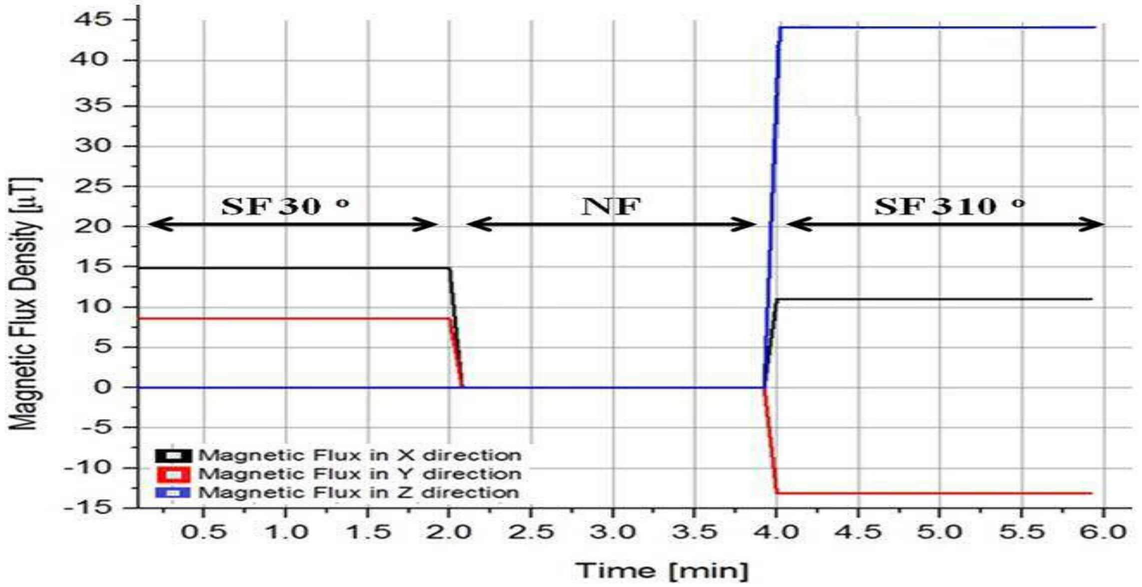


Figure 3-5 Constant field with different field's direction

3.1.4 Flipping Field:

This condition is used to add the inclination factor to the artificial Earth field, i.e. vertical intensity (Z component). In Figure 3-3 fast flipping has been used (1sec per flip) this condition has lasted for 30 second. As shown in the graph, the vertical intensity that the system has produced is 44μT which matches the Earth inclination component.

3.2 Actual Field Measurements:

A MAG-03 MC sensor was placed inside the controlled magnetic environment and moved systematically at 10 cm intervals to check the field uniformity that the system producing.

The starting point was the centre of the system as it is the region of our interest where the pigeon's head will be located.

As the field profile is identical from the centre up to ±15cm, it is clear that the pigeon box, which is ±15cm in size, will be placed in a uniform magnetic field environment, which the system is controlling. The system maintains (48.3 µT), which is identical to Earth magnetic field. See Figure 3-6.

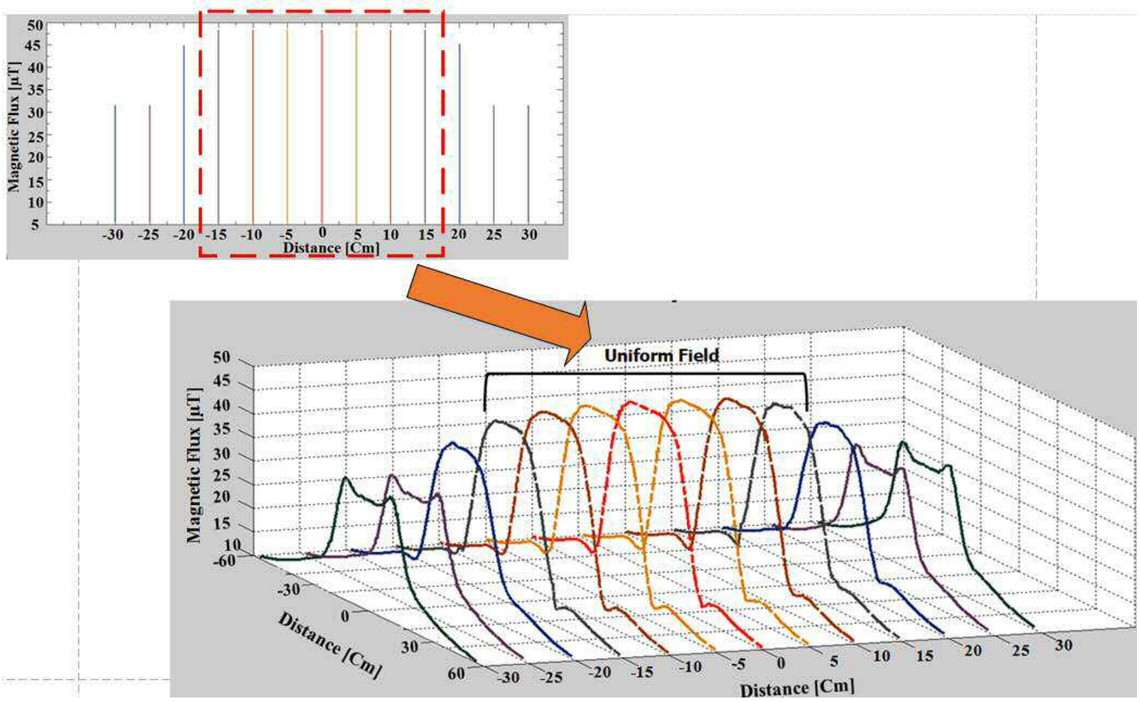


Figure 3-6 Measured magnetic field profile by scanning magnetometer through the centre of the coils. (Through horizontal plane).

To confirm the uniformity of the field, the sensor measurements were shifted 15 cm above and below the horizontal plane through the centre of the field, which contains the bird's head by using the same sensor to record the magnetic flux density at 10 cm intervals.

Also the same kinds of measurements were achieved 15 cm down which confirmed the 15 cm up result, i.e. the field is uniform from centre to ±15cm.

As it is showing in Figure 3-7, at ±40cm and ±50cm (x direction), the field is uniform in the middle (±15cm) but the more the sensor away from the centre the less uniformity which is shown here as sparks due to the influence of each two coils placed 90 degree on the edge of each other. Although the measurement has been taken 15 cm above the central plane, the field is still maintained (48.3 µT), i.e. the same as the Earth field.

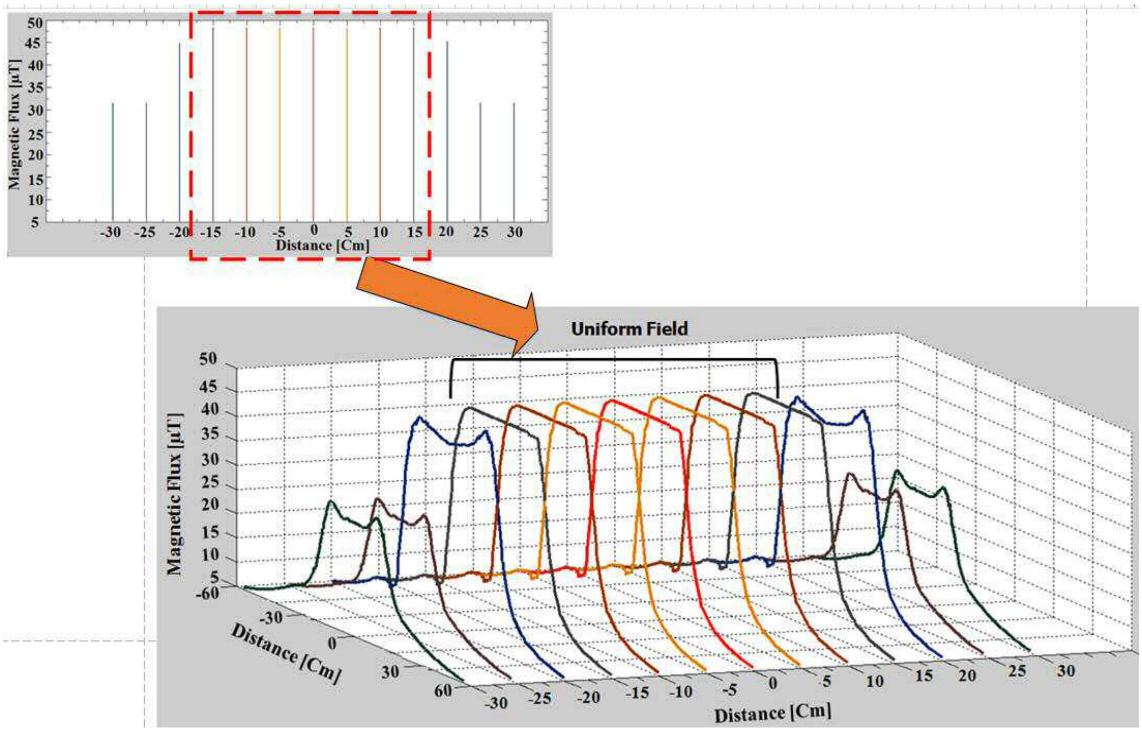


Figure 3-7 Measured magnetic field profile by scanning magnetometer above of the horizontal plane.

The measurement which is below the horizontal plane was shown the same results of the above horizontal plane. Thus, the system is capable of producing a cube of uniform magnetic field, with cube leg length 15 cm.

3.3 Modelling Results:

The system is capable of accurately creating and measuring magnetic fields as low as 10nT in a cube (±15 cm3). COMSOL Multiphysics [198] was employed to identify non-uniform magnetic field regions as well as to determine the coil configuration needed for producing the ideal uniform magnetic field used in this investigation.

The middle square in Figure 3-9 below is the area of interest in which the animal will be placed. Figure 3-8 shows that this area has a uniform magnetic field, the magnetic flux values of which match the Earth field value, which is 48.5 µT. Hence, the coils are producing the same total field intensity as the Earth field in this geographical location. . COMSOL Multiphysics was used to verify the field values along individual components, such as X, Y and Z, which are produced as a result of passing the current through the coils.

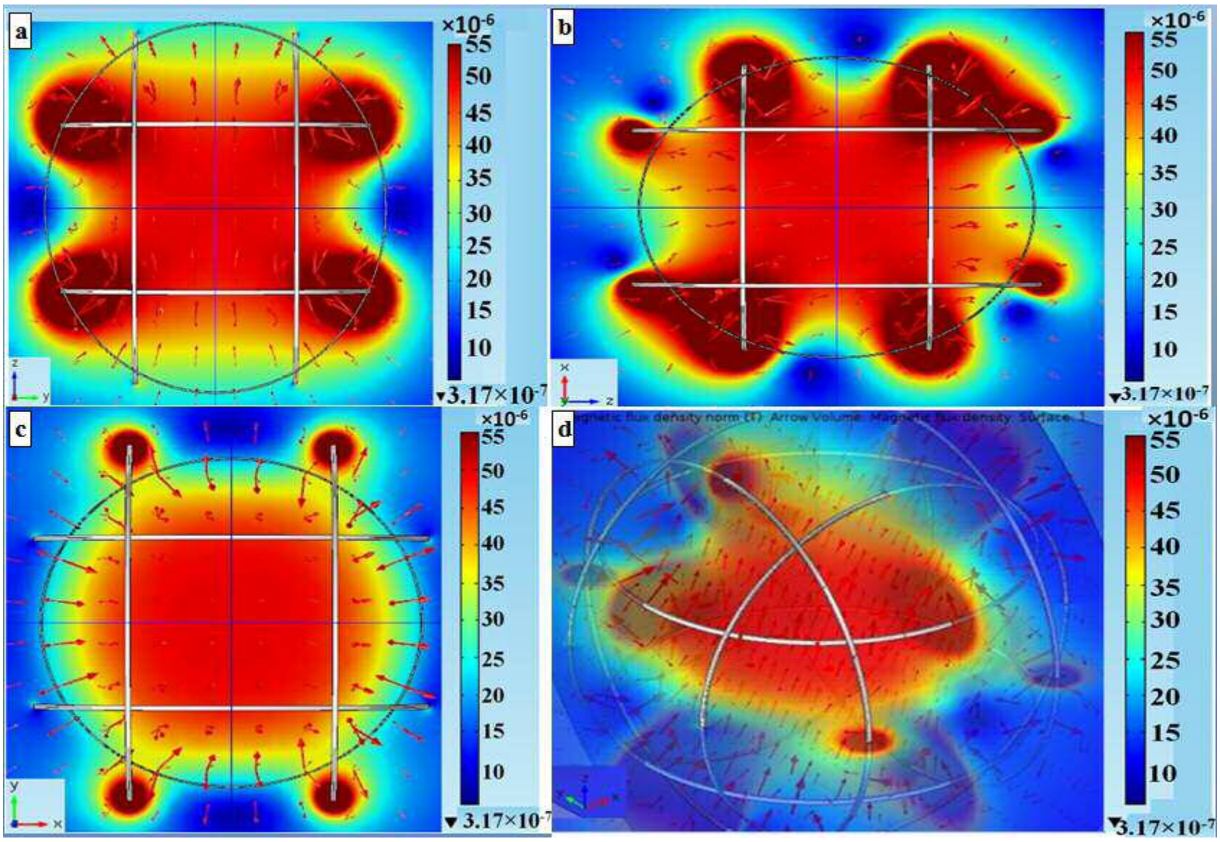


Figure 3-8 (a) A side view of the magnetic field profile along the x coils (b) A side view of the magnetic field profile along the y coils c) A top view of the magnetic field profile along the z coils d) Overview of the magnetic field profile inside 3d Helmholtz coils.

Modelled results indicating the uniformity Magnetic Field Profile through the centre which matches the actual Earth's field intensity in Cardiff UK. See Figure 3-9.

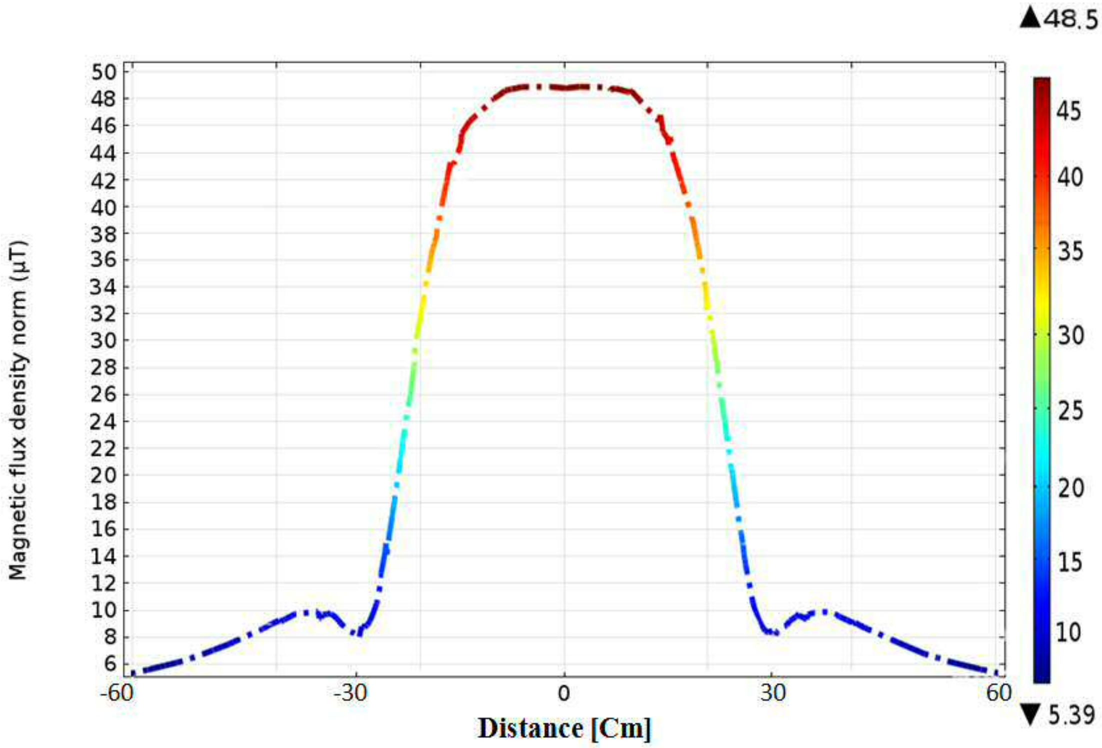


Figure 3-9 Modelled results indicating the uniformity magnetic field profile through the centre which matches the actual Earth's field intensity in Cardiff UK.

Each coil should produce a specific field value to achieve the total field that is same as the Earth's field (48.5 µT). As shown below that the coils in X direction have produced a field which is identical to Cardiff X component. This has been carried out by driving a very low current (0.09Amperes) from the amplifiers through to the coils. See Figures 3-10, 3-11, and 3-12.

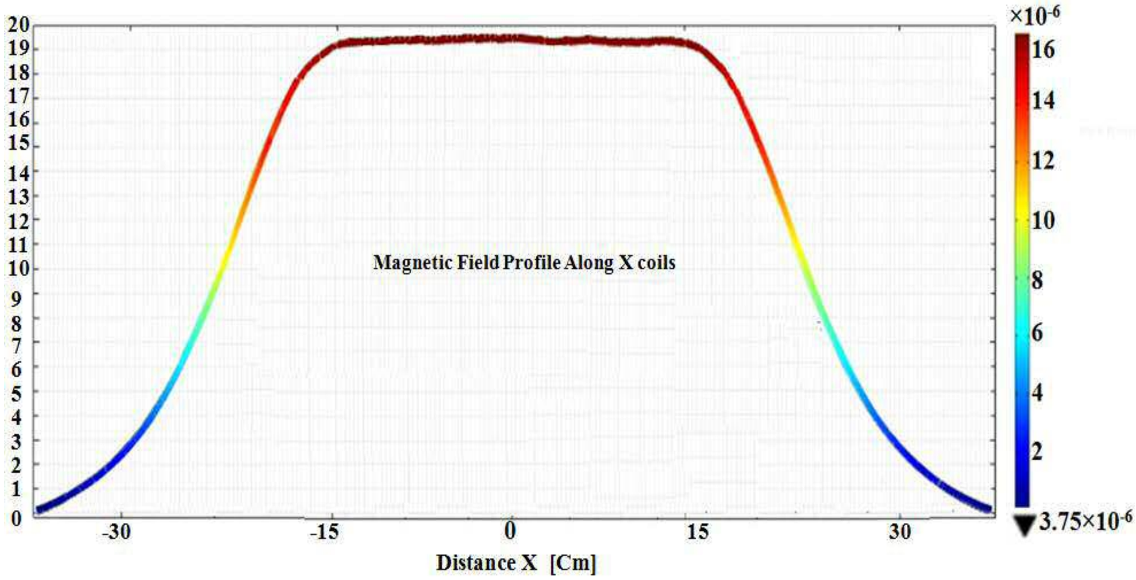


Figure 3-10 Modelled magnetic field profile along the X coils

As shown below that the coils in Y direction have produced a field which is identical to Cardiff Y component. This has been carried out by passing a very small current 0.004 A using COMSOL Multiphysics.

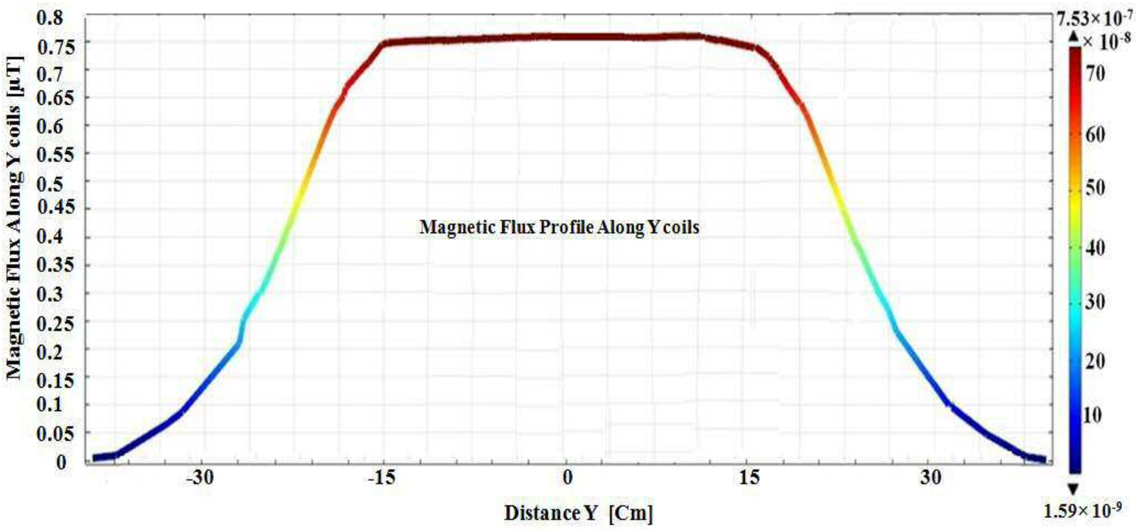


Figure 3-11 Modelled magnetic field profile along the Y coils

As shown below that the coils in Z direction have produced a field which is identical to Cardiff Z component. This has been carried out by passing a very small current 0.22 A. Z coils are fed with higher value of current due to its less number of turns as compared with X and Y coils.

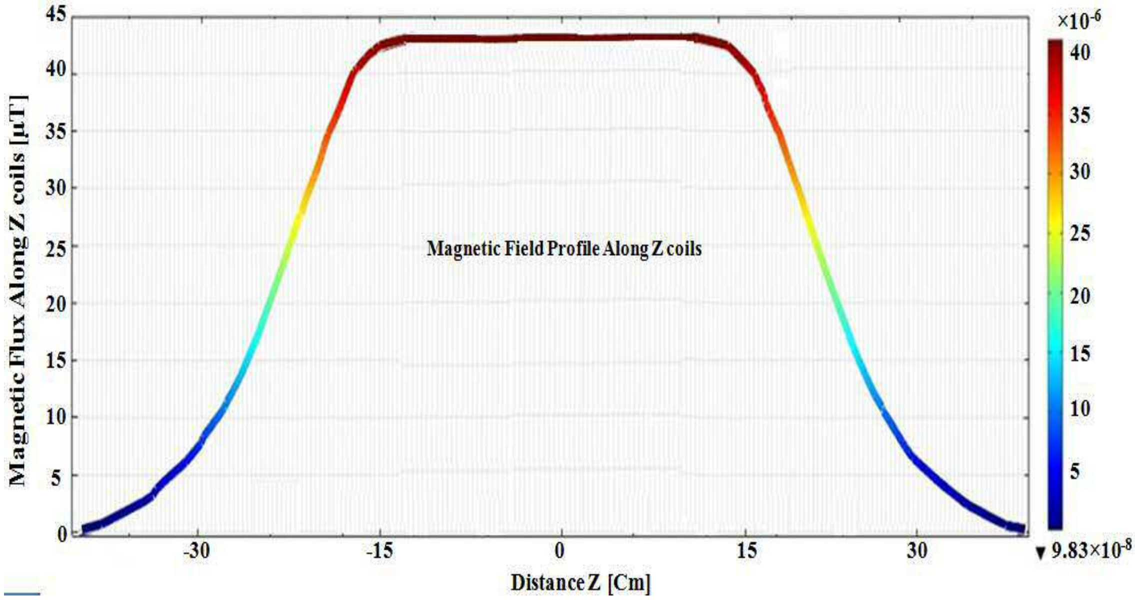


Figure 3-12 Modelled magnetic field profile along the Z coils

The field uniformity has been checked through the coils, this has been achieved by adding multiple planes ±15 cm above and bottom the horizontal plane. Figure 3-13 shows the multiple layers of magnetic field which shows that the system is maintaining 48.5 μT in the middles square of each layer.

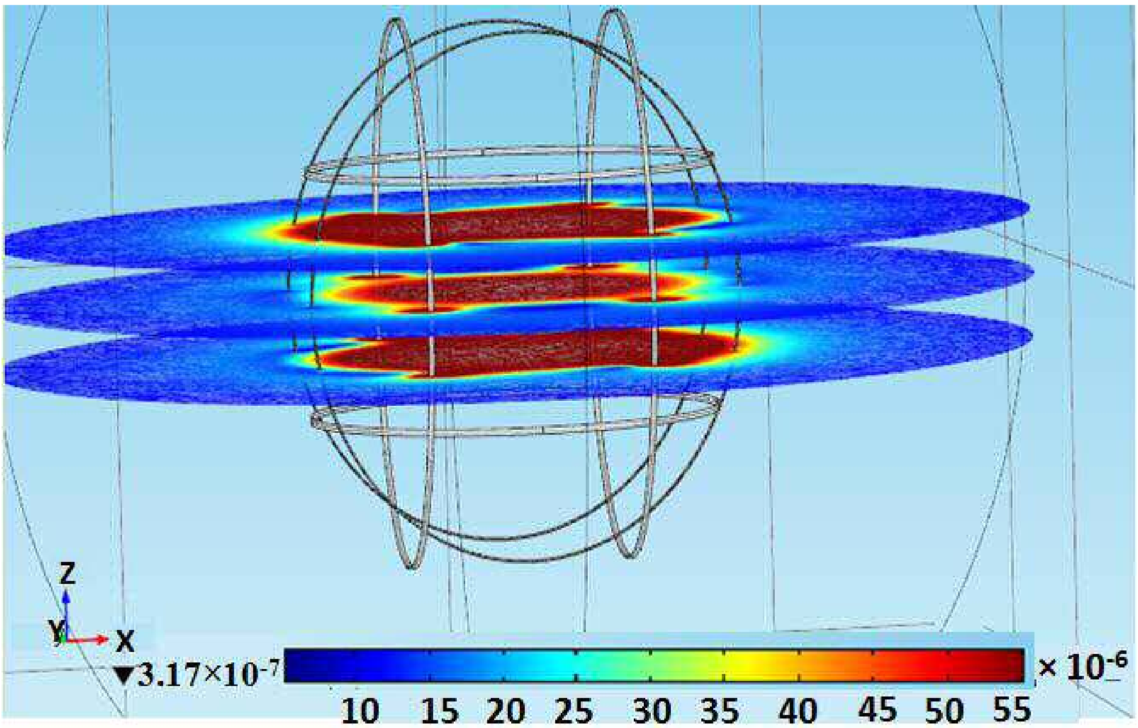


Figure 3-13 Multiple layers above and bottom the horizontal plane

3.4 External Stimuli With Earth Field and Our Field:

As discussed earlier, the aim of this research is to design and develop an instrumentation that can be used to investigate an animal's perception of the Earth's magnetic field. Therefore, the system was constructed with a table in the middle of the three sets of coils so that the head of the animal (i.e. pigeon) will be located exactly at the centre of the uniform field. Head movement analysis is then used to analyze the pigeon's behavioural responses, if any, which depends on the use of an overhead camera to record the angular position of the head at a frequency of 60 Hz or higher while the pigeon is exposed to different controlled artificial magnetic field conditions. A fibre optic ring-light illuminated the bird's head inside the box, and its light source and power supply were covered with a Mu-metal shielding tube to prevent any external magnetic influence within the system. Also, if one or more LCD screens need to be introduced to display visual stimuli to the bird, any impact on the controlled field needs to be determined. For that reason, NI LabView programming was used to measure and subtract from the Earth field as well as taking account of the ambient fields generated by light source, camera system, piezo-electric sensors, nearby electrical and electronic, and other magnetic field generating sources. Hence, this system has advantages over passive shielded systems. Even though the standard deviation and standard error of the repeatability measurements of each source are extremely small (i.e. both much less than 0.001%), the system has the ability to cancel even such external magnetic field disturbances (Figure 3-14.).

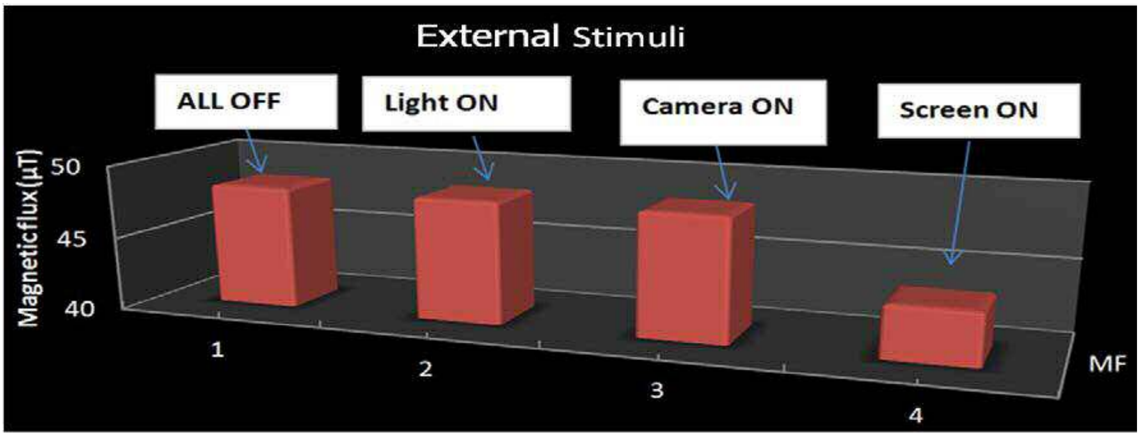


Figure 3-14 Actual measurement of controlled Earth's magnetic field at the centre of the system to verify the influence due to the external stimuli.

CHAPTER 4

EXPERIMENTAL DESIGN AND METHOD

4.1 METHOD PRECEDING EXPOSING THE PIGEON TO MAGNETIC FIELD:

There are few steps should be taken prior to each experiment. Specifically, habituation is one of the essential steps as it offers enough time for the pigeon to habituate to the new environment which surrounding it while the experiment is running. In order to keep the pigeon unstressed and natural, handling the pigeon attentively is necessary.

4.1.1 LODGING AND TRANSPORTATION OF THE PIGEONS

All pigeons were kept in the animal facilities of Cardiff University (Redwood) building. Each pair has been kept in standard cages. Each cage has numbered according to the each pigeon's ring. Qualified staff has taken the responsibility of providing food, water and health check every morning. Also they have ensured to keep the temperature and light in the facility is convenient for the pigeons. To start with each experiment, each pair of pigeon was removed from their housing cages and transported to the laboratory ,which is in the same building and same floor, in a spacious ,and well ventilated carrier (pet taxi) Figure 4-1 . Because the lab has been divided into two sections by using wall divider. So the system has been placed in one side , also the user and computer in the other side. The bird not being used was kept in the carrier in the user side. Ad libitum water was still available to the bird not being used, but food was not. The presence of the pigeon in the carrier box while the user preparing the program condition and steps is considered a good duration of the pigeon to habituate.

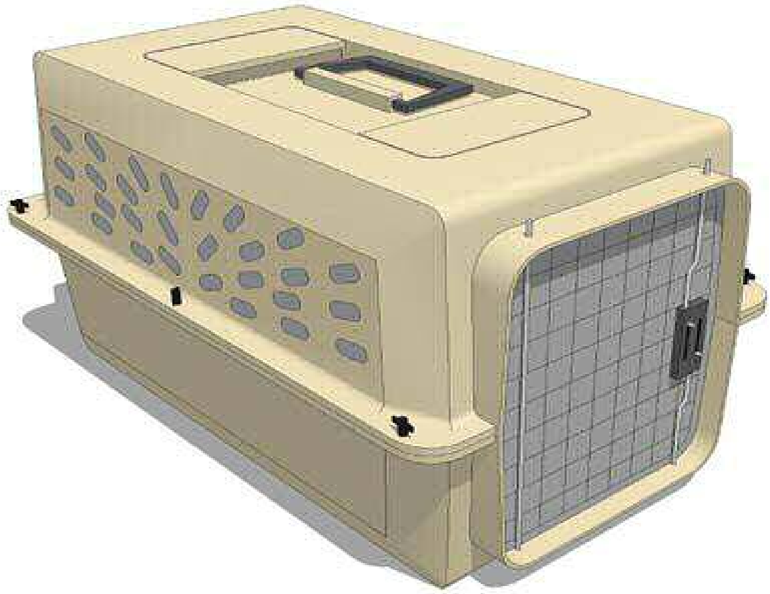


Figure 4-1 Portable pet taxi

4.1.2 Preparing the Pigeons for the experiments

The laboratory in which all experiments were carried out was located in the isolated area of the building. As mentioned above that a standard divider has been added to the lab where the user, Controlling computer and the unused pigeon are placed in one side and the system with experimental pigeon are located on the other side. This divider was used while the experiment is running in order to prevent the computer light to interfere with the experiment condition. Also it minimizes any noise that might occur in the user side. Pigeon were monitored from the computer while they are in the experiment experiencing the magnetic field. Locating the pigeon in the lab will habituate the pigeon to the lab environment. The laboratory has blackout installed to doors and windows allowing us to carry out experiments in darkness, but also to accurately control lighting for experiments generally using a fibre optic lamp. Before each experiment, a head marker has been placed at the top of each pigeon's head. The marker used was white foam rectangle with two black marker pen lines. The marker was asymmetrical to allow better recognition by the analysis software. Head marks were also altered slightly by painting them with matt ‘Tipex’ to avoid reflections, which had obscured some frames in the previous video analysis. After attaching the head marker to pigeon's head, the bird was placed in either transparent of non transparent Perspex experimental box with its lid

(See section 2.3.1.1 point number 8 for more details about the experimental box). The pigeon were left for few minutes habituating to the box environment. Black coloured foam block has been placed in the box to stop the pigeon from moving forward and resting against the front wall of the box and to ensure that the pigeon's head aligned with box hole and subsequently the camera can record all the head movements. First experiment has been occurred with non transparent Perspex box to limit the area that could be viewed by the pigeon, but it was noticeable that the pigeon intend to look out from the chimney as it is only the exist for them this might affect the pigeon behaviour and response to Earth magnetic field. So it was worth to place the pigeon in transparent box which will give the pigeon opportunity to view the outer world normally as it is in the cage or transport box.

4.2 Arrangements and Method During the Experiments

Keeping the pigeon unstressed and handling the pigeon as well as the pigeon box carefully is the essential arrangements to start the experiments with. By doing that, the pigeon will attend the Earth's magnetic field in the experiment. The same procedure should be followed in each experiment.

4.2.1 Placing a Pigeon Inside the 3D Helmholtz Coils

After the few minutes of habituation, the pigeon's box then handled carefully to be inserted in the coils and placed on the top of the mini-table which will make sure that the pigeon's head is experiencing the uniform magnetic field. In order to keep the pigeon calm to experience the magnetic field normally, the sound disturbance has been controlled in several ways. Firstly the lab is located in the isolated area of the building and signs were then placed in the surrounding corridors to ask other facility users to keep noise to a minimum. Secondly, after placing the dividers, all supplementary equipment, such as controlling computer, amplifiers are isolated on the other side to ensure that the pigeon is the only object in the room which can move during the experiment. Lastly a white noise file was then played through speakers in the same room 65dB to mask any sound pollution which will minimize any pigeon's distraction. In order to ensure that the pigeon's head is recordable in most experimental duration, additional cube has been added as some pigeon curiosity intending to raise their heads up. So placing that cube will keep the pigeon in region of interest where the camera can track the head movements clearly.

4.2.2 Starting the Magnetic Field Experiments

As the system of Helmholtz coils is quiet big so it cannot be moved easily, this is one of the advantages. Because no need to align the coils so that the generated magnetic North coincided with the natural magnetic North before each experiment by using a compass. After deciding the scenario of the magnetic field that the pigeon will experience, including the sequence of the magnetic field and the duration of each condition, which can be achieved in Createprogram.vi program (for details see section). During the habituation period of leaving the pigeon in the carrier box, a light meter was then placed inside the Perspex box inside the coils, and the fibre optic ring was adjusted to give a reading of 220 lux from the light meter. A sound meter then was placed into the Perspex box and the speakers were adjusted to 65dB. Then the camera focus has been adjusted. All blinds and doors were fully shut. Pigeon then handled gently and placed in the Perspex box then inserted at the top of the mini-table in the middle of the Helmholtz coils. The lab divider then placed. These arrangements will ensure that the pigeon will be exposed to the artificial Earth's magnetic field which will be run and controlled via Createprogram.vi.

4.2.3 Mask the Extraneous Auditory Stimuli

4.2.3.1 What do Birds Hear?

Birds are exceptionally vocal. Their vocaliztion range from very simple calls to amazingly changed melodies, they communicate by numerous vocalizations, which are frequently likewise wonderful to our ears. Song of the bird can serve to perceive other birds individually and should also be understood well, like human speech. To numerous birds, the sense of hearing has special meaning even in beyond communications. To discover their way dark caves, some birds use echolocation [56]. However, there are large numbers of myths about the birds' hearing. The assertion that ultrasound, frequencies too high for human beings to hear, can be heard by the birds is perhaps the most notorious one. Basing on broadcasting ultrasound, bird scare devices can scare a lot of mammals, but birds are unable to hear them. However, ultrasonic components can also be the part of the songs of some birds [132], but they do not hear those themselves. Nearly lower than hearing of a human, hearing of an avian normally stays restricted to below 10kHz (Shown in Figure 4-2).

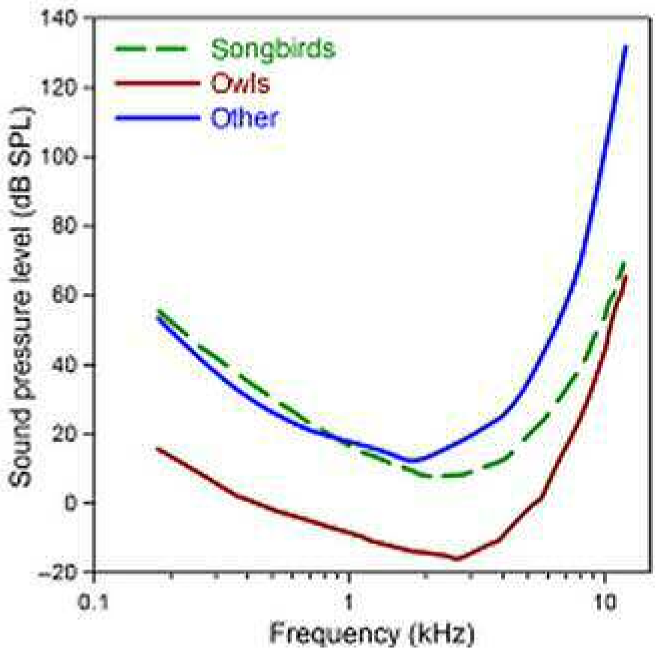


Figure 4-2 Median behavioural audiograms for three groups of birds [56]

Small birds, at high frequencies, hear better over bigger birds and vice versa [133, 134]. Including humans, birds do just as well as a normal mammal in numerous standard behavioural tests related to hearing, such as intensity or frequency discrimination [133]. The birds often perform better than mammals on different measures of sensual resolution, particularly in the tasks requiring perception of rapidly changing fine structures [133]. Localization of sounds is more of a difficulty for birds due to the fact that with limited upper hearing range, their normally small sized head allow only small interaural cues [135,136]. Birds, as a rule, in comparison with any mammal of similar size can localize sounds in azimuth. But, birds cannot localize in elevation. Clearly, hearing has vital part in lives of birds and their auditory world is rich. A summary which describes the sensory aspects of hearing in birds will be included in this research.

4.2.3.2 Cochlear Specializations: Auditory Foveae, Infrasound Hearing

It is known that the basilar papilla is tonotopically sorted out. Low frequencies are most sensitively responded by apical regions, and towards the basal end this characteristic frequency increases step by step. A near-logarithmic function—in which, a more or less equivalent length of papilla is corresponded to every duplication of frequency (equal to one octave), describes his tonotopic map well in a normal avian basilar papilla (Figure 4-3 below) [137]. Nonetheless, this rule has interesting exceptions as well. Regions of enhanced frequency representation, in analogy to visual foveae, have been termed *auditory foveae*, where, along the basilar papilla, there are frequencies that possess disproportionally more space.

It is found in pigeons that they have an amazing specialization for very low-frequency hearing. It is known for some time that pigeons are more perceptive to infrasound (frequencies lower than those which are audible to humans i.e., <15 Hz), from behavioural experiments [138].

Root of this sensitivity to hair cells was traced by Scherm and Klinke, which was located in the pigeon basilar papilla in extreme apical-abneural areas [139]. Normal acoustic frequencies, connecting to these hair cells, were not responded by the afferent fibers, but responses, at similar levels to the behavioural thresholds has been previously shown to infrasound [140]. Conversely to the foveae of high-frequency, the receptive region of infrasound seems to coincide with basilar papilla over the most apical 1mm, along the side of a conventional representation of logarithmic frequency (Figure 4-3 below green) [141]. For behavioural significance of infrasound listening remains obscure. The conclusive evidence is still lacking despite the fact that it is proposed that pigeons utilize it as a navigational cue [138,142]. To pigeons, infrasound sensitivity might likewise not be unique [56].

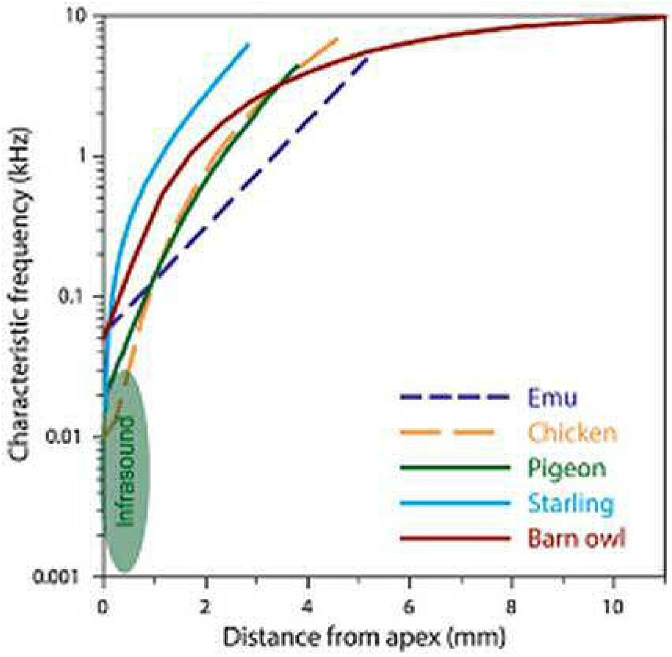


Figure 4-3 Tronotopic frequency representation along the avian basilar papilla [56]

4.2.3.3 Controlling the Noise through the Experiment

Careful control of sound was maintained throughout the experiment. However, some small noises made in the neighbouring laboratories could not be avoided entirely, and may account for some behaviour. In this project any loud noises heard by the observer caused the video to be aborted and smaller noises were overcome by playing white noise throughout. White noise is a random signal with a constant power spectral density. The relevant range of the white noise is between 20 to 20,000 Hz. So the white noise was played before the habituation period i.e. before the pigeon arrives to the laboratory until the pigeon were taken back to the cages. This will ensure that the pigeon will be isolated from any extraneous auditory effect from outside the lab. Some supplementary equipment such as the amplifiers is producing some acoustic noise. Therefore, has been located at the side which is different from the coils side then close the standard dividers. These dividers will minimize any the amplifiers noise which is low volume sound and remained constant regardless of the supplied signal switching. Consequently, the amplifiers will not affect the pigeon perception. Surely, if it can be heard by the pigeon, it simply will contribute to the white noise and together will isolate the pigeon from any external effect.

In the previous research (Szymon Migalski) [114], relay (switch) was used to generate the inclination (field through Z coils). This relay was responsible of producing a noise while switching them on and off. But the white noise was more than enough for masking that noise. In this research, it is no longer to worry about the relays as it has been substituted by using power amplifier as in the X and Y directions.

A sound proof room could overcome this in the future if it was found to be necessary.

4.2.3.4 Conditions of the Pigeon While the Experiment is Running:

Passive shielding might have lack in the ventilation factor which in turns might affect the pigeon perception and stress the pigeon. So this research has improved the environment for the pigeon by using active shielding as it is more convenient and ventilated continuously while the experiment is running. Also the pigeon box has few of holes which ensure that the pigeon is ventilated. Also the top hole of the pigeon box (which gives the camera access to track the head movements) is another source to keep the pigeon fresh and well ventilated while it is in the box. However, most of the experiments have not exceed more than 3 minutes which is not long time for them.

In this research, we do not need to worry about the heating factor because the coils are much bigger than the previous research. Consequently, the current value is much less which will not generate any heating in the pigeon's surrounded area. The maximum current has been pass through the coils was 0.22 A thus the wiring during the experiment remained cool. The laboratory where the experiments are carried out is well ventilated with fresh air by the air-condition system which keeps the lab temperature constant at$22^{o}C$. The air-condition is operating without any generation of a noise that might be audible to the pigeon.

4.2.3.5 PROJECT LICENCE

Under the Animals (Scientific Procedures) Act 1986, project licence has been granted by the Secretary of State which specifies a programme of work. Although, the Home Office licence should be used by those people who carry out a scientific procedure that might cause PSDLH (Pain, Suffering, Distress and Last harming), which is not the case in this research. Because handling and experimenting the pigeons were not in any way PSDLH to them. In addition to that Home Office licence has been obtained in order to covering some protocols that have been carried out in this research. These protocols have been covered in Chapter 5 and Chapter 6. So the project licence holder is responsible for the overall implementation of the programme of work and for ensuring that the programme is carried out in compliance with the conditions of the licence.

4.3 TRACKING THE HEAD MOVEMENTS SOFTWARE

Behavioural experiments often require continuous monitoring of the head movements and position in response to various stimuli. In most of the low cost system for video recording has been used which has been developed by Tomasz Kutrowski [143].

The instrumentation described in this section developed by Tomasz Kutrowski. It consists of the following pieces of equipment:

- An average specification PC (CPU:i5, RAM:8GB) with an SSD to allow saving of video data at speeds up to 120 fps;
- Camera 1, FireWire CCD Monochrome DMK 21BF04 (TheImagingSource) capable of maximum 60 fps, lens:M0814-MP;
- Camera 2, USB3.0 CCD Monochrome DMK 23U618 (TheImagingSource) maximum 120 fps, lens:YV3.3x15SA-2 supplied by Alrad Instruments, Newbury, UK.

Both cameras allow recording at night under infrared illumination. An infrared LED torch emitting 850nmwavelength light was used in the experiments conducted in darkness.

Therefore, the above instrumentation based on the visual programming language, LabView (version 2012SP1) of National Instruments (NI) together with the NI Vision package has been utilized in our investigations. Essentially, its convenience to be used with our test system setup. In this project, frame by frame recording has been achieved with frame rate of 60 frames per second, which ends up with lots of number of frames; manual data extraction can be very time-consuming. However, recording of rotational movements of a bird's head is required for the sake of the analysis, under both light and dark (i.e. infrared) conditions.

Independent of the Camera.vi used to control video recording, the Extract_data.vi was developed for extracting the animal's activity from the video data. On the execution of this VI, the user is required to select the video file that is to be analysed.

The main feature of the program:

- The user can establish the number of the start and finish i.e. the total number of the frames that is desired for analysis.
- The user can select the desired template. However, the program displays the first frame (Figure 4-4). Any frame of the video can be viewed by manipulation of the 'Select frame' control. As can be seen in Figure 4-5, it is necessary to load or define a template image in order to perform an analysis. A template is an idealized representation of the object of interest (head marking in our case). Head marking has been used to provide a suitable target for the image recognition algorithm, a strip of adhesive light foam with an asymmetric marking is placed on top of the bird's head. This allows the search algorithm to detect the two-dimensional orientation of the bird's head in each successive frame of the video. The size and weight of the strip are such that it has no effect on the bird's head movement. The asymmetry of the marking is vital to avoid the potential ambiguity inherent in 180º rotated head positions. A guide on appropriate markings can be found in [144]
- The software allows a template to be tested on any single frame and an adjustment of crucial parameters can then be made, if required. Once the template and settings are deemed appropriate, the user initiates batch processing of all frames, or a selected range of frames. Upon completion, the extracted head angle data is automatically saved to a file.

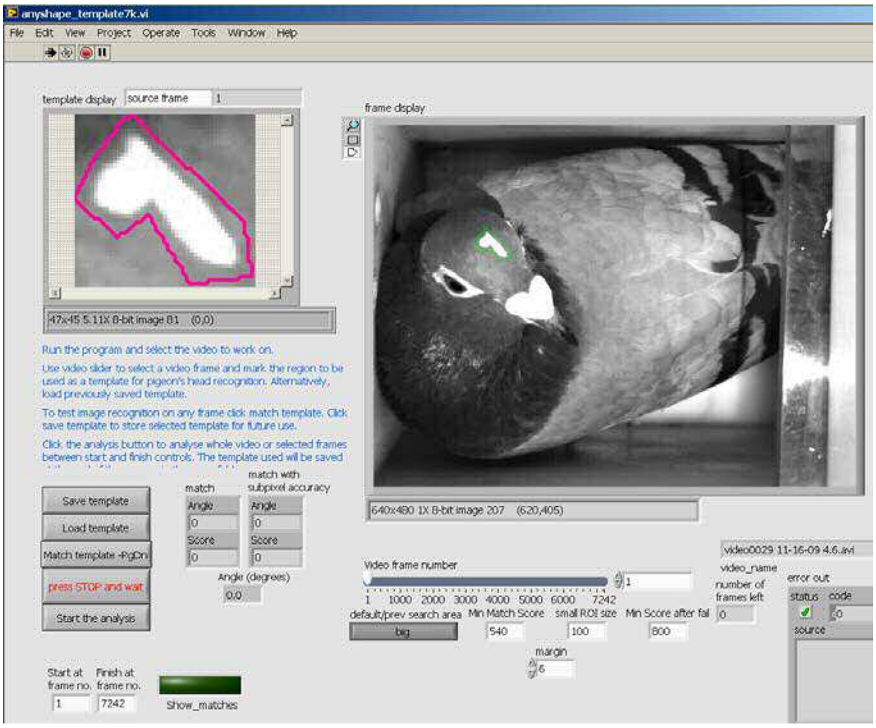


Figure 4-4 User interface of the data extract_data.vi application.

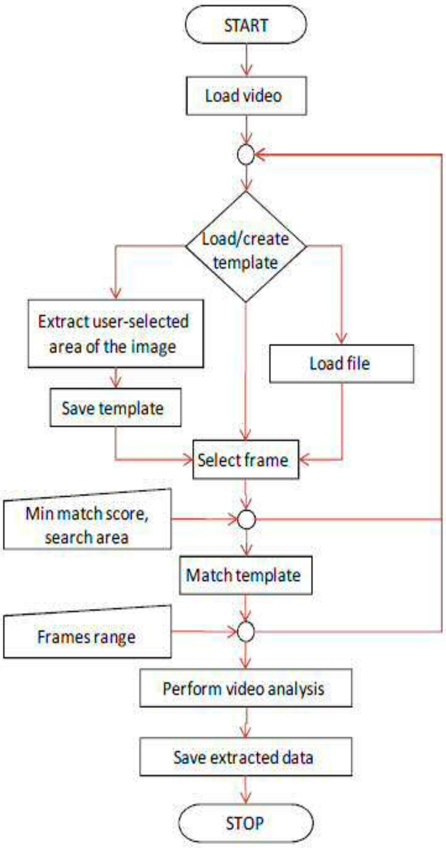


Figure 4-5 Simplified flowchart of the extract data.vi software.[143]

CHAPTER 5

EXPOSING PIGEONS TO EARTH'S MAGNETIC FIELDS

5.1 INTRODUCTION

The experimental results from exposing the pigeon to different field conditions are going to be discussed in this chapter. Individual pigeons were exposed to changes in Earth-like magnetic field conditions (i.e. moving field, static field) and the absence of any field. Pigeon's head movements represent a reference for studying the pigeon's behavioural reaction to the external magnetic field. The results reveal a correlation between these head movements with the induced changes in the magnetic field environment surrounding the birds. All experiments have been conducted in the light due to the possibility of the involvement of vision in magnetoreception. Because the head is considered to be the location of the magnetic sensor in the homing pigeons, it was ensured that its head was located in the canter of the system where the manipulated magnetic field is uniform. So, in this chapter, head movement analysis was presented to provide a complete representation of the pigeon's activity. Detailed analysis of the head movements cannot, of course, precisely locate magnetic sensor to the pigeon's eye. This does not mean that the pigeon's eye has the magnetic sensor to percept the alterations in magnetic fields.

The basis for choosing particular pigeon to include in the behavioural experiment will be explained below.

5.2 Types of Eye Movements and Their Functions

There are five fundamental types of eye movement, which can be assigned to one of two functional categories: (i) those that shift the direction of the gaze, and (ii) those that stabilize the gaze. Shifts of eye position are required in order to foveate new targets, and to follow any foveated targets that are moving within the visual space. To maintain foveation, stabilising eye movements are actioned when either the head moves or there are large-scale movements of the overall field of view. Saccades, vergence, and smooth pursuit movements all shift the direction of the gaze, while optokinetic and vestibulo-ocular movements stabilise the gaze. Saccades are quick, ballistic eye movements that sharply alter the direction of fixation. Their amplitude can range from small movements up to very large movements. Saccades can be implemented voluntarily, but also can occur reflexively when the eyes are open, even when eyes are fixated on a target [145].

Figure 5-1 shows a saccadic eye movement over time. After the initial introduction of a target for a saccade (in this example the movement of a pre-fixated target). The eye movement begins after around 200 milliseconds. Within this delay period, the target's position is computed with respect to the fovea (i.e. how far the eye is required to move).

The difference between the intended position and the initial position determines a motor command that activates extraocular muscles in order to rotate the eyes the appropriate distance in the appropriate direction. Saccadic eye movements are said to be ballistic due to the saccade generation system usually not responding to additional changes in the position of the target during time taken to execute the eye movement. If the target does indeed move again during that time (which is around of 15-100ms), the saccade movement will miss the target, meaning that a second saccade is required to correct the error [145].

Pigeons, just like other animals, try to maintain a picture of objects in a steady location on their retina when tracking a moving target. However, their eye and head geometry leaves little space for eye movements, so they tend to move their entire head rather than just their eyes. This explains the peculiar head movements that many birds exhibit while walking [146].

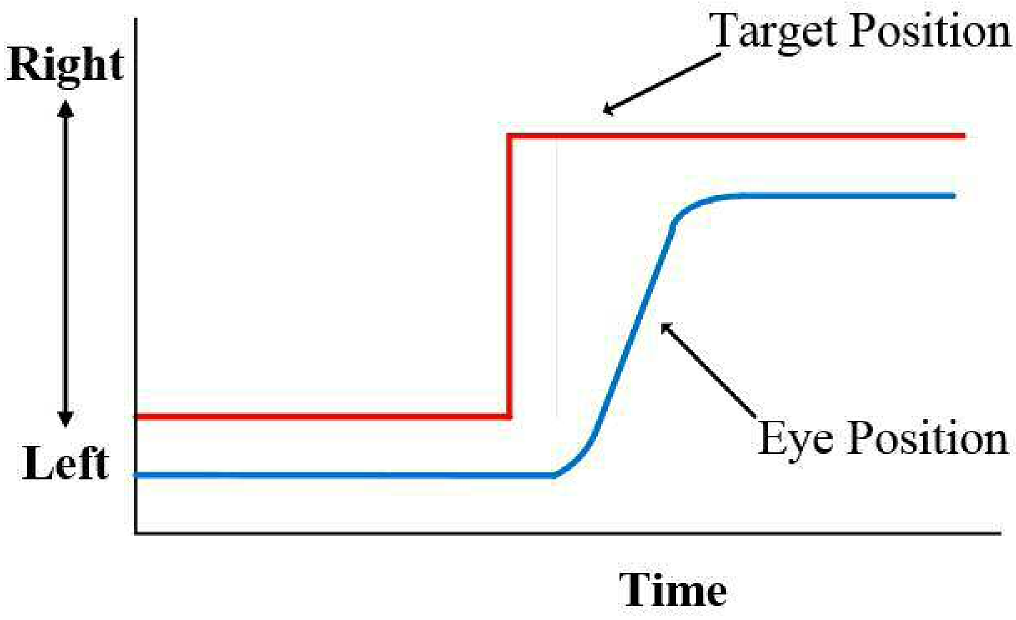


Figure 5-1 Metrics of a saccadic eye movement. the red line here indicates the position of a fixation target, while the blue line indicates the position of the fovea. when the target moves quickly to the right, there is a temporal delay of around 200 ms prior to the eye beginning to move toward the new target position . Adapted from [145].

5.3 Using sequence of Variety of Magnetic Field

As mentioned previously in this project pigeons were used, as they are known to use the Earth's magnetic field to navigate, are widely available, can be relied upon to respond in all seasons, and have been the subject of a large body of research using them as a test subject [147, 148]. It was important as a part of the data analysis to link the pigeon's response to the specific timing in changes in the magnetic field , the long term goal is to provide a better understanding of magnetorecption in the Homing Pigeons.

5.4 Experimental Design

The experiments conducted in this study have been carried out in collaboration between the School of Engineering and the School of Optometry and Vision Sciences in Cardiff University supported by Leverhulme Trust funding (RPG-146). The research encompasses engineering aspects as well as behavioural science in researching "Magnetoreception in Pigeons". In this study I have concentrated on the engineering aspects of this research. The experimental data presented here are of my own research data and analysis. However, there is another PhD student registered in the School of Optometry and Vision Sciences who concentrated on behavioural analysis through in depth statistical analysis.

5.4.1 Experimental Pigeons

The first experimental design was performed in two conditions a set of experiments performed during the daytime, which is between (10:00 till 16:00) as the sunset starts, and a set of experiments carried out in the night time between (20:00 till 00:00). Monitoring the pigeon's magnetic perception will give a more comprehensive picture of the pigeon's response to magnetic field and the influence of time of day.

Six experimental scenarios with two control experiments were carried out for 12 days and 12 nights, see Figure 5-2. Each day, 10 pigeons were run in two consecutive trials. Each pigeon received 1 control trial and an experimental trial each day alternating between control 1 and control 2 and whether the experimental condition came 1st or 2nd. Pigeon order was alternated every day. Also they were kept in pairs during the first habituation period, i.e. while they were in the carry box during the experiment preparation. Two pigeons were excluded from the daytime experiments because of their hyperactive reaction. Thus, they were replaced by another pair. In this thesis, morning set have been used and analysis. However, a comparison between evening and morning set has been demonstrated in John Barnes thesis. In this thesis, results of daytime experiments have been demonstrated .So the magnetic field conditions have divided into six experiments as we want the pigeon to experience each condition by itself to avoid the interference of the other conditions on the pigeon's perception.

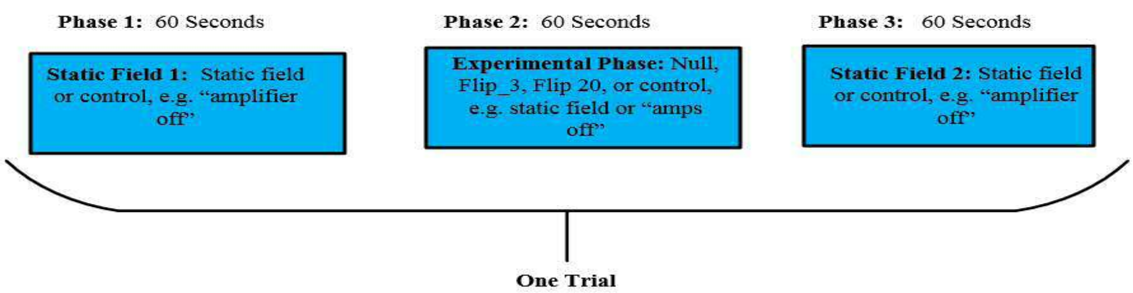


Figure 5-2 Experiments procedure for single trial, for example Phase1=SF1, Phase2=Null, Phase3= SF2

Two types of control:

- Static field control when amplifier ON
- Static field control when amplifier OFF

5.4.1.1 Control Trials that Have Been Used:

Two baseline trials were used with 10 pigeons. The duration of these control trials was matched to the duration of the experimental trials. The main aim of these baseline trials is to carry out analysis on the birds' behaviour in the absence of a magnetic field. This means that the control trials lasted for 180 seconds as did the experimental trials. These trials carried out, prior to the experimental trail or after the experimental trial.

1. Control 1 (Static field with Power amplifier turned off)

This is the first baseline experiment which lasts for 180 seconds in total while the power amplifiers are turned off, i.e. the pigeons are experiencing the actual Earth's magnetic field. See Figure 5-3. This condition was used in order to investigate the pigeon response by comparing their behaviour during the baseline and experimental magnetic field.

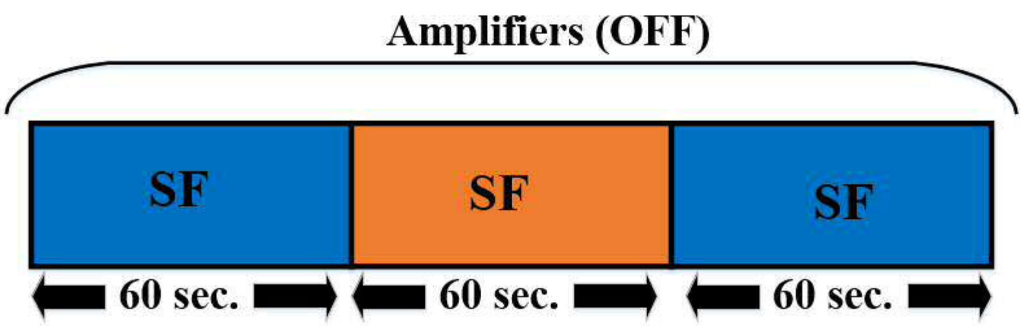


Figure 5-3 Control 1 (static field with power amplifier turned off)

2. Control 2 (Static field with Power amplifier turned ON)

This is the second control experiment which lasts for 180 second in total while the power amplifiers are turned ON, i.e. the pigeons are experiencing the artificial Earth's magnetic field with the inclination factor ON and a zero North angle. See Figure 5-4.

This condition was used in order to see whether the pigeon will be affected by artificial control magnetic field and compare their response with the experimental magnetic field.

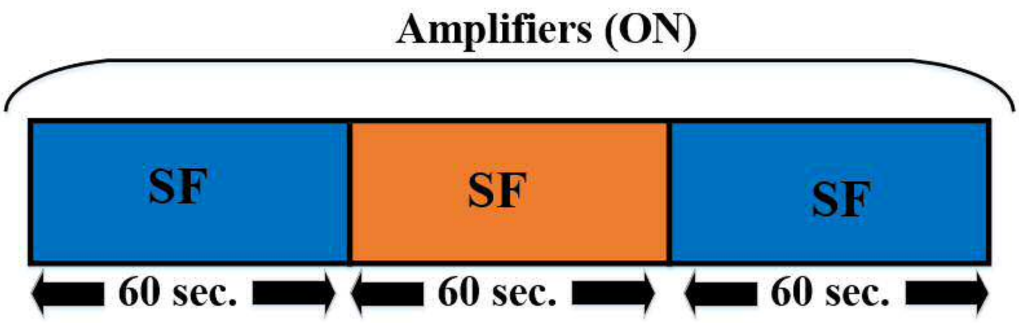


Figure 5-4 Control 2 (static field with power amplifier turned on)

5.4.1.2 EXPERIMENTAL TRIALS:

In the experiments trials below, the static field was presented before any other condition to provide a baseline for any change in the pigeon's behaviour when the field is altered. Although, the axis of the constant field can be controlled to be aligned with any angle required, we have assigned the angle to zero North direction. Also the inclination factor has been turned ON.

1. Experiment 1: Null field

See section 3.1.1 for more explanation about the Null field condition. As shown in Figure 5-5 that each step was divided into three sections because it is aimed to compare the first and last 10 second of each section. So it is preferred to use static field before and after the Null field in order to investigate the pigeon behaviour during each section, also to check whether the pigeon will behave differently during Null experiment.

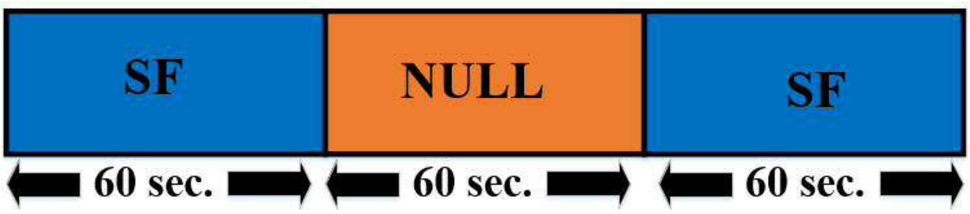


Figure 5-5 Null field

2. Experiment 2: Fast Flipping Field (One flip every 3 seconds)

Field will be flipped every 3 seconds, see Figure 5-6. Also see section 3.1.4 for more explanation about the flipping field condition.

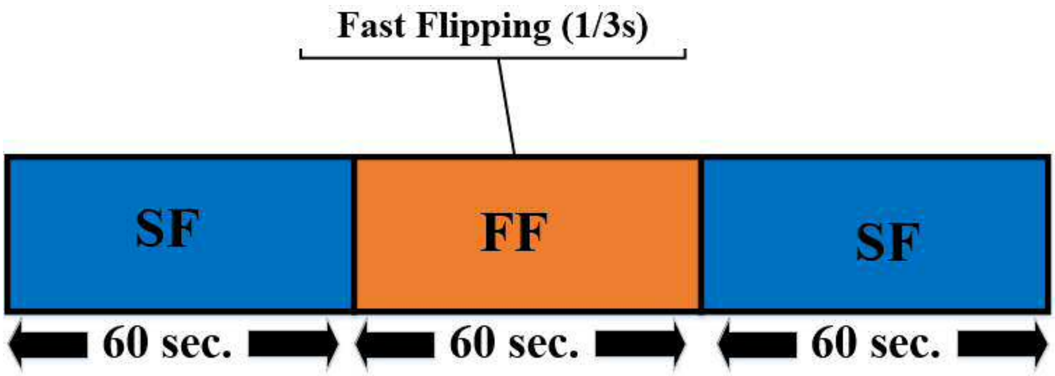


Figure 5-6 Fast flipping field

3. Experiment 3: Slow Flipping Field (One flip every 20 seconds)

The inclination of the field was flipped every 20 seconds, see Figure 5-7. Also see section 3.1.4 for more explanation about flipping field condition.

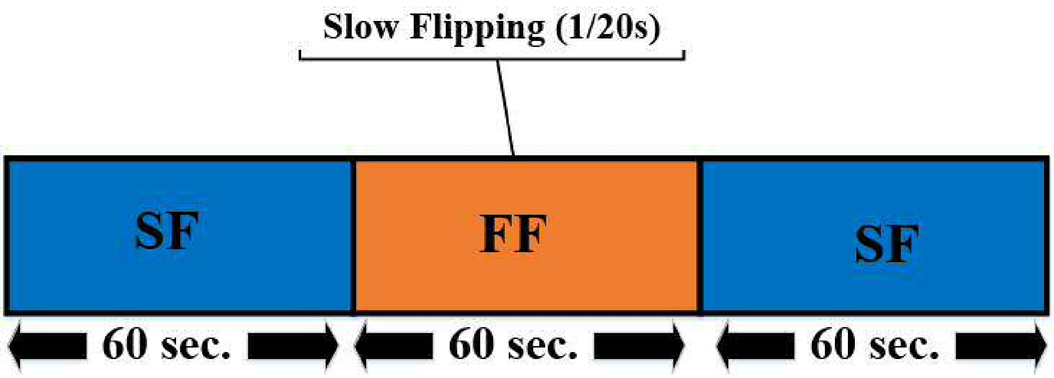


Figure 5-7 Slow flipping field

4. Experiment 4: Sweeping Field Clockwise direction (Rotation 20 deg/s)

This field was rotated 10 cycles in the clockwise direction, which means 18 seconds per cycle. Thus, this magnetic field condition lasted for 180 seconds, which means it lasted longer than the other magnetic field conditions. Inclination was ON while exposing the pigeon to the sweeping field, see Figure 5-8. Also see section 3.1.2 for more explanation about the sweeping field condition.

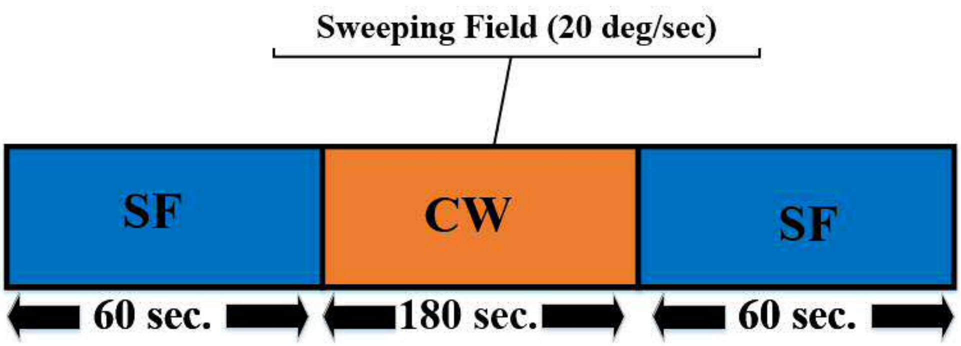


Figure 5-8 Sweeping field clockwise direction

5. Experiment 5: Sweeping Field Counterclockwise (Rotation 20 deg/s)

This field was rotated 10 cycles in the counterclockwise direction which means 18 seconds per cycle. Thus, this magnetic field condition lasted for 180 seconds, which means it lasted longer than the other magnetic field conditions. Inclination was ON while exposing the pigeon to the sweeping field, see Figure 5-9. Also see section 3.1.2 for more explanation about the sweeping field condition.

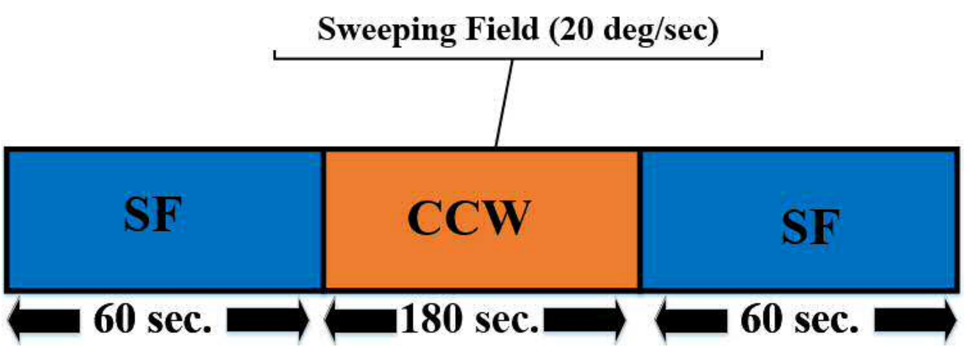


Figure 5-9 Sweeping field Counter clockwise

6. Experiment 6: Control

In this trial, the pigeon was exposed to a control trial in addition to the baseline trial. Also see section 3.1.3 for more explanation about the static magnetic field condition.

5.4.2 Selection of the Experimental Pigeon

The individuality in the pigeon's behaviour has an impact on the procedure of the experiments and the data analysis. Thus, the behavioural characteristics of the pigeons varied despite being exposed to the same field conditions. These different aspects can include:

A- Headshakes: Fast movements of the head.
B- Preening: Can be an indication of boredom so they use their beak to smooth and clean their feathers,
C- Pecking: Pigeons tend to peck the black foam, while they are kept in the experimental box, which is used to keep the pigeon from resting on the wall.
D- Yawning: Pigeons have a tendency to yawn. The reason for this unknown but could even simply be as sign of boredom.

The above individual behaviours could be accompanied by head tilting, and in consequence, the mark at the top of the head, which was tracked by the camera, was lost from the camera viewing area. Thus, these behaviours resulted in missing video frames which affected the matching score. Hence, as mentioned above, two pigeons were excluded from video analysis due to their hyper-activity.

5.4.3 Obstructions Appear While Deciding the Magnetic Field Sequence:

Different magnetic field conditions with various sequences have been tried. Also, the duration of each magnetic step has been varied. These trials were useful as they have led to the choice of the final sequence that has been explained in this chapter.

While doing these trials, many problems were encountered, but a solution has been found for each problem in order to keep the experiment convenient for both subject (Pigeon) and for the user.

One of the challenges experienced while doing different trials of experiments is the head marking that was tracked by the camera to give head angle data. Thus, many pilot trials were carried out included using pattern matching and colour matching in LabView software, but these were often unsuccessful. For that reason, geometric matching in LabView software was considered for tracking the shape of the head marking. Consequently, different shapes, symbols and characters were tried on the white foamplaced on top of the pigeon's head such as using a "L" character which was not successful as it looks similar from all directions (i.e. when the pigeon elevates/ depresses or tilt its head). Also one of the symbols used was a double ended arrow so even when the pigeon's head tilted to extreme angles the software did not seem to have an issue with locating it or recognizing it. Then, it was thought worth while to incorporate various curves and edges in order to take advantage of the features within the geometric feature analysis. However, using this shape was unsuccessful due to LabView confusing the orientation of the symmetrical marking causing it to record a number of frames 180° away from their true value, in effect, flipping the resultant angle in the opposite direction to the heads true position. Once identified, the erroneous values could be multiplied by -1 to correct them but doing that manually would be time wasting. Figure 5-10 shows one of the unsuccessful trials.

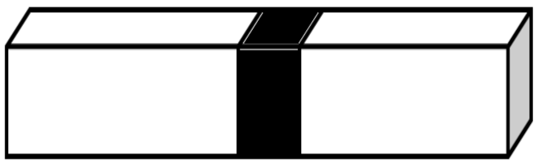


Figure 5-10 Pigeon's head mark

To provide a suitable target for the image recognition algorithm, couple of asymmetries were employed to avoid the potential ambiguity inherent in 180º rotated head positions.

a) A strip of adhesive light foam with an asymmetric “V” shape in which one line was longer than the other and there was an acute angle between them was placed on top of the bird’s head. This allows the search algorithm to detect unambiguously the two-dimensional orientation of the bird’s head in each successive frame of the video, see Figure 5-11.

Figure 5-11 Pigeon's head mark asymmetric “V” shape

b) An asymmetrical marking was employed specifically the black pen mark was moved toward one end of the white rectangular strip placed on the bird's head. This configuration turned out to give the most reliable results with the template matching software. The size and weight of the strip are such that it has no effect on the bird’s head movement, see Figure 5-12.

c) A second issue was keeping the pigeon's head within the white painted Perspex box, as the pigeon , perhaps out of curiosity, tended to raise their heads out of the experimental box. Consequently, the pigeon's head will be out of the camera recordings area. So the first solution was using an additional Perspex cube to be placed at the top of the hole the box cover, which will keep the pigeon head in the region of camera recording. Also an alternative solution has been suggested to use a transparent perspex box which will allow the pigeon to view its surroundings while it is in the experimental box. This in turn will reduce the pigeon's motivation to look outside the experimental box.

d) The duration of most of the experiments exposing the pigeon to the artificial magnetic field lasted for 3 minutes, i.e. three 60 seconds steps.

Figure 5-12 Pigeon's head mark (white rectangular mark)

5.5 Analysis of The Collected Data

The pigeon head angle, obtained with the aid of Video_analysis.vi, was utilized in order to monitor the head's responses. This has been achieved subjecting these data to a number of analyses in order to look at different parameters defining the birds' activity (e.g. Saccades amplitude and number of saccades) during a given magnetic field condition within the sequence. Furthermore, the aim was to present the findings graphically in a clear and concise way, but most importantly to determine significant responses of the birds to the variations in the magnetic field, which in turn would lead to a final validation of the established approach, i.e. to get accurate answer about pigeons' ability to predict Earth's magnetic field.

Hence, several types of analysis were carried out using the Matlab program, which was specifically written for the purposes of this research. This software served as the backbone, i.e. the starting point, for the analysis. The program is capable of the following:

- The user inputs the sampling rate of the specific camera used in order to define the precise intervals between frames.
- Input data appear to be two dimensional, i.e. assuming this represents horizontal head angle data and setting all vertical axis data to zero
- Calculating velocity profile.
- Calculating acceleration profile.
- Removing artefacts (i.e. missed data), see Figure 5-13.
- The software is capable of providing the characteristic of each head movements (as highlighted in Figure 5-14). Subsequently, two files obtained out of this program: a) Saccades output, where the head saccades amplitude, peak velocity, and duration are obtained for further analysis. b) False saccades output, which provides identification of the periods of any lost frames in order to get an accurate analysis of the saccade frequency.

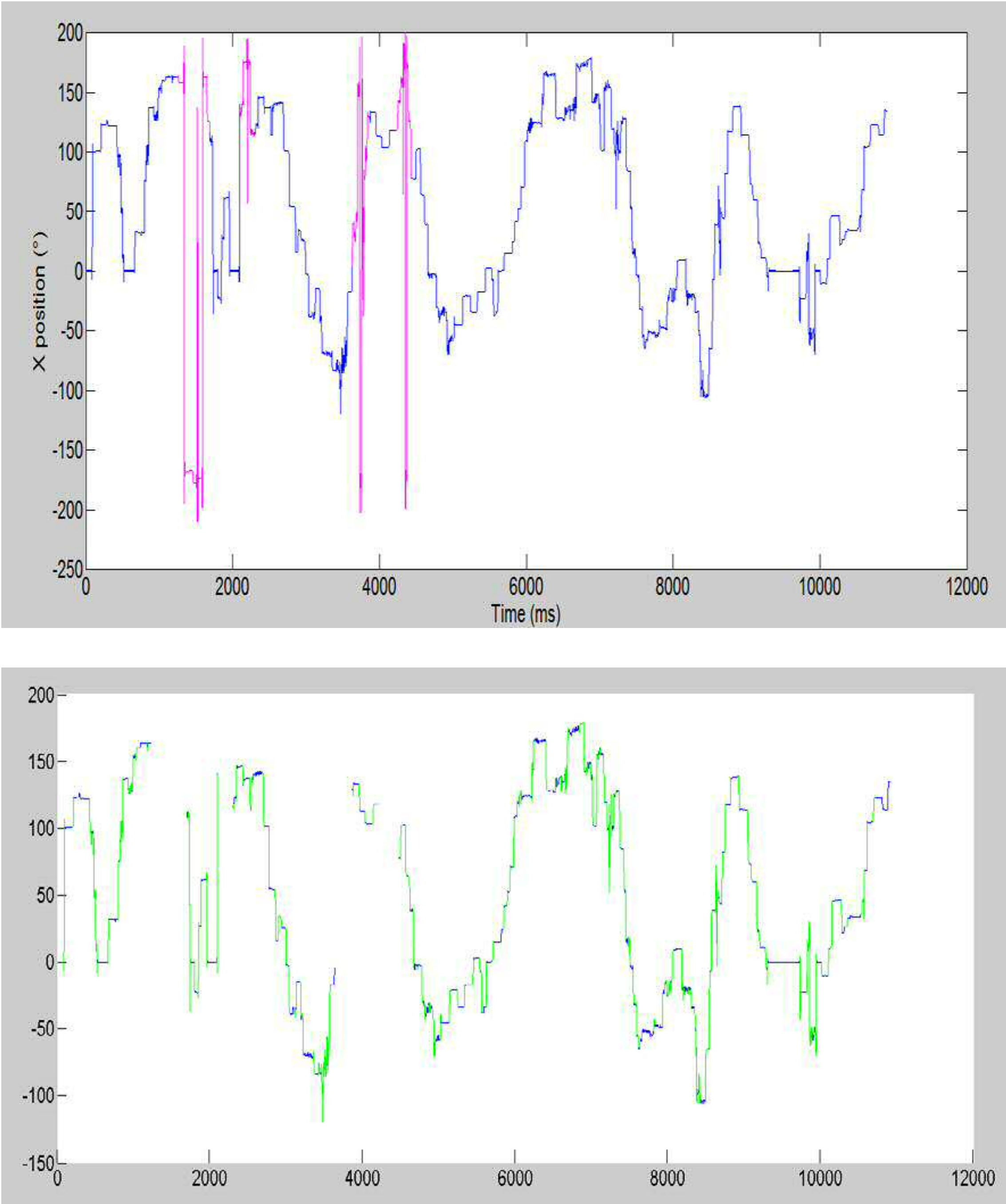


Figure 5-13 a) Head movements data with distortion, b) Head movements data with removed distortion

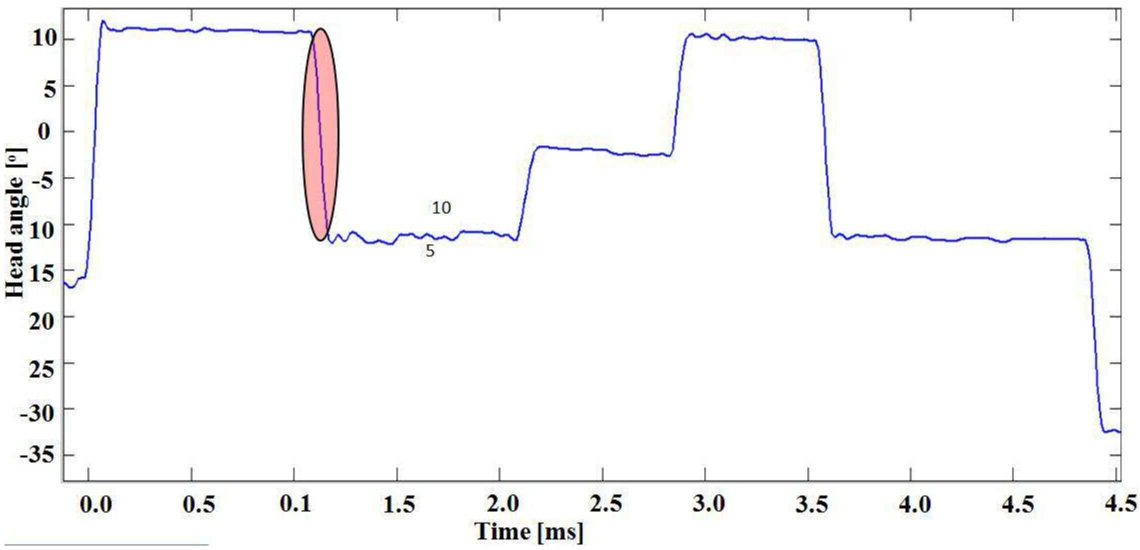


Figure 5-14 An example of saccadic head movement data obtained from the pigeon's head analysis.

The following analysis procedures were also prepared especially for this investigation:

5.5.1 Saccades Amplitude Analysis

This type of analysis has been carried out in order to look more closely at the amplitude of the birds' reactions in every single condition of magnetic field, whether this was a control or experimental condition. After obtaining the head angles and the matching score from video_analsys.VI, the head angles have been processed in MATLAB (MathWorks) program saccades_extraction.m in order to extract the head saccades. The processed file included the saccades amplitude, duration, peak velocity, time of occurrence and the Q value (peak velocity *duration), which has been analysised in details by John Barnes [149]. Any saccade with an amplitude over 4^o is considered a possible reaction to the magnetic field condition (see section 5.5.1.1 for more information). All those saccades smaller than 4^o have been removed, as they do not carry any information about the pigeon's reaction to the magnetic stimuli.

5.5.1.1 THRESHOLD OF THE SACCADES AMPLITUDE

The sampling rate of the camera used to record head position is equal to 60 frames per second. This means that within each second there are 60 measurements of head position, which in turn give accurate insight into the head movements. Software programmed to extract the data from the video picks up the difference in head alignment from frame to frame with an accuracy of 1^{o}. This means that almost every head movement or its tilt can be detected. However, for the purposes of analyzing the data, a threshold was introduced to filter out the very small head movements regularly and spontaneously made by awake birds that may not be associated with reactions to magnetic field changes. Although not necessary when analysing the birds' total activity, removing this behavioural "noise" was required to improve the analyses of the birds' responses. For example, an examination of all head movement data indicated that a reasonable "noise" filtering threshold could be set at $<4^{o}$ in order to more readily identify the saccades more likely to have been made in response to change in the magnetic field, such as number of saccades and the amplitude, etc.

5.6 HYPOTHESIS TESTING

In our study, we attempted to detect the effect of a magnetic field on pigeons. To do so, we measured the number of head saccades and their amplitudes under a given magnetic field, and we then compare them to the same measurements under the baseline conditions.

Two baselines were used during the experiments, the artificial baseline and the natural magnetic field.

Since the same pigeon had measurements taken under the baseline conditions and under one of the magnetic fields, the experiment is a repeated measures experiment, and therefore the paired sample t-test is appropriate [199].

5.7 Paired Samples T-test

The paired samples t-test, or the dependent samples t-test, is a parametric test used to determine whether the mean difference between two measurements taken in the same subjects is significantly different from zero.

5.7.1 Hypothesis

The hypothesis of the paired samples t-test can be written as follows:

$$\boldsymbol{H_0 : \mu_1 = \mu_2} \quad \text{Equation 5-1}$$

$$\boldsymbol{H_1 : \mu_1 \neq \mu_2} \quad \text{Equation 5-2}$$

Where:

μ_1 is the variable (or measurement) mean under conditions 1

μ_2 is the variable mean under conditions 2

5.7.2 The Test Statistic

The paired samples t-test is mathematically equivalent to the one sample t-test applied to the difference between the two measurements (compared to zero). Thus, the statistic of the test, denoted t, follows the same formula:

$$\boldsymbol{t = \frac{\bar{x}_{diff} - 0}{S_{\bar{x}}}} \quad \text{Equation 5-3}$$

Where:

$$\boldsymbol{S_{\bar{x}} = \frac{S_{diff}}{\sqrt{n}}} \quad \text{Equation 5-4}$$

And:

- $\bar{x}_{diff}$ Sample mean of the difference
- n Sample size
- S_{diff} Standard deviation of the difference
- $S_{\bar{x}}$ Estimate standard error of the mean

The t statistic follows the t-distribution. The calculated value should be compared to the critical value of the t-distribution with n-1 degrees of freedom. If it proves to be greater, we reject the null hypothesis and conclude that the means are statistically different. Equivalently, we could find the p-value of the calculated statistic and compare it to the desired degree of significance. P value is the value that is used in statistics analysis in order to determine the level of the significance of the results.

If the p-value is lesser than the degree of significance, then we reject the null hypothesis and conclude that the data in hand suggest that the measurements under condition 1 and under condition 2 are significantly different.

In this study, two levels of significance were considered, 5% and 10%.

To carry out the hypothesis tests, we used the software R which provides us with the function t.test() that implements different Student t-tests. We present the findings of our analysis in the following section.

5.8 Experiment Description

In total, twelve experiments were conducted. Six magnetic fields were studied for each one of the two baselines. See table 5-1 below to show the experiment's division.

Table 5-1: Experiment description

Baseline	Magnetic fields studied
The natural magnetic field	Fast Flipping, Slow Flipping, Null, CW, CCW, Control OFF
The artificial baseline	Fast Flipping, Slow Flipping, Null, CW, CCW, Control ON

For a given baseline and a given magnetic field, the experiment is divided into two parts. In the first part, the pigeons were exposed to the baseline and measurements were taken, whereas for the second part, the same measurements were taken but the pigeons were actually exposed to the magnetic field.

Each part of an experiment consists of three phases, the first static field phase (SF1), the main phase (MF) and the second static field phase (SF2). During each phase, the number of saccades and their amplitudes were measured, then we computed the following values for the three phases:

- The number of saccades during the whole phase
- The average amplitude of the saccades during the whole phase
- The number of saccades during the first 10 seconds of the phase
- The average amplitude of the saccades during the first 10 seconds of the phase
- The number of saccades during the last 10 seconds of the phase
- The average amplitude of the saccades during the last 10 seconds of the phase

Thus, for a given baseline and magnetic field, we carried out 6 different t-tests for each one of the three phases, adding up to 216 tests.

5.9 Test Results

As mentioned before, during the experiments we used two different baselines, the natural magnetic field (AMP OFF) and the artificial baseline (AMP ON), and for this reason, we are going to present the results in two parts.

Because pigeons respond differently to the various types of magnetic field stimuli, using different baselines prior the main experiments helps to reveal whether or not the pigeon response to the change in magnetic field.

Since the number of tests carried out is rather large, we will only report the experiment for which the t-test proved to be significant with either 5% level or 10% which will be presented in table format.

Also, the figures will present the pigeon's response for each magnetic field based on its baseline magnetic field.

5.9.1 Natural Magnetic Field Baseline (Amp Off)

The magnetic fields studied, using the natural magnetic field as a baseline, are the following: Fast Flipping, Slow Flipping, Null, CW, CCW and Control OFF. We are going to present the results of the tests by magnetic field.

Two sets of analysis will be presented below:

5.9.1.1 Identify the significance level of Saccades amplitude and number of saccades

Saccade amplitude and number of saccades data have been analysed carefully to investigate the effect of the magnetic field on pigeon's behaviour as well as to determine whether pigeons perceive the external magnetic field or not.

The saccade amplitude and the number of saccades of each pigeon which occurred while exposing the pigeons to the experimental magnetic field condition were compared with the saccade amplitude and the number of saccades of each pigeon which occurred while exposing the pigeons to the baseline magnetic condition, which is the natural magnetic field when the amplifier is switched off. Table 5-2 and Table 5-3 present the P values to find the significance level of the saccades amplitude and the number of the saccades, respectively. Each cell in the tables represents a comparison between the experimental magnetic field conditions compared with its Baseline magnetic field, which is the Natural magnetic field when the amplifier is switched off.

The red parts represent the absence of a significant effect, suggesting that the pigeon has not perceived the magnetic field. It can be noticed that these p-values are above 0.05 which are not significant at a level 5%.

Light green: means that 90% confident that the field has an effect. So the light green cells are the significant with a level 10%.

Dark green: 95% confident that the field has an effect so the dark green cells are the only ones significant at the level of 5%. So the p-values less than 0.05 are significant at a level of 5%.

5.9.1.2 SIGNIFICANT TESTS BASED ON DIFFERENCE BETWEEN THE MEANS

This test is based on comparing the difference between the mean values.

In the figures below, it can be noticed that the x axis are the pigeons' number (pigeon 39, pigeon 40, pigeon 45, pigeon 46, pigeon 47, pigeon 49, pigeon 50, pigeon 53, pigeon 54, and pigeon 73).

The green dots represent the number of saccades (or saccade amplitudes) of each pigeon for the Baseline (the control) condition.

The red dots are the number of saccades (or saccade amplitudes) of each pigeon but this time, for the experiment (such as: slow flipping field, CW etc…)

The green line is the mean of the number of saccades (or saccade amplitudes) for the Baseline, and the red line represents the mean of the number of saccades (or saccade amplitudes) for the experiment.

Consequently, the space between the two lines is the difference between the means, which appears to be substantial.

Each experimental magnetic field has been preceded and followed by static magnetic field in order to compare the pigeon's response to the magnetic field with the static magnetic field. However, the whole steps have been analysed carefully but it was preferred to compare first and last 10 seconds of each step with other steps. This scenario has been chosen because first 10 seconds will be effective period to be analysed to see whether pigeons have shown any response to the magnetic field. Also the last 10 seconds will show whether the pigeon is still perceiving the magnetic field or it habituated to the external stimuli.

In this research, significant impact at a level of 5% will be taken in account. However, significant impact at a level of 10% has been taken to extend the profile of the statics analysis.

Table 5-2: P values to find the signifi

a) Saccades Amplitude (Middle Phase)

AMP OFF					
All phase		First 10s		last 10s	
Experiment	**p-value**	**Experiment**	**p-value**	**Experiment**	**p-value**
Null	0.139	Null	0.885	Null	0.067
CW	0.598	CW	0.540	CW	0.531
CCW	0.847	CCW	0.556	CCW	0.905
Control_off	0.808	Control_off	0.135	Control_off	0.340
Fast_Flipping	0.951	Fast_Flipping	0.792	Fast_Flipping	0.973
Slow_Flipping	0.547	Slow_Flipping	0.260	Slow_Flipping	0.730

Al
Experimer
Null
CW
CCW
Control_o
Fast_Flippi
Slow_Flipp

c) Saccades Amp

AMP		
All phase		First
Experiment	**p-value**	**Experimen**
Null	0.400	Null
CW	0.007	CW
CCW	0.220	CCW
Control_off	0.564	Control_of
Fast_Flipping	0.697	Fast_Flippir
Slow_Flipping	0.270	Slow_Flippi

95% confident that the field has an effect

Table 5-3: P values to find the signifi

a) Number of Saccades (Middle Phase)

AMP OFF					
All phase		First 10s		last 10s	
Experiment	p-value	Experiment	p-value	Experiment	p-value
Null	0.366	Null	0.1070	Null	0.270
CW	0.931	CW	0.076	CW	0.218
CCW	0.176	CCW	0.405	CCW	0.710
Control_off	0.748	Control_off	0.230	Control_off	0.377
Fast_Flipping	0.804	Fast_Flipping	0.903	Fast_Flipping	0.246
Slow_Flipping	0.0974	Slow_Flipping	0.693	Slow_Flipping	0.303

c) Number of Sac

AMP		
All phase		First
Experiment	p-value	Experiment
Null	0.330	Null
CW	0.007	CW
CCW	0.001	CCW
Control_off	0.533	Control_off
Fast_Flipping	0.6208	Fast_Flippin
Slow_Flipping	0.926	Slow_Flippin

95% confident that the field has an effect

90% confident that the field has an effect

No significant effect

A) THE SLOW FLIPPING FIELD

The tests on the amplitudes of the pigeons saccades when subject to the slow flipping field all proved to be not significant, thus suggesting that the slow flipping field, when considering the natural magnetic field as the baseline, has no impact on the amplitudes of the pigeons' saccades.

The slow flipping field only proved to have a 10% significant impact on the number of saccades of the whole main phase (MF) and of the last 10s of the second static field phase (SF2). Which means that the slow flipping has not shown any effect on the pigeon at any point.

B) THE NULL FIELD

The Null field proved to have a significant impact at a level of 5% on the number of saccades of the main phase shown in Figure 5-15.

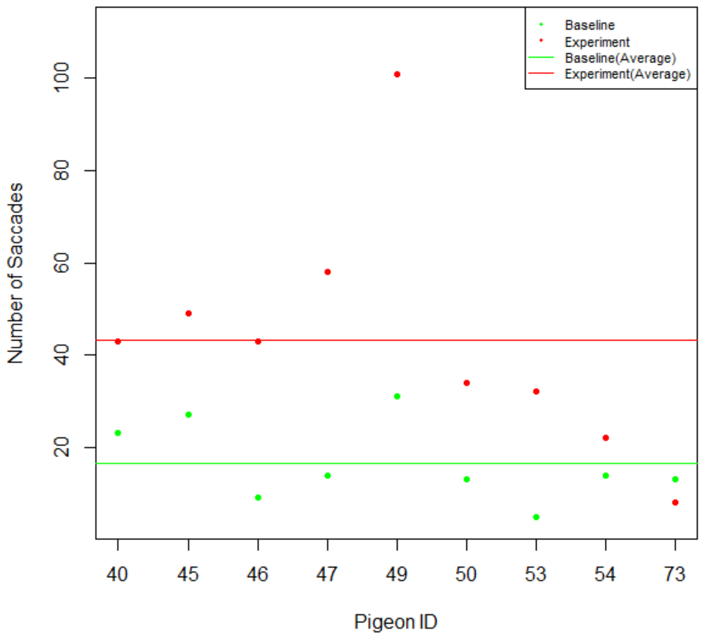


Figure 5-15 The effect of the null field on the number of saccades of the SF1 phase

The pigeon has taken 50 seconds until they realized the absence of the field. For that reason they have shown significant response in the number of saccades.

As for the amplitudes of the saccades, the impact was significant for the last 10 seconds of the SF2 phase but only at a level of 10%.

C) The CW Field

In contrast with the aforementioned magnetic fields, the effect of the CW field proves to be significant at many levels. Starting with the number of saccades, the test for the last 10 seconds of the SF1 phases was significant at a 10% level.

As for the main phase, only the first 10 seconds data yielded a significant test statistic at a level of 10%.

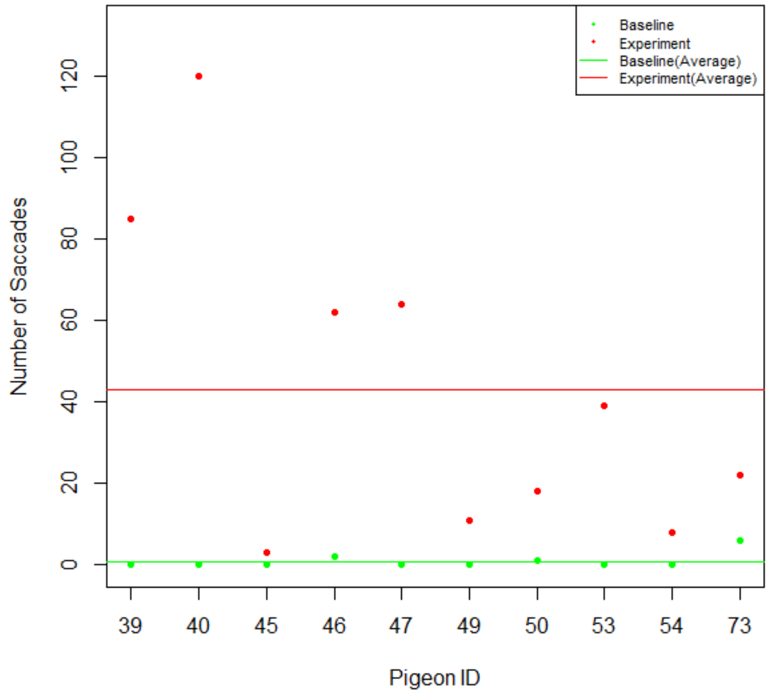


Figure 5-16 The effect of the CW field on the number of saccades of the sf2 phase

Finally, all the tests for the number of saccades of the SF2 phase turned out to be significant, at a 5% level for the whole period and for the first 10 seconds, and at a 10% level for the last 10 seconds, (see Figure, 5-16).

Figure 5-16, pigeon's number of saccades have been effected during the whole phase of the SF2. Hence, pigeons showed a change in their behaviour lasted for 60 seconds even after the CW field has stopped.

On the other hand, the tests for the effects of the CW on the amplitudes of the head saccades for the SF2 phase all resulted in significant values of the test statistic at a level of 5% for the whole phase and the last 10s and at a 10% level for the first 10 seconds of the phase. This means that the amplitude of the saccades has been affected by CW but this effect did not start immediately like what happened in the number of saccades. The effect on number of saccades has started after experiencing the pigeon to CW field which is lasted for 180 seconds. (See Figures 5-17).

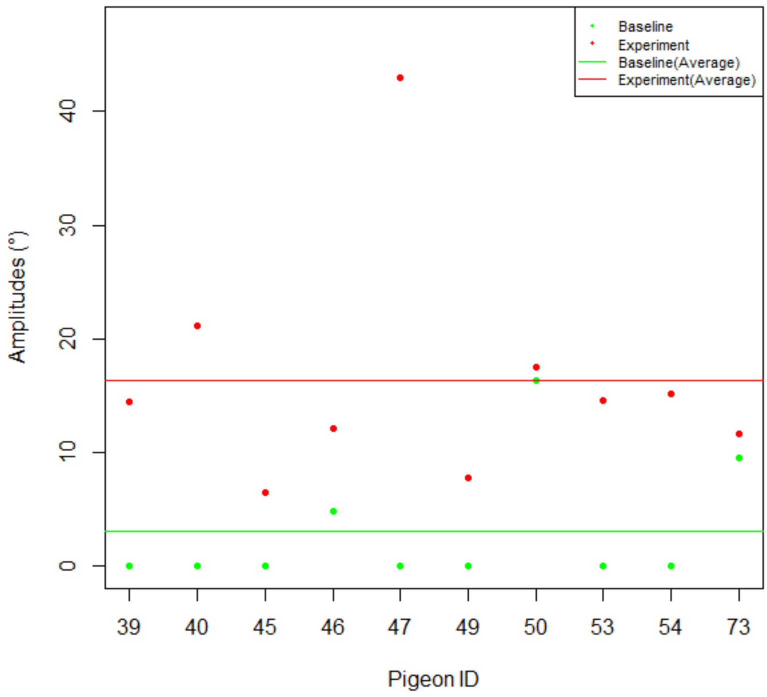


Figure 5-17 The effect of the CW field on the amplitudes of the sf2 phase

D) The CCW Field

Five of the tests for the effects of the CCW field proved to be significant, only one of which is related to the amplitudes, the test on the data of the last 10 seconds of the SF2 phase, which is significant at a 5% level, (see Figure 5-18) .

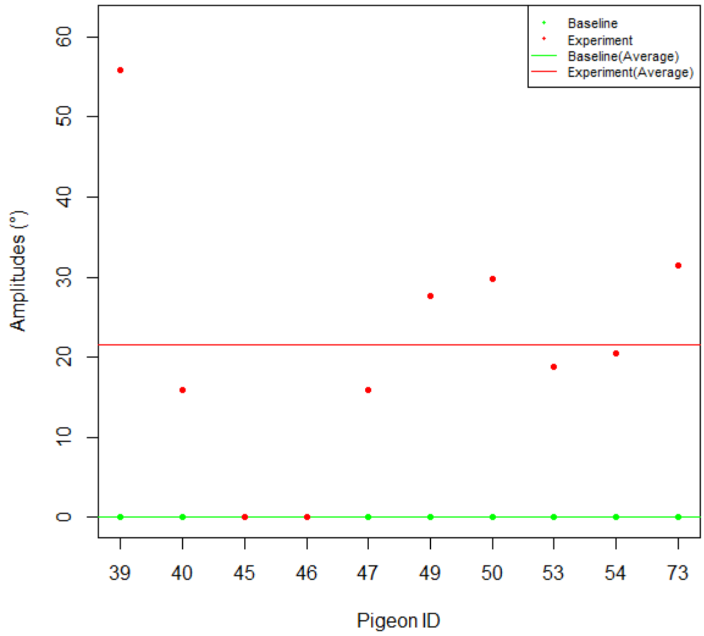


Figure 5-18 The effect of the CCW field on the amplitudes of last 10s of the sf2 phase

The above graph has shown that the pigeons gave responded to magnetic field 230 seconds.

And finally, a significant effect has been shown by the pigeon due to CCW. This response has been started after 180 seconds haven passed. So all the tests on the number of saccades of the SF2 phases showed a 5% significance level, (see Figures 5-19).

As for the number of saccades, the test on the data of the first 10s of SF1 phase yielded a significant test statistic at a 10% level.

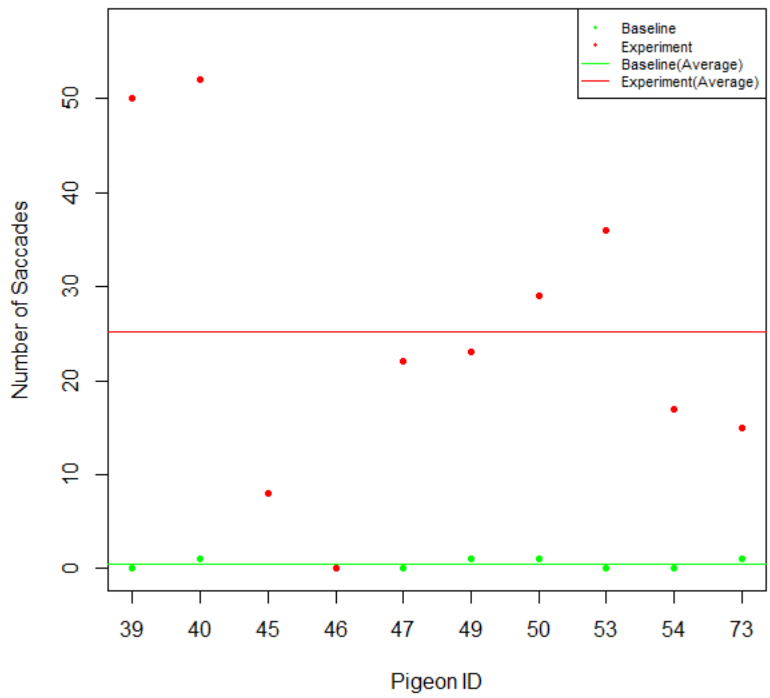


Figure 5-19 The effect of the CCW field on the number of saccades of the sf2 phase

5.9.2 ARTIFICIAL MAGNETIC FIELD BASELINE (AMP ON)

The magnetic fields studied considering the artificial magnetic field as a baseline are the following: Fast Flipping, Slow Flipping, Null, CW, CCW and Control ON. We are going to present the results of the tests by magnetic field.

5.9.2.1 IDENTIFY THE SIGNIFICANCE LEVEL OF SACCADES AMPLITUDE AND NUMBER OF SACCADES

Tables 5-4 and 5-5 show respectively, the P values which are obtained from saccade amplitudes and the number of saccades of each pigeon, which occurred while exposing the pigeons to the experimental magnetic field condition, and the saccade amplitudes and the number of saccades of each pigeon which occurred while exposing the pigeons to the Baseline magnetic which is the Artificial magnetic field when the amplifier is switched ON.

Table 5-4: P values to find the signifi

a) Saccades Amplitude (Middle Phase)

AMP ON					
All phase		First 10s		last 10s	
Experiment	p-value	Experiment	p-value	Experiment	p-value
Null	0.857	Null	0.869	Null	0.781
CW	0.073	CW	0.82	CW	0.763
CCW	0.571	CCW	0.640	CCW	0.783
Control_on	0.276	Control_on	0.012	Control_on	0.888
Fast_Flipping	0.058	Fast_Flipping	0.706	Fast_Flipping	0.042
Slow_Flipping	0.027	Slow_Flipping	0.621	Slow_Flipping	0.458

c) Saccades Ampl

AMP ON			
All phase		First 10s	
Experiment	p-value	Experiment	p-
Null	0.53	Null	0
CW	0.345	CW	0
CCW	0.001	CCW	0
Control_on	0.221	Control_on	0
Fast_Flipping	0.406	Fast_Flipping	0
Slow_Flipping	0.406	Slow_Flipping	0

95% confident that the field has an effect

90% confident that the field has an effect

No significant effect

Table 5-5: P values to find the signifi

a) Number of Saccades (Middle Phase)

AMP ON					
All phase		First 10s		last 10s	
Experiment	p-value	Experiment	p-value	Experiment	p-value
Null	0.972	Null	0.059	Null	0.972
CW	0.265	CW	0.350	CW	0.598
CCW	0.044	CCW	0.112	CCW	7.47E-01
Control_on	0.717	Control_on	0.381	Control_on	0.264
Fast_Flipping	0.820	Fast_Flipping	0.775	Fast_Flipping	0.416
Slow_Flipping	0.904	Slow_Flipping	0.878	Slow_Flipping	0.308

c) Number of Sac

AMI		
All phase		First
Experiment	p-value	Experiment
Null	0.202	Null
CW	0.005	CW
CCW	0.002	CCW
Control_on	0.104	Control_on
Fast_Flipping	0.205	Fast_Flipping
Slow_Flipping	0.572	Slow_Flipping

95% confident that the field has an effect

90% confident that the field has an effect

No significant effect

5.9.2.2 SIGNIFICANT TESTS BASED ON DIFFERENCE BETWEEN THE MEANS

See section 5.9.1.2 for more details.

A) THE NULL FIELD

As mentioned before, for a given field and baseline, there are 18 tests. In the case of the Null field and the artificial baseline, only 2 out of 18 tests resulted in a significant effect, and both are on the number of saccades. The two tests are during the first 10 seconds of the main phase and the last 10 seconds of the SF2 phase, but they are only significant at a 10% level.

B) THE CW FIELD

The CW field produced a significant impact on the amplitudes of the saccades during the main phase at a 10% significance level and the last 10 seconds of the SF2 phase at a 5% significance level, (see Figure 5-20) .Effect of CW field on pigeon was not an immediate effect as it has been shown after 230 seconds has passed.

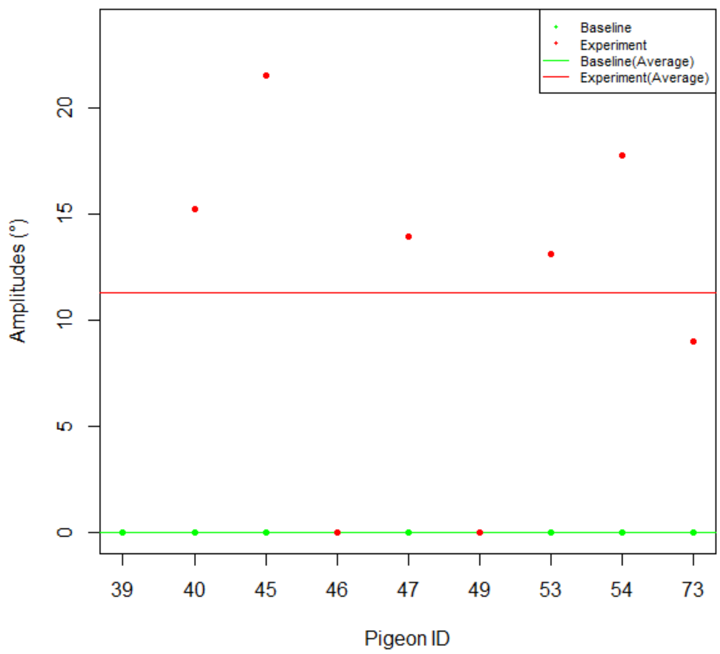


Figure 5-20 The effect of the CW field on the amplitudes of the last 10s of the SF2 phase

In term of the number of saccades, the CW field resulted in a significant effects during , the first 10 seconds of the SF1 phase, the SF2 phase, with significance levels of 10% and 5% respectively. The results of the tests are illustrated in the following graphs, (see Figures, 5-21). The number of saccades has not been affected immediately by CW magnetic field. So the pigeons have perceived this magnetic field condition after the field stopped I.e., after180 seconds have passed.

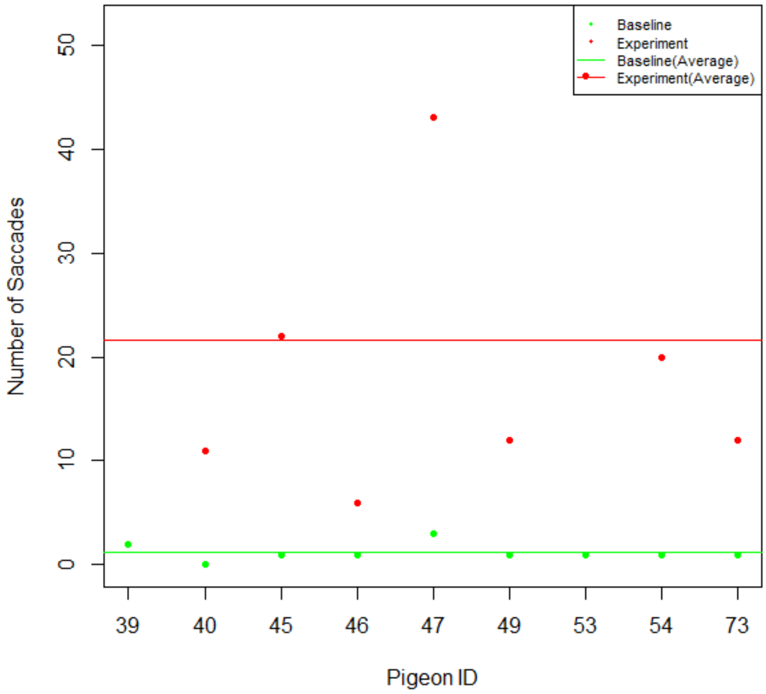


Figure 5-21 the effect of the CW field on the number of saccades of the SF2 phase

C) The CCW Field

Eight of the tests on the data collected during the CCW field experiments with the AMP ON baseline gave significant results, of which seven at the level of 5%. Concerning the amplitudes, the significant tests were on data from the first 10 seconds of the SF1 phase and the whole SF2 phase, both at a 5% level, (see Figure 5-22)

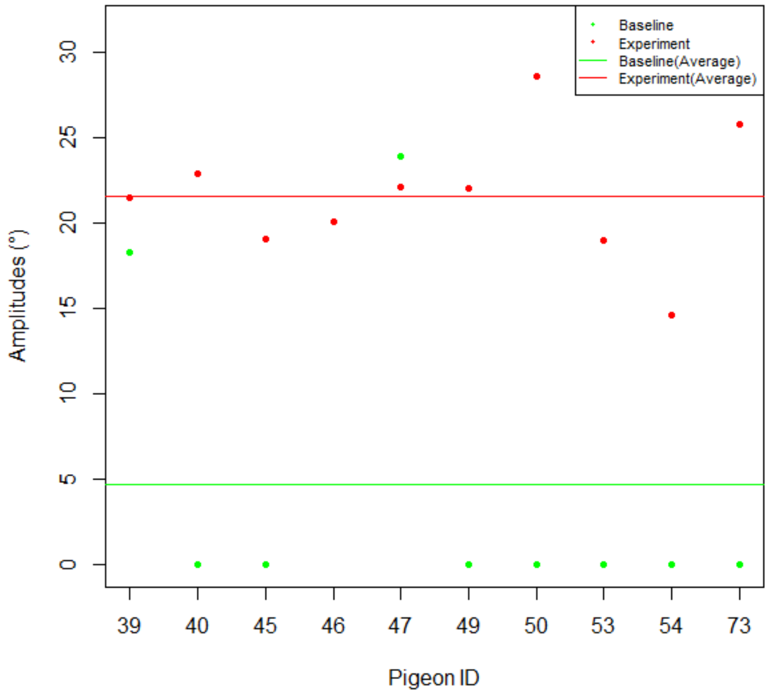


Figure 5-22 the effect of the CW field on amplitudes of the SF2 phase

Pigeon's saccades amplitudes have increased after exposing them to CCW magnetic field, this effect has not been shown immediately as it has started after 180 seconds and lasted for 60 seconds.

As for the tests on the number of saccades, the last 10 seconds of the SF1 phase showed a significance level of 10%.

Also, the test on the number of saccades of has showed that pigeon's number of saccades have been affected directly by turning on the CCW magnetic field. This response has been detected by significant statistic at a level of 5%, (see Figure 5-23).

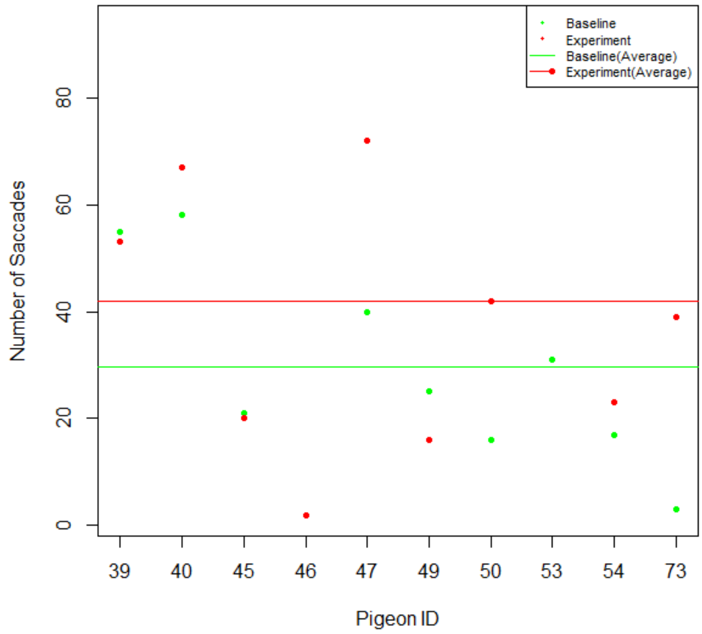


Figure 5-23 The effect of the CCW field on the number of saccades of the main phase

The effect of CCW magnetic field has been lasted for 240 seconds, which means this effect has been continuous even after stopping the CCW magnetic field. Hence, the tests on the saccades of the SF2 phase were significant at a 5% level as shown by the graph, (see Figures 5-24).

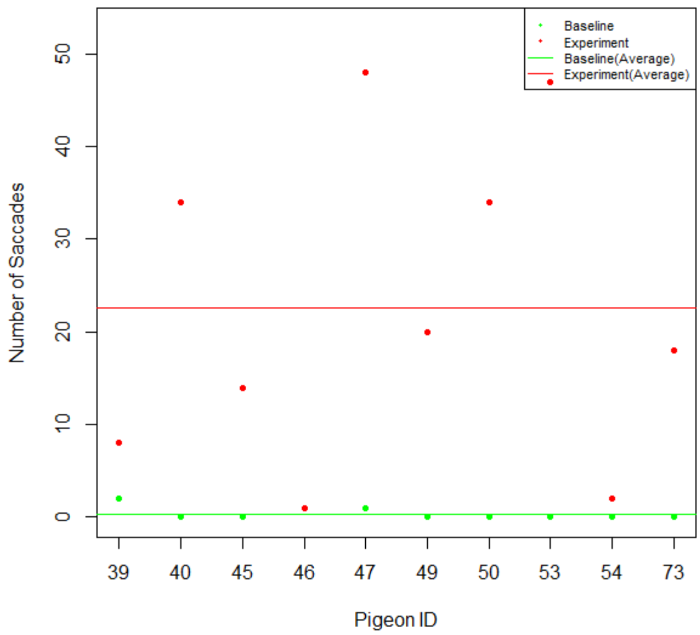


Figure 5-24 the effect of the CCW field on the number of saccades of the SF2 phase

D) Control On Field

The tests on the amplitudes for the Control On field showed significance in four situations. the whole SF1 phase at level 10%, the first 10 seconds of the SF1 phase the first 10 seconds of the main phase and the last 10 seconds of the SF2 phase, all with significance level of 5%. These results are shown in the following graphs, (see Figures 5-25).

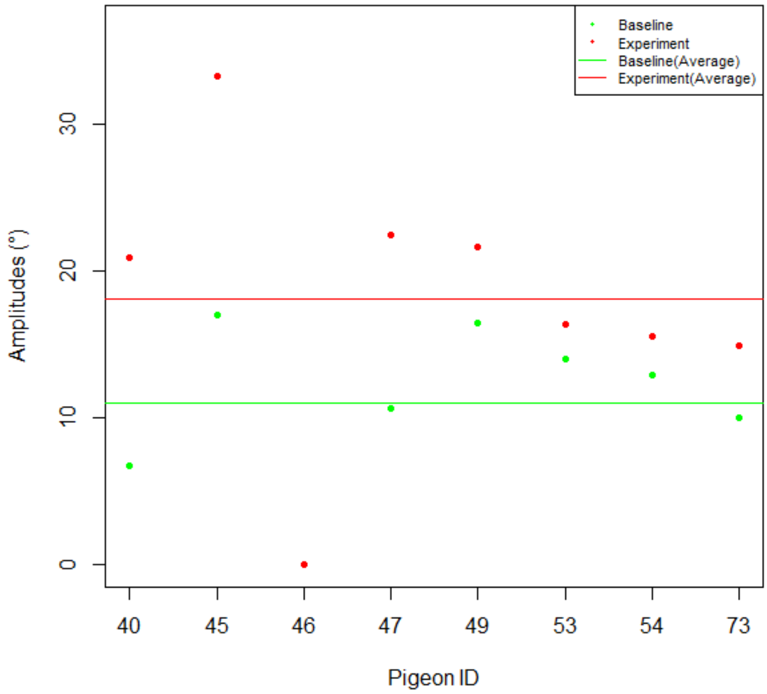


Figure 5-25 the effect of the control on field on the amplitudes of the first 10s of the main phase

So using artificial Static magnetic field has shown some effect on the pigeon response which was significant on the first 10 second of this step of magnetic field. However pigeons get used to this magnetic field type as it is consider being duplicated to the Earth's magnetic field.

Concerning the number of saccades for the Control On field, the tests proved to be significant for the first 10 seconds of the SF1 phase, at a level of 5%, and for the last 10 seconds of the SF2 phase at a level of 10%.

E) Fast Flipping Field

Only two out of the eighteen tests on the data of the Fast Flipping field showed significant impact, and both relate to the amplitudes, namely the tests on the whole main phase (at a level of 10%) and its last 10 seconds (at a level of 5%). The results are illustrated in the following plots, (see Figures 5-26).

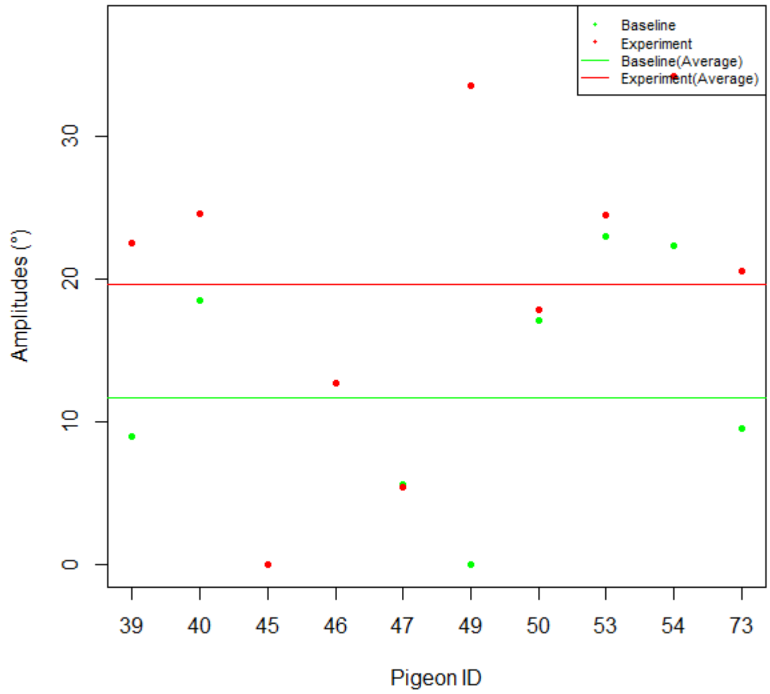


Figure 5-26 the effect of the fast flipping field on the amplitudes of the last 10s of the main phase

This figure is showing that the pigeon can perceive the inclination when it is added to the scenario of magnetic field to be flipped fast. Which means that the inclination is playing an important role as they affect the pigeon's behaviour especially during the last 10 seconds of the main phase of this magnetic field condition.

F) Slow Flipping Field

The Slow Flipping field proved to have a significant impact on the amplitudes of the saccades during the first 10 seconds of the SF1 phase and during the whole main phase, both impacts with a 5% significance level. The results are illustrated in the following graphs, (see Figures 5-27).

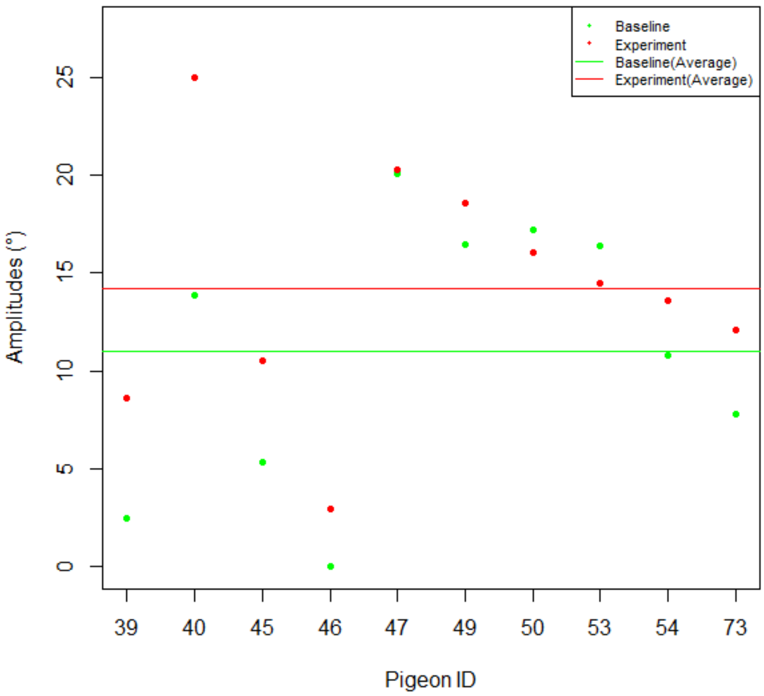


Figure 5-27 the effect of the slow flipping field on the amplitudes of the main phase

The slow flipping magnetic field has a significant effect on the pigeon's behaviour more than the fast flipping. The reason behind that is the pigeon's might need time to realise the change in the inclination. This effect was lasted for 180 seconds continuously.

With respect for the number of saccades, the Slow Flipping showed significant effects during two instances, the whole SF1 phase and the last 10 seconds of the SF2 phase, both at a level of 10%. The graphs below illustrate the results of the tests.

5.10 Summary of Chapter 5

The remarkable ability of diverse animals to orientate and navigate during migration and homing over long distances has fascinated scientists for years. However, how the birds sense and process the magnetic field information in their brains is not known [150]. Recent advances have brought new insights into how field direction, intensity, and polarity are neurally encoded by single cells [151]. Several bird species are believed to possess physiological mechanisms that enable them to navigate using the magnetic field.

Therefore, our experiments have shown that pigeons respond differently to the various types of magnetic field stimuli. Using different baselines prior the main experiments has revealed that the Pigeon's respond is affected by this factor.

Also, each experimental magnetic field was preceded and followed by static magnetic field. The aim of using static magnetic field is to compare pigeon's behaviour while it is exposed to magnetic field conditions and to baseline. However, pigeon's response to magnetic field during the whole magnetic field has been analysed carefully. It was suggested to study the first and last 10 second of each step of each magnetic field. This period of time considered to be a critical as it is the transition between one condition to other one and it is important to look at pigeon's behaviour prior and after the effect of magnetic field condition.

In the case of using natural baseline, it was noticed that slow flipping field and fast flipping field has not affected the pigeon's behaviour. However, pigeon's has perceived Slow flipping magnetic field.

In the absence of magnetic field pigeons' have detected that change in magnetic field, this has happened in the case of using natural baseline. However, pigeons' habituated to the artificial baseline so they could not perceive the null field.

Free flying pigeons tend to fly in circle path before they start their way to their home. So this will give the explanation for the reason of pigeon's detection to sweeping field in CW and CCW direction. Also, pigeons have perceived the sweeping field in both cases of baselines.

The above results are promising which has given a strong base for the researchers in Optometry school to focus on Sweeping and flipping field to be considered for further behavioural analysis which will be tacked by John Barnes.

CHAPTER 6

IMPROVE THE TRACKING SYSTEM USING INERTIAL MEASUREMENT UNIT

6.1 INTRODUCTION FOR TRADITIONAL TRACKING METHODS

The ability to orientate and navigate is critically important for the survival of all migratory birds and other animals [152]. Progress in understanding the mechanisms underlying these capabilities and, in particular, the importance of sensitivity to the Earth's magnetic field has, thus far, been constrained by the limited number of techniques available for the analysis of often complex behavioural responses. Methods used to track the movements of animals, such as birds, have varied depending on the degree of accuracy required.

Manual observations of such behaviours continue to be an effective technique for qualitative accounts of an animal's behaviour in a given condition. However, such an approach is not suitable for studies that require a more accurate, quantitative account [153].

At present, the gold-standard technique for the quantitative evaluation of complex behaviours is video tracking analysis (VTA), which, as the name suggests, relies upon post-hoc digital signal processing with the aid of sophisticated image processing algorithms [143].

While effective, this technique also has a number of limitations: 1) VTA provides only a two dimensional view, meaning that data from any out of plane movements, i.e. tilting or rolling of the region of interest, cannot be analysed and such out of plane movements often bring the fiducial mark used to track the head out of the field of view of the camera resulting in the loss of data; 2) VTA has large memory storage requirements, which become even more demanding with the use of high frame-rate cameras; 3) VTA requires time-intensive post-hoc frame-by-frame analysis. Reducing the frame rate of the camera can counter the issues of storage and time, however this is often at the expense of the spatial and temporal resolution of the data. Alternative automated behavioural tracking systems exist. For example, infrared devices for detecting locomotor activity [154], laser-based instruments for recording behaviour and providing the location of freely moving animals [155], as well as a microwave Doppler radar activity monitor [156]. Additionally, 3D recording of movements is possible using video tracking systems however complex and expensive multi-camera arrangements are required, which are still limited by many factors (e.g. the size of the field of view, data storage capacity and tracking inaccuracy due to the complexity of synchronising multiple cameras). These alternatives all have associated complex difficulties that are not applicable to the new novel that is proposed in this thesis (IMU technology), which will be used in our study.

6.2 Inertial Measurement Units as Precise Tracking Method

6.2.1 Introduction

Traditionally, accurate but costly and cumbersome standalone inertial sensors have been used in aviation, shipping, and aerospace applications. Inertial sensing components have become very small and inexpensive due to the relatively recent development of micro-machined electromechanical system (MEMS) technology. This has been at the expense of reduced accuracy, but as a result of these ongoing developments, many new applications for inertial sensors have been enabled [166], including the one presented here. Focusing on using IMUs in orientation estimation will be presented in this chapter.

Inertial measurement units (IMUs) contain three dimensional (3D) accelerometers as well as 3D rate gyroscopes, and in many cases 3D magnetometers are also included. The functionality of MEMS sensors are based upon simple mechanical principles.

By exploiting the Coriolis Effect of a vibrating structure, the angular velocity can be measured; a secondary vibration is induced from which the angular velocity can be calculated when a vibrating structure is rotated whilst with a spring suspended mass, acceleration can be measured, as the mass will be displaced when subjected to acceleration [166].

6.2.2 Estimation of Orientation

Rate gyroscopes, magnetometers, and accelerometers comprise the sensing components in an IMU. Angular velocity, or rate-of-turn, is measured by the gyroscopes. The accelerometers measure the outer particular force, a specific force that is contributed to by linear acceleration as well as by the Earth's gravitational field, from which acceleration can then be derived. The local magnetic field is measured by the magnetometers. Both accelerometers and gyroscopes are affected by biases that vary slowly with time (i.e. drift). Information about an IMU's 3D orientation is provided by the combination of all of these sensors. The angular velocity can be integrated to get orientation, which is measured by the gyroscopes. Using the outputs of the accelerometers and magnetometers, the integration drift and noise can be compensated for [166]. Previous research [167] has shown that use of a Normal Kalman Filter in DCM (Direction Cosine Matrix) technique helps to avoid the first order approximation error. In the DCM method (1), which is used for attitude and orientation estimation, vectors are rotated by multiplying them by a matrix of direction cosines:

$$\mathbf{Q_G} = \mathbf{RQ_P} \qquad \text{Equation 6-1}$$

$\mathbf{Q_P}$= a vector measured in the frame of reference of the plane.

$\mathbf{Q_G}$= a vector measured in the frame of reference of the ground.

R= Rotation matrix

IMUs which are available commercially are calibrated ordinally at production.

6.2.3 Previous Work With IMUs

6.2.3.1 Animal and Human Behaviour Measurements

Inertial measurement units have been integrated into a number of devices, e.g. mobile phones, and are now available as chipsets. Similarly, very small wireless IMUs have been produced that can be used as components in an extensive array of applications, including human or smaller animal body mounted devices, allowing for accurate recording of an animal's responses [166], including, as in this instance, pigeon head movements.

The implementation of such remote sensor systems allows comprehensive behavioural response data to be collected with minimal involvement of the researcher. Inertial measurement unit (IMU) technology has been used previously in animal behaviour research. For example, a data logger incorporating a 3-axis gyroscope, a 3-axis accelerometer, and a 3-axis magnetometer was developed and externally attached to Japanese amberjacks to assess the validity of MEMS technology for monitoring the movement performance of the fast-start behaviour of fish in the field [168]. Additionally, 3D accelerometers have been used to enable the monitoring and classification of behaviour patterns in cattle, crucially without human interference, which cannot be achieved by the human observation as the natural behaviour is normally disturbed by the traditional observation [169]. Three-dimensional accelerometers have also been used for automated recording and classification of grazing behaviours in goats [170] as well as vertical movement symmetry (MS) in clinically lame horses through attachment of the IMU over the sacrum and the left and right tuber coxae [171].

Three-dimensional accelerometer has been used as automatical method for measuring and recognising several behavioural patterns of cows[172]. Similarly, MEMS sensors have been identified as a technical solution to assist clinical research into the early detection of gait disturbance in horses [173].

Also, recording human movements has proven to be very valuable for the monitoring of personal activity for health purposes, e.g. by detecting steps, estimating stride lengths and the directions of motion, etc. [174], as well as for clinical applications [175] where the clinical specialist or physiotherapist is able to assess the movement of an orthopedic outpatient [176].

IMU has been used in the researches by utilizing human activity and motion in order to build approximate maps of structured environments. A data suit (equipped with several IMUs) used to detect and record movements of a person and door opening and closing occurrence [177].

Inertial tracking device has been used to measure the angular rotations of the pelvis and lumbar spine as well as their sagittal rhythm during forward flexion and backward extension in upright standing of eight asymptomatic males [178].

The researchers were capable to track the motion of the lower limb by covering the lower limb segments with 7- Inertial Measurement Units (motion capture system) [179].

Localization and the human velocity in sports training and exercises have been tracked by using 3 IMU sensors [180].

6.2.3.2 Birds Behaviour Measurements

A number of major challenges exist when implementing MEMs technology for the quantification of animal behavioural dynamics, particularly when the species in question place an evolutionary premium on being lightweight. Birds in general are well adapted to flying, e.g. with low bone density as well as an extensive respiratory system that apportions a number of airfilled sacs within the mediastinum. Many birds must also balance their energy intake with their body mass in order to maintain an optimum flying weight. With this in mind, it is necessary, when implementing body or head mounted sensors, to be aware that normal behavioural responses may be significantly affected by the addition of even a small amount of extra weight. Although a number of studies have used MEM technology to mount devices to the back of pigeons and other birds, without impairing their ability to fly, head mounted devices have been attempted far less often.

A study into how pigeons interact with one another in their hierarchies and flocks, for example, involved the use of small backpacks, which housed GPS receivers. Specifically, a backpack weighing 16 grams was attached to pigeons using elasticized fabric bands [180].

Another study, investigating flight dynamics in raptors, used an MTi-G IMU, 58 x 58 x 22 mm in dimensions and 58 gram in mass, which was attached to the birds with Velcro straps [181].

Another example [182] that serves as a useful reference is an investigation of how pigeons navigate through forests. In that study, a GPS recording device, consisting of a hybrid GPS receiver board, a data logger, a GPS patch antenna, a DC-DC converter, a power supply, a status display, and a connector were mounted on the birds' back, all of which had a combined mass of just 33 grams [182]. Additionally, a camera was mounted on the pigeon's head, connected with cables to the body mounted backpack, all of which were found not to impair normal behaviour [182].

There can be found many examples of placing IMU's on the back of birds. An IMU with GPS logger, a camera, and integrated SD writer was placed on the back of an eagle by American Institute of Aeronautics and Astronautics in a study. With the dimensions of 100 x 50 x 35mm, a custom designed unit, and weighed 104 grams, IMU was used in this study. At a rate of 25Hz the data was captured. The weight and size of the IMU in this experiment is very greater than the pigeon's limits (individual component sections discuss it further) yet this study proves that high movement speeds can be registered by the IMU [183].

Another study of devices and packages being mounted to pigeons, the Sim cards connected to GSM (mobile network signals) weighing 28 grams and GPS receivers on their backs, pigeons were released for the company Fogg 4 as a publicity stunt. As they flew over major European borders and cities, via their own twitter accounts, these pigeons would then send Tweets. Due to the size and weight of the device according to the imposed limitations in this project, this application is of interest. Demonstrating that if smaller or similar sizes can be obtained in this report, with packages of this size on their backs pigeons can fly and the movements of pigeon will not be confined in such a way as to affect the results of the testing [184].

A white water rafting centre in Colorado America, and a more simplistic application, is utilizing homing pigeons to carry memory cards to the base station towards end of the rafting route. Only a few grams weight is used in this application, the interest however is the way backpacks were developed as this was made totally from elasticized fabric and after completion of this project, a comparable design could be implemented [26].

Harvard University has done very similar experiment to the one being performed in this project which is based on how pigeons navigate through forests. Placing a recording device on the pigeons back and a camera on the pigeons head is involved in their study. This research shows that it is possible to have two devices mounted to a pigeon connected through cables and the head movement is not restricted in an unnatural way [185].

A study has been performed to investigate wing morphing, vision-based guidance, automatic flow control, control on free-flying birds, and navigation eagle [28]. From falconry enthusiasts, different trained birds of prey were available locally. Locally available computerized camcorders have been utilized as a part of flight feature recordings, which is industrially accessible as a spy cameras in stereo arrangement. For recording the deployment of automatic flow control devices and using fiducial markers, these cameras have been utilized for photogrammetric reconstruction of wing morphology. To provide information on the physical and visual context of the mechanisms that is recorded, onboard video has been utilized in this study [186].

Researchers was capable of developing very small low-cost strap-down inertial navigation platforms due to latest advance in MEMS technology. A miniature (50g) attitude and heading reference system which is n Xsens MTi platform has been used that calculates almost drift free 3D orientation using magnetometers, rate gyros, and accelerometers. Calibrated 3D acceleration, 3D Earth-magnetic field data, and 3D rate of turn are also provided by its low-power signal processor. To obtain position data (<3m accuracy), low-power Fastrax Upatch100 GPS receiver have been used with integrated antenna (10g). Using a Sensortechnics HCXM100D6 DPT (5g) airspeeds have been measured. Using a PIC 16F877 microcontroller, seamless integration of these three sensors has been achieved, that take samples of information from the three sensors and either send it to a base station up to 900m away by means of a Maxstream 9Xtend radio modem (18g) and/or store it on a multimedia memory card (through an inherent SPI interface). Total weight of <110g, including battery was included in the system, which is <5% body mass for the species with which the work has been accomplished. For studying feather deployment in automatic flow control, frequency of the wing beat, and owing to the high speed of attack manoeuvres, high-speed video was essential. With storage capacity for 2048 frames, which records full frame (1280 by 1024 pixels) at 500 frames per second, a MotionScope M3 was used. Camera possesses a built-in rechargeable battery and is ruggedized for field utilization. In the Southwell Laboratories and Zoology Department of Oxford University, flow visualization facilities and a range of low turbulence, low-speed wind tunnels equipped with force transducers, etc. are available. To test wing morphing devices and models of the automatic flow control, these have been used.

A lightweight (35 g including battery and casing; 40x68x18 mm) GPS data logger which can record paths of flight of pigeons and dogs with an accuracy of ± 12 m. It has been attached to the back of the animal [187].

6.3 Instrumentation Details

6.3.1 VTA System

Video tracking analysis is a method that relies upon post-hoc digital signal processing (DSP) of behavioural responses and is often dependent upon sophisticated image processing algorithms. Our custom-designed VTA system comprises a general specification PC (CPU:i5, RAM:8GB) with an SSD (Solid State Drive) to allow saving of video data at speeds of up to 60 fps as well as a digital video camera (FireWire CCD Monochrome DMK 21BF04 [181]) capable of 60 fps, with a specialised lens (M0814-MP). The camera is mounted facing down into the centre of the optocollic reflex (OCR) (see 6.3.1.1 for explanation) arena comprising six LCD monitors for presenting visual stimuli (drifting vertical grating). A white hard plastic fiducial marker (14mm × 5mm × 3mm), with parallel, perpendicular black lines offset towards its front end, is affixed to the top of the pigeon's head with double-sided sticky tape. Control of the camera recording during experimental trials and the post-hoc frame-by frame extraction of head angles are performed using custom National Instruments LabView software. VTA has proven to be particularly effective in the analysis of complex head movement responses but, as described previously, has a number of inherent limitations. Figure 6-1 depicts the general operation of the tracking system used in this research. The microcontroller is used to acquire the signal, which has been obtained from the tracking system (IMU or camera), and which is then passed to the PC where the data can be analysed, calibrated and displayed for the user.

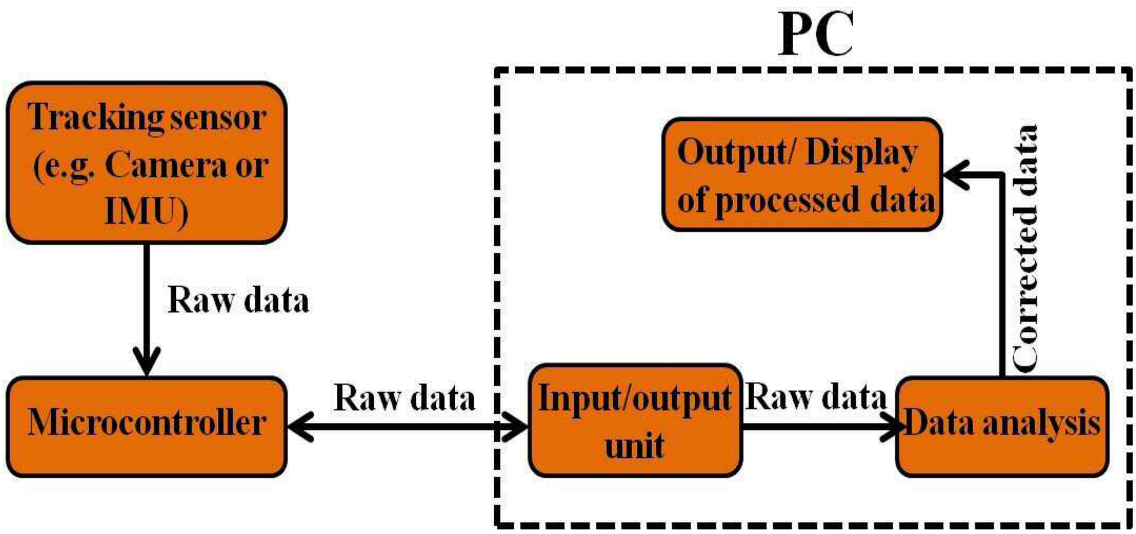


Figure 6-1 General schematic diagram of a tracking systems (camera or imu)which are used in this research.

6.3.1.1 OKN/OCR

When travelling through our environment, or when moving our heads, the visual scene around us is translated across the retina, which causes retinal slip. This engages a reflexive compensatory eye movement process, which acts to stabilise the image reaching the retina. There are two types of stabilising movement apparent in mammals: the vestibular ocular reflex (VOR) and the optokinetic nystagmus (OKN).

OKNs are involuntary eye reflex movements that enable almost all vertebrates to reliably track a moving stimulus whilst their head remains steady. This enables a stable and clear perception of the world around us.

The OKN gaze stabilising function occupies a crucial role in the production of a clear perception of the visual world as we move through it, or as it moves around us. All types of self-initiated motion can cause the visual world to appear to move in a specific direction which depends upon the direction of the self-movement. This is known as optic flow [188]. With no OKN response, the optic flow patterns render the visual world an unclear perception in relation to the optic flow lines. OKN avoids this by tracking the speed and direction of the optic flow in a smooth motion, before quickly moving against the optic flow via use of a saccade, in order to reset the eye's position. During this resetting phase, no blurring of the visual world occurs. This is because of a phenomenon known as saccadic suppression, which maintains the stability of the surrounding environment [189]. Without this reflex we would not be able to accurately move through our environment, and we would become disorientated during self-motion very easily.

The OKN response is initiated by large scene image motion across the retina [190]. It is an innate response involving a 'slow' (tracking) phase, i.e. a smooth pursuit, followed by a faster (resetting) phase, i.e. a saccade [191]. These two components repeat in a cyclic fashion so as to maintain fixation [192] (see Figure 6-2).

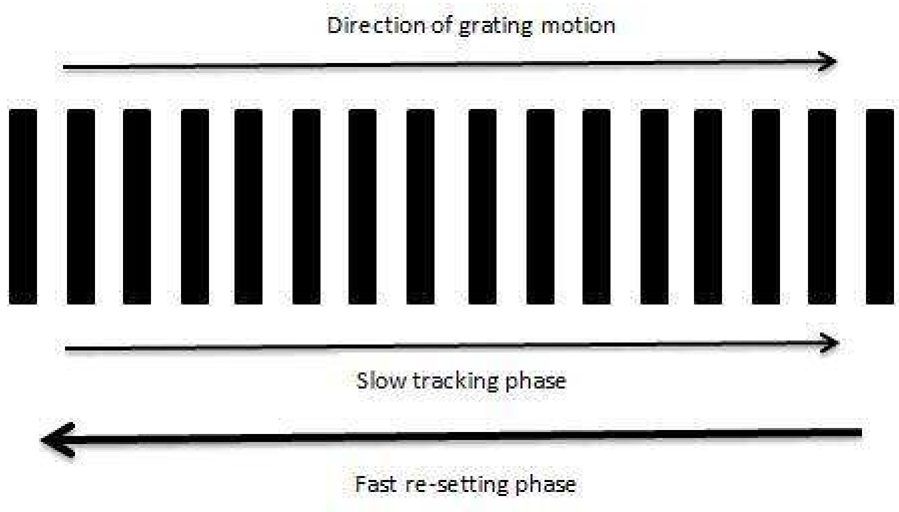


Figure 6-2 Direction of the smooth pursuit, and of the saccade, in relation to a moving square wave grating stimulus that was used to invoke an OKN response [193]

The smooth pursuit movements occur in the same direction and at a similar velocity to the stimulus motion. However, although the eye velocity does not perfectly match the stimulus velocity it is accurate enough to ensure stable perception. The difference between the stimulus velocity and the eye velocity is known as the 'gain' of the optokinetic system. This gain prevents complete retinal stabilisation from occurring. The saccadic movements occur in the opposite direction [195], against the optic flow, and direct the eyes to a location slightly eccentric to the fovea to allow them to track further before becoming restricted by the action of the extra-ocular muscles and the structure of the orbit.

Birds rely much more on head movements. Gioanni found that in the unrestrained position OCR (Oculocardiac Reflex) accounted for 80-90% of gaze stabilising movements. OCR is a reflex mechanism that makes small, involuntary head movements in response to optokinetic stimuli in order to keep the visual world stable. This is very similar to human OKN and the traces produced when measuring both are very similar [196] (see Figure 6-3).

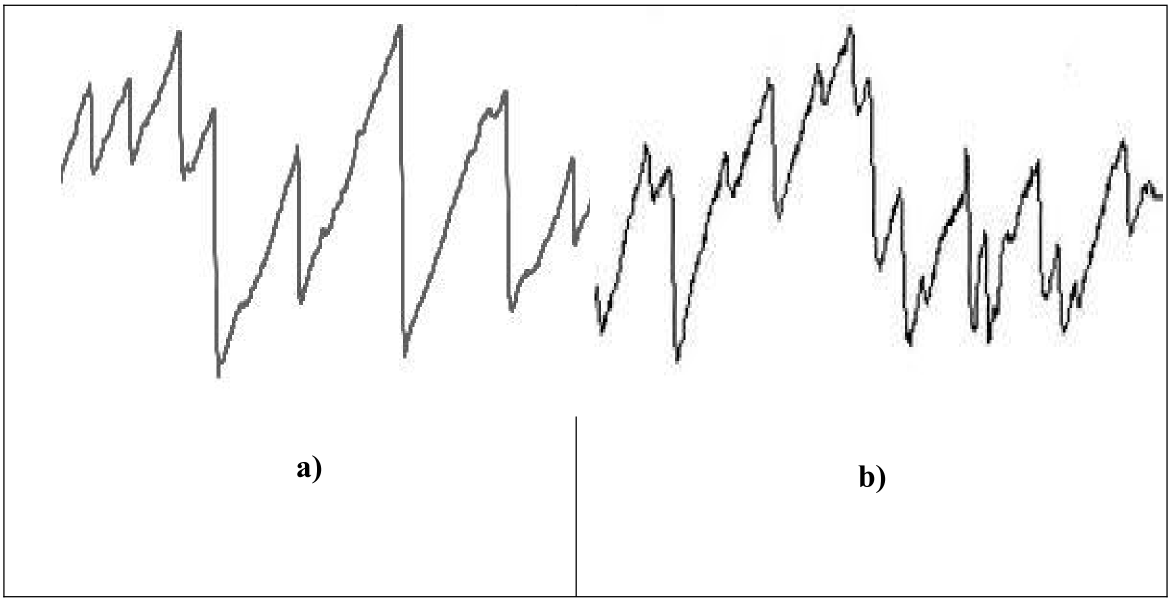


Figure 6-3 a) Pigeon OCR trace, b) Human OKN trace [194]

6.3.2 Inertial Measurement Unit (IMU) System

To address these limitations, we have developed a novel method that is based on the use of an IMU with 9 degrees of freedom (DOF), i.e. a triple axis gyroscope (G) measuring angular velocities around Gx, Gy, and Gz; a triple axis accelerometer (A) measuring acceleration along Ax, Ay, Az; and a triple axis magnetometer (M) measuring magnetic field along Mx, My and Mz, all of which are required for the IMU unit to be able to detect and measure the orientation of the pigeon's head in 3D space. Real time measurements are obtained at 512 Hz (128 samples/sec) from the combined data of all of these sensors, encased within the IMU.

In order to ensure that the Attitude and Heading Reference System (AHRS) algorithms are able to function correctly, all three sensors (magnetometer, accelerometer and gyroscope) must be correctly calibrated. Hard-iron calibration has been established for the magnetometer to compensate for the hard iron biases introduced to the magnetometer by any surrounding metal or electronic devices. The IMU was calibrated based upon a maximum angular velocity head movement of 200˚/s, and thus, the measurement range of the gyroscope has been set to ±2000°/s. This value was chosen as this easily deals with the maximum head movement speed These algorithms have been programmed to use the outputs from the gyroscope, accelerometer and magnetometer to obtain angular velocities of the pigeon's head (gyroscope), the direction of gravity (accelerometer), and the Earth's magnetic field (magnetometer) in the x, y and z axes. The measurements derived from these three components contribute to an overall 3D representation of the IMU unit (in space). Angular velocity measurements derived from the gyroscope are used to filter errors in the estimated orientation, i.e. those caused by linear accelerations and temporal magnetic distortions. In parallel, accelerometer measurements provide an absolute reference for the pitch and roll components of the estimated orientation. Earth's magnetic field measurements, obtained from the built in magnetometer, are used to provide an absolute reference for the heading component of the estimated orientation.

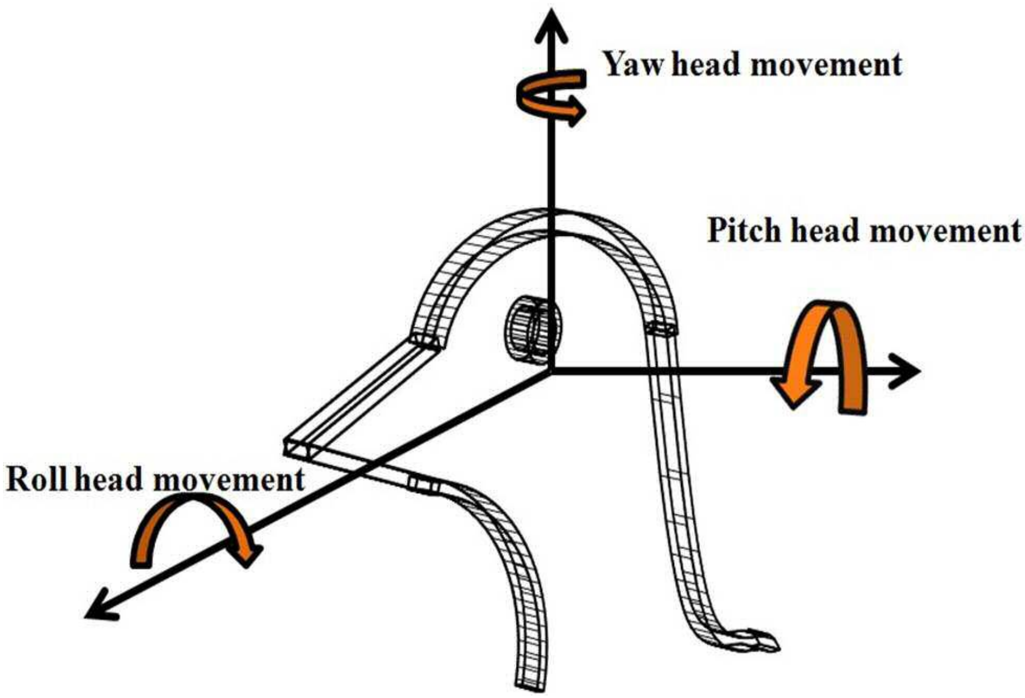


Figure 6-4 The three axes of pigeon head movement

The IMU device outputs 12 variables in total: three rotational angles (roll, pitch and yaw Figure 6-4 , as well as acceleration, angular velocity and magnetic field density, all of which are output in three dimensions and are free from drift. Custom software has been designed to display real-time raw and calibrated data graphically to allow the experimenter to be able to monitor the behaviour of the subject in real time.

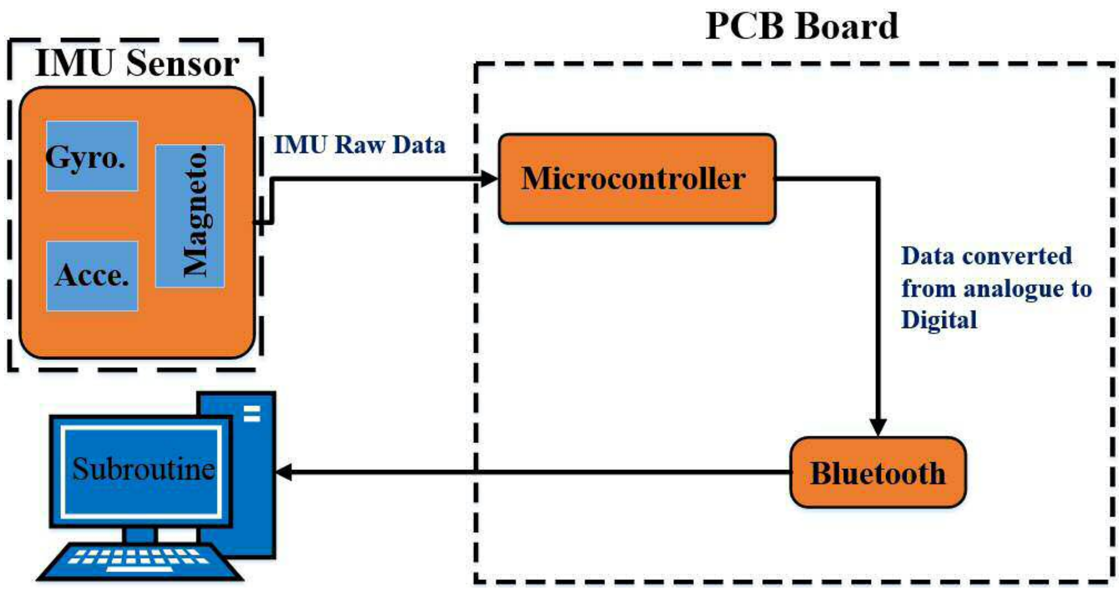


Figure 6-5 A schematic illustration of the new tracking system. the IMU sensor is placed on the head of the pigeon, and the PCB is placed on the back of the pigeon. the microcontroller is used to acquire the raw data (signal), which are obtained from the IMU. the microcontroller then converts the signal from analogue to digital. in turn, the data (in digital format) are passed to the pc via a bluetooth connection.

The size limit of the IMU, imposed by both the biological and physical constraints of the pigeon, is found to be 20.3 × 12.7 × 3 mm. The basis of this limitation is derived from the aforementioned adverse influences there might be on normal behaviour if the device were to hang over the edge of the head and obstruct vision. A weight limit of ≤10 grams is imposed for similar reasons.

As illustrated in Figure 6-5, the IMU board communicates with the microcontroller, which processes the raw data from the three sensors within the IMU and derives an estimate of IMU's absolute orientation. In turn, these data are output through the microcontroller's serial interface. In order to minimize any disturbance of the normal behaviour of the pigeon, the system is fully managed via wireless communication. Power is provided to the system by lightweight rechargeable lithium polymer cells (400mAh). In order to create a backpack unit as small and light as possible and with no unnecessary wires, a PCB board was printed to host all of the components, including the batteries, independently of the IMU.

As with the size and weight restrictions associated with the IMU, a weight restriction of <30 grams is imposed on the backpack.

6.3.2.1 Experimental Set-up

An optocollic reflex (OCR), which comprises a ‘slow’ (tracking) phase and a ‘quick’ reset phase (or saccade) in response to optic flow, was elicited in two restrained adult homing pigeons using a 0.16 cycle/^{o} square-wave stimulus at different levels of contrast, presented within a 360^{o} hexagonal arena consisting of six 17 inch LCD screens (see Figure 6-6). The angular velocity of the stimulus was increased from stationary to 60^{o}/sec stepwise in 4^{o}/sec increments every 20 seconds. All stimuli were generated and controlled using custom MATLAB code. Food and water were constantly available ad libitum other than the duration of stimulus presentation, which did not exceed 10 minutes. Pigeon restraint was in the form of an elasticated tubular bandage. Once restrained, the pigeon was placed on a firm foam base and covered with a matte black sheet, except for its head. Two computer fans were used to generate airflow through the arena to prevent the pigeon from overheating. All blinds and doors in the laboratory were shut in order to eliminate any natural light, and the entire arena was covered with a black matte drape. All experimental procedures involving animals complied with the U.K. Home Office legislation and the European Communities Council Directive 86/609/EEC (1986).

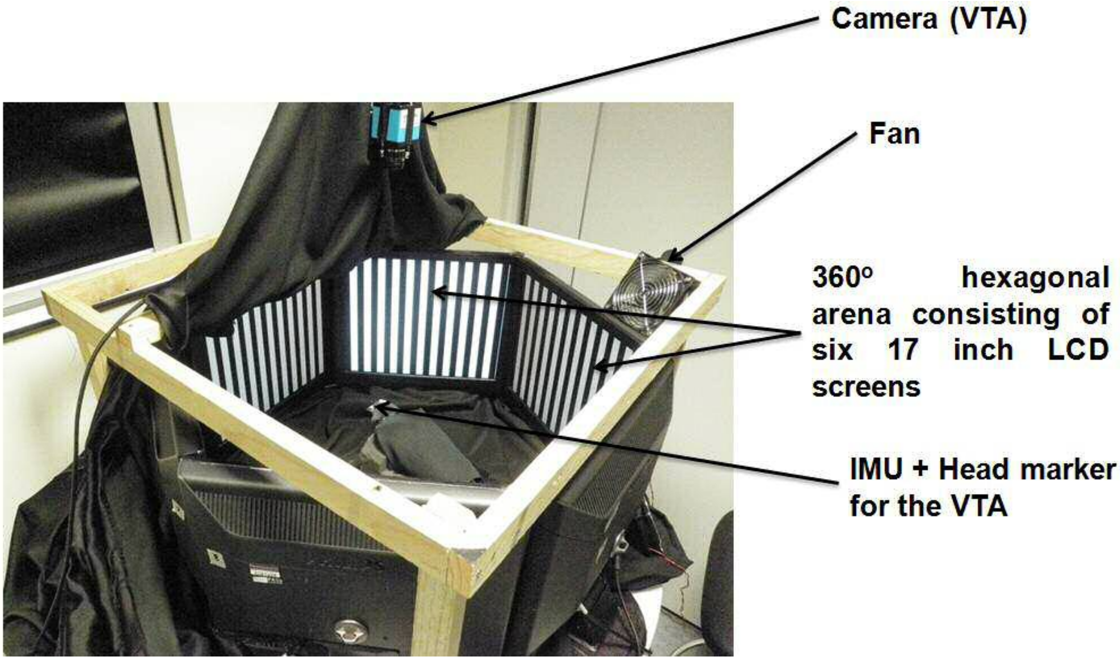


Figure 6-6 Experimental apparatus to elicit the optocollic reflex (OCR) consisting of a ring of 6 LCD monitor screens on which square wave grating stimuli are presented to pigeons retrained on a central platform

6.3.2.2 IMU Placement

For data collection using the IMU, a custom-made lightweight leather hood was placed over the head of the restrained pigeon and gently secured with adjustable straps. In turn, the IMU was attached to the hood with Velcro, Figure 6-7. The backpack unit (PCB board), connected to the IMU, was placed in a lightweight casing, which was gently strapped to the back of the pigeon. For trials in which both IMU and VTA analyses were carried out simultaneously, the hard plastic fiducial marker was attached to the top of the IMU. Note that the pigeon's eyes were completely unobstructed.

Figure 6-7 IMU placement on the pigeon's head. a custom made leather hood is used to velcro-mount the IMU to the pigeons head without obscuring its field of view.

6.5 Validation of The New System

Even with the limitations described previously, VTA is the gold-standard tracking method used to record behavioural responses in the yaw plane. As a result, it was important to first determine whether the IMU effectively replicates this capability. Critically, this validation was necessary to determine whether the position of the mounted IMU on the pigeon's head affected the activity of the pigeon, in addition to checking the overall reliability. As VTA measures only horizontal movements, this validation stage involved comparing the yaw angle measurements using both approaches. To compare the two approaches effectively, data were obtained using both VTA and IMU measurements during the same behavioural session, as well as independently.

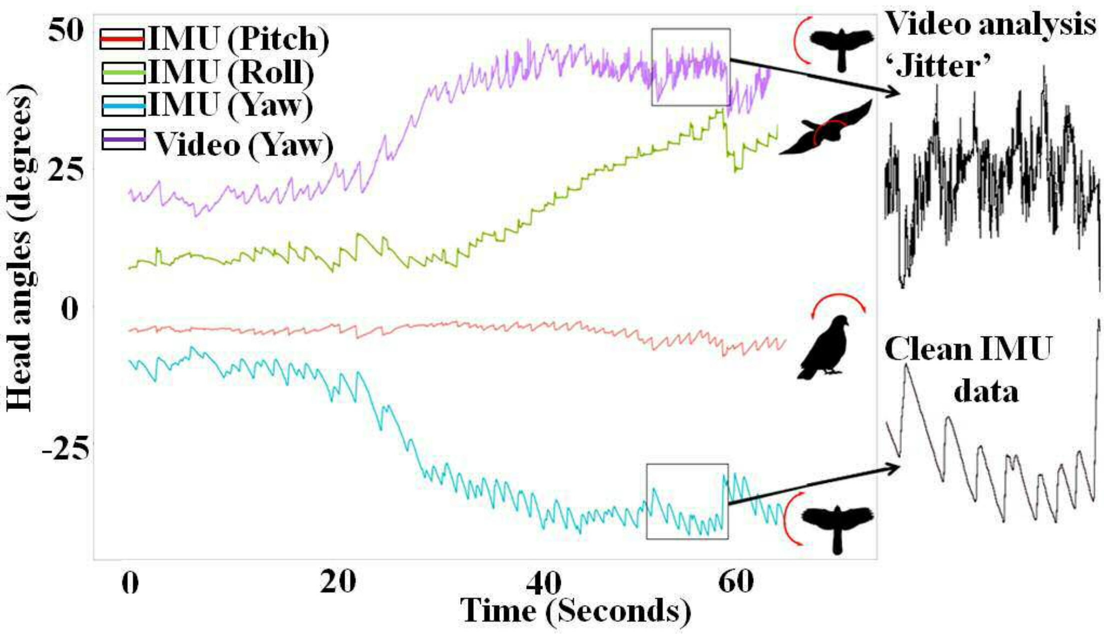


Figure 6-8 Comparison of raw head angle data from both systems: VTA (purple- yaw) and IMU (red- pitch; blue- yaw; green- roll). VTA data exhibit noticeable jitter that is not apparent in that derived from the IMU

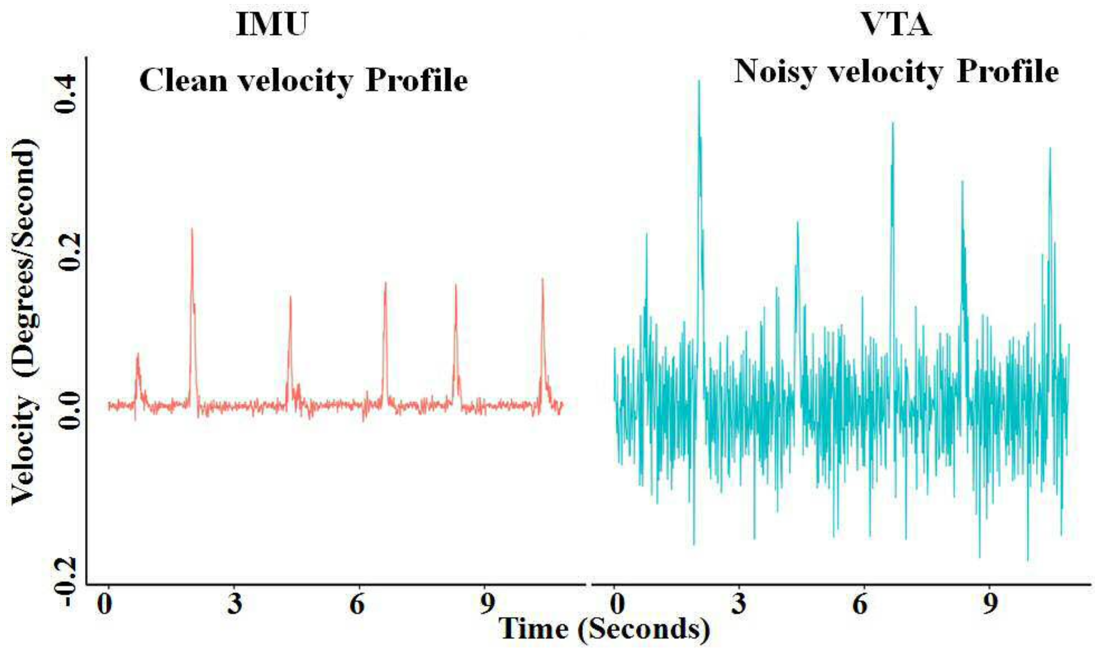


Figure 6-9 A comparison of velocity profiles recorded by the IMU (left facet) and VTA (right facet). the data from the IMU have a considerably higher signal to- noise ratio.

Figure 6-8 illustrates that the signal-to-noise ratio is better from the IMU as compared with VTA, as VTA is influenced by roll head movements. Data obtained from VTA were found to be much noisier than IMU data (Figure 6-9), largely as a result of tracking inaccuracies, i.e. ‘jitter’. Furthermore, atypical head movements (e.g. the bird turning its head to look up) resulted in significant loss of VTA data. Crucially, these did not affect IMU analysis, and indeed, this change in head position could be documented.

The adverse effects of tracking jitter in head angle waveforms, which resulted from differences in tracking accuracy between the two systems, were compounded by the inferior rate of data capture in the VTA system, i.e. the camera delivers images at a constant frame speed of 60 fps whereas the data obtained using the IMU captures 128 readings/second. While this issue could be overcome by using a higher frame rate camera, the benefits would be offset by increased data storage demands as well as considerably increased time required for post-hoc image analysis, all of which are not applicable when using the IMU.

In general, post-hoc analyses revealed that the IMU system approach is capable of giving far more precise data with considerably less tracking ‘jitter’. Moreover, the IMU provided positional information about the pigeon's head movements along an additional two axes of motion (i.e. pitch and roll; Figure 6-8), providing data that greatly facilitate and enhance the analysis of behavioural responses.

Post-hoc analyses of behavioural responses recorded using any given tracking method is often dependent upon the identification of saccadic movements and subsequently, in the case of the optocollic reflex, the separation of saccadic and slow phase movements. Given that ‘tracking jitter’ often has saccade-like characteristics, it is necessary to apply a filter to the data in order to remove this noise. Inevitably, however, during this process true data, e.g. low amplitude saccades or microsaccades, are lost. Through circumventing this issue with the use of the IMU, no such filter is necessary and the output information becomes both more accurate and comprehensive. The presence of jitter in head movement data is of even greater detriment to the analysis of head movement velocity plots (Figure 6-10), the profiles of which can be used to provide insight into both the nature of the response and the impact of the stimulus presented. Again, these issues are typically overcome through discarding sections of particularly noisy data but such non-ideal removal of data is made unnecessary with the IMU.

Subroutines written for the analysis of the slow phase velocity showed that the yaw angle produced by the IMU exhibited an almost identical measurement of the bird's head movements, spatially and temporally, Figure 6-10, but without data loss and with greater precision, smoothness and appreciably fewer anomalies.

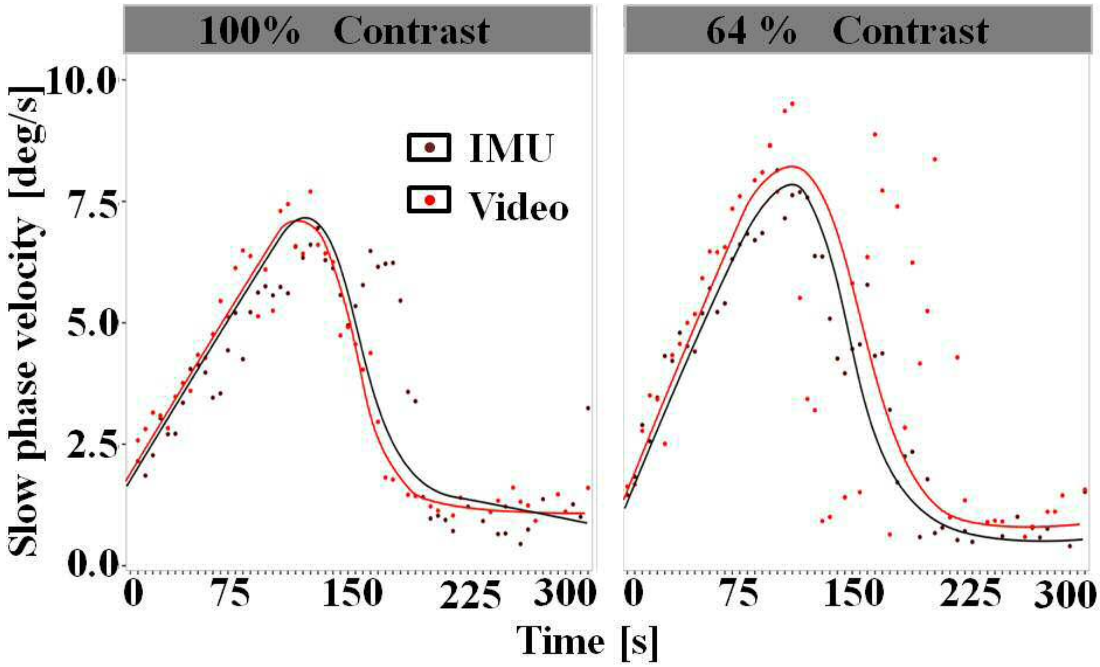


Figure 6-10 Validation of agreement between yaw data from the IMU (black) and VTA (red) systems at two different visual contrasts.

6.6 Additional Benefits of IMU Tracking

Head movements, like eye movements, consist of multiple components, i.e. yaw, pitch and roll. While VTA is limited to a single dimension, i.e. yaw, the IMU has the capability of recording movements in all three planes. Analysis of these additional pitch and roll components in a typical optocollic reflex reveal their temporal and spatial characteristics in ways that were not previously possible with conventional VTA, Figure 6-11.

Indeed, temporal analysis of the yaw, roll and pitch components together, within a complete optocollic reflex, reveals a far more complex sequence of events than previously appreciated. It is apparent from the 3D data obtained from IMU tracking (Figure 6-11) that, when a saccade initiates, the dominant yaw movement is accompanied by a roll movement in the same direction of the saccade as well as a vertical pitch movement that resets during the course of the slow phase movement.

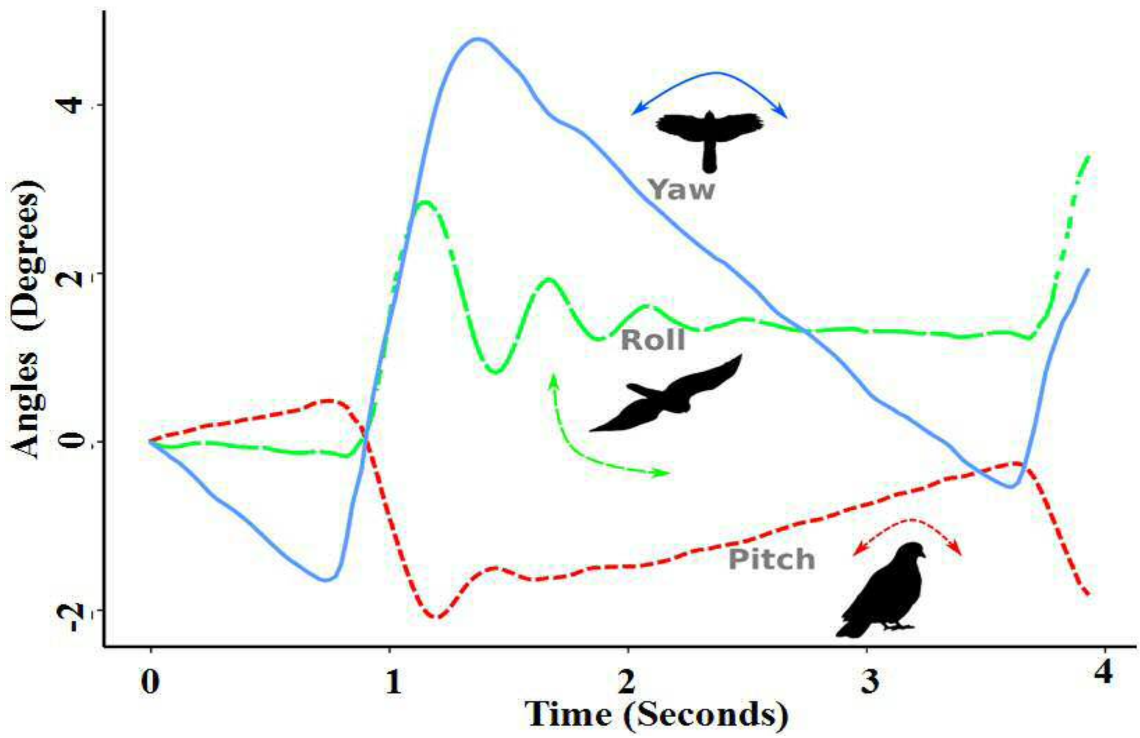


Figure 6-11 Three rotational angles: yaw (blue), roll (green long dashed) and pitch (red dashed) obtained from IMU tracking. Only yaw data are obtained from video tracking analysis.

6.7 Summary Of Chapter 6

The ability of birds to migrate accurately over large distances is, in part, due to their capacity to use the Earth's magnetic field for orientation. At present, laboratory-based research directed towards understanding the intricacies of migratory and/or homing behaviours (e.g. in response to magnetic cues) is limited by the efficacy of techniques that are suitable for the analysis of complex behavioural responses. An example of one such response is the optocollic reflex (OCR), a sequence of head movements often observed in birds that are analogous to the optokinetic eye movements seen in humans when viewing the world through the window of a moving train. This response in birds comprises an alternation between a 'slow' phase following head movement and a 'quick' saccadic reset head movement. Together, these visually driven head movements act to stabilize the image of the world on the retina to ensure good vision [197].

The present study describes the novelty of using (<10g) inertial measurement technology to detect behavioural responses in pigeons. The IMU approach was validated through a comparison with VTA, the current gold standard in quantitative behavioural analyses. The results demonstrate not only that the IMU is a fully capable alternative to the conventional VTA approach but also that it offers considerably more information (in the form of extra dimensions) as well as far greater accuracy and precision. This system programmed and calibrated to provide measurements of the three rotational angles (roll, pitch and yaw), these angles have been detected even they are outside the defined areas of tracking.

Inertial measurement technology holds great promise for the accurate, quantitative measurement of complex head movements, which, in turn, provide important information relating to how animals, including humans, respond to sensory stimuli and, ultimately, navigate. Our preliminary investigation confirms the potential of inertial measurement technology, which clearly overcomes many, if not all, of the limitations and shortcomings of video recording technology, while providing a range of additional benefits, including:

- Generating far more precise data, i.e. no jitter, by removing the requirement for the post-hoc frame-by frame analysis of video recordings of a fiducial marker. Providing lossless data sets, i.e. generating data from the entirety of each behavioural trial and not being limited by the need to keep the head in a set field of view.

- Minimising the requirement for large amounts of data storage
- Eliminating the need for post-hoc data extraction and enabling near real-time observations.
- Providing head angle measurements in all 3 dimensions (pitch, roll and yaw) rather than being limited to just one plane
- Producing additional outputs, such as acceleration, angular velocity and magnetic field flux measurements has enabled an improvement in temporal precision by IMU as high as 53% as compared to VTA.

Moreover, the provision of head angle data in 3D is critical to understanding the complexities of sensorimotor responses that are not confined to the yaw plane, e.g. head bobbing and orientation responses to changes in magnetic field.

CHAPTER 7

CONCLUSIONS AND FUTURE WORK

- 3-D Helmholtz coils were designed, modelled and constructed to generate a uniform magnetic field in the centre of the constructed system into which the pigeon (or other animal) could be placed. The coils were constructed with large dimensions to enable the investigation of the magnetoreception ability of different sized animals. In addition, their large size will enable the application of external stimuli and/or the use of a camera with various lighting systems. The system is fully capable of re-generating magnetic field conditions at any global location, which are [Null field, static field, and rotating field]. The system paves the way for a thorough investigation of magnetoreception in some animal species.
- The modelled data and experimental results prove that the system can maintain a uniform magnetic field of (±15 cm^3) volume at the centre of the 3-D Helmholtz coil system. The uniform magnetic field section is adequate for the animal species it is designed for.
- Video camera was employed to record pigeon's behaviour while exposing them to different conditions of manipulated magnetic field conditions.
- Video tracking analysis has been used to analysis pigeon's behaviour. Control condition was set as the baseline which plays an important role in the pigeon's response to the magnetic field condition. This enabled for the pigeons to respond to the slow or fast flipping field conditions when the artificial baseline has been used but not natural baseline.

However, in the case of Null field condition, it was revealed that the artificial baseline has affected number of saccades whereas the natural baseline has affected the saccades amplitude.

Pigeon's response to CW field, which proceeded by artificial or natural baselines, has the same response pattern.

On the other hand CCW has affected number of saccades more than saccades amplitude in both baselines.

- A novel way of improving the tracking system by using IMU technology for the head movement of the pigeons proposed, designed and implemented in this research study. IMU has been used to overcome the limitations of the VTA (video tracking analysis). The designed system has given more precise data as the user does not need to be concerned in keeping the head of the pigeon in the field of view of the camera. The user friendly IMU system has the added advantage of minimising the data storage requirements of VTA system. Furthermore the head position angles in three dimensions were recorded rather than 2D of the camera system.
- IMU has improved the tracking accuracy by %53, so it can be used to study other behavioural analysis such as head bobbing

Future work:

- 3D Helmholtz coil system could be used with other animal species as well as in humans in order to investigate the possibility of exploring magnetorecetion in species.
- A smaller version of IMU with wireless communication could be used for free flying pigeons for further studies of magnetoreception or other behavioural responses.

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