

A Novel Method for Investigating Magnetoreception in the Homing Pigeon

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Summary

The ability of migratory birds to perceive and orient to the earth's magnetic field has received a great deal of attention in recent years. However, there are only a few methods that enable the investigation of this complex paradigm of avian navigation based on magnetoreception.

A novel method has been developed for investigating birds' reactions to alterations in the ambient magnetic field under laboratory conditions, based on measuring head movements, and this approach is not dependant on season of the year, i.e. the birds' hormonal state.

Homing pigeons, when exposed to a magnetic field sequence of various magnetic field conditions (i.e. moving, static or the lack of any field), were shown to be aware of the changes, with the most abrupt reactions occurring at the appearance of the first sweeping magnetic field (which moved around the bird's head). Furthermore, the timing of the response was investigated, and the first head movement was often found to occur within only a few seconds (sometimes <1s) especially after the most salient transition, i.e. to a sweeping magnetic field.

The nature of the head movements made in response to magnetic field changes was scrutinized and found to be saccadic. The characteristics of these saccadic head movements appear to be similar across different bird species as well as being almost indistinguishable from human head saccades.

Recent studies have indicated that avian magnetoreception is based on vision, being both wavelength and luminance sensitive. Using this novel behavioural assay, the ability to respond to changes in magnetic field conditions was tested in the dark and the light. Darkness was found to have an adverse impact on birds' abilities to perceive magnetic field changes. Magnetoreception as measured by reactivity was virtually eliminated in the dark as compared with the same birds' behaviour in normal light conditions.

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Chapter 1. Introduction – theoretical background

1.1. Birds – machines for flying and the unsolved mystery of navigation

Over millions of years all animals, as well as plants, have undergone extensive adaptation in the course of evolution to be able to live in the often very different conditions of the ecological niches they occupy. This process of adaptive evolution is a tremendous wonder of nature that produces the miracle of the highly diverse species of the biological world. Indeed, looking at the flora and fauna, there are so many astonishing forms of life, each with their own distinctive features. How many human inventions are, in essence, nothing more than a copy of one or another natural “miracle” that has been adopted on a macroscopic scale (e.g. Velcro based on the burr plant). From simple living organisms like bacteria, through all invertebrates to the vertebrates, from fish, amphibians and reptiles, to birds and finally mammals, life covers almost every conceivable environment on the earth. Moreover, different species are able to survive in even very extreme conditions, often coping with them in amazing ways.

The group of animals that is focus of interest in this research is the class Aves, the birds. Birds are bipedal, warm-blooded, egg-laying vertebrates. There are around 9,000 to 10,000 different known bird species, which makes them the largest group among terrestrial vertebrates. Birds first appeared on the earth in the Jurassic period (around 150 million years ago) when they arose from the theropod dinosaurs. Since then, birds started to spread, conquering ever more parts of our planet. At the present day, these predominantly flying animals are present in almost every nook and cranny of the earth.

The principal feature of birds that makes them such a fascinating group of animals is their ability to fly. The human race from ancient times was trying to reach the sky. In the

end, we managed to take off from the ground into the air, but it would not have been possible without the inspiration from birds that flight was possible and without studying their features in detail and then implementing them in practice. All of these features (e.g. empty bones, plumage – unique to birds, a highly efficient respiratory system, and very strong muscles) together make birds able to fly. Thus, all parts of the bird's body are specially adapted for flight.

All of these properties (Whittow 2000) make them perfect machines for flying, especially when covering the long distances sometimes necessary either to find food or move to a different part of the earth when the local climate becomes harsh. However, this displacement, called migration when covering long distances or homing, which involves shorter-range flights, would not be possible without the ability to navigate. In the air, information about direction is limited as compared with that available when wandering on the ground. Nevertheless, birds cope with this challenge again in a masterly way. In fact, they are one of the most impressive of migratory animals, as they can navigate from one distant place to another with outstanding accuracy. The farther north birds live during the summer, the farther south they need to travel in the autumn, like the longest distant flier, the Arctic Tern, *Sterna paradisaea*, which flies as much as 48,000 miles a year, or the Wandering Albatross, *Diomedea exulans*, which might fly 36,000 miles between breedings in one year. The accuracy of migration is also extraordinary. Every year birds fly from their breeding site to the opposite (south or north) hemisphere and then come back to exactly the same spot that they left from. Taken together, these abilities indicate that birds must have a navigation system that is comparable to our GPS (Global Positioning System) guideline or possibly even better.

Similar to the intense interest shown over the ages to flight, the phenomenon of bird migration (and other migrating animals) continues to be investigated intensively. However,

although this behaviour has been known for a long time, the first publications concerning the means by which they can move from one distant place to another with such incredible precision first appeared only at the end of the 19th century. In contrast to solving the enigma of flight, the answer to animal navigation, in spite of numerous studies over the last 100 years, is still a mystery and the question of how birds use their orienting “devices” remains unanswered. Before humans became airborne, everyone had been dreaming about conquering the sky, and so it is today with our interest in the mechanism(s) underlying navigation during migration or homing. We look at creatures that can wander over the earth without the need of the aid of any conventional navigational devices and hope to find the solution to this ability, if only to be able to exploit another of the miracles from the animal world. All of the research in this field is motivated by the possibility that, one day, all the complicated machinery used in planes, as well as other means of transport, may be improved by an adaptation of one or more aspects of the animal navigational system. At the very least, such an understanding will provide insight into behavioural ecology and conservation of birds.

1.2. Cues used by birds in navigation

What exactly is meant by the term navigation? The Oxford English Dictionary offers several definitions including:

- To go from one place to another in a vessel; to sail. Also: to steer, control, or direct the course of a vessel; to sail a vessel
- To sail, manage, steer, control, or direct the course (of a vessel)
- To direct one's own course; to make one's way by walking, to move

Reference to the sail in the above examples is because of the Latin origin of the word navigation; *navigare* - "to sail", that is in connection with "to move" or "to direct." Therefore, navigation is an action used by any person, animal or machine in order to

guide their movements from place to place. This is, of course, a rather broad definition and would need to be more specific when referring to animal navigation. The term navigation, in the context of a bird's navigation as referred to in this thesis, will follow that introduced by Baker (1984): "Navigation is the art of finding one's way to a known destination across *unfamiliar* terrain, by whatever means."

However, as stated in the above definition, navigation has to be based on some directional information. We, as humans, use a large range of devices that tell us about direction, such as compasses, maps and/or very sophisticated GPS systems. In addition, to navigate in a less accurate way, it is possible to extract directional information from natural indicators like the sun (or shadows), the stars, or even moss on the trees, which indicates geographical north. Animals obviously cannot use artificial instruments to navigate; instead, they are limited to natural cues. However, this turns out not to be a drawback for them. On the contrary, they make incredibly elegant use of all the available cues that may contain directional information, reflecting the capacity of adaptive evolution. But how birds utilize different cues to navigate in such a remarkable way, is still an open question. Nevertheless, studies of this mystery have progressed to the point that mankind may be approaching a fundamental understanding of the structural and psychophysical basis of this phenomenon.

The earliest hypothesis concerning bird navigation was proposed by Viguiier (1882), who suggested that birds orient in space using the earth's magnetic field intensity and inclination. Other early studies concerned remembering the route of the outward journey and using this earlier obtained information on the return journey (Reynaud 1898). Since that time, the number of published works about this topic has been constantly increasing. Many different theories have been developed which are based on the various possible cues

for navigation. In the search for a solution to a bird's ability to navigate, the following principal candidates can be differentiated:

- landmarks
- olfactory cues
- sun (or stars in the case of - night migratory birds (Able 1982))
- polarized light (which indirectly specifies the sun's location)
- the ambient Earth's magnetic field

In all probability, navigation relies not just on one factor alone, making the subject of birds' navigation so challenging to investigate. Interaction between the different possible cues has its impact on experiments designed to examine the processes underlying avian navigation. When one of the cues is altered in different ways, others may still be available to a bird and that is why the experiment may not achieve the intended effect. Therefore, the problem arises of how to exclude all possible alternative sources of directional information available to birds.

In the mid-twentieth century, it was suggested that navigation is necessarily constructed on the basis of at least two factors. This intriguing theory, which was well received by other scientists, is Kramer's two-step "map and compass" model. In the first step, a displaced bird determines the location of its goal based on an internally constructed map relating to their present position and thus determining the correct desired direction. In the second step, one or more compass mechanisms is involved in identifying the exact direction of flight (e.g. Kramer 1957; Kramer 1961). The map and the compass are believed to be constructed based on one or more of the different cues listed above.

1.2.1. Landmarks

The possibility of using landmarks as a source of navigating information has generated wide interest. It started with the three "step" theory by (Griffin 1952) in which he proposed

that birds rely on reference points. He distinguished different levels of complexity in using reference points. In the first step that operates in a familiar territory, birds rely on visual landmarks. The following step concerns flying in unknown areas, which is called “one directional orientation” and for which a good example is the homing of racing pigeons to their loft. Finally, the third step involves a bird’s ability to find the correct flying direction precisely in a territory far away from breeding sites that has never been explored before (called “true navigation”). This theory became the subject of interest in many subsequent publications (Keeton 1974; Able 1980)., although some controversy about this model remained (Keeton 1974). A study by Schmidt- Koenig and Schlichte (1972), in which birds were equipped with frosted lenses to impair, and ipso facto, to check the importance of vision in homing, showed that birds could home from a distance of 15 and even as far away as 130 kilometres. This result strongly suggests that landmarks play only a minor role among the possible navigational cues. In contrast, however, a recent study has claimed that birds may use linear objects, such as rivers, roads, etc., as references (i.e. landmarks) for guiding their flight path (Biro et al. 2004; Lau et al. 2006). Nevertheless, the navigation based on landmarks alone cannot possibly be involved in the case of migration over very great distances or when flying over unknown areas when birds must be forced to switch to different navigation cues. Moreover, as shown by Schmidt-Koenig, visual cues – if disrupted in any way – may be substituted by others. Nevertheless, the idea that landmarks can guide navigation in familiar territories is still accepted.

1.2.2. Olfactory cues

Studies on olfaction as a possible navigational cue started with Papi and his co-workers (Papi et al. 1971) in an investigation of pigeons, *Columba livia*, with sectioned olfactory nerves. Subsequently, a number of studies have been performed to determine the influence, if any, of olfaction on navigation (Wallraff 2004; Nevitt and Bonadonna 2005). Many

studies have shown that birds' ability to navigate deteriorates considerably when their sense of smell is disrupted, suggesting that odours can be important cues. In general, olfaction is believed to be used when flying to near- to medium-distance locations. The present knowledge about the role of olfaction in navigation, including both its drawbacks and its strengths, is well reviewed by Able (1996). Currently, there is an agreement that this cue is involved in the map rather than the compass in birds.

1.2.3. The sun as a navigational cue

The hypothesis that has attracted the greatest interest over the years concerns the sun as a factor that helps birds in navigation. According to this view, birds are believed to construct a sun compass and, on its basis, orient in space (Kramer 1957). This concept has been confirmed in clock-shift experiments that consist of manipulating the birds' internal clock. The birds' internal clock controlling circadian rhythms is based on the sun location over the day. This interaction is so tight that birds, combining information on the sun's position and the time of the day, are able to find the proper azimuth that indicates a specific geographical direction (e.g. north, south, etc.) on which basis they construct a sun compass. Experiments confirmed that even a small change in the birds' internal clock was manifested in misjudging the sun's azimuth and led to a predictable deviation of the birds from the correct home direction (e.g. Kramer 1961; Keeton 1974).

However, the exact way by which birds use the sun as a reference is not known. It is possible that they can use the position of the sun itself, polarized light patterns in the sky, various patterns of sky light intensity or even the direction and length of shadows. Another interesting explanation of how birds might use the sun involves the pecten, located in the eye over the optic disc. The pecten is a peculiar structure in the avian eye that is heavily vascularised. All retinal blood vessels are concentrated within this heavily pigmented structure that reduces the reflection of scattered light inside the eye, thus improving vision -

another extraordinary adaptation. One intriguing idea is that the pecten acts as a gnomon, which produces a shadow on the retina and, in respect to its length and location, provides the birds with directional information that is extracted (Pettigrew 1978).

1.2.4. Polarized light

It is also important to highlight the possible role of polarized light in navigation (reviewed in Able (1982); Horvath and Varju (2004)). The distribution of polarized light in the sky is connected to the position of the sun. Birds (in this case sparrows, *Passer domesticus*) have been reported to use polarized light to recalibrate their magnetic compass. This was indicated in the studies of Able and Able (1993) in which they investigated sparrows in a shifted earth magnetic field together with depolarizing filters, that prevented birds from seeing the natural polarized light patterns in the sky. They found that, in the case of a shifted field but continued availability of a natural view of the sky, birds shifted their migration direction in accordance with the field shift. However, when they were exposed to the same conditions but with adaptation of depolarizing filters that deprived them of observing skylight polarization patterns, the birds' migration direction remained unchanged. Skylight polarization patterns in the sky just above the horizon are believed to be used to obtain directional information. The confirmation of this assumption was given by similar studies, in which birds (Savannah sparrows, *Passerculus sandwichensis*) were exposed to polarized light patterns shifted 90° with respect to natural conditions at sunrise and sunset. The results show that this alteration of the polarized light direction shifted the birds' migration direction by the same 90° (Muheim et al. 2006). The integration of the patterns at sunrise and sunset, by providing indirect information about the position of the sun, seems to give reliable information about geographic north/south.

In the case of polarized light perception in homing pigeons, there are arguments for and against the ability of pigeons to do so. Delius et al. (1976) showed that pigeons indeed can

discriminate between different axes of the polarized light vector. On the other side of the argument, there are more recent studies contradicting pigeons' ability to perceive polarized light (Nuboer et al. 1994; Hzn et al. 1995).

1.2.5. Stars

For the sake of completeness, night migratory birds appear to use star locations to derive compass information. The problem in determining the position of, for example, the North Star, is that stellar motion is very slow and ipso facto hard to detect (Emlen 1975). However, numerous studies, some involving manipulations of a planetarium star map, indicated that such birds can locate the normal or an artificially displaced north or pole star (Emlen 1970).

All the above mentioned cues used for constituting avian navigation have some drawbacks or limits, not least that none of them is constantly available. The remaining cue - the earth's magnetic field, however, is the only cue to which access cannot be removed under natural conditions. That makes this cue of special interest, and thus considerable attention has been drawn to the geomagnetic field as a navigational cue and its detection by birds.

1.3. Complexity and problems in birds' navigation studies

Despite the vast array of studies, there is only a partial solution so far. This demonstrates that the subject is challenging. Indeed, several difficulties arise from the nature of birds. In particular, as mentioned previously, their navigation probably does not rely on only one factor but is rather based on at least two factors at any time. For instance, the sun is said to be used by the birds together with the earth's magnetic field, where directional information is extracted from the sun's location, as an east/west reference with respect to their internal (circadian) clock, and from the magnetic field, which provides

north/south information according to its inclination (Wiltschko and Wiltschko 2003). The evidence for the contribution of these two cues, the sun and the geomagnetic field, is given by Walcott and Green (1974) and Walcott (2005). Walcott proved this by attaching to the bird's head a pair of coils generating a magnetic field equal to 60 μ T in a north or south direction. Under overcast skies, birds with coils generating a north-directed magnetic field have their accuracy of migration decreased significantly, whereas birds with coils generating a south-directed magnetic field oriented normally. An interesting observation is that those birds with a magnetic field towards north still oriented correctly when provided with a brief view of the sun.

The next difficulty is that a role for olfaction was observed in birds that grew up in a loft with an access to the wind, whereas those whose loft was sheltered from the wind seemed to use different cues. So, it is likely that the cues from which birds extract directional information depend on their availability, which seems to be required in order to learn how to utilize them (Wiltschko and Wiltschko 1989).

Another difficulty is that the cues that birds rely on vary from one species to another. In particular, birds may use cues that are best in their location (Able 1982). Also, at different ages, birds might use different sources of directional information. Such a situation is present in juvenile birds – their ability to utilise a magnetic compass is innate but the magnetic map is learnt only during adolescence (Wiltschko and Wiltschko 2003).

The other issue contributing to the complexity of studies on birds' navigation, but not focused on their sensory capabilities, is also their internal spatial representation. This approach has not been considered in much detail as the ethologists have understandably focussed on the ecological stimuli that underpin navigation. However, for concise information on spatial representation in homing pigeons, see Bingman (1998). Although, the topic is interesting, the approach of the present study concentrates on enhancing our

understanding of the earth magnetic field stimulus and the pigeons' ability to sense the field.

All the above difficulties mean that the studies concerning magnetoreception are numerous and diverse in their approaches, techniques, the animals tested, and so on. For the present research, the intention was to focus solely on the cue constituted by the earth's magnetic field and the birds' ability to detect it. To better understand why this cue was chosen, it is desirable to have an insight into the nature of terrestrial magnetism.

1.4. Earth magnetic field (EMF)

The first remarks about the magnetic field of the earth appeared in ancient Greece in 600 B.C. and are attributed to the Greek philosopher Thales. The next references are found in Chinese literature from 200 B.C. onwards concern the properties of loadstone, which consists of the mineral magnetite. This discovery was presumably connected to the invention of the compass that is likely to have taken place around the same time in China. In Europe, however, the compass arrived much later, the first mention appearing in 1300 A.D. It can be seen that it took a long time from the recognition of the earth's magnetic field to the development of the capacity to utilise it. Nevertheless, since that achievement, it is obvious that the spread of civilization and further acceleration in technological development was facilitated by the magnetic compass and its measurement of the geomagnetic field.

1.4.1. Nature of the magnetic field

The origin of the earth's magnetic field is not fully understood. The explanation almost certainly lies inside the earth's core, which is mostly composed of ferromagnetic iron and nickel. Nevertheless, the ferromagnetism of these elements is not the explanation of the

origin of the magnetic field. According to the Curie temperature¹ theory, iron should no longer be ferromagnetic above its Curie temperature, which is around 770°C. In contrast to this, the temperature of the earth's core is much higher and reaches even 5,000°C. Therefore, exactly how the earth acquires its magnetic field remains unclear.

The geomagnetic field is thought to be associated with the dynamo effect. The dynamo effect explains the formation of the magnetic field around a celestial body. It also predicts the astonishingly long life of the magnetic field. To understand this effect, it is necessary to know the details of the structure of the earth's core.

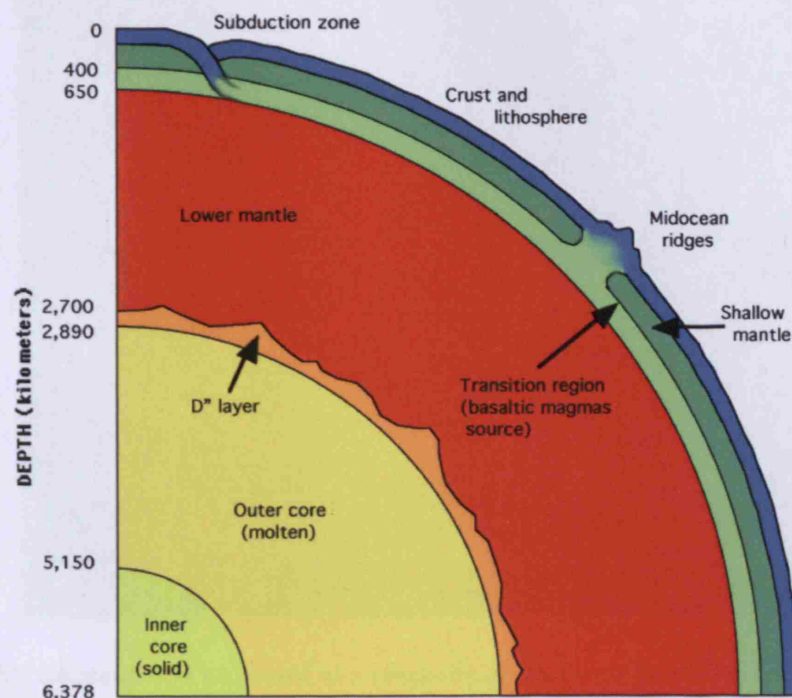


Figure 1.1 The earth's core map (www.chianti.geol.ucl.ac.uk)

Figure 1.1 illustrates a section of the earth's core map. Convection of the melted iron situated in the molten outer core plus the Coriolis force² are believed to be the origin of the earth's magnetic field. The Coriolis force aligns currents of molten iron in a whirl along the poles' axis. Then, conductive liquids cross the existing magnetic field, which induces

¹ Curie temperature – temperature above which ferromagnetic material becomes paramagnetic, i.e. no longer shows magnetic properties.

² Coriolis force – force which causes deflection from a straight path of the moving object on the rotating surface (e.g. wind on the earth).

electric currents, and these currents generate a consequent magnetic field. The dynamo effect takes place when the latter magnetic field amplifies the first one, thus allowing the resultant magnetic field to last as long as these two components exist.

The magnetic field is produced inside the earth, but the issue that is more important in this case is how the magnetic field acts *on* the earth. A good graphical approximation is shown in **Figure 1.2**.

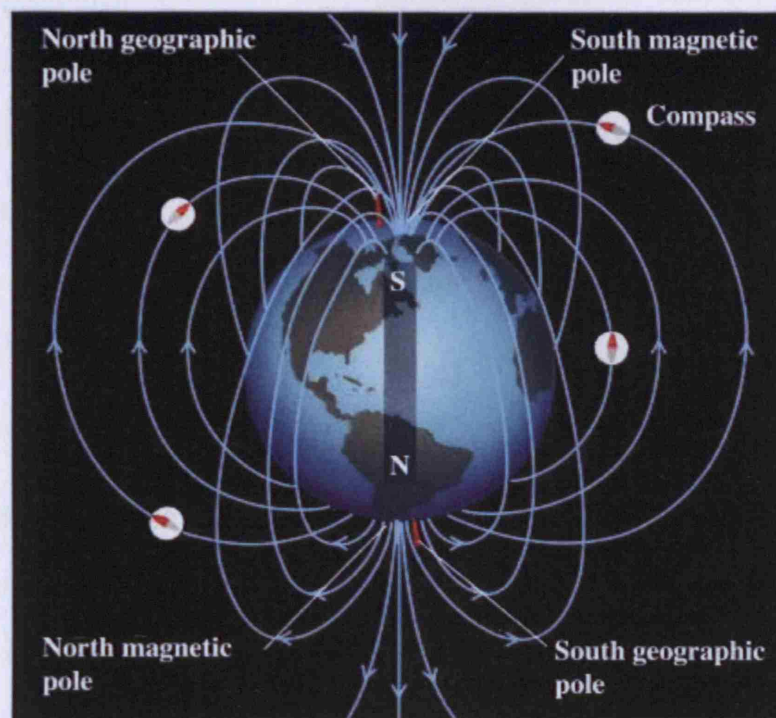


Figure 1.2 Earth's magnetic field presented as a magnetic dipole (www.bu.edu/core/cc105/lectures).

1.4.2. Characterization of the magnetic field

To a good approximation, the field is analogous to a magnet having two poles. One is situated near the geographic North Pole and the other near the geographic South Pole. Magnetic field lines radiate between the North and South Pole, making a detour around the earth at different heights, thereby creating the three dimensional magnetosphere. Magnetic and geographic poles do not coincide with each other, and in contrast to the geographic poles, the magnetic ones are not stable. At the moment, the magnetic field axis is tilted

11.3° with respect to the planet's axis of rotation. From the point of view of earth's history, the exact locations of its magnetic poles change continuously. Furthermore, the North and South Poles actually exchange positions with each other, which happens in intervals of tens of thousands of years; the last one occurred around 800,000 years ago (Lund et al. 1998). In terms of the effect such a reversal may have on animals, it is hard to draw any reliable conclusions due to the fact that the last reversal was so long ago, and the data available today are not accurate or complete. But it might be speculated that, because of the long intervals between reversal as well the fact that the process of reversing might take as long as several thousands years, such a change may not exert a high impact on most fauna, especially on those species that use the earth magnetic field as a cue for navigation. Another fact worth mentioning is that the magnetic poles are in the opposite hemispheres with respect to the geographical poles (i.e. north/south magnetic pole is in the south/north hemisphere), as depicted in **Figure 1.2**.

The magnetic field enables us to orient using a compass. The compass tells us where the north is but does not tell us any more information about the field, such as its strength. The components that are often used to describe the magnetic field vector at a particular earth location are: magnetic field intensity, magnetic field inclination, magnetic field declination and these are different in different locations on the earth.

The strength of the magnetic field ranges from 30 μ T (i.e. in South America and South Africa) to 60 μ T (i.e. around the magnetic poles in southern Australia and northern North America) as depicted in **Figure 1.3**.

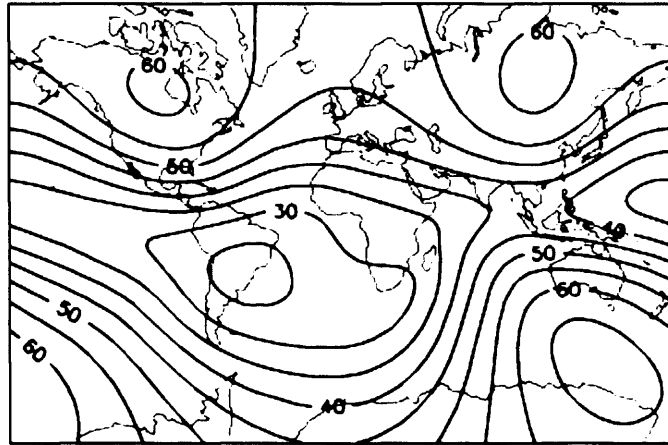


Figure 1.3 The earth's magnetic field intensity (in μT) in 1990 (U.S. Department of the Interior 2005).

The “strength” of the earth's magnetic field is a bit of an abstract concept. To have an idea of its values, it is helpful to compare it, for example, with permanent magnets, which are very popular magnetic materials. The strength of a permanent magnet's field is measured in T (Tesla), whereas that of the earth is measured in μT , which underlines the fact that the geomagnetic field is six orders of magnitude lower in strength.

Geomagnetic field inclination, sometimes also called dip, is the angle constructed between the horizontal plane of the earth and the plane, which is constituted by the field vector. This angle is 0° along the equator and 90° in the vicinity of magnetic poles. To distinguish between the northern and southern hemispheres, inclination is measured with a negative value in the southern hemisphere (see **Figure 1.4**).

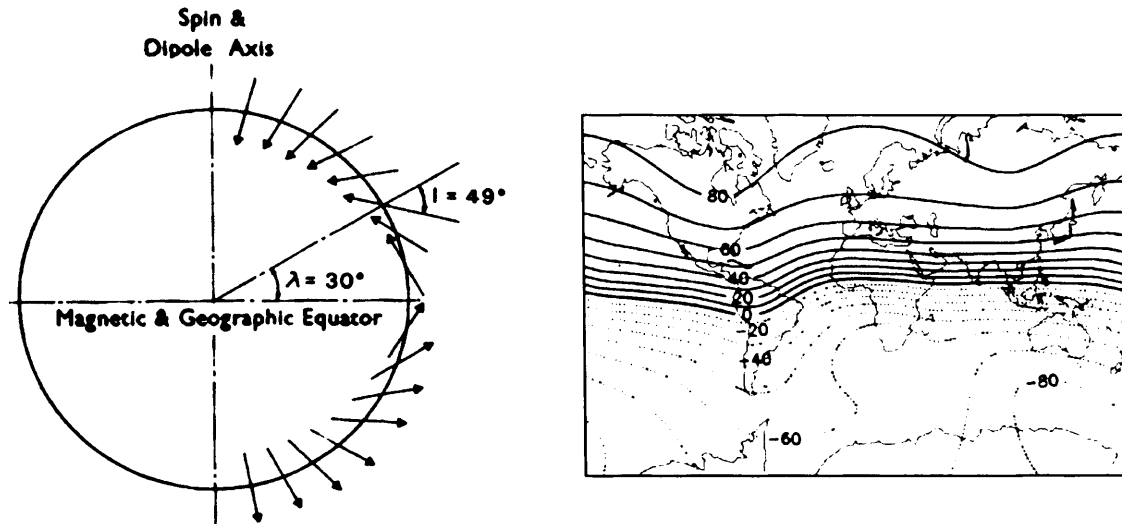


Figure 1.4 Left: inclination draft (www.geo.umn.edu), right: earth's magnetic field inclination in 1990 (U.S. Department of the Interior 2005).

The last component, declination, is the deviation of magnetic north from the geographic north, and it is positive when the deviation is to the east and negative to the west (see Figure 1.5).

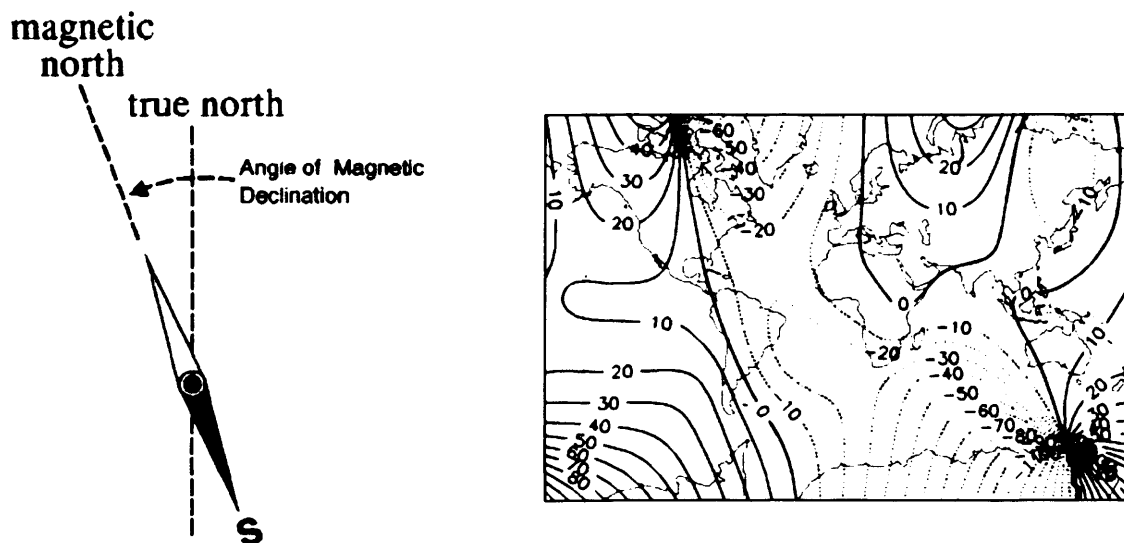


Figure 1.5 Left: schematic illustration of declination, right: the earth's magnetic field declination in 1990 (U.S. Department of the Interior 2005).

For example, the values, which characterize the earth's magnetic field in Cardiff, UK, are shown in the table below. These values were approximated in our experiments when reproducing the magnetic field of the earth.

Table 1.1 Local earth magnetic field condition in Cardiff (National Geophysical Data Center 2006)

Lat: 51°28'48'' Lon: -3°12'36'' Elev: 0 m	<u>Declination</u> + East - West	<u>Inclination</u> +down – up	<u>Horizontal</u> <u>Intensity</u>	<u>Vertical component</u> +down – up	<u>Total Field</u>
11/3/2009	-2°55'	66°23'	19,446.1 nT	44,491.5nT	48,555.6 nT
Change per year	9' per year	- 0' per year	16.3 nT/year	25.8 nT/year	30.1 nT/year

1.5. Biological perception of the earth magnetic field

1.5.1. Theories and up to date studies on magnetoreception

The theoretical assumptions made in the literature about the avian magnetic compass are reasonably consistent (Wiltschko and Wiltschko 1972; Ranvaud et al. 1991; Wiltschko and Wiltschko 2002a). Furthermore, it is known that birds can use the magnetic compass in their navigation. However, additional knowledge concerning magnetoreception, such as how birds are able to navigate by deriving information from the magnetic field, and precisely what part of the body is responsible remains to be determined. Specifically, following Kramer's model, birds are believed to possess distinct mechanisms for a magnetic field map and a magnetic compass. Currently, the two main theories that are used to explain what underlines magnetic perception are:

- magnetite in the beak (forming the neural basis for a magnetic map)
- radical pair process in the head or possibly eyes (forming the basis for a compass that may relate to vision)

The difference between these two components contributing to avian navigation is that the magnetic map indicates position rather than directional information, which is assigned to the magnetic compass (Wiltschko and Wiltschko 2002a). The nature and function of the magnetic map and compass are both introduced below.

1.5.2. Magnetite

Magnetite, the most magnetic material in the animal world is a member of the ferrimagnetic³ group of materials. Magnetite is a mineral having the chemical composition Fe_3O_4 , and its ferrimagnetism makes it vulnerable to changes caused by the ambient magnetic field. Although the magnetic properties of magnetite are not that distinct, as compared to ferromagnetic materials (e.g. iron, cobalt or nickel, which are basis of the magnetic industry), they are high enough to respond to a magnetic field even as low as that of the earth's. This is why birds' magnetic perception could possibly be based on magnetite, assuming there is an associated structure that permits transducing the effect of the field on magnetite into a sensory neural signal (Mora et al. 2004).

To have an idea of how such a magnetic map system might function in birds, a brief description is provided: The magnetodetection system is believed to rely on a magnetite-based magnetic field receptor. The receptors consist of chains aligned along the dendrites of neurons that attract clusters of magnetite, which are then associated with the nerve cell membrane, through which the magnetic information is conveyed to the brain. Thus, a magnetoreceptor is a single chain with a magnetite cluster. These magnetoreceptors are located in the birds' beak (e.g. Walcott and Green 1974) and, being aligned in different directions, are able to sense magnetic field strength in different directions. This in turn, is suspected to form a kind of 3-axis magnetometer, i.e. a device that measures magnetic field strength/intensity in 3 dimensions, forming an overall sense or measure of the local magnetic field strength. Furthermore, the sensing of the magnetic field is believed to be finely tuned, allowing birds to differentiate between magnetic field changes as low as 50nT. This is the process that enables birds to form a magnetic map, or in other words an

³ Ferrimagnetic materials- a group of magnetic materials that possess antiparallel alignment of magnetic moments but do not completely cancel out each other. This makes ferrimagnetic materials very weak in comparison to ferromagnetic materials, which possess a parallel alignment of magnetic moments and thus have distinctive magnetic properties.

“image” of the local magnetic field, which is distinctive to a specific place on the earth’s surface (Fleissner et al. 2007).

Magnetite particles were found in the beaks of bobolink, *Dolichonyx oryzivorus*, (Beason and Brennan 1986) and pigeons (Walcott and Green 1974). Interesting studies have been performed by applying strong magnetic pulses to other birds (Australian silvereyes, *Zosterops l. lateralis* and European robins, *Erithacus rubecula*) (Wiltschko et al. 1994; Wiltschko and Wiltschko 1995; Wiltschko et al. 1998). Using the magnetic pulse (intensity of 0,5T), they intended to change the magnetization of the magnetite-based receptor, and to provide evidence that such a sensor exists. Results showed that, after exposure to such pulses, migrating birds become disoriented, but around ten days after the pulse treatment, birds could again navigate with the same accuracy as before treatment. These findings are difficult to interpret. However, they suggest the existence of a magnetic-based receptor, which can be disrupted by a magnetic pulse.

According to recent research, iron (magnetite)-based receptor is involved in the avian magnetic map rather than the compass. To show that dependence, young juvenile birds (i.e. Australian silvereyes), which only have an innate compass, were subjected to a magnetic pulse (Munro et al. 1997). In contrast to the results with experienced birds (Wiltschko, Munro et al. 1994; Wiltschko and Wiltschko 1995; Wiltschko, Munro et al. 1998), the pulse did not affect their direction of migration; the birds’ headings did not differ from the headings in control experiments. This is consistent with the findings that such young migrants do not yet possess a magnetic map sense. Therefore, the mechanism responsible for the magnetic map formation has been attributed to magnetite.

1.5.3. Radical pair process that may mediate “magnetic vision”

The radical pair process is a reaction between radicals, molecules with one or more unpaired electrons that are highly reactive and usually interact with other radicals. Hence,

the lifespan of these molecules is very short, with magnitudes of μs . This reaction is termed a radical pair process and was first suggested as a possible geomagnetic transducer by (Leask 1977, Schulten 1982). A simple example that illustrates how a radical pair process might proceed is given by Ritz et al. (2000). At the beginning, the excited donor (stimulated by a photon of light) passes an electron to the molecule called an acceptor, causing formation of the radical pair of donor and acceptor in either a triplet or singlet state, depending on the radicals' spin orientations. Interconversions between the triplet and singlet states can be influenced by magnetic fields in a way that is manifested by a change in the reaction's yields (**Figure 1.6**). Ritz proposed and described this process as a possible geomagnetic compass system, which operates in the range of the intensity of the geomagnetic field, i.e. around $50 \mu\text{T}$.

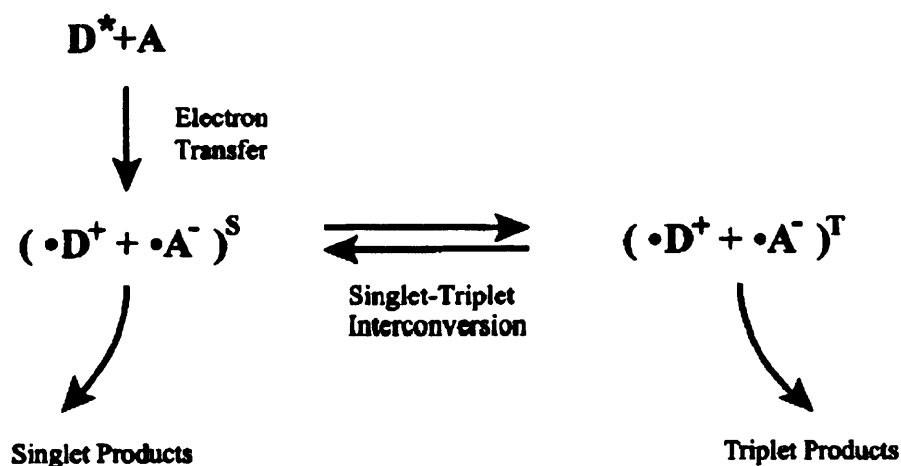


Figure 1.6 Reaction scheme for a radical pair reaction with magnetic field-dependent reaction products. The radical pair is generated by an electron transfer from a donor molecule, D , to an acceptor molecule, A . An external magnetic field affects interconversion between the singlet and triplet states of the radical pair (Ritz et al. 2000).

A radical pair process can be disturbed by a high frequency magnetic field in the Megahertz range. To prove that such a mechanism really takes place in birds, two experiments were performed on European robins. A magnetic field of either 1,315 MHz (Thalau et al. 2004) or 7,0 MHz (Ritz et al. 2004) was applied to them. These two frequencies were chosen, as it was expected that resonance might occur between these

oscillating magnetic fields and the radical pair process, and as a result, the radical pair process would be perturbed. Interestingly the birds could orient perfectly when the high frequency magnetic fields of the above values were produced along the same angle as the geomagnetic field, but when the field was shifted by a 24° or 48° angle, the birds were totally disoriented (Ritz et al. 2004). These findings endorse Ritz's radical pair model theory as these specific frequencies, presumably being in resonance with the radical pair process, do perturb magnetic field detection.

The next important issue is to define in which part of a bird's body (and in which organ or organs) magnetoreception is located. In the studies concerning either magnetite or the radical pair process, the location of the magnetic transducer is believed to be situated somewhere in the head. This is well substantiated by the study of Mouritsen et al. (2004a), i.e. that birds use characteristic movements of their head, or scans, in an apparent effort to detect the magnetic field. Garden warblers, *Sylvia borin*, which are night migratory birds, were investigated. In the normal earth's magnetic field, in which birds chose the appropriate migration direction, the migration movement was preceded by head scans. But in the case where there was no magnetic field, the frequency of head scans increased three fold. This strongly suggests that the "magnetic sensor" is indeed situated in the bird's head. Another type of research, the outcomes of which implicate the head as a location of a magnetic transducer, are studies in which small magnets were glued to the bird's head (i.e. Homing pigeons that underwent such a manipulation were disorientated (Keeton 1971)).

That the sensor is situated in the bird's head can also be confirmed by experiments that reveal a bird's dependency on the ambient light wavelength during magnetoreception. Birds in normal earth conditions and in white light navigate with excellent accuracy. However, in monochromatic light the situation is quite different. The range of visible light from blue to red wavelength was investigated to determine the influence of colour on birds'

navigation behaviour. The results demonstrate, that under an intensity 7×10^{15} quanta $\text{s}^{-1} \text{m}^{-2}$, and in the wavelengths from 424 nm (blue light) through 510 nm (turquoise) to 565 (green), birds do not show any deflection from the correct direction (see **Figure 1.7**). Yet at higher wavelengths, i.e. 590 nm (yellow) up to 635nm (red), birds are no longer able to choose their migratory direction correctly (Wiltschko and Wiltschko 1995; Wiltschko and Wiltschko 1999; Wiltschko and Wiltschko 2001).

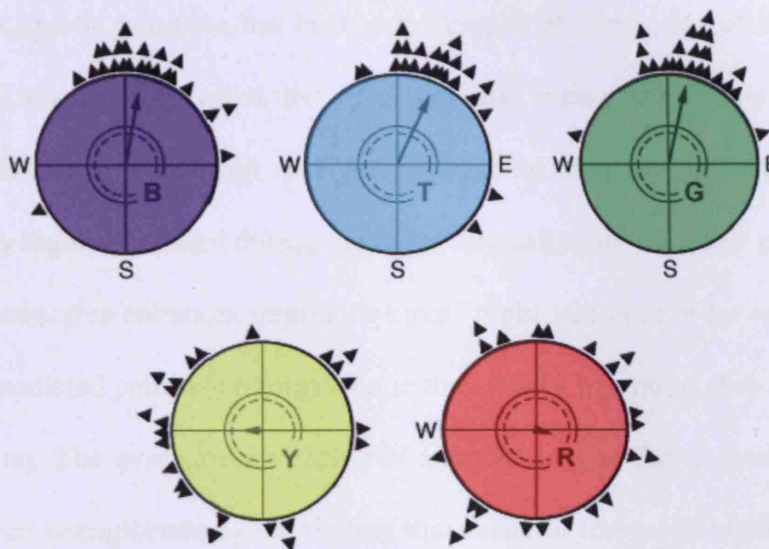


Figure 1.7 Orientation of the European robins under different wavelengths of light. (Black triangles around the circles are individual average headings of each tested bird, the arrow in the middle is a grand mean vector of all headings, circles inside are the statistical significance of the Rayleigh test; 5%-dashed lined circle and 1%. Adapted from (Wiltschko and Wiltschko 2002a).

Moreover, the intensity of the light has a significant effect on birds' magnetoreception. Under an intensity seven times higher than the foregoing studies, a large disorientation effect was observed. Surprisingly, this increased light intensity was still not high when compared to the normal day light intensity of light (Wiltschko et al. 2003a). Thus, not only is there good evidence that the reception of the magnetic field occurs in the head, these studies also confirm a light-dependant magnetoreception mechanism, strongly suggesting a role for the eye, i.e. birds may well "see" the earth's magnetic field. The nature of such perception, however, is still unknown. Further confirmation of the importance of the eye

comes from a study investigating any difference in perception by the left or right eye alone (using occluders). Tests were performed on the European robin and revealed that orientation by means of only the left eye was less accurate than in the case of the right eye (Wiltschko and Wiltschko 2002b). This evidence for lateralization is additional support for involvement of the eye (in this instance the right) in the magnetoreception process. A detailed review of lateralization in the homing pigeon is provided by Bingman et al. (2006).

The other very recent documentation of light being necessary for the proper functioning of the magnetic compass has been put forward by the group of Mouritsen (Zapka et al. 2009). They had discovered that a region in the brain called cluster N, which was found to be involved in night vision in night migrants in their previous studies (Mouritsen et al. 2005), is highly activated during the night migration period. They proposed that this newly found brain area enhances migratory birds' night vision in order to facilitate access to the vision-mediated compass information, either star or magnetic, that is needed for night-time navigation. The evidence for cluster N involvement in the magnetic compass system has since been strengthened by the finding that bilateral lesions of cluster N in garden warblers impaired their magnetic compass. In contrast, their orientation behaviour remained intact when navigation was performed on the basis of star and sunset compass (Zapka, et al. 2009).

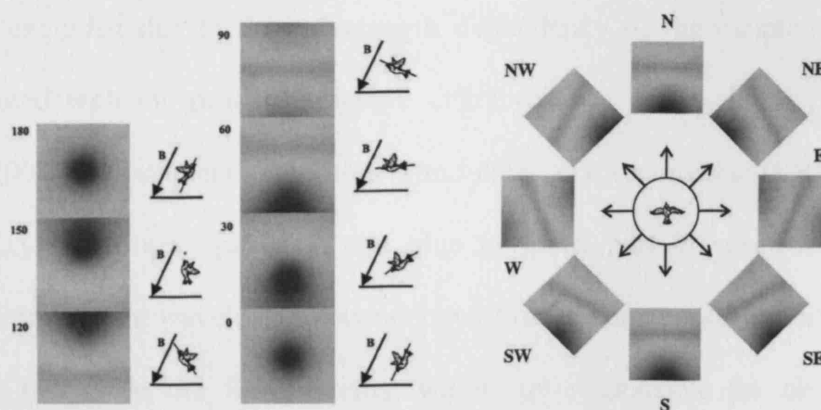


Figure 1.8 Proposed way of seeing a magnetic field by birds according to the radical pair process theory (Ritz et al. 2000).

Taken together, the evidence implicating the eye has led to a growing consensus that magnetoreception, at least relating to a compass sense, is based on vision. If true, photoreceptors are the most likely elements to be involved in the process of magnetic perception. Ritz's photoreceptor-based model, constructed on the basis of the radical pair process theory, involves only the eye. Photons of light excite macromolecules, and hence a radical pair is generated. Any occurring single pair can be interconverted to the triplet state by a hyperfine interaction⁴, the effect of which is a triplet yield. The magnetic field plays its role by influencing this singlet-triplet transition. Depending on the geomagnetic field strength, the yield varies with respect to the alignment of the magnetic transducers radical pair. Simultaneously, comparing the triplet yield in various directions, birds can derive information about direction from the magnetic field (Ritz, et al. 2000). This is the principal reason that implicates the eye, as it contains sensory cells (i.e. the photoreceptors) systematically oriented in all spatial directions, forming an array that is equivalent to a three-dimensional sensor grid. Ritz has suggested that this process, producing a differential output from the photoreceptor aligned or nearly aligned with the magnetic field, leads to an actual perception of the field by birds (illustration is given in **Figure 1.8**).

Therefore, many investigators have recently begun a quest for a magnetic transducer photoreceptor in the retina. Early in this search, the first most likely candidate, i.e. retinal cones, were excluded due to the wavelength dependency of the magnetic compass not being correlated with the peak wavelength characteristics of the cones (Wiltschko and Wiltschko 2005). Subsequently, another candidate, cryptochromes (CRYs), has been suggested. Cryptochromes operate in the blue to green part of spectrum, which is in agreement with the light wavelength dependency of magnetoreception condition mentioned above. They belong to the flavoproteins, which are responsible for circadian rhythm

⁴ Hyperfine interaction – the interaction between a nucleus and its surrounding environment.

regulation in animals and plants (Cashmore et al. 1999; Sancar 2003). Cryptochromes have also been found in the avian retina (Moller et al. 2004; Mouritsen et al. 2004b). However, as there are various kinds of cryptochromes, this does not help in locating the one possibly responsible for magnetoreception. As a result, despite the extensive studies concentrated on this issue, thus far the actual molecule and retinal cell type that mediate magnetoreception have not yet been found.

1.6. Utilization of the magnetic field map and compass

According to the previously mentioned theory of Kramer (1957) and (1961), both the magnetic map and compass are constructed on the basis of directional information extracted from the geomagnetic field. The field at each place on earth has a characteristic inclination and intensity, and by recognizing these factors, birds may be able to construct (i.e. learn) a field-based map. Map detection in birds is believed to be made possible by magnetite, and thus vision is probably not involved in the internal map.

Birds' magnetic compass is believed to be quite different from the compass that we use. The first difference lies in how birds derive magnetic information from the magnetic field, namely by navigating based on the inclination of the magnetic field, instead of its polarity as in the case of our technical compass (Wiltschko and Wiltschko 2002a). Birds, when subjected to a change in the polarity of the ambient magnetic field, respond in the same way as before the change. This result led to the assumption that they orient on the basis of the distinction between “poleward, the direction where the field lines axis constitutes an acute angle with gravity, and equatorward, where the field lines run horizontally” (Wiltschko and Wiltschko 1972). That is why birds, which cross the equator, are forced to switch from the equatorward to the poleward strategy (Wiltschko and Wiltschko 1972; Beason 2005). Secondly, the low range of magnetic field intensity perceived by birds differs from that of a technical compass. Birds can orient to a geomagnetic field with an

intensity of $46\mu\text{T}$ and in the limited range between $43\mu\text{T}$ to $54\mu\text{T}$ (Wiltschko and Wiltschko 1972; Merrill and McElhinny 1983; U.S. Department of the Interior 2005) (see also **Figure 1.9**), reflecting the highly specific adaptation of this sensory system to the earth's magnetic field.

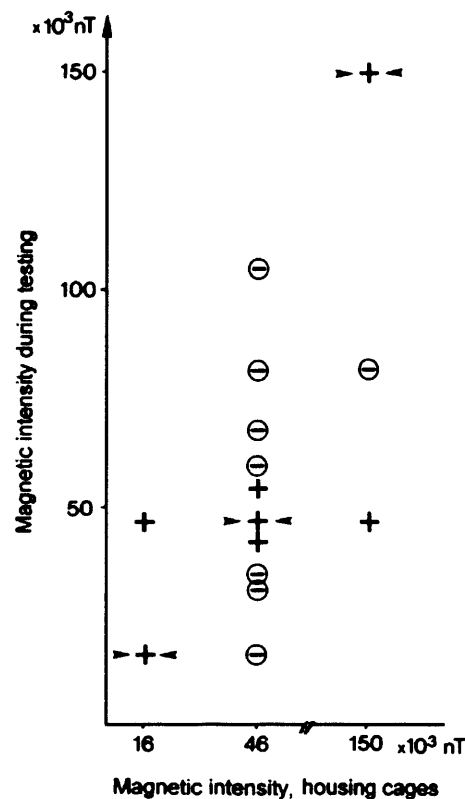


Figure 1.9 The orientation (marked with +) or disorientation (encircled -) of birds in different magnetic field intensities. Arrows mark the magnetic field in the housing condition. Adapted from Wiltschko and Wiltschko (2002a)

1.7. The basis for a novel approach to investigate magnetoreception

Although, research in the magnetoreception of birds has been going on for several decades, there are only a few methodologies allowing investigations of this phenomenon. The main methodologies used are analyses of the vanishing bearings of homing pigeons released away from their home loft and a laboratory based measurement of migratory restlessness (i.e. urge to fly/move in a particular direction) in migratory birds.

1.7.1. Vanishing bearings

Until the 1950s, the investigation of birds' orientation was usually confined only to theoretical considerations because there was no known method for a practical determination of birds' orientation. At the time, following birds from a plane was only a very crude, impractical and unreliable approach. That situation changed when the vanishing bearings method was introduced for the first time by Matthews (1951). He found that the vanishing direction of pigeons released after being displaced from their home loft (i.e. the last moment when the bird was seen with binoculars) is well coordinated with the true home direction. In other words, the correlation between the vanishing bearing at the release site with the arrival aim of a bird is a good enough approximation to serve as a scientific method for investigating the accuracy of avian orientation, either in normal conditions or after different experimental manipulations (e.g. clock shifts, exposure to magnets, etc.).

1.7.2. Migratory restlessness

The second method was invented a bit later in 1966. This approach depends on a phenomenon called migratory restlessness, which consists of the seasonal eagerness of migratory birds to move in a specific direction that corresponds to the location of their migration goal. This tendency is so compelling that, even in an enclosed area, the behaviour is still present. The use of this fact was first exploited by Emlen and Emlen (1966), who built special cages, now called Emlen funnels (see **Figure 1.10**). This approach involves placing the migrant in the funnel, and when the bird moves in the migration direction, it leaves marks (footprints or scratches) on the paper that covers the surface of the funnel. The sheets are then examined to determine the mean orientation bearings of each bird.

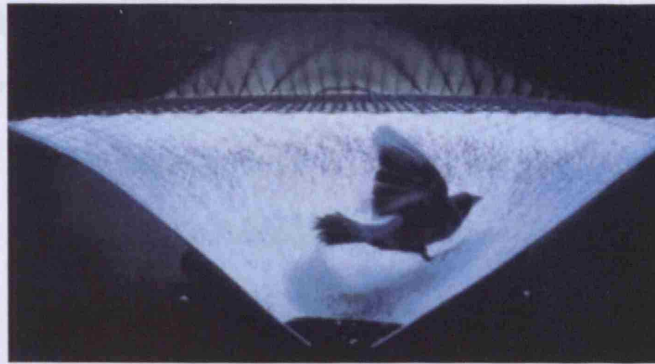


Figure 1.10 Emlen funnels used to measure the migratory restlessness in migrant bird species (www.paulnoll.com)

Both of these methods enabled the quantitative study of avian navigation, although they have constraints that limit their usage for studies of magnetoreception. For example, determining vanishing bearing involves releasing homing pigeons and tracking them in the field. Not only is this difficult and time consuming, but also it does not easily permit the manipulation or elimination of one or more of the cues other than the magnetic field (i.e. sun, polarized light, odours, landmarks, etc.). Thus, the birds' orientation may not be based only on the earth magnetic field. The second method is confined to migrants only, which in turn constrains experimentation to relatively short periods during the year when birds display migratory restlessness.

Finally, both of these techniques monitor a bird's *orientation* with respect to, for example, the geomagnetic field. Prior to this behaviour, however the bird must first *detect* the field. Almost nothing is known about this stage of magnetoreception; for example how quickly birds can perceive and thus respond to the field or the psychophysical limit of such detection. The goal of the present research was to develop a novel approach that could address this issue in the laboratory. Two observations reported in the literature ultimately suggested how this might be achieved, as discussed in the following sections.

1.7.3. Operant conditioning

When describing methods used in avian magnetoreception studies, another technique that should be mentioned is operant conditioning. This approach relies on training a bird on specific tasks, involving a discrimination between two options, by reinforcement (when a correct choice was made) and punishment (in the case of a wrong choice), which is often the presence or absence of a food reward. However, not many studies achieved convincing outcomes using this method in investigations of magnetoreception in birds (e.g. Mora et al. 2004). As a result, operant conditioning is still questioned as an effective method for the study of magnetic field detection in birds (Wiltschko and Wiltschko 2007) and thus is not widely utilised.

1.7.4. Avian magnetic compass is an inclination compass

The investigation that provided the evidence that the avian compass depends on inclination, not polarity in the man-made compass, was based on flipping one of the magnetic field components. It turned out that birds were turning their heads according to the change in the resultant field vector only after the horizontal component of the field was flipped. Interestingly, when both components (i.e. horizontal and vertical) of the field vector were changed, the headings of the subjects remained unaltered (Wiltschko and Wiltschko 2002a).

1.7.5. Head scans serve as a magnetic field calibration tool

Subsequent intriguing research by Mouritsen and his co-workers (2004a) reported a marked increase in the number of head movements by night migratory songbirds (garden warblers) when exposed to the absence of the earth magnetic field. The increased number of head movements was attributed to the lack of the magnetic field and, ipso facto, the directional information provided by the magnetic field (Mouritsen et al. 2004a). Therefore,

head movements were indicated as a potentially useful measure of the functional characteristics of the magnetoreception that underlies the birds' magnetic compass.

The constraints of magnetoreception research characteristic of these two above-described methods as well as the fact that head movements were possibly a sufficient way of measuring responses to magnetic field changes paved the way for establishing the novel methodology that was constructed for the purposes of the research described in this thesis.

1.8. A novel method for investigating magnetoreception

With a view to being able to conduct year-round research on avian magnetoreception in a controlled laboratory environment, and hence increasing the power and efficiency of such studies by avoiding the need of field work and a dependency on birds' seasonal migration periods, a new method was developed.

Magnetic field changes clearly affect birds' behaviour (i.e. both orientation accuracy and frequency of head movements). Therefore, manipulations of the ambient magnetic field conditions are the basis for the approach. In previous research, manipulations of the magnetic field consisted simply of either changing the direction of the field vector or switching off the magnetic field altogether. Nevertheless, these alterations were sufficient to produce a response in the birds. In the present research, the approach was to expose birds to more intense spatiotemporal changes within the magnetic field and then explore how their behaviour was affected. In this case, "more intense" involved presenting subjects with a sequence of different magnetic field conditions, or steps, consisting of three types: normal static field, a field sweeping (i.e. rotating) with a speed of $144^{\circ}/s$ in either direction (clockwise or counterclockwise) around a bird's head, and lastly the absence of any field. Each of the steps within the sequence lasted only 30 to 60s, depending on the experiment, to allow sufficient exposure but minimise habituation. 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 amount of time that each step is present within the sequence, and even the speed of the sweeping field could be altered. The entire procedure for creating and controlling the earth magnetic field was fully automated. The artificially generated magnetic field used was as exact a replication of the local earth magnetic field with its intensity and inclination values as possible.

Based on the work of Wiltschko and Wiltschko (2002a) and Mouritsen, et al. (2004a), it would appear that head movements are relevant indications of the changes in the magnetic field direction or its presence/absence. For that reason, head movements were chosen as the measure in this novel method for the birds' detection of, and responses to, the controlled variations in the ambient magnetic field.

Homing pigeons, *Columba livia*, were selected as subjects for several reasons. Firstly, pigeons are well known for their ability to use the magnetic field for orientation and to navigate year round when homing, as opposed to migratory birds whose navigational behaviour is seasonal. Secondly, pigeons are readily available and, as a popular species for laboratory investigations, are well studied. Knowledge about their brain, especially their visual pathways is extensive (Granda and Maxwell 1979; Gunturkun 2000).

Furthermore, this approach would make it possible to eliminate other navigational cues, described earlier in this chapter, in the setting where birds are investigated (i.e. the laboratory). In other words, it has to be ensured that the only cue that is available to the tested birds during an experiment is the cue that is the focus of the study, in this case the magnetic field.

A rising awareness among investigators in this field that a new method was needed was also an important motivation for this research. After many years of study, major questions concerning magnetoreception remain. Thus, it seems almost vital that new experimental

paradigms are required (Kirschvink et al. 2010) (Lohmann 2010).

1.9. The approach explanation in the light of other research in the field

The main difference between this approach and those used in most of the previous studies of magnetoreception is that, here, the birds' orientation and/or navigation abilities are not under investigation. The present method is designed to observe behavioural reactions, manifested as head movements, that occur when changes in the magnetic field are presented to the tested birds. Thus, the level of magnetic field perception is determined by the presence or absence of a reaction. Therefore, it is important to make clear that, in this research, only real time behavioural responses are analyzed, without suggesting that the results involve navigational orientation to the magnetic field(s) presented.

1.10. Hypotheses and aims

Before this study was started, a number of questions were formulated as the basis for this work. These hypotheses included:

1. Investigation of magnetoreception in homing pigeons may be based on head movements as a reliable measure of their responses to the artificially induced alterations in the ambient magnetic field.
2. Pigeons (birds) are able to detect and are sensitive to sudden, significant changes within magnetic field, and this detection is rapid.
3. The characteristics of the head movements, which are not expected to be of any specific type, will provide insight into the nature of the birds' responses to changes in the magnetic field.
4. Darkness highly attenuates or eliminates the magnetic field sensing capabilities of

pigeons (and possibly other birds) indicating that light is needed for magnetodetection.

The overall aim of the research described in this thesis is to validate the method developed and demonstrate its usefulness for studies of magnetoreception. The experiments conducted using this novel approach are described, analyzed and discussed in the light of up-to-date theories and findings concerning magnetic field perception in birds.

1.11. Thesis outline

Immediately following this introduction, the next two chapters are devoted to explaining the system developed and built for investigating homing pigeons in a controlled laboratory environment, and subsequently, in the third chapter, the procedures involved for investigations of the birds' responses to changes in the magnetic field are presented.

The main experimental chapter (i.e. **Chapter 4**) contains the results of the main experiment involving the use of magnetic field sequences. Different analytical approaches are employed to present the data, documenting the birds' activity, i.e. head movements, from several different perspectives in the case of both real and control experiments. This analysis indicates that the behaviour of the birds is dependant on the magnetic field conditions and, in particular, on specific changes in the ambient magnetic field.

The most noteworthy observation when performing the magnetic field sequence experiments was the way in which birds reacted to changes in the magnetic field, particularly the increase in activity and nature of their head movements. In order to characterise precisely whether the head movements (i.e. responses to transitions in the magnetic field) fall into a specific category of visuomotor behaviours, a high-speed camera system was used to record these movements at specific transitions. The results of this examination of the characteristics of the head movements are presented in **Chapter 5**.

Finally, the problem of the importance of light for magnetoreception, which seems to be a vital factor allowing birds to perceive magnetic field (see **Section 1.5.3**), was explored. Therefore, an experiment informed by the findings from the main experiment (Chapter 4), in which the same birds were exposed to a relatively abbreviated magnetic field sequence, was performed in either light or total darkness. The outcomes of this experiment are presented and discussed in **Chapter 6**.

The last chapter contains a consideration of the overall conclusions that can be drawn from the careful analyses of the experimental results obtained.

1.12. References for Chapter 1

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Chapter 2. System overview

For the purpose of this study, the experimental setup was composed of the following: a component for the generation of the magnetic field, a field controller, video recording of the birds' behaviour, and subsequent computer analysis of data from the recorded videos. The coils needed to be designed and constructed to enable magnetic field generation to accommodate an overhead digital camera and illumination inside a magnetic field shielding chamber. Finally, the software had to be written for automation of the magnetic field generation system and subsequent data analysis.

2.1. System for generating and controlling a magnetic field

To generate an appropriate magnetic field, manipulate its temporal and spatial properties and also record the birds' behaviour within the magnetic field, a special system has been designed and constructed by the author. The system consists of four main parts:

- computer with controlling software
- signal amplifying system (number 1 in **Figure 2.1**)
- set of Helmholtz coils (number 4) placed in a magnetic shielding chamber (number 6)
- camera recording system together with illumination (number 3)

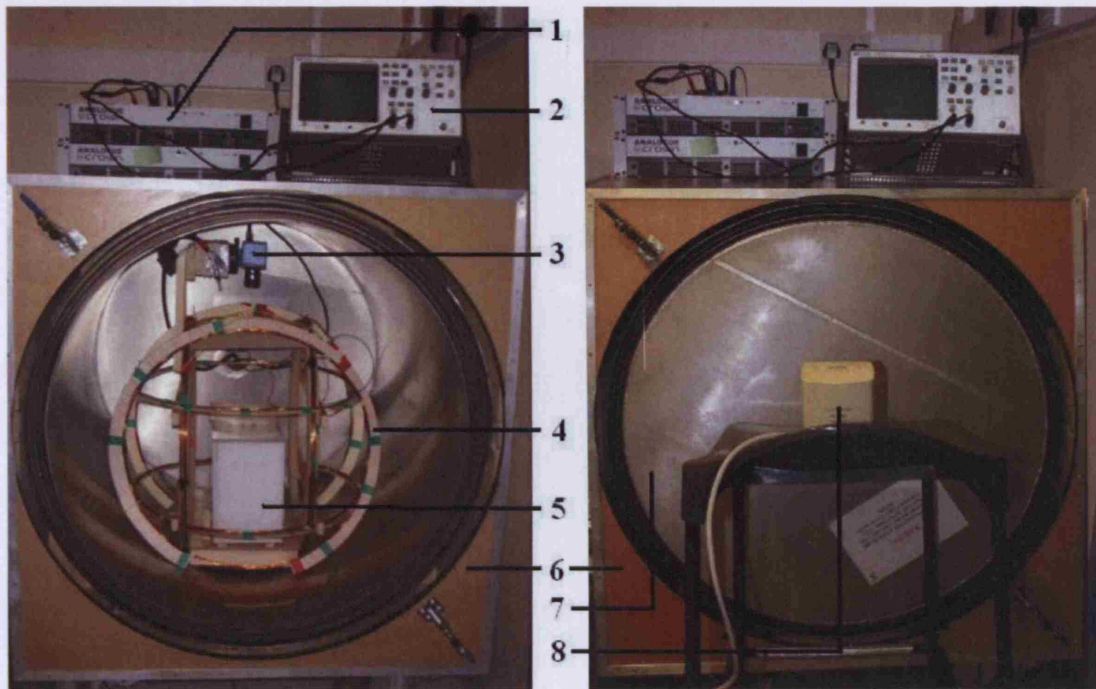


Figure 2.1 System where experiments are performed (1- amplifiers, 2- oscilloscope, 3- camera and illumination, 4- Helmholtz coils, 5- experimental box, 6- shielding chamber, 7- shielding lid, 8- white noise speaker)

2.1.1. Computer with controlling software

The computer acts as a master, which controls the amplitude and both the spatial and temporal settings of the magnetic field as well as the recording facility, and synchronizes both.



Figure 2.2 Front panel of the Labview mainprocedure.vi programme

Labview (short for Laboratory Virtual Instrumentation Engineering Workbench) is a platform and development environment produced by National Instruments (Austin, Texas, USA) and differs from common programming languages as it is based on the graphical “G” language. Any programme written in this language consists of two parts. The first one is the Control Panel, in which all programming is done. The second one is called the Front Panel, in which all parameters are set before the programme is run as well as allowing the user to observe all of the results live while the programme is running. The interface is very user friendly, by not requiring code to be written for each command as in most other popular programming platforms. Instead, there is a library of blocks in which commands are already embedded. An example of the block programming in Labview is shown in **Figure 2.3**.

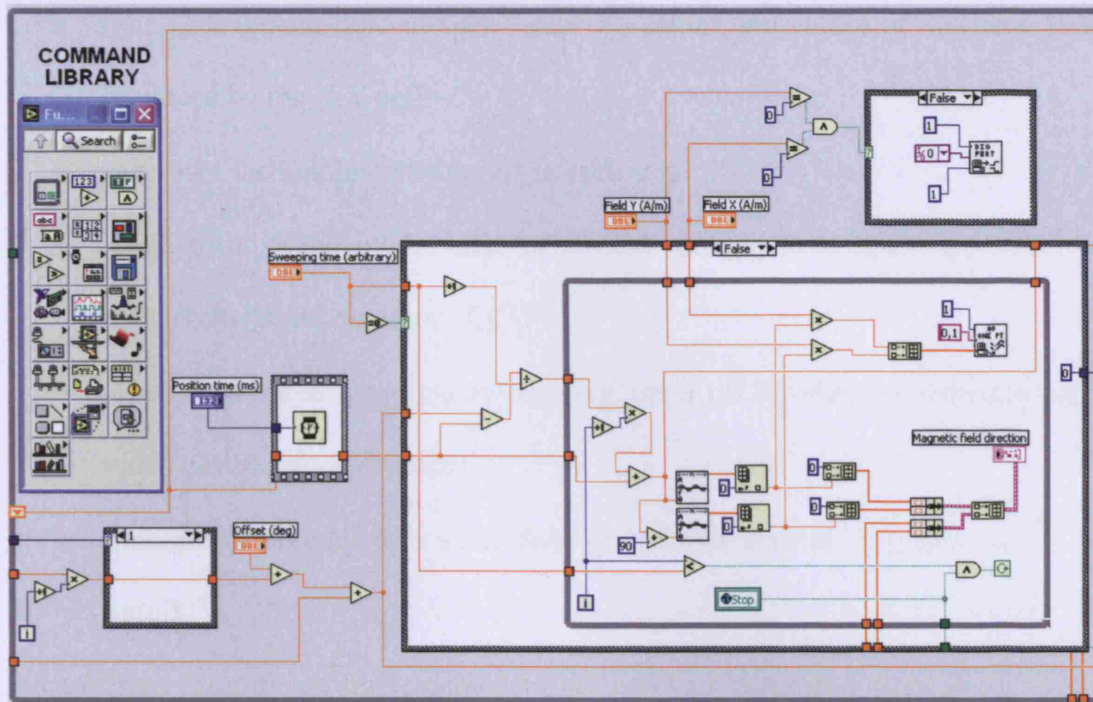


Figure 2.3 Extraction of a Labview control panel from the `mainprocedure.vi` programme with command functions library highlighted

Due to these advantages, the Labview environment was the basis for all programming within this study, including the software for controlling the magnetic field, recording the birds' behaviour and analysis of the results. The operational software was fully designed and purposely written by the author, for this study with the exception of `mainprocedure.vi`, which had already been programmed by Dr Stan Zurek (Cardiff University) for trial experiments that had been carried out before this PhD research was started.

The program, named `mainprocedure.vi` (for a front panel see **Figure 2.2**), was designed to encompass whatever was needed for the smooth running of the experiments, and was capable of programming the:

- length of a magnetic “step” (i.e. time period during which one of the types of magnetic field condition was generated)
- number of “steps” within each experiment's sequence

- type of magnetic field in each “step” by setting the values of magnetic field produced by the X,Y coil
- earth-like inclination to be on/off in each step
- speed of a sweep in the case of a field rotating in a clockwise (CW) or counterclockwise direction (CCW)
- state of the video recording system (e.g. on or off for trial experiment when a video coverage is unwanted)
- presence or absence of a static field prior to the start of an experiment to be on/off

All of these features are implemented in the software through a user-friendly front panel (**Figure 2.2**). Moreover, the camera controls are incorporated in the software – camera switches on after the prior static field (SF) is finished and switches off at the end of the last step in the experimental sequence. However, the camera on and off setting may be easily changed to meet the user’s particular needs. Furthermore, the software enables recording of the experiment to be omitted (e.g. in the case of trial experiment), and a pre-exposure to a field condition before an experiment can be removed. Another useful feature of the mainprocedure.vi programme is that it stores all of the details about an experiment (i.e. procedure, speed of a sweep, time, date, etc.) and automatically saves them, as a .txt file, in a given directory, with a date and time in the filename, which is presented in a Saved File box in **Figure 2.2**. This, in turn, is particularly useful when many experiments are performed, as the details of each experiment are labelled and saved. These data may then be accessed any time when information about a particular experiment is required.

In this configuration, there are four possibilities for setting the magnetic field of a step during an experimental sequence:

- static field (SF)
- sweeping field (with a speed of $144^\circ/\text{s}$) in a clockwise direction (CW)
- sweeping field (with a speed of $144^\circ/\text{s}$) in a counterclockwise direction (CCW)
- null field (NF)

Also, in the case of a sweeping field, the speed of sweep can be adjusted, however in most experiments it was left set at $144^\circ/\text{s}$ (n.b. the choice of this particular speed was somewhat arbitrary, not having been selected on the basis of any particular biological phenomena), unless stated otherwise. Any given sequence can be built from the steps noted above. Although the duration of the step can be altered, the one limitation is that all of the steps in a given sequence of magnetic field conditions need to be of the same length.

All communication between the computer and the amplifiers, which then send signals to the coils generating the magnetic field, is performed with the help of a National Instrument's NI PCI-6711 12-bit high-speed analogue output board (National-Instruments 2002), which is manufactured by the same company that produces Labview and thus is fully compatible with this software. High-speed analogue inputs on the board are essential to ensure the smooth controlling of the current that is sent to the amplifiers and hence generates the magnetic fields that are presented to the birds.

2.1.2. Signal amplification system (number 1 in Figure 2.1)

From the control card that is fully controlled by the software, programmed in Labview, appropriate control signals are sent to the two power amplifiers (Crown-Audio 2004), one on each channel. Their role is to magnify the low signal from the computer to the higher level that is sent to the X and Y direction magnetic field

producing coils. The output impedance of the amplifiers was changed in this investigation, by changing both the resistance and capacitance values, in order to adjust them to the new load (set of coils), thus making it possible to generate smoother signals (waveforms). This, in turn, ensured that the change in the field that the birds may detect was not abrupt but more gradual, although always less than 100ms.

The Z coil, which is the coil responsible for generating the inclination factor of the earth-like magnetic field, is connected to a power supply that feeds the coil with a constant value of current. It is connected through a relay that opens or closes the circuit of the Z coil and thus switches the current flow on and off. This operation is done with the help of the relay circuit (**Figure 2.4**). The relay is controlled by the digital output from the control card that in turn is controlled by the mainprocedure.vi. When the output is activated, a 5V signal appears on the output that drives a current flow, which is then amplified by the common emitter transistor so that the current reaches a value capable of activating the relay (i.e. $I_C > 72\text{mA}$) (i.e. producing an inclination in the generated magnetic field in the cases of SF, CW and CCW). When the digital output is set by the software not to send any signal, the relay is off and thus the current flow through the Z coil is stopped and the inclination component is removed – that is in the NF condition.

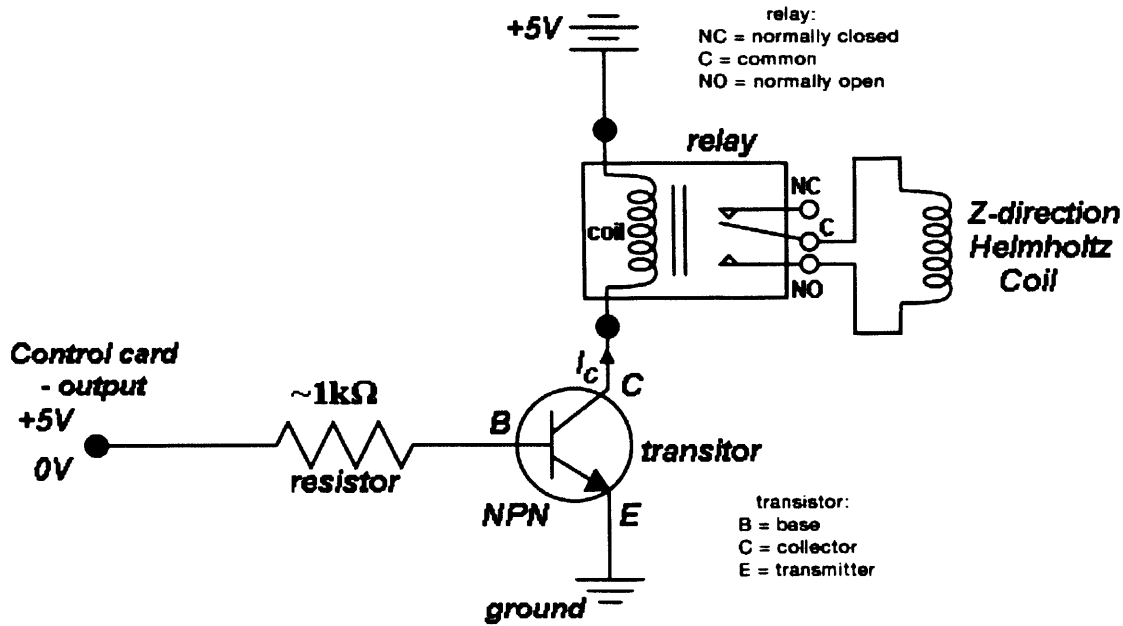


Figure 2.4 Block diagram of the relay circuit that was embedded in the Z-coil control system

2.1.3. Magnetic field generation system

The system to generate and control magnetic field was specially designed and built on the basis of the Helmholtz coil principle.

2.1.3.1. Helmholtz coil principle

A Helmholtz coil is a coil constructed of a pair of identical coils, made of the same material (current conducting wire) with the same number of turns and also with the same diameter. These two coils are connected in series and placed parallel to each other at a distance of the radius of the coil (**Figure 2.5**). Such a system is very commonly used to generate a uniform magnetic field.

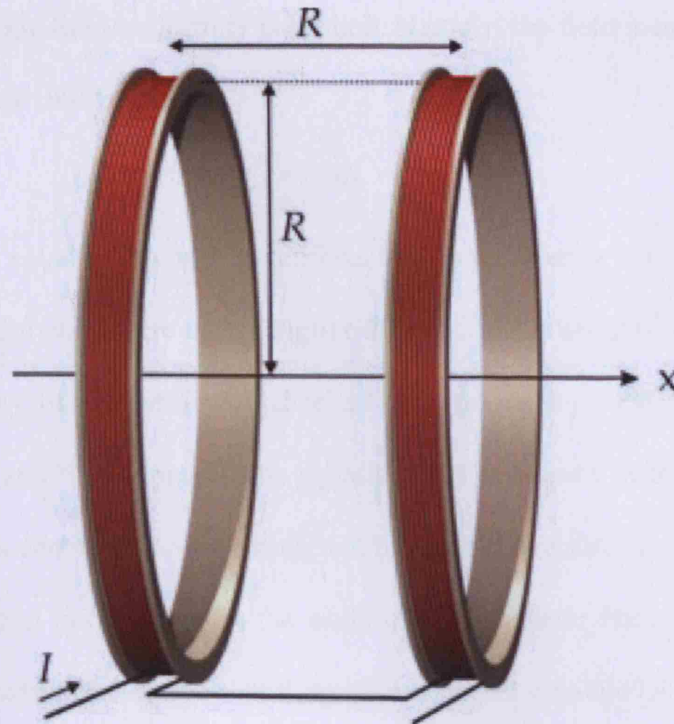


Figure 2.5 Outline of the Helmholtz coil (http://en.wikipedia.org/wiki/Helmholtz_coil)

The strength of the magnetic field inside of Helmholtz coils can be calculated using the formula:

$$H = 0.715 \frac{NI}{r} \quad (2.1)$$

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

N – number of turns [-]

I – current inside the coil [A]

r – radius [m]

For the full derivation of the above equation see **Appendix 3**. In this case, as it was intended to produce a magnetic field strength equivalent to that of the earth, the coils have a radius of 25cm, 10 turns of wire and the current is between 1 and 2 amps.

However, the magnetic field uniformity is limited. Namely, the field is uniform within the region up to (Jiles 1998):

$$H_{uniform} = 0.4R \quad (2.2)$$

This, in our case, is equal to 10cm. The uniform area is therefore a cube with a 10cm side, as 3-dimensional coils were used (**Figure 2.6**). For the coils used in the present study, the uniformity of the field was checked with the help of the finite element modelling method, and the output of this calculation is presented in **Figure 2.6** (the uniform field area is denoted with an orange colour; as this simulation was introduced for visual presentation purposes only, the scale of the magnetic field distribution is arbitrary). It is important to mention that there were limiters installed on the bottom of the coils in order to be sure that every time the experimental box, in which the bird is placed for the time of an experiment (see in **Figure 2.1** on the left hand side the experimental 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.

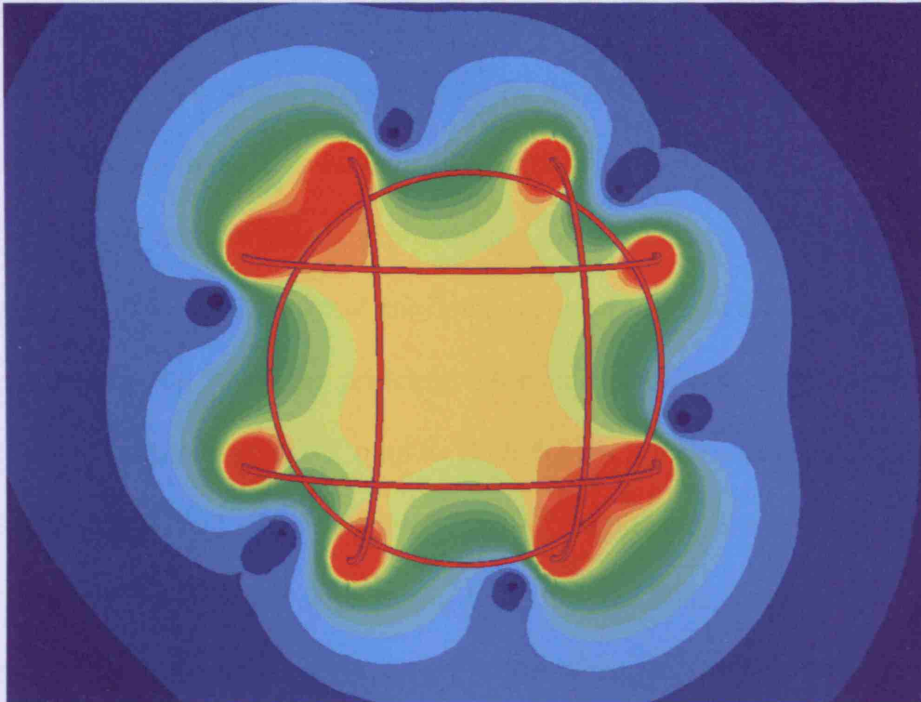


Figure 2.6 Approximate finite element method simulation of 3-dimensional set of Helmholtz coils (red line grid) as used in this study in order to show the uniformity of the field inside the coils. Magnetic field strength's variation is colour-coded: the highest intensity being denoted by red and the lowest by blue. The scale of the magnetic field strength in this simulation is arbitrary. (The dimensions of the coils correspond to the real dimensions of the coils used in the setup)

2.1.4. Three-dimensional Helmholtz coil system to produce earth-strength magnetic fields

A set of 3-dimensional coils means that there is one pair of coils producing a field in each of 3 orthogonal (XYZ) directions. In order to produce the magnetic field in the horizontal plane, two Helmholtz coils were situated orthogonally to each other, i.e. the X and Y coil. The computer software controls two output channels on the control card (National Instruments NI PCI-6711) connected to two amplifiers, which in turn, send a signal to the coils. One coil is responsible for producing a field in the X direction and the other in Y. They are connected together to the system that controls the direction and/or movement of the generated magnetic field inside, as was described above when explaining the software. In addition, the third coil in the Z direction is responsible for introducing the inclination component of the field in the system. The inclination component is added when there is a static or moving replica of the earth

magnetic field (i.e. SF, CW or CCW) generated or removed when the field is totally switched off (i.e. NF). That is controlled by the digital output in the control card and the relay embedded in the Z coil circuit (see **Section 2.1.2**).

A graphical approximation, representing the generation of a field in three dimensions – XYZ – and the resultant field, is shown in **Figure 2.7**. Generation of a 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'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 $19,4\mu\text{T}$ in order to meet the conditions of the local magnetic field in Cardiff (National Geophysical Data Center 2006). Such a signal, when combined on the XY plane, results in a circle (blue circle in **Figure 2.7**) with a radius of the length of $19,4\mu\text{T}$ (blue vector in **Figure 2.7**). The resultant field was only the horizontal component of the field. Therefore, to facilitate generation of a more accurate replica of the local field, the third Z coil is fed with a current to add a vertical component of the field, resulting in an inclination of the field being introduced equal to $44,4\mu\text{T}$ (red vector in **Figure 2.7**). Therefore, the resulting field in three dimensions (XYZ) was equal to the local earth magnetic field in Cardiff (i.e. $48,5\mu\text{T}$) – and its inclination of $66,4^\circ$. All three coils together, i.e. 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 2.7**) – within which all the experiments took place, excluding the situation of a null field when the field was off. Note that the bird is always isolated from the ambient magnetic field by the enclosing shielding chamber.

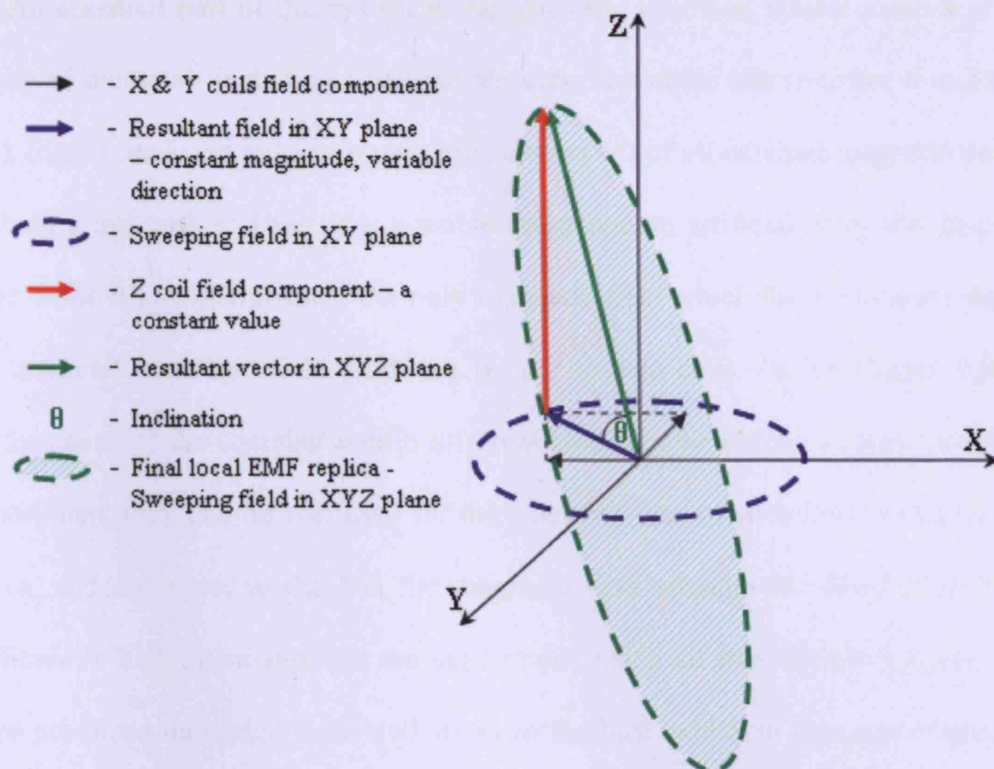


Figure 2.7 Representation of the XYZ plane components and the resultant magnetic field

All of these operations, controlling the three Helmholtz coils, are performed within the software. The user simply needs to specify the sweeping speed in each step, the steps' duration, and the magnetic field intensity. As mentioned previously, the programme also saves all of the settings to a .txt file. 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 produced magnetic field was checked with a 3-axis fluxgate magnetometer (Bartington-Instruments 2009), which measures magnetic fields of μ Tesla magnitude. Thus, it is confirmed that the features of the field produced inside the chamber are in close agreement with the local earth magnetic field (EMF) at Cardiff.

An essential part of the system is the shielding chamber, which consists of five layers of mumetal⁵ including five separate superimposable lids (number 6 in **Figure 2.1**). Such a structure ensures a very efficient cut off of all external magnetic fields – including the earth's. Thus, it is possible to operate an artificial, very low magnetic field without any disturbance; the only magnetic field which the bird experiences is the artificial earth-like field produced by our system coils. In the **Figure 2.8**, the performance of the chamber within different shielding conditions is depicted. In our experiment, only one lid was used for the shielding factor (earth field is reduced 826 times, and therefore, in the NF, the magnetic field strength is $\sim 50\text{nT}$), but this is sufficiently high attenuation for the experiment; using all five lids every time would have produced unwanted noise and stress to the bird, which in turn could affect the bird's behaviour.

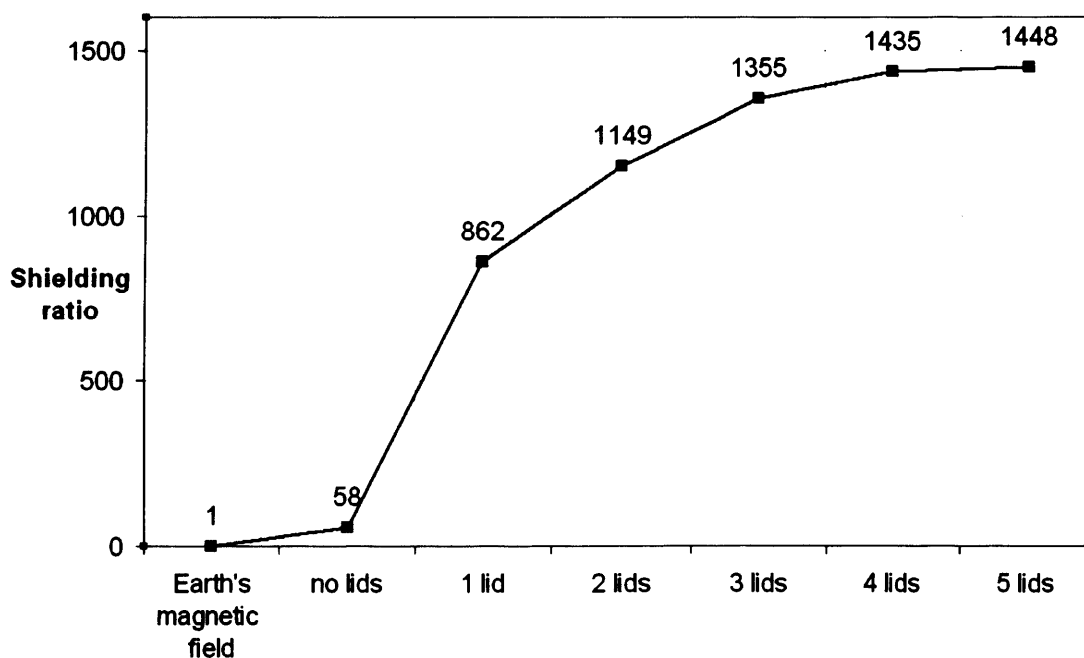


Figure 2.8 Plot showing shielding factor versus number of lids of the shielding chamber (Kulsoom 2005)

⁵ Mumetal – high permeability magnetic material, used in shielding applications.

The final part of the entire “magnetic field system” is the oscilloscope (number 2 in **Figure 2.1**), which shows on the display the magnetic field being produced at the moment. This facilitates correlating the bird’s behaviour in time with respect to the state of the magnetic field while an experiment is being performed.

2.1.5. Camera recording system and illumination (number 3 in Figure 2.1)

The other important part of the setup is the equipment responsible for recording the bird’s behaviour. This consists of a high quality, black & white, firewire video camera (Imaging source DMK 21BF04) with a Sony Diagonal 4.5mm (Type 1/4) Progressive Scan CCD Image Sensor (SONY 2008). Notably, the progressive scan feature of the image sensor makes sure that not even one frame is lost. The camera is synchronised in a parallel manner with the magnetic field generation part of the setup by means of the aforementioned Labview software, mainprocedure.vi. The software ensures that video recording starts precisely together with the beginning of the first step of the generated magnetic field and stops together with the end of the last step. This, in turn, makes it possible to perform time-locked video analysis of the bird’s responses, which can then be correlated with the state of the magnetic field at any point during the experiment.

The camera is capable of recording a video with different frame rates in a range of 3.75 to 60 frames per second (fps). The video recording rate of 30 frames per second was found to be accurate enough and, ipso facto, was used in most experiments. Therefore, there were 30 samples in each second, on which basis the orientation of the bird’s head position could be specified precisely.

Inside of the chamber, the interior of the experimental box was specifically illuminated by a fibre optics device (Schott KL 1500 LCD – (Schott-Inc. 2010)). The

light consists of all colours of a spectrum, being a white light. The light condition, illuminance, in the box where a bird sits during an experiment was approximately 158lux as measured with an ISO-TECH ILM350 luxmeter. Also, as the light source is fibre optic-based, it does not produce any heat in the chamber when switched on.

2.1.5.1. Camera setup and illumination for dark experiments

For the darkness experiments, the prerequisite was a camera recording system that could operate in total darkness. The most common solution for making observations in the dark is to use the infrared range of the light spectrum, which is not visible to either the human or bird eye and then have a camcorder operating in this bandwidth. Following this approach, the operational frequency range of the camera sensor was checked (**Figure 2.9**) to determine that its light sensitivity extended to the near infrared (IR), i.e. up to 1000nm. Nevertheless, in order for the camera to “perceive” in the near IR, an infrared source excitation is indispensable. Therefore, an infrared LED torch with a wavelength 850nm was mounted on the top of the coils next to the already present visible light source, which obviously was switched off. Observing the spectra of homing pigeons confirms that they are unable to see at wavelengths higher than 700nm (Bowmaker 1977) (for more see **Section 6.1.1**). Thus, it validates that the IR torch, which emits at a wavelength of 850nm, is beyond pigeons’ visible light spectrum; i.e. the birds are in total darkness. Additionally, it should be pointed out that there was no evidence found in the literature to suggest that IR light might have any effect on magnetoreception.

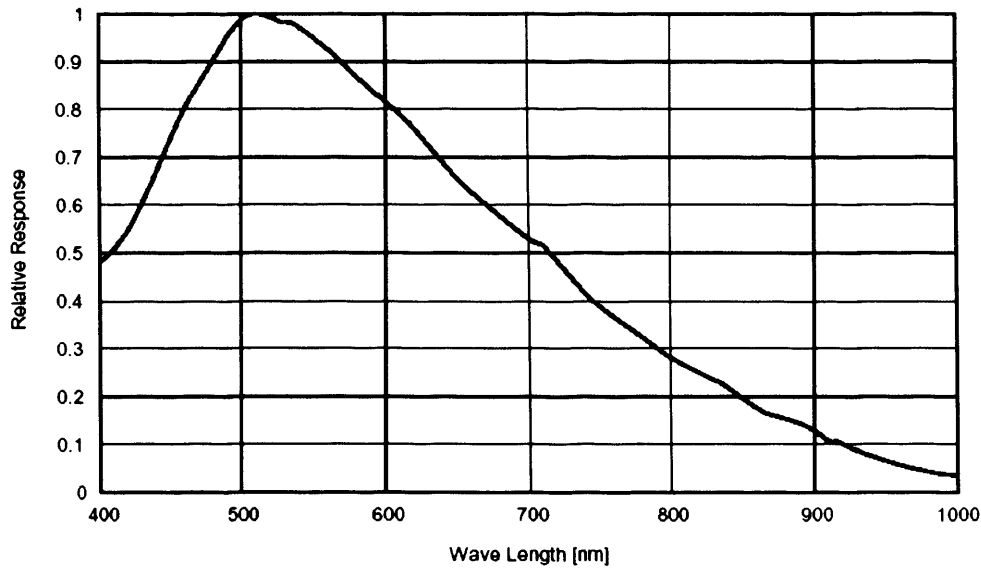


Figure 2.9 Spectral sensitivity characteristics of the Sony image sensor (SONY 2008) used in the digital camera

2.2. Adaptations in the system for darkness experiments

For the purpose of performing experiments in the dark, the same camera was used due to its near IR operational bandwidth. The camera sensor is capable of viewing in the dark under the excitation of an infrared light source (for more on the sensor's characteristic, see **Section 2.1.5.1**). During the dark experiments, the IR torch is on all the time so that the camera can view the bird in total darkness. There is no remote access to the torch and hence the infrared light cannot be switched off unless the lid is taken off. Nevertheless, as switching on/off the visible light (i.e. fibre optics light source can be controlled by the system), darkness can be achieved during the same experiment. Although on all the time the infrared source does not affect the light condition in any way, but allows recording to take place in darkness. A frame extracted from one of the darkness experiments is shown below (**Figure 2.10**) to illustrate the clarity of the video in this condition.



Figure 2.10 Example frame from a darkness experiment illustrating the clarity of the picture.

2.3. Adaptations in the setup for the high speed camera

For the majority of the experiments, the sufficient requirement for the frame rate was deemed to be 30 fps. However, in the last experiment, the temporal dynamics of the head movement itself became our interest and therefore, the recording frame rate for that purpose needed to be much higher. In order to fulfil this prerequisite, a high performance, high-speed digital camera was acquired from the EPSRC Engineering Instrument Pool and incorporated into the system. The Fastcam MC1 camera (Photron) was capable of recording a video with 2000 fps, which was more than fast enough for these purposes. More information and features of the fast speed camera system will be described in **Chapter 5**.

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Chapter 3. Experimental procedures

3.1. Procedure prior to experimenting with a pigeon

Before an experiment, each subject needed to go through a couple of steps. In particular, to transport a bird to the laboratory and prepare it for an experiment (i.e. make sure the bird had enough time to habituate to its new surroundings and is not distracted by unfamiliar noises, etc.), careful handling was required.

3.1.1. Housing and transportation of pigeons

All of the pigeons were cared for in the animal facilities of Cardiff University. They were obtained and housed as pairs in standard cages. Adequate suitable food and water as well as a daily health check, were ensured by the trained staff of the facility.

For each experiment, a pair of pigeons was transported individually, by car, to the laboratory site and then returned to their cage after no more than 6 hours. During the time outside of their home cage, each bird was kept in a white translucent Perspex carrier box with a transparent lid and provided with ad libitum water, except during the actual experiment, but was deprived of food. The carrier box was designed in a way to be very similar to the experimental bird box in order to reduce the subject's habituation time.

3.1.2. Subject preparation before an experiment

The laboratory in which all the experiments were carried out was located within a high-frequency shielding room, which was the size of an average room. The walls were covered with a specially designated metal, and there were no windows. Birds in their carrier boxes were placed inside the room immediately after arrival to allow them to acclimatize to the new environment. Before each experiment, a non toxic, non

permanent marking (**Figure 3.3**) was painted on the pigeon's head to make it possible to identify each bird, but also allowing determination of the orientation of its head, thus allowing an analysis of its movement. White, water-based acrylic paint that is not harmful in any way to animals was used. After this marking had been applied, the bird was placed inside a white translucent Perspex experimental box with a transparent lid (**Figure 3.1**), very similar to the carrier box. 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. The bird was left in the box for a few minutes to habituate to its surroundings. On the top of the experimental holding box, an additional white Perspex cube around a hole in the transparent lid was placed so that even particularly active birds were restricted from extending their neck to look out of the box or even escaping from the box (**Figure 3.1**). The other reason for this precaution was the need to have an opening in the lid to ensure adequate ventilation to the bird.

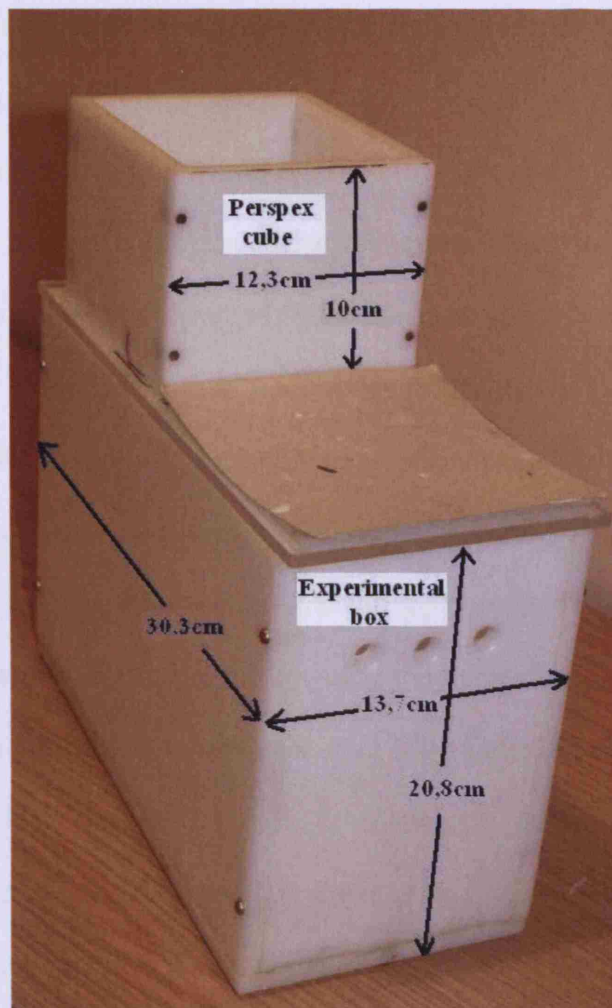


Figure 3.1 Experimental box equipped with ventilation holes on both sides. On the top of the box is a Perspex cube, placed around the hole in the covering lid, which prevents birds from looking outside the box, as well as an opaque white paper covering the remaining part of the transparent lid

3.2. Procedure during the experiments

The procedure followed during each set of experiments was essentially the same and was carried out with much care to keep the subject as calm as possible and not stressed or distracted by any noises, mishandling of the bird's box, etc.

3.2.1. Placing a bird inside the magnetic field producing setup

After the habituation period spent in the Perspex experimental box, the box was then carefully positioned inside the coils. It was ensured that the bird's head was in

the middle of the coils so that the surrounding field was uniform with the help of the aforementioned limiters (**Section 2.1.3**).

A very important objective was to keep the bird relaxed and eliminate anything that could stress or distract a subject during the experimental process, which may result in reducing its ability to attend to the magnetic field. In order to mask any extraneous auditory stimuli, and thus minimise any distractions to the bird, white noise was played through a speaker into the experimental chamber (see number 8 in **Figure 2.1**), resulting in a noise level in the bird's cage of ~60dB. In addition, the 10cm high Perspex cube (see **Figure 3.1**) (without top or bottom) placed on the top of the cover around the hole cut in the lid covering the experimental bird box allowed camera access to the bird's head movements even if the bird raised its head above the lid in an attempt to look "outside" the box. Consequently, the head was always at a similar distance from the camera, and thus it could be video-recorded clearly and in proper focus. This in turn made the further analysis of the bird's responses easier to perform. Specifically, the cube was placed on the lid of the box and the remaining part of the transparent lid was covered with an opaque sheet of white paper in order to limit area above that could be viewed by the bird (see **Figure 3.1**).

3.2.2. Procedure in setting magnetic field steps

The user decides on the specification of the sequence to which a given bird is going to be exposed by setting all of the parameters in the mainprocedure.vi programme (for details see **Figure 2.2**). After a habituation period of several minutes, which was usually limited to 2 minutes (however, in the case of a bird being too active, this period could be lengthened to 3-4 minutes), the box with a subject was placed inside the set of Helmholtz coils, which in turn were situated in the magnetic field shielding chamber. Then, the chamber was closed with a shielding lid to ensure

that the only magnetic field the birds were exposed to was the magnetic field produced by the magnetic field generating system.

3.2.3. Background white noise

To eliminate any extraneous disturbance during the experiment, the bird was exposed to continuous white noise⁶. The white noise was presented in the background with the help of a speaker that was placed against the lid's open air hole (see **Figure 2.1**– number 8). This white noise was played throughout as well as shortly before and after each experiment. Therefore, the bird was isolated from any accidental noises coming from the outside. Another potential source of acoustic noise was the amplifiers, which were located on top of the chamber (number 1 in **Figure 2.1**). However, this low volume sound remained constant regardless of the supplied signal switching, and ipso facto even if perceptible could not have any effect on the birds' behaviour. Indeed, if audible to the bird, it simply contributed to the white noise and together helped to mask any transient noises that might have occurred during the experiment.

A recurrent concern mentioned in the literature, dealing with methods for investigating magnetoreception, is especially well summed up in the paper of Kirschvink et al. (2010), i.e. the possibility that the clicking of the relay that switches on and off the field produces an audible sound.

For the purposes of this study, the Helmholtz coil system was designed so that it incorporated only one relay, i.e. the switch controlling the coil that produced the field in Z-direction (inclination). The two other coils are controlled by the Labview control card, and hence switching them on and off could not generate any noise. Nevertheless, there remains the remote possibility of a low switching noise when the relay is

⁶ White noise – acoustic noise consisting of randomly changing signals' parameters. It was named white by the analogy to the white light that is a mixture of different colours of the visible spectrum.

activated (i.e. when the Z coil – inclination – is being switched on or off). To overcome this problem and eliminate the possible perception of any click by the pigeons, the volume of the white noise was more than sufficient for that purpose. Moreover, constant noise of the amplifiers as well as enclosing the birds in the shielding chamber with five layers of metal on the outside with insulating air gaps between each of them and the wooden frame on the top together ensured that the noise shielding factor was high enough to result in the exclusion of any noise being detected from this one relay. This was confirmed by measuring the noise level inside the chamber when the relay was turned on and off, which resulted in no detectable increase (or change) in the noise level, which was consistently measured to be 60dB.

3.2.4. Conditions inside of the shielding chamber

The chamber is kept opened at all times, except when experimenting, and therefore the air flow is almost constant, which in turn ensured that bird inside was in a fresh and well ventilated area. For all experiments, a bird was never kept inside the chamber with the lid closed for more than 10 minutes. Moreover, the box in which the bird sits is equipped with holes on the sides on both ends (see **Figure 3.1** – holes in the front side) as well as there being a large opening in the lid covering the box, through which the camera clearly views the bird's head. Consequently, a sufficient level of ventilation is assured.

One possible problem that might occur is that, due to any heating of the wiring of the coils, the temperature inside the shielding chamber might rise. In order to avoid this problem, the current load capacity of used wires was selected to be much higher (10A) than the current passing through them (up to 2A). Thus, the wiring during the experimentation remained cool, and could not produce any increase in the chamber's internal temperature. The room in which the experiments were carried out is also

equipped with a ventilation system that maintains the temperature between 21 and 22°C. The air conditioning of the room also produces a noise; however, its constant level of the volume is much lower compared with the white noise and the noise of the amplifiers, which entirely masks the air conditioning noise.

3.2.5. Animal procedures

The procedure involves the placement of birds in a constrained space (experimental box) for no longer than 15 min, and was reviewed by the University's Biological Standards Officer and the assigned UK Home Office Inspector. The Inspector's judgement was that the proposed experimental protocols did not fall within the regulations that govern licensed procedures with animals, which only apply when the animal may be caused stress or lasting harm. However, the ethical guidelines with respect to handling and restraining birds required for licensed procedures in the UK (UK Animals (Scientific Procedures) Act 1986) were followed.

3.3. Software for recorded video analysis

The head movements of the birds are the main focus in this study with respect to the data analysis, the main measure of response upon which rests both data gathering and subsequent analysis. Therefore, it was essential to be able to extract information about any change in the bird's head position throughout each entire recorded video, and ipso facto, frame-by-frame analysis was vital. In most cases, where video was recorded for a few minutes with a frame rate of 30 frames per second, the total number of frames was in the thousands, and hence manual analysis was not possible in these circumstances. The only option in such a case was to digitize the frames and use the computer to analyse head position. In order to complete this task, an additional analysis software was programmed in Labview. This frame-by-frame analysis

programme was written specifically for this investigation by the author. The main role of the dataextraction.vi was to access directional information from the recorded movie as accurately as possible. The main features of the dataextraction.vi are:

- setting frames per second rate according to the recorded video's frame rate
- specifying the number of frames to be analysed and the number of a start frame (i.e. which part of video is to be analysed)
- choosing a template using the identifying mark on the top of the head on any frame in a straightforward way to determine changes in head orientation
- saving the results in any convenient file type (e.g. .xls, .txt)

3.3.1. Extracting the data by means of the video analysis software

As mentioned above, a frame-by-frame analysis was performed. Therefore, the video must be converted into a set of frames, numbered in ascending order, so that it is convenient for the software to look for the template on each subsequent frame and output the result in an ordered way. Having accomplished this, the Labview programme is used to search for the template on the chosen frame; this is the special marking placed on the top of the bird's head (see top on **Figure 3.3**). The template is defined by a user within the software, usually on the first frame; however, any other frame can also be used. Then, when the programme is started, each frame is loaded in an ascending order, opened and the template is searched for. When the template is located (see **Figure 3.3**), its rotational translation is logged to memory. Rotational translation is an angle of rotation between the position of the specified template ((a) in **Figure 3.3**) and its position in the investigated frame ((b) in **Figure 3.3**). Here it should be mentioned that, of course, the bird's head does not move only in the XY plane, but may also pitch or/and roll. However, in the present setup (i.e. with only one

overhead camera), it is only possible to access the rotation in the XY plane. After all frames have been processed to determine the bird's head position in each, the whole data set is then calibrated to the 0° reference axis. Resultantly, each head movement to the right (with respect to the reference axis) of the birds becomes "+" value and movements to the left are assigned with "-" (see **Figure 3.2**). The data then can then be saved in different formats. Since subsequent analysis is needed (see **Section 3.4**), the file for this purpose was saved as .txt.

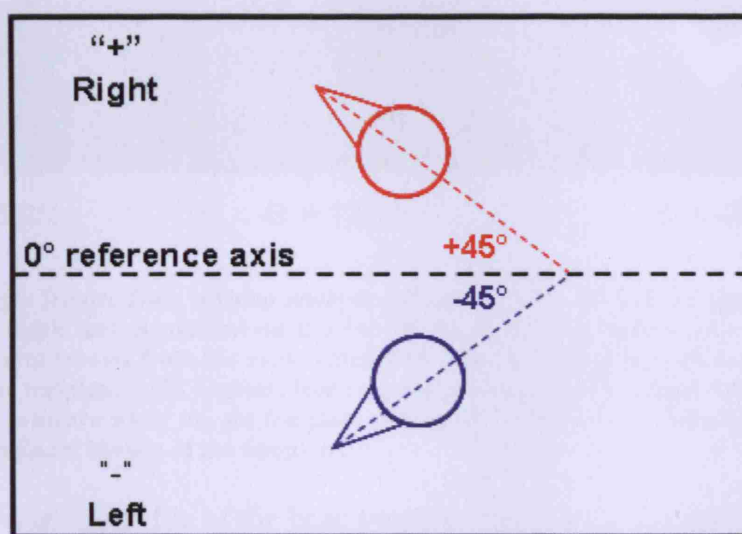


Figure 3.2 Sketch showing schematically the situation captured by the digital camera of the bird's head movement to its right (red) and to its left (blue) calibrated with respect to the reference 0° axis of the box.

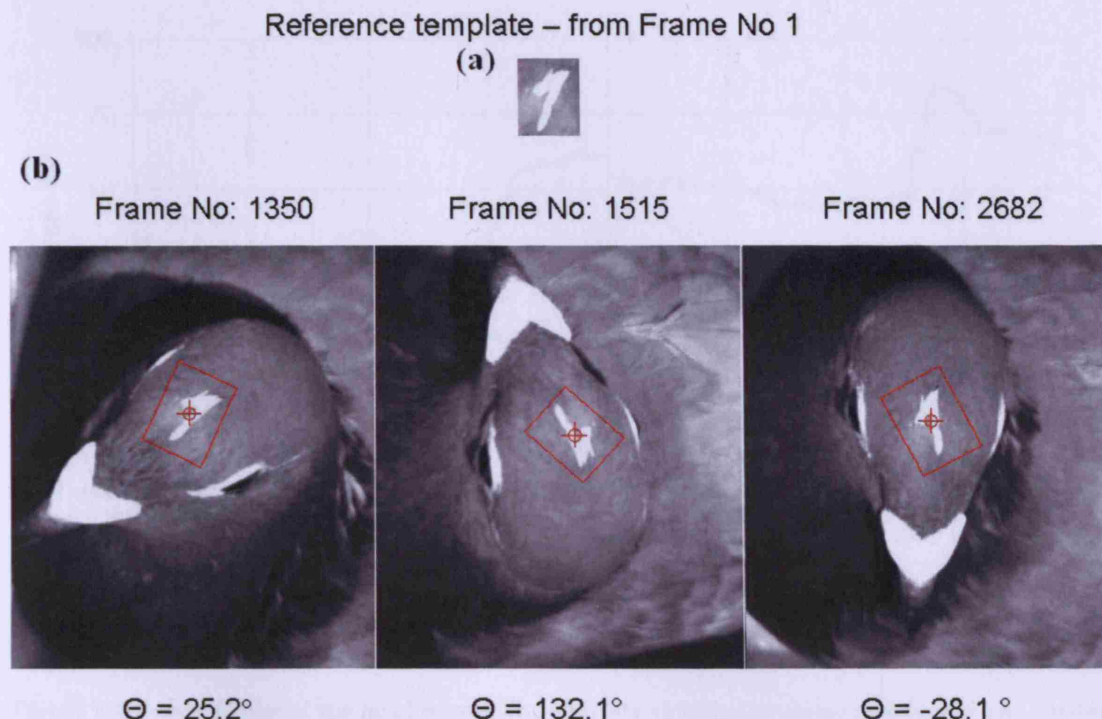


Figure 3.3 Example frames from a video analysis performed with the help of `dataextraction.vi`, (a) the template mark that is painted on the top of the birds head before an experiment, (b) examples of different frames from the same video with angle Θ – rotational change of the frame with respect to the template (a) in degrees. Red rectangles on the pigeon's head are automatically displayed by the software when the set template is located on the frame, and at the same time, they show the rotational change of the template.

Furthermore, an Excel file of the head position data can be also saved in order to facilitate plotting XY charts, on which the X axis represents time and Y axis represents the head position (angle) in each frame. This enabled the graphical presentation of the data from each experiment, which in turn provides a good overview of the bird's activity with respect to temporal changes in the generated magnetic field, i.e. one of the four main possibilities (CW, CCW, SF, and NF) (Figure 3.4).

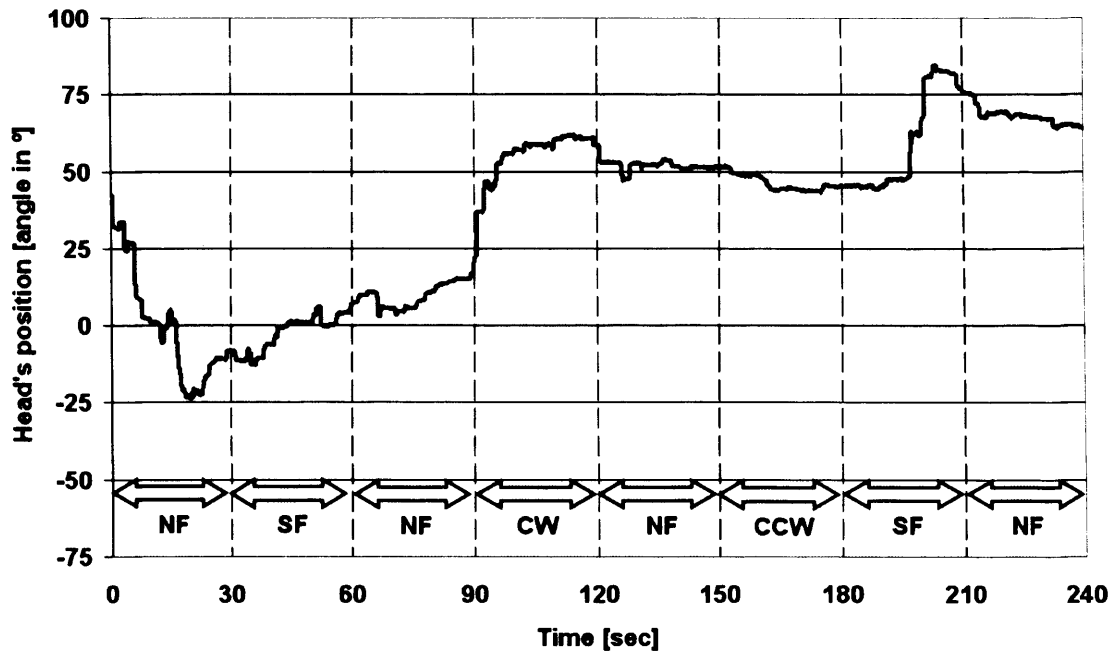


Figure 3.4 Example plot of the head movement analysis obtained by dataextraction.vi and plotted in Excel.

3.3.2. The head template becomes invisible

As live pigeons were being recorded, any number of behaviours (e.g. hyperactivity, bowing, head tilting, etc) could result in the head marking being lost from the view of the camera. Although such behaviour was present from time to time, a specific procedure was applied when the head marking became briefly invisible. Two different actions were taken depending on the length of time during which the marking was hidden from the camera and hence could not be picked up by the software. Firstly, in the event that the marking was lost for only few frames, the data was corrected manually. Manually here means that the plotted chart of the data was examined for the lost/missing frames, made easy by the fact that the software automatically marks frames on which the template cannot be located. Then, when the area of the data loss is identified, each such frame from the data analysis is located in the video and the value of the rotational change of the head is corrected manually. Secondly, if the top of the head was out of camera view for a longer period during an

experiment or a manual correction was not possible to introduce, such cases were rejected and not analysed. Importantly, there was no one specific procedure that could be followed in the case of data loss and hence the correction of the video, if needed, was performed on a case by case basis.

3.4. Analysis of the collected data

The data obtained with the aid of dataextraction.vi (for example see **Figure 3.4**) were next subjected to a number of analyses in order to look at different parameters defining the birds' activity (e.g. total activity, number and size of head movements, distribution of head movements, latency of the response, etc.) 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. do the birds respond to transitions?

Hence, several programmes were written for data analysis purposes using the already described Labview programming environment, and the dataextraction.vi was specifically written for the purposes of this research. The following analysis programmes were also prepared especially for this investigation by the author.

3.4.1. Software for the initial analysis

The first programme written for analysis purposes was dataanalysis.vi, which was programmed so that the data obtained earlier from dataextraction.vi were loaded and an initial differentiation was performed on the whole data set. The differentiation was done by implementing the equation below:

$$\frac{1}{2} \left(\frac{y_{i+1} - y_i}{x_{i+1} - x_i} + \frac{y_i - y_{i-1}}{x_i - x_{i-1}} \right) \quad (3.1)$$

As the camera frame rate used in the experiments was 30fps, the number of data points was 1800 for each minute of the experiment. In most cases, the experiments lasted for 4 minutes, which produces 7200 data points in each case. In order to reduce the number of data points to be analysed, without losing any important information, 5 consecutive points (i.e. 1/6s) were averaged before differentiation took place.

After differentiation, the next step was integration, which has the purpose of obtaining the birds' activity in digital values for each second of the experiment; this involved summing the integrated activity over 6 time points. At the end, the data file could be saved in either a .txt or .xls file.

Before data file analysis was begun, several starting conditions using the control buttons on the front panel had to be specified (e.g. number of magnetic steps, length of the experiment, row in the source .txt file, etc.). This was due to the need to specify precisely where transitions occurred within the whole sequence of the data. Once all of the data were integrated, which was a measure of the activity of a bird, transitions were the chief subject of interest, since it was expected that the birds would react most abruptly immediately, or soon after, changes in the magnetic field conditions. The area of 10s before and after each transition was chosen (box 2 in **Figure 3.5**), although the software allowed this time window to be altered. This led to obtaining, automatically, the activity of a bird within a specified time period around each transition. This was the first approach to the data analysis with the hope of paving the way for determining whether the birds actually respond to the transitions in a time dependant manner.

There is also the possibility, using the same programme, of changing the output region of interest when analysing. Namely, instead of only 10s either side of a transition, the activity of birds over each entire step during the experiment could be scrutinised. Accordingly, the information on the activity of each bird within each magnetic step may also be obtained and examined by the software.

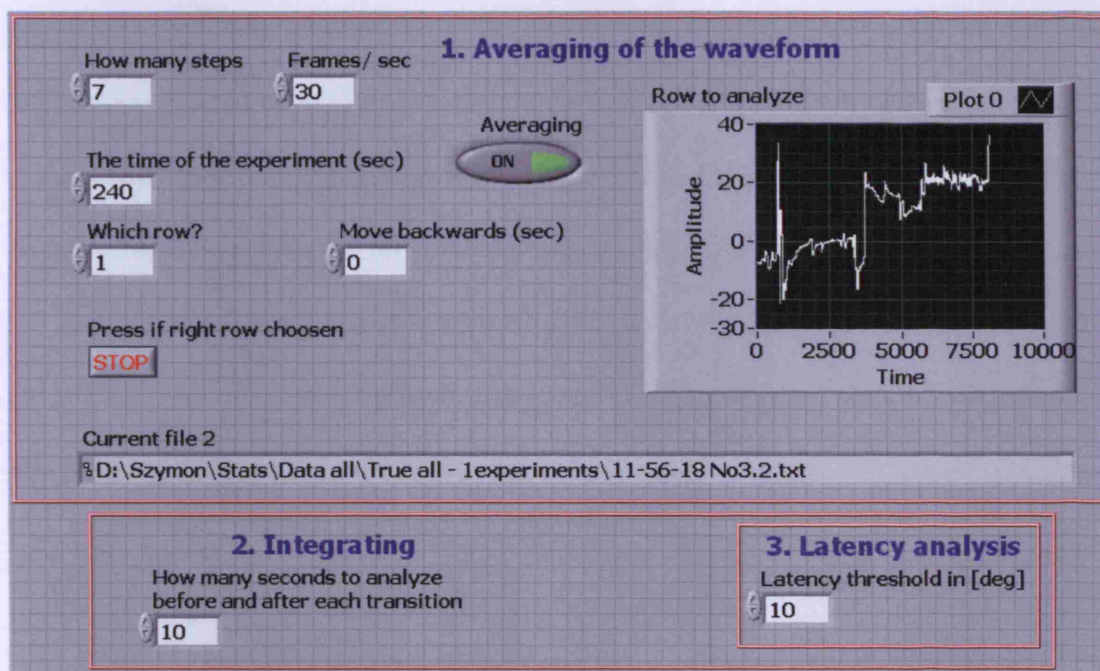


Figure 3.5 Control panel of the dataanalysis.vi. (Which row? – specifies the row within the spreadsheet containing the data to analyze, Move backwards – gives the possibility of moving the data along the time axis)

3.4.2. Field transitions analysis – integration method

The first idea that was developed in order to look more closely at the birds' reactions to changes in magnetic field conditions was a simple integration method, which was applied to the results that were obtained in the form of a chart of head position versus time to show changes in head angle during the experiment (**Figure 3.4**).

As there is an abrupt change in the field conditions at each transition between different magnetic field steps, it might be expected that a bird's most salient reactions will be present at these time points in the experimental sequence. For this reason, the

first approach in the analysis of the results was focused on examining the pigeons' behaviour around transitions. Because the transitions were fixed (time locked), i.e. they occur in the same places within the experimental sequence, the analysis could be automated and focused on the 10s period before and 10s after each transition (**Figure 3.6**). The integration method consists of two steps. The integration of that area was calculated from the absolute value of the results after differentiation of the data. Removing the sign of the integrated value eliminates the problem of subtracting head movements of different direction from each other and then summing up total activity irrespective of whether the movements were to the right (+ on the below plot) or to the left (on the below plot) (for allocating signs to the head movements see **Figure 3.2**). Then, the difference between integrated head movement activity 10s after and 10s before each transition was calculated.

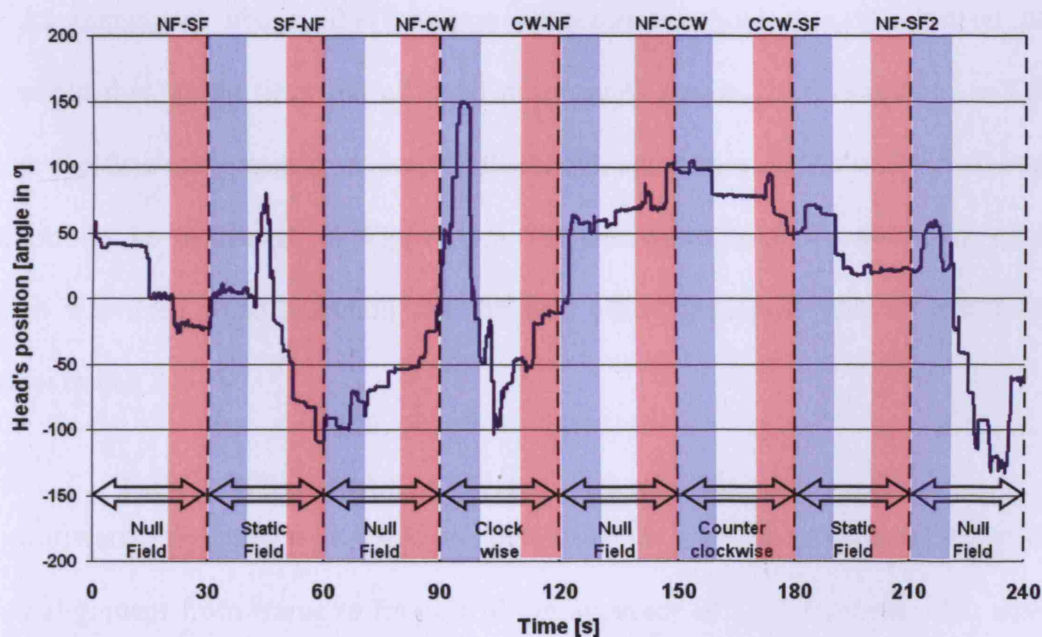


Figure 3.6 Example sequence with marked 10s regions before – red- and after – blue – each transition. Above each transition there is a name that each transition was assigned in the short form.

3.4.3. Latency of the response analysis

Another interesting approach when analysing the data would be to look at how quickly the birds reacted (by changing head position angle) to transitions between magnetic field and then characterizing these reaction times. In order to find out the timing of the expected responses, the first head movement over a 10° threshold (for more explanation on the threshold see **Section 3.4.3.1**), defined as a reaction, after each transition was used. To be able to automatically access the data in a search for the latencies of the reactions, further software was written. The software described in **Section 3.4.1** served as the backbone for the approach, i.e. a starting point for analyses of response latencies as implemented in subsequent programmes. Therefore, within dataanalysis.vi, latencyfinder.vi was embedded to pick up the latency of the birds' reactions to each transition.

As mentioned above, this analysis approach involved the introduction of a threshold that would filter out all head movements below a set amplitude and thus allow the first movement over the 10° threshold, referred as “reaction” (see the next section) to be measured. In **Figure 3.5**, the minimum head movement threshold, which was used when searching for the first head movement after a transition, is shown in box 3.

3.4.3.1. Threshold of birds 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° . 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 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 in precise 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 $<10^\circ$ in order to more readily identify such parameters as response latency, number of head movements, etc. The full rationale behind setting such a threshold is presented in **Section 4.3.3.1**.

One could doubt that this analysis may not cope with the situation of a slow drift of the bird’s head. However, this problem is not present in this analysis, as the sampling rate with which the video is checked for the change in head movement position is equal to 30 frames per second. This means that within each second there are 30 measurements points, which in turn give accurate insight in the head movements. Even in the situation of a slow drift there have to be at least short intervals in the movements, which, due to the sampling rate, will be detected.

3.4.4. Number of head movements analysis

In the following analysis programmes, dataanalysis.vi was used and changed or developed as necessary to allow further analysis of the data depending on the subject of interest. For instance, to facilitate calculating the number of head movements present in each step, the dataanalysis.vi was modified. Namely, integration was removed and all of the head movements above the threshold (e.g. abrupt change in the head angle at 90s in **Figure 3.4**) were detected rather than just the first, as in the latency analysis. Resultantly, another programme analysing the number of head movements over a certain threshold in each step, i.e. headmovements.vi, was designed.

3.4.5. Head movements distribution

In the final programme headmovementdistribution.vi, not only the number of head movements was under scrutiny but also the amplitude and timing of each and every single head movement of a bird during the experimental sequence. The entire trajectory of a bird's head movement (e.g. XY plot **Figure 3.4**) was searched for changes in head angle – i.e. head movements –, including the exact time at which it occurred as well as its magnitude. All of this information across all head movements was then saved. In this analysis, however, very small head movements, i.e. less than 3° were omitted and not taken into account. As these head movements were “omnipresent”, they would not carry significant information in terms of specific reactions and the removal of so many “noisy” measurement points made the preparation of the maps of head movements, e.g. as in **Figure 3.7**, much more convenient and less time consuming. The remaining head movements above were put in bins of $3\text{-}5^\circ$, $5\text{-}10^\circ$, $10\text{-}15^\circ$ and so on to $25\text{-}30^\circ$ and the last bin contained all the movements over 30° and then saved into an .xls file. This had the purpose of depicting the data in such a way as to better discriminate the temporal distribution of the birds' responses. An example of this approach, programmed in the headmovementdistribution.vi, is presented in **Figure 3.7**, where each and every head movement of a particular bird is denoted with a dot that represents the magnitude and time of its occurrence.

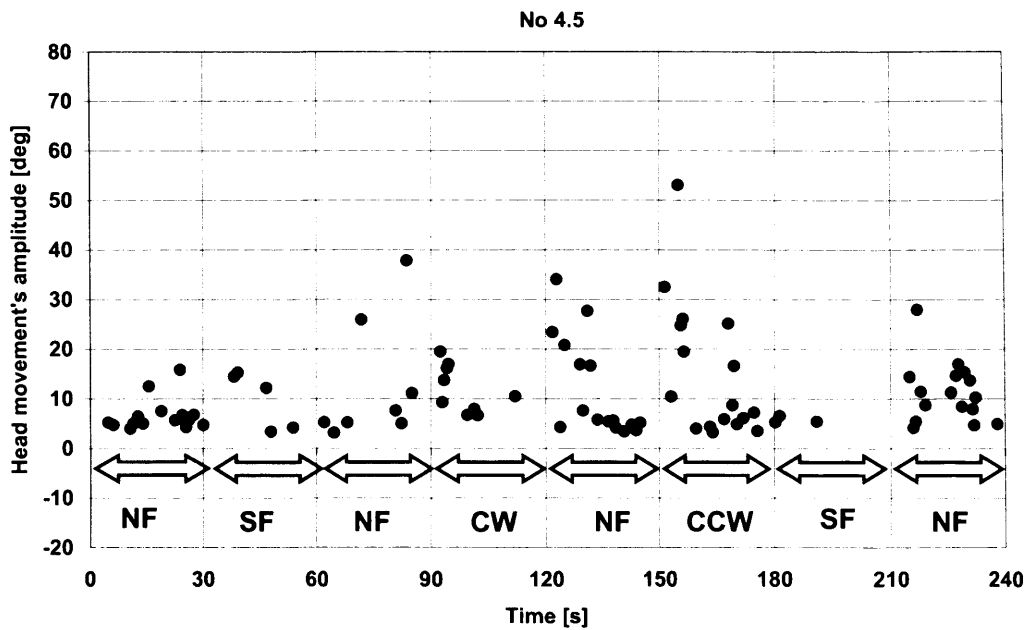


Figure 3.7 Example of the plotted analyzed data in headmovementdistribution.vi.

3.4.6. Mean and median values

The data was, depending on the analysis, presented as a mean and median value calculated across all subjects within the experiment. Due to various levels of activity among the tested birds that were sometimes very different from each other, the need for including the mean as well as median values emerged. For example, one or two birds being much more active than the rest would affect the mean value disproportionately. However, when in the same situation the median value was used, the result, in spite of an outstanding bird or two, was a better indication as the birds as a group. For this reason, the graphic presentation of the following analyses, i.e. transition analysis, number of head movements, total activity, and latency, incorporated both the mean and median results.

3.4.7. Statistical analysis

3.4.7.1. General Linear Model

In order to determine whether there is a significant trend in the response after magnetic field transitions, as observed in a majority of birds, statistical analysis was carried out. The main statistical tool used in this study was the general linear model (GLM).

The GLM model is an established statistical method, which was developed as a widely used statistical tool in the 20th century. “Linear model” means that the method is calculated with the help of a regression line, and the model refers to the equation describing this regression line, which is fitted to the analysed data. In the GLM model, there can be numerous variables; either categorical variables (factors), which in turn may be fixed (i.e. the same for all experiments, e.g. type of transition in this study) or random (i.e. loosely correlated with the experiment, e.g. particular birds used in the experiments) as well as continuous variables (covariates). The GLM model enables an overview of all the variables and covariates, if present, and then specifying those that exhibit a significant association with the dependent variable. Therefore, this method seems the most appropriate in the statistical validation of the effects of changing magnetic fields on the birds.

When calculating the final GLM model, a stepwise procedure was used, with the help of SPSS software, and thus only single variables as well as the significant interactions between two variables (or the interaction with least p value) were included in the resultant table.

3.4.7.2. T-test

The other statistical approach used in the present study was the well known Student's t-test, which will be referred to simply as t-test. The concept of the two sample t-test, which was used when analysing the results obtained in **Chapter 4** and **Chapter 5**, is based on testing the null hypothesis that the means of the two tested data sets are equal. The t-test is a parametric test, which means that it is valid only when applied to data which are (or nearly) normally distributed. The type of t-test used was an unpaired two-sample, or in other words, an independent samples test.

3.4.7.3. Fisher's Exact test

The third statistical method, Fishers' Exact test, was used to test the statistical significance of the possible directionality in the birds' responses to magnetic field sweeping in one of two opposite directions (i.e. CW and CCW) (**Section 4.3.8**). This test uses contingency tables, which include different treatments and their different (or not) outcomes. Fisher's Exact test is usually used instead of the more common Chi-square test when the sample sizes are relatively low. It is named "exact", because the exact value of the degree of the deviation from the null hypothesis can be calculated.

3.5. References for Chapter 3

(1986). Animals (Scientific Procedures) Act 1986 OPSI. London, Her Majesty Stationary Office.

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Chapter 4. Birds' responses to alteration in EMF - magnetic field sequence experiment

The new method that has been developed and described in detail in the previous chapter has been implemented by conducting experiments with homing pigeons as subjects. There were several groups of pigeons used in the experiments, which were undertaken sequentially as explained below.

4.1. Aims of the magnetic sequence experiment

The main purpose of this experiment was to validate the method for investigating magnetoreception in homing pigeons by establishing whether the birds respond to changes in the ambient earth-like magnetic field. The parallel objective was to analyse results for both the timing (i.e. in view of the radical pair mechanism – how fast birds are able to detect and react to an alteration in the magnetic field) and properties of any behavioural response in the hope of shedding light on magnetic field perception in birds.

4.2. Experimental design

4.2.1. Experimental birds

The magnetic sequence experiment was performed in two runs with two different groups of birds. The first group consisted of six pigeons that were investigated following the procedures described in detailed in the previous chapter.

The next experiment was carried out with a second group of 16 birds, from which three were ultimately excluded because they did not show any responsiveness, i.e. they “froze” in the experimental box and made no head or body movements. Of the

remaining 13, three more were rejected due to hyperactivity or lack of responses (for the full criteria of bird selection see **Section 4.2.3** and **Appendix 1**). Thus, for both experiments, a total of 16 experimental birds were included in the analysis, the results from which are presented in this chapter. The results were looked at from different perspectives in order to make the most sufficient use of the data obtained.

There is one difference between the sequence of magnetic fields experienced by the first and second groups of birds. Specifically, the first group began with a null field (NF) and the second group with a static field SF, which were the starting conditions of the respective experiments (for more information see **Section 4.2.5**) As a consequence, the first transition that birds within the two groups experienced was different. This slight variation in conditions, however, did not affect the birds' behaviour significantly (**Section 4.3.9**), and thus the results of the two groups were eventually combined, as presented and described in this chapter.

4.2.2. Birds used in the control experiment

In the case of the control experiment, initially 14 birds were selected out of which 4 were rejected due to difficulties in data extraction either due to a wrongly painted mark on a bird's head, hyperactivity or inactivity (see **Appendix 1**). The remaining 10 control birds were analyzed in the same way as the experimentals, i.e. with respect to the same timing of the field "transitions" but in the absence of any magnetic field as the Helmholtz coils were not fed with any current. Note that the birds used in the control experiments were not the same as those used in the real experiments (see **Appendix 1**) due to the fact that the birds were no longer available.

The main goal of this control experiment was to investigate the birds in the absence of any magnetic field changes, in contrast to the main experiments, and thus act as a basis for a comparison of their behaviour in the two situations. For this

reason, the control experiment was designed to be the same duration (4 minutes) as the real experiment, but in the absence of any field (NF). Thus, the behaviour of the birds could be measured in the chamber for the same length of time but when there are no field changes or indeed any field at all. This represents a “matched” control experiment in which the only difference was the presence or absence of *changes* in the magnetic field conditions.

4.2.3. Criteria for bird selection

The use of live behaving animals of course, influenced the process of data acquisition. Namely, as the homing pigeons can be quite individual in their behaviour, there was an intrinsic variability in their responses. Thus, although the conditions were the same for all subjects, the birds at times exhibited extremely different behaviours – from falling asleep, to turning around in the box, to preening their feathers and even to escaping from the experimental box! Therefore, in order to obtain meaningful data, consistent selection criteria had to be applied.

Due to this individuality in behaviour, the data from some of the birds that underwent the experimental or control procedure could not be used in the analysis. This was mainly owing to the missing head movement data that could not be extracted from the video record. Specifically, data on the rotational change of the head could not be obtained from the video for one or more following reasons:

- the bird was hyperactive, which lead to the situation where the mark on the head was invisible because the image of the head was too blurred;
- the bird bowed or tilted its head and in consequence, the mark on the top of the head, which was tracked for rotational changes in head position, was lost from the camera's view;

- the mark was put in the wrong position, or its particular shape prevented the software from recognizing the mark on every frame.

If any of the above conditions was present, the behavioural data from the experiment was either rejected straight away, or unless the head mark was invisible on a reasonably small number of frames, the head positions were corrected by hand (see **Section 3.3.2**). On the other hand, a bird could be inactive during most of the experiment. In both cases, i.e. hyper- or inactive birds, the data from such birds were rejected and the experiment was repeated. If, in the second run, the situation was similar, such a bird was excluded from further experiments.

All of the information for birds in all experiments performed for this study that were included in the analysis, as well as rejected cases and, if it was rejected, the reason(s) for exclusion can be found in **Appendix 1**.

4.2.4. The process of framing the basis for the experiments

Before the experiments presented in this chapter were performed under the procedure described in the previous chapter, trial experiments were carried out. These trials were designed to specify a possible magnetic step order that would be particularly salient for the tested birds. Therefore, different sequences of the magnetic steps, as well as their time durations, were tried. But during these trials, other problems arose that needed to be addressed.

The first was caused by birds occasionally raising their heads and looking outside the experimental box, which resulted in the camera being out of focus, making it impossible to extract the data from those recorded frames. To eliminate this problem, a box was placed over the hole in the lid preventing the bird from looking above the lid but still allowing the camera a view of the birds. For this reason, a white Perspex

cube (without top and bottom) was always placed on the top of the experimental box (see **Figure 3.1**) for subsequent trials as well in the proper experiments.

Another difficulty that was encountered in the course of the trial experiments involved deciding the best way to mark the birds' heads to allow a determination of the rotational position of the tested bird's head throughout the whole experiment, which was intended to be carried out using frame-by-frame analysis. The first idea was to use a strip of "post-it" paper lightly glued to the bird's head. However, this attempt failed because birds were distracted by the strip on their heads and kept trying to get rid of it, thus interfering with their attention to changes in the magnetic field. A subsequent idea was to use white acrylic paint (or a black whiteboard marker on birds with white heads). It appeared to be a good solution as it did the birds no harm, and they were not aware of anything on their heads. Nevertheless, it was not sufficient to specify the kind of marker used; the shape of that mark was also very important as it had to be of a specific shape in order to enable accurate software extraction of head alignment. Finally, after trying out various shapes and running the software on each to see whether the accuracy was high enough, a suitable configuration was found (see **Figure 3.3**). This comprised a "V" shape in which one line was longer than the other and there was an acute angle between them. This enabled the software to recognize any rotational change, removing the problem of a possible "symmetry mistake" of the software in recognizing the mark's angle which would lead to a 180° error.

It took almost 9 months, 6 birds and trying many different sequences to achieve what is described above as well as to find out that, when the sequence was longer than 4 minutes, the birds became increasingly agitated. Furthermore, 30s steps were sufficiently long exposures to a given magnetic condition; shorter steps did not appear

to allow enough time between magnetic steps for birds to accommodate and fully experience the effect of the changes in the magnetic field.

4.2.5. SF preceding magnetic field sequence

Originally, the sequence in the first experiment began with the coils not fed with any current (i.e. NF), but after the first group had finished, this starting condition was reconsidered. It seemed advantageous that SF should be present from the moment the bird was placed in the chamber, in order to minimise any initial “magnetic shock” that the birds might have experienced had they been placed in the shielded chamber with the coils not producing any magnetic field. After all, this initial NF might well be the first time in these pigeons' lives that they were completely unable to sense an ambient magnetic field. As birds were being investigated under changing magnetic field conditions, it seemed preferable to eliminate *any* changes in the field that birds could experience *before* the proper experimental sequence that was recorded and analysed. This alteration of the starting condition to the sequence was introduced in the remaining birds (Group 2). However, as the results will show in **Section 4.3.9**, there was no significant difference in the overall pattern of behavioural responses in the experiments with (Group 2) or without (Group 1) SF prior to the initiation of the magnetic field sequence, and thus the data from both first and second groups were merged, analysed and presented together.

This investigation incorporating two experiments, one involving exposure to changes in magnetic field and the other a control under NF, were the base of this research. Therefore, they served as the basis for subsequent experiments to investigate the nature of the birds' head movements and the influence of light on their responses to field transition. All birds used in these investigations underwent the procedures described in **Chapter 3**.

4.3. Results

4.3.1. Transition analysis

As the transitions between magnetic field conditions were the times at which the reaction of the experimental birds could be expected with the highest probability, the first analysis approach was to concentrate on the birds' behaviour after each transition and compare it with their activity prior to this transition (**Section 3.4.2**). Therefore, as a starting point, the difference between the pigeons' activity (using integrated head movement) within 10s after and before each transition was obtained, revealing the findings presented in **Figure 4.1**. Namely, two transitions stand out as clearly different from zero, i.e. the expected value in the case where there is, on average, no reaction displayed by the majority of birds to the change in the magnetic field conditions. Specifically, in the transitions NF-CW and the immediately following CW-NF, the differences in the activity (see **Section 3.4.2**) have high positive values, which means that there was much higher overall activity after these transitions than before. The increase was not present in only a minority of the 16 tested birds, as one might infer from the reasonably low mean values (blue bars on **Figure 4.1**). In fact, such a trend was present in *most* subjects, which is indicated by the fact that the median values (red bars) also have highly positive values in these two transitions. Therefore, the median value of this difference in activity is especially well suited for denoting the transition in which a significant change in behaviour existed in a large proportion of birds. Besides the NF-CW and CW-NF, none of the remaining transitions is higher than 20, which suggests that these field changes were not sufficiently apparent and/or stimulating to attract the attention of most of the birds and provoke an increase in head movements.

Results from an analysis of the control birds show that the differences in activity on either side of the transitions (i.e. “fake” or virtual transitions in the case of controls that were set for comparison purposes) are even lower *throughout* the experiment. Although the mean values (yellow bars) of the activity around each transition are high in some cases, the median values (turquoise bars) are much closer to zero, which suggests the absence of any stimulus affecting the birds' attention. This is in contrast to the experimental birds investigated under changing magnetic fields, which displayed a significantly higher overall amount of head movement activity after transition than before in the case of NF-CW and CW-NF (paired t-test, see **Table 4.3**), as indicated in **Figure 4.1** by both mean and median values.

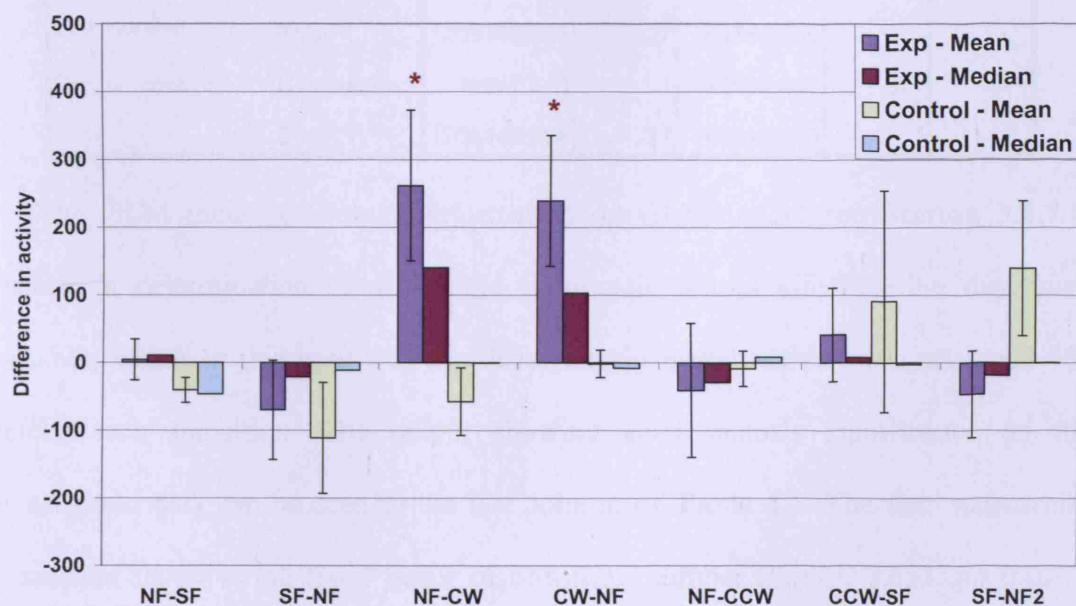


Figure 4.1 Mean and median difference in the birds' activity 10s after and before each transition in experimental and control cases, *- statistically significant paired t-test ($p < 0.05$) (performed on the true (not average) values of activity before and after each transition). Error bars represent standard errors. (NF-SF – transition from NF to SF, SF-NF – transition from SF to NF, and the same convention with the others)

Table 4.1 Analysis of variance table of the general linear model (GLM) for difference in activity at transitions of the experimental and control birds. (Transition_number*Bird_number is an interaction between the two variables that represents the prediction of the pigeons' head movements response to transitions depending on the specific bird; Exp_or_control is a variable comparing the birds subjected to the real magnetic field alterations (Exp group) and birds that did not experience any change in the magnetic field (Control group)) (df – degrees of freedom, F – variance ratio of which Sig. gives significance value (p value))

Tests of Between-Subjects Effects

Dependent Variable: Difference_in_activity

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	119094.823	1	119094.823	1.561	.222
	Error	2073503.773	27.179	76289.693		
Transition_number	Hypothesis	1642198.657	6	273699.776	2.653	.018
	Error	1.360E7	131.822	103163.939		
Bird_number	Hypothesis	1782543.786	21	84883.037	.805	.709
	Error	1.350E7	128.053	105393.081		
Transition_number * Bird_number		1.340E7	125	107210.398	2.396	.005
Error		1208146.298	27	44746.159		
Exp_or_control	Hypothesis	16867.845	1	16867.845	.377	.544
	Error	1208146.298	27	44746.159		

The GLM model (for more information on GLM model see **Section 3.4.7.1**) enabled a determination of all of the significant factors affecting the dependant variable, which in this case was the difference in birds' activity 10s after and 10s before each transition. The output showing each factor's significance on the investigated data can be seen in the last column of **Table 4.1**. The first statistically significant factor is the fixed factor of transition number ($F_{6,132} = 2.653$, $p = 0.018$). That carries the information that the birds reacted in a significantly different manner to different transitions; in other words, it means that different transitions had different impacts on a given bird's reactivity. The other factors (i.e. fixed factor experimental versus control and random bird number) did not have any effect on the birds' reactions. The fact that the Exp_or_control value was not significant indicates that there is no variability between the true and control experiment in terms of a difference

in activity across all the birds and transitions. This could be expected from the results in **Figure 4.1**, where only two middle transitions differ between experimental and controls, while in the remaining five transitions there is hardly any difference between them, especially when medians are compared. The interaction of transition number and bird number factors had a significant impact, which most likely is a result of the large variability in activity/responsiveness between the individual tested birds, specifically, individual differences in responsiveness to magnetic field changes.

Nevertheless, this outcome only tells us that the transitions did indeed exert different impacts on the birds, but the information about which transition(s) significantly affected birds is not available from the analysis within **Table 4.1**. A post-hoc multiple comparison between all the transitions indicated that the two transitions with the highest difference in activity at the transition (**Figure 4.1**) are the only transitions significantly different from the others (the cases of the transitions that are significantly different from each other are highlighted in **Table 4.2**).

Table 4.2 Table of GLM post-hoc analysis of multiple comparison of the difference in activity 10s after and 10s before each transition for experimental birds, main experiment (Section 4.3.1) (*The mean difference is significant at the .05 level. and the p (Sig.) value for such cases is highlighted)

Multiple Comparisons

Difference_in_activity: LSD

(I) Transition_number	(J) Transition_number	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1- NF-SF	2- SF-NF	73.00	58.669	.224	-47.38	193.38
	3- NF-CW	-151.97*	58.669	.015	-272.35	-31.59
	4- CW-NF	-159.54*	58.669	.011	-279.92	-39.16
	5-NF-CCW	15.55	58.669	.793	-104.83	135.93
	6- CCW-SF	-73.36	58.669	.222	-193.74	47.02
	7- NF-SF2	-35.25	59.252	.557	-156.82	86.33
2- SF-NF	1- NF-SF	-73.00	58.669	.224	-193.38	47.38
	3- NF-CW	-224.97*	58.669	.001	-345.35	-104.59

	4- CW-NF	-232.54*	58.669	.000	-352.92	-112.17
	5-NF-CCW	-57.45	58.669	.336	-177.83	62.92
	6- CCW-SF	-146.36*	58.669	.019	-266.74	-25.99
	7- NF-SF2	-108.25	59.252	.079	-229.83	13.32
3- NF-CW	1- NF-SF	151.97*	58.669	.015	31.59	272.35
	2- SF-NF	224.97*	58.669	.001	104.59	345.35
	4- CW-NF	-7.57	58.669	.898	-127.95	112.80
	5-NF-CCW	167.52*	58.669	.008	47.14	287.89
	6- CCW-SF	78.61	58.669	.191	-41.77	198.98
	7- NF-SF2	116.72	59.252	.059	-4.86	238.29
4- CW-NF	1- NF-SF	159.54*	58.669	.011	39.16	279.92
	2- SF-NF	232.54*	58.669	.000	112.17	352.92
	3- NF-CW	7.57	58.669	.898	-112.80	127.95
	5-NF-CCW	175.09*	58.669	.006	54.71	295.47
	6- CCW-SF	86.18	58.669	.153	-34.20	206.56
	7- NF-SF2	124.29*	59.252	.045	2.72	245.87
5-NF-CCW	1- NF-SF	-15.55	58.669	.793	-135.93	104.83
	2- SF-NF	57.45	58.669	.336	-62.92	177.83
	3- NF-CW	-167.52*	58.669	.008	-287.89	-47.14
	4- CW-NF	-175.09*	58.669	.006	-295.47	-54.71
	6- CCW-SF	-88.91	58.669	.141	-209.29	31.47
	7- NF-SF2	-50.80	59.252	.399	-172.37	70.78
6- CCW-SF	1- NF-SF	73.36	58.669	.222	-47.02	193.74
	2- SF-NF	146.36*	58.669	.019	25.99	266.74
	3- NF-CW	-78.61	58.669	.191	-198.98	41.77
	4- CW-NF	-86.18	58.669	.153	-206.56	34.20
	5-NF-CCW	88.91	58.669	.141	-31.47	209.29
	7- NF-SF2	38.11	59.252	.525	-83.46	159.69
7- NF-SF2	1- NF-SF	35.25	59.252	.557	-86.33	156.82
	2- SF-NF	108.25	59.252	.079	-13.32	229.83
	3- NF-CW	-116.72	59.252	.059	-238.29	4.86
	4- CW-NF	-124.29*	59.252	.045	-245.87	-2.72
	5-NF-CCW	50.80	59.252	.399	-70.78	172.37
	6- CCW-SF	-38.11	59.252	.525	-159.69	83.46

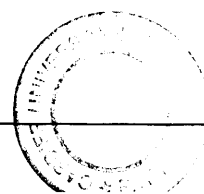
The multiple comparison analysis revealed that the expected transitions based on **Figure 4.1** (i.e. NF-CW and CW-NF) indeed stand out among the rest (**Table 4.2** - e.g. in the first column ((I) Transition_number) and in the first row (1- NF-SF), the two transitions – NF-CW ($p=0.015$) and CW-NF ($p=0.011$) are significantly different from this NF-SF transition).

Furthermore, the observed greater difference in activity just after, with respect to the activity just before, these transitions could also be confirmed statistically by a parametric (the data appeared to be normally distributed) paired t-test (**Table 4.3**). The results confirm what was indicated by the post-hoc analysis of the GLM model, and could be observed in **Figure 4.1**; the only statistical differences in activity between 10s after and 10s before a transition were found in NF-CW and the immediately following CW-NF transitions in experimental birds. The other transitions in the case of experimental birds and indeed all the transitions in control birds were found to have no impact significant impact on the birds' reactions as measured by integrated activity (t-test analysis of all control transitions together revealed the lack of any significant pattern ($p= 0.99$)).

Table 4.3 The comparison of paired 2-tailed t-test's p values between experimental birds (exposed to true magnetic field transitions) and control birds (did not experience any magnetic field changes). * marks significant cases of $p<0.05$

Transition	Experimentals	Controls
NF-SF	0.888	0.054
SF-NF	0.356	0.278
NF-CW	0.032*	0.745
CW-NF	0.014*	0.220
NF-CCW	0.686	0.207
CCW-SF	0.558	0.939
SF-NF2	0.492	0.592

However, one puzzling result can be seen in the first “transition” in controls where the result is close to significance. Comparing this result with the significant transitions within the experimental birds (i.e. NF-CW and CW-NF) indicates that both the mean



and median values are much smaller (3-4 times less, in the case of the non-existent “NF-SF” transition in the controls). Also, the mean and median values are actually negative, meaning that control birds, if anything, tended to move more just before than after this transition. Therefore, as the control birds were not exposed to any actual transition, this result seems accidental, i.e. a random variation in birds' behaviour that might reflect a gradual calming after being placed in the box.

It has to be remembered that multiple pairwise comparisons, as in the above t-tests, may spuriously produce apparently significant results, of which an example may be the control NF-SF t-test, which has a nearly significant p value (0.054).

In summary, the results presented in **Figure 4.1** show that these two transitions, where significant reactions to an alteration in the ambient magnetic field occurred, are the first transition to a sweeping field (NF-CW) and the immediately succeeding transition to a null field (CW-NF). The absence of any such change in behaviour in the control experiments, confirmed by statistical analysis (**Table 4.3**), underlined the significance of these changes in the experimental birds' activity after each of these two transitions.

4.3.1.1. The influence of low activity birds on the analysis

The experiments were repeated for four of the 16 experimental pigeons, due to the generally low behavioural activity throughout the first run. Thus, for these birds, the second run, which manifested more active behaviour, was then included in the overall analysis. In order to determine whether this decision had a significant impact on the overall results, the statistical analysis was repeated by including only the low activity experimental data for these four cases (see **Appendix 1** table cases mark – low activity in the first experiment, 1st group and 2nd group of birds).

Table 4.4 Analysis of variance table of the general linear model (GLM) for difference in activity at transitions of the experimental including 4 low activity birds and control birds. (Abbreviations as in Table 4.1)

Tests of Between-Subjects Effects

Dependent Variable: Difference_in_activity

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	65372.105	1	65372.105	.897	.352
	Error	1972000.720	27.060	72873.945		
Transition_number	Hypothesis	1237599.742	6	206266.624	2.163	.050
	Error	1.258E7	131.929	95341.019		
Bird_number	Hypothesis	1706989.951	21	81285.236	.835	.673
	Error	1.247E7	128.103	97375.446		
Transition_number * Bird_number	Hypothesis	1.238E7	125	99034.021	2.356	.006
	Error	1134702.433	27	42026.016		
Exp_or_control	Hypothesis	24797.871	1	24797.871	.590	.449
	Error	1134702.433	27	42026.016		

The GLM model was run on these (normally distributed) data, and the results are presented in **Table 4.4**. The results are very similar to those described above. The only difference is in the effect of transition number, which is of interest in this case. The factor affecting the difference in activity at transitions is now right at the edge of significance level ($F_{6,132} = 2.163$, $p=0.05$). To follow the same trend of statistical analysis as before, paired t-tests were applied to verify the effect that each transition had on experimental and control birds separately. The collation of the statistical analyses performed on the main data (1st row from **Table 4.3**) and on the results that included the data from the four low activity birds is presented in

Table 4.5.

Table 4.5 The comparison of paired 2-tailed t-test's p values between experimental birds and the same birds but with the 4 low activity birds included. * marks significant cases of $p < 0.05$

Transition	Experimentals	Experimentals including 4 low activity birds
NF-SF	0.888	0.110
SF-NF	0.356	0.363
NF-CW	0.032*	0.051
CW-NF	0.014*	0.015*
NF-CCW	0.686	0.757
CCW-SF	0.558	0.513
SF-NF2	0.492	0.369

Similar to the result obtained with the GLM model (**Table 4.4**), t-tests on individual transitions confirmed that the involvement of low activity birds weakens the impact of the two transitions, especially observed in the NF-CW transition, to the level of significance ($p=0.051$). This behaviour could have been predicted as the lower difference in the behaviour at the transitions had to be expected in the low activity birds. This effect was strongly seen in the NF-CW transition, but not in the other significant transition CW-NF, in which the level of significance was affected only slightly.

The influence that low activity birds had on the data analysis was predictable given that lower activity overall would be expected to lead to smaller differences in activity after and before each transition. Nevertheless, this weakening effect was present only in one of the two significant transitions (i.e. NF-CW), and even after introducing low activity birds, the statistical power of this transition still falls right at the border of significance level. Thus, the level of the responses of the other birds to the two significant transitions was high enough that it was only slightly diminished in one transition by including the four low activity birds, whose reactions, if any, were not as marked. Additionally, the intention of this study was to explore characteristics of the pigeons' reactions to alterations in the magnetic field. Therefore, in the

following analysis, the data from generally more responsive birds will be used and discussed.

4.3.2. Number of head movements

The foregoing analysis was based on using an integrated measure of the total head movements activity. An alternative approach was focused on determining the number of head movements that were larger than a set threshold of 10° (see **Section 4.3.3.1**), i.e. those identified as measurable responses to magnetic field changes. The number of head movements was calculated by the software for each 30s step in the sequence of magnetic field changes, and the results are presented in **Figure 4.2**.

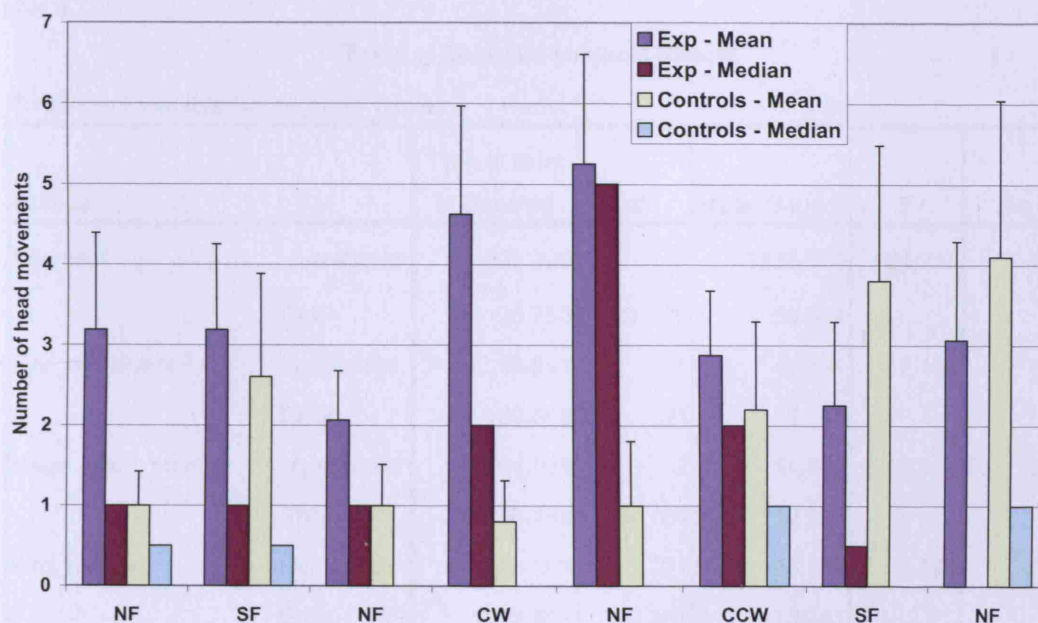


Figure 4.2 Number of head movements in each step in the sequence of experimental and control cases. Error bars represent standard errors. (NF- null field, SF – static field, CW – field sweeping clockwise, CCW – field sweeping counterclockwise). (In the last experimental step, NF, the data includes 15 birds as one bird was hyperactive at the end of this step)

Just as in the earlier analysis approach, the two middle magnetic “steps”, CW and NF, stand out against the “background” of others. Clearly, focusing on the median values reveals that there are at least twice as many such head movements as in the previous or remaining steps. A particularly large increase in head movements is seen

in the NF in between the two sweeping fields. It is also worth noting that the second sweeping field, CCW, still has a high median of two head movements per step.

In the control experiment, the overall distribution of head movements across magnetic sequence is very different. The lowest activity is observed in the middle of the experiment, i.e. in virtual "CW" and the following "NF", during which the maximum number of head movements was observed in the actual experiment. However, there is a distinct trend for the number of head movements to increase towards the end of the control experiment.

Table 4.6 Analysis of variance table of the general linear model (GLM) for number of head movements of the experimental and control birds. (Magn_step_number*Bird_number is an interaction between the two variables that represents the prediction of the number of head movements in a specific magnetic step by a specific bird (number); other abbreviations as in Table 4.1)

Tests of Between-Subjects Effects

Dependent Variable: No_of_head_moves

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	1233.020	1	1233.020	24.213	.000
	Error	1126.756	22.126	50.924		
Exp_or_control	Hypothesis	43.891	1	43.891	7.138	.012
	Error	190.609	31	6.149		
Magn_step_number	Hypothesis	104.039	7	14.863	1.152	.334
	Error	1996.149	154.704	12.903		
Bird_number	Hypothesis	1325.501	21	63.119	4.796	.000
	Error	1972.867	149.897	13.161		
Magn_step_number * Bird_number	Hypothesis	1952.093	146	13.371	2.175	.007
	Error	190.609	31	6.149		

Comparing both experiments exposes very different activity patterns. Firstly, overall, there are many more head movements in the birds investigated under changing magnetic field conditions. In addition, the distributions of maxima in both experiments do not coincide. These observations clearly point out the disparity in the behaviour of birds tested under changing magnetic fields and those that did not

experience any change in magnetic conditions, i.e. the control birds. This fact is confirmed by the statistical analysis again performed with the help of a GLM model. The results (**Table 4.6**) indicate the significant difference between the number of head movements in experimental and control birds ($F_{1,31} = 7.138$, $p = 0.012$) (the data are normally distributed, hence the use of GLM analysis). Other significant factors affecting the number of head movements are, firstly, bird number ($F_{21,150} = 4.796$, $p < 0.001$), which certainly confirms a vast variability in the reactions of individual tested birds, some being significantly less active than others. Secondly, there was a significant interaction of bird number and transition number factors ($F_{146,31} = 2.175$, $p = 0.007$). This large variability in the birds' activity may have affected the magnetic field number factor alone (i.e. the consecutive number of each magnetic field step within the sequence), which is not significant as in the earlier transition analysis (**Section 4.3.1**).

4.3.3. Distribution of head movements

The preceding approaches considered only the difference in activity at a transition or the total number of head movements in each magnetic “step”. Yet another method employed was to investigate where *each* of the head movements made by each tested bird occurred along the time axis of the experiments, as well as denoting its magnitude. The resultant distribution for the experimental birds, i.e. a kind of head movement map, is presented in **Figure 4.3**.

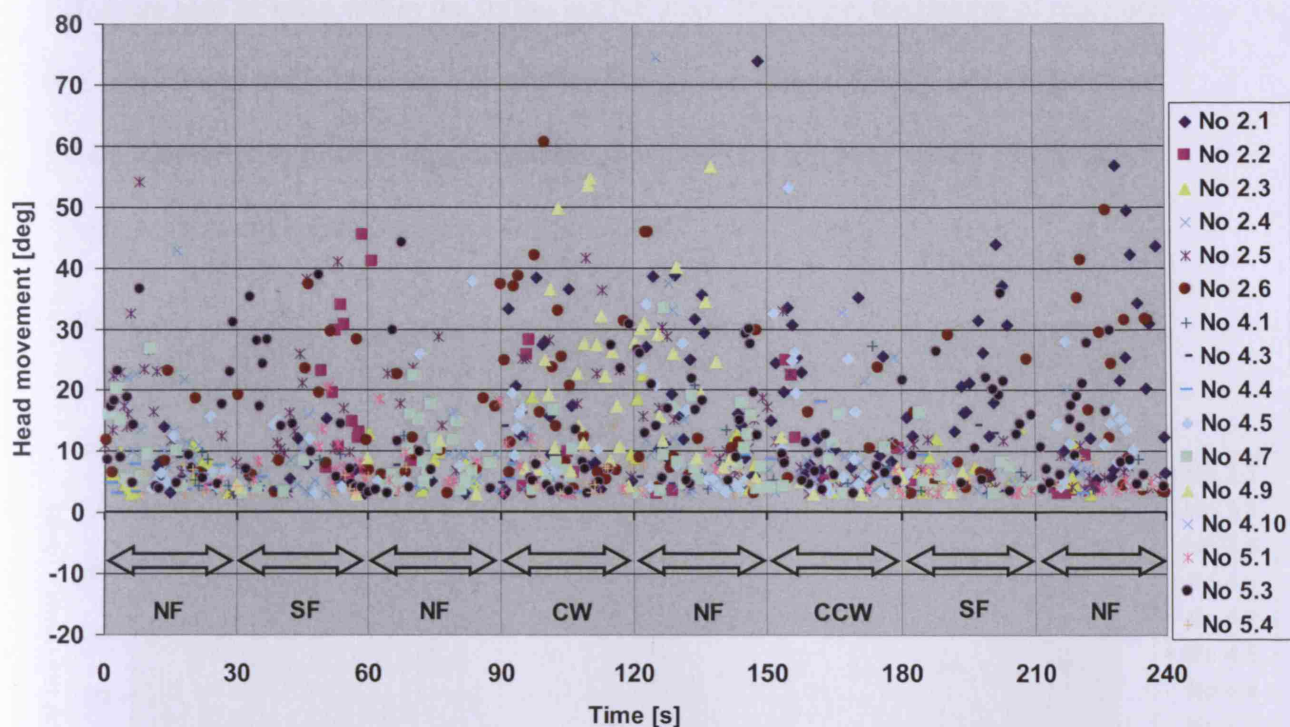


Figure 4.3 Distribution of all head movements of all 16 experimental birds. Each point denotes a head movement with its amplitude and the time when it occurred within the experimental sequence. (Abbreviations as in Figure 4.2)

The head movements map displays the behavioural results as measured by the number of head movements in a different way. It can be observed that, in both analytical approaches, the maximum occurrence of head movements is focused in the middle CW and NF steps. However, this method is more illustrative, as not only is the overall concentration of head movements shown but also the head movements of each of the tested birds can be distinguished. This underlines the significance of the reactions for the majority of the tested pigeons.

Thus, the striking feature in **Figure 4.3** is the differentially high concentration of head movements in specific steps. Most of the birds are particularly active just after the transition to CW and the subsequent one to NF. These are the only transitions with such a high density of head movements across the majority of subjects, which in turn suggests the existence of consistent reaction. This reaction is highlighted even more by the fact that, just before CW step, there are hardly any head movements over 10° .

This can also be observed in the following NF step. Therefore, the pattern of reactions (if one occurs) tends to be an increase in activity just after the magnetic field change, and lower activity prior to this transition constituting a kind of “valley”, best seen in the case of the NF-CW transition in **Figure 4.3**.

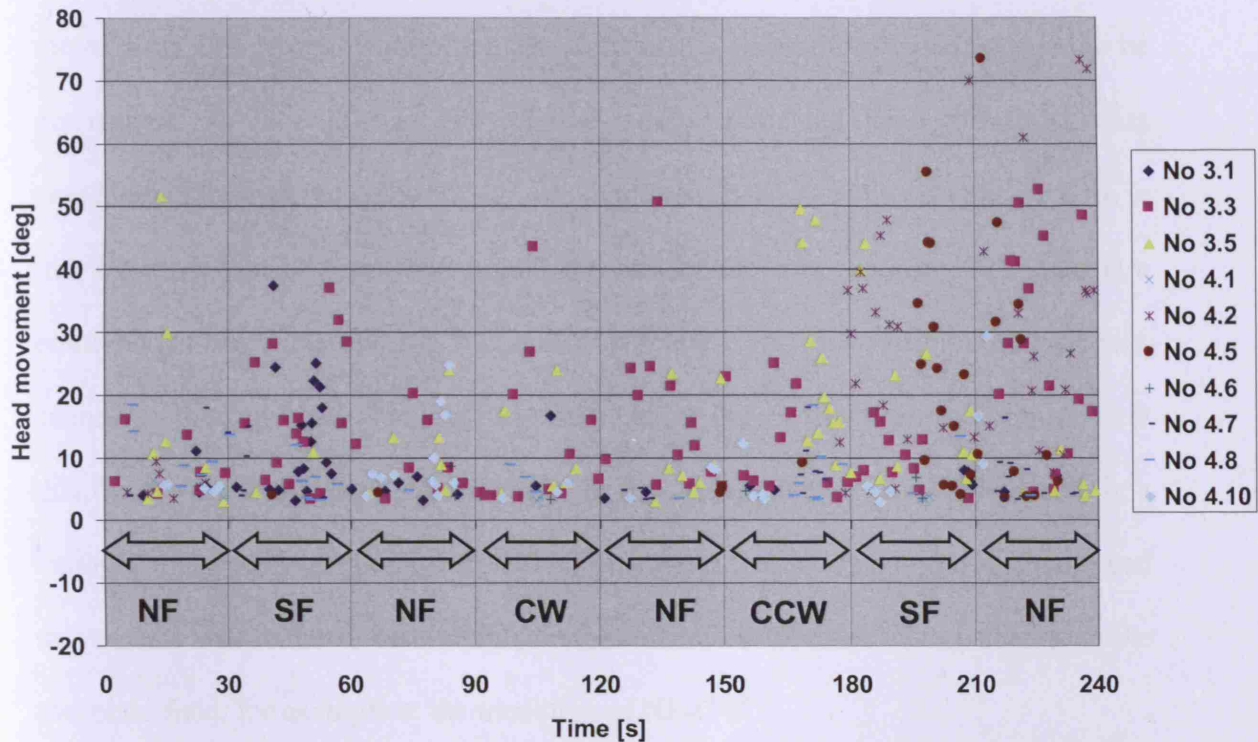


Figure 4.4 Distribution of all head movements of all 10 control birds. Each point denotes a head movement with its amplitude and time when it occurred within the experimental sequence. (Abbreviations as in Figure 4.2)

Using this head map approach, the experimental birds can be directly compared with those in the control experiment (**Figure 4.4**), where birds did not experience any changes in magnetic field. As before, the distribution reveals the relative absence of head movements during the middle of the experiment, i.e. the middle “steps” are characterised by relatively low activity, whereas the maximum head movement activity is towards the end of the 4 minutes that the experiments lasted. Furthermore, the other dissimilarity is that there is no evident effect of a transition as head movements are rather evenly distributed without any significant “valleys”.

4.3.3.1. Threshold of birds' head movement responses

The software programmed to extract the data from the video, i.e. frame-by-frame analysis, picks up any difference in head alignment with an accuracy of 1° . This means that almost every head movement or its tilt is captured. However, in order to differentiate stimulus-evoked reactions from the ordinary generally small head movements that pigeons normally make over time, a threshold response needed to be determined. A decision was made based on the collected head movement data presented in the form of a distribution or “map” across all 16 subjects (**Figure 4.5**). In this figure, it can be seen that, below the dashed red line marking 10° , there is a relatively uniform distribution of head movements, and any differential reactions cannot be distinguished amongst head movements below this size. In contrast, over this threshold the evident “valley” effect in the distribution of head movements in a majority of subjects can be observed. This change in frequency or occurrence of head movements was found to be the main feature of the birds' reaction to a change in the magnetic field, for example at the transition of NF-CW.

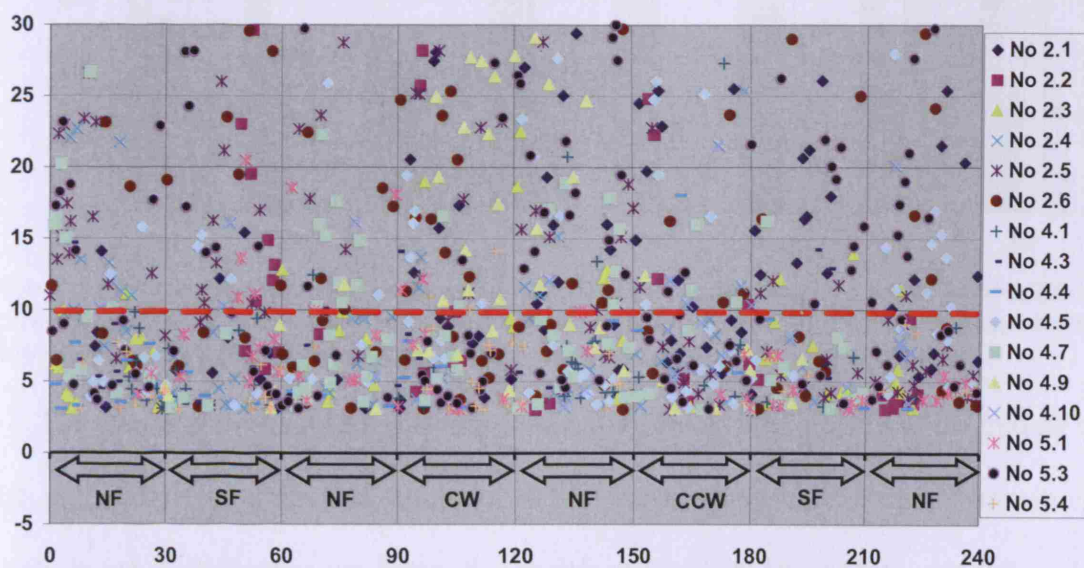


Figure 4.5 Set threshold of 10° for analysis (red line) based on the distribution of all head movements in the case of all 16 experimentals from Figure 4.3

For this reason, i.e. because there was a need to remove small ($<10^\circ$) spontaneous head movements, this threshold was introduced to facilitate analysis of the number of head movements and the latency of the birds' response to a given transition.

4.3.4. Total activity

In this analysis, the method of collecting and presenting the data was similar to the integration approach used in investigating the responses to transitions. However, instead of integrating movement within the 10s on both sides of a transition, here the entire period of 30s of each step was taken into account. Therefore, the total integrated activity within each step of the magnetic sequence was obtained (Figure 4.6).

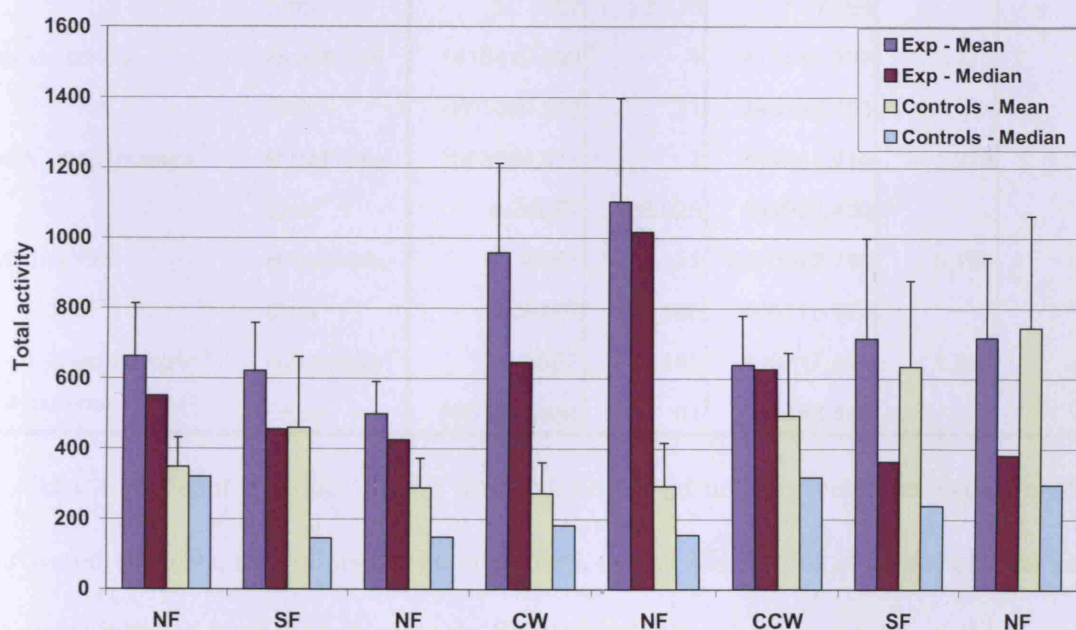


Figure 4.6 Total activity within each magnetic step in 16 experimental and 10 control birds. Error bars represent standard errors. (Abbreviations as in Figure 4.2) (In the last experimental step, NF, the data includes 15 birds as one bird was hyperactive at the end of this step)

The results show again two clearly outstanding steps, CW and NF in the middle of the sequence, that were also found to be the most active in the earlier transitions analysis of a difference in integrated head movement 10s before and after the transition. The comparison of the experimental with control birds shows that the

overall activity is almost always higher in each step in subjects exposed to changes in the magnetic field (**Figure 4.6**), and this difference was confirmed to be statistically different ($F_{1,31} = 5.773$, $p = 0.022$ in **Table 4.7**) (the analyzed data have a normal distribution). As before, within the control experiment, there is an evident trend for activity to increase in the course of the experiment, reaching its maximum in the last two steps – “SF” and “NF”.

Table 4.7 Analysis of variance table of the general linear model (GLM) for total activity of the experimental and control birds. (Abbreviations as in Table 4.1)

Tests of Between-Subjects Effects

Dependent Variable: Total_activity

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	6.279E7	1	6.279E7	37.558	.000
	Error	3.747E7	22.415	1.672E6		
Exp_or_control	Hypothesis	1415419.699	1	1415419.699	5.773	.022
	Error	7601089.958	31	245196.450		
Magn._step_number	Hypothesis	3393984.411	7	484854.916	1.212	.299
	Error	6.240E7	156.028	399958.459		
Bird_number	Hypothesis	4.348E7	21	2070372.740	5.102	.000
	Error	6.092E7	150.140	405775.570		
Magn._step_number *	Hypothesis	5.955E7	145	410717.412	1.675	.047
Bird_number	Error	7601089.958	31	245196.450		

The significant p value for the difference in total activity between experimental birds and controls, as well as the other factors, mirror the output of statistical analysis of the number of head movements (see **Section 4.3.2**).

4.3.5. Reaction time to the transitions in the magnetic field

The reaction time of birds to a magnetic field change or transition, in other words, how quickly birds moved their heads in response to a transition, was also investigated. The aim of this latency analysis was to look at the first significant head movement above the previously defined threshold for reaction ($>10^\circ$) and compare this timing

across all the transitions (**Figure 4.7**). However, the first head movement (which here defines latency) may not always be a clear reaction to a change in the magnetic field condition, but can instead be a spontaneous head movement. In such a behavioural experiment as this, it is not possible to have more insight into the exact cause of each head movement. Nevertheless, an analysis of the timing of the first head movement after each transition across birds, as compared with the results from the control experiments, has at least the potential to provide information about how fast birds may detect alterations in the magnetic field. Nonetheless, this uncertainty should be kept in mind.

The main trend of the experimental birds' latencies during the experiment is the inverse of the trend found in the number of head movements and total activity. That seems reasonable as the more active birds might be expected to have a shorter response time and therefore the shortest latency.

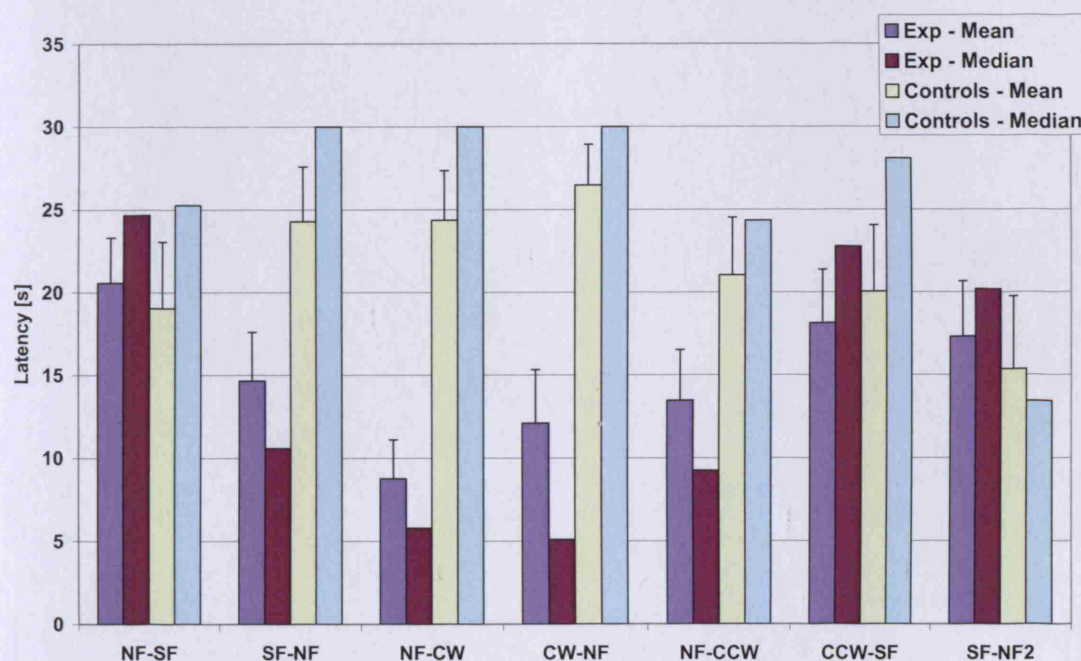


Figure 4.7 Mean and median values of latency of responses to each transition in magnetic field within the sequence for 16 experimental and 10 control homing pigeons. Error bars represent standard errors. (Abbreviations as in Figure 4.1)

In fact, the distribution for all birds reveals that the shortest latency is to the previously identified significant transitions, NF-CW and CW-NF, i.e. median values of 6 and 5s, respectively, whereas all the other transitions are associated with much longer response times. This difference in reactivity to different transitions in the experimental birds is indeed confirmed by the GLM model ($F_{6,47}= 3.549$, $p= 0.003$ in **Table 4.8**). The means also follow the same tendency; however, the values are higher, which is most likely due to one or two birds, which either did not respond (i.e. a maximum value of 30s was used) or did so with a much higher latency, producing a considerable increase in mean values. Therefore, as in earlier analyses, the median value would appear to be more informative as it reflects the responses of more birds.

The corresponding values from the control birds are again clearly different from those from the experimental birds ($F_{1,147}= 15.003$, $p< 0.001$ in **Table 4.8**) (the data have a normal distribution). Throughout the duration of the experiment, the latencies are much higher, except for the transition to the last step SF-NF2 where, as has already been highlighted, the overall activity of the control birds has increased. Another important noteworthy difference is the very small variation in latencies across the different control transitions. Whereas in the experimentals, the median latency ranged over a factor of three in the extreme case, in controls the difference between latencies (except the last transition) is negligible. That relatively uniform distribution of latencies strongly suggests the lack of any source of distracting or perceived stimulus during the control experiments in contrast to the differential responsiveness of the experimental birds to changes in the magnetic field (i.e. NF-CW and CW-NF transitions). The other trait of the control experiment that suggests low overall activity is the much higher median values for latency. Median values of 30

mean that more than half of the birds did not react *at all* within the entire 30s of the magnetic step (turquoise bars on **Figure 4.7**).

Table 4.8 Analysis of variance table of the general linear model (GLM) for reaction time latency of the experimental and control birds. (Abbreviations as in Table 4.1)

Tests of Between-Subjects Effects

Dependent Variable: Latency

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	47591.173	1	47591.173	122.096	.000
	Error	9060.506	23.245	389.784 ^a		
Exp_or_control	Hypothesis	1356.326	1	1356.326	15.003	.000
	Error	13289.572	147	90.405 ^b		
Transition_number	Hypothesis	324.867	6	54.144	.599	.731
	Error	13289.572	147	90.405 ^b		
Bird_number	Hypothesis	9900.078	21	471.432	5.215	.000
	Error	13289.572	147	90.405 ^b		
Exp_or_control *	Hypothesis	1925.147	6	320.858	3.549	.003
	Error	13289.572	147	90.405 ^b		

4.3.6. Latency distribution

Error! Reference source not found. depicts the frequency distribution of latencies, as defined in the previous analysis, for each transition. The bars represent the percentage of the 16 experimental birds or 10 in the control experiment and are merged into 5s bins for better clarity of presentation. The last bars marked as “no reaction” represent the cases where birds did not react during the whole step; in other words, such birds did not move their heads more than 10° at any time within the 30s of a step.

The information that characterises the degree of the birds' responsiveness is the concentration of bars towards the left of the X-axis (i.e. shorter latencies) on Error! Reference source not found.. Therefore, for most transitions, the experimental birds

clearly demonstrate a higher concentration of reactions in the first two bins, which simply means that the birds' first significant head movement occurred close to the time of the transitions. Whereas, control birds have exactly the reversed concentration pattern – higher bars are evident in the last two bins (i.e. much longer latencies). This difference in the distribution of latencies is most prominently seen in the previously identified significant transitions, NF-CW and CW-NF. What is also characteristic for the control birds is that the frequency distribution of latencies denoting birds' reactions is more evenly spread, when the last bin (i.e. no reactions) is omitted.

All of the data presented in Error! Reference source not found. strongly support the conclusion that the experimental birds are behaving in response to the transitions in the magnetic field, most prominently NF-CW and CW-NF. These reactions are manifested as intensified, larger head movements that occur earlier after a transition as compared with the control birds, which did not experience any alteration in the magnetic field.

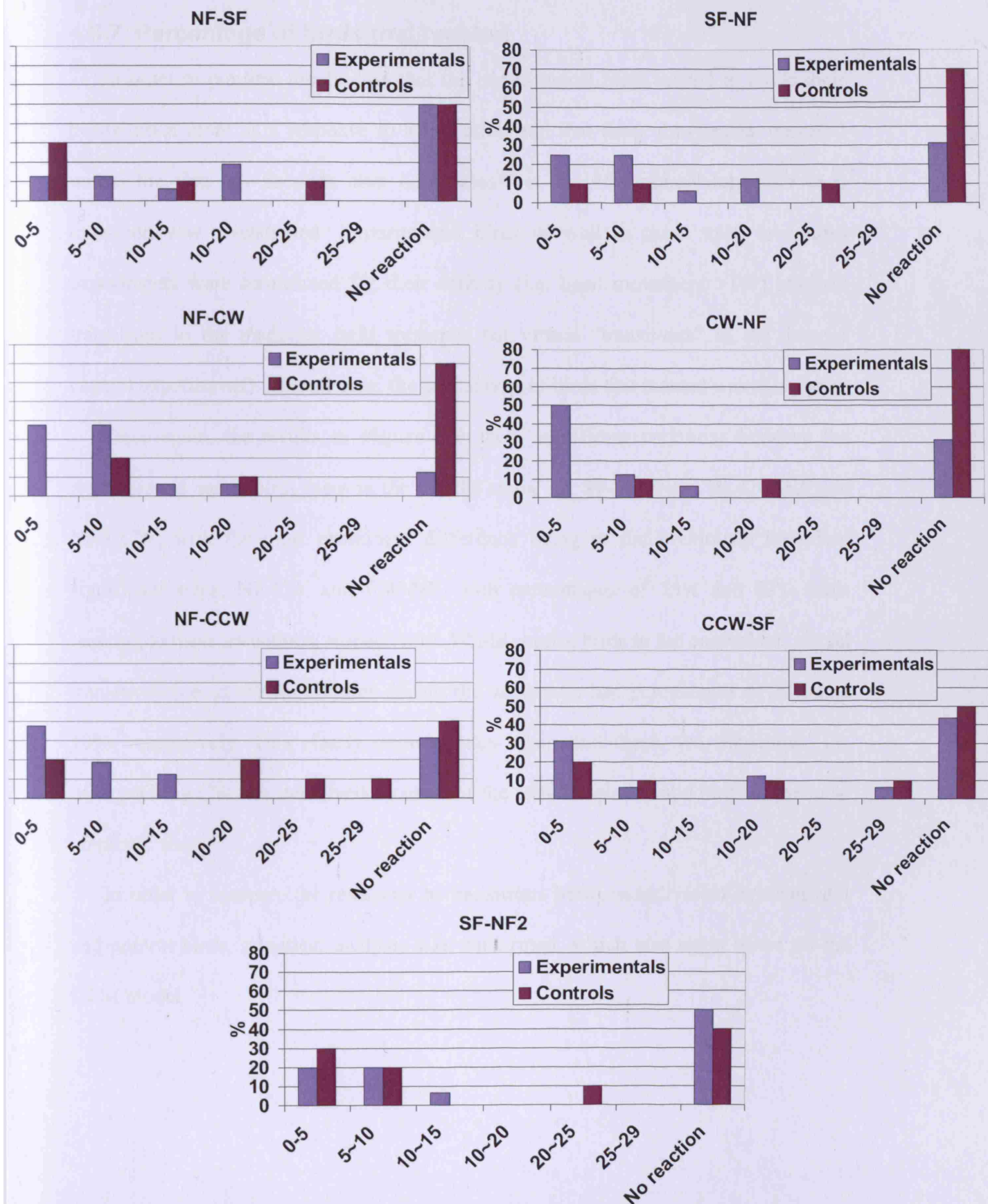


Figure 4.8 Percentage frequency distributions of latencies (in 5 second bins) of all the transitions for the 16 experimental and 10 control birds. Comparing the experimentals and controls by Chi-square test revealed that, in all situations, these frequency distributions are significantly different (in all cases $p < 0.001$)

4.3.7. Percentage of birds that reacted

In order to confirm the finding that the experimental birds tended to move their heads much more as a response to a transition and that those movements occurred within the first few seconds after each transition, the 10s immediately after each transition was investigated. Experimental birds as well as those used in control experiments were scrutinized for their activity (i.e. head movement $>10^\circ$) after all transitions in the magnetic field sequence (or virtual “transitions” in the case of control experiments). In each case, the percentage of birds that reacted was calculated.

Once again, the results in **Figure 4.9** show significant variations between the experimental and control cases in the middle steps, i.e. SF-NF, NF-CW, CW-NF and NF-CCW, with the most prominent difference being in the previously identified significant steps, NF-CW and CW-NF, with percentages of 75% and 63% birds reacting to these transitions, respectively. While control birds in the equivalent, virtual transitions (i.e. at the same times during the sequence) had percentages of 20% and 10%, respectively. This clearly demonstrates again that these two transitions, i.e. incorporating CW, are perceived by most of the tested experimental birds in the most emphatic way.

In order to compare the reactivity to transitions between individual experimental and control birds, statistical analysis was performed, which was again based on the GLM model.

Table 4.9 Analysis of variance table of the binomial general linear model (GLM) for birds' reactions to each transition of the experimental and control birds. (Abbreviations as in Table 4.1)

Tests of Between-Subjects Effects

Dependent Variable: Reacted_or_not

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	18.779	1	18.779	34.088	.000
	Error	13.128	23.830	.551		
Exp_or_control	Hypothesis	1.446	1	1.446	9.142	.003
	Error	23.257	147	.158		
Transition_number	Hypothesis	.884	6	.147	.932	.474
	Error	23.257	147	.158		
Bird_number	Hypothesis	13.818	21	.658	4.159	.000
	Error	23.257	147	.158		
Exp_or_control *	Hypothesis	2.577	6	.429	2.714	.016
	Error	23.257	147	.158		

The results of the statistical analysis undoubtedly show (**Table 4.8**) that there is a significant difference between both groups of birds ($F_{1,147} = 9.142$, $p = 0.003$). In addition, the interaction of exp_or_control and transition_number factor was found to be significant ($F_{6,147} = 3.714$, $p = 0.016$). This finding indicates that, the difference in reactions to different transitions across the magnetic sequence differed between experimental and control birds, and vice-versa. Collating this result with **Figure 4.9** and earlier findings revealing an overall higher variability in responsiveness to different transitions in experimental birds, suggests that the significance of this factor can be assigned to experimentals and not control birds.

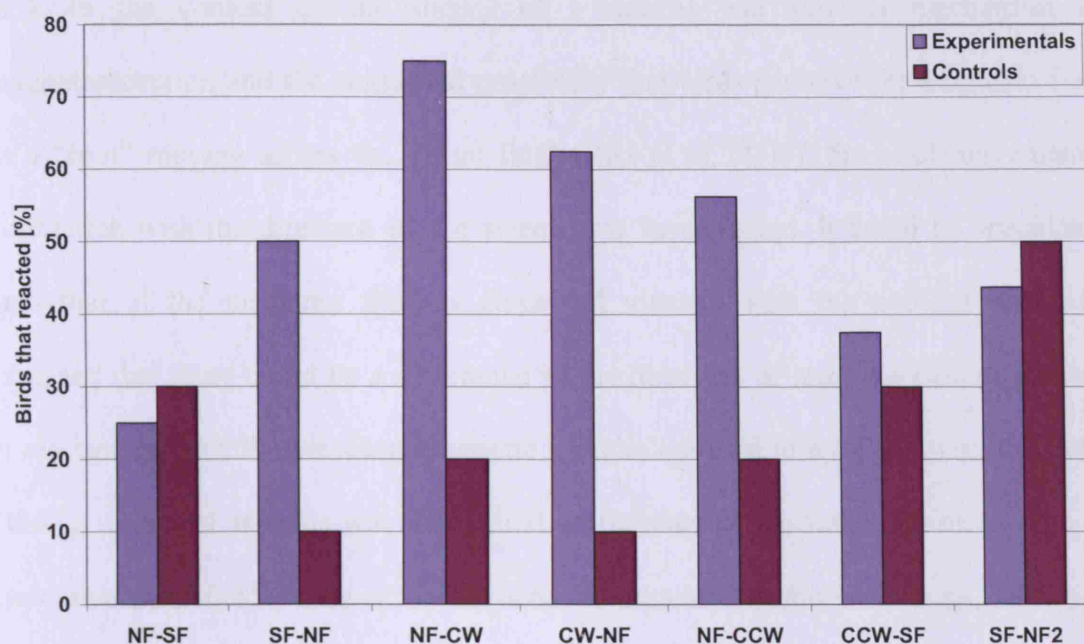


Figure 4.9 Percentage of birds that reacted to each transition within the first 10 seconds for 16 experimentals and 10 control birds. (Abbreviations as in Figure 4.1) (in NF-CW there is one bird included that reacted with a clear head movement of a magnitude of 9.8°)

The other evidence that a real transition in magnetic field conditions is perceivable by birds is the marked contrast between the distributions of the percentage of experimental birds responding and control birds that “reacted” (with the exception of the last step). The increase in apparent responsiveness of control birds toward the end of the sequence is most likely a manifestation of the birds becoming “bored” after spending more than 3 minutes of experimental time in the absence of any sensory stimulation. This effect is not seen in the experimental birds, where the reactions actually decreased towards the end of the experiment, presumably as they become habituated to changes in magnetic field conditions.

4.3.8. Directionality of responses in sweeping fields

The first transition to a sweeping field (i.e. CW) has undoubtedly been shown to be the first especially salient magnetic field change for pigeons, which was manifested by the huge increase in the number of head movements (e.g. see **Figure**

4.3). In the context of the theory of a radical pair process mechanism of magnetoreception and the suggested possibility that birds perceive the magnetic field as a “spot” moving across the visual field (Ritz et al. 2000), the head movements' correlation with the direction of the sweep was investigated. It could be speculated then that, if the magnetic field is perceived visually with the eyes, it might be expected that there could be a difference in the direction of head movement patterns in reaction to the CW sweeping magnetic field as opposed to a CCW sweeping field. Thus, a statistical analysis was performed on the data of the first reaction to each of these sweeping fields, as well as all head movements within both steps, and their correlation was tested against each other to verify whether any differences were present.

Table 4.10 Fisher's exact test on the directionality of the 1st head movement >10° of responses in 16 experimental birds

Sweeping field direction	right "+"	left "-"	Fisher's exact test, p
CW	7	8	0.265
CCW	10	4	

Table 4.11 Fisher's exact test on the directionality of the majority of head movements within the step of responses in 16 experimental birds

Sweeping field direction	right "+"	left "-"	Fisher's exact test, p
CW	7	5	0.449
CCW	6	9	

The Fisher's exact test (for more information on this test see **Section 3.4.7.3**) was used to reveal if there was any significant difference between the expected and the observed distribution of head movement directions, in this case, with respect to the opposing directions of the magnetic field stimulus, i.e. CW and CCW. The results of both of these directionality analyses, i.e. 1st head movement after transition (**Table 4.10**) and the majority of all head movements within steps (**Table 4.11**), showed that there is no significant association between the direction of the stimulus of the magnetic field (CW and CCW) and the directionality of the birds' head responses.

4.3.9. Influence of starting magnetic field condition

In the first part of the experiments, the first group of 6 birds was exposed to an initial NF, whereas the subsequent birds began with a SF prior to each experiment, which was designed to reduce any magnetic “shock” that the birds would experience when first placed in the shielded chamber (for more information see **Section 4.2.5**). In order to investigate whether this starting condition could have any significant influence on birds, another statistical analysis was performed. The ideal would be to compare both groups of birds directly. However, due to the low number of birds in the first experimental group, the statistical GLM model used in the analysis was not possible to perform. Therefore, an alternative approach was chosen; namely, the 2nd group of experimental birds was compared with the control birds, and on this basis, the extent to which the overall output of studies was affected by the 1st group of birds was judged.

Table 4.12 Analysis of variance table of the general linear model (GLM) for number of head movements of the 2nd group of experimentals and control birds. (Abbreviations as in Table 4.1)

Tests of Between-Subjects Effects

Dependent Variable: No_of_head_movements

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	816.500	1	816.500	16.555	.001
	Error	749.604	15.198	49.322		
Exp_or_control	Hypothesis	43.891	1	43.891	7.138	.012
	Error	190.609	31	6.149		
Magn._step_number	Hypothesis	66.437	7	9.491	.892	.515
	Error	1204.997	113.279	10.637		
Bird_number	Hypothesis	774.334	15	51.622	4.755	.000
	Error	1172.048	107.967	10.856		
Magn._step_number * Bird_number	Hypothesis	1146.137	104	11.021	1.792	.032
	Error	190.609	31	6.149		

Table 4.13 Analysis of variance table of the general linear model (GLM) for total activity of the 2nd group of experimentals and control birds. (Abbreviations as in Table 4.1)**Tests of Between-Subjects Effects**

Dependent Variable: Total_activity

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	4.029E7	1	4.029E7	34.238	.000
	Error	1.808E7	15.365	1.177E6		
Exp_or_control	Hypothesis	1415419.699	1	1415419.699	5.949	.016
	Error	3.022E7	127	237922.532		
Magn._step_number	Hypothesis	744300.092	7	106328.585	.447	.871
	Error	3.022E7	127	237922.532		
Bird_number	Hypothesis	1.854E7	15	1235784.352	5.194	.000
	Error	3.022E7	127	237922.532		
Exp_or_control *	Hypothesis	3012063.140	7	430294.734	1.809	.091
Magn._step_number	Error	3.022E7	127	237922.532		

Table 4.14 Analysis of variance table of the general linear model (GLM) for latency of the 2nd group of experimentals and control birds. (Abbreviations as in Table 4.1)**Tests of Between-Subjects Effects**

Dependent Variable: Latency

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	48569.629	1	48569.629	109.716	.000
	Error	6769.844	15.293	442.686		
Exp_or_control	Hypothesis	1356.326	1	1356.326	15.164	.000
	Error	9928.004	111	89.441		
Transition_number	Hypothesis	332.581	6	55.430	.620	.714
	Error	9928.004	111	89.441		
Bird_number	Hypothesis	6905.222	15	460.348	5.147	.000
	Error	9928.004	111	89.441		
Exp_or_control *	Hypothesis	1536.145	6	256.024	2.862	.012
Transition_number	Error	9928.004	111	89.441		

The three GLM models on the total number of head movements, total activity and latency of the response for 2nd group of experimentals and controls are presented in,

Table 4.12, Table 4.13, Table 4.14, respectively (all three data sets are normally distributed). The comparison between experimental and control birds (i.e. exp_or_control) is in all three cases significant ($F_{1,31} = 7.138$, $p = 0.012$; $F_{1,127} = 5.949$, $p = 0.016$; $F_{1,111} = 15.164$, $p < 0.001$, for number of head movements, total activity and latency GLM analysis, respectively), as it was in the data incorporating 1st and 2nd group of birds together (see **Sections 4.3.2, 4.3.4 and 4.3.5**). Another important finding of this analysis is the fact, that without the 1st group of experimental birds, the latency analysis revealed the same outcome of the interaction between bird or control and transition number factors ($F_{6,111} = 2.862$, $p = 0.012$ in **Table 4.14**), which means that the variability in the responsiveness of the individual experimental birds to different transitions remains.

These very similar findings when considering the data including all experimental birds or only the 2nd group revealed little difference between them. That in turn would mean that an influence of the starting condition (i.e. lack of initial SF in the 1st group and its presence in the 2nd group of experimental birds) was not evident and the observed trend in the behaviour of the experimental birds, particularly the significant difference from the control birds, as well as some transitions being more salient than others, was not affected by the starting condition in the magnetic field. This may simply be because the salient transitions were not immediately at the beginning of the magnetic sequence but in the middle of the sequence (NF-CW and CW-NF 3rd and 4th transitions, respectively).

For this reason, the data from both groups of investigated birds were combined for the purpose of analysing and presenting the behavioural responses to subsequent transitions in the magnetic sequence experiment.

4.4. Discussion

In this experiment, only one sequence was used, which was selected on the basis of the preliminary study in which different possible experimental sequences were tried (see **section 4.2.4**). For this reason, a limitation stemming from following the same sequence in the experiments is important to address, i.e. a possible time/order effect. Namely, using the same sequence for all of the birds could have affected their attentiveness in the same way due to habituation, particularly in the case of the two transitions to sweeping field (i.e. NF-CW and NF-CCW). Therefore, the interpretation of this difference could be explained in two ways: either there is a real difference in salience between both sweeping fields or simply the first transition to a sweeping field (i.e. in either direction) attracted high attention and thus exposure to a second sweeping field (NF-CCW) was not so “shocking” any more.

Of course there are many variations possible in the sequence order that could be applied based on the same magnetic field steps (NF, SF, CW, CCW) and their duration, which would allow a more robust comparison of the effects of each respective transition. However, it should be remembered that, in this study, which involved the introduction of a novel method, the main intention was to validate the approach and show its possible usefulness in studies of magnetoreception. Thus, while there were different approaches possible (e.g. in terms of changing the sequence in any number of ways), in this situation the experiments were performed based on the one chosen sequence (a strategy also made necessary by the limited time available to complete this study) as the departure point and from which other avenues of investigation were followed (see chapter 5 and 6). Nevertheless, in future experiments, it should be essential to follow up this study by experiments involving systematic changes in the sequence (e.g. swapping the sweeping fields (CW & CCW)

with each other to be able to determine whether the difference in the birds' responsiveness is due to the CW sweeping field only or is simply a matter of the first exposure to a moving magnetic field, regardless of its direction) that could confirm the present findings and/or further progress the development of this method.

Consequently, these points should be kept in mind while considering the outcomes of the present experimental work in this and subsequent experimental chapters, in which results are compared, directly or indirectly, with the findings from this chapter.

4.4.1. The abrupt and recurrent reaction to specific magnetic field transition

All of the different analytical approaches confirmed that homing pigeons can detect changes in the ambient magnetic field and this magnetoreception is manifested as an increase in the birds' behaviour, specifically in response to the first sweeping field that appears in the sequence – CW. Regardless of the analysis method, i.e. transition analysis of integrated head movement, the number of head movements, the distribution of head movements or latency of the reaction, the CW sweeping field stands out as the most salient against the background of all the others field conditions. Therefore, this pronounced behavioural response clearly indicates that the transition, NF-CW, made a consistent impact on birds. The change in the nature of the field from no field (NF) to sweeping field appears to be particularly distinctive for pigeons perhaps because a moving field is highly unusual and/or the fact that the field is moving makes it easier to perceive. The following transition CW-NF (**Figure 4.1**) also provoked a statistically significant increase in head movements. Considering that, before those significant transitions, there had already been a transition to or from NF and the fact that none of the other transitions prior the ones including CW, i.e. a

sweeping field, was as salient for the birds, it is clear that the presence of the CW made the difference. Thus, it can be safely stated that the sweeping field was perceived in the most pronounced way. Moreover, the first sweeping field attracted more birds' attention than the second one in the opposite direction. However, there is still a considerable increase in activity after the transition to CCW, but it was somewhat masked by the already high activity of the pigeons in response to the earlier NF step.

Another noteworthy result is that the NF between sweeping fields is also characterized by the especially quick reactions that are evident from the "valley" (i.e. a decrease before and an increase just after a transition in the distribution of head movements) at the CW-NF transition (see **Figure 4.3**), although not as clear as at the NF-CW transition. However, what is even more remarkable is the large number of head movements, exceeding those in all the other steps, even CW (**Figure 4.2**). Similar behaviour has been observed previously in night migrants – garden warblers (Mouritsen et al. 2004a).

The final evidence validating this finding is the control experiment, which was focused on investigating the birds' behaviour in constant (null field) magnetic conditions and then comparing this outcome with their responses to actual changes in magnetic field conditions in the real experiment. Although the NF was chosen as the magnetic field condition under which the control birds were tested, the other possibility would have been to perform this control experiment in SF, which could be closer to the natural condition for the birds. Nevertheless, the purpose of the control experiment was achieved: the birds were tested in constant NF, and their behaviour was different from that of the birds tested in the real experiment. The results of the control experiment clearly show that there were no significant changes in the birds'

behaviour at any of the artificially set transitions (**Figure 4.1**). The only essential change across the magnetic sequence experiment was the alteration of the magnetic field, which was entirely absent in the control experiment. Thus, birds in this situation remained in a NF and did not experience any perceivable change during entire experiment. Therefore, the difference in the behavioural results between these two experiments had to be entirely due to the birds' perception of the magnetic field alterations.

In summary, these results all shed some light on how birds perceive the magnetic field or, more precisely, on how the magnetoreception system works. Specifically, the birds' ability to detect the magnetic field may depend on the field changing. In other words, the homing pigeon's magnetic field sensing system may be designed in such a way that it picks up gradients in a magnetic field. In particular, this would explain the frequent head movements observed in the situation where the magnetic field was totally removed (i.e. NF), which may be the birds' effort to induce a change in the magnetic field and thus detect it. The involvement of movement in the magnetoreception seems not to be specific to birds; such a phenomenon has also been reported to take place in honeybees (Walker and Bitterman 1989). This apparent need for motion in order to detect changes in the magnetic field may be reflected by the homing pigeons' differentially high responsivity to moving the field around (i.e. CW or CCW), which in turn also stimulated head movements. However, in addition to head movements, in natural conditions the movement is most likely to be achieved when birds are flying, and thus their movement with respect to stable magnetic field leads to detecting it.

4.4.2. Behaviour observed in NF

Together with the first sweeping field, CW, another step in the sequence during which the birds exhibited high responsiveness and activity, was the NF immediately following the CW field. Within the experimental sequence, the NF condition was present four times, but the one NF that stands out is in the middle of the sequence, i.e. immediately after CW, while during the other NFs no noticeable change in the birds' behaviour was indicated. From the birds' point of view, the zero magnetic field could be assumed to be "shocking", as all of the tested pigeons experienced this total absence of a field for the first time during these experiments. As shown by (Mouritsen et al. 2004a), who were investigating migratory birds, garden warblers, the absence of a magnetic field (NF), among all the other field conditions, triggered the highest activity of birds as measured by head movements. Therefore, it might be expected to observe similar behaviour in homing pigeons. Although the conditions under which migratory birds were investigated and the methods used were considerably different from the present research, there is a striking similarity in that the number of head movements was again highest in NF. Mouritsen et al. explained that these movements could have been made in order to recalibrate the birds' intrinsic magnetic compass. In the situation where the magnetic field was removed (NF), the head "scanning" was considered to have increased, most likely due to numerous unsuccessful attempts to locate an ambient magnetic field.

However, the present results show that the behaviour of the birds was not significantly different during the first NF but only after the first sweeping field. This difference between these two studies may result from the different species tested, i.e. migrants versus homing birds; in addition, the conditions of the tests undertaken were considerably different from each other, i.e. in this study no kind of orientation

response was studied, only behavioural activity in the form of head movements. Indeed, being enclosed in a small box would not have been conducive to the pigeons trying to determine a particular direction based on the magnetic field. Perhaps for this reason, reactions were not evident in the three other non-significant NF steps. However, the significant reactions after a transition to NF from sweeping field would point to such a change in magnetic conditions having an especially high impact on the experimental pigeons.

Although subjecting birds to a zero magnetic field seemed to be very interesting approach in investigating their capabilities for sensing a magnetic field, there are too few studies using this paradigm to allow a meaningful comparison with the outcomes of this study and then draw firm conclusions about the precise effect that the absence of any magnetic field might have on birds.

4.4.3. Perception of the magnetic field alterations

The head being the centre for magnetoreception has been demonstrated by studies in which Helmholtz coils were placed around a pigeon's head and reversal of the magnetic field polarity led to reversal of the bird's orientation (Walcott and Green 1974). Moreover, further evidence that the head is the location of the magnetic sensor can be found in the study of Mouritsen et al. (2004a) which suggested that head movements are a measure of birds' reactions to magnetic field changes. The results of the present study are consistent with this view that the sensor is located in the head and that moving the sensor may be important for sensing the magnetic field. Thus, the frequent head movements observed after a transition to a sweeping field (CW) and during the following NF step support the findings of Mouritsen et al. (2004a) and the conclusion that the magnetic sensory system is located in the bird's head.

Head movements were specifically hypothesised to be the tool for recalibrating the magnetic compass in migratory birds just before their outward journey (Mouritsen et al. 2004a). In turn, the basis of the avian magnetic *compass*, an inclination compass (Wiltschko and Wiltschko 1972; Wiltschko et al. 1993), has been proposed to be the radical pair process (Wiltschko and Wiltschko 2002a; Ritz et al. 2004) that is located in the retina. In contrast, the magnetic *map* is said to be based on the magnetite system (Munro et al. 1997), which is located in the beak. Although both sensory systems are in the head, a link has been suggested between the compass sensor and visual perception. The final experiment in the present study (see **Chapter 6**) supports the idea that the head movements observed here do depend on the presence of light and thus relate to the compass mechanism.

In view of the growing evidence that light is needed for magnetoreception (Wiltschko and Wiltschko 2001; Wiltschko et al. 2005a; Johnsen et al. 2007; Wiltschko et al. 2007) to take place, as well as for the birds' visual system being responsible for magnetoreception (Heyers et al. 2007; Zapka et al. 2009), the obvious question to ask would be how the results of the present study fit into that hypothesis.

The sweeping field being especially salient may be explained in the context of the radical pair process theory and its suggestion that birds perceive the magnetic field as a dimmer dot or area within the visual field (Ritz et al. 2000). If the magnetic field is truly visible, the increase in behavioural activity observed in the case of first sweeping field (CW) may have an intuitive explanation. When the stimulus, magnetic field, is constantly changing its position (i.e. sweeping around bird's head), there would be a corresponding movement of the visual consequence of the magnetic field across the pigeon's visual field. This "image" movement might reasonably be expected to trigger corresponding movements of the eyes (i.e. head in the case of birds with restricted eye

movements (Wallman and Pettigrew 1985)). This response is also concordant with the findings that birds, European robins, reverse their head position when the horizontal component of the field was suddenly reversed (Wiltschko and Wiltschko 1972), as seen in the orientation reversal of homing pigeons (Walcott and Green 1974). Whether the head movements are a reflection of an urge to track the moving stimulus is unclear (see **Chapter 5**). Nevertheless, the finding here of a significant increase in birds' behaviour – and the rapidity of this response – to a NF-CW transition may well be a reflection of some sort of visual perception of the magnetic field.

A logical test to determine whether magnetic field sensing is visually mediated and thus dependant on light would be to investigate pigeons in the same conditions but in light or total darkness (or even under specific wavelengths of light). The final experiment of this study (**Chapter 6**) examines the question of the importance of light for the behavioural reactions of pigeons to changes in the magnetic field.

4.4.4. Timing of the reactions to magnetic field alterations

An interesting question when considering the psychophysics of magnetoreception is how rapidly the birds are able to become aware of any change in the ambient field. In other words, how quickly can the sensory system responsible for detection of the magnetic field update the information.

In the case of the experimental birds studied here, the variation in their response latency across all the transitions is very high. Even though some birds could have much slower reactions than the median values, the maximum difference between the fastest reaction (i.e. in NF-CW and CW-NF – median of 5s) and the slowest (i.e. in the case of NF-SF – median equal to 25s) is a factor of five indicating a clear difference in birds' reactions to different magnetic field transitions. A comparison of the mean values, which were less informative about reaction time due to the influence

of a few birds with very long latencies, still indicates a high variability between the same transitions – 3 times difference (see **Figure 4.7**). Whereas in the control experiments (**Figure 4.7**), where no stimuli are present, head movements of magnitude $>10^\circ$, which were used to define reactions in the genuine experiment with magnetic stimuli present, are less frequent and their occurrence seems to be more of an effect of random movements made by live birds over time. The clear difference in the size of latencies between the genuine and control experiments indicates that the magnetic stimulus did indeed affect the birds' attention, which was most likely stimulated by the magnetic field sensory system that in turn drove the reactions, i.e. head movements just after the significant transitions, NF-CW and CW-NF.

Clearly, the results strongly suggest that the magnetic field stimulus' salience has an effect on the time in which birds reacted to the transitions, which in turn may reflect the time differences in the actual perception of the magnetic field alterations by the avian sensory system. Thus, response latency is likely to be an important psychophysical measure of magnetoreception by pigeons. It could be expected to give some insight into the time needed for a bird to become aware of perceivable alteration in the ambient magnetic field. The evidence here indicates that such detection takes only a few seconds, or possibly even around a second, as some (i.e. 4 in the case of most salient transition NF-CW) were almost instantaneous.

In conclusion, the timing of the reaction to magnetic field alterations, i.e. the time apparently needed for a bird to become aware of a change in the magnetic field, has been investigated. For the first time, to the best of our knowledge, the time needed for pigeons, and presumably other birds as the sensory system may be expected to be similar across avian species, to notice the change in the magnetic field change and become aware of that change, as manifested by their reaction measured by increase in

head movements, has been determined. This finding (and approach) may help in understanding the magnetic field sensory system as a whole because the overall timing of the reactions (i.e. from the sensory level of perception to the manifestation of the magnetic field change recognition by a bird's head movement) may give important insights into the radical pair process mechanism that is currently believed to underlie magnetic field sensing (Ritz et al. 2009).

4.4.5. Validation of the method

Recently, there has been a large emphasis placed on the importance of finding a new method that could enable psychophysical measures for determining the as yet unknown features of the magnetic sensory capability in birds (Kirschvink et al. 2010; Lohmann 2010). In view of this need, it is proposed that the novel approach developed here could provide the basis for exploring the sensory parameters of magnetoreception in birds.

When comparing this method with the two most popular, i.e. noting the vanishing bearings of freely flying birds (Matthews 1951) and a method based on the seasonal orientation of migratory restlessness in migrating birds (Emlen and Emlen 1966), one main difference is that, in the present approach, orientation to a field could not be measured. However, the behavioural responses used to monitor birds' reactions to magnetic field changes are not dependent on the birds' motivational state (i.e. migratory restlessness) and can be used to determine the timing and other parameters of magnetoreception.

This experiment, carried out on 16 birds, shows that the most prominent behaviour response consistently occurred in the same field transition (i.e. NF-CW and CW-NF). The integration method, which focused on the transitions, the number of head movements, the distribution of head movements, response latency analysis and its

distribution, all identify the same significant result. Moreover, the control experiment, in which 10 birds were investigated, showed that the behaviour of birds where no change in the field is present was completely different. That marked contrast between the results of the control and genuine experiments strongly supports the contention that this method can indeed be used to measure reactions to magnetic field alterations. Thus, subjecting pigeons to a sequence of magnetic field conditions can identify the birds' reactions to the most salient transition(s), which can then in turn be used as a diagnostic tool for testing the psychophysics of magnetoreception using different approaches (e.g. different light regimes, varied magnetic steps setting, etc.). This also supports the suggestion that head movements are useful as a potential technique for measuring the sensory parameters of magnetic detection in birds (Mouritsen et al. 2004a).

The proposed method allows experiments to be performed at any time and under controlled laboratory conditions, simply triggering the birds' responses by temporal and/or spatial manipulations of the ambient magnetic field. In contrast to using migratory restlessness, this approach can be employed throughout the year for investigations of various aspects of birds' magnetoreception. In addition, it should also be possible to apply this method to other bird species, being just a matter of accommodating the system for their different body size, by adjusting the size and position of the experimental box accordingly.

All of the above leads to the conclusion that this validated paradigm may in the future allow further development of our knowledge about the phenomenon of magnetoreception, the basis of which remains as yet largely unknown.

It should be noted that, as in all studies using awake animals, unbiased criteria for rejection of those subjects for which either no data could be accessed or several runs

were necessary to obtain clear behavioural reactions, need to be established. That is the main limitation of the present method, but this is similar to that of any behavioural paradigm unless very large sample sizes are used. Therefore, despite its drawbacks, the method can be further developed, with a view towards reducing situations in which no or overreacting behaviour interferes with data collection.

4.5. Summary

The experiment conducted showed that homing pigeons are aware of the changes in the ambient magnetic field, especially when the alterations are abrupt, which in this case involved the transition from a null field (NF) to a sweeping field in a clockwise direction (CW) and the immediately following transition from a moving field (CW) to its absolute removal (NF). Analysis of these head movement responses showed that the reaction time varies, apparently with respect to the saliency of the transition in the field. Thus, the quickest perceived transition was that involving the first exposure to a sweeping field in the experimental sequence following a null field (NF-CW), being “noticed” by the birds within a few seconds.

In summary, for the first time, experiments under laboratory conditions on a non-migratory bird were performed, allowing the observation of birds' responses to a sequence of alternating magnetic field conditions in real time. Therefore, this newly introduced method should pave the way for further investigation of different aspects of psychophysics of magnetoreception in different birds' species and possibly other animals.

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Chapter 5. Characterisation of the head movement responses

5.1. Introduction

The results of the experiment performed thus far have shown that homing pigeons undeniably react to changes in the ambient magnetic field and that these reactions can be detected and measured by means of the bird's head movements. In this study, the head movements serve as the main reference for judging pigeons' behavioural responses to changes in the ambient magnetic field, and they were found to be a time-correlated measure of the birds' magnetoreception. As a result, the head movements themselves became of significant interest, especially in light of the possibility that vision is involved in magnetoreception. The head movement being a reliable response to alterations in the magnetic field already lends support to the head as the location of magnetic sensor in birds. However, this does not establish that the eye is the location for magnetoreception. Nevertheless, this part of the project became a quantitative analysis of these head movements with the goal of understanding their nature and dynamics.

5.1.1. The aims of the experiment

The first aim was a thorough characterisation of the head movements that are present during sweeping magnetic fields. These are the field changes that provide the largest and most significant responses, i.e. the transition to first sweeping field in the sequence (CW), as well as to the field sweeping in the opposite direction (CCW). It was deemed important to determine whether these are normal head movements in most cases or whether they have special properties. Secondly, it would also be desirable ideally to compare and contrast these head movements with those of other

animals. Finally, in light of the theory that the visual system is involved in magnetoreception, the possibility of a correlation between these head movements and a need to move the eyes could be explored.

5.2. Description of the head movement characterisation experiments

Since the approach of this part of the project is concerned specifically with the head movements themselves, some changes needed to be incorporated into the measuring system. First and foremost, a high-speed camera capable of a much faster frame rate was required to capture the temporal properties of such rapid movements, and therefore a small adaptation had to be made in the system in order to attach this different camera in the proper place above the bird's head. Moreover, owing to the characteristics and specifications of the new camera system, there was a need to modify the experimental sequence and subsequent analysis of the collected data. All of these adaptations are described below.

5.2.1. High-speed camera description

In order to gather information on the nature of the head movements, a camera with a much higher temporal resolution than the original (i.e. 30 frames per second) was needed. Consequently, a camera capable of recording with such a high sampling rate – frames per second rate - was obtained through a loan from the EPSRC Engineering Instrument Pool. The loan period was limited to three months. This monochromatic Photron Fastcam MC-1 camera (**Figure 5.1**) was capable of recording video up to 2,000 frames per second (fps) in standard resolution (512x512 pixels) or even up to 10,000fps but with the trade off of a lower resolution (**Table 5.1**).



Figure 5.1 The Photron Fastcam MC-1 camera with its processor-controlling unit

The camera had an internal memory of 2GB that could be used for recording all of the frames within a given experimental sequence. However, the hard disk capacity was also a limitation on the number of frames that could be stored in one experiment and so, depending on the chosen frame rate, head movements for different lengths of time could be recorded. The frame rate versus recording time dependency is shown in **Table 5.1**. Furthermore, a higher frame rate than 500fps required brighter illumination which in, the present setup, was not possible.

Table 5.1 Recording durations of the Photron FASTCAM MC-1. (Photron 2008)

Frame Rate (fps)	Max. Resolution (pixels)	Recording Duration (seconds)	Recording Duration (frames)
250	512x512	32.0	8,184
500	512x512	16.0	8,184
1000	512x512	8.0	8,184
2000	512x512	4.0	8,184
3000	512x352	3.8	11,368
4000	512x256	4.0	16,368
8000	512x128	4.0	32,736
10000	512x96	4.4	43,648

Taking into consideration the above constraints and yet allowing for a frame rate that would be sufficient for analysing the head movements in this case, a frame rate of 500 was chosen; thus, the buffer could hold 8,184 frames, which in turn meant that the maximal time for one recording was 16 seconds. Furthermore, this frame rate seems reasonable as it is found to be used in studies on human head movements (Liao et al. 2005) and in investigations of head saccades in the barn owl, *Tyto alba*, in which the frame rate was 468fps (Whitchurch and Takahashi 2006).

5.2.2. Adaptations introduced in the high-speed camera experiments

The procedure under which these high-speed video experiments were performed was essentially very similar to the earlier experiments. The basic protocol was unchanged but for a few minor but important variations. The main alterations in these experiments with respect to the experiments conducted earlier with the low-speed camera are:

- a different sequence of magnetic field steps
- unequal durations of recorded videos

Bearing in mind the above-mentioned calculations and the resultant specification with which the camera operated during experiments, the experimental sequence obviously needed to be altered. In particular, the sequence had to be shortened, as the maximum time that could be recorded was only 16 seconds. Thus, each bird tested with the high-speed camera was placed in the chamber and exposed to only *one* transition in the field from NF to sweeping field, either in the CW or CCW direction depending on the experiment. In the earlier experiments, transition to a sweeping field produced the most abrupt reactions in the pigeons. Indeed, the first statistically significant transition in the sequence under which the birds were tested was the transition to a CW

sweeping field (**Section 4.3.1**). Therefore, a transition to either CW or CCW, i.e. expected to have a similar effect on birds when placed first in the experimental sequence, was chosen to increase the probability of the birds' responding and thus make optimal use of the short period of 16s available to record.

Another way to maximise the ability to capture one or more head movements during the brief 16s recording time was to use the option of delaying the start of the saved recording. The Fastcam processor stored, in a continuously reused buffer, all 16s of the video. For this reason, it was possible to programme the trigger so that the real start of the recording could be moved back in time (i.e. in this case, the recording started 8 seconds before the trigger fired). That enabled first observing the birds' reactions after the transition and if one or more head movements were present, then the decision could be made to save the recorded video tested bird. Moreover, the user had the opportunity to better assess the behaviour of the birds, in terms of responsiveness, and thus record most if not all of the bird's response within the 16s recorded period. The camera recording could be remotely started with a trigger connected to the processor-controlling unit, thus the camera could be switched on at any time during the experiment.

The other difference from the previous experiments in which all sequences were 4 minutes in length is that the duration of each of these recordings could be different. This was made necessary because of the large amount of memory required by the video when recorded at such a high frame rate (500fps). Specifically, in the case where the reaction lasted less than 16s, the "silent" (i.e. head still) portion was deleted to reduce the number of frames stored and investigated and thus the time taken for data processing.

5.3. *Data processing*

Although the number of frames per second was significantly increased as compared with the earlier experiment, the data extraction from the video was based on the same template matching software that had been designed with an eye to the initial experiment with the low-speed camera. Therefore, adjustments were needed; i.e. the `dataextraction.vi` was adapted for the higher frame rate. An example output of the `dataextraction.vi` is presented in **Figure 5.2**.

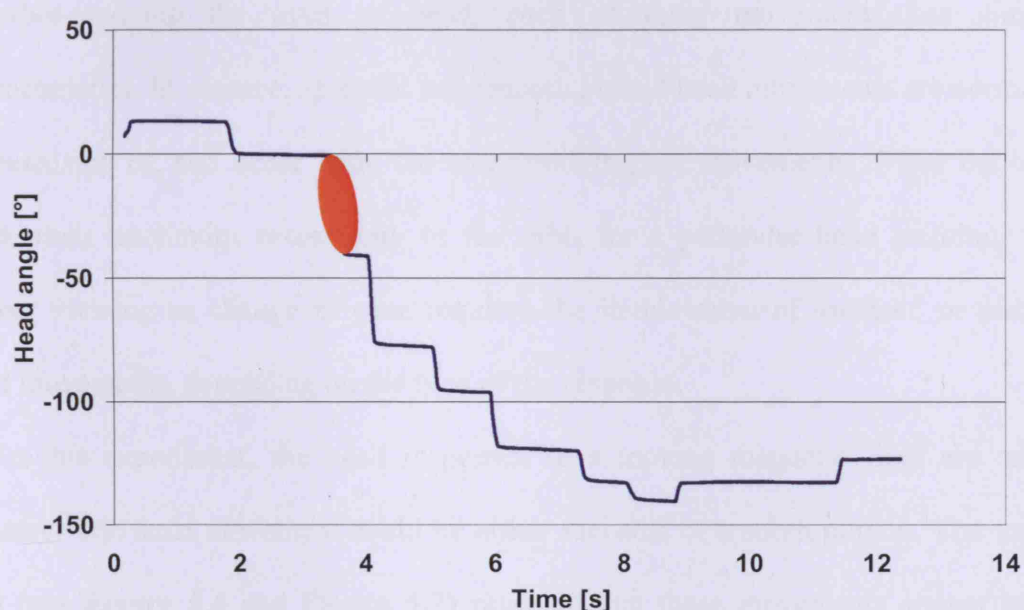


Figure 5.2 An example of saccadic head movement data obtained with the high-speed camera to be processed. Highlighted area marks a single head movement.

Subsequently, in order to be able to determine the characteristics of each head movement (for example see the highlighted area in **Figure 5.2**), a new analysis tool for these specific data based on software kindly supplied by Professor Chris Harris (personal communication) was incorporated. This software gives, for all head movements, the values of amplitude, duration and peak velocity.

5.4. Saccadic movements

The two principal voluntary targeting movements of the eye as well as the head are:

- Saccades- very fast movements to change abruptly the point of fixation (i.e. gaze), accompanied by saccadic suppression of vision
- Smooth pursuit- slower movements, intended to keep the image of a small, slowly moving stimulus on the fovea (Purves et al. 2008)

Whether moving the eyes or head, each of these movements has similar characteristics. In essence, saccadic and smooth pursuit head movements are normally an extension of, and occur with, the corresponding eye movements. When the eyes reach their maximum eccentricity in the orbit for a particular head position, any further viewing or change in gaze requires the involvement of saccadic or pursuit head movements, depending on the type of the response.

In this experiment, the head responses to a moving magnetic field are under scrutiny. The head movement could be either saccadic or smooth pursuit. The initial data (see **Figure 5.6** and **Figure 5.7**) revealed that these movements appear to be saccadic in nature.

The sense of vision can play a major role in the animal world as well in humans. This is particularly true of birds, in which vision could be argued to have reached its highest degree of development. Most birds largely rely on vision for survival. In some vertebrate retinas, the neural tissue for transduction of light and initial processing centre for vision, contains a depression in the inner layers called the fovea (fovea centralis). There is usually one fovea in each eye (e.g. in humans), however there are exceptions in some birds species whose eyes can possess a central and temporal fovea in each eye. The fovea is characterised by being associated with the highest density of

photoreceptors across the retina. As a consequence, the image perceived by this part of the retina has the highest acuity. Therefore, at least in humans and birds, an important aspect of vision is the ability to make fast gaze shifts movements and/or hold the eye still (on a stationary or moving target) so that the object of interest can be viewed by the fovea. For that reason, the eye and head movements serve to provide stable fixation and alignment, as well as tracking of a visual stimulus or shifts in gaze in the fastest possible way to sustain good acuity of vision all the time. This principle is present in all foveate animals. Although birds are capable of eye movement, the eye rarely remains in an eccentric position but normally quickly returns to its default straight ahead orientation in the orbit (Wallman and Pettigrew 1985). As a consequence, birds tend to move their heads rather than their eyes to accomplish long term gaze shifts. Interestingly, birds' eye saccades may serve another function: helping to increase the nutrition and respiration of the retina (Pettigrew et al. 1990).

Saccades then are normally thought of as fast movements of the eyes and/or head; however, in principle, movements of any part of an animal body or device can also be saccadic. The main parameters describing eye saccades are faster than those of head movements, due to the larger size and weight of the head – i.e. inertial mass. However, the relationship between these characteristics remains proportional and therefore can be cross-correlated.

The amount of the literature on animal saccades is vast and encompasses many different animals: different species of birds (chicken, *Gallus gallus* - Pratt 1982; barn owl, *Tyto alba* - Whitchurch and Takahashi 2006), cats, *Felis catus* (Evinger and Fuchs 1978), monkeys (Fuchs 1969; Ron et al. 1972) and humans (Stark et al. 1980). Even saccadic movements in insects have been investigated: e.g. mantis, *Tenodera*

aridifolia (Yamawaki 2000). However, there is surprisingly little detail reported about eye or head saccades when considering only birds.

Eye saccades are one of the fastest movements in the animal world. Indeed, they are the fastest movements that can be made by the human body. It is reported that maximum eye velocities approach $1000^{\circ}/s$ in monkey (Fuchs 1969) and $700^{\circ}/s$ in humans (Garbutt et al. 2001). Whereas, head saccades may reach the speed of $800^{\circ}/s$ in barn owl (du Lac and Knudsen 1990), $500^{\circ}/s$ in humans (Stark et al. 1980) and $400^{\circ}/s$ in mantis (Lea and Mueller 2004). For example, humans perform numerous eye saccades even when reading – moving quickly from one line of text to the next. Saccades are also found in humans and animals while viewing objects that appear or are located in random locations.

5.4.1. Main sequence of saccades

When specifying the type of a particular eye or head movement, the following parameters are examined:

- amplitude (i.e. the angle that the eye or head travels) of each movement –
- duration (i.e. the time the travel takes) of the movement
- peak velocity (i.e. maximum speed that is reached) of the movement

(Figure 5.3).

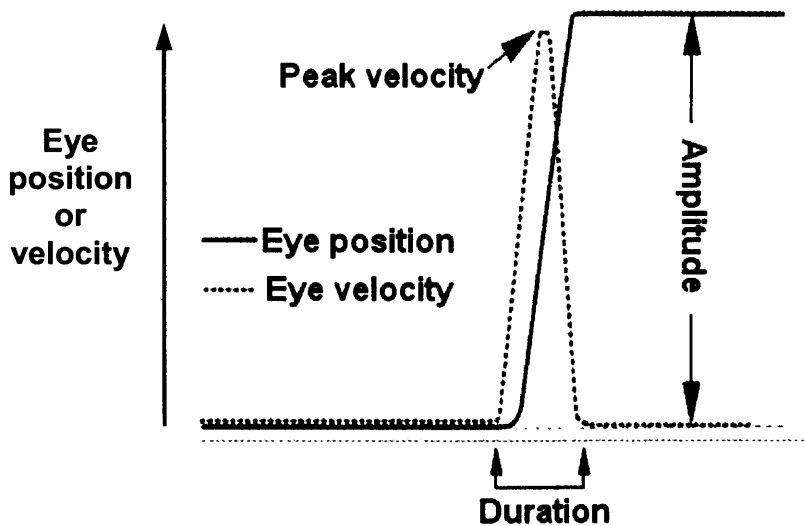


Figure 5.3 Depiction of the main parameters of a saccadic movement

When considering saccades, the most commonly defined correlation is the main sequence. This is characterized by a systematic relationship between the amplitude, duration and peak velocity of the movement. When plotting the amplitude of saccadic head movements against their duration or peak velocity, a linear correlation is observed. The relationship is sufficiently tight that it is possible to judge whether the movement of the eye or head is purely saccadic or not. Moreover, the phenomenon of the main sequence in saccades is so sensitive to particular alterations in the body that it can be used to assess the effects of drugs, disease, brain lesions, etc., on human subjects (Tedeschi et al. 1986; Henik et al. 1994). As an example of a saccadic main sequence for human eye and head movements see **Figure 5.4**. For comparison, **Figure 5.5** presents the main sequence correlation between the amplitude and peak velocity of observed head movements in owls.

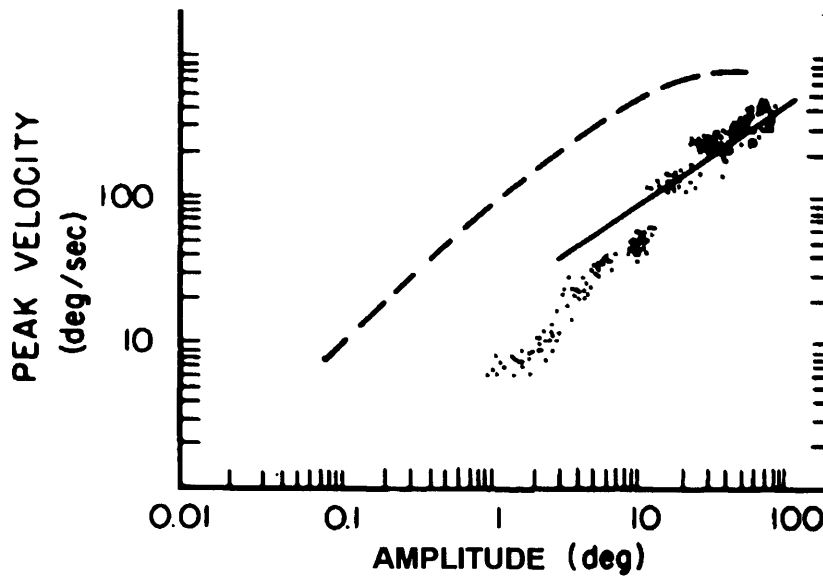


Figure 5.4 Peak velocity as a function of amplitude for the saccadic eye main sequence (dashed line) and the head main sequence (dots and solid line – another subject for comparison) in human (adapted from (Stark et al. 1980))

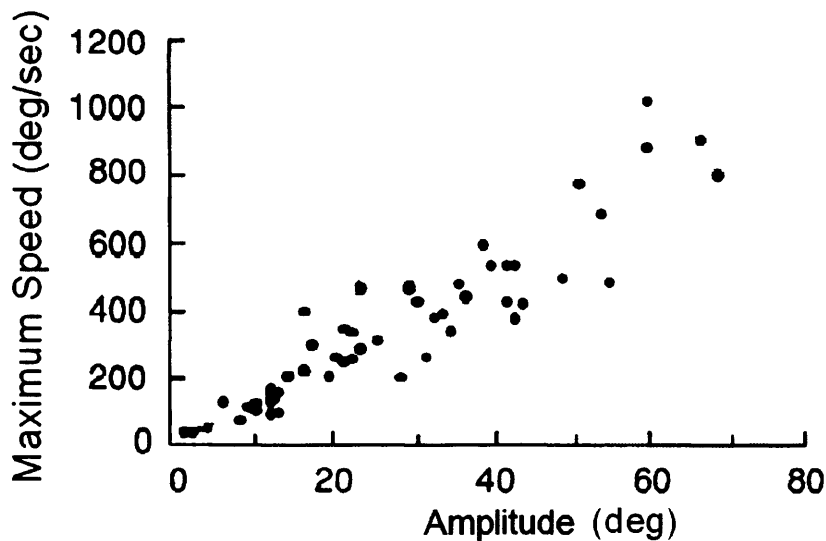


Figure 5.5 Head movement peak velocity versus amplitude relationship, comprising the main sequence, of the barn owl; adapted from du Lac and Knudsen (1990)

5.4.2. Saccades in response to auditory stimulus

It is important to point out that saccades may be voluntary, but they are also frequently driven by any perceived sensory stimulus. For example, in humans, the addition of an auditory stimulus to a visual one actually speeds up the reaction time of the eye saccades. Interestingly, when the situation was reversed, i.e. when the human subjects' saccades were driven by an auditory stimulus and a distractor visual

stimulus was added, this resulted in prolonging the saccadic head movements (Kirchner and Colonius 2004). On the other hand, when the saccadic eye movements are driven by only an auditory stimulus, they are reported to be slower than those driven by only visual cues (Zambarbieri et al. 1982).

However, in comparison to the volume of research on visually driven saccades, there is much less investigation focused on auditory saccadic head movements, especially in animals. In the investigation of how a bisensory stimulus – visual and auditory – facilitates the head saccades of barn owl, it was found that, in contrast to humans, “auditory saccades characteristically had shorter reaction times but were less accurate than visual saccades” (Whitchurch and Takahashi 2006). That study, however, did not attempt to define the characteristics of birds’ saccades to an audible stimulus but rather investigated how a bisensory stimulus affected the accuracy of head saccades and then made the comparison between audibly and visibly evoked saccades. To the best of our knowledge, there is no other study tackling the problem of audibly evoked saccades in birds and their detailed properties. Why this potentially interesting and important area of research has not yet been explored remains unclear.

5.5. Introduction to results

5.5.1. Preliminary investigation

Before obtaining the high frame rate camera, the various parameters of the head movements recorded by the 30fps camera in the previous experiments were analysed. Although the temporal resolution of this camera was clearly not sufficient to fully characterise such rapid head movements, this preliminary investigation might at least suggest the nature of this response to magnetic field changes. As the first sweeping field in the experimental sequence was shown to most reliably provoke a head

movement, a couple of clearly responsive birds were chosen, and each head movement's amplitude, duration, and peak velocity were separated by hand; thus, its precision was not high. However, precision was not the ultimate aim at this stage, but rather an attempt to shed light on the basic properties of the head movements and to investigate whether there is any consistency in the behaviour.

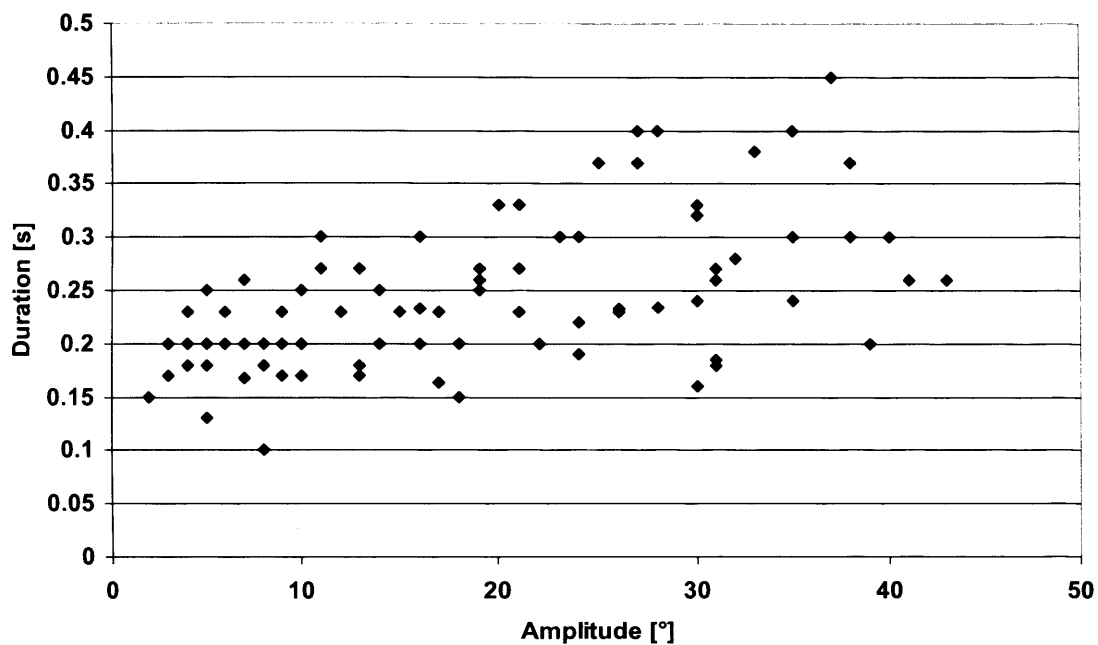


Figure 5.6 Correlation of amplitude and duration of head movements – data from the low-speed camera system

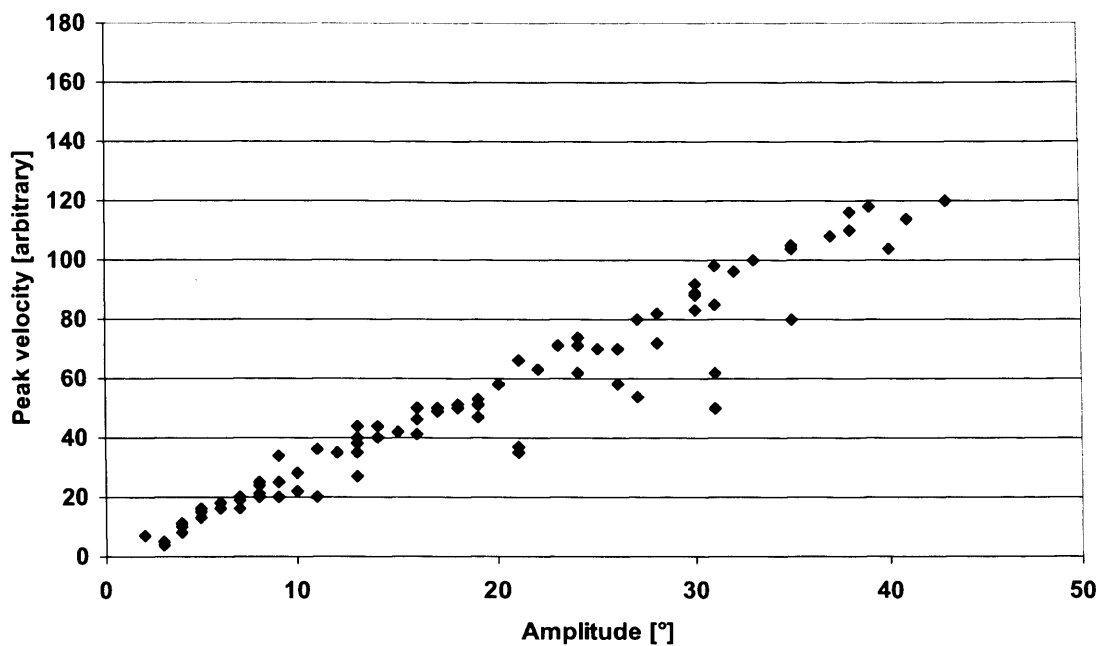


Figure 5.7 Correlation of amplitude and peak velocity of head movements – data from the low-speed camera system

The results (**Figure 5.6** and **Figure 5.7**) exhibit a trend that suggests that there is such a consistency across head movements. However, what can easily be seen in these graphs is evidence of a very low sampling rate, which is manifested by runs of data points at a given peak velocity and the discrete gaps between such groups of data with the same Y-axis values. Therefore, the low sampling rate led to a correspondingly low accuracy of measuring the changes in head position, especially in the cases of brief head movements or those with high velocities. Nevertheless, a cautious conclusion can be drawn: the apparent linear correlation of peak velocity and amplitude suggests that these head movements are saccadic as such a relationship is characteristic of saccadic movements. In saccadic eye movements, for example, there is typically a linear relationship between movement amplitude and its duration as well as between amplitude and peak velocity – that is the called main sequence (see **Section 5.4.2**). On the other hand, the visible trend could still be an artefact of the low sampling rate.

However, in order to be sure about the nature of head movements, more in-depth investigation is needed, which means that a higher resolution measuring system is vital. Therefore, the following sections will be devoted to the results gathered with the help of a very high-speed camera system and will include a discussion of the data obtained.

5.5.2. Initial results from the high-speed camera experiments

In this section, only the initial results from the high-speed camera experiments will be displayed. The intention is to identify and introduce the topic of the specific head movements made by the birds during the experiment.

To serve as an initial example, the results from one of the experiments were chosen. In this experiment involving nine pigeons exposed to a field sweeping in a clockwise direction (CW) and recorded at a sampling rate of 500fps (for justification of this chosen frame rate, see **Section 5.2.1**), both duration and peak velocity were plotted versus the amplitude of each movement. The aim was to establish whether the observed head movements are actually saccadic in their nature, as suggested by the preliminary analysis of the low frame rate recordings.

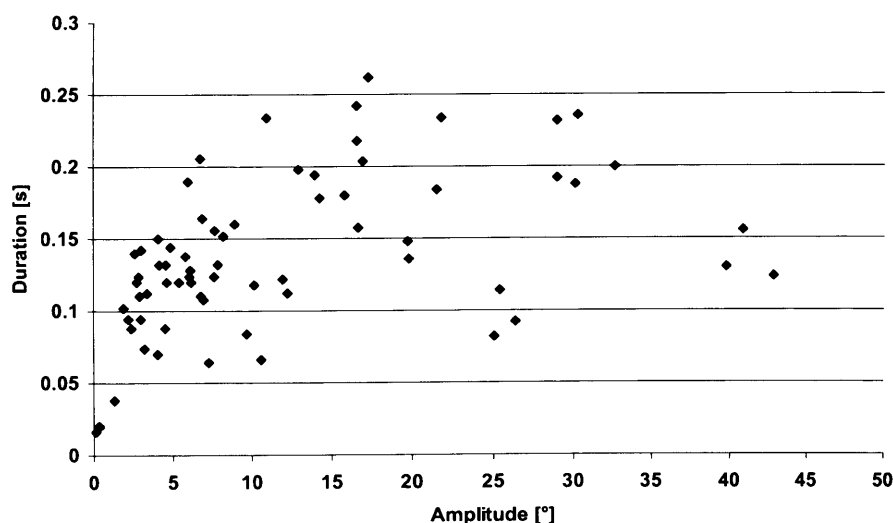


Figure 5.8 Correlation of amplitude and duration of head movements – data from high-speed camera collected for CW

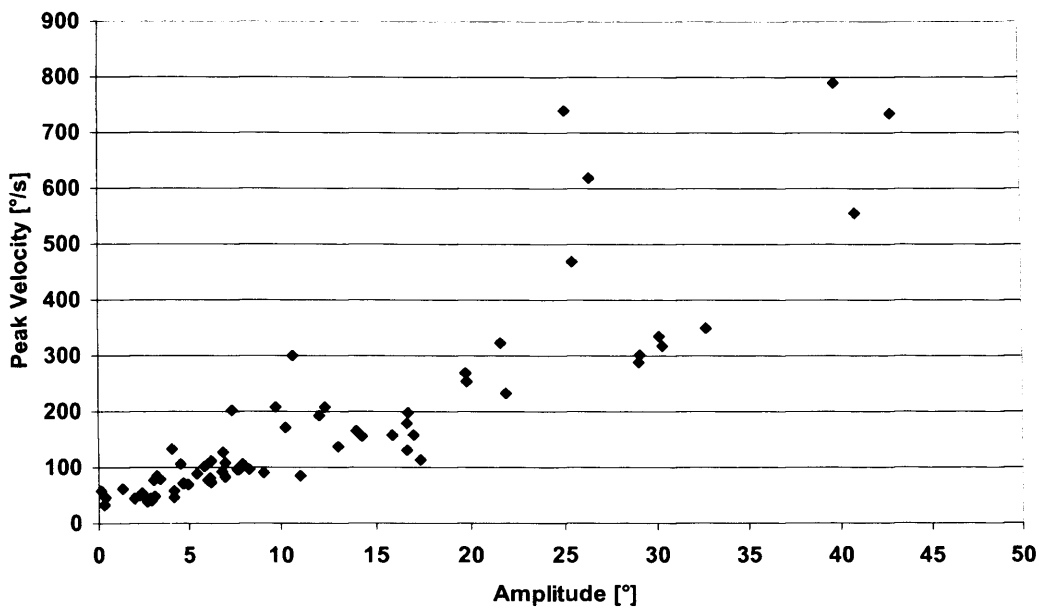


Figure 5.9 Correlation of amplitude and peak velocity of head movements – data from high-speed camera collected for CW

Both of the graphs (**Figure 5.8** and **Figure 5.9**) suggest the presence of a specific systematic relationship between duration, peak velocity and amplitude without the artefacts evident in low frame rate data. Although the data are variable, especially in the duration versus amplitude plot, there is a clear similarity between these trends and the previously mentioned typical characteristics of saccades. Saccadic movements are characterised by the main sequence, i.e. a correlation between amplitude, duration and peak velocity. In normal human saccadic head movements, the duration versus amplitude correlation has a curvilinear shape and the relationship between peak velocity and amplitude is nearly linear. In our case, in the duration versus amplitude graph (**Figure 5.8**), a monotonic linearity is present in smaller head movements (i.e. up to 20°) but at higher values, duration becomes more or less constant. Whereas, in the plot of the peak velocity versus amplitude (**Figure 5.9**), peak velocity continues to increase fairly linearly with amplitude. These results confirm that the characteristics of the birds' head movements correspond well with the known properties of saccades.

Ipso facto, it can be concluded that the preliminary results from albeit a low-speed camera, which suggested a saccadic relationship, were indeed not an artefact but simply a poor quality representation of the true nature of the examined head movements.

However, there remain some uncertainties, which in light of the unusual magnetic stimulus used, will be addressed later in this chapter.

5.6. Results

Three kinds of experiments were performed with the high-speed camera, using two different stimuli. The first two were experiments in which birds' responses were driven by a sweeping field, either in a CW or CCW direction, as in the previous low-speed camera experiments. In the third experiment, a different stimulus was used to drive the birds' responses. Instead of manipulations of the magnetic field stimulus, a noise was induced by gently knocking on the mumetal lid that covers the magnetic field shielding chamber.

In order to determine the characteristics of head movements as a result of birds' responses to either a magnetic field stimulus or an audible noise, the data were gathered, analysed and presented on three main graphs, which is standard procedure when investigating whether movements are saccadic in nature, as was suspected in the case of the birds' head movements in this study: (1) the correlation of each head movement's duration time plotted against its amplitude; (2) a plot of peak velocity for each head movement as a function of amplitude; and (3) the product of peak velocity multiplied by duration of each head movement, which is also used in investigating the main sequence properties, was plotted against amplitude. These graphs are presented in the following results sections.

5.6.1. Selection of experimental birds

A variable number of birds were used in this experiment. The main criterion for including a bird in further data analysis was that a significant reaction was made within the first 8s of the onset of a sweeping field. A significant reaction in this case is understood as a reaction that consists of at least two observable head movements of variable amplitude. The characterisation of the nature of head movements (i.e. the main sequence) has to be based on a considerable number of measurement points (i.e. discrete head movements) of various amplitudes in order to investigate its properties over the whole range. Therefore, a sufficiently wide distribution of head movements had to be obtained. As the camera recording time was limited (i.e. 16s for each sequence), only birds that clearly reacted in that time frame and made significant head movements were selected for further analysis. All together, there were 17 different homing pigeons used in this experiment, but the same birds were not used in all three treatments (i.e. exposure to CW, CCW and AUD stimulus). For the detailed summary of the birds tested and included in the analysis, see **Appendix 2**). Also, note that the birds were subjected to the experiments in a randomised order.

5.6.2. Saccades in response to a magnetic field stimulus

The first experiment that was done with the help of the high-speed camera was to record birds' responses to sweeping magnetic fields in one of two directions (CW or CCW). The responses observed here are, on the basis of the main experiment (i.e. significant transition to the first sweeping field NF-CW), believed to be reactions to alterations of the magnetic field, the only stimulus present during these experiments. However, as in any behavioural experiment, it cannot be excluded that some of the movements observed here could be just spontaneous movements of the pigeons irrespective of changes in the magnetic field. Therefore the ideal approach would be

to measure the frequency and characteristics of spontaneous head movements in SF (i.e. not made in response to any stimulus change) in order to find out whether they are saccadic as well and if so compare them with the dynamics of the saccadic head movements studied here.

5.6.2.1. Characteristics of responses to field sweeping in CW direction

Birds were exposed to a sequence of SF, NF and CW, each of 30s, and the period of sweeping field during which the birds were reacting (i.e. moving its head) was recorded for further analysis. Following the criteria for selecting reactive birds (see **Section 5.6.1**), 9 birds were used in this experimental analysis.

The three characteristics of the main sequence were plotted. The first, duration versus amplitude, is presented in **Figure 5.10**, and shows a trend that consists of two regions. In the first region, at small amplitudes of head movements $<10^\circ$, the trend increases linearly. In the second region of higher amplitudes, it reaches saturation and then remains constant. Although, in the second part especially, the data points are scattered, this does not obscure the overall pattern of the head movements.

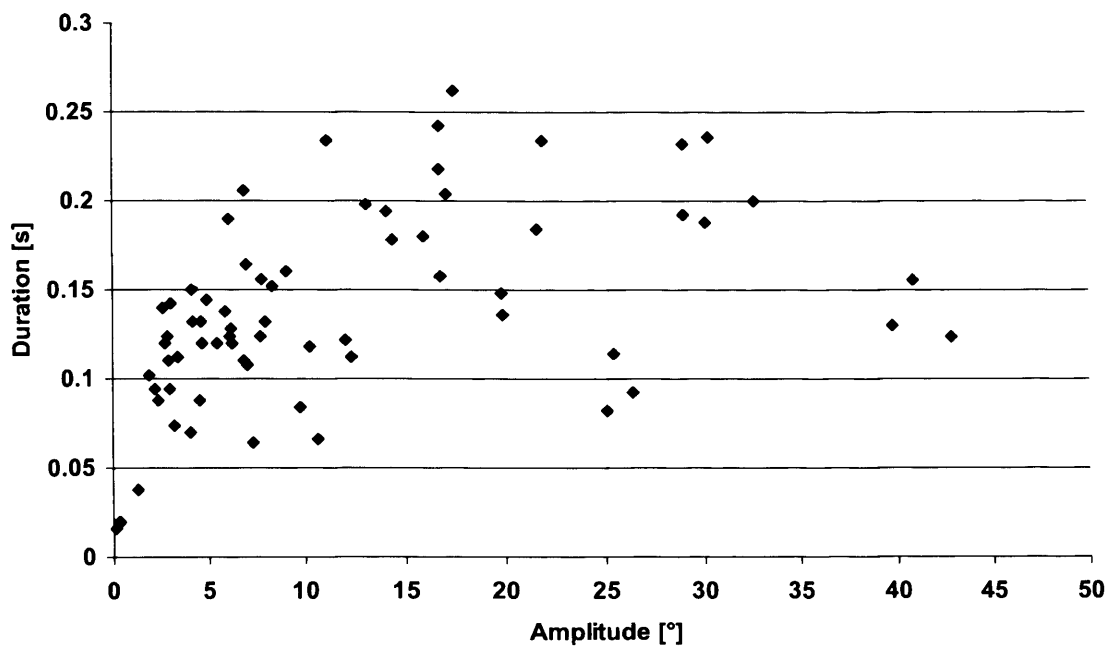


Figure 5.10 Correlation of duration and amplitude of head movements comprising the main sequence as responses to CW

The second characteristic, peak velocity as a function of amplitude of each head movement, shows the linearity of this correlation over a large range of amplitudes (**Figure 5.11**). In addition, the scatter is considerably smaller than in the duration plot.

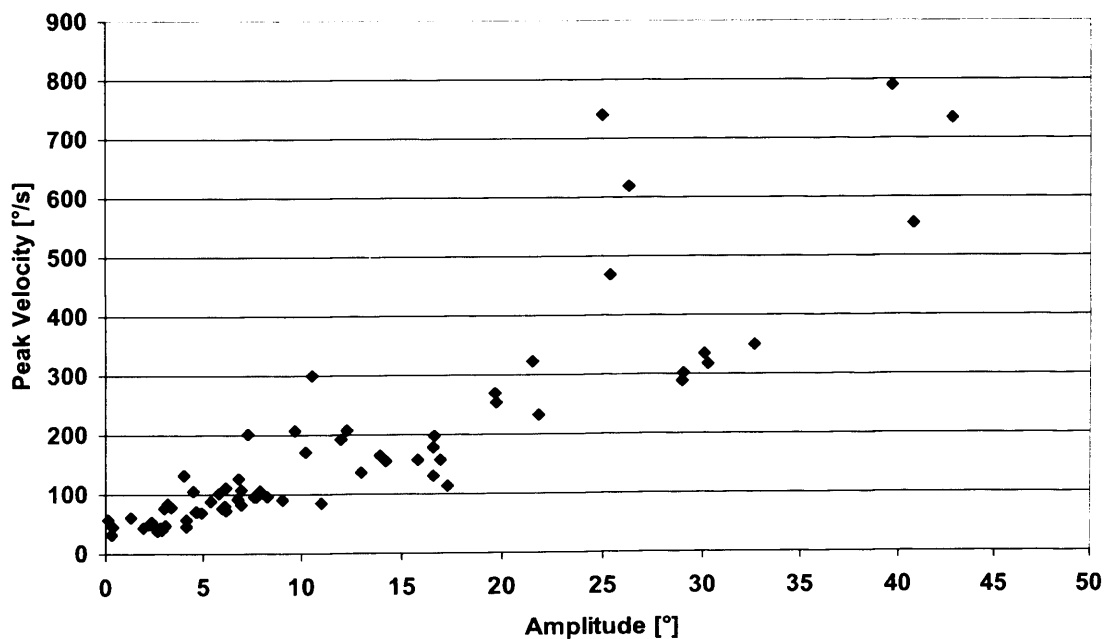


Figure 5.11 Correlation of peak velocity and amplitude of head movements comprising the main sequence as responses to CW

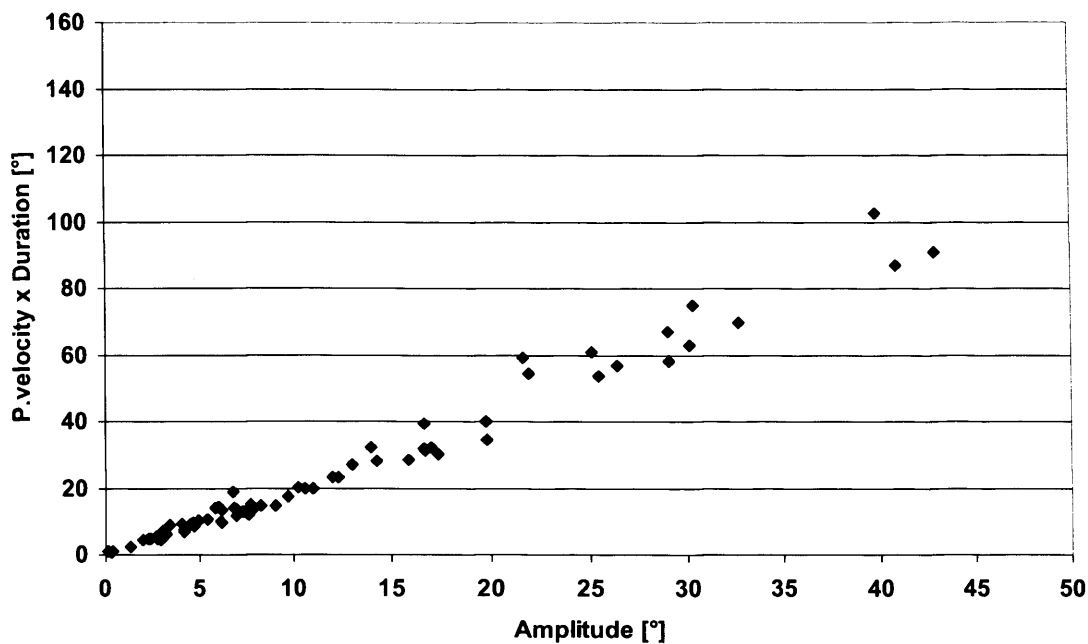


Figure 5.12 Correlation of product of peak velocity and duration versus amplitude of head movements as responses to CW

The final characteristic, peak velocity x duration against amplitude (**Figure 5.12**), reveals a highly linear correlation. A similar type of relationship is seen in human saccadic eye movements and is thought to reflect internal feedback control during the movement (Sparks 2002). For example, any increase in peak velocity will be compensated by a shorter duration movement, and vice versa, and thus the product of velocity x duration has a much lower variance. In other words, the system is designed to produce a target head movement (of a given amplitude), which has an organised internal representation that is achieved with internal feedback control. The target movement is produced by the saccadic system with the help of a velocity profile that the system generates and tries to follow (Harris and Wolpert 2006) (for velocity profiles of the saccadic head movements as responses to magnetic as well as auditory stimulus, see **Figure 5.20**). The implication is that these are stereotyped head saccades albeit, different from eye saccades, presumably because of the difference in inertial mass and/or elasticity of the muscles or due to independent systems being responsible for saccades of the eye and head, respectively (Liao et al. 2005).

The internal feedback responsible for controlling these movements and enabling achievement of such high accuracy has not yet been characterised. What is known, due to numerous studies on saccadic eye movements, is that the feedback cannot be visual, as the high speed of saccades precludes this option (Sparks 2002; Harris and Wolpert 2006).

5.6.2.2. Characteristics of responses to field sweeping in CCW direction

In the next experiment, the sweeping direction of the stimulus field was counterclockwise (CCW). As in the high-speed camera CW experiment, the same selection criteria were followed, resulting in a total of 10 birds being chosen. The same sequence was applied to the birds with the exception of one bird in which case the sequence was composed of SF and CW steps of 60s each.

The data were analyzed to obtain the same three plots that define the main sequence of head saccades. The results are very similar to those obtained when exposing the birds to the CW sweeping field.

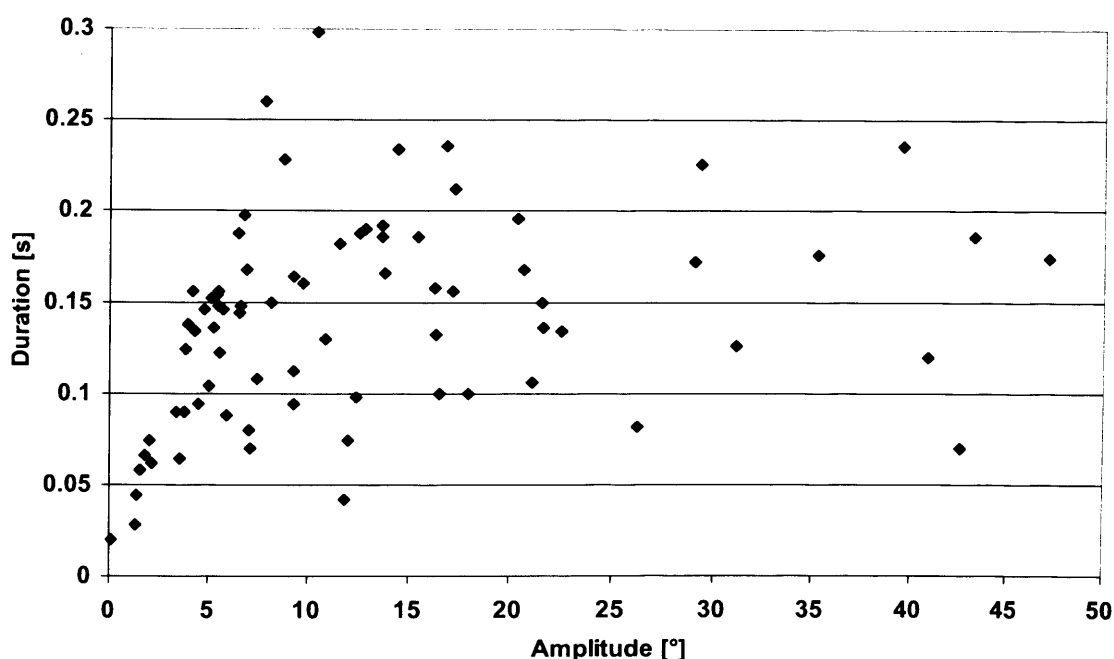


Figure 5.13 Correlation of duration versus amplitude of head movements comprising the main sequence as responses to CCW

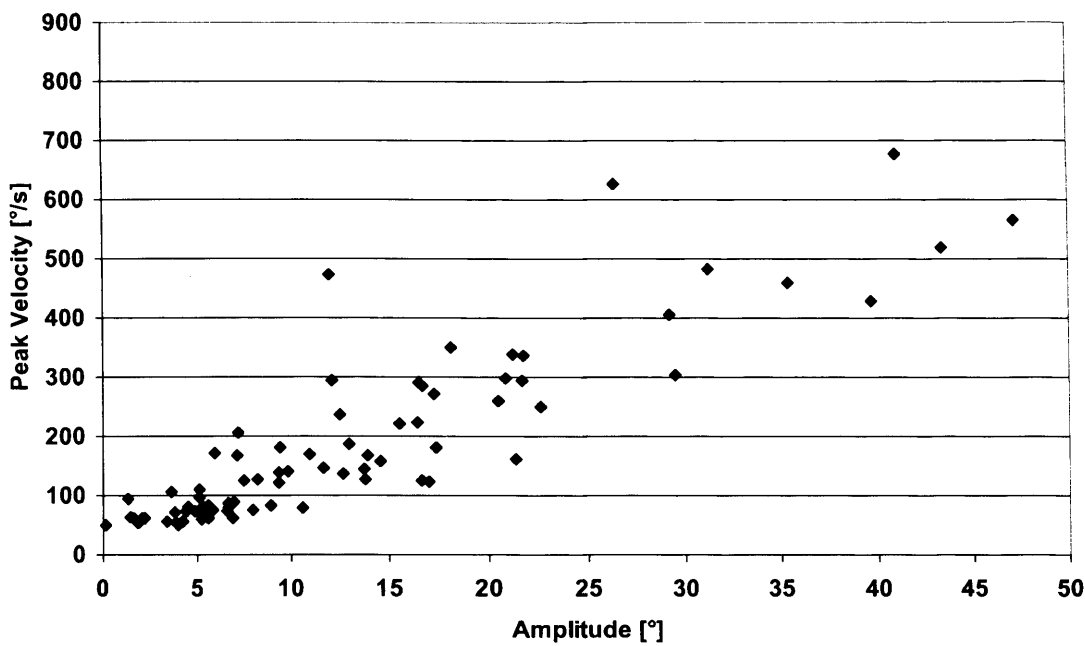


Figure 5.14 Correlation of peak velocity versus amplitude of head movements comprising the main sequence as responses to CCW

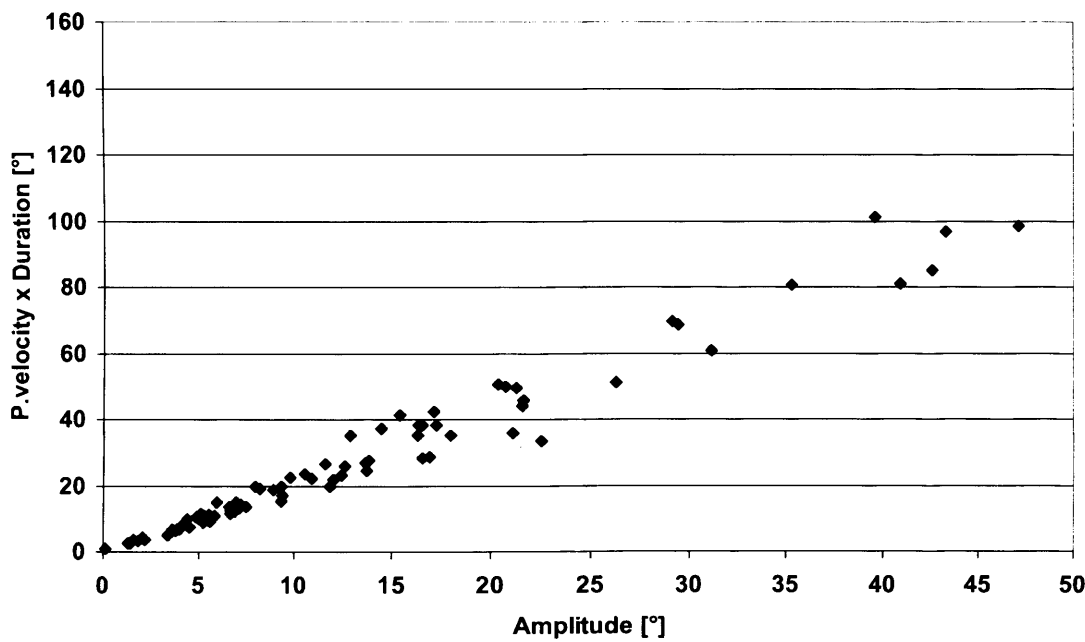


Figure 5.15 Correlation of product of peak velocity and duration versus amplitude of head movements as responses to CCW

In all three cases depicted in **Figure 5.13**, **Figure 5.14** and **Figure 5.15**, the trends are a clear reflection of what happened in CW. Indeed, this is confirmed by the statistical analysis (see **Section 5.6.4**). Therefore, it can be assumed that properties of head movements evoked by CW and CCW stimulus are very similar and thus they are not influenced by the direction of the sweep of the stimulus. Moreover, it can be

clearly stated, on the basis of the above characteristics, that the head movements are saccadic in nature.

As little to no information about main sequence analysis of head movements in birds could be found in the literature, the results from the CW- and CCW-directed stimulus experiments were compared with data from studies of eye saccades in humans. This comparison reveals that the main sequences are different from each other, most prominently in the case of the duration versus amplitude plot. In human eye saccades, duration linearly increases up to amplitudes of 40° (Harris and Wolpert 2006), while in the case of bird head movements in response to CW or CCW (**Figure 5.10, Figure 5.13**), saturation is reached at around 10°, after which head movement duration remains close to constant with respect to amplitude. On the other hand, a previous study of patterns of head movement saccades in humans, reveals an initial linear increase followed by an apparent saturation at low amplitudes (Stark et al. 1980) in agreement with the birds' head movements analyzed in this investigation. This indicates that the dynamics and hence the neural control of pigeons' head saccades and human head saccades are highly similar.

In a previous study of saccadic head movements in the barn owl, which are the closest to this investigation, the peak velocities reported (**Figure 5.5**) highly resemble the peak velocities obtained in the present study in response to both the CW and CCW stimulus (**Figure 5.11** and **Figure 5.14**, respectively).

5.6.3. Saccades in response to an auditory stimulus

The induction and analysis of auditory driven saccades had the purpose of gathering data on pronounced head movements in response to an auditory stimulus that was known to be perceived by the birds and could be easily recognized and characterized, in order to validate the method as well as to obtain a reference

comparison for the high-speed camera magnetic stimulus experiments described in the previous section.

The saccadic head movements that the birds make in response to an auditory stimulus might be expected to be similar to human saccadic head movements in response to a visual stimulus as they are both reacting to a perceived sensory input. Therefore, a comparison of magnetically-induced head movements with auditory saccades (and/or human head movements) may help reveal whether magnetically driven saccades are typical of head movements in response to a sensory stimulus. This would also provide further support for the idea that the birds indeed perceive the change in magnetic field conditions.

In this case, no selection criteria for subjects were required to be applied due to the very reliable and immediate response of all the birds to the stimulus. Although the knocking was rather brief and gentle, the enclosed environment in which birds were placed might understandably lead to an auditory stimulus being somewhat alarming for the pigeons. That in turn, might explain why the birds always reacted with head movements in a clear and prolonged manner, which allowed the gathering of a considerable number of measurement points. Consequently, in order to have a similar number of subjects as in the earlier experiments, 10 birds were used in the analysis.

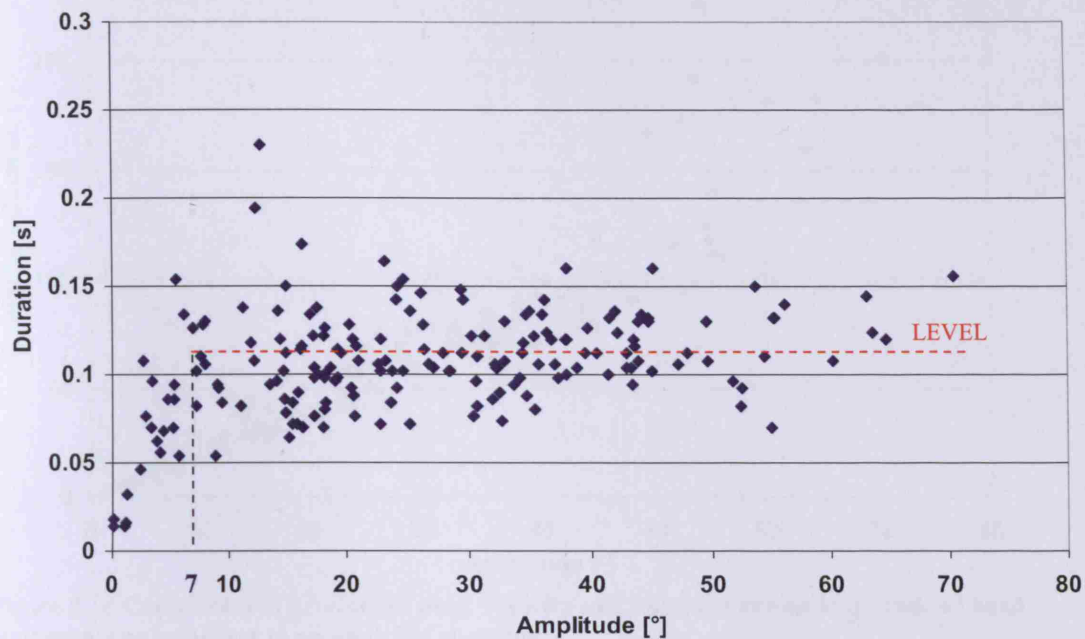


Figure 5.16 Correlation of duration versus amplitude of head movements comprising the main sequence as responses to an auditory stimulus. (Red dashed line denotes the mean duration for head movements larger than 7°)

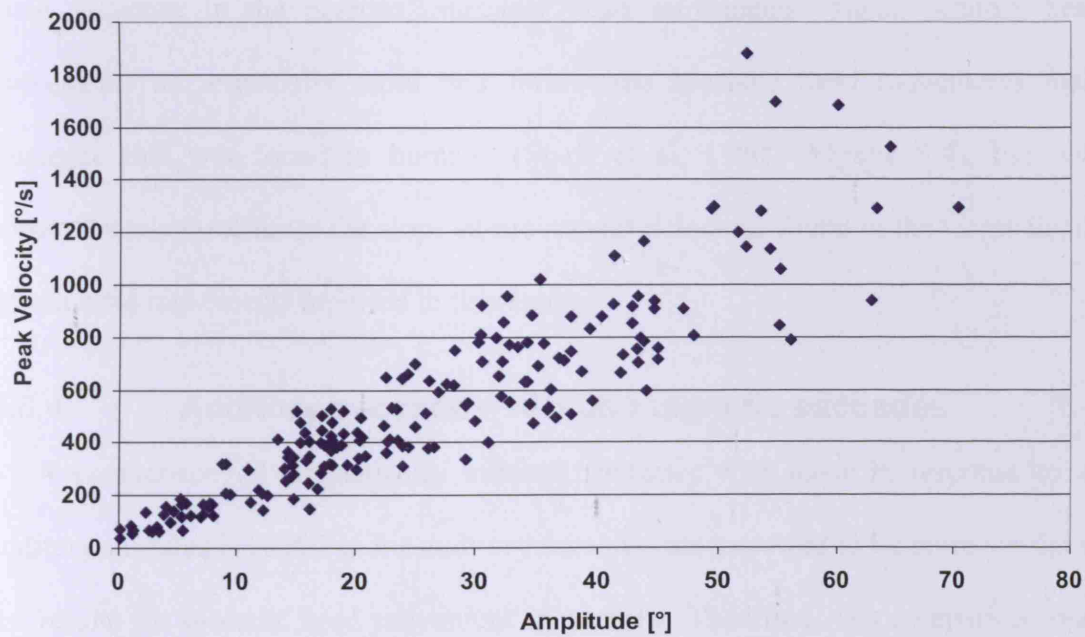


Figure 5.17 Correlation of peak velocity versus amplitude of head movements comprising the main sequence as responses to an auditory stimulus

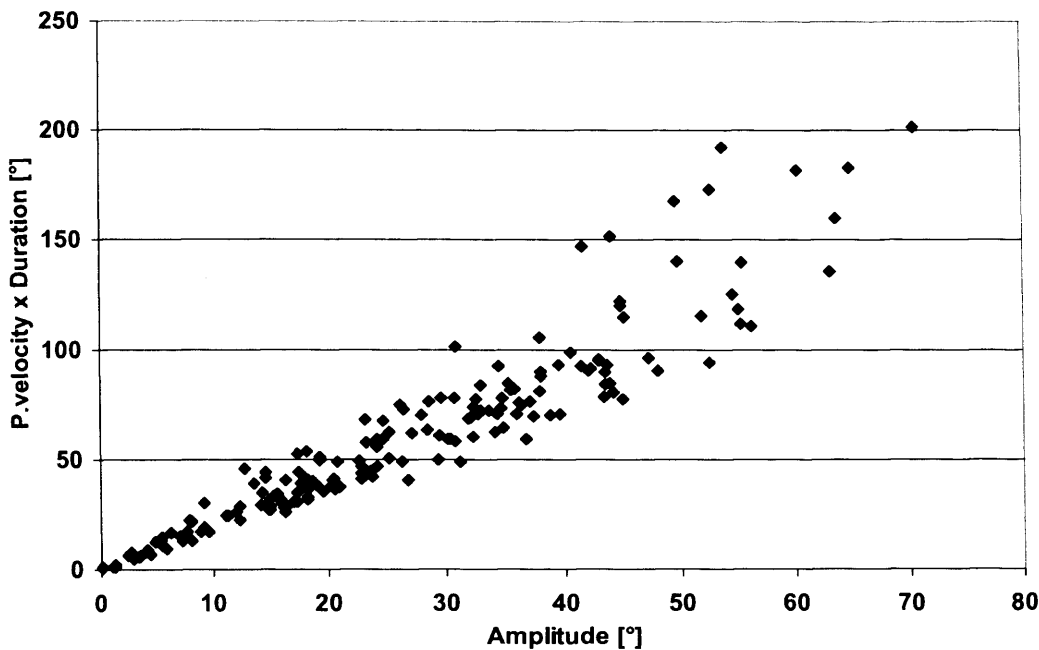


Figure 5.18 Correlation of product of peak velocity and duration versus amplitude of head movements as responses to an auditory stimulus

Similar to the magnetic stimulus experiments, the results depict a clearly defined main sequence in the pigeons' saccadic head movements. These auditory head movements are especially rapid and follow the saccadic head movements main sequence that was found in humans (Stark et al. 1980) (**Figure 5.4**), but with approximately three times the slope of movement velocities found in the magnetically driven head movements reported in this study.

5.6.4. Auditory saccades versus magnetic saccades

A comparison of magnetically induced saccades with those in response to an auditory stimulus is useful as the auditory saccades are expected to be more similar to the results for saccadic head movement in humans. Therefore, this comparison may help in defining the relationship between magnetic saccades and those head movements in response to a normal visual stimulus.

The first obvious difference when examining the CW and CCW main sequence graphs, as opposed to the auditory main sequence graphs, is that there is much less

variability in the scatter of the distribution of the measurements of auditory head movements (compare auditory duration and peak velocity characteristics with the magnetic ones). The reason for that is likely to arise from the stimulus' nature. Namely, the auditory stimulus, induced by knocking on the lid of the chamber, arguably had a much higher impact on the birds' reactions – the perception was always nearly instantaneous and involved more head movements as compared to the magnetically induced reactions, even though a similar number of birds was included in the analysis. Following this argument, the perception of the magnetic stimulus was less salient for the pigeons' sensory system and thus the performed head movements were less accurate (i.e. the scatter in the duration and peak velocity characteristics was clearly higher).

Table 5.2 T-test (done independently from GLM model statistics) performed on the level (i.e. the mean value of duration for head movements larger than 7° only, for illustration see Figure 5.16) in the case of duration and slope and intercept in the case of peak velocity and the product of both, which were calculated for each bird based on all head movements made by a particular bird.

Main sequence	Parameter	CW vs. AUD	CCW vs. AUD	CW vs. CCW
Duration versus amplitude	Level	p< 0.001	p= 0.002	p= 0.50
	Slope	p< 0.001	p< 0.001	p= 0.59
Peak velocity versus amplitude	Intercept	p= 0.74	p= 0.39	p= 0.35
	Slope	p= 0.38	p= 0.24	p= 0.62
Peak velocity x duration versus amplitude	Intercept	p= 0.74	p= 0.36	p= 0.52
	Slope	p= 0.38	p= 0.24	p= 0.62

The statistical analysis performed on all of the head movement data revealed that there are unequivocal significant differences in responses between either of the magnetic stimulus (i.e. CW or CCW) and the auditory stimulus in the case of duration and slope of the peak velocity (p<0.001 in all cases, except for duration in CCW versus AUD: p= 0.002 (**Table 5.2**)). Durations, peak velocities and the product between them were not statistically different for the two different directions of

sweeping magnetic stimulus. This again confirms that the birds reacted in the same manner regardless of the magnetic field stimulus direction. Therefore, the statistical analysis clearly shows the difference between durations (compare **Figure 5.10**, **Figure 5.13** and **Figure 5.16**) and peak velocities (compare **Figure 5.11**, **Figure 5.14** and **Figure 5.17**) of birds' responses to a magnetic versus an auditory stimulus. However, this is not true in the case of the product of duration and peak velocity (**Figure 5.19**). As the head movement durations were shorter when in response to an auditory stimulus and the peak velocities faster when compared to reacting to a magnetic stimulus, the output of the product of the duration and peak velocity could be expected to be more similar across magnetic and auditory stimuli, and indeed, this is the situation observed when comparing this product in the case of CW, CCW and AUD (see **Table 5.2**).

Associated with the plot of peak velocity product versus amplitude is a parameter denoted by Q , which is the ratio of peak velocity to mean velocity. It is based on the equation of a regression line that is constrained to intersect through the origin of the graph. The Q value is thus a slope parameter of the line (Harwood et al. 1999). Calculation of the Q ratio for the three experiments is shown in **Figure 5.19**. The Q values are very similar to each other in all three cases reflecting the fact that the same mechanism is likely to be controlling head saccades in response to either magnetic or auditory stimulus.

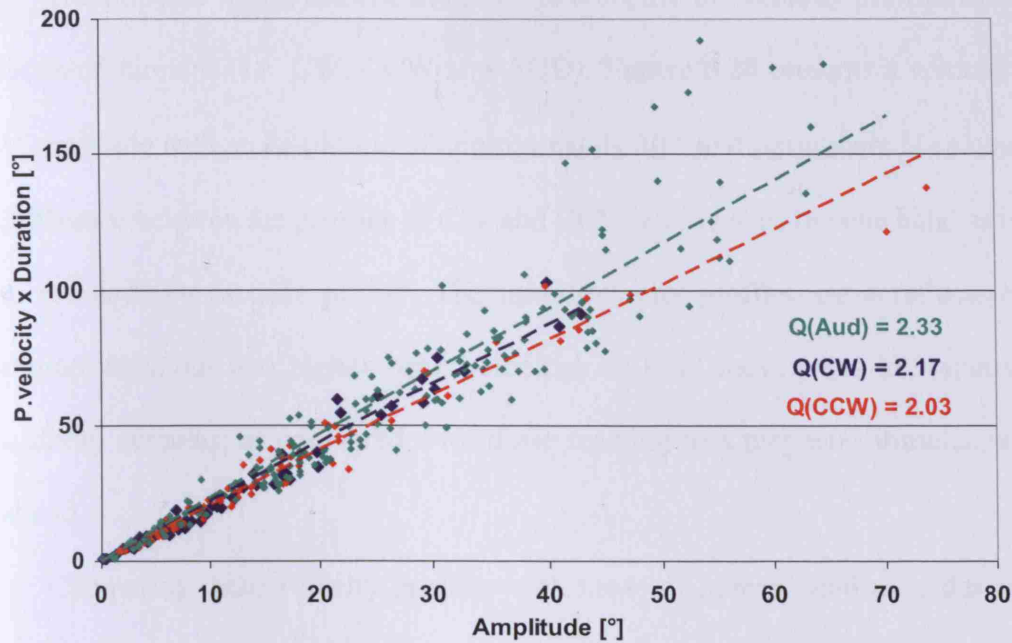


Figure 5.19 Correlation of peak velocity x duration versus amplitude with ratios of peak velocity to mean velocity, Q for CW, CCW and auditory stimulus

Comparing the above results with previously reported human eye-saccades data reveals that Q values range from 1.64 (Liao et al. 2005) to 1.72 (Harwood et al. 1999), which are considerably lower than the birds' head movement peak velocity to mean velocity dependence found here. However, in the case of head saccades in humans, the reported values of the Q factor are 2.03 ± 0.18 (Liao et al. 2005) and 1.85 (Evinger, personal communication), which cover the Q range reported in the present investigation.

Very similar plots of peak velocity x duration versus amplitude in all three cases also show that, even though there were significant differences between duration and peak velocity of auditory and magnetic saccades, overall there is not much difference in the features of the saccadic movements driven by these two stimuli. Therefore, it can be stated that both kind of saccades are likely to be controlled in the same manner by similar systems that possess internal feedback driven by a velocity profile.

Therefore, it would also be desirable to compare the velocity profiles of all three kinds of stimulus (i.e. CW, CCW and AUD). **Figure 5.20** presents a velocity profile of a saccade with an amplitude of approximately 30° , and again there is an observable difference between the profiles of CW and CCW saccades on the one hand as opposed to the auditory saccade profile. The latter velocity profiles are a reflection of the shorter durations and higher peak velocities of head movements in response to an auditory stimulus, as compared with those reacting to a magnetic stimulus, as stated above.

Comparing these velocity profiles with those of human head saccades reveals a close similarity between both (Liao et al. 2005), again strongly suggesting that the overall characteristics and mechanisms underlying saccadic head movements in humans and birds are almost indistinguishable.

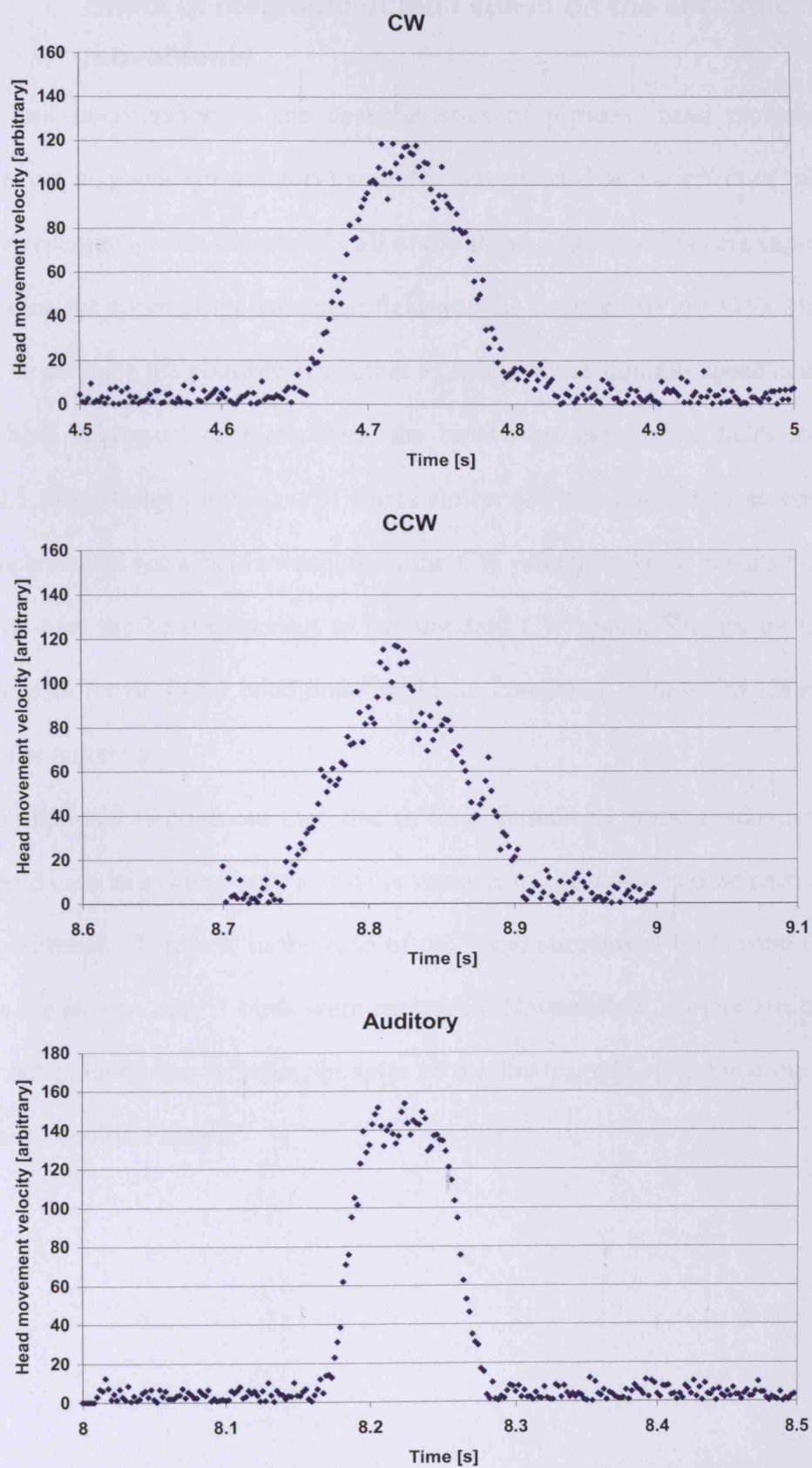


Figure 5.20 Velocity profiles of an example saccadic head movement of approximately 30° in response to a CW, CCW and auditory stimulus

5.6.5. Effect of magnetic stimuli speed on the saccadic head movements

The last investigation of the characteristics of pigeons' head movements in response to a magnetic (or auditory) stimulus was focused on the effect of the speed of the sweeping magnetic stimulus. In all of the above high-speed camera experiments to this point, the speed of the sweeping field was the same, equal to $144^{\circ}/s$. However, in order to examine the possible effect that a change in the stimulus speed could have on the head movement characteristics, the birds were exposed to fields sweeping either 2.5 times faster ($360^{\circ}/s$) or 4 times slower ($36^{\circ}/s$). Both of these conditions were implemented for a field sweeping in the CW direction. These results were then compared with the head responses to the standard CW speed. This meant that data from three different speed conditions could be compared in order to identify any differences observed.

It is important to point out that, due to time limitations in the availability of the high-speed camera system, only a smaller number of pigeons could be used in these last experiments. Therefore, in the case of the faster stimulus, 6 birds were recorded and, for the slower, only 3 birds were measured. Nevertheless, an analysis could be carried out to determine whether, in spite of the low sample size, some meaningful conclusions could be drawn.

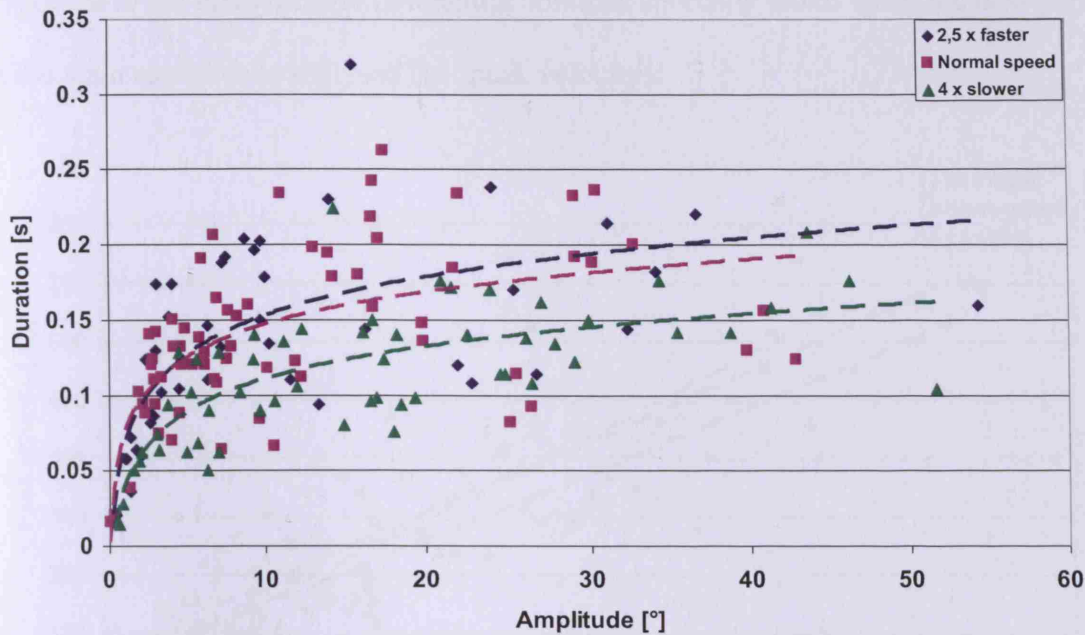


Figure 5.21 Correlation of duration versus amplitude of head movements comprising the main sequence of different stimulus speeds as responses to CW

The first graph from the main sequence, duration versus amplitude, shows that the faster and slower speed stimulus again provoked characteristics resembling saccadic movements. Moreover, there are differences in the overall trend that are shown by the regression lines for each of the three cases (**Figure 5.21**). Those trends appear to follow the rule that the slower the stimulus, the shorter the overall duration of head movements of a given amplitude. Ipso facto, there would appear to be an inverse correlation between the duration of a given amplitude head movement driven by these stimuli and stimulus speed. However, the statistical analysis did not reveal any significant difference between the faster and normal stimulus ($p=0.6$, **Table 5.3**). The slowest stimulus, which has shorter durations from the remaining two, was not included in the statistical analysis because only three subjects used. However, the difference in durations is not as noticeable as that between the movements in response to a magnetic or auditory stimulus, which were statistically different (see **Section 5.6.4**), and therefore, it could be concluded that durations for the different stimuli speeds are reasonably similar. Further evidence for the similarity of the saccadic

responses to the three different sweeping stimulus speeds is found when the next part of the main sequence is analysed (i.e. peak velocity).

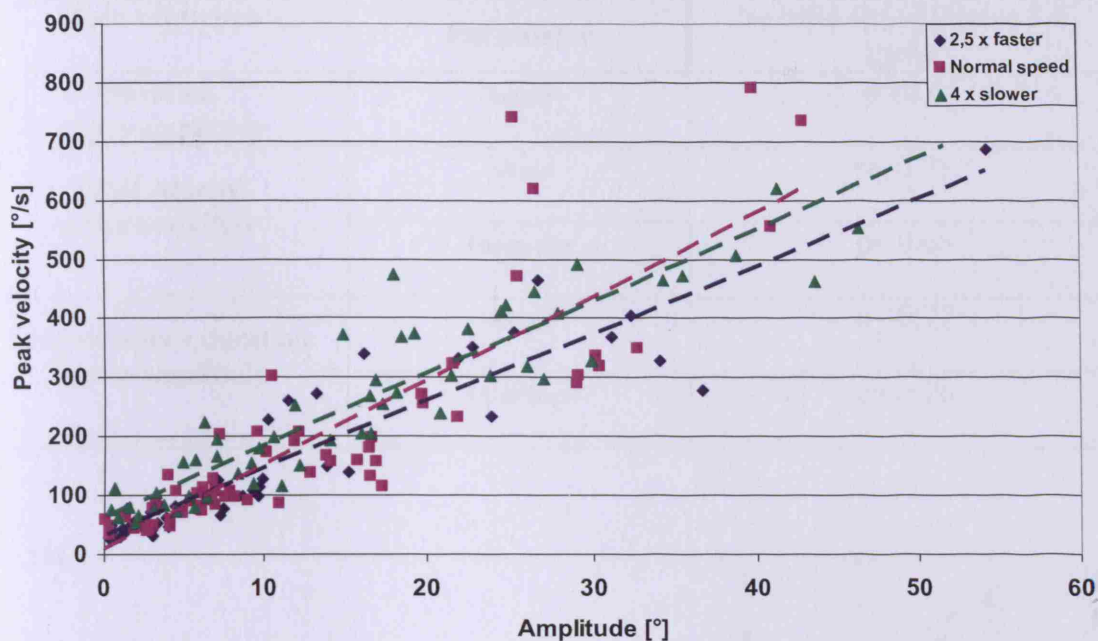


Figure 5.22 Correlation of peak velocity versus amplitude of head movements comprising the main sequence of different stimulus speeds as responses to CW

Examining **Figure 5.23** reveals that there is no difference between any of the stimulus speeds. Indeed, more in-depth statistical analysis showed no difference between velocities of the head movements as response to the faster (i.e. $360^{\circ}/s$) and normal stimulus speeds ($144^{\circ}/s$) (slope comparison, $p = 0.58$, and intercept comparison, $p = 0.68$, in **Table 5.3**). The slower stimuli (i.e. $36^{\circ}/s$) peak velocities are also very close to those seen at normal stimulus speed and therefore, it can be concluded that there is no evident difference in peak velocity among all three different magnetic field stimulus speeds.

Due to the low number of subjects in the case of the field sweeping 4x slower (i.e. 3 subjects were used), only the data from the field moving 2,5x faster were compared statistically with those obtained from the field at normal speed (**Table 5.3**).

Table 5.3 T-test (done independently from GLM model statistics) performed on the level (i.e. the mean value of duration for head movements larger than 7° only, for illustration see Figure 5.16) in the case of duration and slope and intercept in the case of peak velocity and the product of both, which were calculated for each bird based on all head movements made by a particular bird.

Main sequence	Parameter	Normal speed versus 2,5 faster
Duration versus amplitude	Level	p= 0.6
Peak velocity versus amplitude	Slope	p= 0.58
	Intercept	p= 0.68
Peak velocity x duration versus amplitude	Slope	p= 0.77
	Intercept	p= 0.26

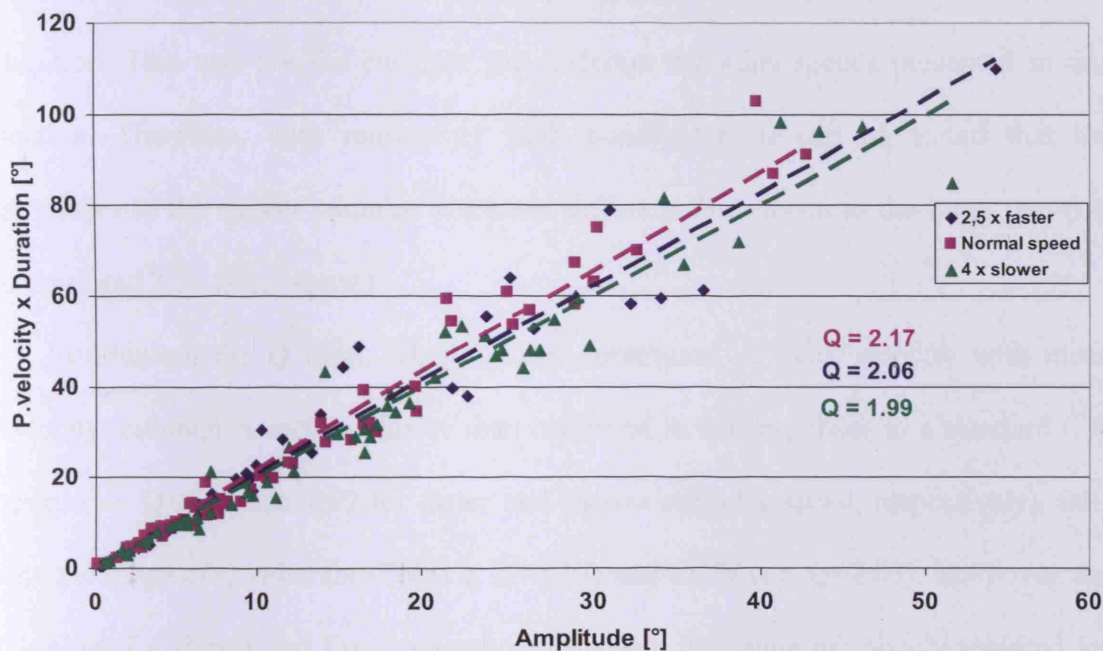


Figure 5.23 Correlation of peak velocity x duration versus amplitude of head movements of different stimulus speeds as responses to CW

Similar to the peak velocity characteristics, the product of peak velocity and duration in all three cases follow the same pattern as the statistical analysis of the CW, CCW and AUD. No difference was found in the case of the three magnetic stimulus speeds (see Figure 5.23; statistical analysis in Table 5.3).

Comparison of the faster and normal speed data revealed that none of the parameters in the three plots of the main sequence were significantly different from each other. Even though the 4x slower stimulus could be not included in the statistical analysis, it seems unlikely that a larger sample size would reveal a significant difference from any of the other two stimulus speeds. The only observable difference on the main sequence plots is in duration (**Figure 5.21**), in which the slower stimulus has shorter durations. However, the remaining plots of the main sequence, do not exhibit any noticeable differences. In the case of the auditory stimulus, the durations were significantly shorter than the durations of the magnetic stimulus, but at the same time, auditory saccades had much faster peak velocities than in the case of shorter duration. This was not the case for the different stimulus speeds presented in this section. Therefore, with reasonably high confidence, it can be stated that the responses to the slower stimulus were not different from those to the other two (i.e. normal and 2,5x faster speed).

In addition, the Q ratio, which is the correlation of peak velocity with mean velocity, although somewhat lower than observed in the responses to a standard CW speed (i.e. $Q=2.06$ and 1.99 for faster and slower stimulus speed, respectively), falls into the range of Q value for CW (i.e. $Q=2.17$) and CCW (i.e. $Q=2.03$). Moreover, the Q values for slower and faster stimulus are within the range previously reported for human head movements, i.e. 2.03 ± 0.18 (Liao et al. 2005).

The birds appear to react in similar manner to the different stimulus speeds tested or at least there is no evident significant difference in the characteristics of the saccadic head movements in response to the different speeds of field rotation. Therefore, the birds would appear to have reacted in a similar manner to all three stimulus speeds, which in turn would lead to the conclusion that varying the speed

within the investigated range did not have any significant impact on the birds' perception of, or reaction to, the sweeping magnetic field and that the nature of the head movements was unaffected and remained clearly saccadic, regardless of stimulus speed.

5.7. Discussion

The results of the high-speed camera experiments demonstrate that the head movements, as a response to magnetic stimulus as well as to an auditory stimulus, are saccadic in nature. All three relationships, i.e. of duration, peak velocity, and the product of these two versus amplitude, resemble the key feature of saccadic movements, i.e. a main sequence.

As mentioned before, it was not possible to measure spontaneous head movements made by homing pigeons when there is no any stimulus present, due to the fact that the high-speed camera system was only available for a limited period. The ideal situation would have been to measure spontaneous head movements and compare them with the saccadic movements as reactions to magnetic field alterations. Nevertheless, based on the main experiment and its finding that the birds' reaction was most abrupt to the first transition to a sweeping magnetic field (i.e. NF-CW), the head movements measured here and found to be saccadic in nature are discussed as characteristic reactions to this most salient transition.

5.7.1. Head saccades in response to a magnetic field stimulus

The main purpose of these experiments was to confirm the nature of the head movement responses and, furthermore, to connect that with a possible visual perception of the magnetic field. The first aim seems to have been accomplished;

namely, head movements in response to a transition to a sweeping field (in either direction) revealed a main sequence relationship between their duration, peak velocity and amplitude.

The second aim was not as straightforward to attain. Specifically, the connection between the perception of alterations in the ambient field and vision cannot be established from the experimental results presented thus far. Saccadic movements of the head or eye whether by a primate, bird or insect, are normally performed in response to sensory (i.e. visual, auditory, etc.) stimuli. Following the approach taken here, the first goal was to restrict the birds' exposure to only the generated magnetic fields (e.g. using a magnetic shielding chamber, a special designed experimental box, white noise, etc.). Thus, no auditory, visual, or any other stimulus that is known to us could impact on a bird during the experiments and thus interfere with its magnetoreception. The major advantage of this method is that we can be reasonably sure that timing of the response can only be linked to the timed transitions in the magnetic field sequence. In response to a field actually sweeping around the bird's head (i.e. transition NF-CW not the second significant transition, CW-NF, the birds' response to which is more likely to be the result of the abrupt change of the magnetic field conditions from moving to none), one might expect a smooth pursuit movement that is a reflexive automatic response to a stimulus moving across its visual field with a constant speed (or even an optokinetic nystagmus⁷ (OKN) responding to a diffuse patterning in the visual field that moves). However, the evidence for a saccadic response could suggest that the perception of the sweeping stimulus by pigeons is not continuous. This may ultimately shed some light on how homing pigeons, and presumably other birds, may perceive a moving magnetic field in this case or

⁷ Optokinetic nystagmus (OKN) – rhythmic and oscillating movement of the eyes of humans, birds and other vertebrates, which occurs as a response to patterned (stripped) stimulus moving across the visual field

magnetic fields in general. Namely, the mechanism that allows them to sense or even see it may have a certain threshold that updates the information about the magnetic field in time intervals, i.e. at a certain frequency.

The other option is that these head movements could just be spontaneous movements carried out as a result of a bird finding itself in an enclosed space-limited environment – i.e. the experimental box. However, this interpretation is contradicted by the fact that, indeed, the most apparent head movements, as well as an increased number of head movements occurred only at specific transitions when compared with others (see **Figure 4.2**). These movements also tended to happen much more quickly after the onset of the transition. However, in order to check whether these movements are spontaneous or (at least partly) evoked by the magnetic stimulus, it would be useful to characterise homing pigeons' normal spontaneous saccadic head movements in the absence of a stimulus and compare them with the movements analysed here. However, this was not part of the original design of this study, and there was insufficient time to continue using the high-speed camera system making it impossible to perform this additional experiment. Another option might have been to record at least some spontaneous head movements within this setup by starting the recording of the birds before the onset of the NF-CW(CCW) transitions. However, due to the above mentioned limitation of the high-speed camera system (i.e. the video could only be a maximum of 16s in length), recording the birds before the transitions would unavoidably limit the time recorded after the transitions to either of the sweeping magnetic fields. This, in turn, would make it impossible to gather enough data to investigate the nature of the head movements in response to the experimental stimulus.

The other conclusion, stemming from the fact that CW and CCW saccades were found to be similar, is that the direction of the stimulus does not affect birds' perception of it. This is consistent with the finding in **Section 4.3.8**, that there was no difference in the directionality of the birds' responses to a field sweeping in either a CW or CCW direction.

To summarize, saccadic head movements were observed in reaction to changes in the magnetic stimulus, especially when the field was rotated around the birds' head. Whether this response was visually driven, in other words the birds' saccades were stimulated by "seeing" the magnetic field rotating, or due to another non-visual way of perceiving magnetic field alterations, cannot be demonstrated from the results of this experiment. However, with higher confidence, it can be stated that the observed head movements were evoked by the changes in magnetic field stimulus, i.e. transitions.

5.7.2. Effect of magnetic stimulus speed on the saccades characteristics

The number of subjects in the experiments involving changing the speed of the sweeping field stimulus was low; 3 and 6 birds in the exposures to the faster and slower speeds, respectively, and thus the results cannot be treated as definitive. Yet, some conclusions may be drawn, albeit cautiously, from the results.

There is an important point to be made about the speeds of the sweeping magnetic field used here in the context of the birds' perception. As stated earlier, the initial 144°/s speed was chosen because, in the pilot experiments, head movement reactions were noted at transitions to this sweeping field condition. However, it cannot be concluded that this speed is actually biologically meaningful or even optimal for provoking a magnetoreceptive response. It is difficult to know how to resolve this

point, but a comparison with other research may be helpful. In previous studies involving investigations of optokinetic nystagmus in pigeons, the stimulus speeds are much lower than the 144°/s used here, e.g. 36°/s (Gioanni et al. 1981). This suggests that future experiments might include the use of a lower range of speeds when presenting a sweeping magnetic field.

The overall data and subsequent statistical analysis do not reveal any consistent trend that changes according to the speed of the sweeping field, which if present should be most clearly evident as a significant difference between the fastest and the slowest stimulus. Therefore, such changes in the magnetic field sweeping speed do not appear to have an impact on the birds' perception of, or reaction to, the stimulus. Analysing the results would rather tend to suggest that, within the speeds used in the present study, there was no observable effect of the variation of the rotation speed of the magnetic field stimulus on the saccadic main sequence of the head movement responses.

However, in order to be sure whether there is any effect of the sweeping field speed on the salience of the stimulus and on the birds' perception, which would be manifested by consistent changes in the main sequence, further study would be necessary with more subjects and perhaps also with larger alterations in the stimulus speed.

5.7.3. Differences between auditory and magnetically stimulated saccades

The auditory part of the experiment was, in a way, a control experiment, the results from which could serve as a basis for comparing the data obtained from exposures to the sweeping magnetic fields. The auditory stimulus, as described in **Section 5.6.4**, clearly startled the birds. This undoubtedly led to a very quick

response, as seen in the results. In contrast, the “magnetic” saccades are much slower than auditory-induced head movements, which suggest that the magnetic stimulus is less arousing. Indeed, for a given amplitude auditory saccades are much faster almost by a factor of two. At the same time, the durations of the auditory saccades were significantly shorter, and thus the product of peak velocity and duration was almost the same. Thus, it suggests that saccades as a response to both stimuli are controlled by the same system, which in turn is modulated by the animal’s level of arousal. This system, which is believed to possess internal feedback, generates velocity profiles that it then tries to follow (velocity profiles were also found to have the same symmetrical shape, which is characteristic for head saccades, in both magnetically and auditory-induced saccadic head movements).

The differences in the dynamics between the two classes of saccadic head movements are almost certainly due to the difference in salience between these two stimuli - the auditory perhaps being much more “alarming” to the birds. The variability in the responses, as a function of arousal, suggests the influence of the reticular formation, a part of the brainstem in all vertebrates, including humans and birds. The reticular formation is responsible for determining levels of reactions (both attentional and motor) to different stimuli. Thus, differential responses to auditory and magnetic stimuli may reflect the involvement of the reticular formation, which is confirmed to be involved in triggering saccadic movements in humans (Graf and Ugolini 2006) as well as in birds (Masino and Knudsen 1993).

To sum up, the difference between the dynamic properties of auditory and magnetic saccades is almost certainly due to the difference in salience between these two stimuli - the auditory perhaps being much more “alarming” to the birds.

5.7.4. Head saccades main sequence resembles human saccadic head movements

As the head movements in both cases were defined as “saccadic” head movements, it would be desirable to compare their parameters with the ones found in analogous studies. Thus, an important consideration is to ascertain how the dynamics of the observed saccades, i.e. possibly visual saccades, compare with the characteristics of head and/or eye saccades in other birds or even humans. The only experiment that was found to have investigated birds’ saccadic head movements are those of du Lac and Knudsen (1990) on the barn owl, which were elicited by both an auditory stimulus, i.e. noise burst, as well as a visual stimulus, i.e. flash of light. The one characteristic of the main sequence that is shown is the peak velocity versus amplitude relationship (**Figure 5.5**), which closely resembles that obtained in this investigation in response to a magnetic stimulus (**Figure 5.11** and **Figure 5.14**). Although, only involving a comparison of two species of birds, this agreement suggests that saccadic head movements across birds generally share similar features.

The most extensive research on saccadic movement of the eyes in most cases but also at times including head movements seems to be focused on primates, particularly humans. When compared with human head saccades, characteristics of the head movements in response to a magnetic stimulus from the present study are very similar. In particular, there is a very close agreement between the data from the present pigeon experiments and the human head saccades study reported by Liao et al. (2005), as shown in **Figure 5.24**. Two other studies that analysed human head saccades (Stark et al. 1980; Evinger et al. 1994) reveal similar trends in the relationship between peak velocity versus amplitude of the movements. However, as reported here, the pigeons’ head saccades are twice as fast as human ones – i.e. the

slopes of peak velocity versus amplitude trend lines are two times higher in the case of homing pigeons (compare **Figure 5.11** and **Figure 5.14** with **Figure 5.24**).

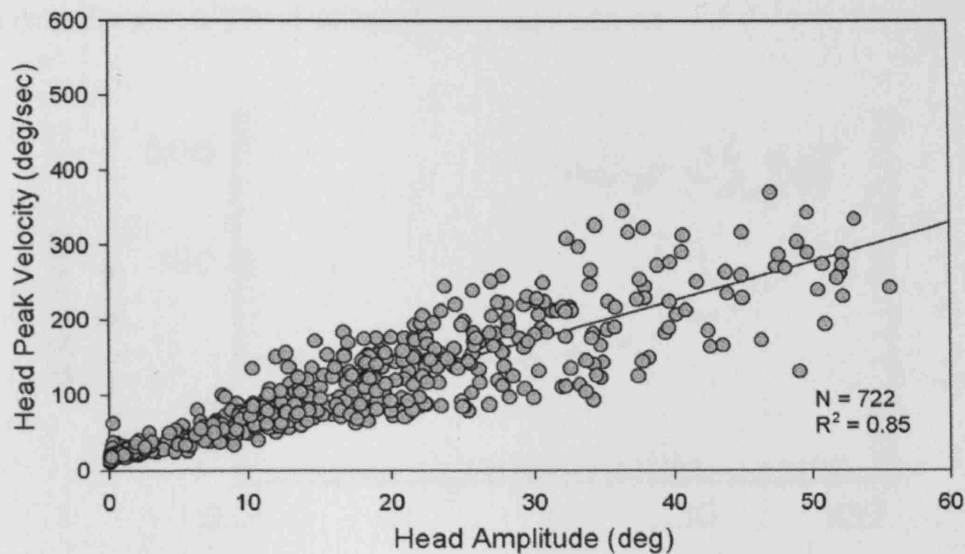


Figure 5.24 Plot of peak velocity versus amplitude for head saccades from 10 human subjects (adapted from Liao et al. (2005))

The main sequence plot of duration against amplitude in birds also resembles those observed in human head movements (**Figure 5.25**). However, only the study of Stark et al. (1980) presents the complete profile of the main sequence. In conclusion, it is clear that the homing pigeon head saccades are faster than those in humans, most likely because of the difference in the inertial mass of the head, but nevertheless the dynamic properties of the overall main sequences of pigeons and humans closely resemble each other.

It is important to emphasise that, like pigeon head saccades, human head movements do not follow the main sequence of saccadic eye movements. In both human and bird head movements, generally the first component of the main sequence, duration, remains mostly constant across amplitudes higher than 10° (**Figure 5.10**, **Figure 5.13** and in humans **Figure 5.25**). In contrast, the same correlation for eye saccades increases logarithmically across almost the entire range of eye movements (dashed line in **Figure 5.25**). Why such a qualitative difference is present between

head and eye movements is intriguing. One possible explanation may be that the motor system controllers responsible for eye and head saccades, respectively, are independent (Liao et al. 2005), although they may interact.

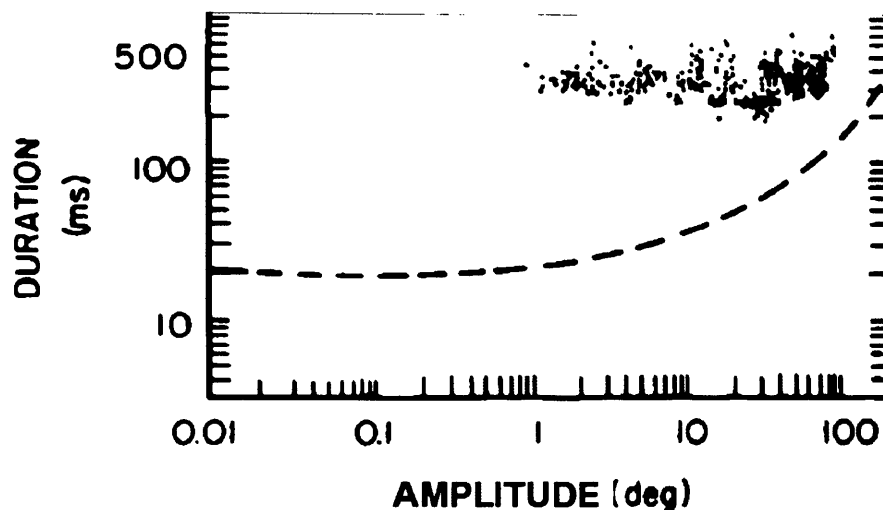


Figure 5.25 Duration as a function of amplitude part of the saccadic main sequence. Dots represent single head movement of a subject – man. Dashed line is the same characteristic but for eye movement for comparison (adapted from (Stark et al. 1980))

Interestingly, studies of the movement of eyelids in humans have produced similar results (Evinger et al. 1984). Namely, the nearly constant duration and linearly increasing peak velocity as a function of amplitude followed the characteristics of human and homing pigeons head saccades reported here. This may indicate that, at least in humans, eyelid movement is controlled by mechanism(s) that have very similar characteristics to those involved in producing head movement in humans or birds.

Taken together, these findings demonstrate that the characteristics of human and bird head saccades are very similar, notwithstanding the birds' head saccades being much faster. Surprisingly, this suggests that the nature of saccadic control is similar across bird and primate species, even though both groups diverged evolutionarily hundreds of millions of years ago.

5.8. Summary

Saccadic head movements have not been very well studied in vertebrates, including birds, and thus, much remains to be investigated within this interesting, although largely neglected, area of motor behaviour.

In this high-speed camera study, the saccadic head movements were observed when the pigeons responded to a sweeping magnetic field stimulus, which the earlier experiment had proved to be most effective in attracting the birds' attention. Although, the perception of the magnetic field with the help of vision cannot be confirmed, the data supporting the saccadic nature of the head movement response provide further evidence that the presence of, or changes to, the ambient magnetic field can be sensed quite quickly.

Furthermore, it was shown that the reaction to an auditory stimulus, which was introduced partly as a reference, were also saccades, although even faster and more numerous than those in response to the magnetic field. The fact that the analysis of the head movements in response to the audible stimulus confirmed their saccadic nature demonstrates that such responses are made in reaction to a sensory stimulus.

Finally, it was also shown that the data from magnetic saccades very closely resemble those from human head saccades in response to a visual stimulus. However, the pigeons' head saccades are faster, presumably as a consequence of their much smaller head size. In addition, although there are characteristic differences between eye and head saccades in humans, the essential distinguishing properties of head saccades are also seen in birds.

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Chapter 6. Influence of light on the behavioural responses to changes in magnetic field conditions

6.1. Introduction

The first report concerning the effect of darkness on navigation involved homing pigeons (Wiltschko and Wiltschko 1981). It was found that young inexperienced pigeons, after being transported in total darkness to the release site, could not orient towards their home loft. Those moved in light had no such difficulties. Interestingly, young inexperienced homing pigeons have not yet developed a magnetic map, for it is only learnt from experience with time; only their magnetic compass seems to be available to them as innate (Wiltschko and Wiltschko 2003b). The purpose of choosing inexperienced birds was to rule out the use of the magnetite system, which is believed to be responsible for pigeons' map sense (Munro et al. 1997), and thus to investigate the birds' magnetic compass alone, which in turn is believed to be constructed on the basis of a radical pair process (Ritz et al. 2004). Since then, there has been not much exploration of the influence of darkness on birds' navigation until very recently when a study was conducted on the orientation of European robins (Stapput et al. 2008). In the dark, these migrants displayed a variable orientation as compared to their robust orientation during daylight conditions. However, they do seem to have an orientation pattern described as a "fixed direction", an unusual direction of migration, which is only present in unnatural light regimes (Wiltschko et al. 2000; Wiltschko et al. 2003a) or darkness. Since there are indications that birds have two magnetic field sensing centres: magnetite (map) and the presumptive "eye-based system" (compass), the fixed direction response is attributed to the still functioning magnetite system, on the basis that the radical pair process is not able to convey directional information in darkness.

In addition, the proposal that a radical pair process in the eye provides a possible explanation for magnetic compass operation suggests that light may be a crucial factor for functioning of the avian compass. Consequently, if the magnetoreception underlying the avian magnetic compass does not depend on the presence of light, the effect of darkness on the behavioural paradigm developed here needs to be investigated, even if its effect on directional orientation cannot be addressed.

6.1.1. Perception of light of homing pigeons in near infra-red

To investigate the effects of darkness (i.e. the absence of visible light), the birds' reactions to magnetic field changes had to be monitored using infra-red illumination (to which the camera was sensitive). However, it is important to highlight the pigeons' range of wavelength sensitivity in order to make sure that they cannot see in the chosen wavelength of the infra-red light source (i.e. 850nm). Maximal spectral sensitivity of the long-wavelength sensitive (LWS) cone pigments in homing pigeons is equal to $\lambda_{\text{max}} = 567\text{nm}$ (Varela et al. 1993), (Bowmaker et al. 1997), and the best colour discrimination behaviourally was found to be at 595nm (Emmerton and Delhis 1980). However, the maximum wavelength at which homing pigeons are able to perceive *any* light is around 700nm (Bowmaker 1977). For a better understanding of the perception of the homing pigeon at different wavelengths of light, the spectral sensitivity across all the photopigments, in pigeons as well as humans, is depicted in **Figure 6.1**. Furthermore, light above 850nm is impossible to perceive owing to the fact that photons of light at this wavelength, and higher, are not energetic enough to excite organic molecules (Bowmaker 1991). Therefore, any perception of light in the 850nm infrared light source can be ruled out, and thus, the homing pigeons were tested in total perceptual (subjective) darkness.

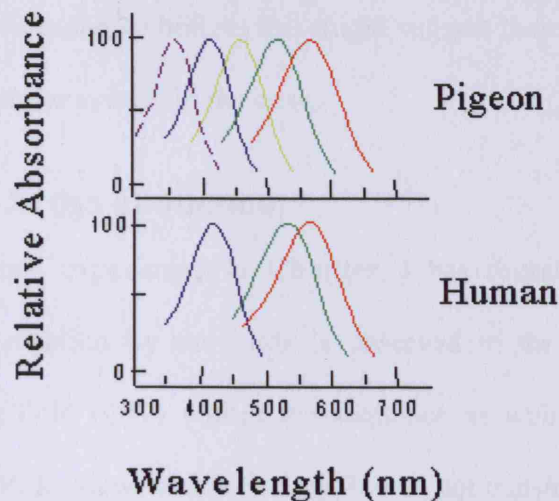


Figure 6.1 Photopigments absorption spectra for homing pigeon and human (adapted from (Bowmaker 1991))

6.1.2. Purpose of dark experiments.

The results of the main experiment, described in **Chapter 4**, investigating the behavioural responses (i.e. head movements) to changes in the ambient magnetic field are consistent with the magnetic compass being involved in magnetoreception (for more see **Section 4.4.3**). In turn, the possible involvement of vision in magnetic field perception may be manifested by head movements that are used as the response measure of the response in this study. In order to determine whether the observed responses to changes in the magnetic field in homing pigeons require visible light, undertaking experiments in the dark seemed the most direct approach, at least as a first step. Therefore, birds were tested in total darkness in an attempt to confirm (i.e. validate) our method and determine if the birds' responses are in accordance with findings from previous studies. The order of the exposure of the birds to the light and darkness experiments was randomised.

In summary, the aim of this experiment is to compare the birds' behavioural responses in normal light regime conditions with the activity of birds in total darkness. The absence of a reaction to magnetic field transitions would be consistent with magnetoreception depending on light (i.e. visually mediated magnetoreception).

However, if the birds do respond as before, this might suggest their head movements are mediated by the magnetite system in the beak.

6.1.3. Change in the sequence.

The magnetic sequence experiment in **Chapter 4** has revealed that the most pronounced and abrupt reaction by the birds is observed in the case of the first transition to a sweeping field (CW) within the sequence as well as the following transition from CW to NF. In view of this finding, it was not considered necessary to run as long an experiment as before (i.e. 4 min). Moreover, keeping this in mind, the number of steps was reduced to only three magnetic field conditions: SF, NF and ending with a sweeping field in the clockwise direction (CW). This alteration focused on the transition that produced significant responses in the earlier, long sequence experiments. In addition, the time of each step was doubled to 60 seconds (**Figure 6.2**) to prolong the time within each step in order to ensure that the bird was able to get used to each new magnetic environment before the next transition, in the hope of enhancing this response.

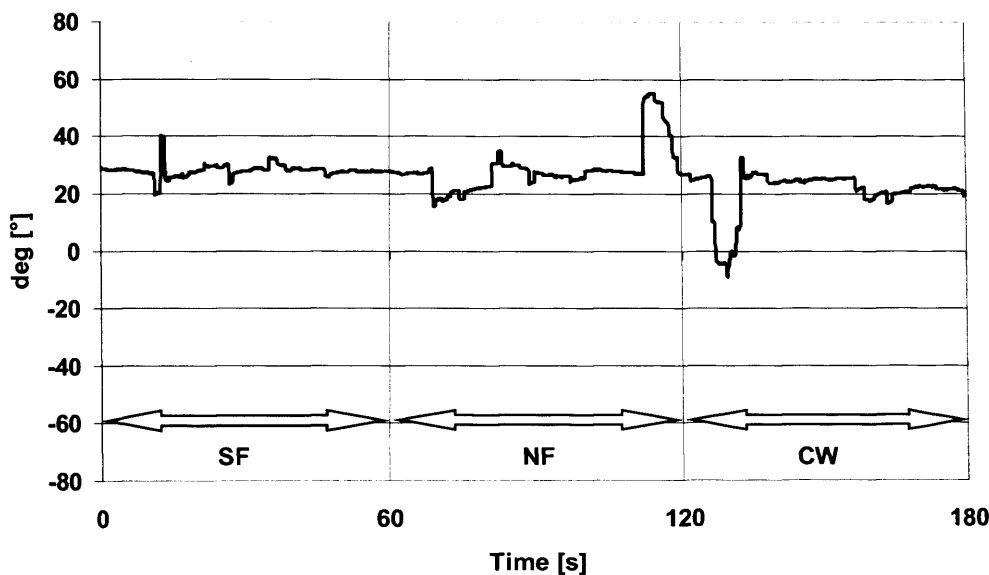


Figure 6.2 Example showing new, shortened magnetic field sequence used in the light and darkness experiments.

6.1.4. Procedure prior, during and after experiments

The procedure before, during, and after each experiment was the same as the one established during the earlier experiments (see **Chapter 3**). Each bird was investigated in the dark and light condition on the same day. If a bird did not react or was hyperactive, the experiment was repeated on another day. Then, if in the second experiments the bird's behaviour was not analysable, its data were not included in the analysis. "Not analysable" in this case means that the pigeon's hyperactivity prevented extraction of its head position from the recorded video or that either very low, or the lack of any, activity throughout the experiment (e.g. bird falls asleep as observed in the video).

6.1.5. Subjects used in the dark experiments.

A total of 14 different homing pigeons were investigated, a pair each time. The sex of birds was not confirmed, however pigeons were supplied and housed in pairs so, in most cases, one male and one female was used on each day. Out of the total of

14 subjects, four were rejected for one of the reasons discussed in the preceding section. Consequently, 10 birds were investigated in the normal light conditions used in the main, longer sequence experiment (i.e. 158 lx) and in total darkness.

6.2. Results

The arrangement of the data presentation and the analysis of the results will mirror these in **Chapter 4**.

6.2.1. Transition analysis

The first analytical approach again focuses on the most abrupt changes in the magnetic field conditions, the most pronounced birds' reactions being expected to occur at these transitions. There were only two transitions in the case of these shorter light and darkness experiments. An additional nonexistent (i.e. virtual) "transition" was defined in the middle of the first SF step – denoted as "SF middle" – as a basis for comparison (i.e. control) purposes. Exactly the same analysis was carried out for the results of the light and dark experiments for each bird.

The results, presented in **Figure 6.3**, clearly highlight the difference between the light and dark conditions. In the case of birds investigated in total darkness, there is hardly any difference in their activity before and after transitions. In the light, however, the difference in the two transitions SF-NF and NF-CW is positive, which means that birds were more active after transitions than before. That is especially evident when looking at median values for all 10 tested birds, as the mean values are more vulnerable to be affected by the extremes of individual birds' behaviour. In the case of the fake transition, SF middle, the median value is lower and of different sign meaning that birds were moving slightly more before than after this virtual transition.

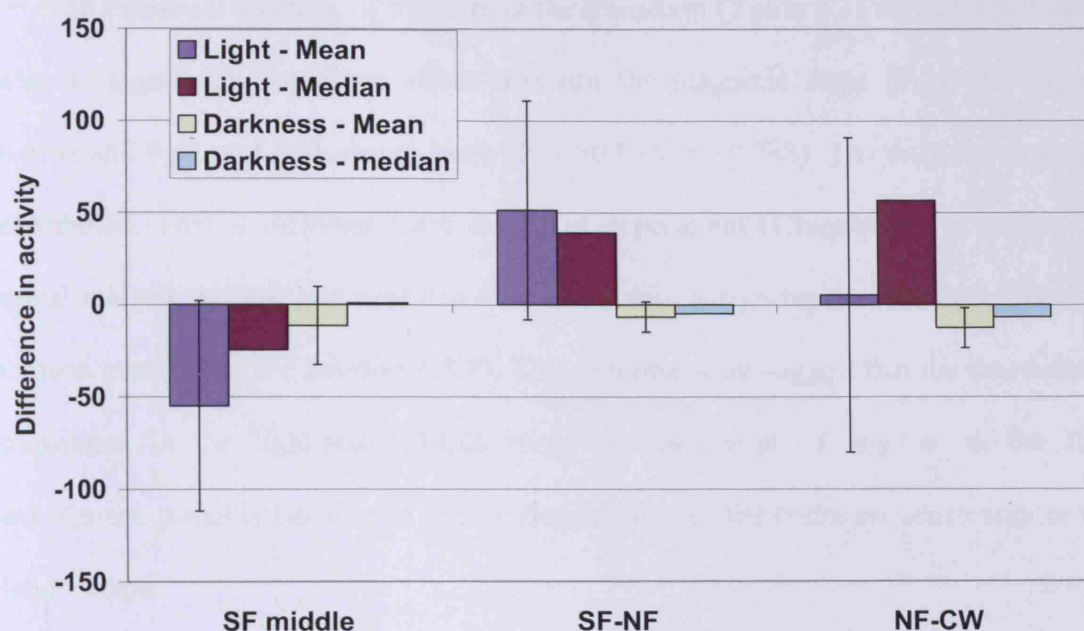


Figure 6.3 Difference in activity 10 seconds after and 10 seconds before each transition in the case of experiments in light and in darkness. (SF middle is an artificial transition set as middle of the first step in the sequence SF in order to compare the remaining two transitions; SF-NF transition from static field to null field, NF-CW transition from null field to field sweeping clockwise)

Table 6.1 Analysis of variance table of the general linear model (GLM) for difference in activity at transitions of the light and dark-tested birds. (Magn_step_number*Bird_number is an interaction between the two variables that represents the prediction of the head movements response in the specific magnetic step depending on specific bird number; Light_or_dark is a variable comparing the birds investigated in the light (Light group) and darkness (Dark group)) (df – degrees of freedom, F – statistical power on which basis p value is given)

Tests of Between-Subjects Effects

Dependent Variable: Difference

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	1401.251	1	1401.251	.055	.819
	Error	227886.641	9	25320.738		
Light_or_dark	Hypothesis	1746.459	1	1746.459	.074	.788
	Error	686087.675	29	23658.196		
Bird_number	Hypothesis	227886.641	9	25320.738	.807	.616
	Error	564706.079	18	31372.560		
Magn_step_number	Hypothesis	30905.546	2	15452.773	.493	.619
	Error	564706.079	18	31372.560		
Magn_step_number * Bird_number	Hypothesis	564706.079	18	31372.560	1.326	.243
	Error	686087.675	29	23658.196		

The statistical analysis of the data at the transition (**Table 6.1**) revealed that there was no significant transition effect between the magnetic steps ($F_{2,18}= 0.493$, $p= 0.619$) and light and dark-tested birds ($F_{1,29}=0.074$, $p= 0.788$). The data are normally distributed. This is different from the main experiment (**Chapter 4**), in which the same analysis carried out revealed that there was a significant difference between various transitions (see **Section 4.3.1**). This outcome may suggest that the reactions to transitions in the light-tested birds were not as abrupt, if any, as in the first experiment, possibly because of the shorter duration of the entire sequence and/ or the longer steps.

In order to check whether the birds' behaviour in this experiment was affected by the alterations in the magnetic field as well as by darkness, further analyses of the results from different perspectives are required.

6.2.2. Number of head movements

When analysing the total number of head movements presented in **Figure 6.4**, the striking point is the much higher concentration of head movements in the light-tested birds over those tested in darkness, where the mean of all 10 birds is lower than one head movement ($>10^\circ$) per step in all three conditions (SF, NF and CW). That clearly indicates that hardly any activity occurred during the sequence when the birds were investigated in darkness.

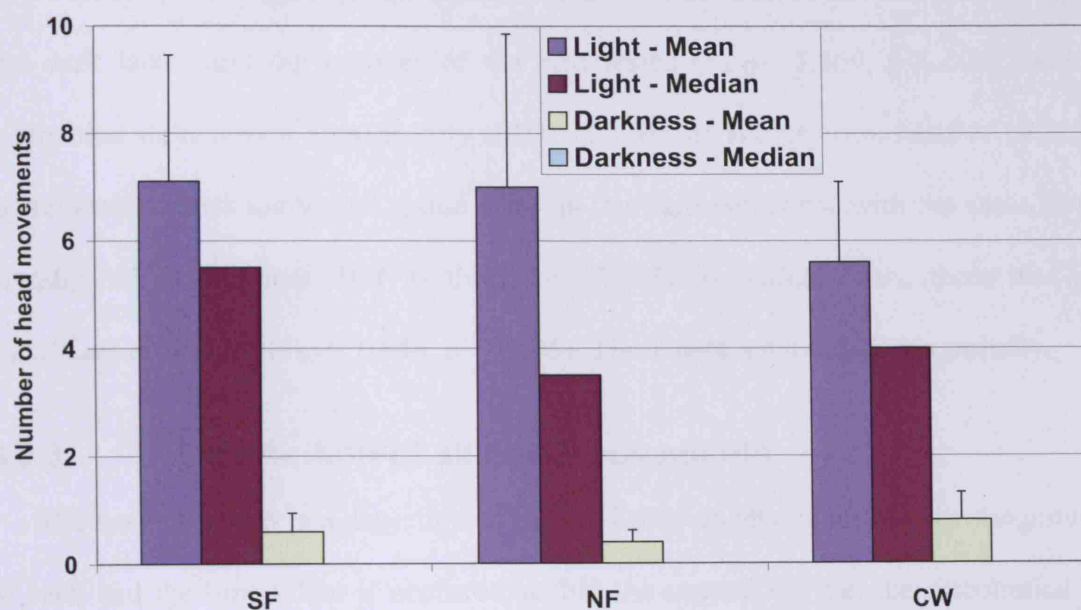


Figure 6.4 Number of head movements in all steps of 60s in light and darkness part of the experiment; SF- static field, NF- null field, CW- field sweeping in a clockwise direction

Table 6.2 Analysis of variance table of the general linear model (GLM) for number of head movements of the light- and dark-tested birds. (Abbreviations as in Table 6.1)

Tests of Between-Subjects Effects

Dependent Variable: Number_of_head_moves

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	770.417	1	770.417	8.780	.016
	Error	789.750	9	87.750		
Light_or_dark	Hypothesis	534.017	1	534.017	8.811	.016
	Error	545.483	9	60.609		
Magn_step_number	Hypothesis	4.633	2	2.317	.301	.742
	Error	292.700	38	7.703		
Bird_number	Hypothesis	789.750	9	87.750	1.448	.295
	Error	545.483	9	60.609		
Light_or_dark *	Hypothesis	545.483	9	60.609	7.869	.000
	Error	292.700	38	7.703		

The difference in the number of head movements between birds' tested in light and darkness, which can be observed in **Figure 6.4**, is confirmed statistically using a GLM model (**Table 6.2**), which indicated significant variability between both groups

($F_{1,9} = 8.811$, $p = 0.016$). Another significant term is the interaction between the light and dark factor and the number of the bird tested ($F_{9,38} = 7.869$, $p < 0.001$), which means that there was a significantly different level of activity (i.e. number of head movements) across individual tested birds in the light compared with the same birds investigated in darkness. But in the case of only individual birds, there was no significant difference ($F_{9,9} = 1.448$, $p = 0.295$). These data are distributed normally.

6.2.3. Distribution of all head movements

The next approach is a depiction of all head movements, including the magnitude of each and the time when it occurred within the sequence – i.e. the distribution of head movements over time. The distributions of head movements for the light and dark experiments are presented in **Figure 6.5** and **Figure 6.6**, respectively. Comparing both figures, it can clearly be seen that there is a huge difference in the overall number of head movements in favour of birds tested in light, which has already been observed in the earlier analysis of the number of head movements.

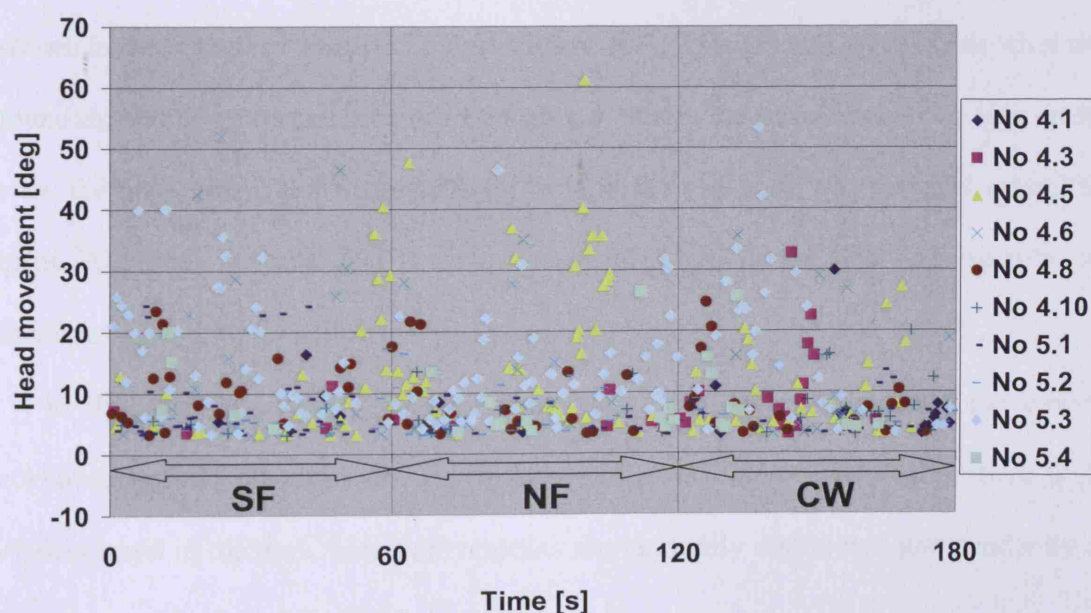


Figure 6.5: Distribution of all head movements of all tested birds in light; abbreviations as in Figure 6.4

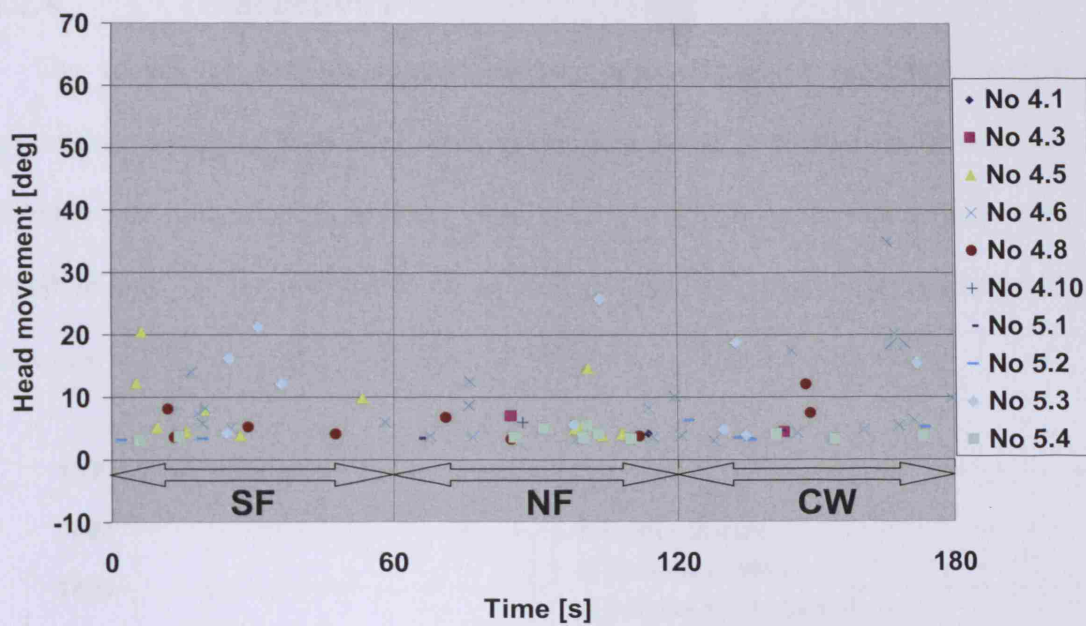


Figure 6.6 Distribution of all head movements of all tested birds in darkness; abbreviations as in Figure 6.4

In the case of the light experiments (**Figure 6.5**), looking at the transitions reveals that there is an increase in activity at the beginning of both the NF and CW steps. However, this is more observable, at the NF-CW transition, as more head movements of different birds are evident (i.e. on the plot each bird has its own identifying marker shown in the legend of **Figure 6.5** and **Figure 6.6**). This finding agrees with what was found in the main experiment from **Chapter 4**, where the same conclusion was drawn – i.e. the transition to the first sweeping field in the sequence was the most salient for birds. Although, in this case it was not as abrupt as in the first experiments, the increase in head birds' activity is still observable.

In the dark experiment (**Figure 6.6**), there are only a few head movements acknowledged as reactions ($>10^\circ$). In contrast to the light-tested birds, there is no evident trend in the dark. Head movements are randomly distributed independently of the timing of the magnetic field changes.

6.2.4. Total activity

The results of total integrated activity analysis, as in the other analytical approaches presented thus far, again reveal a very large difference between birds tested in light and darkness. Indeed, **Figure 6.7** shows that the overall activity of birds tested in light is on average 4 times higher than the *same* birds investigated in darkness.

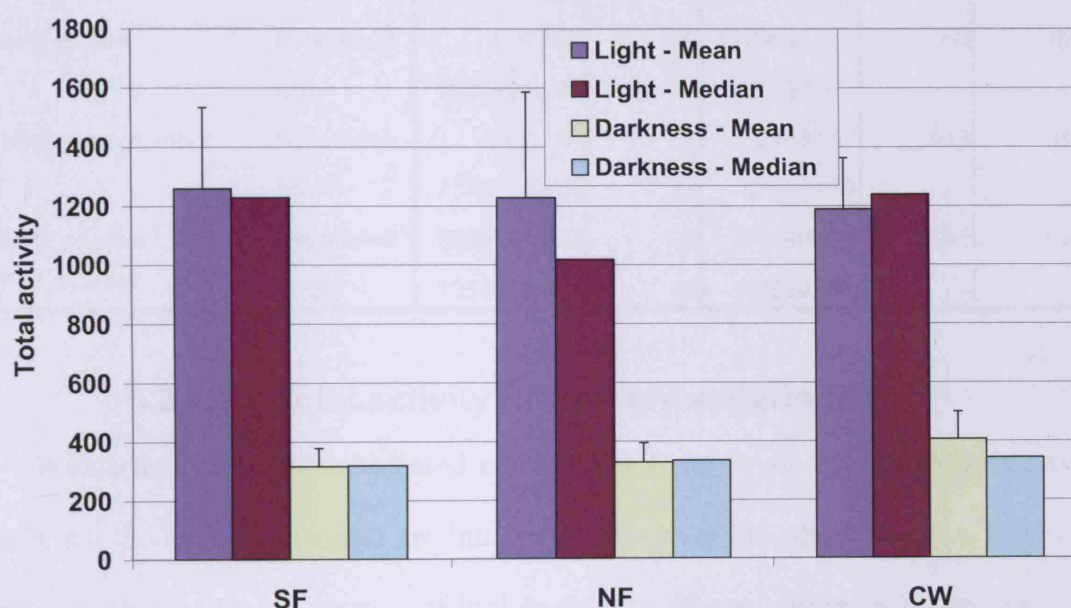


Figure 6.7 Total activity within each magnetic field step in the sequence for light and darkness-tested birds; abbreviations as in Figure 6.4

The statistical analysis revealed the same results as in the case of the number of head movements' analysis **Table 6.3**. The light-tested birds were far more active than when the same birds were investigated in darkness ($F_{1,9} = 19.088$, $p = 0.002$). In addition, individual birds were characterized by totally different activity patterns at specific transitions ($F_{9,38} = 3.965$, $p = 0.001$). Similar to the earlier cases, the data also have a normal distribution.

Table 6.3 Analysis of variance table of the general linear model (GLM) for total activity of the light and dark-tested birds. (Abbreviations as in Table 6.1)

Tests of Between-Subjects Effects

Dependent Variable: Total_activity

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	4.367E7	1	4.367E7	36.711	.000
	Error	1.071E7	9	1.190E6		
Light_or_dark	Hypothesis	1.465E7	1	1.465E7	19.088	.002
	Error	6905161.892	9	767240.210		
Bird_number	Hypothesis	1.071E7	9	1189602.760	1.550	.262
	Error	6905161.892	9	767240.210		
Magn_step_number	Hypothesis	4907.734	2	2453.867	.013	.987
	Error	7354019.874	38	193526.839		
Light_or_dark * Bird_number	Hypothesis	6905161.892	9	767240.210	3.965	.001
	Error	7354019.874	38	193526.839		

6.2.4.1. Total activity in the light experiment

Within the results of light-tested birds, the activity in all three steps varies very little, but the head movements are integrated here over the whole step (i.e. 60s) and this may obscure the occurrence of higher activity, if any, within each step just after each transition. Also as the key reactions identified in the previous experiment were head movements of magnitude $>10^\circ$, it would be interesting to see whether, using this measure, there were more distinctive differences involving those steps where the highest activity might be expected based on the findings from the main experiments (i.e. sweeping field - CW). Therefore, in **Figure 6.8**, the data concerning only the first 30 seconds of each step and only including head movements larger than 10° (i.e. previously acknowledged as a response) are presented. The main difference from the data in **Figure 6.7** is over twofold increase in the activity of birds in the first half of the CW step compared with SF and NF. This suggests, indeed, there was a marked

increase in the birds' activity (characterized as head movements over 10°) at the NF-CW transition, although not as high as in the main experiment (**Chapter 4**).

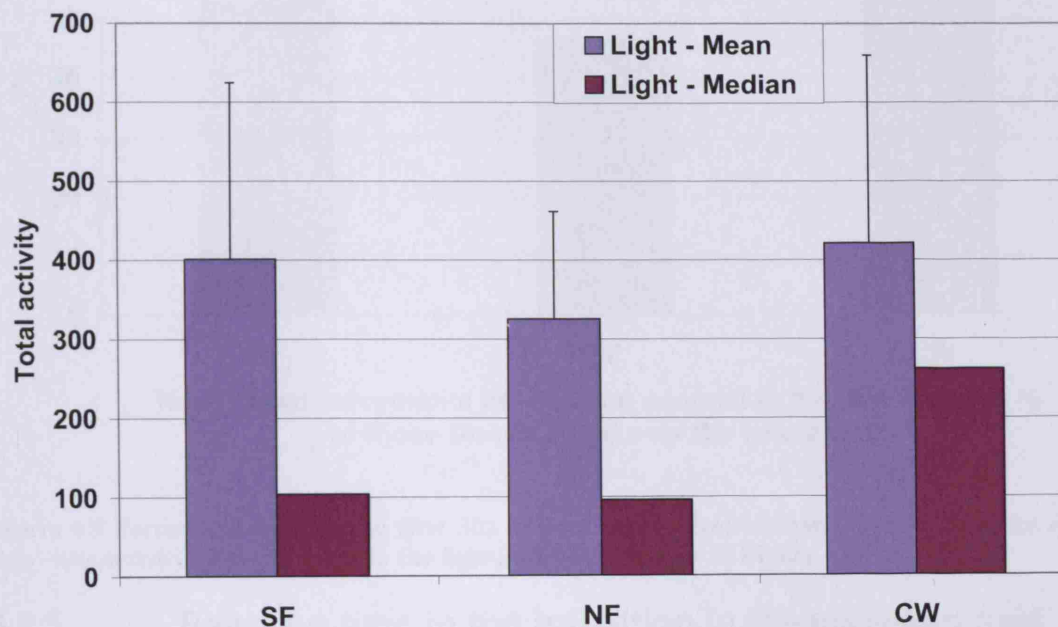


Figure 6.8 Total activity of head movements over 10° within 1st 30s of each step; only light- tested birds; abbreviations as in Figure 6.4

Furthermore, the analysis of the distribution of significant activity (i.e. head movements $>10^\circ$) revealed that this concentration in the case of CW is shifted towards the beginning of the step, in which over 70% of all head movements occurred within the first 30 seconds. Whereas, in the two remaining magnetic field steps, SF and NF, the concentration of the significant head movements is even – the ratio of the first 30s to the whole 60s oscillates around 50% (see **Figure 6.9**).

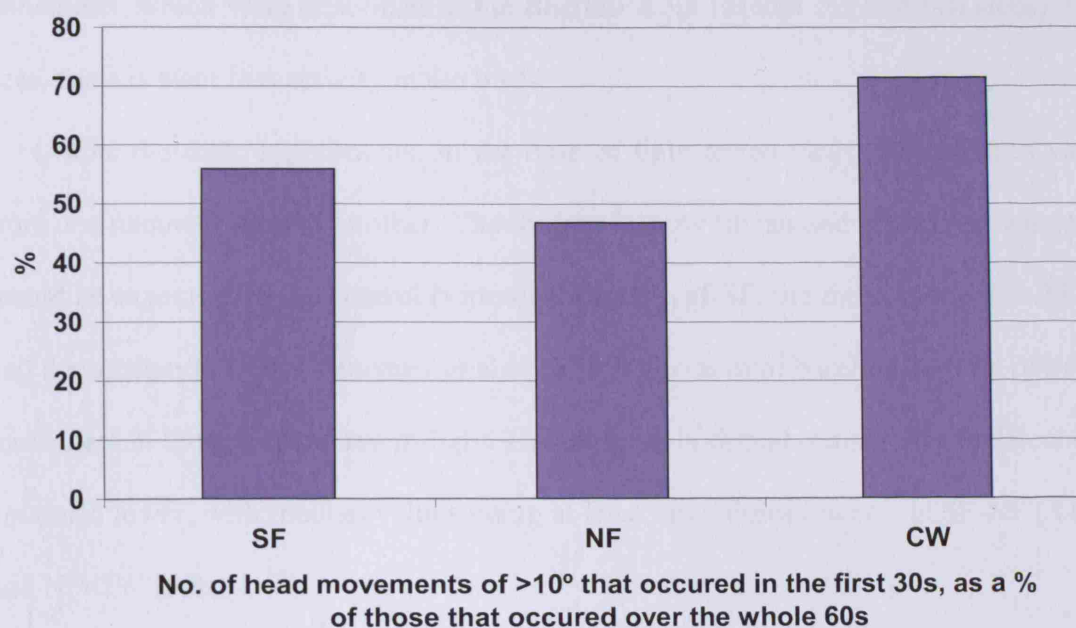


Figure 6.9 Percentage ratio of the first 30s of each step overall activity over 10° and the whole step – 60s activity of birds tested in the light; abbreviations as in Figure 6.4

6.2.5. Reaction time to the transition in the magnetic field

The response (or reaction) times of the 10 tested birds, in the light and in the dark are presented in the **Figure 6.10**. The reactions are responses to two real transitions (i.e. SF-NF and NF-CW) and a “reaction” to an imaginary (virtual) transition introduced to serve as a control, that is at the beginning of the first SF step marked sf-SF, where sf indicates the static field that was present before the experiment started (different from the control transition in **Section 6.2.1**, which was set in the middle of the first SF step).

The first striking result is a big discrepancy between the latencies in the response in the two experiments, which is consistent with the findings from the above analysis of only very low activity in dark-tested birds. The median latency in the darkness experiments is equal to 60s in all three transitions, which means that more than half of the tested birds did not make a single head movement larger than 10° . This resembles the latency results of the control light experiments involving no magnetic field

transitions, which were described in the **Section 4.3.5** (except the two last steps) but here, there is even less activity in the birds.

Unlike the dark experiments, in the case of light-tested birds, the latencies vary from one magnetic step to another. The longest latency (mean and median) is where it would be expected; in the control (virtual) transition, sf-SF, the mean is equal to 28.3s and the median is 18.6s. This presumably reflects the normal baseline activity of head movement in these birds when in light. However, in both real transitions, the latencies are much lower, with median values being at least three times lower – in SF-NF (3.8s) and NF-CW (6.8s).

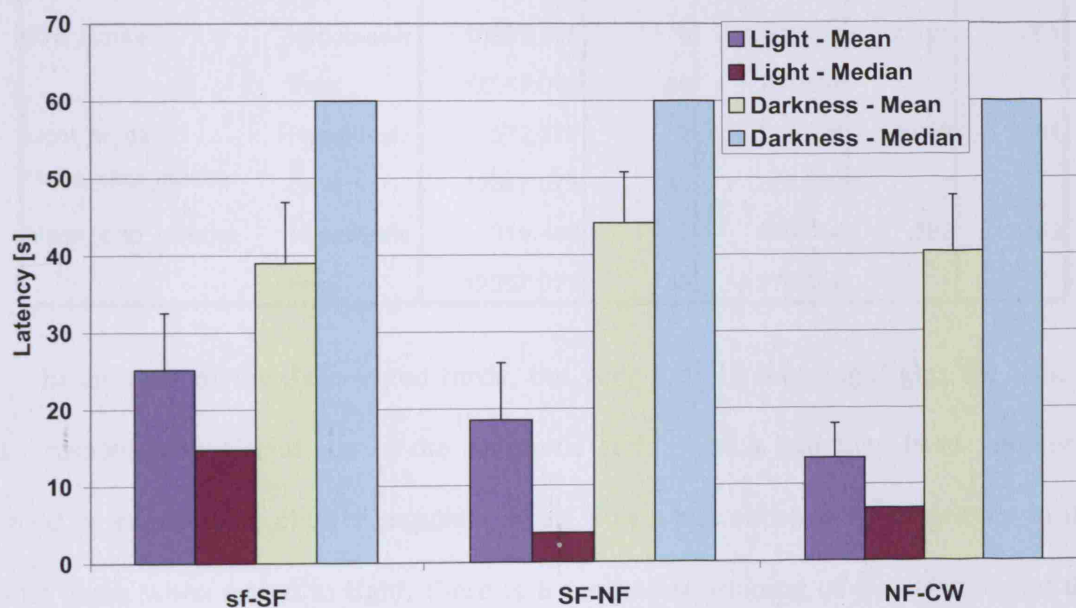


Figure 6.10 Latency of the response in the case of light and darkness experiments in the case of each transition; sf-SF – artificial transition set as a kind of control to compare with the two real transitions, SF-NF and NF-CW, as in Figure 6.3

From the statistical point of view, the results of running the GLM model on the latency data (see **Table 6.4**) mirror the outcomes of this analysis in the two earlier analytical approaches (i.e. number of head movements and total activity) to the effects of light and darkness on the latency of head movements of tested birds ($F_{1,45} = 50.301$, $p < 0.001$). This time however, the individual tested birds are distinguished by

significant differences not only in their responses to specific transitions but overall (i.e. for Bird_number $F_{9,45} = 4.191$, $p = 0.001$). Again, the data are normally distributed.

Table 6.4 Analysis of variance table of the general linear model (GLM) for latency of the light and dark-tested birds. (Abbreviations as in Table 6.1)

Tests of Between-Subjects Effects

Dependent Variable: Latency

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	70221.704	1	70221.704	61.020	.000
	Error	10357.268	9	1150.808		
Light_or_dark	Hypothesis	13812.801	1	13812.801	50.301	.000
	Error	12357.073	45	274.602		
Bird_number	Hypothesis	10357.268	9	1150.808	4.191	.001
	Error	12357.073	45	274.602		
Light_or_dark *	Hypothesis	572.277	2	286.138	1.042	.361
	Error	12357.073	45	274.602		
Magn_step_number	Hypothesis	319.488	2	159.744	.582	.563
	Error	12357.073	45	274.602		

In the case of the dark-tested birds, the very long latency highlights the lack of any responses to alterations in the magnetic field, which had they been perceived would be expected to elicit a response, even if in a less abrupt way. However, in the same birds, when tested in light, there is a marked shortening of the latencies of the responses to the apparently perceivable changes in the magnetic field. That in turn coincides with the findings from the main experiment that birds can perceive transitions in the ambient magnetic field.

6.2.6. Latency distribution

Following the analytical approach taken in the first results chapter, the distribution of reaction times for both light and darkness-tested birds in response to all three

magnetic field steps in the sequence are depicted in Error! Reference source not found.11.

Again, the same finding that there are virtually no responses to changes in the magnetic field in birds tested in the dark is indicated by the red bars in Error! Reference source not found.11. In the sf-SF control “transition” and in the actual SF-NF and NF-CW changes, most of the birds did not react at all, while those reactions that did occur are spread evenly across the entire 60s. This suggests the absence of responses triggered by the magnetic field transitions.

However, the same birds in the light display a very different pattern in their responses, which are distributed much less evenly (blue bars Error! Reference source not found.11). Namely, there is hardly any case, where a bird did not perform any head movement greater than 10°. Those head movements that did occur are evidently especially concentrated in the first 15s of the step, such as in the case after the NF-CW transition (i.e. 80% of reactions). In addition, the other real transition, SF-NF, also has a high concentration of reactions just after the transition – 70% in the first 10seconds. While the artificial transition, sf-SF, results in a much more evenly spread temporal occurrence of head movements across the 60s step.

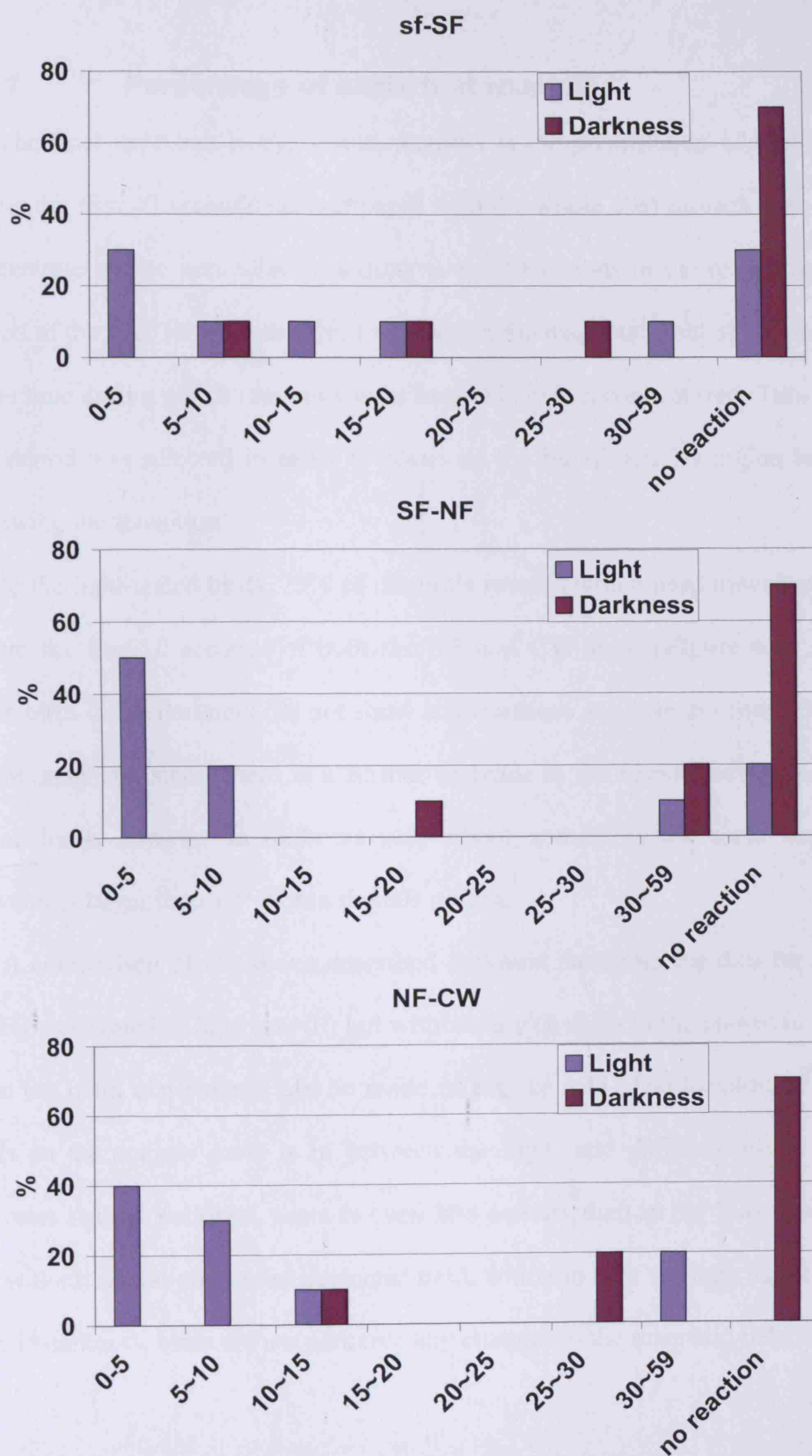


Figure 6.11 Percentage distribution of latencies of light and darkness-tested birds in 5 seconds bins up to 30s, the 30-59s bin and the bin including the cases where birds did not react at all during the entire 60s step. Comparing the experimental and controls by Chi-square test revealed that, in all situations, these frequency distributions are significantly different (in all cases $p < 0.001$)

6.2.7. **Percentage of birds that reacted**

The final approach in the results analysis is the presentation of birds' reactions within the first 10 seconds (as compared with the whole 60s) of each step in order to concentrate on the immediate reactions to the transitions in the magnetic field. The period of the first 10 seconds after transition in the magnetic field of NF and CW step is the time during which reactions were more likely to have occurred. Thus, the initial 10s period was selected in order to focus on the birds' activity region immediately following the transition.

In the light-tested birds, 75% of the birds reacted with a head movement over 10° within the first 10 seconds of both the NF and CW steps (**Figure 6.11**), while the same birds in the darkness did not show any reactions at all. In the entire 60s duration of the magnetic steps, there is a further increase in the responsiveness of the light-tested birds, however in darkness only about a third of the birds made a head movement larger than 10° within the 60s period.

A comparison of the above described darkness data with the data for the control birds investigated in light (in NF) but without any changes in the magnetic field, taken from the main experiment, can be made in **Figure 6.11**. The location of the control birds on the activity scale is in between the light- and darkness-tested birds. That indicates that, in darkness, there is even less activity than in the birds tested in light but without alterations in the magnetic field, which in turn strongly suggests the fact that, in darkness, birds did not perceive any changes in the magnetic field.

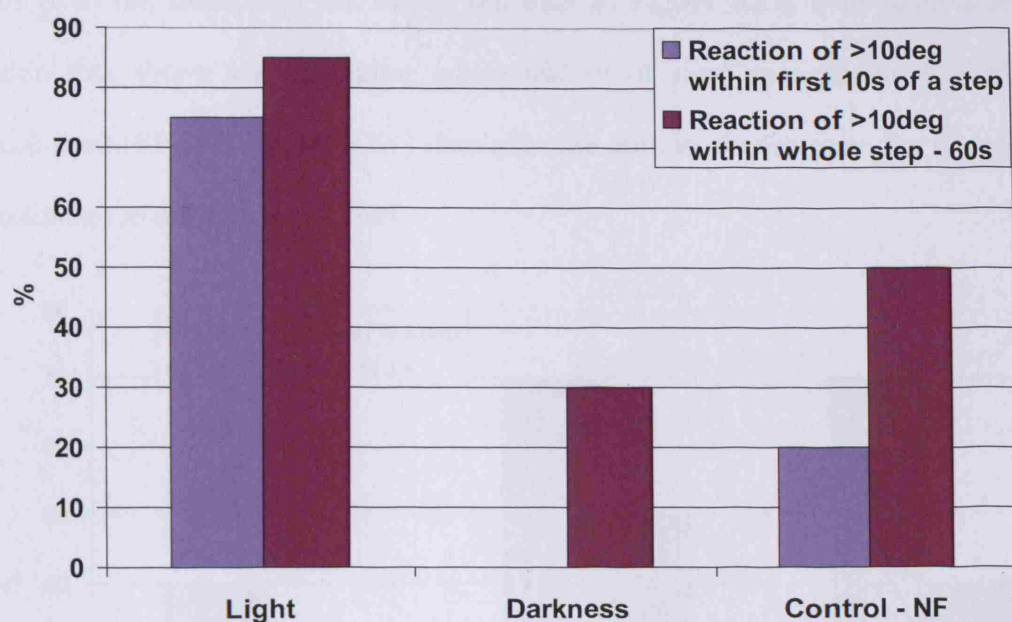


Figure 6.11 Combined percentage of birds that reacted to transitions that appeared in the magnetic field sequence (SF-NF and NF-CW) in light darkness experiment and within control experiment (i.e. Control – NF – means that the control experiments were performed in NF – data taken from the control experiment in Chapter 4).

Finally, it would be desirable, in light of the findings from the main experiment, to look more closely at only light-tested birds within this light and darkness experiment to find out whether there was any increase in birds' activity after the real transitions (SF-NF and NF-CW). In **Figure 6.12**, there are data showing the percentage of birds that reacted within the first 10s of each step, including a kind of a control case, as the first sf-SF is not a transition. The results clearly show that both real transitions, SF-NF and NF-CW, are characterized by the fact that 70% of birds reacted, while in the fake transition the activity was much lower – 40%. This indicates that there is higher activity just after a real transition which in turn strengthens the argument that birds are able to perceive the presented changes in the magnetic field. In addition, there was another kind of control- “semi-control” – case added, which was based on moving the analysis to a virtual transition at the middle of each step, i.e. 30s after a real transition, and looking at the percentage of birds performing significant head movements within

10s from the middle of the step – red bars in **Figure 6.12**. This approach showed again that there was a higher concentration of head movements just after real transitions (SF-NF and NF-CW) than after the middle of each step, i.e. where there is no change in the magnetic field.

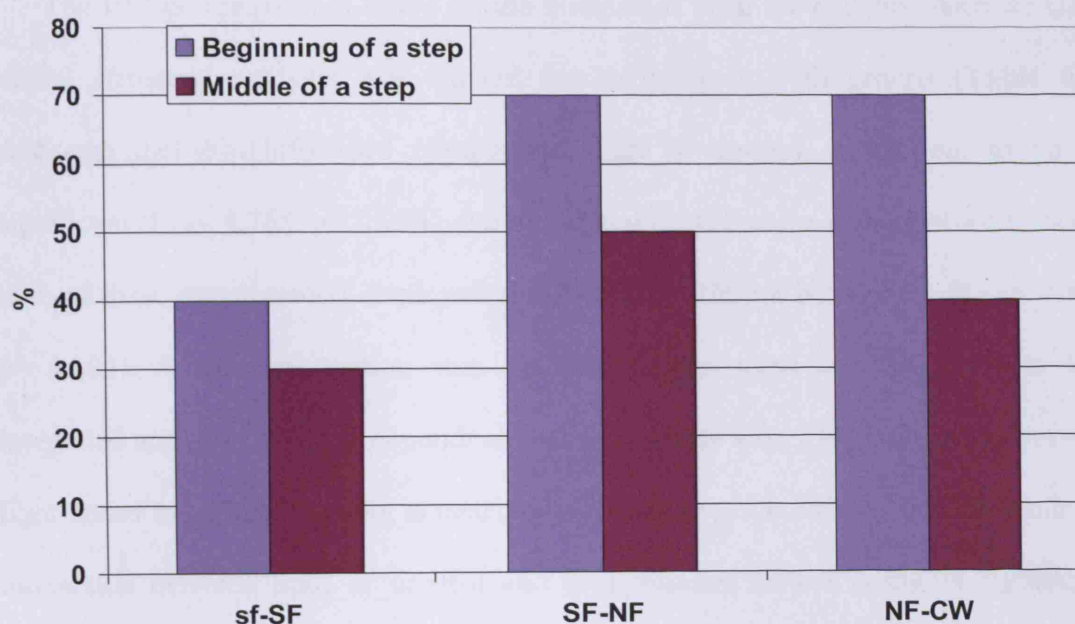


Figure 6.12 Light tested Birds that reacted to the transition – SF-NF and NF-CW and beginning of the experiment – sf-SF where no transition was present (blue bars). Red bars are in essence control cases as this transition is an artificial (i.e. virtual) one in the middle of each step.

6.2.8. Reactions to alterations in the magnetic field in Light-tested birds

The above analysis has undoubtedly shown that the behaviour of birds tested in light and dark is different. Ipso facto, the influence of darkness on birds' responsiveness to any alterations in the magnetic field should be interpreted as highly attenuating any magnetoreception or general responsiveness to external stimuli. However, there remains one more important question to be addressed, namely whether the same birds, which did not show any signs of magnetoreception taking place in darkness, did indeed react to alterations in magnetic field conditions in light. For that purpose, additional analysis has to be introduced, in which the light-tested

bird's results were compared with the control birds from the first (light) experiment that did not experience any change in the magnetic field, remembering that there will be limitations to this comparison because other aspects of the experimental conditions had also changed.

The first comparison is based on the number of head movements. Another GLM model statistical analysis was carried out to compare both groups (**Table 6.5**). Although the straightforward comparison, `light_or_control`, turned out to be not significant ($F_{1,4} = 4.763$, $p = 0.094$), the individual birds (i.e. the same that were used in both of these experiments) displayed significantly different behaviours ($F_{4,38} = 4.846$, $p = 0.003$). A similar situation was observed in the same analysis but with total integrated activity being the dependent variable (**Table 6.6**). The comparison between light-tested birds and controls is nearly significant ($F_{1,5} = 6.282$, $p = 0.054$), while the interaction between `light_or_control` and `bird_number` factors is highly significant, indicating again that the same; individual birds (i.e. some of the birds used in the compared experiments were the same, see **Appendix 1**, last two columns) reacted in a significantly different manner in the light-tested and control experiments ($F_{5,38} = 4.951$, $p = 0.001$). These results suggest that a larger sample size might be required to establish a significant reaction to changes in the magnetic field given the variability of individual birds.

Table 6.5 Analysis of variance table of the general linear model (GLM) for number of head movements of the light- tested and control birds. (Abbreviations as in Table 6.1)

Tests of Between-Subjects Effects

Dependent Variable: No_of_head_moves

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	1407.900	1	1407.900	13.155	.003
	Error	1472.991	13.763	107.028		
Light_or_control	Hypothesis	252.300	1	252.300	4.763	.094
	Error	211.867	4	52.967		
Magn_step_number	Hypothesis	12.700	2	6.350	.581	.564
	Error	415.300	38	10.929		
Bird_number	Hypothesis	1485.667	14	106.119	2.004	.263
	Error	211.867	4	52.967		
Light_or_control * Bird_number	Hypothesis	211.867	4	52.967	4.846	.003
	Error	415.300	38	10.929		

Table 6.6 Analysis of variance table of the general linear model (GLM) for total activity of the light- tested and control birds. (Abbreviations as in Table 6.1)

Tests of Between-Subjects Effects

Dependent Variable: Total_activity

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	6.692E7	1	6.692E7	44.734	.000
	Error	1.905E7	12.736	1.496E6		
Light_or_dark	Hypothesis	8603338.613	1	8603338.613	6.282	.054
	Error	6847077.617	5	1.369E6		
Magn_step_number	Hypothesis	204802.680	2	102401.340	.370	.693
	Error	1.051E7	38	276619.345		
Bird_number	Hypothesis	1.943E7	13	1494466.261	1.091	.500
	Error	6847077.617	5	1.369E6		
Light_or_dark * Bird_number	Hypothesis	6847077.617	5	1369415.523	4.951	.001
	Error	1.051E7	38	276619.345		

A comparison of latencies across light-tested birds and control birds from the main experiment (**Chapter 4**) using the GLM model revealed that, in this case, the difference between these groups is statistically significant ($F_{1,4} = 14.444$, $p = 0.019$, **Table 6.7**). This result points out that there is indeed a marked difference between the responsiveness of the birds that underwent alterations in the magnetic field (i.e. light-tested birds) and those that did not experience any changes in the ambient magnetic field (i.e. control birds).

The fact that an analysis of latency revealed the largest difference between light-tested birds and control subjects whereas the total activity analysis indicated a smaller difference and the number of head movements was even lower may suggest that the type of reaction was unlike that observed in the first (i.e. main) experiment. Namely, the first reaction at the transition was present, but after the first reaction, the activity of the light-tested birds in the present experiment was not as high as in birds during the first experiment.

Table 6.7 Analysis of variance table of the general linear model (GLM) for latency of the light-tested and control birds. (Abbreviations as in Table 6.1)

Tests of Between-Subjects Effects

Dependent Variable: Latency

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	34349.037	1	34349.037	42.768	.000
	Error	10993.983	13.688	803.155		
Light_or_control	Hypothesis	7533.681	1	7533.681	14.444	.019
	Error	2086.369	4	521.592		
Magn_step_number	Hypothesis	32.524	1	32.524	.179	.677
	Error	3449.518	19	181.554		
Bird_number	Hypothesis	11177.926	14	798.423	1.531	.366
	Error	2086.369	4	521.592		
Light_or_control *	Hypothesis	2086.369	4	521.592	2.873	.051
Bird_number	Error	3449.518	19	181.554		

When investigating the latency difference in light-tested birds only, i.e. between the virtual transition (sf-SF) and the identified as the first salient transition in the magnetic sequence NF-CW, no statistical difference ($F_{1,9} = 1.809$, $p = 0.211$, **Table 6.8**) was found. However, this fact was due to two birds that exhibited very long latencies to this transition (these bird cases are denoted clearly in the NF-CW latencies distribution in Error! Reference source not found.11 – bin 30-59s with value of 20% - two birds). The fact that the majority of light-tested birds had latencies much shorter than in the case of a virtual transition within the same magnetic sequence suggests a real perception of the magnetic field change.

Table 6.8 Analysis of variance table of the general linear model (GLM) for latency of the light-tested birds of only sf-SF virtual transition and real NF-CW. (Abbreviations as in Table 6.1)

Tests of Between-Subjects Effects

Dependent Variable: Total_activity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	7449.761	1	7449.761	16.748	.003
Error	4003.230	9	444.803		
Magn_step_number	684.438	1	684.438	1.809	.211
Error	3404.449	9	378.272		
Bird_number	4003.230	9	444.803	1.176	.407
Error	3404.449	9	378.272		

Furthermore, looking at the data already presented within this chapter related to only the light-tested birds gives further support to the conclusion that these birds reacted to the alterations in the magnetic field. Some of the results pointing towards a perception of the magnetic field changes by the light-tested birds can be seen in **Figure 6.8**, in which an evident increase in activity during the first 30s of a step is present in CW. Similar information is conveyed in the following figure (**Figure 6.9**), where the ratio of the first 30s activity to the activity within whole step is presented and is clearly highest in the CW step. Also, the latency values at real transitions

compared with the control transition set at the beginning of the first SF step (sf-SF) are much lower (**Figure 6.10**). Furthermore, the distributions of latencies vary between real transitions (i.e. SF-NF and NF-CW) as compared with the “control one” (i.e. sf-SF). The latter has even distribution across the time axis, whereas the real transitions’ distributions are clearly shifted towards the beginning of the time axis, which can be seen in Error! Reference source not found.11. Lastly, analysis of the percentage of birds that reacted also confirms the presence of reactions by light-tested birds to the two real transitions (see **Section 0**).

6.3. Discussion

As homing pigeons are diurnal birds, it could be argued that, in darkness, their alertness may decrease, and thus, their activity may be largely diminished or they can even go to sleep. In the most similar study investigating birds’ magnetoreception in darkness (Stapput et al., 2008), European robins were under investigation. These are also diurnal birds, and therefore there was a concern that darkness would inhibit the birds’ alertness sufficiently that such a study would not be possible. However, the authors state that darkness suppresses only higher activity, i.e. more complicated tasks that require photopic conditions, such as jumping between perches in this case. Consequently, such an investigation of orientation behaviour was possible and produced meaningful results.

Nevertheless, in few cases birds did appear to go to sleep but these subjects were excluded from the analysis in this experiment. The remaining birds’ alertness was checked by a gentle knocking on the chamber’s lid *after* the experiment was finished. In all cases, the remaining birds reacted immediately to the noise, which indicates their continued alertness even though under dark conditions. Therefore, it could be expected that reactions to the magnetic field changes should also be present, if the

stimulus was perceived. Furthermore, head movements that served as a response measure here, which were of low activity in the context of the European robins darkness study, should not be suppressed.

6.3.1. Light versus darkness experiment

The essential aim of these experiments was to investigate the role that light plays in the pigeon's magnetoreception. For that purpose, this experiment was performed in two parts: The first part involved the birds being exposed to a sequence of magnetic field changes in normal light, and the second part where all the conditions stayed the same except that darkness was introduced. In both parts of this experiment, the same ten homing pigeons were used.

The main difference revealed, when looking at all of the results – i.e. number of head movements (**Figure 6.4**), distribution of all head movements (**Figure 6.5** and **Figure 6.6**), total activity within each step (**Figure 6.7**), as well as the latency and latency distribution results (**Figure 6.10** and Error! Reference source not found.11) and, finally, the percentage of birds that reacted (**Figure 6.12**) – was a very high discrepancy between the activity and responsiveness of the birds when tested in light and darkness. All of these results were confirmed statistically (GLM model – see specific results sections) indicating that indeed there was a large difference in responsiveness and activity between the same birds tested in light and darkness conditions. There was a clear reduction in overall activity as well as an increase in the lack of almost any responses in the darkness situation. Taken together, this evidence strongly suggests that darkness totally attenuated the ability of pigeons to perceive any changes in the ambient magnetic field. Although, the behaviour of homing pigeons in the dark, or in low-level light conditions, is generally characterized by remaining still, in this experiment the birds did not fall asleep (cases where birds did

fall asleep were rejected and not included in the analysis) and were still attentive to another sensory stimulus, i.e. gentle knocking on the chamber's lid at the end of the experiment to which all birds reacted immediately, as they do in the light. Thus, the lack of responses in the dark appears to reflect a significant reduction in the ability of birds to perceive changes in the ambient magnetic field, even a previously identified salient transition to a sweeping field, i.e. NF-CW (as seen in the light experiment).

6.3.2. The impact of darkness on homing pigeons' magnetic field perception

This experiment – an attempt to investigate the influence of darkness on the perception of the magnetic field – strongly suggests that darkness is a major cause of attenuation in pigeons' perception of alterations to the ambient magnetic field. An obvious possibility is that, in darkness, magnetoreception is no longer activated in the same way as in the light. There are two possible mechanisms for the magnetic field sensing that provide directional information to birds. Firstly, there is the inclination compass, which is believed to be based on vision and specifically a radical pair process in the eye that requires light to be activated (Ritz et al. 2000). Secondly, there is the iron-based, i.e. magnetite, system, which is located in the upper beak (Kirschvink et al. 2001), and is also said not to be light dependent (Stapput et al. 2008). If the beak-based system were responsible for the head movements (as observed in all of these experiments) as a response to changes in the magnetic field in light, then the birds' behaviour should not disappear in dark-tested birds, as seen in these results, because light is not required by this magnetic field sensing system. Therefore, it is a reasonable deduction that the radical-pair mechanism may provide the information here about changes in the earth's magnetic field. Thus, the birds' responses, which are manifested as head movements, are not observed in darkness

most likely because of a lack of the photons of light needed for triggering or mediating magnetoreception (Rodgers and Horea 2009).

As previously mentioned, it has been shown that juvenile homing pigeons could not home when they were transported to the release site in total darkness (Wiltschko and Wiltschko 1981) or when the magnetic field was altered during the outward journey (Wiltschko and Wiltschko 1978). Taken together, these findings show that both light and magnetic field information are needed for juvenile pigeons to obtain the correct information that will be used when homing. Moreover, it can be deduced from these studies that the magnetic compass used by young pigeons is innate (Wiltschko and Wiltschko 2003b). The magnetic compass, in turn, is believed to be based on the radical pair process and hence is light activated (Wiltschko and Wiltschko 2002a; Ritz et al. 2009). The present results showing a lack of responses (i.e. head movements) in the dark are consistent with this previous research. If the magnetic compass light-mediated mechanism is responsible for the reactions to changes in the magnetic field, the lack of birds' reaction in the dark is due to the unavailability of such magnetoreception in the absence of light.

On the other hand, if magnetite is involved, it is hard to explain the large difference in the pigeons' activity between light and dark. This iron-based mechanism does not require light for magnetoreception to take place. Stapput et al. showed for the first time that oriented movements in migratory species (i.e. European robins) do occur in total darkness, and in the same paper, they concluded that these darkness-oriented movements do not involve the radical pair process mechanism (Stapput et al. 2008). In fact, Stapput et al. (2008) reported a fixed direction of migration in birds when in total darkness. This fixed direction of migration was very different from the normal spring and autumn headings and was attributed to the magnetite system.

However, in the case of the present research, the approach was quite different, as migratory birds were not under investigation nor were birds in a position to orient to the artificially generated field. Thus, only head movements were used as a measure of the response not orienting. Therefore, although these results are not fully comparable, darkness clearly has an impact on the reactions of birds, regardless of the method used.

In summary, the present results show that darkness clearly limits birds' ability to demonstrate magnetic field perception, but whether it eliminates magnetoreception completely cannot be confirmed. However, it seems evident the avian vision system is likely to be involved, and may be crucial, for the operation of the magnetic compass.

6.3.3. Light-tested birds' results compared with earlier experiments

The purpose of this series of experiments was to investigate the perception of the magnetic field by the same homing pigeons in light and total darkness. Although the sequence was different from that in the main experiment described in **Chapter 4**, those homing pigeons were also subjected to a magnetic field sequence in the light. Thus, it would be desirable to compare the outcomes of both otherwise similar experiments.

The most striking finding of the main magnetic sequence experiment was the especially significant reactions to the transition to a sweeping field (NF-CW) (see results section in **Chapter 4**). In the light and darkness experiments, the sequence was altered, i.e. shortened to only two transitions with second being NF-CW. That alteration was introduced in order to concentrate the analysis on the transition judged to be most salient to the bird.

In the light part of this latter experiment, the response to the NF-CW transition was not as dramatic as in the main experiment. Nevertheless, a closer look at the birds' reaction to this transition, as well as during the CW step, and to the first transition in the sequence, SF-NF, and then comparing these data with the data from the control experiment step clearly indicates that the birds did perceive the alterations in the magnetic field when in the light.

Section 6.2.8 was devoted to the analysis of the light-tested birds only and comparing the results to the control experiment, which was carried out as a part of the main experiment (**Chapter 4**). The results show that there is a difference in behavioural activity as compared with these control birds. The difference is not as clear as between the control birds and the birds from the real experiment, both from **Chapter 4**. The light-tested birds here are characterized by similar reaction times, especially in the NF-CW transition, but the behaviour later in this longer step is not as active. The other contrast between these birds and ones from the main experiment is the somewhat faster reaction time to the SF-NF transition in the light-tested birds.

In **Section 6.2.4.1**, the analysis of only the light-tested birds was focused on significant head movements (i.e. $>10^\circ$) and the 30s period immediately after the transition in order to match the length of the step in the main experiment. The median values indicate that there was almost three times as much activity as compared to the two remaining steps, i.e. SF and NF (see median values in **Figure 6.8**). This outcome was the first indication that reactions to this transition were present. Further confirmation of this conclusion can be found in the analysis of latencies in **Figure 6.10**, showing a difference between the light-tested and the control birds (also tested in the light) (**Table 6.8**). In the light-tested birds, the latency of the response to the NF-CW transition as well as to SF-NF was at least half that of the not-existent

transition, sf-SF. This latency result is in accordance with the analysis of the latencies shown by the experimental birds in the main experiment. A reflection of this situation is shown in Error! Reference source not found.11, which depicts the distribution of latencies. This distribution is similar to that found in the main experiment (see transitions NF-CW and CW-NF in Error! Reference source not found.). Such a temporally correlated finding strongly suggests that the change within the magnetic field is indeed recognized by the birds as manifested by their head movements. In light of Mouritsen and his co-workers' paper, the head movements observed in the present experiments might be explained in a similar way. Recalibration is suggested as the purpose of the head movements in garden warblers (Mouritsen et al. 2004a), and this is conceivably also a reason for the increased pigeons' head movement activity shown here; if not recalibration in the case of pigeons, then at least an attempt to accommodate themselves to a new magnetic field environment.

Finally, the comparison of the percentage of birds that reacted to real transitions with the artificial one (sf-SF) as well as with the "semi-control" (i.e. transition artificially set in the middle of each 60s magnetic step) revealed that there was higher activity at the real transitions (SF-NF and NF-CW) than in both the control transitions (see **Figure 6.12**). All of this reveals that the birds were indeed more active after real transitions, which in turn confirms the findings presented earlier (**Chapter 4**) that pigeons are able to sense alterations in the ambient magnetic field.

The observed difference between the main experiment and this one, i.e. a higher level of responsiveness to the NF-CW transition in the main experiment and a lower response latency in the first transition of this light experiment, SF-NF, is most likely explained by the changed conditions of the magnetic field sequence, as this was the only difference between the main experiment and here. In particular, the elongation of

each step from 30 to 60s as well as the reduction of the number of magnetic field steps could have led to reducing the birds' attention to what would otherwise be the most salient transition (i.e. to a sweeping field), instead of increasing the impact of the transition as was intended when introducing these changes in the sequence. Therefore, it is suggested that, in further experiments of this kind, the longer magnetic sequence applied to the birds during the main experiment should be used.

6.4. Summary

The findings of this investigation of the same birds tested in the light and in darkness within the same magnetic field conditions revealed that light appears to have a great impact on magnetic field perception, manifested behaviourally in this study as time-locked head movements. In addition, the overall activity in the light was far higher than in the darkness, in which pigeons did not show any signs of recognition of the magnetic field alterations. In the light, both transitions (i.e. SF-NF and NF-CW) produced evidence of magnetoreception, while, in the dark, there were few if any head movements near the transitions, indicating at least a much lower level of perception.

The results of the light part of the experiment alone when compared with the main experiment showed that the reactions to the transitions were not as abrupt, which could be due to the changes made in the magnetic field sequence. However, a closer analysis of these data indicated that these magnetic field alterations were indeed perceived.

In conclusion, the ultimate goal of the light and darkness experiment has been accomplished – the effect of total darkness on magnetoreception in homing pigeons has been investigated and found to highly attenuate magnetoreception or at least responsiveness to magnetic field changes.

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Chapter 7. Conclusions and future work

7.1. Conclusions

- Homing pigeons are able to discriminate between various changes in the ambient magnetic field. Using head movements as the response measure, the most abrupt reactions were observed in the magnetic sequence at the transition to the first sweeping field (i.e. NF-CW) as well as at the immediately following transition, i.e. from the sweeping field to null field (CW-NF). These results might have been anticipated as these were the most abrupt transitions in terms of magnetic field change and thus were most likely to elicit a significant response from the birds.
- The latency of the reaction to changes in the magnetic field (i.e. magnetoreception) was investigated and, to the best of our knowledge, is reported here for the first time. The first head movement larger than 10° after the transition was used to determine the reaction time and was found to occur within 6s and 5s (median values for the NF-CW and CW-NF transitions, respectively), with some responses being almost instantaneous (≤ 1 s). This finding sheds some light on previous discussions in the literature about how quickly birds might be able to perceive magnetic field change, or more specifically, how much time is needed for the avian magnetic field sensory system to distinguish a change in the EMF.
- Investigation of the results with respect to any possible directionality in the birds' responses to magnetic fields sweeping in opposite directions (i.e. CW or CCW) revealed that there was no apparent bias in the direction of the head

movement reactions. Therefore, it was concluded that the direction of movement of the magnetic field does not appear to affect the birds' sensory system in a significant way. Put another way, the putative magnetoreceptive system in the head does not need to move in a particular way with respect to motion of the magnetic field to facilitate detection.

- Taken together, the results demonstrate that head movements can serve as both an indicator and measure of the homing pigeon's reactions to magnetic field changes.
- The technique developed here for investigating magnetoreception in the homing pigeon has been partially validated and thus may prove to be a useful tool for year round study, performed under controlled laboratory conditions, of avian magnetoreception. Indeed, this approach will potentially allow repeated investigations of the same individual animals in order to explore the detailed psychophysics of magnetoreception.
- The investigation concerning the nature of the observed head movements as responses to the previously identified highly salient transition(s) for the birds (i.e. transitions to sweeping field) revealed that they were saccadic in nature. Indeed, their dynamic properties turn out to be very similar to saccadic head and eye movements in other animals, including humans. This result may shed some light on our understanding of magnetic field perception in pigeons, i.e. if magnetoreception involved a continuous *following* response to the continuously changing sweeping field (i.e. moving around a bird's head) smooth pursuit head movements might be expected rather than the saccades observed here.

- Further evidence that the type of response was not correlated with the direction of the sweeping magnetic field was found when analysing the properties of the responses to a magnetic field sweeping either CW or CCW. The quantitative characteristics of the saccadic head movements driven by these two magnetic stimuli were found to be not significantly different from each other.
- The introduction of an auditory stimulus to induce saccadic head movements in birds allowed a comparison of the magnetic saccades with auditory saccades, which were expected to be more similar to those described in the literature. Differences in the dynamics between auditory and magnetic saccades were found. Auditory saccades were demonstrably much shorter and faster, which almost certainly reflected the difference in the perceptual salience between these two stimuli, i.e. the magnetic being less “alarming”. At the same time, the overall mechanisms controlling these saccadic head movements driven by both stimuli were found to be virtually identical.
- Saccadic (body, head and/or eye) movements are normally made as a response to a perceived stimulus. The only stimulus presented to the birds during the experiments was a sequence of changes in the magnetic field condition. Therefore, the saccadic head movements observed here are clearly responses to these imposed changes. Thus, the saccadic nature of the response provides further evidence, i.e. in addition to the changes in activity and the time correlated aspect of the head movement(s), that birds were indeed aware of (i.e. sensed or perceived) the alterations in the ambient magnetic field.

- The fact that the head movements observed in homing pigeons in this study were found to possess very similar dynamic properties when compared with the head movements of humans is a striking finding in terms of the underlying control neural systems that produce saccadic movement. Birds and mammals diverged (i.e. have not shared a common ancestor) hundreds of millions years ago. This strongly suggests that saccadic movements evolved early in vertebrate evolution and that their control mechanisms have been remarkably conserved ever since.
- Finally, the exposure of the same homing pigeons to the same changes in magnetic field conditions in light and in total darkness revealed that the expected reactions to those changes, still evident in the light, were not observed in the dark. Thus, darkness seems to adversely affect the process of magnetoreception in homing pigeons. Future work is required to determine if the absence of light inhibits birds from responding to changes in magnetic field conditions or, more consistent with other recent studies, interferes with the sensory process that underlies magnetoreception.

7.2. Future work

The approach developed and presented here to investigate behavioural responses to changes in the ambient magnetic fields is both novel and important, providing the basis for future studies of the psychophysics of magnetoreception. However, there are a number of possible improvements or extensions to this method, which could be introduced in order to investigate magnetoreception more broadly, from different perspectives or simply to improve the system itself. Some ideas for future

enhancements, which have arisen in the course of this PhD investigation, are presented below.

7.2.1. Making the system more movable

In order to improve the system's mobility and thus enlarge the possible settings where investigation may take place, the magnetic field shielding chamber could be removed. Eliminating the need for such a chamber, which is both expensive and immovable because of its heavy weight, is possible if other ways of compensating for the present natural magnetic field are introduced. This could realistically be accomplished by negating the field using the existing 3D Helmholtz coils and thus producing either a net null, static and/or sweeping field.

To ensure such dynamic shielding, the first step would be to measure the ambient field in all three planes. This can be done with the help of the 3-dimensional fluxgate magnetometer that is already used in the system at the moment to check the correctness of the generated magnetic field. Once the values have been determined, the second step is to generate the same magnetic field in each direction as the measured values but with a negative sign with each of the three coils. This entire negation process can also be automated by computer control, thus making the system easier to use.

7.2.2. The use of the pre pulse inhibition (PPI) method within the present system

Another possible avenue that may be investigated using the present approach is exploiting the phenomenon of prepulse inhibition (PPI). The use of PPI to investigate sensory perception is an interesting and useful tool (Larrauri and Schmajuk 2006). PPI is a neurological property of the central nervous system (CNS) that consists of the inhibition of the startle response to a large sudden onset stimulus (pulse) by an

immediately preceding (prepulse) stimulus with smaller amplitude. A simple example might be the case in which a person is subjected to an unexpected, very loud noise. Usually after such a stimulus, the person is scared and makes an involuntary body movement (i.e. 'jumps'). For reasons that are not entirely understood, any prepulse stimulus (i.e. something which can be perceived- touch, flash of light or sound) will invariably and measurably inhibit the startle response to the proper stimulus presented immediately afterwards. This effect does not depend on behavioural conditioning, and the response is not easily habituated. Normally, a weak prepulse stimulus is presented around 20-500 ms before the appropriate startle-inducing stimulus (Braff et al. 2001). In Braff's review, PPI subjects are human, but the same can be implemented in investigations of animals, including birds (Stitt et al. 1976), as PPI occurs in many vertebrates (Yoemans et al. 2002), and also, interestingly, in invertebrates (Riede, K. 1993). Our intention would be to use PPI as another objective and possibly more consistent approach for investigating magnetoreception in birds. The experimental plan is to subject a bird to any startle-inducing stimulus, measure its response(s) using a movement sensing cage apparatus (e.g. a non metallic version of the system produced by San Diego Instruments, Inc.), and then repeat the stimulus using a generated magnetic field as a prepulse, inhibitory stimulus. If birds can detect the magnetic field presented, then an inhibition of the startle response should reliably be observed. A further advantage of this method should be the more straightforward analysis of the birds' responses as compared to that in the present study.

7.2.3. Altering visual conditions, e.g. investigating under different wavelengths of light.

Another modification or different use of the method is to investigate birds' responses under different light regimes. For example, the investigation of birds may

be carried out under one or more specific wavelengths of light, instead of using full spectrum white light as presented in this research. This idea was taken from a publication by the Wiltschko group, the outcome of which became the first strong evidence in favour of the theory that vision plays a role in magnetic field sensing in birds (Wiltschko and Wiltschko 2002). The advantage of using the method developed here is that such tests can be made on the same individual birds, allowing more powerful within subject analysis.

7.2.4. Operant conditioning introduction to the present system.

Another concept that might be introduced in conjunction with our method is so-called operant conditioning. This is a well-known learning task in which animals are “asked” or conditioned to learn certain responses to different changes in the parameters under investigation. For example, food is given as a reward for the *right* choice and punishment is given in the case of a *wrong* choice, which can be withdrawal of the reward – food, or even a mild shock, followed by a time penalty.

Operant conditioning (OC) is a popular technique for investigating the visual and other abilities of animals. The idea is to train the subject to perform a task that is contingent on its ability to make a particular discrimination. In this case, training will depend on the perception of an ambient magnetic field or its absence. The principle of this research is based on the reinforcement and punishment rule. Reinforcement is achieved by rewarding a desired response from the animal in the form of food. The failure to deliver the right response results in punishment for the animal in the form of a time delay (i.e. no food). This procedure is usually preformed in an operant conditioning chamber, often called a Skinner box. This approach has had mixed success in previous studies of magnetoreception, but the use of more salient magnetic

field conditions, i.e. sweeping field, that have been identified here could enhance its effectiveness.



Figure 7.1 Pigeon inside of the Skinner box (www.jsu.edu/depart/psychology/sebac).

An illustration of a typical operant conditioning chamber (i.e. Skinner box) is presented in the **Figure 7.1**. Birds signal their choice by pecking one of the two buttons/holes. Buttons are different in some way, for example in colour, so that the bird can distinguish between them.

Studies with the help of the Skinner box paradigm are much easier in terms of analysis in comparison to the experiments performed here. There is no need for sophisticated statistics of the bird's movements. It will simply be obvious from the birds' choices whether or not they are able to distinguish between the presence and absence of the magnetic field or temporal changes to that field.

The use of OC to gauge birds' responses to the magnetic field has yielded few successful investigations in the past. One of the significant studies in the area was

done by Mora et al. (2004). In their research, there was a modification introduced, namely, instead of buttons to peck birds were trained to jump on one platform when an anomaly in the magnetic field was present or jump on the opposite platform when there was no anomaly. This study demonstrated that birds are able to distinguish between an anomalous magnetic field of intensity of $189\mu\text{T}$ from the earth's magnetic field (Mora et al. 2004).

In our case, OC might be used to “teach” birds to distinguish between various magnetic fields (i.e. Static Field from Null Field, Sweeping field from Static Field etc.). Furthermore, it may be possible to determine birds' thresholds for magnetic field intensity or the speeds of the field sweep below which birds are no longer able to perceive changes within the field. The introduction of a Skinner box would mean that a major modification of the magnetic generation system (i.e. set of Helmholtz coils) is needed.

7.2.5. Further investigation of the saccadic head movement phenomenon.

The experiment regarding investigation of the head movements nature (**Chapter 5**) needs to be pursued further. In particular, it would be advantageous to measure spontaneous head movements, present (if any) when the birds are not subjected to any stimulus, and then compare the nature of these movements with the movements investigated here, which are believed to be responses to changes in the magnetic field.

Furthermore, another suggestion for broadening our analysis of the birds' reactions to magnetic stimuli would be to investigate the dynamic properties of saccadic head movements made in response to broader variations in the sweeping magnetic field speed. This proposed experiment would verify and extend the results obtained in the present study.

Indeed, another avenue that could be investigated in more depth is the phenomenon of saccadic movements in birds, an area that seems to be largely neglected. Ultimately, extending our knowledge about pigeons' saccadic head movements as responses to magnetic field may shed some light on the psychophysics of magnetoreception.

7.3. Literature for Chapter 7

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Appendix 1

[illegible]

2.5	11-44 24.01.08																
2.6	24.01.08- Wrong head mark	10-25 08.02.08															
3.1			11-42 23.06.08														
3.2			12-29 23.06.08 Hyperacti ve	14-41 23.06.08 Hyperacti ve													
3.3			12-22 25.06.08														
3.4			12-36 25.06.08 Wrong head mark	No experim ent													
3.5			12-46 25.06.08														
4.1			12-26 28.07.08		11-43 21.10.08 Low activity	12-35 3.11.08	14-34 17.02.09								14-47 17.02.09		
4.2			12-58 28.07.08		11-23 21.10.08 Hyperactive	11-54 3.11.08 hyperactive	Hyperactive	No experiment								No experim ent	

4.3				10-54 28.11.08		12-12 22.01.09 Low activity	14-13 22.01.09	15-27 23.02.09	
4.4			12-39 29.07.08 – Low activity	No experim ent	10-39 28.11.08 Wrong head mark	14-27 28.11.08		Falls asleep	Falls asleep
4.5			12-22 1.08.08		11-33 23.10.08 Very low activity - “Falls asleep”	14-16 23.10.08		12-43 16.02.09	
4.6			12-06 1.08.08		11-16 23.10.08 Wrong head mark	14-28 23.10.08 Hyperactive		12-34 16.02.09	
4.7			11-42 1.08.08		14-12 17.10.08				
4.8			11-29 5.08.08		11-49 17.10.08 Hyperactive	13-47 17.10.08 Hyperactive	13-50 5.02.09	12-08 5.02. 09	
4.9					11-45 2.12.08		10-39 4.02.09 Wrong head mark	Falls asleep	No exp.

4.10			11-41 5.08.08		11-56 2.12.08		10-28 4.02.09			11-44 4.02. 09	
5.1					10-53 3.10.08 Low activity	11-00 3.10.08	14-27 19.02.09			14-34 19.02.09	
5.2							15-22 19.02.09			15-27 19.02.20 09	
5.3					11-47 3.10.08 Hyperactive	10-37 24.11.08	11-18 12.02.09 Head unseen	11-22 12.02.09 Wrong head mark	14-38 18.02.09	12-30 12.02.09	
5.4					11-58 09.10.08 Hyperactive	14-12 9.10.08	11-47 12.02.09 Hyperactive	11-51 12.02.09		12-40 12.02.09	

Appendix 2

Table presenting birds' selection for high speed camera experiments (i.e. for magnetic field sweeping in counterclockwise (CCW) and clockwise direction (CW) (wrong head mark denotes situation in which the head template was put in a way that prevented from valid data extraction, no responses- was the situation when there was lack of any reaction or the reaction was subtle)

Bird number	CCW		CW	
	1	2	1	2
4.3	06.05.09 no reactions	06.05.09	-	
4.4	07.05.09 wrong head mark	-	-	
4.5	28.04.09		26.06.09	
4.6	28.04.09 no responses	28.04.09	26.06.09	
4.7	30.04.09 no responses	30.04.09	30.06.09	
4.8	30.04.09 no responses	30.04.09	30.06.09 no responses	-
4.9	06.05.09 no responses	06.05.09	-	
4.10	07.05.09 no responses	-	-	
5.1	21.05.09		01.07.09	
5.2	21.05.09		01.07.09 no responses	01.07.09
5.3	22.04.09 no responses	22.04.09	-	
6.1			02.07.09 no responses	02.07.09
6.2			02.07.09 no responses	02.07.09 no responses
6.3	23.04.09	23.04.09		

Appendices

6.5			03.07.09	
6.6			03.07.09	
6.7			06.07.09 no responses	06.07.09

Appendix 3

Derivation of the equation describing the magnetic field in the middle of a Helmholtz coil (Section 2.1.3.1)

On the basis of Biot-Savart law, the magnetic field on the axis of a single coil, which contains N turns, a radius r , and carries a current I at a distance x from the plane of the coil is equal to:

$$H = \left(\frac{NI}{2r} \right) \left(1 + \frac{x^2}{r^2} \right)^{-1.5}$$

In the case of the Helmholtz coil (i.e. two coaxial coils) one coil is defined at distance $x=0$ and the other one at distance $x=r$, the magnetic field is equal to:

$$H = \left(\frac{NI}{2r} \right) \left[\left(1 + \frac{x^2}{r^2} \right)^{-1.5} + \left(1 + \frac{(r-x)^2}{r^2} \right)^{-1.5} \right]$$

The Helmholtz coil principle is based on the fact that the radius is equal to the distance between the two coils ($x=r/2$), and so the magnetic field in the middle of the Helmholtz coils is reduced to:

$$H = \left(\frac{NI}{2r} \right) \left[(1.25)^{-1.5} + (1.25)^{-1.5} \right] = 0.715 \frac{NI}{r}$$

Appendix 4

List of publications:

1. **Migalski, S. P.**, Meydan, T., Bingman, V. P., Erichsen, J. T., Developing a Behavioural Assay of Magnetic Field Sensitivity in Homing Pigeons: Is it a Question of Vision?, Proceedings of 6th International Conference on Animal Navigation 2008, RIN 08: Orientation and navigation: birds, humans and other animals, Reading, United Kingdom, April 2008
2. **Migalski, S. P.**, Meydan, T., Bingman, V. P., Erichsen, J. T., Detection of terrestrial magnetism in homing pigeons; Is it a question of vision? Proceedings of International Magnetic Conference INTERMAG 08, Madrid, Spain , May 2008
3. **Migalski, S. P.**, Meydan, T., Bingman, V. P., Erichsen, J. T., Can a homing pigeon's head movements serve as a measure of its behavioural response to changes in a magnetic field?, Proceedings of 8th European Magnetic Sensors and Actuators Conference (EMSA), Bodrum, Turkey, June 2010 (submitted for publication)
4. Erichsen, J. T., Meydan, T., Harris, C. M. Bingman, V. P., **Migalski, S.P.**, Magnetic vision: Magnetoreception in the homing pigeon. Proceedings of 8th European Magnetic Sensors and Actuators Conference (EMSA), Bodrum, Turkey, June 2010 (invited talk, submitted for publication)

