The Effect of Static Electric Fields on *Drosophila* Behaviour

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Abstract

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Electric fields are present in the environment and generated from natural sources, such as a thunderstorm or artificially from electrical devices and transmission lines. The electric field is defined as the space surrounding an electric charge, which exerts forces on other charged objects. In recent years, the existence of artificial electric and magnetic fields (EMF) in the environment has provoked concern regarding potential adverse effects on public health, including childhood leukaemia, brain tumours and cardiovascular diseases. Establishing experimental procedures to investigate causal relationships in a human system is fraught with difficulties. Invertebrate model systems are often used as an alternative for basic research. *Drosophila melanogaster* is one such system. Previous studies have shown that exposure of insects to static and alternating electric fields induces changes in their behaviour in relation to field strength. Unfortunately, the majority of these publications are not comprehensive (focused on either behaviour or harmful effects) and tend to be on invertebrates that are not established model systems.

The present study focused on developing a thorough quantitative analysis of the interactions between static electric fields and *Drosophila melanogaster*. Firstly, this required developing novel bioassay procedures to measure detection and avoidance behaviour to static electric fields, detailed mapping of electric fields in the apparatus and investigating the potential mechanisms of detection. Secondly, to establish a suitable bioassay procedure to test whether the exposure of *Drosophila* to static electric field leads to harmful effects, by measuring knockdown and mortality. Most of the previously published research investigated the EMF component which included both electric and magnetic fields. Thus, it is difficult to separate and identify the individual effect of each of them as they are usually emitted together (for example, AC power lines). In this study, only the static electric field was used in order to identify its effects.

The results showed that *D. melanogaster*, in a novel Y-tube bioassay avoided static electric fields, after applying 0.5 kV as threshold level (corresponding to a modeled electric field strength of 26-34 kV/m). As the applied voltage increased from 1kV to 3kV so did the level of avoidance. Wing movement caused by electrical field forces were associated with avoidance. This became clear when vestigial winged mutants and wild-type flies with cut wings were exposed to these fields. They exhibited avoidance behaviour only when the highest voltage potentials (2 kV and 3 kV) were applied. In addition, the field strength required to raise the intact and excised wing in females was greater than in males due to the bigger size of the female wing. It was found that the field strength required to raise the intact wings in live and dead male flies was similar, indicating that movement of the wing in response to a static electric field is uncontrolled even with live flies. It is postulated that the electric field imposes physical forces on the wings due to polarization between opposite charges, causing wing movement and ultimately inducing a change in behaviour.

To assess the harmful effects of longer term exposure (up to 168 hours), a novel vertical tube design was developed. There was a significant relationship between field strengths and mortality with a (lethal time) LT_{50} value of 6.48 h in males and 13.02 h in females with field strengths between 89-100 kV/m. The results showed that *Drosophila* mortality occurred at higher field strength than those that induced avoidance behaviour.

This research provides new results and experimental designs to underpin future research using *Drosophila* as a model system to understand the other possible effects of sublethal static electric fields, such as the induction of stress proteins. Although not the remit of this thesis, the results also provide evidence for the potential ecological effects of static electric fields on organisms in the environment.

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Declaration

I confirm that the material contained in the thesis is all may own work and where the work of others has been drawn upon, it has been properly acknowledged according to appropriate academic conventions.

No portion of this work has been submitted or is currently being submitted in support of an application of another degree or qualification of this or nay other university.

Signed: M. AL Ghamdi ....
Date: 9 July 2012

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List of Abbreviations

2D	Two Dimensional
AC	Alternating current
AD1	Aluminum Disc 1
AD1	Aluminum Disc 2
AM	Aluminum Mesh
CE	Charged Electrode
CI	Confidence Interval
CR	Cooper Ring
D	Distance (E = V/**D**)
DA	Decision Area
DC	Direct Current
DEHP	Diethylhexy Phthalate
DNC	Digital Nikon Camera
E	Electric Field
EC	Electric Cable
EE	Earthed Electrode
ELF	Extremely Low Frequency
EMF	Electric and magnetic field
EOD	Electric Organ Discharge
ES	Earth surface
EW	Earthed Wire
Fe_3O_4	Magnetite
GC	Glass Chamber
GS	Glass Slide
GT	Glass Tube
hr	Hour
hrs	Hours
HSPs	Heat Shock Proteins
Hz	Hertz
IS	Insulated Socket

kV	Kilovoltage
kV/m	Kilovoltage per meter
L x D	Length x Diameter
LD	Lethal Dose
LS	Light Source
LT	Lethal Time
min	Minute
MLT	Melatonin
mm	Millimeter
µV/m	Microvolt per meter
NM1	Nylon Mesh 1
NM2	Nylon Mesh 2
nV/cm	Nanovolt per centimeter
OW	Operating wire
P4	Progesterone
P	P-value (0.05)
PC	Plastic Cup
PS	Power Supply
PRL	Prolactin
Q	Charge
R1	Rubber 1
R2	Rubber 2
RA	Release Arm
RI	Response Index
ROS	Reactive Oxygen Species
S	Second
SEM	Stander Error of mean
SM	Static Monitor
SL	Spirit Level
V	Voltage
V/m	Voltage per meter
W x D	Width x Diameter

WHO	World Health Organization
X	Fly’s Position
XY	X and Y Axis Grid
ZR	Z Axis and Rotational

Chapter 1

General introduction

1.1 Electric fields within the environment

The electric field between the Earth's surface and the outer atmosphere exists naturally and is generated by a variety of sources (Bering *et al*., 1998; and Roble, 1991). The primary source of this field is a global electric circuit that is created by the global weather system. This causes a potential difference of 250 kV between the outer atmospheres (50 km above the ground) and the surface of the planet. This potential difference generates an electric field at the earth's surface, in fair weather, of 100 to 300V/m (Adlerman and Williams, 1996; Bering *et al*., 1998). The strength of the electric field can increase within the atmosphere, particularly during the winter season when the fractioning of ice particles in clouds contributes to the generation of more electric charges. Discharging activities between the opposite charges at the base of a cloud and the ground atmosphere level, leads electric fields to dissipate within seconds, resulting in lightning (Aldosri, 2007; Rycroft *et al*., 2000).

Other natural sources include volcanic eruption, in which friction between ash particles can generate up to 15kV/m at a distance of five kilometres from the main volcanic crater (Vladimir *et al*., 1998). Dust storms can also generate up to 10 kV/m (Kamra, 1972). Electric fields can be induced in the marine environment as a result of the movement of ocean water through the Earth's geomagnetic field (Sanford, 1971).

Some organisms can generate small electric fields; for example, field strengths of 100- 400V/m can be generated by honey bees during flight as a result of the friction of their wings through the air, and these electrostatic forces can aid in pollination (Vaknin *et al*., 2000). Jackson and McGonigle (2005) showed that electrostatic forces are also produced when the house fly, *Musca domestica,* walks on a dielectric surface.

Electrostatic fields are also produced by friction between materials, such as a person walking on a carpet. This can produce a charge of up to 30 kV/m and cause minor electric shocks (Chubb, 2003). Extremely high electrostatic charges are generated by friction in industrial processes, for example, moving powder through tubes. Subsequent electrostatic discharge can cause serious explosive damage and many safeguards are taken to reduce the build-up of electrostatic charges (Luestgem and Wilson, 1979)

High voltages power lines (National Grid Systems) are an artificial source of electric fields, and are found in urban and rural areas across the globe. They are operated at voltages exceeding 765 kV and produce electric fields in the surrounding environment greater that natural sources (Abdel-Salam and Abduallah, 1995; Fews *et al*., 1999). For example, the field strength underneath power lines at 400 kV can reach approximately 9-11 kV/m at 1 m above ground level (Dezelak, 2010). Most power lines carry alternating current (AC) and this emits both electric and magnetic fields. In addition, electric fields can be produced by electric household appliances (Table 1.1) (Belyeav *et al*., 2006).

Electrical appliances	**Electric field strength (V/m) at 0.3 m**
Stereo receiver, Iron, Refrigerator	180, 120, 120
Mixer, Toaster, Hair dryer	100, 80, 80
Colour TV, Coffee machine, Vacuum cleaner	60, 60, 50
Electric oven, Light bulb	8, 5
Other sources of electric fields	
Railway systems	30 V/m (at 5m)
Plastic welding and moulding	100-300 V/m

Table 1.1 Electric field strengths (V/m) measured near household appliances and other source at various distances.

1.2 The concept of an electric field

An electric field is defined as a vector field that permeates in the region around an electric charge (Q), which exerts an electric force on another charged object (q). The strength of an electric field at any point in that region is directly proportional to the magnitude of the electric charge and inversely proportional to the distance from the charge (Q). The equation for calculating field strength is E = V/D (where V is the voltage and D is the distance). Electric field lines go by convention from positive to negative charge, as shown in Figure 1.1 (Ellse and Honeywill, 1998), so that the direction of electric field lines depends on the central charge (Q). In the case of a positive charge (Q^+), they direct away from the centre, whereas they direct towards a negative charge (Q^-) (Fig. 1.2A, B) (Serway, 1990).

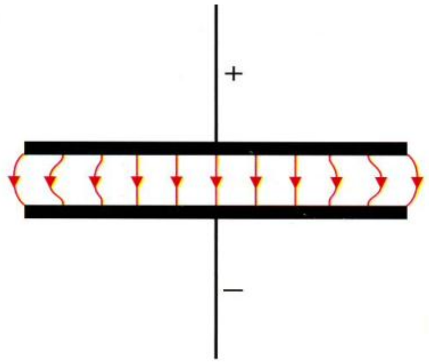

Figure 1.1 Electric field lines between two parallel plates (red arrows). Electric field lines go from positive to negative.

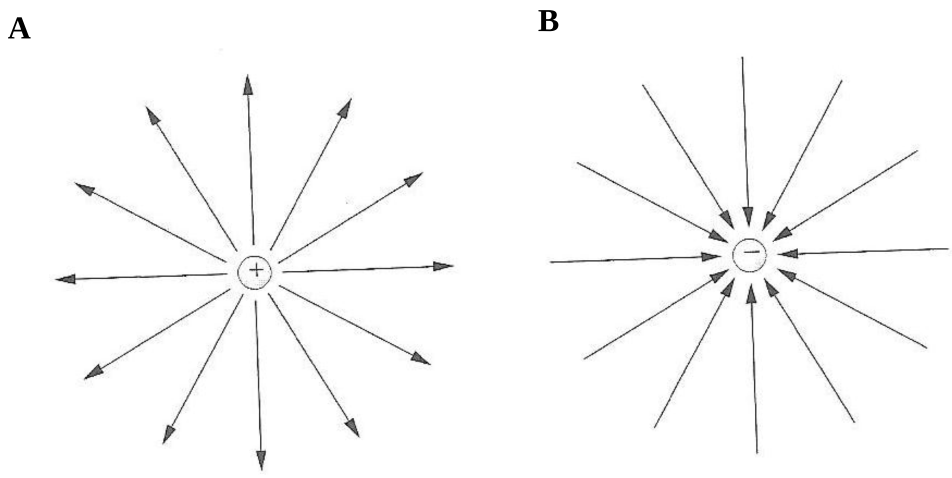

Figure 1.2 The direction of electric field lines at different charges. (A) The direction of the arrows face away from the positive charge (+ve). (B) The arrows point toward the negative charge (-ve).

There are two types of electric field that are produced by static electricity and current electricity; the latter is divided into direct current (DC) and alternating current (AC) (Aldosri, 2007). Static electric fields can be generated by friction between two different materials, and the subsequent transfer of electrons between the two materials. The material receiving electrons becomes negatively charged, while the one that loses electrons becomes positively charged. This negative or positive charge produces an electric field, which can exert physical forces on charges in adjacent objects, causing attraction between unlike charges or repulsion between like charges. For example, rubbing an ebonite rod with fur makes the ebonite rod negatively charged as a result of gaining more electrons (the electron acceptor). The fur is the electron donor, and becomes positively charged. When the negative ebonite rod is brought close to a pith ball, the negative charge of the ebonite rod will produce an electric field, which exerts forces on the electrons in the pith ball causing polarization of the ball, with electrons being repulsed to the opposite side of the pith ball, while positive charges being attracted to the rod (Bhatnagar, 1993) (Fig. 1.3A-C).

Static electric fields can also be generated by triboelectric charging, which occurs when two materials are pressed together. The surface of one material will gain some electrons from the surface of the other (Aldosri, 2007). A high voltage DC power supply, such as a Brandenburg device (Alpha ш), can also be used to produce a static charge on electrodes, which have a voltage range of up to 15 kV.

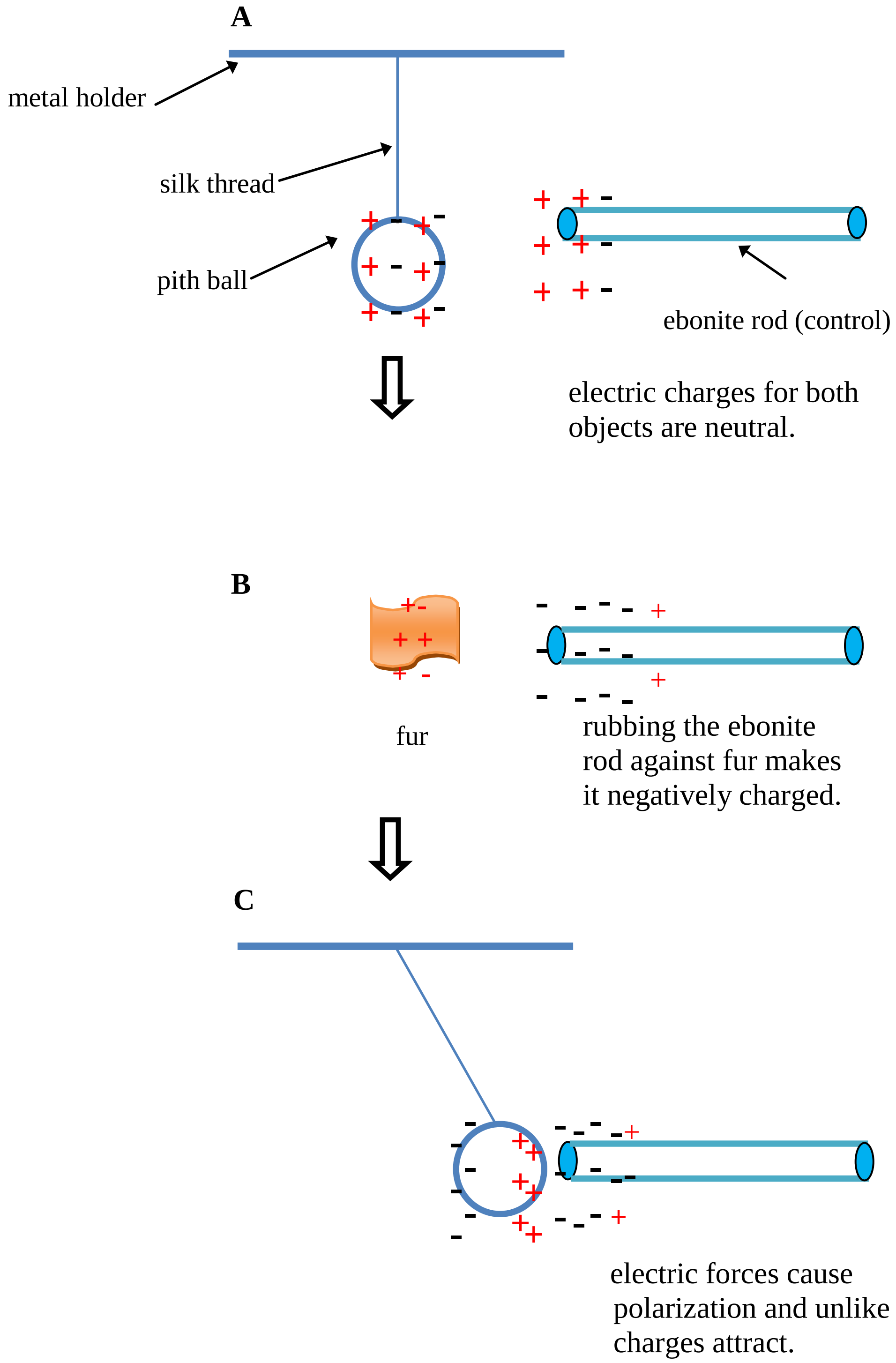

Figure 1.3. Schematic diagram showing electrostatic forces between two matrials. (A) Both the ebonite rod and the pith ball have neutral electric charges. (B) When the ebonite rod is rubbed with fur, it gains some electrons and becomes negatively charged. (C) This produces an electric field, which exerts a force on the pith ball causing polarisation, where negative charges

(electrons) are repelled and move to the other side of the pith ball, whereas positive charges are attracted to the negatively charged rod, causing the pith ball to move towards this rod.

Electrical current occurs when electrons flow through a conductor (Aldosri, 2007). This kind of electricity differs from static electricity because it requires a conductor to enable flow. In a direct current (DC), electrons usually move in one direction, from the negative to the positive pole, e.g. simple electric circuit. This produce static electric and magnetic field (EMF). In an alternating current (AC), the electrons flow one way, then reverse direction. This process repeats 50 or 60 times per second, as in 240 V domestic electrical circuits (Fig. 1.4). AC also produces a fluctuating electric and magnetic field when an electric current flows through a metal wire. This magnetic field has multi-spiral lines, and their directions are perpendicular to the electric field, the force of the magnetic field is proportional to the speed of the moving charge through the conductor (Ellse and Honeywill, 1998; Aldosri, 2007). Electric fields can be blocked by shielding with an earthed conductive material such as copper, steel or aluminium, whereas the magnetic field can penetrate these conductive materials even when they are earthed. It is therefore not straightforward to protect the human body from the exposure to magnetic fields (Belyeav *et al*., 2006).

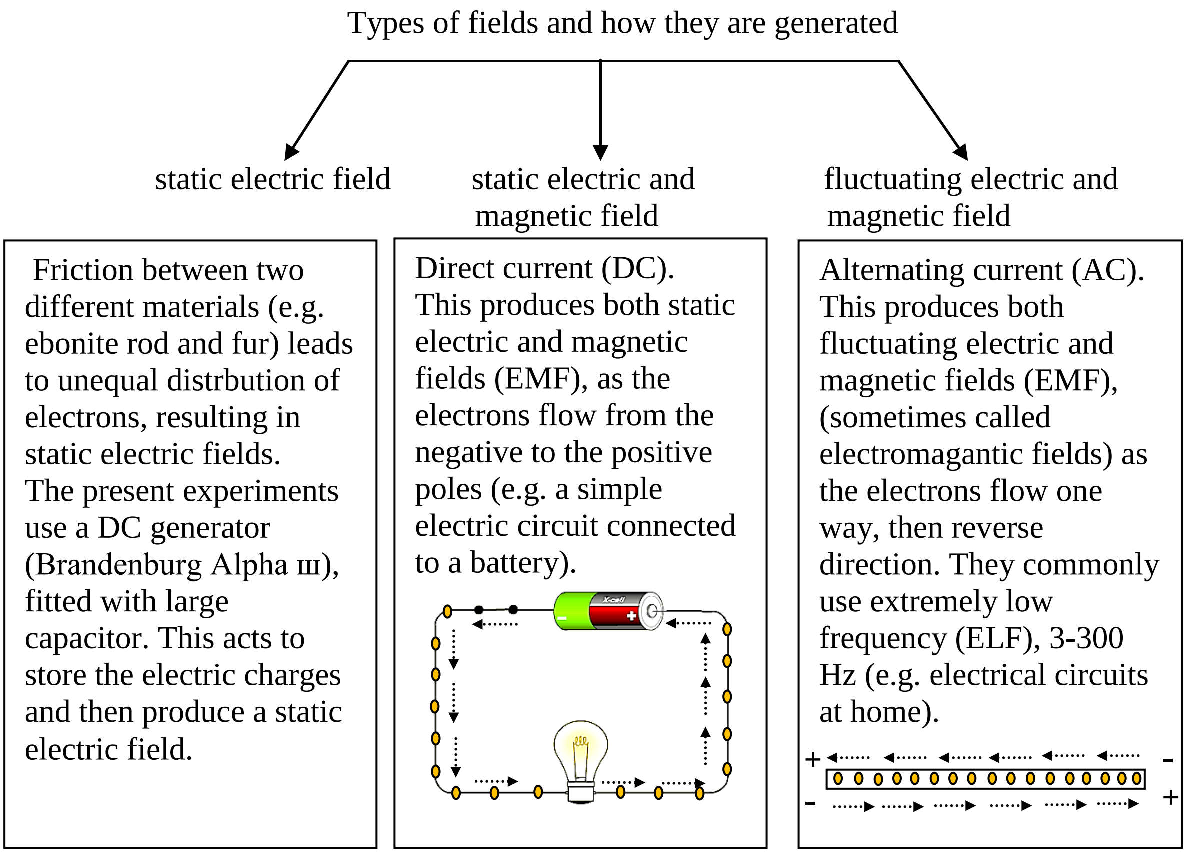

Figure 1.4 Schematic diagram showing the types of electric fields produced by electrcity.

1.3 The effect of electric fields on animal behaviour

Previous studies indicate that an electric field can induce changes in the behaviour of invertebrates (particularly insects), and also vertebrates. For example, rats exhibit avoidance behaviour when exposed to extremely low frequency (ELF) electric fields of 90 kV/m for 45 min (Hjersen *et al*. 1980), in which they prefer to move towards the uncharged side of a shuttle box. Long-term exposure (8 h daily for 56 days) of rats to static electric fields of 35 kV/m, causes a significant reduction of their locomotor activity, whereas there was no change in rats' exploratory activity (Cieslar *et al*., 2009). Coelho *et al*. (1991) demonstrated that the social behaviour of baboons, including tension and stereotypy cases, was affected when they were exposed to ELF electric fields of 30 kV/m for 6 weeks (12 h daily).

The effect of electric fields on insect behaviour has been observed in field and laboratory experiments. Field studies on colonies of honey bees, *Apis mellifera* underneath 765 kV power lines showed an increase in agitation at the entrance of the hive, and a decrease in foraging rates (Greenberg *et al*., 1981). Orlov (1990) found that flying behaviour in flies was affected under power lines emitting 500 kV. Laboratory studies have reported that electric fields lead to a number of altered behavioural responses in insects that depend on the type of electric field and the kind of insects studied (Edwards, 1960; Maw, 1961b; Maw, 1962; Perumpral *et al*., 1978; Watson *et al*., 1997).

It has been reported that insect movements are affected by static and ELF electric fields (Edwards, 1960; Watson, 1984). Perumpral *et al*. (1978) noted a disturbance in the flight behaviour in cabbage loopers (*Trichoplusia ni*) when exposed to static electric fields of more than 20 kV/m. Exposing *Drosophila* for one minute to static and ELF electric fields of up to 200 kV/m resulted in agitation, and when the field strength was raised to 410 kV/m and to 416 respectively, flies could not walk (Watson, 1984). Bee wings and antennae vibrate when exposed to ELF electric field strength of 150kV/m, while bees stopped walking when exposed to field strengths over 300 kV/m (Bindokas *et al*., 1989). Static electric field strengths of 10 kV/m were found to reduce the walking activity in the Ichneumon fly, *Itoplectis conquisitor* (Maw, 1961b). A recent study found that static electric fields reduced the walking speed of cockroaches (Jackson *et al*., 2011).

Static and ELF electric fields have been shown to act as stimuli, causing avoidance behaviour. For example, insects avoid the region under power lines when the field strength is more than 10 kV/m (Orlov, 1990). Perumpral *et al*. (1978) found that when house flies have a choice between untreated and treated areas with high static electric fields (100 kV/m), they avoided the latter. Newland *et al*. (2008) found that applying more than 1kV (40-50 kV/m) of static electric fields led to avoidance behaviour in cockroaches. Adult cigarette beetles and fruit flies avoided static electric fields generated by 0.3 kV- 5.1 kV when they approach a charged screen (Matsuda *et al*., 2011). This avoidance behaviour indicates that the insects are able to detect electric fields.

However, there is no evidence that insects have specific organs for the detection of electric fields unlike certain aquatic animals, as water is a much better electrical conductor than air (Maclver *et al*., 2001). For example, gymnotiform fish have evolved to produce and sense electric fields, as they live in murky water. This field is generated via an electric organ that is located in their trunk and tail. This can cause a discharge of low electric fields of < 100 μV/cm into the surrounding water (Rasnow, 1996). This electric field is used for orientation, navigation, and communication purposes. The gymnotiform fish also uses this field to catch their prey (Fugere, 2010; Bastian, 1994). Sharks and rays are also able to detect weak electric fields of < 5 nV/cm, emitted by small fish during swimming using an electroreceptor known as an ampullary organ, which is distributed along the lateral lines of the head (Adair *et al*., 1998; Stoddard and Markham, 2008).

Studies on invertebrates to understand the detection of electric fields have focused on the direct effects of electrical forces on the sensory appendages, such as the antennae (Yes'Kov and Sapozhnikov, 1976; Bindokas *et al*., 1989; Newland *et al*., 2008), and the wings (Watson, 1984), which may contribute to electric field perception. The sensory appendages of insects include mechanoreceptors that are responsible for detecting and responding to any mechanical distortion in the external environment of the body of insects, including touch, body stretching and pressure.

There are several types of mechanoreceptors including the trichoid sensilla (hairs) that cover the body surface of insects. The dendrites of a sensory neuron are connected to the base of a hair and produce a nerve impulse whenever they receive any influence, such as touch. These receptors are commonly found behind the head, on the legs, near joints and on the wings (Chapman *et al.,* 1998). Another type of mechanoreceptor (campaniform sensilla) is activated whenever a mechanical stress occurs on the exoskeleton (Daly *et al*., 1998). They can be induced when wings are exposed to external deformation (Elson, 1987). They are found on many parts of the body, particularly on the legs and the wings (Dickinson, 1990; Zill *et al*., 1999). Stretch receptors are innervated by multi-polar neurons and are associated with muscles and connective tissue, such as the muscular walls of the digestive system and the ovarian wall (Daly *et al*., 1998). They also play an important role in controlling wing movement during upstroke (Yang and Meng, 1999). Another mechanoreceptor, known as the Tegula, is present at the anterior of the wing articulation of *Drosophila* (Weihe *et al*., 2003) and is responsible for elevator activity in the wings (Wolf and Pearson, 1988), as shown in Figure 1.5 (A-D).

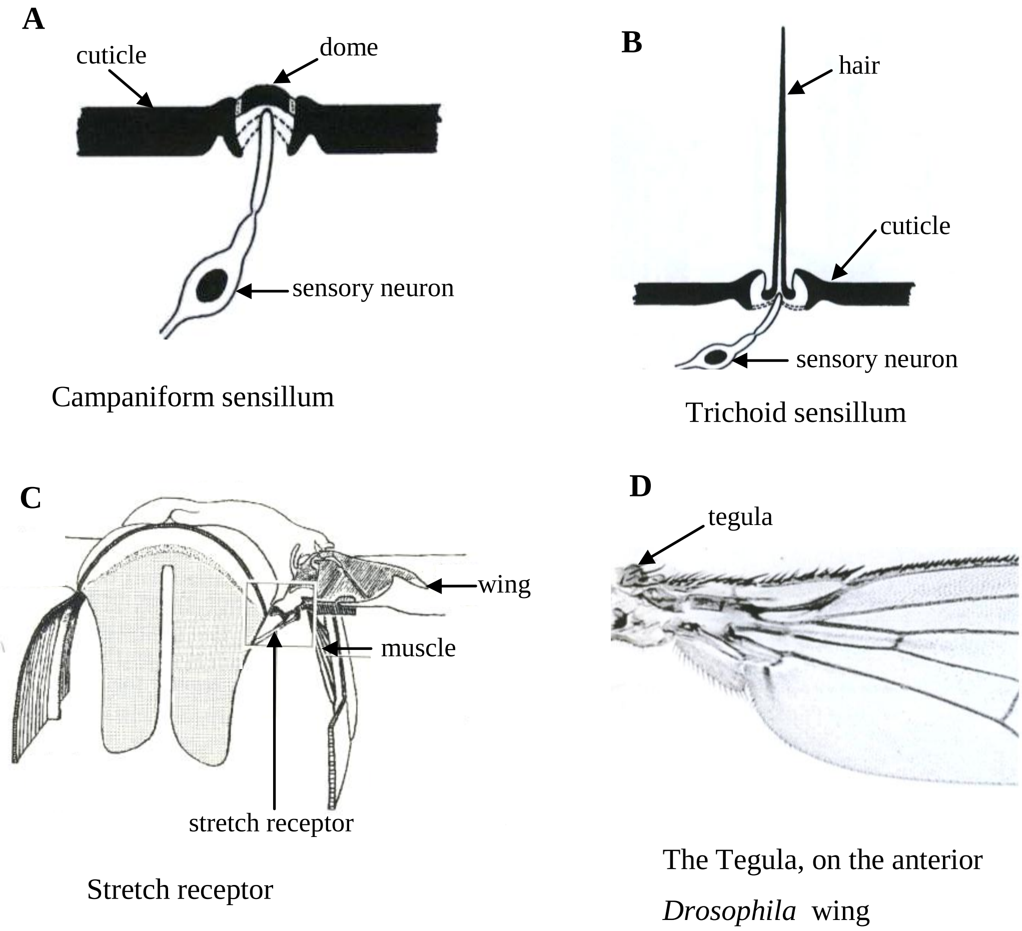

Figure 1.5 Types of mechanoreceptors in insects. (A) Campaniform sensillum (Keil, 1997), located on the legs and wings. (B) Trichoid sensillum (Keil, 1997), located on the head, legs and wings. (C) Stretch receptor (Burrows, 1996), located near wing muscles and connective tissue. (D) Tegula (Weihe *et al*., 2003), located in the anterior part of the wing. These receptors are innervated by one or more sensory neurons that play a key role in signal detection when insect appendages are disturbed by any external stimulus in the environment.

These mechanoreceptors receive stimuli from the insect's environment, and transmit them via sensory axons to a segmental ganglion. The information is processed via interneurons and then transmitted to motor neurons, which are ultimately transmitted to muscles (Fig. 1.6) (adapted from Gullan and Cranston, 2005).

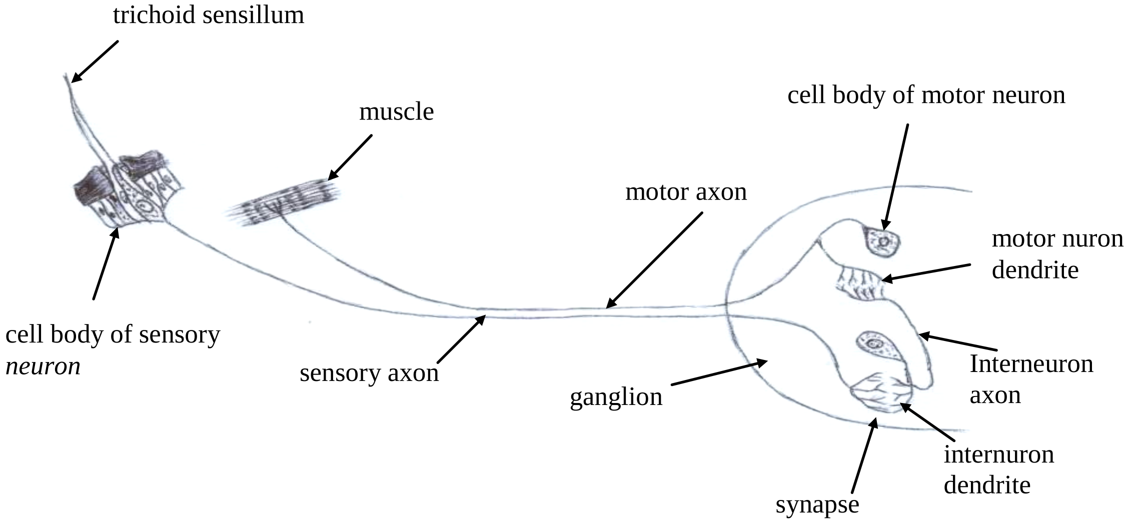

Figure 1.6 A simple reflex circuit in insects. The cell body of the sensory neuron receives stimuli whenever the Trichoid sensillum is disturbed by an external factor such as wind; this leads to the transmission of a signal neural via the sensory axon to the ganglion, This signal is transmitted to the motor neuron through an interneuron and then to the motor axon, ultimately transmitted to muscles, causing a potential response.

The movement of the sensory appendage by electrical forces may act in a similar fashion to environmental stimuli, leading to the activation of the mechanoreceptors, and initiating behavioural responses. For example, Bindokas *et al*. (1989) found that the wing and antennae in bees moved in response to ELF electric field of 150 kV/m. A vibration occurred in *Drosophila*'s wings when exposed to static and ELF electric fields of 500 kV/m (Watson *et al*., 1997). A recent study found that exposure of cockroaches to static electric fields of 40-50 kV/m caused a deflection of the antennae (Newland *et al*., 2008).

It is not only insects in which sensory appendages are affected by electric fields. For example, other studies found that mammalian hairs are also affected. Chapman *et al*. (2005) noted that electric fields can lead to the movement of hairs on the forearms and hands of humans. Additionally, body hairs were shown to bend away from their normal direction under exposure to static electric field of 45 kV/m (Blondin, *et al*., 1969; Shimizu and Shimizu, 2003).

Given that electric fields can affect sensory appendages, a key question to be addressed is whether the movement of *Drosophila* wings by electric fields might lead to the activation of mechanoreceptors and ultimately elicit avoidance behaviour.

1.4 The adverse effect of artificial electric and magnetic fields (EMF)

The effects of electric and magnetic fields (EMF) on biological systems have recently been the subject of much research (Feychting *et al*., 2005; Draper *et al*. 2005). Studies of the health effects of exposure to the EMF report an increased risk of childhood leukaemia, breast cancer and brain tumours (Ahlbom *et al*., 2001; Draper *et al*. 2005), particularly for those people who live close to transmission power lines (WHO, 2002).

The EMF components such as Extremely low frequency (ELF) electric fields, (produce fluctuating electric field in association with magnetic fields), showed other effects including physiological changes, such as in blood pressure and heart rhythm. In one study, volunteers were exposed to field strengths of up to 30 kV/m and exhibited haematological changes (reviewed by Bonnell *et al*., 1980). Saunders *et al* (1991) suggested that the possible adverse effects of ELF electric fields as these fields act on humans by induced charges on the surface of the body and then lead to a weak current flow. This might cause alteration in intracellular, biochemical and physiological functions (Saunders *et al*., 1991; Tenforde, 1991; Hanafy, 2004). However, epidemiological studies do not show sufficient evidence about the possible health effects of exposure to these fields in the residential areas. (Schuz and Ahlbom, 2008).

Many laboratory studies using different animal models have been conducted to assess the possible harmful effect of ELF fields on health, because they are common in the environment through power lines and electric home appliances (Shaw and Croen, 1993). On the other hand, only a few studies have focused on the effect of static electric fields and mainly focused on behaviour aspect.

ELF electric fields have been shown to cause chromosome aberrations in the bone marrow cells of mice exposed to field strengths of 6 kV/m for 30 days (Fawzia, 2002). Goraca *et al*. (2010) found that ELF electric fields affect antioxidant enzymes, leading to reactive oxygen species (ROS) in the heart tissue of rats when exposed for 14 days (60 min/ day). Other studies found that the long term exposure of mice (up to 60 days) to electric field strengths up to 6 kV/m with a frequency of 50 Hz affects the dielectric properties of proteins and increases their conductivity, which might indicate a change in the molecular structure of total serum proteins (Hanafy, 2006). However, exposure of dairy cattle to ELF electric fields of 10 kV/m for 4 weeks did not show any significant effect on the serum concentration of progesterone (P4), melatonin (MLT) or prolactin (PRL), when sample blood was tested (Burchard *et al*., 2004).

It has been reported that EMF can induce the synthesis of stress proteins (Coulton, 2004). This might indicate that EMF has a harmful effect on cells and provides further evidence that these fields interact with cells and tissues (Goodman and Blank, 2000). An in *vitro* study showed that the exposure of human breast cells to 60-Hz EMF for 20 min led to the induction of hsp70, which returns to its normal level after 2 h (Han *et al*., 1998). In contrast, a study conducted by Cieslar *et al*. (2005), showed no significant changes in antioxidant enzyme activity in the tissue of rats exposed for long periods to static electric fields of 16 kV/m and 35 kV/m. This observation suggests that static electric fields generated by high voltage direct current (DC) transmission lines do not cause any serious effect on antioxidant reactions in the human population.

Invertebrates were also affected by ELF electric fields. For example, chromosomal aberrations in *Drosophila* were observed when applying field strengths of 30 kV/m as a threshold level (McCann *et al*., 1998). Long term exposure of ELF electric fields of 0.8 kV/m led to reduced egg laying in *Scambus buoliana* (Maw, 1961b). An ELF electric field of 50-100 kV/m (under wet conditions) was shown to cause mortality in bees when they landed on the surface of a conductive tunnel located around the colony, suggesting that death occurred as a result of an electric shock (Bindokas *et al*., 1988). A field strength of 352 kV/m with a distance of 50 mm between high voltage electrodes and the ground electrodes was able to kill flies within 72 hr (Watson *et al*., 1988).

The majority of research on the effects of electric fields has focused either on behaviour or health effects. This study however, includes the effect of static electric fields, in absence of magmatic fields, on both behavioural changes and the harmful effects of electric field exposure. *Drosophila melanogaster* was adopted as a model system for a number of reasons, since it has been previously used in many studies with different treatments, such as temperature, starvation, crowding and chemicals (Huang and Chen, 2007; Bourg, 2007b; Sorensen and Loeschcke, 2001; Nazir *et al*., 2003). *Drosophila* are cheap to maintain, and it is easy to culture new generations over short periods. This is because of the short life cycle from egg to adult fly of approximately 14 days. *Drosophila* have been widely used in genetics and developmental biology and have aided our understanding of the molecular and genetic basis of some human diseases (Schneider, 2000; Sang and Jackson, 2005).

1.5 Project Aim

This project aims to investigate the effect of static electric fields on behaviour and whether exposure to these fields leads to harmful effects on animal life. We therefore set out to:

1- Investigate whether *Drosophila* are able to detect static electric fields by measuring avoidance behaviour within a Y-tube apparatus.

2- Provide a better understanding of how *Drosophila* can detect static electric fields.

3- To investigate whether long term exposure of *Drosophila* to static electric fields leads to harmful effects, by measuring knockdown and mortality rate.

Chapter 2

The avoidance of static electric fields

2.1Introduction

Avoidance behaviour is a common behavioural response in many animals and is defined as a "type of activity exhibited by an animal exposed to adverse stimuli in which the tendency to flee or act defensively is stronger than the tendency to attack" (Stevens, 2000). In insects, avoidance behaviour can be observed when an animal encounters environmental stimulus and it is typically beneficial for the insect's survival (Bale, 1993; Meyling and Pell, 2006). Avoiding natural enemies is one of these. For example, *Anthocoris nemorum* evolved adaptations to detect and avoid their natural 'enemy' the pathogenic fungus, *Beauveria bassiana*, when they are searching for prey or oviposition sites on plant leaves (Meyling and Pell, 2006). Futami *et al*. (2008) suggested that mosquito larvae avoid their predators such as the wolf spider, *Pardosa messingerae*, by diving into deeper water.

The olfactory system in insects is sensitive to volatile substances (Stortkuhl *et al*., 2005) and many odorant stimuli can elicit attraction or avoidance behaviour (Wang *et al*., 2003). For example, carbon dioxide (CO_2) with a concentration of 0.1% elicits avoidance behaviour in *Drosophila* (Suh *et al*., 2004; Suh *et al*., 2007), as a result of the activation of the olfactory receptor neurons in the antennae (Bruyne *et al*., 2001). Stortkuhl *et al*. (2005) showed that misexpression of the olfactory receptors Or43a leads to the reduced ability of *Drosophila* to avoid benzaldehyde compared to wild-type flies. Tactile stimuli can also cause avoidance behaviour in many invertebrates. For example, a study by Comer *et al*. (1994) showed that the cockroach, *Periplaneta americana*, turns away and escapes when its antenna are touched by different predator species. However, actual physical contact is not necessarily required, as the cockroach also exhibits escape avoidance behaviour triggered by puffs of wind, which cause excitation of the cercal hairs (Dagan and Camhi, 1979).

Odorant and tactile stimuli are not the only way to elicit avoidance behaviour, as it is also observed in insects when the auditory system is stimulated (Miller and Surlykke, 2001). Fullard *et al*. (2008) showed that nocturnal insects such as moths can detect the ultrasonic waves produced by bats, and this causes them to fly directly away from the predators. Additionally, insects can exhibit avoidance behaviour to a thermal stimulus. Cang *et al*. (2006) noted that *Drosophila* jump when different parts of their thorax and abdomen were heated using a laser beam, suggesting that the thermo receptors distributed on their bodies were stimulated, thus triggering their movements.

Furthermore, insects often rely on visual feedback from their environment for a variety of tasks such as moving, flying and landing (Yakovleff *et al*., 1995). They use their visual systems to avoid impending collision with other objects during flight and escape from predators (Tammero and Dickinson, 2002; Warrant and Dacke, 2010).

Besides these stimuli, avoidance and other behavioural changes are observed when insects are exposed to electric fields of various strengths. However, it is not clearly understood why they avoid these fields. This raises several interesting questions: a) Why do insects avoid electric fields and how do they detect these fields? b) Is this avoidance behaviour due to the effect of electrical forces on the insect’s appendages or did insects evolve to detect these fields? c) Moreover, it is interesting to know whether these electric fields have a harmful effect on the insects. To this end, in this study I conduct a comprehensive analysis in which *Drosophila* are exposed to electric fields with varying strengths in an attempt to answer these questions. To achieve this goal, the modelling of electric fields will be used to quantify the strength causing the avoidance behaviour and whether this field strength has a detrimental effect on the insects by measuring their mortality rates (see Chapter 4). There is currently no established bioassay standard to quantify avoidance behaviour due to exposure to electric fields; indeed virtually every report in the literature describes different apparatus and measures of this behaviour. For example, Orlov (1990) demonstrated that flies avoid the area under power lines with electric fields greater than 10 kV/m. Laboratory studies also show that the exposure of insects to static electric fields cause avoidance behaviour. For example, house flies, *Musca domestica* and cockroaches, *Periplaneta Americana* show avoidance behaviour (Perumpral *et al.*, 1978; Hunt, 2005 and Newland *et al*., 2008). A recent study shows that adult cigarette beetles and fruit flies make avoidance responses to static electric fields when they approach a charged screen. This observation suggests that this can potentially be used for protecting crops (Matsuda *et al*., 2011).

Exposure to static electric fields can also cause changes to activity behaviour. Maw (1961) found that the Ichneumon fly (*Itoplectis conquisitor)* can walk quickly across a charged surface with a field strength of 3kV/m in less than 60 s. However, at 100 kV/m it requires 3 min or more to walk the same distance. Wing beat frequency in cabbage loopers, *Trichoplusia ni*, is significantly affected by static electric fields of 20-150 kV/m (Perumpral *et al*.,1978). Watson *et al*. (1984) found that exposure of *Drosophila* to static and ELF electric fields of 200 kV/m caused agitation, while higher fields of up to 416 kV/m affected walking ability. Further studies on *Apis mellifera* exposed to ELF electric fields of 150kV/m led to vibrations of wings and antennae, but body hair movement was less affected. By increasing the field strength up to 300 kV/m, bees appeared to have difficulty in walking (Bindokas *et al*., 1989). Thus, extremely high electric fields may have paralysing effects on insects.

Most of the bioassays used in previous studies did not include a modelling of the electric field. In this study the bioassay design will be accompanied with a modelling of the electric field to allow for a comparison between the electric fields where avoidance behaviour was observed and the electric fields induced by high voltage power lines which continue to be surrounded by the controversy as to whether they adversely affect human health. For example, underneath a 400 kV power line, the electric fields strength can reach between 9-11 kV/m at 1 m above the ground (Dezelak, 2010; www.emfs.info) (Fig. 2.1).

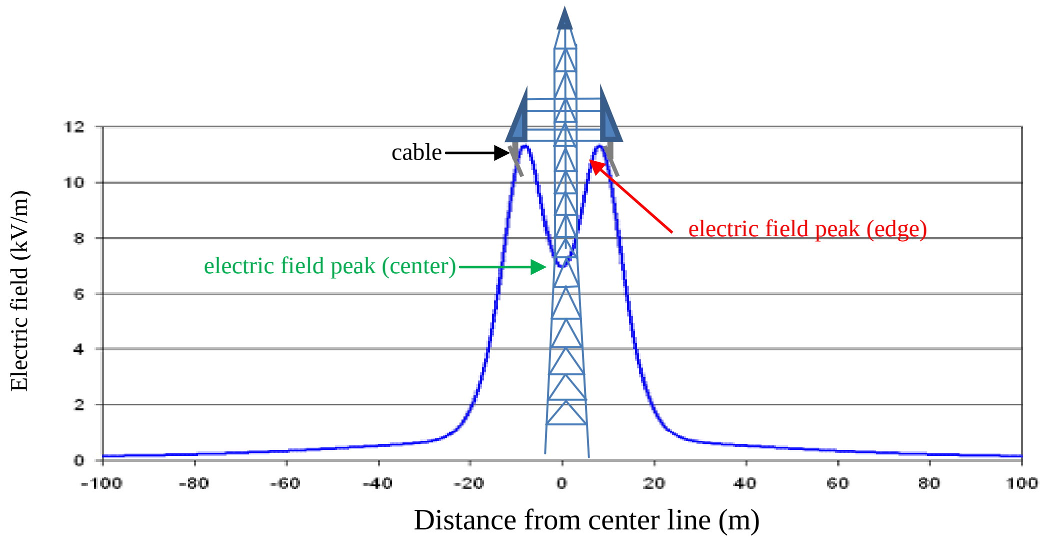

Figure 2.1 Electric field strength under a 400 kV power line. The field strength was calculated at 1 m above the ground, from the centre line and up to 100 m on both sides. The electric field underneath the edge of the pylon at ground level is higher than the centre as shown by the blue curve.

The above studies have shown that electric fields have an effect on insect behaviour, including *Drosophila.* Therefore, the aim of this chapter is to determine whether *Drosophila* are able to detect electric fields by measuring avoidance behaviour within the Y-tube bioassay, and to determine the time at which the majority of flies showed avoidance. Modelling of the electric field within a Y-tube bioassay using Maxwell SV two dimensional software provided a more detailed analysis of the magnitude and distribution of these fields and understanding of how flies respond to electric fields of these various strengths.

2.2 Materials and Methods

2.2.1 Culturing flies

Wild-type *Drosophila melanogaster* (Oregon-R) were obtained from Blades Biological Ltd., Kent. They were maintained on a food medium consisting of Agar (5g), dried active yeast (15g), plain white flour (35g) white sugar (75g) and distilled water (850 ml). The ingredients were mixed together on a hot plate to boiling point, and then removed from the heat for a few minutes. A solution containing ethanol (25ml) and Nipagin (2.5g) was added to prevent fungal growth on the food. The food was poured into bottles (250 ml) and *Drosophila* vials, with approximately 0.15 g dried yeast per vial sprinkled on the surface of the medium, then plugged with cotton wool. Flies developed (from egg to larva to pupa to adult) in 13-14 days at 20 ±2°C.

2.2.2 Preparation of flies for experiments

Wild-type flies (4-8 days old) were anaesthetised with CO_2 and removed from the original cultures. Groups of 20 flies (mixture of males and females) were put into eight new tubes (50 ml). They were starved for 24 h at room temperature, with a small piece of wet tissue placed in the test tube to prevent desiccation. Starvation was used to condition the flies and encourage them to move up towards the treated and untreated arms, (unstarved flies tended to be inactive and remain in the release arm of the bioassay).

2.2.3 Y-Tube apparatus

Y-tube bioassays were used to investigate whether *Drosophila* can detect and avoid electric fields, and to monitor their behaviour under different voltages. Two designs of Y-tube were evaluated; the only difference between the two designs was an earthed aluminium mesh that covered the release arm surface of the Y-tube to help localise the electric field. The Y-tube consisted of three cylindrical chambers of glass, 2mm thick, 150 mm x 30mm (length (L) x diameter (D)), fused together to form a ‘Y’ with an angle of 120° (made by the Chemistry Workshop, University of Southampton). Two copper rings, 5 mm x 28 mm (width (W) x D), were positioned inside each arm (A and B) close to the decision area (14 mm). They were attached to an insulated socket through a small 7 mm hole in the surface (Fig. 2.2 A). One copper ring was connected to a DC power supply (Brandenburg Alpha III, Brandenburg, UK) by an insulated wire and the other was connected to an earth electrode, (Fig. 2.2B). The ends of each arm were covered by two tubes of glass (85 x 35 mm) to prevent insect escape, and a cold light source (40 W) was placed over the end of the arms to encourage upward movement. The third arm (C-arm) was used as a release chamber; it was covered by an aluminium mesh and connected to an earth electrode to prevent the electric field in this area. The area which joins these three arms together is called the decision area. Finally, the Y-tube was secured by a metallic holder and placed inside a dark aluminium cage. This technique was modified from Newland *et al*. (2008).

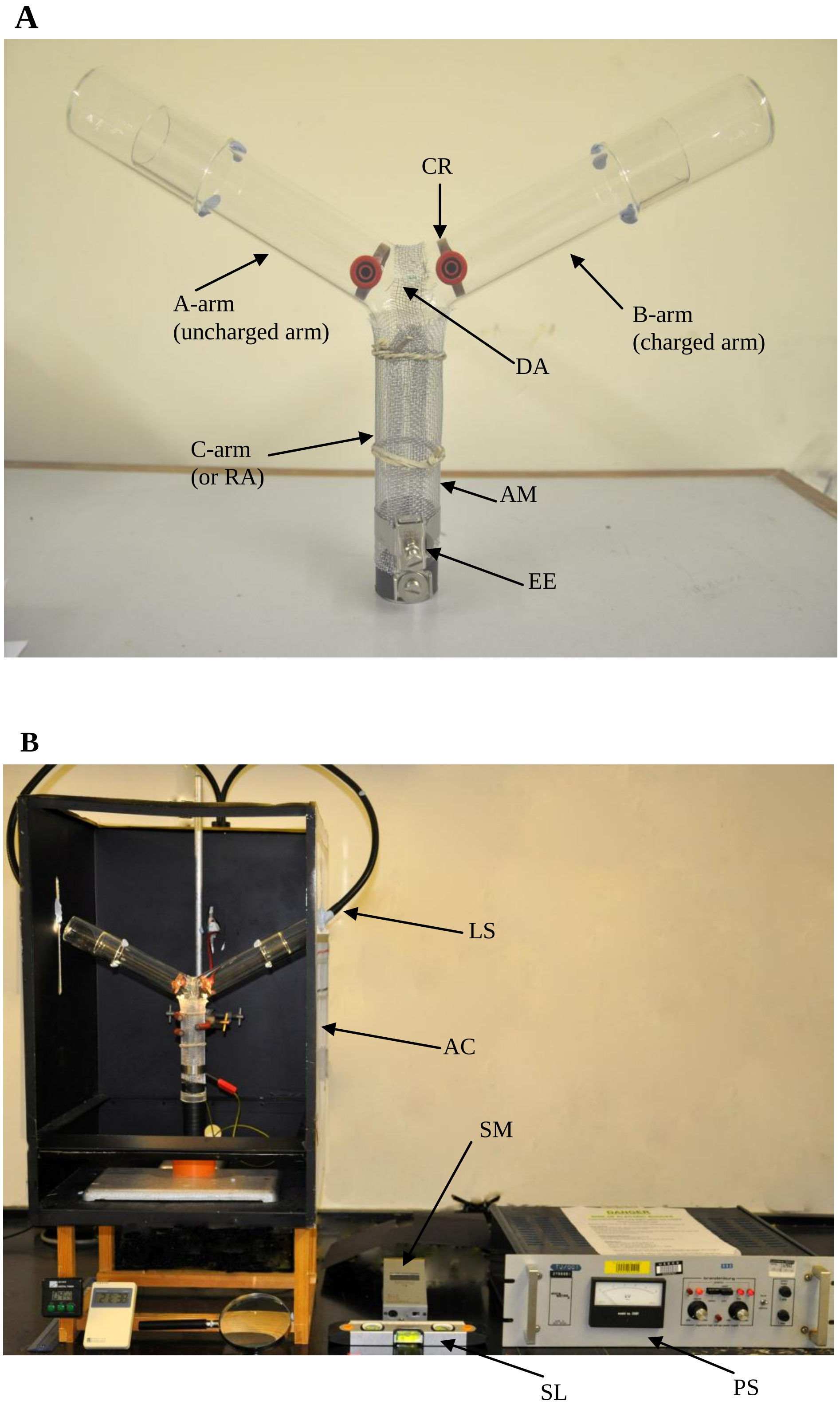

Figure 2.2 Y-Tube apparatus. (A) The Y-tube consists of three glass cylinders, 150 mm x 30 mm (L x D), fused together to form a ‘Y’ with angle 120°. (B) The Y-tube is connected to a Brandenburg device to generate static electric fields and a source of light is provided on both sides. (CR: copper ring; DA: decision area; RA: release arm; AM: aluminium mesh; EE: earth electrode; LS: Light source; AC: aluminium cage; SM: static monitor; SL: spirit level; PS: power supply)

2.2.4 Behavioural bioassays

Before testing the avoidance behaviour, the Y-tube had to be fixed securely inside an aluminium cage to reduce the impact of uncontrolled electric fields. It was also balanced using a spirit level to make sure that both arms were in equal positions. Illumination on both sides was measured using a digital light meter and connected to the arms of the Y-tube through a small hole (5 mm), located on each side of the aluminium cage. The electric field strength was checked around the Y-tube, particularly the treated arm, using a static monitor (JCI 140) to ensure that the apparatus functioned correctly (Fig. 2.2B).

Each sample tube (containing 20 flies, both male and female randomly) was tapped gently. The stopper was opened rapidly, and the tube containing the flies was presented to the base of the Y- tube. Different voltages were applied individually at 0 kV, 0.12 kV, 0.25 kV, 0.5 kV, 1 kV, 1.5 kV, 2 kV and 3 kV in one arm of the Y-tube. Each voltage was applied for 20 min to test each group of flies (for example, the first group was only tested with 0 kV up to 20 min). This experiment was repeated 8 times (n = 8) and the total number of flies was 1280. A magnifying glass (100 mm) was used to monitor the distribution of the insects within the Y-tube. After each experiment, 10 min were taken to remove the flies using CO_2 gas and to refresh the air in the Y-tube for another use with a new group of flies. The charged arm was switched between experiments to check for experimental bias. Avoidance behaviour observations were entered on a data sheet every 5 min and up to the maximum time (20 min). The data sheet also included drawings of the Y-tube to clarify the locations and directions of all flies at the end of each experiment.

2.2.5 Washing the apparatus

The apparatus was washed after each experiment to remove pheromone deposits, in 3 steps. Firstly, it was soaked for 5 min in a solution of 5% Decon and 95% distilled water and then washed with a smooth brush (all arms). Next, it was rinsed with normal water and immersed in a dish of distilled water for 2 min before being washed with acetone for 5 min. Finally, the apparatus was dried in a drying chamber (100 C˚) for 25 min to be ready for the next experiment.

2.2.6 Electric field modelling within the Y-tube apparatus

Maxwell SV two dimensional software (Version 7 for Windows) was used to model the electric field within the apparatus. This modelling was used to help understand the suitability of a particular bioassay design of the Y-tube through visualisation of the distribution of the electric field. In addition, it provides estimates of the magnitude of the electric fields, which could be correlated to the response of the flies at different levels of field strength. In Maxwell SV the Y-tube apparatus was drawn as a simple 2 D, x-y model due to a cross-section that was only taken from each part of the Y tube as shown in Figure 2.3. The electrical properties of the material were assigned to the different materials of the model (e.g. glass, copper, aluminium etc.). Boundary conditions were defined as the electric potential values applied to the charged arm electrode. Different voltage potentials (0 kV, 0.25 kV, 0.5 kV, 1 kV, 1.5 kV, 2 kV and 3 kV) were investigated.

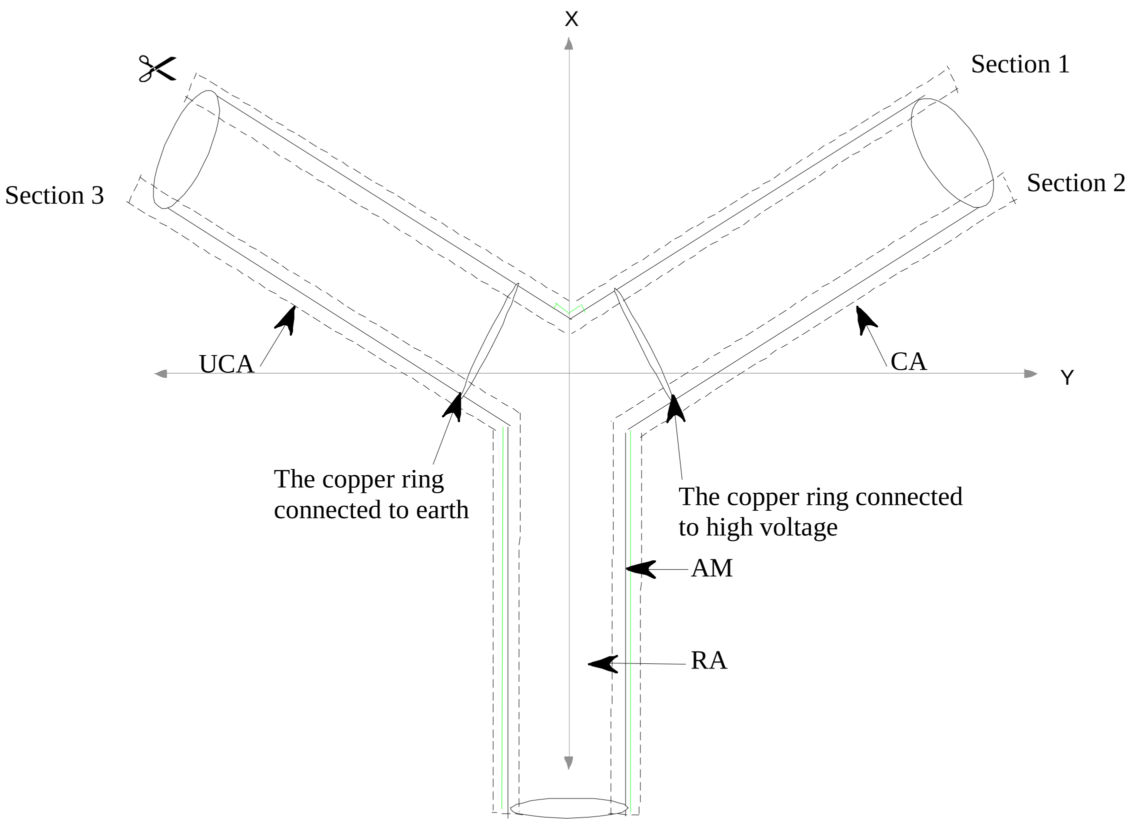

Figure 2.3 A cross-section of the Y-tube was drawn as in the 'x-y' plane in Maxwell SV software to calculate electric field strength. This model assumes that the bioassay is not circular but lies in the same plane. The first section represents the top of the Y-tube, whereas sections 2 and 3 represent the right and left side of the Y- tube. (UCR: uncharged arm; CA: charged arm; AM: aluminium mesh (green line); RA: release arm).

2.2.7 Statistical Analysis

During all experiments the number of flies in the uncharged and charged arms of the Y-tube were counted after each 5 minute period. The response index (RI) was calculated as the number of flies in the uncharged arm minus the number of flies in the charged arm and divided by the total number of flies (Stortkuhl *et al*., 2005; Turner and Ray, 2009). An RI value above zero indicated that the flies avoided the electric fields. Data was tested for normality and homogeneity, and data transformation was not required. The statistical significance of the difference between the mean index values at 0 kV and the other voltages (0.12 kV, 0.25 kV, 0.5 kV, 1.5 kV, 2 kV and 3 kV) were assessed using one-way ANOVA. Regression analysis was further used to clarify the effect of the increase in voltage on avoidance behaviour (RI value).

The number of flies remaining in the release arm was analysed using one-way ANOVA (SPSS software, version 17), to test whether there were significant differences between the number of flies at 0 kV and the other voltages at each time point (5, 10, 15 and 20 min). The constant number of flies at these voltages would suggest that the release arm of the Y-tube is not affected by the electric field.

2.3 Results

2.3.1 Electric field modelling within the Y-tube apparatus

In order to test the avoidance behaviour of flies to static electric fields, two Y-tube designs were assessed using the Maxwell SV modelling software to map field distributions.

The modelling of Design 1 revealed the electric field distribution in the uncharged and charged arms. A 0.5kV potential generated an electric field around the copper ring of up to138 kV/m, whereas the field strength was less (26-34 kV/m) at the entrance of the arms (Fig. 2.4A). Applying a 1.5 kV potential to the charged arm electrode caused high fields around the copper ring of up to 164 kV/m, which dropped to 43-95 kV/m at the entrance to both arms (Fig. 2.4B). Therefore, this design of theY-tube did not show differences in field strength within the entrance area for both arms when 0.5 kV was applied. The same occurred when 1.5 kV was applied. Consequently, flies were not be exposed to differential electrical fields within the two arms of the Y-tube bioassay.

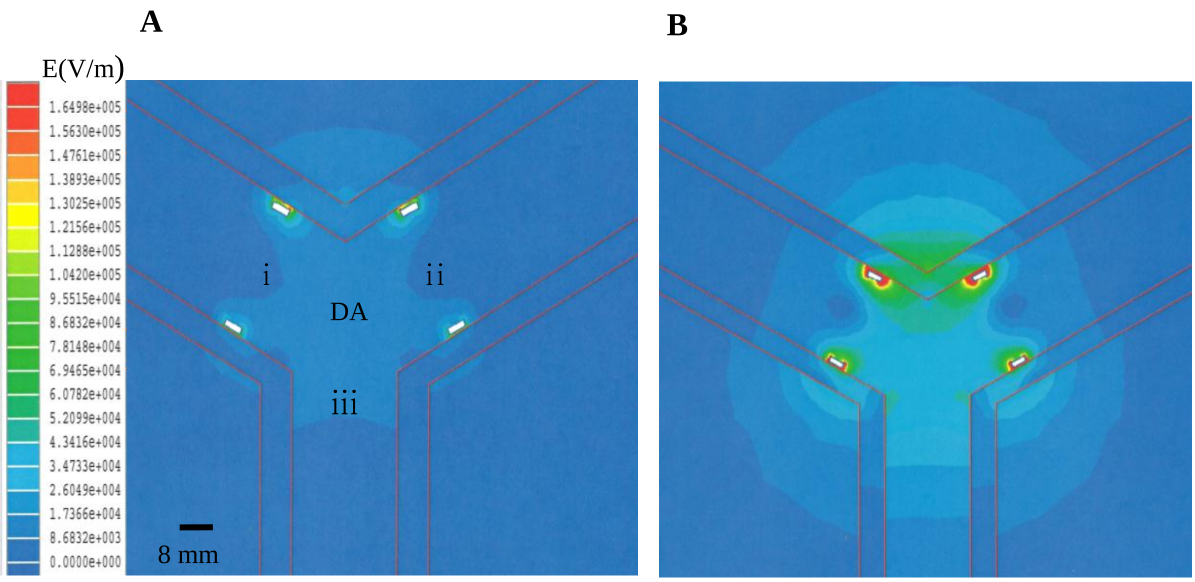

Figure 2.4 The static electric fields distribution within the Design 1 Y-tube apparatus at different voltages using Maxwell SV modelling software. At 0.5 kV (A) and 1.5 kV (B) the electric fields distributed into the entrance of the uncharged (i), charged arm (ii), decision area (DA) and the top of the release arm (iii) .

Modelling the electric field within the Design 2 of the Y-tube showed a localization of electric fields within the charged arm. The highest electric field strength was distributed immediately around the copper ring. For example, a 0.25 kV potential generated an electric field of 104 kV/m around the ring of the treated arm, while the decision area and control arm remained unaffected (Fig. 2.5A).

Applying a 0.5 kV potential led to a slight diffusion of the electric field in the entrance of the treated arm, and the midpoint of the decision area. The field recorded around the copper ring was up to 138 kV/m, whereas in the entrance, the field strength was between 26-34 kV/m (Fig. 2.5B). Raising the voltage to 1 kV and 1.5 kV showed an increasing distribution of the electric field with values between 34-43 kV/m and between 43-95 kV/m respectively in the entrance of the charged arm, whereas the midpoint of the decision area and uncharged were still less with field strengths of 26-34 kV/m (Fig. 2.5C, D).

In addition, in Design 1 the electric field strength through the release arm was higher than Design 2. For example, 1.5 kV generated field strengths between 26-78 kV/m in Design 1, but 17-34 kV/m in Design 2 (Fig. 2.4B and Fig. 2.5D).

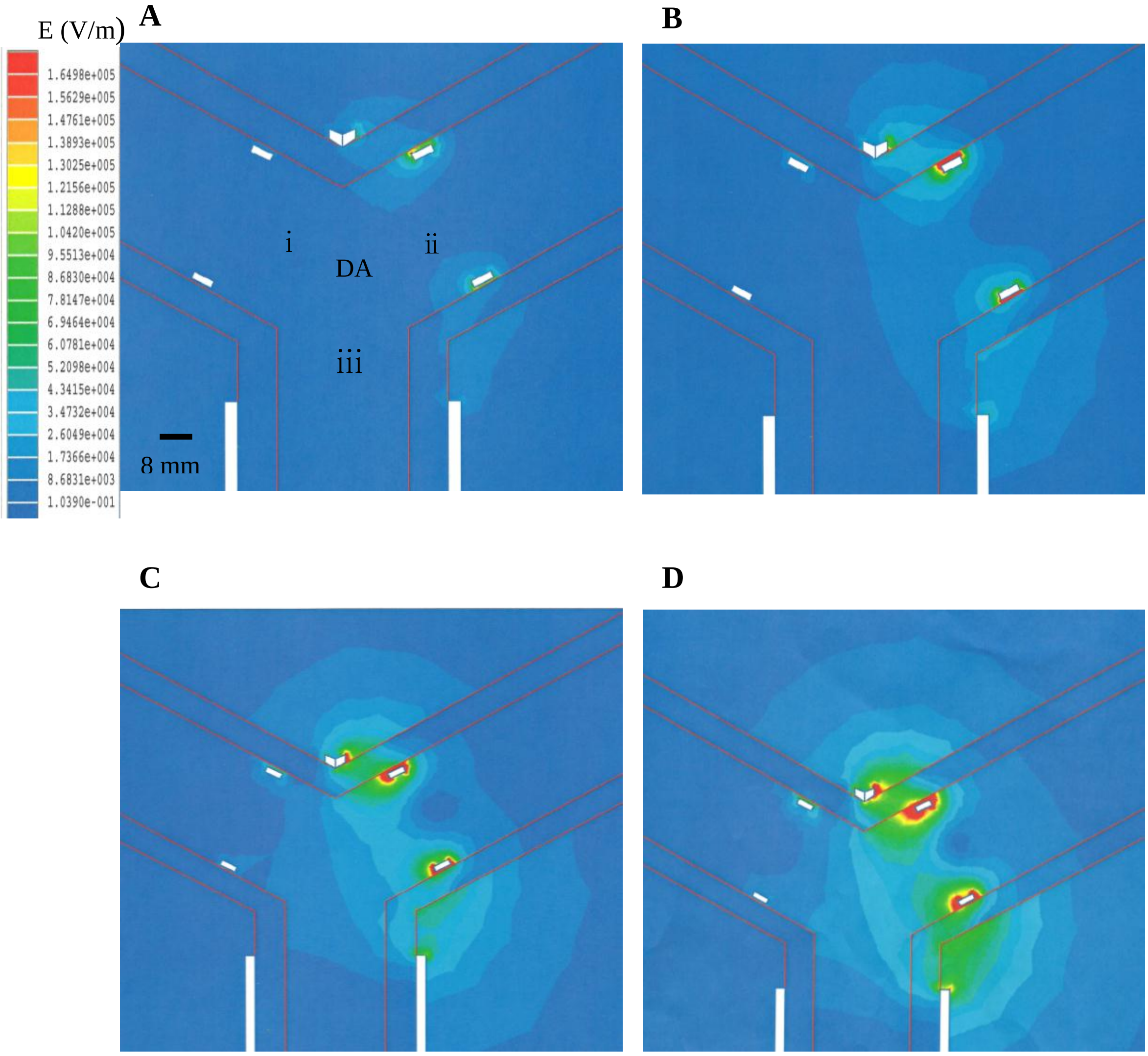

Fig. 2.5 The static electric field distribution within the Design 2 Y-tube apparatus at different voltages using Maxwell SV modelling software. (A) At 0.25 kV the electric field was distributed mainly around the copper ring of the charged arm (ii), but the entrance pathway of this arm and the decision area (DA) were not affected. (B) At 0.5 kV the electric field expanded into the whole of the entrance of the charged arm with a value of 26-34 kV/m. When higher voltages of 1 kV (C) and 1.5 kV (D**)** were applied, the electric field was distributed into the entrance of both arms. The field strength however, remained stronger in the entrance of the charged arm of approximately between 34-43 kV/m at 1kV and 43-95 kV/m at 1.5 kV potential.

2.3.2 Flies in the release arm

The number of flies remaining in the release arm of theY-tube (Design 2) was counted under different voltages to further show the convenient of this design for measuring the avoidance behaviour in *Drosophila* as the release arm was not affected by electric fields even when the highest voltage (3 kV) was applied compared to the decision area of theY-tube.

The results did not show a significant difference across the different voltages applied. For example, after 5 min of exposure to the electric fields, the number of flies in the release arm at 0 kV did not differ significantly from any of the applied voltages (0.12 kV, 0.25 kV, 0.5 kV, 1 kV, 1.5 kV, 2 kV and 3 kV, (n = 8, $F_{7, 56}$ = 0.40, P = 0.89) (n = 8) (Fig. 2.6A). This result suggests that the release arm of theY-tube was protected from electric fields, as the number of flies was nearly constant, even when increasing the field strength.

Similar results were observed when flies were exposed for 10 min, 15 min and 20 min of exposure, there was no significant difference between the number of flies in the release arm at 0 kV and other voltages ($F_{7, 56}$ = 0.54, P = 0.80) (Fig. 2.6B), ($F_{7, 56}$ = 0.85, P = 0.54) (Fig. 2.6C) and ($F_{7, 56}$ = 0.25, P = 0.96) (n = 8 in all cases) (Fig. 2.6D), respectively.

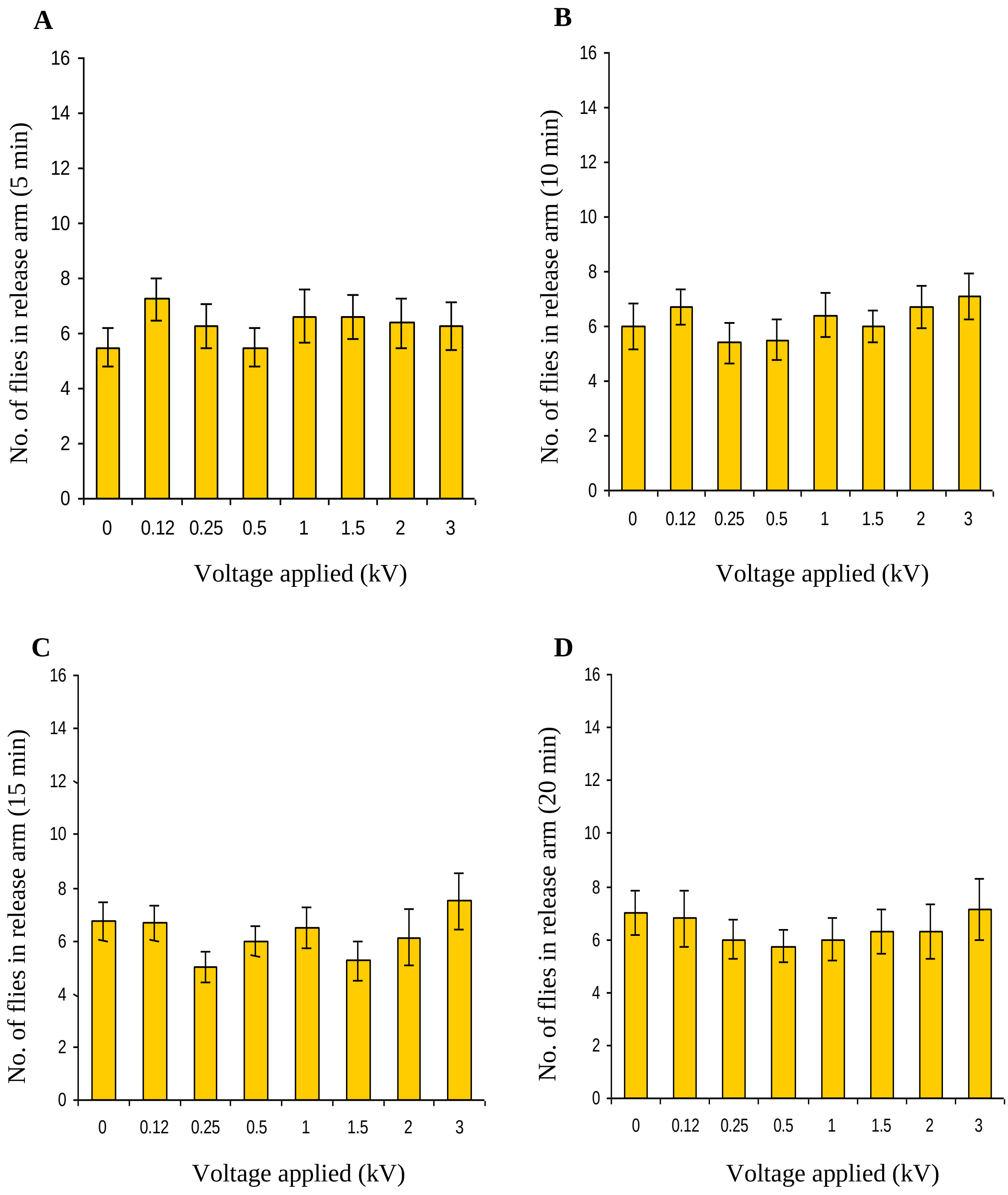

Fig. 2.6 The number of flies in the release arm of the Y-tube apparatus (Design 2) at different voltages. At 0 kV after 5 min (A), after10 min (B), after15 min (C) and after 20 min (D). The number of flies did not differ significantly when exposed to electric fields at different voltages (P> 0.05 in all cases).

2.3.3 Avoidance behaviour

Static electric fields at different voltages were applied to the electrode of one arm of the Y- tube bioassay (Design 2) to determine whether *Drosophila* were able to avoid these fields. The flies in the uncharged and charged arms were recorded every 5 min, and each trial was carried out for 20 min with replication of 8 times (n = 8).

The results indicate that *Drosophila* significantly avoided the electric fields within 5 min (one way ANOVA, $F_{7, 56} = 9.7$, $P < 0.05$). Significant avoidance to electric fields was also observed after 10 min ($F_{7, 56} = 10.5$, $P < 0.05$), 15 min ($F_{7, 56} = 11.8$, $P < 0.05$) and 20 min ($F_{7, 56} = 14.9$, $P < 0.05$).

Post-hoc analysis showed significant avoidance compared to 0kV, was first observed with the application of a 1.5 kV (field strength 43-95 kV/m), after 5 min of exposure (mean = 0.40, SEM ± 0.04 and 0.05 ± 0.05) (Fig. 2.7A) and after 10 min (0.44 ± 0.05 and 0.06 ± 0.07) (Fig. 2.7B).

Significant avoidance of electric fields continued to be exhibited with the application of the higher voltages (2 kV and 3 kV) according to regression analysis. For example, after 5 min ($F_{1, 6} = 21.4$, $P = 0.004$, $y = 0.023 + 0.183x$). However, there were no significant differences between responses at these potential voltages (1.5 kV, 2 kV and 3 kV) ($P > 0.05$ in all cases).

After 15 min of exposure, *Drosophila* avoided the electric field at 1kV (34-43 kV/m). A *post-hoc* analysis showed a significant difference between 0 kV and 1 kV (-0.02 ± 0.10 and 0.39 ±0.06, P = 0.015) (Fig. 2.7C). At 20 min *Drosophila* avoided these fields only when they were exposed to the application of 0.5 kV (26-34 kV/m). There was a significant difference between 0 kV and 0.5 kV (-0.04 ±0.08 and 0.28 ± 0.07, P = 0.04) (Fig. 2.7D). Additionally, the data demonstrated that there were no significant differences of avoidance to electric fields between the highest voltage (3 kV) and the other voltages (1 k, 1.5 kV and 2 kV) when flies were exposed for 15 min and 20 min ($P > 0.05$ in all cases).

To summarize, the results show that *Drosophila* avoided static electric fields. This avoidance compared to controls was observed within 5 min at application of 1.5 kV (43-95 kV/m) potential as a threshold field. However, 20 min of exposure demonstrated that flies were able to avoid the electric fields at 0.5 kV (26-34 kV/m), suggesting that some flies who reached the decision area of the Y-tube made a later decision to avoid these fields. Care should be taken with this interpretation as the control values dropped slightly over time. Direct comparison of response indices within voltage treatments at the different times indicated no significant differences. In conclusion the optimum time to establish avoidance behaviour was established as 5 minutes by which most flies are able to avoid electric fields.

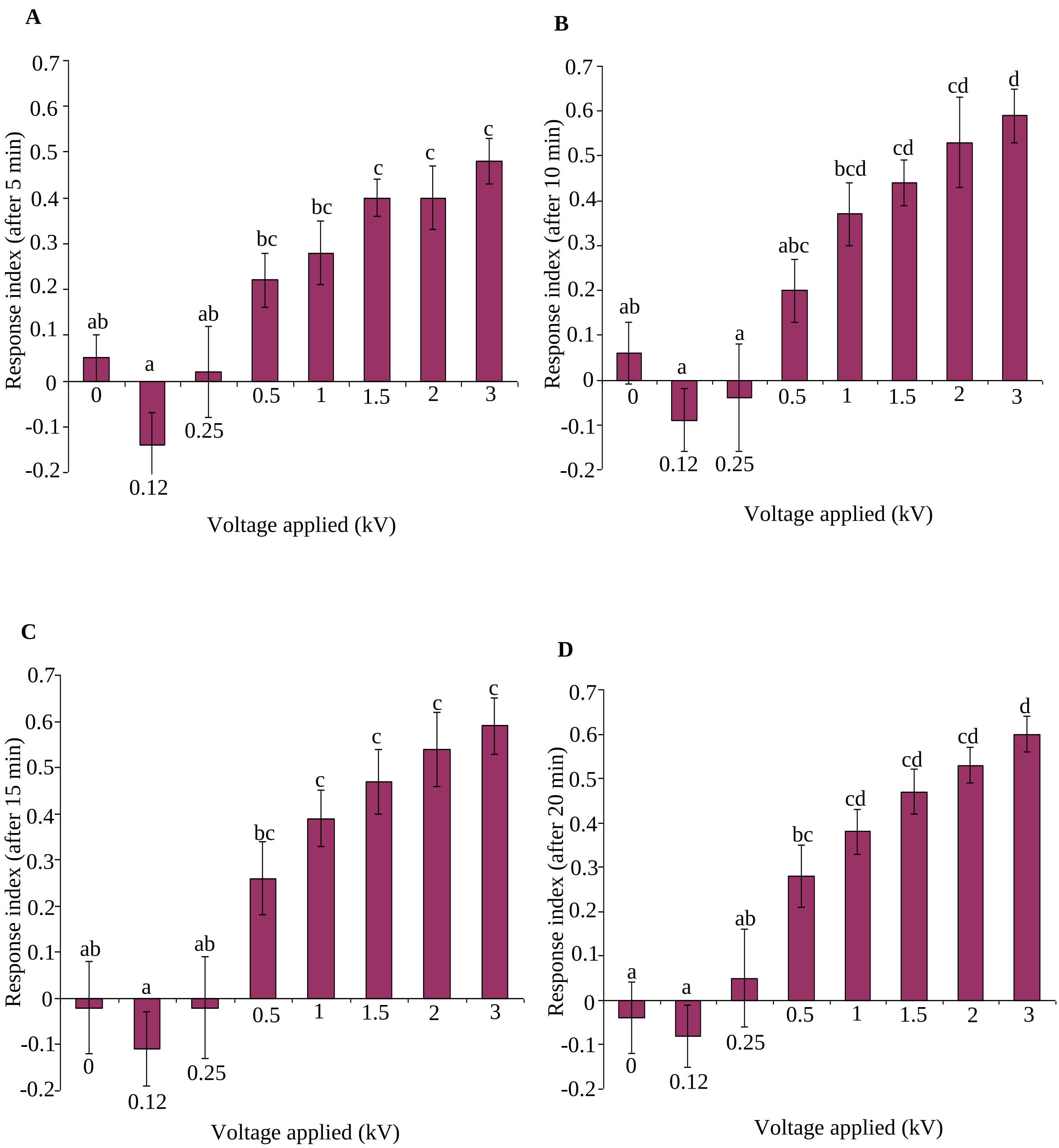

Figure 2.7 The avoidance behaviour of *Drosophila* to static electric fields at different voltages. (A) After 5 min of exposure, a significant difference was found between control (0 kV) and 1.5 kV (field strength approximately of 43-95 kV/m), P = 0.019. (B) After 10 min, flies also significantly avoided fields at 1.5 kV, P = 0.02. (C) Exposure for15 min demonstrated a significant avoidance at 1 kV (34-43 kV/m), P= 0.015. (D) *Drosophila* showed avoidance at 0.5 kV (26-34 kV/m) when they were exposed for 20 min, P = 0.04. (The same letter is not significant).

2.4 Discussion

Many studies have shown that the avoidance behaviour observed when insects encounter an environmental stimulus, such as predators, is important for the survival and fitness of the insect (Bale, 1993; Meyling and Pell, 2006). The present results show that *Drosophila* avoided electric fields of 26-34 kV/m within 5 min of exposure. Therefore, avoidance of an electric field might also be beneficial. Further, this avoidance indicates that *Drosophila* are able to detect the electric fields, although no evidence showed that insects have evolved electrosensory receptors to detect these fields (Newland *et al*., 2008) as have some aquatic animals (Rasnow, 1996).

2.4.1 Electric field modelling within the Y-tube apparatus

Modelling electric fields within the Y-tube apparatus showed the distribution and magnitude of electric fields and provided data on how flies respond to these specific fields at various strengths. The modelling software had been used in a previous study to calculate the electric field that caused avoidance behaviour in cockroaches (Newland *et al*., 2008). However, other studies did not model the electric fields that led to changes or avoidance behaviour (Perumpral *et al.*,1978; Bindokas *et al*., 1989; Maw, 1961; Watson, 1984). Therefore, it was difficult to determine the threshold field effect on insect behaviour or estimate the quality of those bioassay designs.

In the present study, two designs of theY-tube apparatus have been modelled using Maxwell software, to make a comparison between the distributions of electric fields within those designs. The electric field was distributed around the charged and uncharged arms when Design 1 was used. It was found that there were similar magnitudes of field present at the entrances of both the uncharged and charged arms when different voltages were applied. For example, a 1.5 kV potential generated field strengths of 43-95 kV/m at the entrance of both arms suggesting that this design may not be suitable for testing avoidance behaviour in *Drosophila.*

By contrast, modelling Design 2 of the Y-tube showed localization of the electric fields around the treated (charged) arm with the application of different voltages (0.05 kV, 1 kV and 1.5 kV). That is due to the whole surface of the release arm and the midpoint of the decision area being covered and grounded using an aluminium mesh to reduce the penetration of electric fields to the control arm of theY-tube. A 1.5 kV potential generated a field between 43-95 kV/m at the entrance of the treated arm, but only 26-34 kV/m in the uncharged arm. This indicats that the flies faced forces at the entrance of the treated arm stronger than at the untreated arm and ultimately elicit avoidance behaviour.

In addition, the data did not show significant difference (P > 0.05) between the numbers of flies in the release arm after 5, 10, 15 and 20 min and when several voltages (0 kV, 0. 12 kV, 0.25 kV 0.5 kV, 1 kV, 1.5 kV, 2 kV and 3 kV) were applied. This indicates that the electric field had no effect on flies in the release arm even at the highest applied voltage (3kV), thus providing further evidence of the usefulness of the current design for analysing avoidance behaviour in *Drosophila*.

2.4.2 The avoidance behaviour in response to electric fields

Many studies have shown that insects have evolved to detect and avoid several types of external stimuli in the environment. This could be beneficial for survival and fitness (Bale, 1993; Meyling and Pell, 2006). In the current study, *Drosophila* exhibited avoidance to electric fields stimuli. Therefore, these fields could represent one of the external stimuli that might affect insects and therefore, the avoidance should be beneficial for survival. This avoidance behaviour also has been found to increase in relation to the increase in electric field strength. For example, *Drosophila* exhibited significant avoidance behaviour to static electric fields within 5 min with the application of 1.5 kV, corresponding to a modelled electric field strength of 43-95 kV/m as a threshold field. Raising the voltages up to 3 kV showed clear avoidance which confirms that the avoidance is related to the increase in field strength. Significant avoidance was observed early with the application of 0.5 kV when flies were exposed to the electric field (26-34 kV/m) for 20 min, suggesting that some flies might make a delayed decision to avoid these lower electric fields particularly, those flies in the decision area of the Y- tube.

Although avoidance of the electric fields confirms that *Drosophila* are able to detect these fields, no evidence has yet been found to show that terrestrial insects have evolved specialised electrosensory receptors for the detection of electric fields such as those reported for aquatic animals (Newland, 2008). For example, gymnotiform fish live in murky water, and have evolved to produce and sense electric fields that are used for orientation, navigation, communication and for catching prey (Rasnow, 1996). Besides that, water is a much better electrical conductor than air (Fugere, 2010; Bastian, 1994).

In terrestrial invertebrates, particularly insects, previous studies have shown that specific appendages move in response to electric fields. For example, the wings of *Drosophila* vibrate when exposed to a field strength of 500 kV/m (Watson *et al*., 1997) and the antennae of bees appear to be deflected by ELF electric fields of 150 kV/m (Bindokas, 1989). However, those two studies did not measure the avoidance behaviour in response to electric fields, as the experimental design was not fixed within a test chamber, unlike in the present study. In addition, those previous studies used extremely low frequency (ELF) electric fields which produce fluctuating electric field, which in turn might cause the appendages to vibrate.

Newland *et al*. (2008) showed that the exposure of cockroaches to static electric fields of 40-50 kV/m causes a deflection of the antenna that ultimately led to the avoidance of electric fields. Perumpral *et al*. (1978) demonstrated that house flies avoided a treated chamber with only high static electric fields (100 kV/m) compared to low (50 kV/ m). However, the present study showed that between 43-95 kV/m were required to cause *Drosophila* avoidance behaviour after 5 min of exposure. Furthermore, both cockroaches and house flies support the notion that insects in general detect and avoid electric fields. However, they are not suitable for further research to investigate the possible effects on genes or fitness, as they are not model organisms, whereas *Drosophila* have been widely used in experiments where they were exposed to various environmental stressors (thermal, chemical, starvation) to measure detrimental effects (Huang and Chen, 2007; Nazir *et al*., 2003)

A recent study by Matsuda *et al* (2011) also showed that *Drosophila* avoid static electric fields when they approach a charged screen at 0.3kV as a threshold potential. This study suggested using electric fie1ds for protecting the crops. However, the electric field that caused avoidance behaviour was not modelled to understand their strength.

It is known that the natural electric fields in the environment range between 0.1 and 0.3 kV/m (Adlerman and Williams, 1996; Bering *et al*., 1998). Thus the electric fields experienced by insects in their environment is less than those used in our experiments (26 kV/m and above) to elicit avoidance behaviour, except under unusual circumstances such as close to electricity transmission lines (Orlve, 1990).

The low field in the natural environment might explain why insects have not evolved receptors to detect electric fields. This became clear when a high voltage power line of 400 kV was modelled using Maxwell software as the majority of previous research only calculated the electric field at ground level, which is between 9-11 kV/m (Dezelak, 2010). Therefore, the simulation modelling below shows the gradient of electric fields from power lines to the earth's surface, which suggests that *Drosophila* would avoid directly the fields close to power lines (1.5 m away from operating wires) (Fig. 2.8). This corresponds with field strength (43-95 kV/m) observed in avoidance within the Y-tube apparatus when flies exposed for 5 min, and is supported by previous studies that noted that a field strength greater than 20 kV/m caused changes in insect behaviour (Perumpral *et al*,1978; Orlve, 1990; Newland, 2008).

This Chapter has shown that *Drosophila* are able to detect and avoid static electric fields. How *Drosophila* detect the static electric fields and the influences of field forces on *Drosophila* wings will be analysed in the next Chapter.

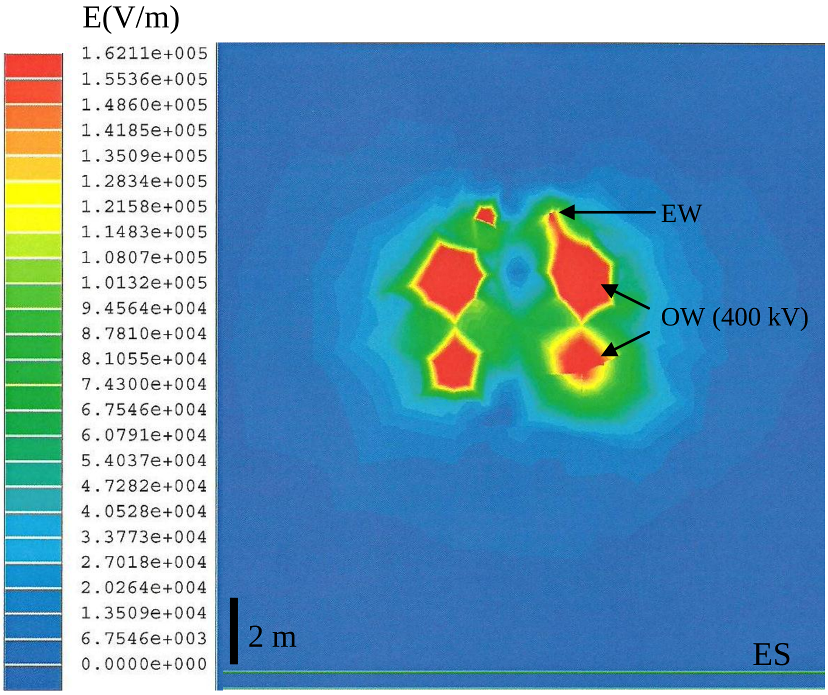

Figure 2.8 A model of the electric fields underneath transmission lines at 400 kV with ground clearance of 8 m. The present study suggests that electric field strength between 43- 95 kV/m (coloured bright blue to green) under these lines can be avoided by *Drosophila*, which corresponds with field strength used within the Y-tube apparatus (EW: Earthed wire; OW: Operating wire; ES: Earth surface).

Chapter 3

The detection of static electric fields

3.1 Introduction

The preceding Chapter showed that *Drosophila* exhibited avoidance behaviour to static electric fields. This avoidance behaviour was dependent on field strength, and the threshold level of the fields that cause avoidance was determined. This chapter aims to determine how the *Drosophila* detect these electric fields.

Many studies have shown that a large number of animals have evolved the ability to detect forces such as the Earth's magnetic and electric fields (Kalmijn, 1971; Lohmann, 2006). For example, the existence of magnetite particles (Fe_3O_4) in the heads of social insects and birds are thought to be involved in the detection of magnetic fields (Wiltschko *et al*., 2002; Wajnberg *et al*., 2010), and these animals are capable of using these fields for orientation, foraging and migration (Jones and McFadden, 1982; Wajnberg *et al*., 2010).

It has also been reported that some aquatic organisms have evolved the ability to generate and detect electric fields via an electric organs (Kramer, 1996). This sense has evolved to enable animals to orientate and navigate in murky water with poor visibility (Fugere and Krahe, 2011). For example, gymnotiform fish have electric organs located in their trunk and tail. These organs consist of excitable cells, known as electrocytes, which are modified from muscle cells (Kramer, 1996). The electrocytes generate a potential difference across the cell membrane, causing a current follow, ultimately discharging an electric field (< 100 μV/cm), called an electric organ discharge (EOD), into the surrounding water, forming fields lines around their bodies (Kramer, 1996; Rasnow, 1996)**.** These fish can therefore sense any alteration that might occur in these fields, allowing them to determine the shape of an object, communicate with other electric fish, catch prey or escape from predators (Fugere and Krahe, 2010; Bastian, 1994). In addition, other aquatic organisms, such as sharks and rays, have evolved the ability to detect weak electric fields (< 5 nV/cm) in the surrounding water generated by muscular contractions of the other animals during swimming using an electroreceptor, known as an ampullary organ, which is distributed within the lateral lines of the animals' head (Adair *et al*., 1998; Stoddard and Markham, 2008).

A recent study also found that crayfish showed changes in their behaviour in response to electric fields of 400 μV/cm in the surrounding water, suggesting that these animals could have an electric sense to detect electric fields (Patullo and Macmillan, 2010).

The evolution of the detection of electric fields, particularly in aquatic organisms, has raised the possibility that terrestrial invertebrates, such as insects, have a similar electric sense. However, evidence has yet to be shown whether insects have evolved specific electrosensory receptors to detect electric fields (Newland *et al*., 2008) or whether electric fields are detected through other means.

Insects possess many types of receptors that respond to environmental stimuli such as smell, sound and heat (Stortkuhl *et al*., 2005; Fullard *et al*., 2008; Cang *et al*., 2006). In particular mechanical stimuli can be detected by the activation of mechanoreceptors that are distributed on the different body parts of insects (McIver, 1975; Chapman *et al*., 1998). Hiraguchi and Yamaguchi (2000) showed that stimulating the tactile hairs on the hind wings of crickets leads to escape behaviour. Similar responses also occur after tactile stimulation of the antennae of crickets (Gebhardt and Honegger, 2001). Touching the cockroach antennae by predators (Comer, 1994) and movement of hairs on the cerci by wind also contribute to escape behaviour (Dagan and Camhi, 1979). The response to wind movement has been also observed in locusts when the filform hairs of the cercal were displaced (Boyan and Ball**,** 1989).

It has been suggested that electric fields could be detected via electrical forces causing deflection of sensory appendages resulting in mechanical stimulation (Newland *et al*., 2008). It is known that any electrically neutral object has a random distribution of negative and positive charges over the surface. When an object enters an electric field region, it will experience forces on the electrons in that object leading to an uneven distribution (polarisation) of electric charges. These electric forces can also generate physical movement of the whole object towards or away from the electric field region as a result of an attraction between unlike charges or repulsion between like charges (Bhatnagar, 1993; Ellse and Honeywill, 1998). For example, deflection of the antennae in cockroaches as a result of exposure to static electric fields of 40-50 kV/m led to the activation of mechanoreceptors (scape hairs) and ultimately caused avoidance behaviour (Newland *et al*., 2008). Other studies have shown that the sensory appendages of insects can be influenced by electric forces when they were exposed to both static electric fields and extremely low frequency (ELF) electric fields. For example, vibration occurred in the wings of *Drosophila* when exposed to field strengths of 500 kV/m (Watson *et al*., 1997). A similar response was shown by bees' wings and antennae when exposed to ELF electric fields of 150 kV/m field (Bindokas *et al*., 1989). Other studies found that the antennae in bees started to move when exposed to static electric fields of 95 kV/m field (Yes'Kov and Sapozhnikov, 1976) and in the Ichneumon fly, *Itoplectis conquisitor*, when it walked over a charged surface (Maw, 1961).

Humans also show responses to electric fields. Movement of hairs on the forearms and hands of humans has been observed when subjects were exposed to static electric fields of 45 kV/m (Blondin *et al*., 1969; Chapman *et al*., 2005). Removing the hairs from a human subject's arm leads to a reduction in the ability to recognise the fields, suggesting that the hairs are responsible for the detection of the electric fields (Chapman *et al*., 2005).

In conclusion, previous studies have shown that electric fields cause deflection of the insects' sensory appendage and human body hairs by electric fields. This Chapter therefore will focus on the effect of field strength on wing movement and how the wing might play a key role in the detection and avoidance of electric fields in *Drosophila*.

3.2 Materials and Methods

3.2.1 Flies

Three groups of flies (*Drosophila melanogaster)* were exposed to static electric fields at different voltages using, the Y-tube apparatus (described in Chapter 2) to assess the role of the wings in the detection and avoidance of electric fields. These groups were:

A) Cut wing flies (wild-type flies with wings physically removed)

The same strain of wild-type flies as described in Chapter 2 was used. However, before testing the avoidance behaviour to electric fields, the flies were gently anaesthetised using CO_2 and their wings removed (Fig. 3.1A) using fine scissors (McPherson-Vannas) (80 mm length, 5 mm blades, 0.1 mm tips) and forceps (0.01 mm thick, 0.05 mm width, 110 mm length). The flies were then grouped into 5 tubes (50 ml), each group consisting of 20 flies (males and females were randomly selected). They were kept in these tubes for 24 h to recover with the addition of a small piece of wet tissue placed in the tube to prevent desiccation. Each group was exposed to an electric field for 20 min at different voltages (0 kV, 0.5 kV, 1 kV, 2 kV and 3 kV) following the same procedures outlined in Chapter 2. The flies were observed every 5 min and the numbers of flies in each arm (charged and uncharged) of the Y-tube were counted. This experiment was repeated 6 times with a total number of 600 flies.

B) Vestigial wing flies

Vestigial winged mutant strains were obtained from Blades Biological Ltd., Kent (Fig. 3.1B). These flies were reared in the same way as the wild-type flies (described in Chapter 2). The vestigial wing flies have smaller wings than wild-type flies and are irregular in shape. The experimental procedure for the vestigial wing flies and the number of flies used was the same as for the cut wing flies (above).

C) Wild-type flies

Wild-type flies of both sexes (Fig. 3.1C) were also tested as wing size is known to be different between the sexes, with males having smaller wings (Gilchrist *et al*., 2001). Male and female flies were exposed separately to static electric fields. In each experiment 20 flies (one group) were tested at each voltage (0 kV, 0.5 kV, 1 kV, 1.5 kV and 2 kV) for 5 min. This experiment was repeated 6 times for each of the sexes, the total number was 600 male flies and 600 female flies respectively.

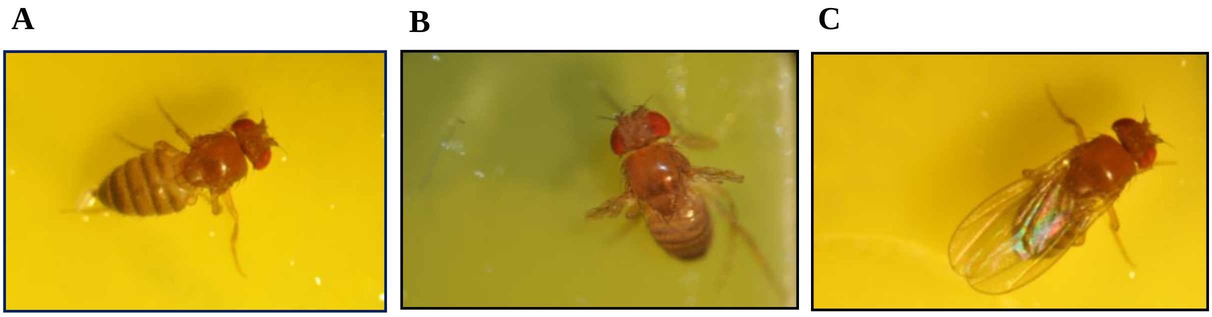

Figure 3.1 Three types of flies were exposed to static electric field to assess the role of the wings in the detection of electric fields and avoidance behaviour. (A) cut wing wild-type *Drosophila* (B) vestigial wing flies and (C) wild-type flies.

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3.2.2 Measuring wing displacement

To measure the direct effects of static electric fields on wing deflection, flies were placed in an apparatus consisting of a chamber (tube) of glass, 2 mm thick, 100 mm x 30 mm (L x D), fixed in a metallic holder. A copper ring electrode 4 x 28 mm (W x D) was placed near the entrance (8 mm) to the chamber which was attached to an insulated socket through a small hole (7 mm) in the glass. This electrode was connected to a DC power supply (Brandenburg Alpha III, Brandenburg, UK) to generate static electric fields (Fig. 3.2).

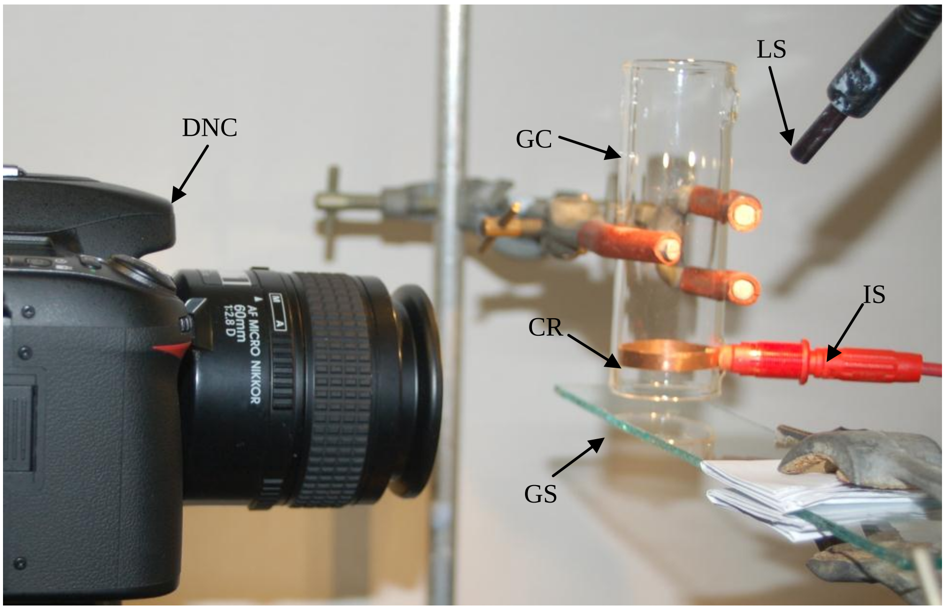

Figure 3.2 The set-up for wing measurement. A glass chamber (100 mm x 30 mm) was fixed in a metallic holder; several photographs were taken at different applied voltages using a digital Nikon camera (DNC). (GC: glass chamber; CR: copper ring; GS: glass slide; IS: insulated socket; LS: light source).

Eight male and 8 female flies were tested individually. First, each living fly was fixed on a glass slide underneath the edge of the charged electrode of the glass chamber (14 mm distance between the electrode and glass slid) using yellow sticky paper (EasiStick' Traps, Fargro Ltd.) and then exposed to static electric fields at different voltages. The fly was then killed using CO_2 by squashing the head and again tested (to compare resistance and response to electric fields between live and dead flies and investigate whether that movement of wing in response to static electric field is uncontrolled among live and dead flies). Flies were photographed at each voltage using a Nikon digital camera (D80, micro-60 mm) using illumination from a cold light source (Schott- kl 1500) to measure wing displacement. All photographs were downloaded onto a computer (Dell-Windows XP), and the Canvas program (ACD system for windows), version 11 was used to calculate the wing angle for each individual voltage by drawing two lines; the first line extended from the mid-point of the head to the base point of the wing and the second between the tip of the wing and the base point of the wing (Fig. 3.3).

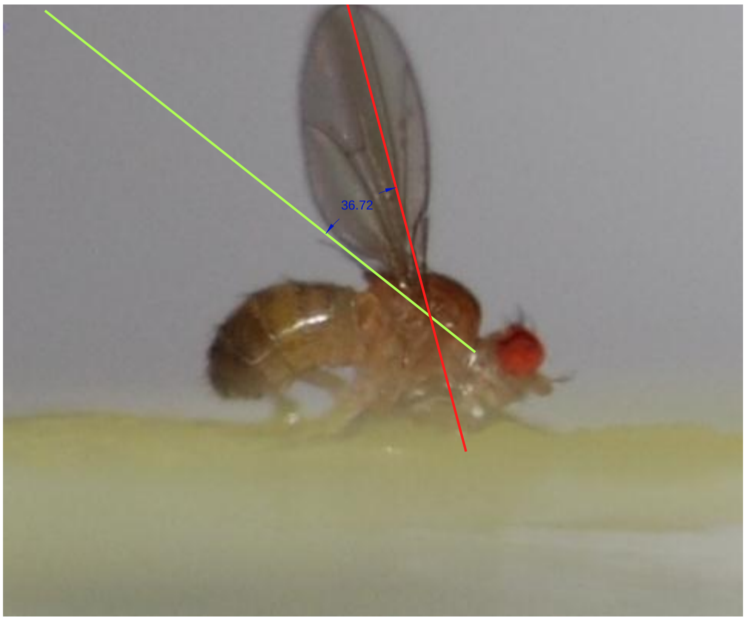

Figure 3.3 Determination of wing angle. Two lines were drawn to the wing base using Canvas to calculate the wing angle. The first (green) line passed between the mid-point of the head and the base point of the wing whereas the second (red) line extended from the tip of the wing to the base point of the wing.

In addition, individual fly wings (male and female separately) were removed and placed on a glass slide to measure the field strength required to raise the wing toward the charged electrode (copper ring). After each trial images of the excised wing were collected (Fig. 3.4) using a compound microscope (Zeiss Axioplot) with a digital camera (Roper Scientific RTE/CCD-1300-y). The width and length of the wings were measured using MetaMorph Imaging Software (Version 6).

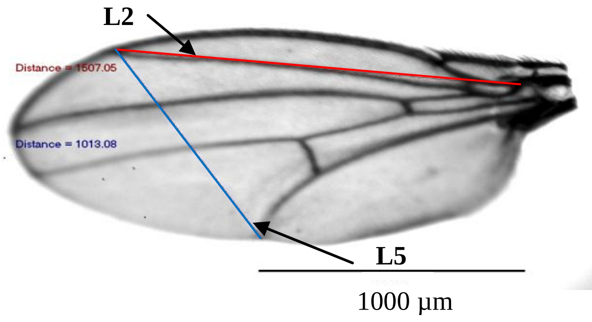

Figure 3.4 Measurement of the length and width of *Drosophila* wing. The red line represents the length to the L2 wing vein (from the tip of the wing to the crossveins) and the blue line represent the width (from the tip of L2 wing vein to L5) (Wolf *et al*, 2000).

3.2.3 Electric field modelling

Maxwell SV two dimensional software was used to model the experimental chamber design and to visualise the spatial distribution and magnitude of electric field strength based on dimensions, material properties and applied voltages. The glass chamber was drawn in ZR view as a symmetric design. Z represents the Z axis and R the rotation around the Z axis. Each part of the design was assigned to its appropriate material and the boundary values were designated depending on the applied voltage, while the glass slide was connected to a ground electrode to limit electric field distribution (Fig. 3.5). The modelling was also used to provide the field strength at the point where an excised wing was placed.

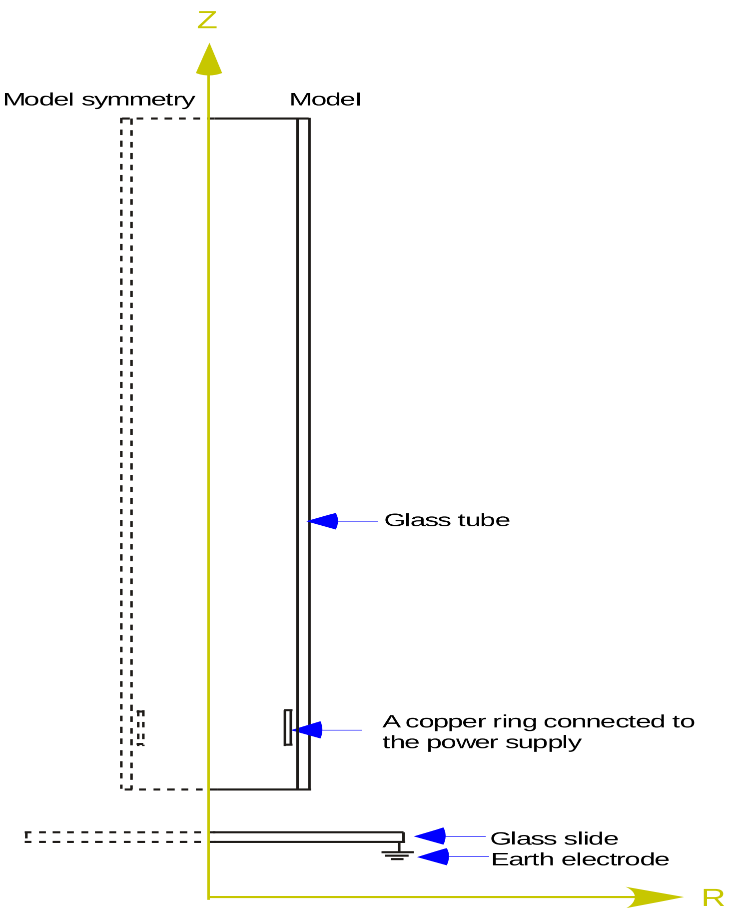

Figure 3.5 The experimental chamber was drawn as a ZR model in Maxwell SV software for electric field calculation. A cross-section was taken from the centre of the chamber (solid line), representing symmetrical design. (Z: Z axis and R: Rotational around Z axis)

3.2.4 Statistical Analyses

The number of flies (wild-type flies with cut wing, vestigial wing and wild-type flies; male and female) was counted in the left and right arms of the Y-tube and analysed using the same statistical tests as in the previous Chapter, to determine the threshold of avoidance of the electric fields when different voltages were applied. Further, a one-way ANOVA with *post hoc* Tukey-test was used to investigate whether there was a significant difference in the avoidance behaviour between wild-type male and female flies.

Three-factors repeated measures ANOVA with *post hoc* t-test were used to compare between the wing angle in male and female live and dead flies at different voltages (0 kV, 0.5 kV, 1 kV, 2 kV, 3 kV, 4 kV and 5 kV). In addition, t-tests were used to investigate whether the field strength that raised excised wings in males and females was significantly different. The Data was tested for normality and homogeneity and data transformation was not required.

3.3 Results

3.3.1 Avoidance behaviour

Drosophila (wild-type flies with cut wing and vestigial wing) were tested to assess the role of the wing in the detection and avoidance of static electric fields. Flies were exposed to a 0-3kV electric field for up to 20 min using the Y-tube apparatus. In all avoidance experiments in this chapter, the number of replicates was limited to 6 due to time constraints in preparing the flies. This has reduced the statistical power. Further replication in the future could help clarify the significance of avoidance effects.

3.3.1.1 Cut wing wild-type flies

The results showed that, after 5 min, the flies with cut wings (n = 6) did not significantly avoid electric fields even with the application of 3 kV, corresponding to a modelled electric field strength of 95-164 kV/m ($F_{4, 25}$ = 1.21, P = 0.33) (Fig. 3.6 A). However, avoidance was observed after 20 min of exposure to electric fields only at 3 kV ($F_{4, 25}$ = 5.68, P = 0.02). There was a significant difference between control (0 kV) and 3 kV ((mean ± SEM (0.21 ± 0.17 and 0.77± 0.11 respectively)) (Fig. 3.6B).

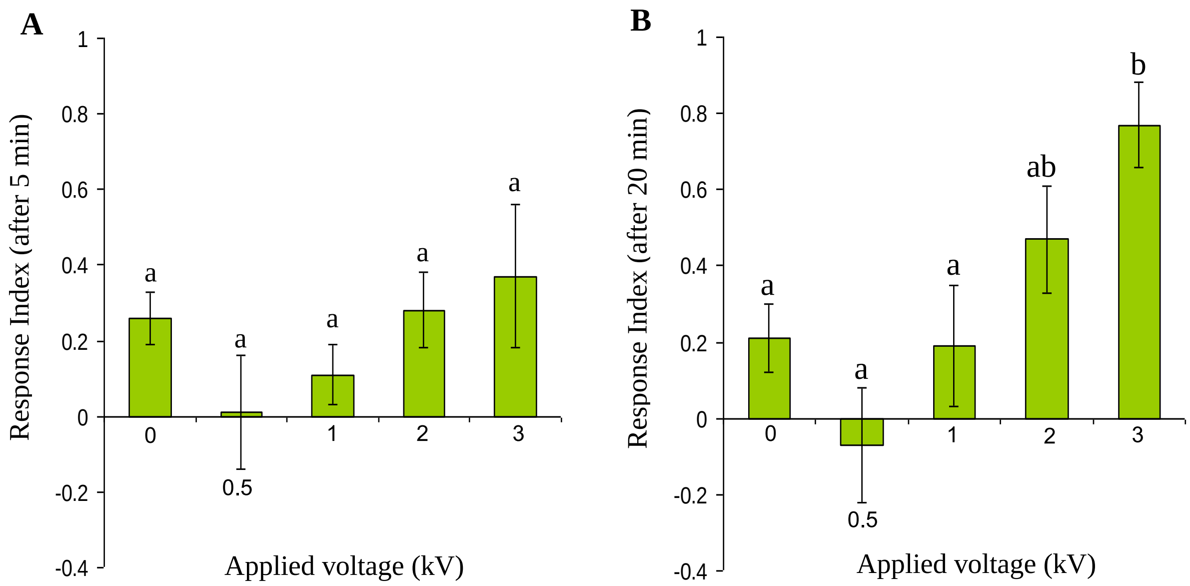

Figure 3.6 Avoidance behaviour of *Drosophila* (cut wing flies) to static electric fields at different voltages. After 5 min (A) of exposure, the results showed no significant difference between the response index value at control (0 kV) and other voltages (P > 0.05 in all cases). After 20 min (B) the flies avoided significantly electric fields only when 3 kV (95-164 kV/m) was applied.

3.3.1.2 Vestigial wing flies

The vestigial winged flies (n = 6) showed significant avoidance at both 5 min (Fig. 3.7A) and 20 min (Fig. 3.7B), when electric fields of 2 kV (field strength 52-104 kV/m) and 3kV (95-164 kV/m) were applied ($F_{4, 25}$ = 4.87, P = 0.005) and ($F_{4, 25}$ = 4.42, P = 0.004). The mean response values and SEM after 5 min at 0 kV and 2 kV were -0.14 ± 0.13 and 0.38 ± 0.12, respectively, and -0.06 ± 0.14 and 0.39 ± 0.06 respectively after 20 min. Raising the applied voltage to 3 kV also showed a significant response difference when compared to 0 kV, (-0.14 ± 0.13 and 0.46 ± 0.08) after 5 min and (-0.06 ± 0.14 and 0.65 ± 0.12) after 20 min respectively (P < 0.05 in both cases).

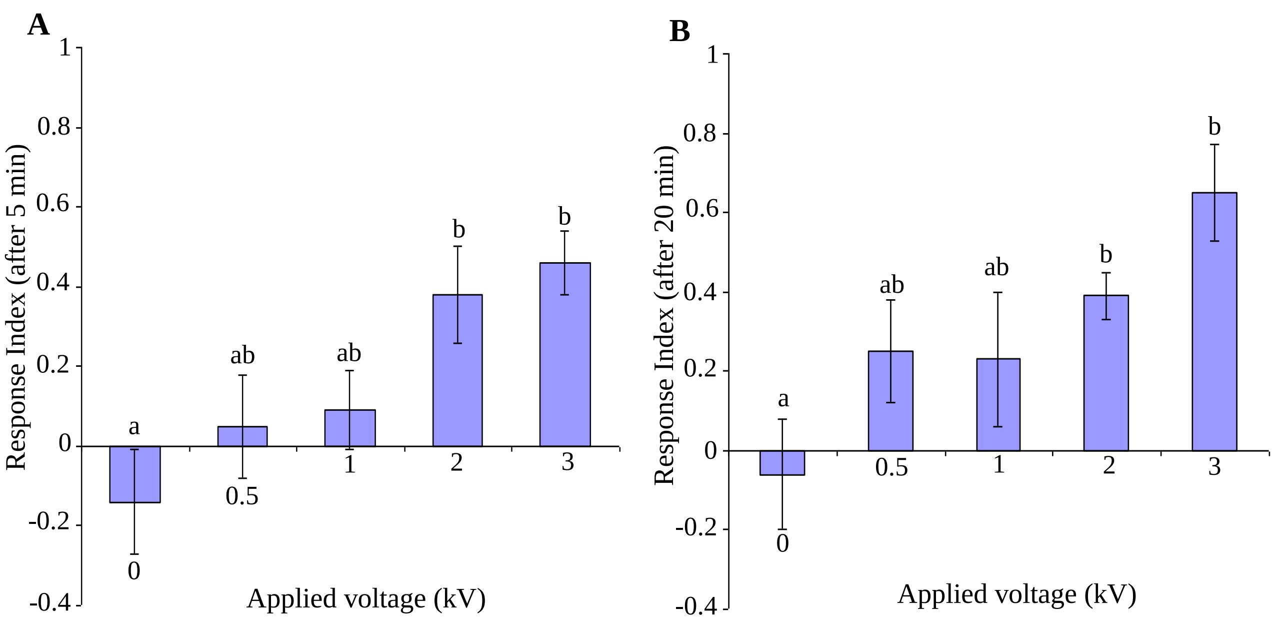

Figure 3.7 Avoidance behaviour of *Drosophila* (vestigial wing flies) to static electric fields at different voltages. After 5 min (A) and after 20 min (B) flies significantly avoided the electric field at 2 kV (52-104 kV/m) and above. There were significant difference in the response index at 0 kV, 2 and 3 kV (P < 0.05 in both cases).

3.3.1.3 Wild-type flies

Separate gender groups of wild-type flies (male and female) were exposed to static electric fields generated at 0 to 2kV for 5 min. These experiments were conducted to provide possible evidence for the role of wings in detecting and avoiding the electric fields. The results showed that both male (n = 6) and female (n = 6) wild-type flies significantly avoided electric fields, ($F_{4, 25} = 6.64$, $P < 0.05$ and $F_{4, 25} = 20.41$, $P < 0.05$).

Post hoc analysis showed that the threshold for avoidance behaviour, in both male and female wild-type flies when 1 kV (field strength of approximately 34-43 kV/m) was applied to the electrode. The mean value of the RI (Response Index) was 0.59 ± 0.06 in males and 0.52 ± 0.05 in females, compared to 0.07 ± 0.14 in male and -0.10 ± 0.10 in female at 0 kV. The avoidance behaviour continued to be exhibited with the application of higher voltages (1.5 kV and 2 kV) (Fig. 3.8A, B). However, no statistically significant difference was found between male and female flies in terms of avoidance of the electric fields ($F_{1, 60} = 0.808$, $P = 0.37$).

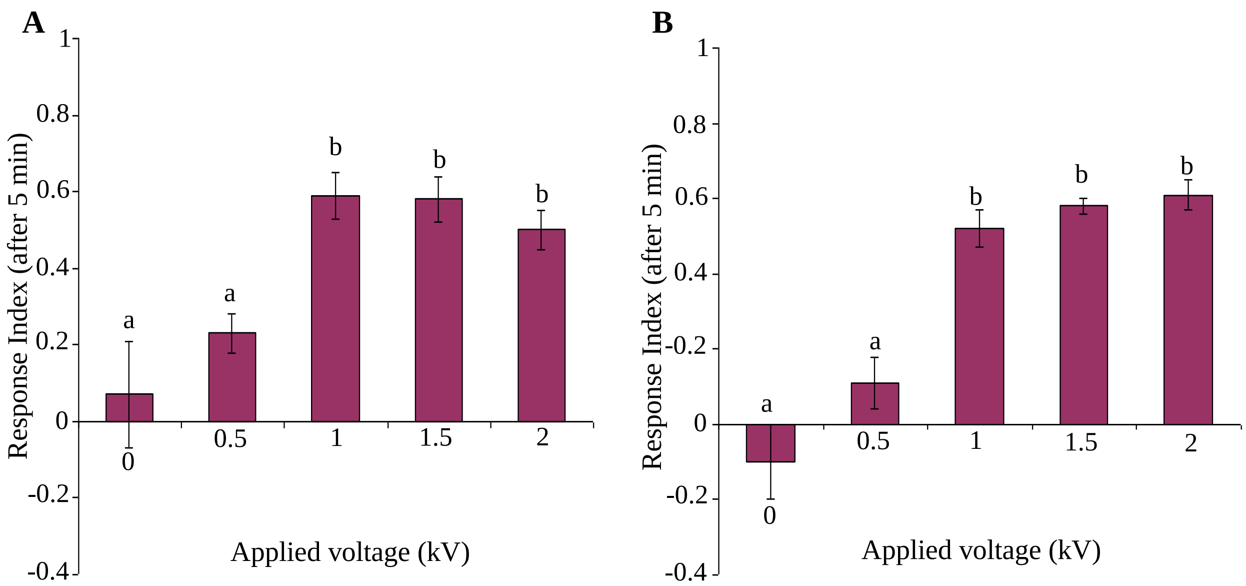

Figure 3.8 Avoidance behaviour of wild-type male and female flies to static electric fields at different voltages. Males (A) and females (B) showed a significant avoidance behaviour to electric fields at 1 kV, compared to control at 0 kV ($P < 0.05$ in both cases). There were no significant differences between male and female flies ($P > 0.05$ in all cases).

To summarize these findings, the results showed that the wild-type flies with removed wings did not avoid the electric field when different voltages (0.5 kV, 1 kV, 2 kV and 3 kV) were applied during the first 5 min of exposure. However, testing this type of fly for 20 min resulted in significant avoidance behaviour at 3 kV (95-164 kV/m). ($P < 0.05$). Vestigial winged flies showed significant avoidance when exposed to electric field at 2 kV (52-104 kV/m) and above after 5 min and 20 min of exposure. Both males and females showed avoidance behaviour at 1 kV (34-43kV/m) and above after only 5 min. There were no differences between the sexes.

3.3.2 Static electric fields and wing deflection

Since avoidance appears to be greater in flies with intact wings, it is possible that the wings of *Drosophila* may play a key role in the detection of static electric fields. Flies were therefore placed underneath a charged electrode using the glass chamber to investigate whether the wing was displaced by static electric fields. Moreover, the wing displacement of male and female flies was analysed to test the hypothesis that the wing plays a role in the detection and avoidance of electric fields.

To understand the distribution of electric fields and to estimate the actual field strength, it was necessary to model the apparatus using Maxwell SV software. Modelling of the static electric field within the glass chamber apparatus showed a maximum value adjacent to the copper ring electrode of 183 kV/m at 2 kV (Fig. 3.9 A). However the electric field strength around the fly's position was between 57 and 96 kV/m, when a 2 kV potential was applied. This field increased gradually around the fly's position with the application of greater voltages. For example, at 3 kV, the field strength was between 96 and 115 kV/m, at 4 kV between 125 and 134 kV/m and at 5 kV between 163 and 183 kV/m, as shown in Figure 3.9 (B-D).

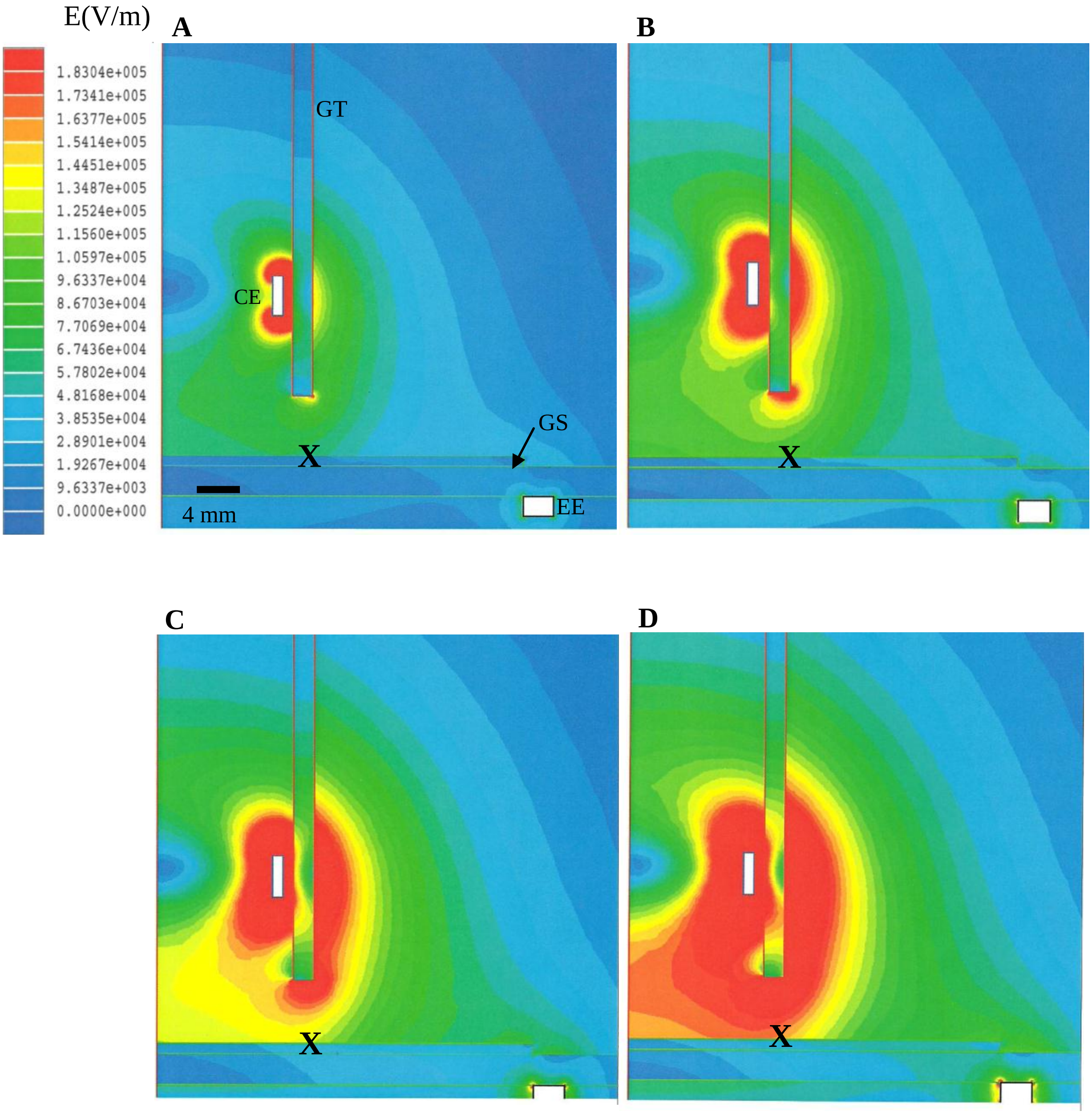

Figure 3.9 The magnitude and distribution of static electric fields around the charged electrode (CE) at different voltages was modelled using Maxwell SV software. (A) 2 kV was applied to the charged electrode causing a field strength between 57-96 kV/m around the fly's position (X). (B) Raising the applied voltages to 3 kV increased the field strength to 96-115 kV/m. (C) and (D) show an increase in field strength around the fly to 125-134 kV/m at 4 kV and 163-183 kV/m at 5 kV. (GT: glass tube; GS: glass slide; EE: earth electrode).

3.3.2.1 The effect of static electric field on intact wings

To measure the effect of static electric fields on intact wing deflection. Separate male and female flies were placed underneath a charged electrode with the application of different voltages (0-5 kV). The tested flies were then killed and tested again to clarify that movement of wing resulted in response to static electric field is uncontrolled.

The results demonstrated that the wings of flies (n = 8) were significantly affected by increasing the electric field strength ($F_{6, 196}$ = 230.80, P = 0.001), with the greater field strength causing a greater displacement (Fig. 3.10).

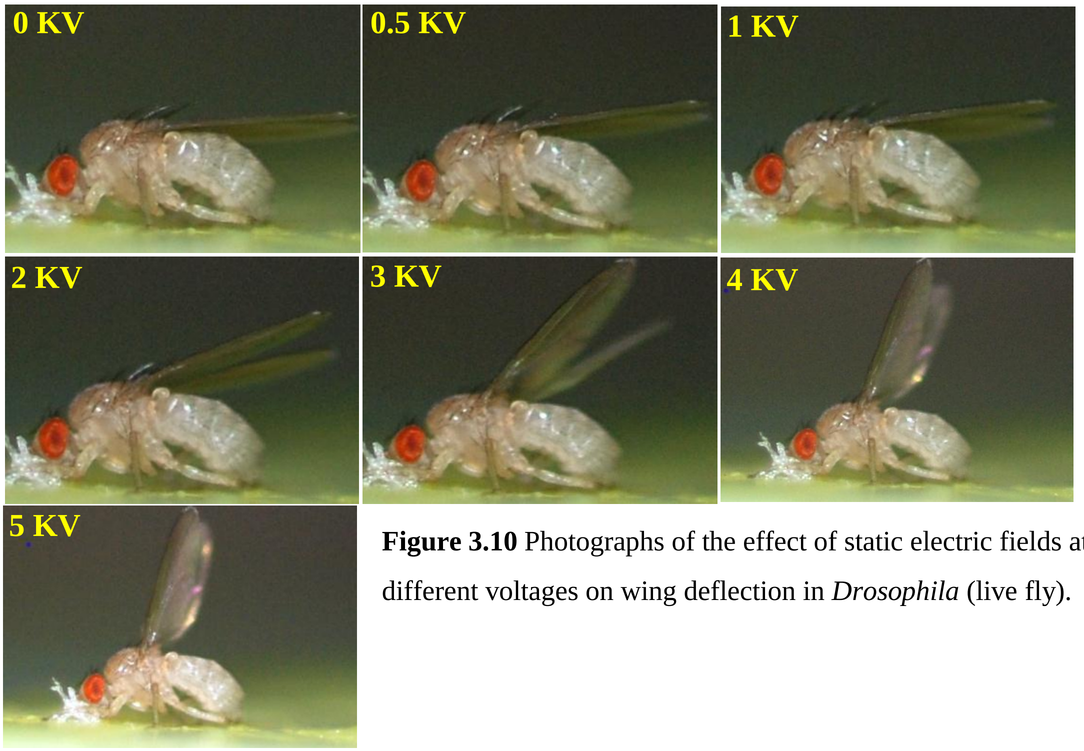

Figure 3.10 Photographs of the effect of static electric fields at different voltages on wing deflection in *Drosophila* (live fly).

Post hoc t-tests showed no significant difference in wing elevation between control (0 kV) and the low voltages (0.5 and 1 kV) (P > 0.05 in both cases). However, applying 2 kV (57-96 kV/m) and above caused significant wing elevation in males (live and dead) when compared to 0 kV (P < 0.05 in all cases). In females (live and dead) a significant wing elevation occurred at 3 kV (96-115 kV/m) and above. There was also significantly greater elevation in live males (14.48 ± 1.90), compared to live females (20.44 ± 4.20) when 5 kV (field strengths of 163-183 kV/m (P < 0.05) was applied. However, the results did not show significant differences in wing elevation between live and dead flies (P > 0.05) (Fig. 3.11).

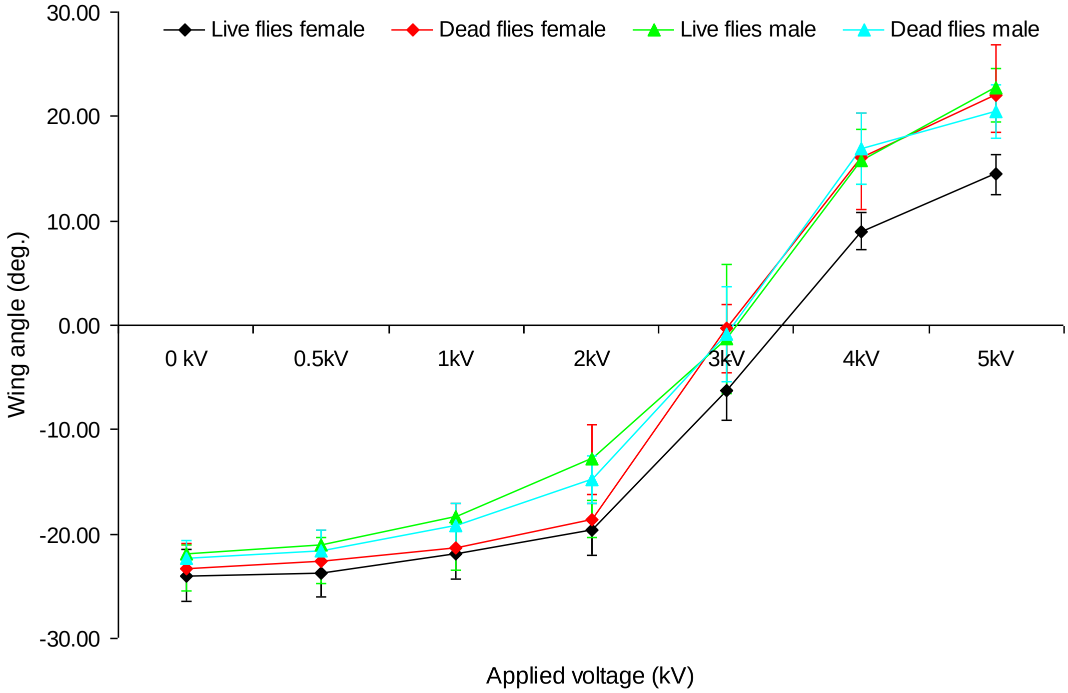

Figure 3.11 The elevation of *Drosophila* wing in response to static electric fields at different voltages. The wings of male flies were lifted significantly at a threshold of 2 kV (57-96 kV/m) compared to the control voltage (0 kV), but at 3 kV (96-115 kV/m) for females. At 5 kV (163-183 kV/m) there was a significant difference but the angle of the wings in live male and female flies. The wing angle of dead and live flies did not show significant differences ($P < 0.05$).

3.3.2.2 The effect of static electric fields on excised wings

Excised wings were exposed individually to static electric fields at different voltages to determine the threshold of field required to lift these wings and whether there are differences between males and females. This experiment again required modelling of the apparatus at mean voltage that raised the excised wing in males and females respectively.

The results demonstrated that the male excised wings ($n = 8$) were raised by application of 0.6 kV, corresponding to a modelled electric field strength of 28-38 kV/m (Fig. 3.12A) compared to 1.1 kV (38-48 kV/m (Fig. 3.12B) for the excised wings of females. The results showed that these differences were significant ($p < 0.05$) between males and females using a t-test (Fig. 3.13).

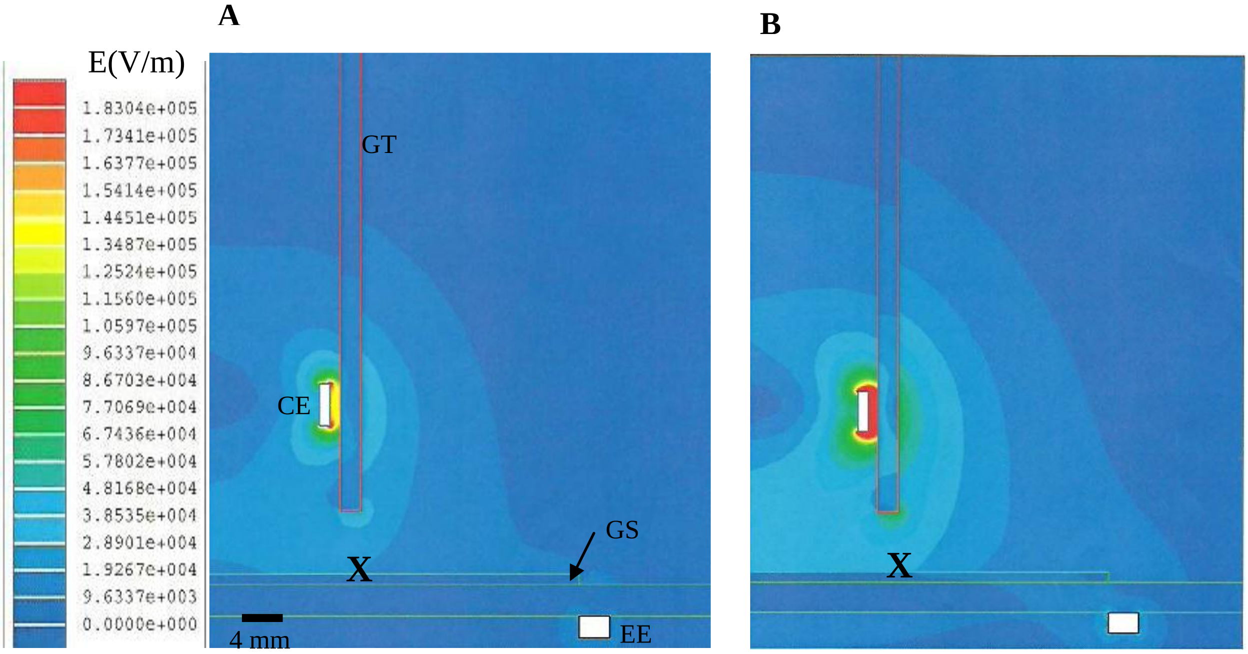

Figure 3.12 Magnitude and distribution of the static electric fields around the charged electrode (CE) at different voltages using Maxwell SV modelling software. (A) Applying 0.6 kV (28-38 kV/m) led to the raising of excised wings (x) in males, whereas (B) Shows that this required 1.1 kV (38-48 kV/m) for females. (GT: glass tube; GS: glass slide; EE: earthed electrode).

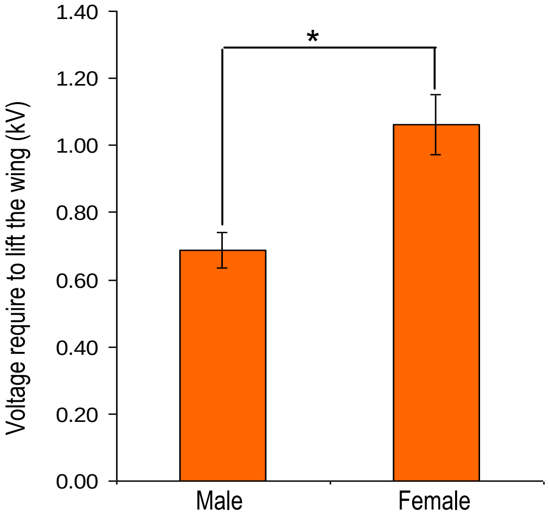

Figure 3.13 Exposure of excised wings to electric fields at different voltages. The mean value of voltage required to lift the excised wing in males was only 0.6 kV (field strength 28-38 kV/m) and in female was 1.1 kV (38-48 kV/m). There was a significant difference between the sexes ($p < 0.05$).

To confirm that the male excised wings required a lower field strength (28-38 kV/m) to be raised in comparison to the female excised wings (38-48 kV/m) and that this was due to a gender difference in wing size. The excised wings' width and length of both genders were measured using MetaMorph Imaging Software (version 6).

These results showed that the wing length and width in males are significantly smaller than the wing in females (Figure 3.14 A&B). Therefore the results were consistent with the hypothesis that smaller male wings require a lower field strength to be moved, as shown in Figure. 3.13.

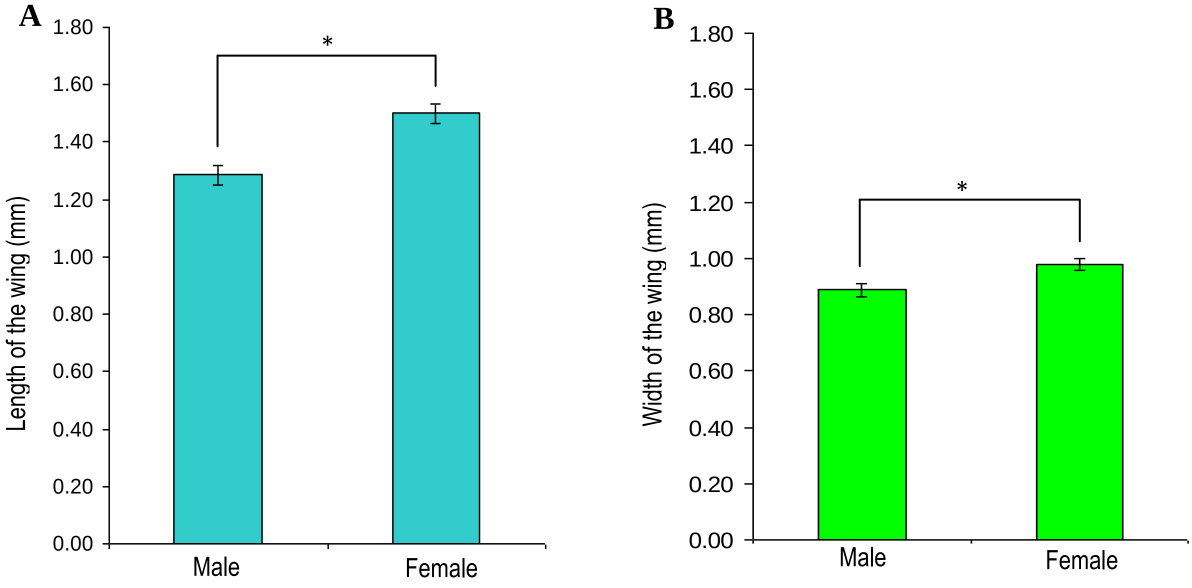

Figure 3.14 Wings measurements in male and female flies. Significant differences between male and female flies were found in terms of the length and width of wings when the t-test was used **(**$p < 0.05$). (A) The mean length of the male wing was 1.29 mm ± 0.03 while in the female it was 1.49 mm ± 0.03. (B) Width of male 0.89 mm ± 0.02 and female 0.98 mm ± 0.02 wings respectively.

3.4 Discussion

3.4.1 The detection and avoidance of static electric fields

The results demonstrate that *Drosophila* wild-type flies significantly avoided static electric fields, indicating that *Drosophila* are able to detect these fields. The results also showed that the wings are displaced by electric field.

These results suggest that the wings are involved in the detection and avoidance of static electric fields by *Drosophila*. For example, after 5 min of exposure, wild-type flies with ablated wings did not avoid the static electric field, even at an applied voltage of 3 kV (95-164 kV/m), while intact wild type flies avoided the static electric field at only 1kV (34-43 kV/m). Vestigial winged mutant *Drosophila* were also tested and results indicated that the vestigial winged flies exhibited a threshold avoidance when 2 kV (52-104 kV/m) was applied. Thus, the avoidance appears to be greater in flies with larger wing.

This may indicate that the intact wing of wild-type flies were subject to attractive forces by the presence of the electric field (Bhatnagar, 1993; Ellse and Honeywill, 1998), leading to a passive raising of the wing towards the copper ring electrode. Movements of the wing are detected by mechanoreceptors such as campaniform sensilla, tegula, and stretch receptors (Daly *et al*., 1998; Elson, 1987; Wolf and Pearson, 1988; Siegler and Burrows, 1986) located in and on the wing. The sensory neuron then transmits this information to the ganglion. Locally, responses are processed by interneurons and motor neurons that control the wing muscles (Gullan and Cranston, 2005). However, intersegmental interneurons also receive stimulus input from the wing, which is ultimately transmitted to the leg muscles to produce a response (Matheson, 2002). For example, in the locust, Matheson (1997) found that tactile stimulation of the wing in locusts caused a scratching movement of one or both hind legs.

Since the vestigial wing is small and twisted compared to wild flies, and lacking a flat surface to hold the electric charge, this could lead to a reduced attraction of the wing by electrical field forces and ultimately less likelihood of activation for the mechanoreceptor located at the base of the wing to detect these movements. This reduced response by the vestigial flies could also be compounded by the fact that these mutants show a pleiotropic degeneration of mechanosensory cells during wing development (Fristrom, 1964), causing low numbers of campaniform sensilla in the wings (Inestrosa *et al*., 1987), and this could lead to an abnormal connection between the mechanoreceptors and their targets that could result in the failure to detect movement of the wing when the flies were exposed to electric fields.

Wild-type male and female flies were tested to evaluate the effect of the wing size within the Y-tube bioassay. The results showed no significant difference in avoidance between males and females, although the size of the female wing is bigger than the male (Gilchrist *et al*., 2001). Both males and females avoided the electric field when 1 kV (34-43 kV/m) and higher was applied to the charged electrode of the Y-tube. This might be explained through the variability in the flies' position around the charged electrode.

3.4.2 The effect of static electric fields on wing elevation

Wing elevation experiments were conducted to determine whether the wings could be moved as a result of the exposure to static electric fields of the same magnitude as those found to have a role in inducing the avoidance behaviour. The results here showed that the angle of the wings increased with increased electric field. As the fly was placed underneath a negatively charged electrode, the static electric field forces cause an uneven charges distribution (polarisation) (Bhatnagar, 1993; Ellse and Honeywill, 1998). This leads to passive elevation of the wing toward the electrode, as unlike charges are attracted.

With live flies, there were significant differences between the field strength required to raise the wings of male and female flies, though the wing of the female is bigger and therefore holds more electric charges. However, the wing of the female is heavier than the male which therefore requires higher field strength to be lifted toward the electrode. Moreover, the difference between male and female flies was more apparent, as they were always fixed at the same distance and orientation to the charged copper ring in the vertical tube, compared to when they were freely moving within the Y-tube apparatus. Exposing the excised wings of male and female flies to static electric fields showed that the value of the voltage required to lift the male wings was only 0.6 kV (28-38 kV/m) compared to female wings at 1.1 kV (38-48 kV/m). This difference is due to the different size of the male and female wing. However, the range of field strength to lift the excised wings is still within the range of field strength (26-34 kV/m) that caused avoidance behaviour in the Y-tube apparatus when flies were exposed to electric fields for 20 min (Chapter 2).

Newland *et al*. (2008) also found that the antennae of cockroach were deflected when approaching electric field of 40-50 kV/m. Previous experiments also showed a defection of the antennae in bees when exposed to electric fields of 95 kV/m (Yes'Kov and Sapozhnikov, 1976) and wing at 150 kV/m (Bindokas *et al*., 1989). Thus, it is thought that deflection in the wing caused by electric field leads to both detection and avoidance of electric fields.

In addition, the current experiments also included the testing of the elevation of wings in live and dead flies (males and females separately) and the results showed no significant differences between the wing angle of live and dead flies when exposed to static electric fields at different voltages. This confirms that the wings move in response to static electric fields and their movement is uncontrolled, even in live flies. However, the field strengths caused significant wing elevation was similar to the field causing avoidance behaviour when wild-type flies were tested through the Y-tube apparatus. This provides further evidence to support the hypothesis of the importance of wings in the detection and avoidance of electric fields.

Chapter 4

The effect of static electric fields on *Drosophila* mortality

4.1 Introduction

The presence of artificial electric fields in the environment and their potential adverse effects on public health has raised concern in the recent years (Feychting *et al*., 2005; Draper *et al*., 2005). AC transmission power lines are considered the main source of both electric and magnetic fields (EMF) with strengths of 9-11 kV/m at 1 m above ground (Dezelak, 2010). A number of studies have reported a possible relationship between EMF and human diseases, such as childhood leukaemia, breast cancer, brain tumours and cardiovascular disease (Ahlbom *et al*., 2001; Draper *et al*., 2005). A recent review by Kheifets *et al.* (2010) also concluded that there was a weak association between EMF and childhood leukaemia, although there was no reported mechanism to underpin the association (Schuz and Ahlbom, 2008).

The EMF include different components, such as extremely low frequency (ELF) electric fields, which produce fluctuating electric field in association with magnetic fields. ELF fields have been studied intensively and it has been suggested that they are responsible for harmful effects on humans, compared to static electric field. For example, a study by Fawzia (2002) found that the exposure of mice to ELF electric field of 6 kV/m for 30 days leads to chromosome aberrations in the bone marrow cells. Hanafy (2006) demonstrated that long term exposure (up to 60 days) to ELF electric field of 6 kV/m affected the dielectric properties of protein in mice cells and increased their conductivity as a result of changes in the molecular structure of the total serum of protein. However, neither of these studies investigated the separate role of magnetic or electric fields.

Therefore, the present study focuses solely on the effect of static electric field in the absence of magnetic fields, to understand its potential role in causing harmful effects to organisms, using *Drosophila* as a model system.

Exposure of invertebrates to ELF electric fields can cause harmful effects. For example, chromosomal aberrations have been observed in *Drosophila* when they were exposed to field strengths of 8 kV/m and above (reviewed in McCann *et al*., 1998). Maw (1961a) demonstrated that exposure of the parasitoid *Scambus buoliana* to ELF electric fields of 0.8 kV/m led to reduced egg laying.

In addition, previous studies showed also that ELF electric fields can cause knockdown or mortality in *Drosophila* (Watson *et al*., 1983). Knockdown is a temporary loss of locomotor function, causing flies to drop to the ground when exposed to a stimulus, whereas removal of the stimulus results in recovery, and has been used to assess responses and resistance to stress factors such as thermotolerance (Folk *et al*., 2006) and resistance to ethanol (Hoffmann *et al*., 1987). For example, exposure of *Drosophila* to ELF electric fields of 410 kV/m for 1 min caused flies to become temporarily paralysed and drop on a grounded electrode but they recovered within a few minutes after the electric field was removed (Watson *et al*., 1983). ELF electric fields of 352 kV/m, caused mortality when flies were exposed to them for 72 hr (Watson *et al*., 1988). In many of these reported studies, it was not clear from the design of the experimental equipment what the relative contribution of electric field exposure and electrical discharge (electric shock) was to knockdown and mortality. Bindokas *et al*. (1988) showed that an ELF electric field of 50-100 kV/m under wet conditions caused mortality when bees landed on the surface of a conductive tunnel located around a hive, suggesting that death occurred as a result of electric shock.

Many environmental stressors are known to cause knockdown or early mortality in insects. For example, *Drosophila* knockdown was observed when exposed to a temperature of 38˚C for 20 min, while 50% of flies were killed (LT_{50}) when exposed for 30 min (Berrigan, 2000). Other studies showed that exposing 10 day old *Drosophila* to a desiccating air flow for 24 hr at 24˚C caused mortality of up to 74% in males and 30% in females (Khazaeli, 1995). Crowded conditions at 25˚C reduced the longevity of *Drosophila* to 35 days in males and 28 days in females, compared to controls of 38 and 30 days, respectively (Joshi and Mueller, 1997). The addition of chemicals to *Drosophila* food can also cause mortality. For example, the average lifespan of female *Drosophila* was reduced when Diethylhexy Phthalate (DEHP 0.2 %) was added to food (Shuguang *et al*, 2010). Carbon dioxide (mixed with 10% potassium hydroxide) resulted in a 50% increase in mortality in *Drosophila* within 15 min of exposure (Perron *et al*., 1972). The exposure to electric field might be another environmental stressor that can cause mortality in *Drosophila*.

One aim of this project is to develop a reliable bioassay design to quantify any harmful effects of static electric fields on *Drosophila* by measuring knockdown and mortality. Different assay designs will help clarify the contribution of electrical discharge (electric shock) and electric field exposure to *Drosophila* mortality, which is unclear in the literature. The electric fields in each design were modelled using Maxwell SV two-dimensional software to estimate the field strength that caused knockdown and mortality flies. Furthermore, this bioassay design might help to understand the effect of sub-lethal electric field on organisms.

4.2 Materials and Methods

4.2.1 Preparation of flies for experiments

Drosophila melanogaster were reared as described previously (Chapter 2). Adult flies 4 to 10 days old were used for all experiments.

4.2.2 Bioassay apparatus

30 glass tubes, 2 x 110 x 30 mm (thickness x length x diameter) were placed vertically on a wooden stand (Fig. 4.1). The ends of each tube were fitted with a rubber bung with a circular aluminium disc electrode, the circumference of which had a layer of insulating tape to prevent electrical contact with the glass tube. The top electrode was connected to a high voltage power supply (DC Brandenburg Alpha III, Brandenburg, UK) and the base electrode connected to a earth. The distance between the electrodes was 30 mm, and an inverted plastic test tube cap 1 x 10 x 16 mm (thickness x length x diameter) was placed in the base filled with food (Fig. 4.2).

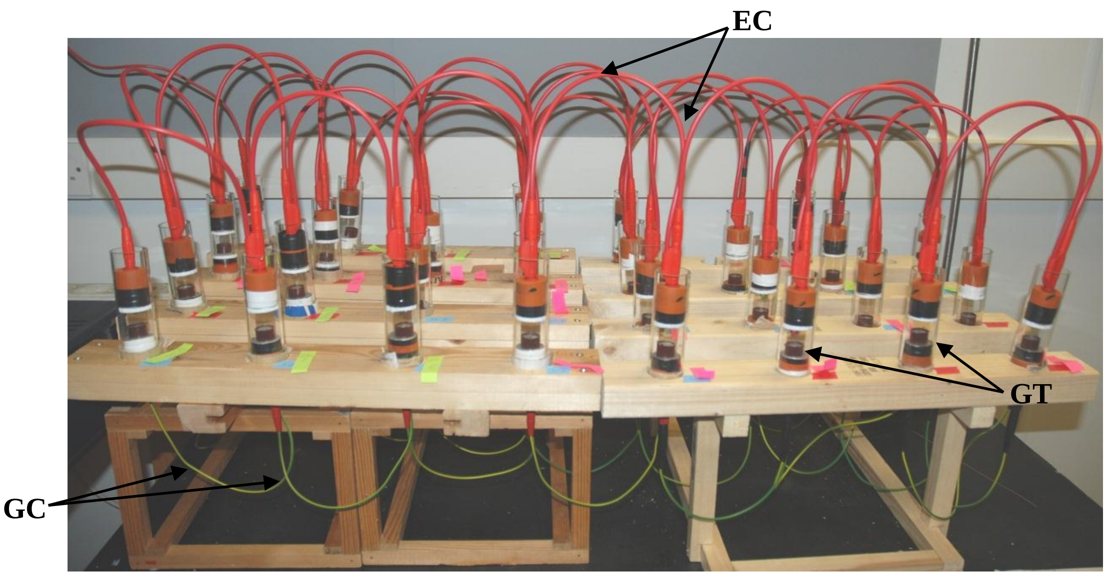

Figure 4.1 Photograph of the setup used to test the effects of static electric fields on the knockdown and mortality of *Drosophila*. Three designs of experimental chamber were used. Each glass tube contained ten flies (males and females separately). A DC generator was connected to the top electrode and the lower electrode was connected to ground (GC: ground cables; EC: electric cables; GT: glass tubes).

There were three different bioassay designs. In the first design, the top electrode only was covered by a Nylon mesh (an insulating material) and the base earth electrode was left exposed. Thus, flies were able to contact the base ground electrode only (Fig. 4.2A). In the second design, both electrodes were covered with an insulating material (Nylon mesh) to prevent flies from receiving an electrical shock (Fig. 4.2B). For the third design, the top electrode was covered by a nylon mesh and the ground electrode was connected directly to food through a plastic cup, instead of using the aluminium disc (Fig. 4.2C).

The glass tubes were washed after each trial (each individual voltage) using 5% Decon and 95% water with a smooth brush, rinsed with distilled water and dried at room temperature to be ready for the next experiment.

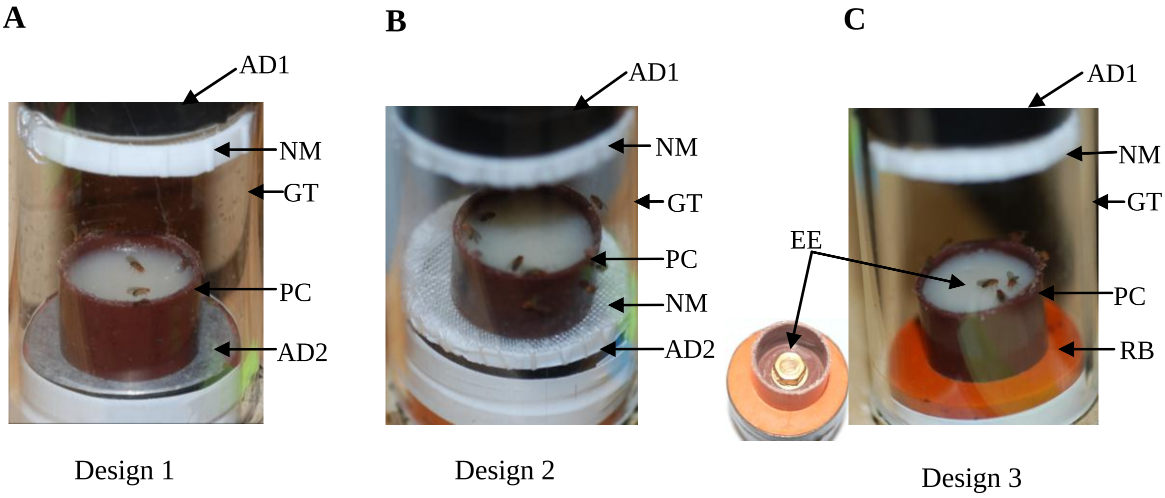

Figure 4.2 The three bioassay designs (A-C) used for exposing flies to static electric fields. (NM: nylon mesh; GT: glass tube; AD1: aluminium disc1 (connected to power supply); PC: plastic cup (contains food); AD2: earthed aluminium disc; EE: Earth electrode (which was filled with food in Design 3); RB: rubber bung.

4.2.3 Experimental treatments

The plastic cups were filled with food and kept at 4˚C for 24 hr. The next day, food cups were placed in the assay tubes. The flies were anaesthetised with CO_2 , sexed and then transferred into the tubes. Each tube contained 10 flies, either males or females, with 5 replicates per treatment (n = 5). The tubes were placed on a wooden stand and the wires connected to the power supply (switched off) and to the ground as appropriate, and then left overnight to acclimatise. Any dead flies were removed and new flies were added from the cultures. The power supply was turned on and the flies exposed to static electric fields at several voltages (5 kV, 4 kV, 3 kV, 2 kV, 1 kV, 0.5 kV and 0 kV) for 7 days under lab conditions: temperature 20˚C, humidity 30-40 %, and white light (40 watt) 16 L : 8 D photoperiod.

Counts of immobile flies on the base of the tubes were taken after 3, 6 and 9 hrs with the power remaining switched on. For the rest of the experiment (12 to 168 hrs), the number of flies on the base was recorded, the power switched off for one hr to allow any knocked down flies to recover and the remaining dead flies were counted.

4.2.4 Electric field modelling

Maxwell SV two dimensional software was used to model the distribution of the electric field based on a drawing of a'Z R' symmetrical model of the glass tube (Z represents the Z axis and R the rotation around the Z axis) (Fig. 4.3 Design 1-3), to enable accurate determination of the field strength causing knockdown or mortality at different voltages. Each part of the apparatus was assigned with an appropriate material and the boundary values were also designated depending on the applied voltage. For example, 0-5 kV was applied individually to AD 1 as the charged electrode, while 0 kV was applied to AD 2 as a ground electrode for all trials. The electric field output was obtained by displaying a vector field plot that includes all the surfaces of the apparatus.

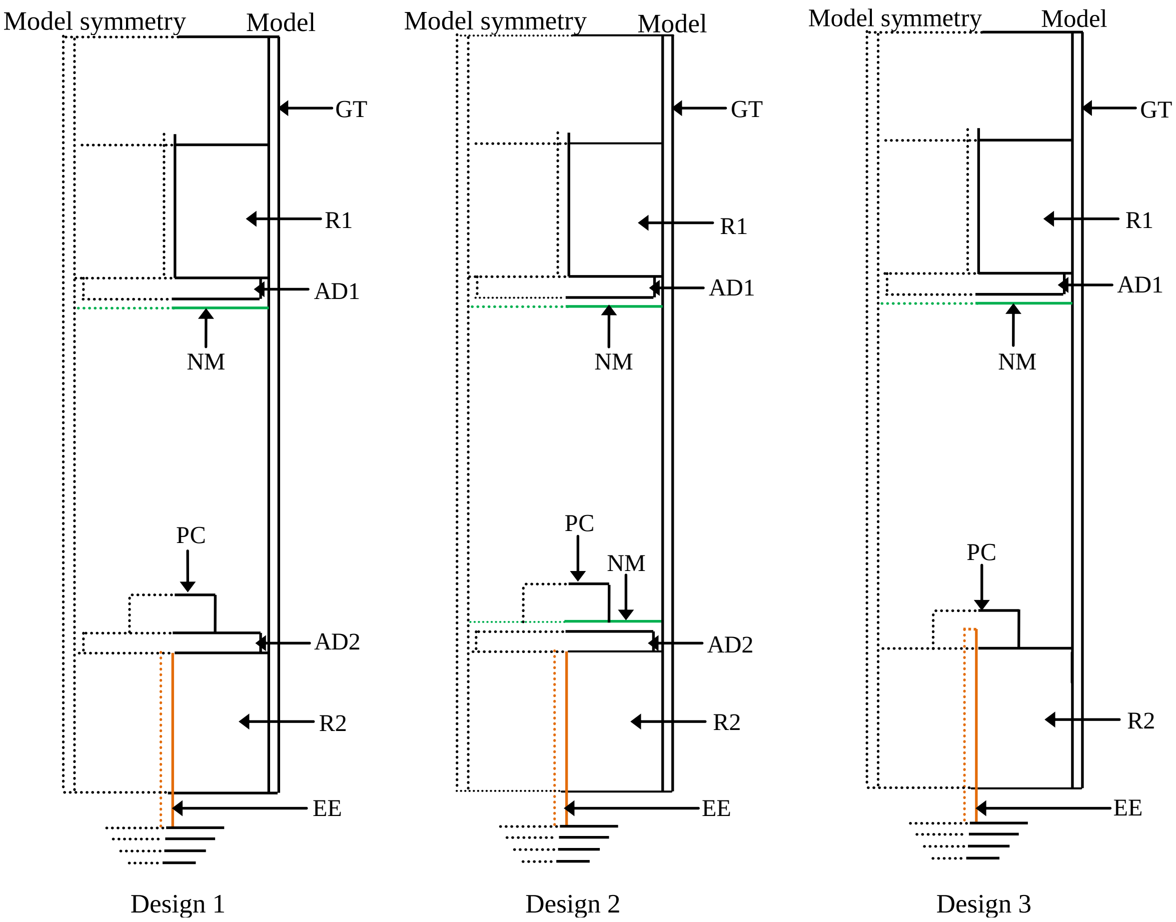

Figure 4.3. Diagram showing the three bioassay designs (1, 2 and 3) used for static electric field modelling. One side of the apparatus was drawn (solid lines) using Maxwell 2D SV Version as 'ZR' symmetry. (GT: glass tube; R1 and R2: rubber bung; AD1: aluminium disc 1 (connected with power supply); AD2: aluminium disc (connected to earth); NM1and NM2: nylon mesh 1 and 2; PC: plastic cup (containing food); EE: earth electrode.

4.2.5 Statistical Analysis

Knockdown and mortality data were analysed using a three-way ANOVA with post hoc (Tukey) analysis to test whether the differences between the numbers of flies were significant between bioassay designs, voltages and gender (0-5 kV) at each individual time point. These data were analysed in interval time (after 3, 6, and 9 hrs) for immobile flies (dead and/or knocked down) and each 12 hr for dead flies. Design 2 summary data was analysed using two way ANOVA also in interval time (12 hr, 24 hr, 36 hr, 48hr and 168 hr). The data was tested for normality and homogeneity and data transformation was not required.

Probit analysis (a type of regression used to analyze binomial response variables) was used to calculate the lethal dose of voltages (LD_{50}) at each time interval and the lethal time (LT_{50}) at each voltage, and raw data were used, as they exhibited a better linearity compared to transformed data.

4.3 Results

4.3.1 Static electric fields and *Drosophila* knockdown or mortality

Flies were exposed to static electric fields for 3, 6 and 9 hrs at different voltages (0 kV-5 kV), to determine the level of the field strength required to cause knockdown or mortality. As the electric field remained switched on, it was not possible to discriminate between knockdown and mortality.

The results showed that static electric fields of 0 and 0.5 kV (22-29 kV/m) did not cause any knockdown or mortality in any of the three designs of the experimental chambers. At 1 kV (50-64 kV/m) knockdown or mortality was only observed in Design 1, while the other voltages ((2 kV (76-78 kV/m), 3 kV (84-100 kV/m), 4 kV (89-100 kV/m) and 5 kV (95-100 kV/m)) showed knockdown or mortality in all designs.

After 3 a hr exposure to static electric fields, three way ANOVA showed that the average number of knockdown and dead flies was affected significantly by voltage ($F_{6, 168}$ = 53.46), design ($F_{2, 168}$ = 37.30) and gender ($F_{1, 168}$= 16.30) (P = 0.001) (Table 4.1).

Sig.	**a**		**b**		**b**		
Voltage (kV)	**Design 1 x (±SEM)**		**Design 2 x (±SEM)**		**Design 3 x (±SEM)**		**Sig.**
	Male	Female	Male	Female	Male	Female	
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
0.5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
1	1.2 (0.73)	0.4 (0.40)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
2	5.6 (1.57)	3.8 (0.49)	1 (1.0)	0.8 (0.58)	3.6 (1.21)	2.4 (1.17)	**II**
3	9.2(0.37)	8 (1.05)	4.2 (1.36)	0 (0.00)	6.2 (2.13)	1.6 (0.93)	**III**
4	9.6 (0.40)	8.6 (.51)	4.6 (2.04)	2.4 (1.03)	4.2 (2.37)	4.2 (2.06)	**III**
5	10 (0)	9.6 (0.40)	6 (1.82)	3.8 (1.11)	9 (0.77)	3.2 (1.83)	**III**

Table 4.1 Mean (±SEM) number of knockdown or dead flies at different voltages in three different designs after 3 hr of exposure to static electric fields. (a,b significant difference between designs and I, II, III, significant difference between voltages).

Post hoc Tukey analysis revealed no significant differences between the number of dead or knockdown flies at 5kV, 4kV and 3kV (P > 0.05). The knockdown and dead flies at these high voltages was significantly higher than at

0kV, 0.5kV, 1kV and 2kV ($P < 0.05$). The data also showed that 2kV caused a significantly higher knockdown or mortality than 0kV, 0.5kV and 1kV ($P < 0.05$), whereas there was no significant difference between 0kV, 0.5kV, 1kV ($P > 0.05$). Comparison of the different designs, showed a significantly higher knockdown or mortality in Design 1 compared to Designs 2 and 3 ($P = 0.001$), however there was no significant differences between Design 2 and Design 3 ($P = 0.06$). Male flies had significantly higher knockdown or mortality than female flies (Table 4.1).

After exposure for 6 hr the results also showed that voltage significantly affected the number of knockdown and dead flies ($F_{6, 168} = 60.51$), design ($F_{2, 168} = 33.37$) and gender ($F_{1, 168} = 10.68$) ($P = 0.001$). The difference between the number of knockdown and dead flies at 5kV, 4kV and 3kV was not significant ($P > 0.05$). However, these voltages cause significantly more knockdown or mortality than at 0kV, 0.5kV, 1kV and 2kV ($P < 0.05$). Knockdown or mortality at 2 kV was significantly higher than at lower voltages (0kV, 0.5kV, 1kV) ($P < 0.05$). There were no significant differences between the number of flies at 0kV, 0.5kV, 1kV ($P > 0.05$). The difference between the number of dead or knockdown flies in Design 2 and Design 3 was not significant ($P = 0.06$). However, these designs exhibited significantly less knockdown or mortality than Design 1 ($P = 0.001$). Male flies again had a significantly higher knockdown or mortality than female flies (Table 4.2).

Sig.	**a**		**b**		**b**		
Voltage (kV)	**Design 1 x (±SEM)**		**Design 2 x (±SEM)**		**Design 3 x (±SEM)**		**Sig.**
	Male	Female	Male	Female	Male	Female	
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
0.5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
1	1.6 (0.68)	1 (0.63)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
2	6.4 (1.60)	5.4 (0.75)	1 (1.00)	0.8 (0.58)	4.2 (1.20)	3.2 (1.39)	**II**
3	9.6 (0.24)	9.2 (0.37)	4.4 (1.36)	0.6 (0.60)	6.2 (2.13)	2.8 (1.39)	**III**
4	9.6 (0.40)	9 (0.55)	6.2 (1.85)	3.2 (1.53)	6 (2.45)	4.8 (2.24)	**III**
5	10 (0)	10 (0)	7.4 (1.36)	5 (1.34)	9 (0.77)	4.8 (1.93)	**III**

Table 4.2 Mean (±SEM) number of knockdown or dead flies at different voltages in three different designs after 6 hr of exposure to static electric fields. (a,b significant difference between designs and I, II, III, significant difference between voltages).

After a 9 hr exposure knockdown and dead *Drosophila* were affected significantly by voltage ($F_{6, 168}$ = 65.03, P = 0.001), design ($F_{2, 168}$ = 29.57, P = 0.001) and gender ($F_{1, 168}$ = 8.22, P = 0.005).

Post hoc analysis showed no significant differences between the number of knockdown and dead flies at 5kV, 4kV and 3kV (P > 0.05). These high voltages were significantly higher than at 0kV, 0.5kV, 1kV and 2kV (P < 0.05). The differences between knockdown and dead flies were not significant at 0kV, 0.5kV, 1kV (P > 0.05), but at 2 kV, there were significant differences from other voltages. The different designs showed significantly higher knockdown or mortality in Design 1 compared to Designs 2 or 3 (P = 0.001), however there were no significant differences between Design 2 and Design 3 (P = 0.10). Male flies again had significantly higher knockdown or mortality compared to females (P < 0.05) (Table 4.3).

Sig.	**a**		**b**		**b**		**Sig.**
Voltage (kV)	**Design 1** ***x*** **(±SEM)**		**Design 2** ***x*** **(±SEM)**		**Design 3** ***x*** **(±SEM)**		
	Male	Female	Male	Female	Male	Female	
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
0.5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
1	1.6 (0.68)	1 (0.63)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
2	7 (1.58)	5.8 (0.73)	1.2 (1.20)	0.8 (0.58)	4.2 (1.20)	3.4 (1.44)	**II**
3	9.6 (0.24)	9.8 (0.20)	4.6 (1.50)	1.2 (1.20)	7 (1.90)	3.2 (1.77)	**III**
4	9.8 (0.20)	9.6(0.40)	7.2 (1.88)	4 (1.58)	6 (2.45)	5 (2.14)	**III**
5	10 (0)	10 (0)	7.8 (1.96)	6.2 (1.46)	9 (0.77)	5.4 (1.89)	**III**

Table 4.3 Mean (±SEM) number of knockdown or dead flies at different voltages in three different designs after 9 hr of exposure to static electric fields. (a,b significant difference between designs and I, II, III, significant difference between voltages).

4.3.2 Static electric field and *Drosophila* mortality 12-168 hrs

In order to understand the effects of chronic exposure to electric fields, flies were continuously exposed to static electric fields at different voltages and bioassays; mortality was recorded every each 12 hr for 7 days. To discriminate between knockdown and mortality, the electric field was turned off for one hour to determine if any individuals had recovered. However, the number of knockdown flies recovering after 12 hr was minimal or zero, with virtually all flies on the base dead or not recovering when the power supply was switched off, as shown in Table 4.4.

Voltage	**Design**	**Flies recovered (out of 50 flies)**		
		after 12 hr	**after 24 hr**	**after 36 hr**
2 kV	1	3	0	0
	2	0	0	0
	3	2	1	0
3 kV	1	0	0	0
	2	2	0	0
	3	0	0	0
0.5 kV, 1 kV, 4 kV and 5 kV no flies recovered.				

Table 4.4 The number of knockdown flies that recovered when the power was switched off for one hour after 12, 24 and 36 hrs respectively.

Mortality was analysed from 12 hr to168 hr and the results showed that static electric fields at 2 kV (76-78 kV/m), 3 kV (84-100 kV/m), 4 kV (89-100 kV/m) and 5kV (95-100 kV/m) caused significant mortality when all designs had been used. The number of dead flies in Design 1 was significantly higher than in Designs 2 and 3 ($P > 0.05$). Indeed, all the flies (male and female) in Design 1 were killed at 4 kV and 5kV within 12 hr of exposure only (Tables 4.5 to 4-9).

After 12 hr exposure, the number of dead flies was affected significantly by voltage ($F_{6, 168} = 64.7$, $P = 0.001$), gender ($F_{1, 168} = 5.82$, $P = 0.01$) and design ($F_{2, 168} = 26.96$, $P = 0.001$). Post Hoc Tukey analysis showed no significant differences between the average number of dead flies at 5kV, 4kV and 3kV ($P > 0.05$). These voltages caused significantly higher mortality than 0kV, 0.5kV, 1kV and 2kV ($P < 0.05$). The results also showed that the 2kV treatment caused significantly higher mortality than 0kV, 0.5kV and 1kV ($P < 0.05$), whereas there were no significant differences between 0kV, 0.5kV, 1kV (P > 0.05 for all cases). Comparing the different designs showed that Design 1 had significantly higher mortality than the other Designs (2 and 3) (P = 0.001), while there was no significant difference between Designs 2 and 3. Male flies had significantly higher mortality than female flies (Table 4.5).

Sig.	**a**		**b**		**b**		
Voltage (kV)	**Design 1 *x* (±SEM)**		**Design 2 *x* (±SEM)**		**Design 3 *x* (±SEM)**		**Sig.**
	Male	Female	Male	Female	Male	Female	
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
0.5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
1	1.4 (0.75)	1 (0.63)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
2	6.8 (1.83)	6.2 (0.73)	1.4 (1.40)	0.8 (0.58)	4.2 (1.20)	3.4 (1.44)	**II**
3	10 (0)	9.8 (0.2)	4.6 (1.50)	1.8 (1.80)	7.2 (1.83)	4 (1.92)	**III**
4	10 (0)	10 (0)	7.4 (1.94)	4.6 (1.47)	6 (2.45)	5.4 (2.14)	**III**
5	10 (0)	10 (0)	7.8 (1.96)	6.2 (1.46)	9 (0.77)	5.6 (1.83)	**III**

Table 4.5 Mean (±SEM) number of dead flies at different voltages in three different assay designs after 12 hr exposure to static electric fields (a,b significant difference between designs and I, II, III significant difference between voltages).

After 24 a hr exposure, the results showed that only voltage and design had significant effects on *Drosophila* mortality (Voltage ($F_{6, 168}$ = 67.1, P = 0.001) and design ($F_{2, 168}$ = 22.7, P = 0.00), whereas gender had no effect ($F_{1, 168}$ = 3.8, P = 0.052).

Post hoc analysis showed that the number of dead flies between 3 kV, 4 kV and 5 kV was not significantly different (P > 0.05). However, mortality at these voltages was significantly higher than at 0kV, 0.5kV, 1kV and 2kV (P < 0.05). the data also showed that mortality at 2 kV was significantly higher than at 0kV, 0.5 and 1kV (P < 0.05) whereas no significant differences were found between 0kV, 0.5kV, 1kV (P > 0.05). The number of dead flies observed in Design 1 was significantly higher than in Design 2 and Design 3 (P = 0.001). However the difference between Design 2 and Design 3 was not significant (P = 0.055). Mortality between male and female flies showed no significant differences for this length of exposure (Table 4.6).

Sig.	**a**		**b**		**b**		
Voltage (kV)	**Design 1** *x* **(±SEM)**		**Design 2** *x* **(±SEM)**		**Design 3** *x* **(±SEM)**		**Sig.**
	Male	Female	Male	Female	Male	Female	
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
0.5	0 (0)	0.2 (0.20)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
1	1.6 (0.93)	1 (0.63)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
2	7 (1.82)	6.2 (0.73)	1.6 (1.60)	0.8 (0.58)	4.8(1.50)	3.4 (1.44)	**II**
3	10 (0)	10 (0)	4.8 (1.53)	2 (2.00)	8.4 (1.60)	5 (2.05)	**III**
4	10 (0)	10 (0)	7.6 (1.75)	5.6 (1.83)	6 (2.45)	6.2 (2.33)	**III**
5	10 (0)	10 (0)	7.8 (1.96)	6.4 (1.57)	9 (0.77)	7.6 (1.60)	**III**

Table 4.6 Mean (±SEM) number of dead flies at different voltages in three different designs after 24 hr exposure to static electric fields (a,b significant difference between designs and I, II, III significant difference between voltages).

After a 36 hr exposure to static electric fields, the results also showed that both voltage and design significantly affected *Drosophila* mortality, whereas gender did not(voltage ($F_{6, 168}$ = 79.5, P = 0.001), design ($F_{2, 168}$ = 22.1, P = 0.001) and gender ($F_{1, 168}$ = 2.06, P = 0.15)).

A *post hoc* Tukey test showed that there were no significant differences in the number of dead flies at 5kV, 4kV and 3kV (P > 0.05). However, these voltages caused significantly higher mortality than 0kV, 0.5kV, 1kV, 2kV (P < 0.05). Mortality at 2 kV was significant higher than at 0kV, 0.5kV, 1kV, while there were no significant differences between the number of flies at 0kV, 0.5kV and 1kV (P > 0.05).

The number of dead flies in Design 3 was significantly higher than in Design 2. (P = 0.02), while these two designs (2 and 3) showed significantly less mortality compared to Design 1 (P = 0.001) (Table 4.7).

Sig.	a		b		c		Sig.
Voltage (kV)	**Design 1 *x* (±SEM)**		**Design 2 *x* (±SEM)**		**Design 3 *x* (±SEM)**		
	Male	Female	Male	Female	Male	Female	
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
0.5	0 (0)	0.2 (0.20)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
1	1.6 (0.93)	1 (0.63)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
2	7.4 (1.89)	6.4 (0.87)	1.8 (1.56)	0.8 (0.58)	4.8(1.50)	3.4 (1.44)	**II**
3	10 (0)	10 (0)	4.8 (1.53)	2.6 (1.94)	8.4 (1.60)	6.6 (2.09)	**III**
4	10 (0)	10 (0)	8 (1.38)	6.6 (1.54)	6.2 (2.33)	7.8 (1.96)	**III**
5	10 (0)	10 (0)	7.8 (1.96)	6.6 (1.54)	9.2 (0.80)	8 (1.55)	**III**

Table 4.7 Mean (±SEM) number of dead flies at different voltages in three different designs after 36 hr of exposure to static electric fields (a,b significant difference between designs and I, II, III significant difference between voltages).

After a 48 hr exposure, the results showed that different voltages and designs significantly affected *Drosophila* mortality ($F_{6, 168}$ = 92.03, P = 0.001) and ($F_{2, 168}$ = 21.02, P = 0.001) respectively, while gender had no significant effect ($F_{1, 168}$ = 1.9, P = 0.16).

Post hoc Tukey analysis showed that there were no significant differences between fly mortality at 5kV, 4kV and 3kV (P > 0.05), however, the number of dead flies at these voltages was significantly more than at 0kV, 0.5kV, 1kV and 2kV (P < 0.05). There were significantly more dead flies at 2 kV than at 0kV, 0.5kV, 1kV (P < 0.05), but the number of dead flies between these low voltages (0kV, 0.5kV, 1kV) did not exhibit significant differences as the time of exposure increased. Mortality in Design 3 was significantly higher than in Design 2, while Design 1 caused significantly higher mortality than Designs 2 and 3 (P = 0.001) (Table 4.8).

Sig.	a		b		c		Sig.
Voltage (kV)	Design 1 *x* (±SEM)		Design 2 *x* (±SEM)		Design 3 *x* (±SEM)		
	Male	Female	Male	Female	Male	Female	
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
0.5	0 (0)	0.2 (0.20)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
1	1.6 (0.93)	1.2 (0.58)	0 (0)	0 (0)	0 (0)	0 (0)	**I**
2	7.6 (1.69)	6.6 (1.80)	1.8 (1.56)	0.8 (0.58)	4.8(1.50)	3.4 (1.44)	**II**
3	10 (0)	10 (0)	5.4 (1.6)	2.6 (1.94)	8.4 (1.60)	6.6 (2.09)	**III**
4	10 (0)	10 (0)	9.2 (0.58)	7.6 (1.36)	6.2 (2.33)	8 (2.00)	**III**
5	10 (0)	10 (0)	7.8 (1.96)	7.2 (1.32)	9.2 (0.8)	8.4 (1.17)	**III**

Table 4.8 Mean (±SEM) number of dead flies at different voltages in three different designs after 48 hr of exposure to static electric fields (a,b significant difference between designs and I, II, III significant difference between voltages).

For the remaining period (60-168 hrs) of exposure to static electric fields, ANOVA showed that *Drosophila* mortality was significantly affected by voltages and design but not gender. The number of dead flies between different voltages and designs were similar to those at 48 hr exposure. However, the number of dead flies continued to increase with time of exposure (Table 4.9).

Sig.	a		b		c		Sig.
Voltage (kV)	Design 1 *x* (±SEM)		Design 2 *x* (±SEM)		Design 3 *x* (±SEM)		
	Male	Female	Male	Female	Male	Female	
0	0 (0)	0.2 (0.20)	0 (0)	0 (0)	0 (0)	0.2 (0.20)	**I**
0.5	0 (0)	0.2 (0.20)	0 (0)	0 (0)	0 (0)	0.2 (0.20)	**I**
1	1.8 (0.86)	1.6 (0.68)	0 (0)	0.2 (0.20)	0 (0)	0.4 (0.24)	**I**
2	9.4 (0.40)	7.4 (0.08)	1.8 (1.56)	0.8 (0.58)	5.6(1.72)	6.4 (1.83)	**II**
3	10 (0)	10 (0)	5.8 (1.62)	6.2 (2.01)	8.8 (1.20)	6.6 (2.09)	**III**
4	10 (0)	10 (0)	10 (0.00)	8.6 (0.87)	6.4(2.23)	10 (0.00)	**III**
5	10 (0)	10 (0)	8.6(1.40)	9.8 (0.20)	10 (0.00)	10 (0.0)	**III**

Table 4.9: Mean (±SEM) number of dead flies at different voltages in three different designs after 168 hr of exposure to static electric fields (a,b significant difference between designs and I, II ,III significant difference between voltages).

4.3.3 Effects of voltage applied and time on *Drosophila* mortality

4.3.3.1 Lethal dose of voltage (LD_{50})

The bioassay design had a significant effect on LD_{50} when flies had been exposed to static electric fields at different voltages (2-5 kV). For example, the LD_{50} in Design 1 was significantly lower than in Design 2 and 3 in all experiments (12 to 168 hrs) based on the confidence intervals (CI) (Liang, 2005; Negahban *et al*., 2006). For example, an applied voltage of 1.69 kV was sufficient to kill 50% of male flies in Design 1 compared to 3.46 kV in Design 2, and 2.97 kV in Design 3 when exposed to static electric fields for 12 hr. There was no overlap in CI values between Design 1 (1.54-1.84), Design 2 (3.22-3.71) and Design 3 (2.72-3.22).

Within individual designs, the results showed that the LD_{50} for flies in Design 1 after 12 hr of exposure was not significantly different from that at 168 hr. For example, after 12 hr exposure, the LD_{50} value in males was 1.96 kV and 1.41 kV at 168 hr. In females the LD_{50} value at 12 hr was 1.81 kV and at 168 hr was 1.61 kV in Design 1. However, in Design 2 the LD_{50} value was significantly higher at 12 hr than at 168 hr of exposure (at 12 hr was 3.46 kV and at 168 hr was 2.95 in males, and 4.35 kV and 2.96 in females. The LD_{50} in Design 3 showed no significant differences between 12 hr and 168 hr in males (2.97 kV and 2.52 kV), but significant differences were observed in females at 12 hr (3.97 kV) and at 168 hr (2.17 kV). Thus, the LD_{50} of Design 2 has an intermediate value between that of Design 1 and 2.

For Design 1 there were no significant differences in the LD_{50} between male and female flies. For example, at 12 hr the LD_{50} value in males was 1.69 kV while in females it was 1.81 kV. In Design 2, however, the LD_{50} was significantly higher in females than in males at 12-48 hr exposure to static electric fields. For example, at 12 hr, the LD_{50} in males was 3.46 kV and in females it was 4.35 kV, but after 168 hrs exposure, there appeared no significant differences between males (2.95 kV) and females (2.96 kV). In Design 3, the LD_{50} for males was significantly lower than female flies at 12, 24 and 36 hrs while no significant difference was found at 48 and 186 hrs (Table 4.10).

Time (hr)	Design	Gender	LD_{50}	95% CI
12	1	M	1.69	1.54 – 1.84
		F	1.81	1.66 – 1.97
	2	M	3.46	3.22 – 3.71
		F	4.35	4.05 – 4.74
	3	M	2.97	2.72 – 3.22
		F	3.97	3.62 – 4.42
24	1	M	1.65	1.51 – 1.81
		F	1.78	1.63 – 1.93
	2	M	3.40	3.16 – 3.66
		F	4.16	3.88 – 4.50
	3	M	2.81	2.57 – 3.06
		F	3.41	3.14 – 3.71
36	1	M	1.61	1.47 – 1.77
		F	1.74	1.59 – 1.90
	2	M	3.34	3.10 – 3.59
		F	3.95	3.69 – 4.26
	3	M	2.76	2.53 – 3.01
		F	3.21	2.97 – 3.48
48	1	M	1.59	1.45 – 1.74
		F	1.70	1.56 – 1.86
	2	M	3.17	2.93 – 3.40
		F	3.75	3.51 – 4.02
	3	M	2.76	2.53 – 3.01
		F	2.96	2.20 – 2.73
168	1	M	1.41	1.29 – 1.55
		F	1.61	1.46 – 1.77
	2	M	2.95	2.73 – 3.16
		F	2.96	2.76 – 3.15
	3	M	2.52	2.30 – 2.74
		F	2.17	1.98 – 2.36

Table 4.10 LD_{50} (kV) in *Drosophila* with the application of different voltages after 12, 24, 36, 48 and 168 hrs of exposure to static electric field using the three different bioassay designs (M: males; F: females).

4.3.3.2 Lethal Time (LT_{50})

The results showed that as the applied voltage increased, the LT_{50} tended to decrease. For example, in Design 2 and 3 the LT_{50} for flies (male and female) at 3 kV was significantly more than at 5 kV.

Within individual designs, LT_{50} values were calculated for Design 1 at 2 kV only, as the flies at higher voltages (3 kV- 5kV) showed 100% mortality even at the shortest exposure time of 12 hr. By contrast, the LT_{50} for Design 2 could not be calculated at 2 kV, since more that 50% of the flies survived at this voltage (and below). However, mortality of more than 50% was observed when 3 kV (and above) was applied, which allowed calculation a value for LT_{50} in this case. In Design 3, the LT_{50} was calculated at 2 kV- 5 kV and this clearly indicated that Design 3 is the intermediate design in terms of the LT_{50} value.

The results also showed that the LT_{50} was significantly less in Design 1 than in Design 3. For example, the LT_{50} values at 2 kV in Designs 1 and 3 for male flies were 5.40 hr (1.74-10.20) and 73.99 hr (45.50-119.31), and for female flies 10.43 hr (3.58-18.77) and 99.06 hr (75.49-138.28), respectively. The LT_{50} values at 3 kV in Design 3 were significantly less than in Design 2; for male flies was 1.98 hr (0.46-4.57) compared to 50.91 hr (28.69-78.67), and for female flies was 21.18 hr (9.56 – 33.13) compared to 202.3 hr (151.2-348.8).

The LT_{50} was not significantly between male and female flies in Design 1 when different voltages were applied. However, the LT_{50} for males were significantly shorter than for female flies in Designs 2 and 3 at 3 kV and 5 kV, as shown in Table 4.11.

KV	DESIGN	GENDER	LT_{50}	95% CI
2	1	M	5.40	1.74 – 10.20
		F	10.43	3.58 – 18.77
	2	M	dead flies less than 50% during entire experiment	
		F		
	3	M	73.99	45.50 – 119.31
		F	99.06	75.49 – 138.28
3	1	M	100% of flies killed within 12 hr	
		F		
	2	M	50.91	28.69 – 78.67
		F	greater than 168 hr	
	3	M	1.98	0.46 – 4.57
		F	21.18	9.56 – 33.13
4	1	M	100% of flies killed within 12 hr	
		F		
	2	M	6.48	3.04 – 10.26
		F	13.02	6.44 – 19.82
	3	M	16.22	6.02 – 28.65
		F	11.31	5.87 – 16.95
5		M	100% of flies killed within 12 hr	
		F		
		M	2.20	0.52 – 5.03
		F	14.16	8.60-19.61
		M	0.68	0.13-1.83
		F	11.82	7.54-15.93

Table 4.11: LT_{50} (hr), calculated when flies were exposed to static electric fields at 2kV, 3kV, 4kV and 5kV for the three different bioassay designs (M: males; F: females).

4.3.4 Design 2 results – Effect of electrostatic fields

The results showed that Design 2 was the best design as flies were only exposed to static electric fields, whereas in the others designs (Design 1 and Design 3) the flies experienced both static electric fields and electric shocks. Exposure to static electric fields caused mortality and two way ANOVA established the number of dead flies was affected significantly by voltage ($P < 0.05$). Gender had no significant effect when flies were exposed to electric fields for different periods of time or voltage ($P < 0.05$). Although the value for males was often higher than females. This lack of significance was probably due to the mortality averages at 0 kV, 0.5 kV (22-29 kV/m), 1 kV (50-64 kV/m) being nearly always zero. (Table 4.12). However, further evidence for a significant gender effect was demonstrated by LD_{50} and LT_{50} results (Table 4.10 and Table 4.11).

Post hoc Tukey analysis showed there was no significant differences between the number of dead flies at 0 kV, 0.5 kV, 1 kV and 2 kV voltages and this was significantly lower than that at 4 kV (89-100 kV/m) and 5 kV (95-100 kV/m) at all different times. There was no significant difference between 4 kV and 5 kV.

Data analysis at each point was presented. For example, after 12 hr of exposure to static electric fields, the number of dead flies was significantly affected by voltage ($F_{6, 56} = 12.71$, $P = 0.001$), while gender had no significant effect ($F_{1, 56} = 3.08$, $P = 0.08$). *Post hoc Tukey* analysis showed. Mortality at 3 kV (84-100 kV/m) was significantly lower than at 5 kV.

After 24 hr of exposure to static electric fields, the number of dead flies was also affected significantly by voltage ($F_{6, 56} = 12.56$, $P = 0.001$), but no significant effect was caused by gender ($F_{1, 56} = 2.23$, $P = 0.14$). *Post hoc* analysis showed the number of dead flies at 3 kV did not show any significant difference with other voltages.

After 36 hr of exposure voltages showed also significance on mortality ($F_{6, 56} = 15.97$, $P = 0.001$) while gender did not show significance ($F_{1, 56} = 1.73$, $P = 0.15$). *Post hoc* analysis indicated that the number of dead flies at 3 kV was not significant difference with those flies at 3 kV and at 5 kV.

After 48 hr of exposure, the number of dead flies was also affected significantly by voltage ($F_{6, 56} = 22.19$, $P = 0.001$), while gender showed no significance ($F_{1, 56} = 2.13$, $P = 0.15$). *Post hoc* analysis showed the number of dead flies at 2 kV and 3 kV showed lower significance compared to the number of dead flies at 4 kV and 5 kV.

After 168 hr of exposure, the Mortality was significantly affected by voltage ($F_{6, 56}$ = 43.43, P = 0.001) whereas gender did not show any significant effect ($F_{1, 56}$ = 0.05, P = 0.82). *Post hoc* analysis showed that the number of dead flies at 3 kV was significantly lower than 4 kV and 5 kV, and significantly higher than 0 to 2 kV as shown in Figure 4.12.

Volt (kV)		**Flies mortality (out of 10 flies)**									
		12 hr		**24 hr**		**36 hr**		**48 hr**		**168 hr**	
		M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
0 kV	M	0	0	0	0	0	0	0	0	0	0
	SEM	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
.5 kV	M	0	0	0	0	0	0	0	0	0	0
	SEM	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
1 kV	M	0	0	0	0	0	0	0	0	0	0.2
	SEM	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.20)
2 kV	M	1.4	0.8	1.6	0.8	1.8	0.8	1.8	0.8	1.8	0.8
	SEM	(1.40)	(0.58)	(1.60)	(0.58)	(1.56)	(0.58)	(1.56)	(0.58)	(1.56)	(0.58)
3 kV	M	4.6	1.8	4.8	2	4.8	2.6	5.4	2.6	5.8	6.2
	SEM	(1.50)	(1.80)	(1.53)	(2.00)	(1.53)	(1.94)	(1.6)	(1.94)	(1.62)	(2.01)
4 kV	M	7.4	4.6	7.6	5.6	8	6.6	9.2	7.6	10	8.6
	SEM	(1.94)	(1.47)	(1.75)	(1.83)	(1.38)	(1.54)	(0.58)	(1.36)	(0.00)	(0.87)
5 kV	M	7.8	6.2	7.8	6.4	7.8	6.6	7.8	7.2	8.6	9.8
	SEM	(1.96)	(1.46)	(1.96)	(1.57)	(1.96)	(1.54)	(1.96)	(1.32)	(1.40)	(0.20)

Table 4.12: Mean (±SEM) number of dead flies at different voltages in Design 2 after 12 hr, 24 hr, 36 hr, 48 hr and 168 hr of exposure to static electric fields. The number of dead flies was significantly affected by voltage while gender did not show any significant differences among the flies (two way ANOVA).

4.3.5 Design 2 (LD_{50} and LT_{50})

Results showed that the value of LD_{50} and LT_{50} decreased significantly with increasing time and voltage respectively. Gender had a significant effect with LD_{50} and LT_{50} values lower in male than female flies based on the confidence interval (CI).

Data analysis in more detail showed the LD_{50} value was 3.46 kV (3.22-3.71) in male and 4.35 kV (4.05-4.74) in female flies at 12 hr exposure to static electric fields which is significantly higher than the LD_{50} value at 186 hr; 2.65 kV (2.73-3.13) in male and 2.96 (2.76-3.15) in female flies.

LT_{50} at 2kV could not be calculated as mortality was less than 50%. Raising the voltage to 3 kV and above led to killing the flies in few hours. For example, LT_{50} at 4 kV was significantly higher than at 5 kV for male flies, with a value of 6.48 hr (3.04-10.26) and 2.20 hr (0.52-5.03) at 5 kV.

Both LD_{50} and LT_{50} were significantly lower in male flies (Table 4.10 and Table 4.11). For example, the LD_{50} value was significantly lower in male flies at 12 hr (3.46 kV and 4.35 kV), 24 hr (3.40 kV and 4.16 kV), 36 hr (3.34 kV and 3.95kV) and 48 hr (3.17 kV and 3.75kV). However increasing the time up to 168 hr did not show significant difference between males and females due to mortality results reaching a plateau.

4.3.6 Electric field modelling

The three bioassay designs were modelled using Maxwell SV software to understand the distribution and magnitude of the static electric fields and to determine the actual field strength that caused mortality within the different designs at all voltages.

The results showed that the electric field strengths within the bioassay designs (Designs 1, 2 and 3) increased as higher voltages were applied. The distributions of electric field strengths were similar within the three bioassay designs. For example, a voltage of 0.5 kV caused electric field strength between 22 and 29 kV/m within the exposure area (EA) in the vertical tube (Fig. 4.4A, Fig. 4.5A and Fig. 4.6A). This field strength did not show significant knockdown mortality, as there was no significant difference between control (0 kV) and 0.05 kV ($P < 0.05$).

1 kV, applied to the charged electrode, generated electric field strengths between 50 and 64 kV/m (Fig. 4.4B, Fig. 4.5B and Fig. 4.6B). This value of electric field caused mortality in Design 1.

Electric fields increased to 76 and 78 kV/m with the application of 2 kV (Fig. 4.4C, Fig. 4.5C and Fig. 4.6C) which caused significantly higher knockdown or mortality than at 0.5 kV and 1kV. However, the number of knockdown or dead flies was significantly higher in Design 1 compared to Designs 2 and 3. The electric field strength of 94-100 kV/m that was generated by 5 kV potential (Fig. 4.4D, Fig. 4.5D and Fig. 4.6D) and showed significantly higher knockdown or mortality than at 2 kV.

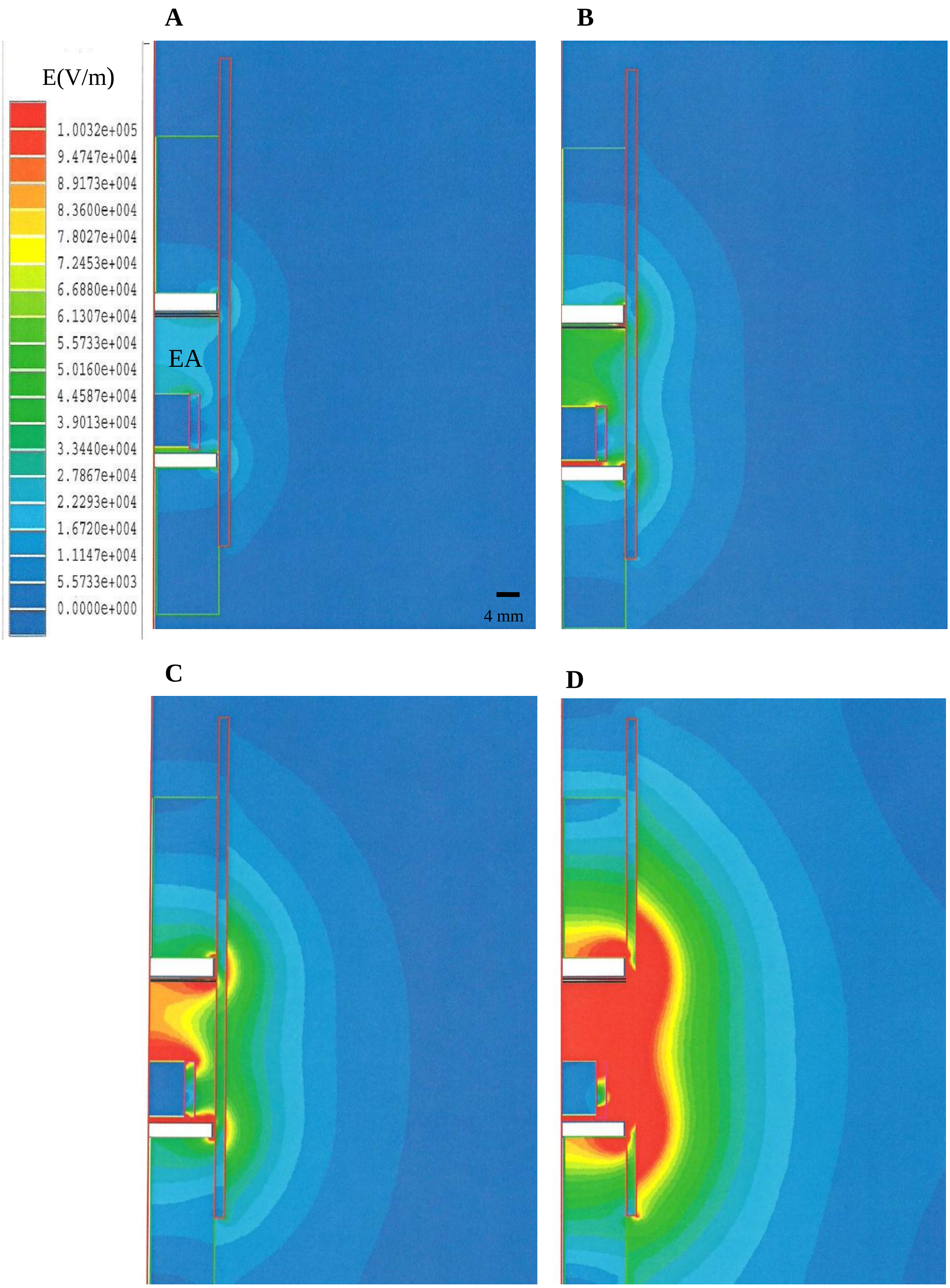

Figure 4.4 Electric field plots of a 'Z R' model of the Design 1 bioassay (without mesh). (A) a 0.5 kV applied potential generated an electric field strength of 22 kV/m within the exposure area (EA), (B) at 1 kV (50 kV/m), (C) at 2 kV (78 kV/m) and (D) at 5 kV (100 kV/m).

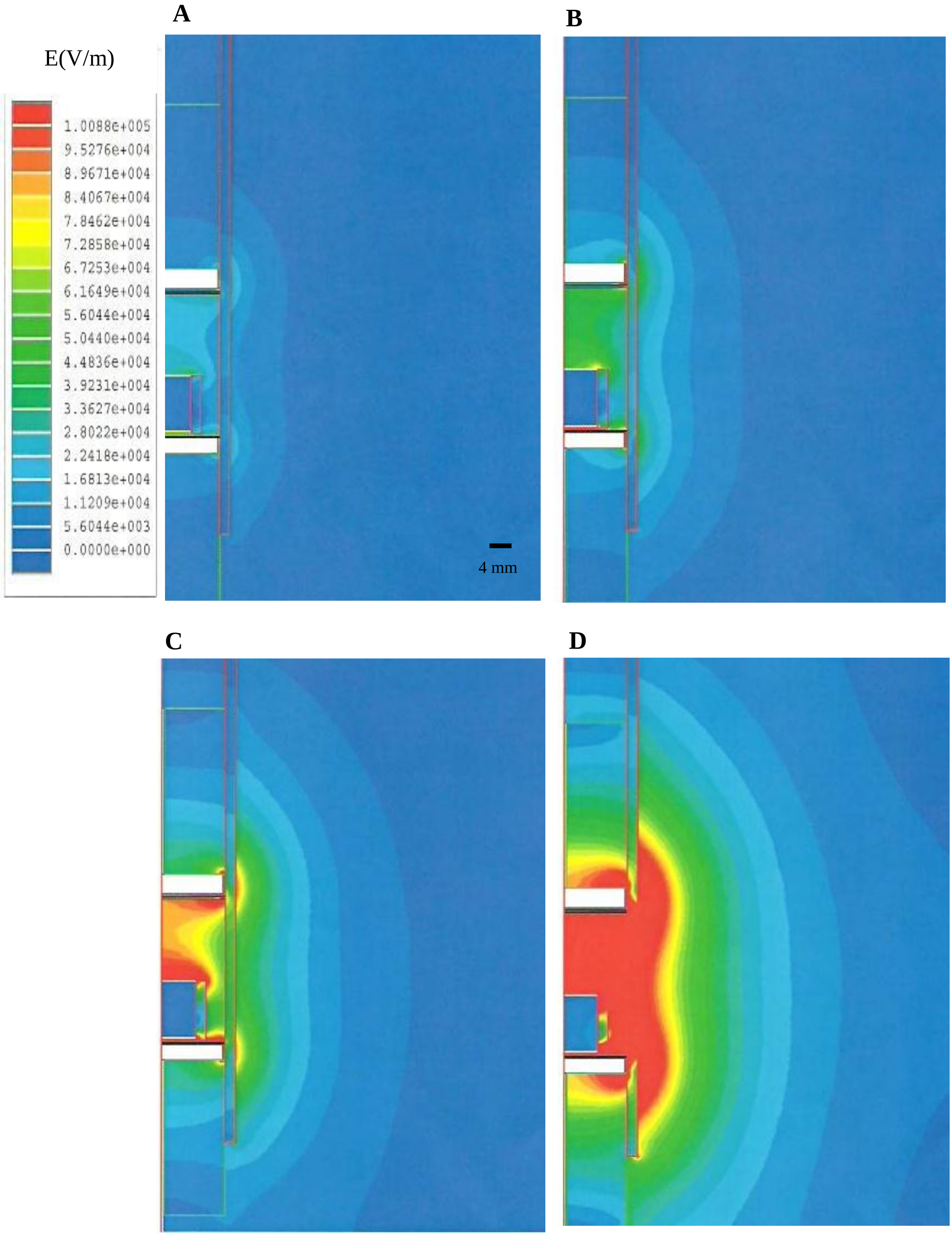

Figure 4.5 Electric field plots of a 'Z R' model of the Design 2 bioassay (with mesh). (A) a 0.5 kV applied potential generated an electric field strength of 28 kV/m within the exposure area (EA), (B) at 1 kV (56 kV/m), (C) at 2 kV (78 kV/m) and (D) at 5 kV (95 kV/m).

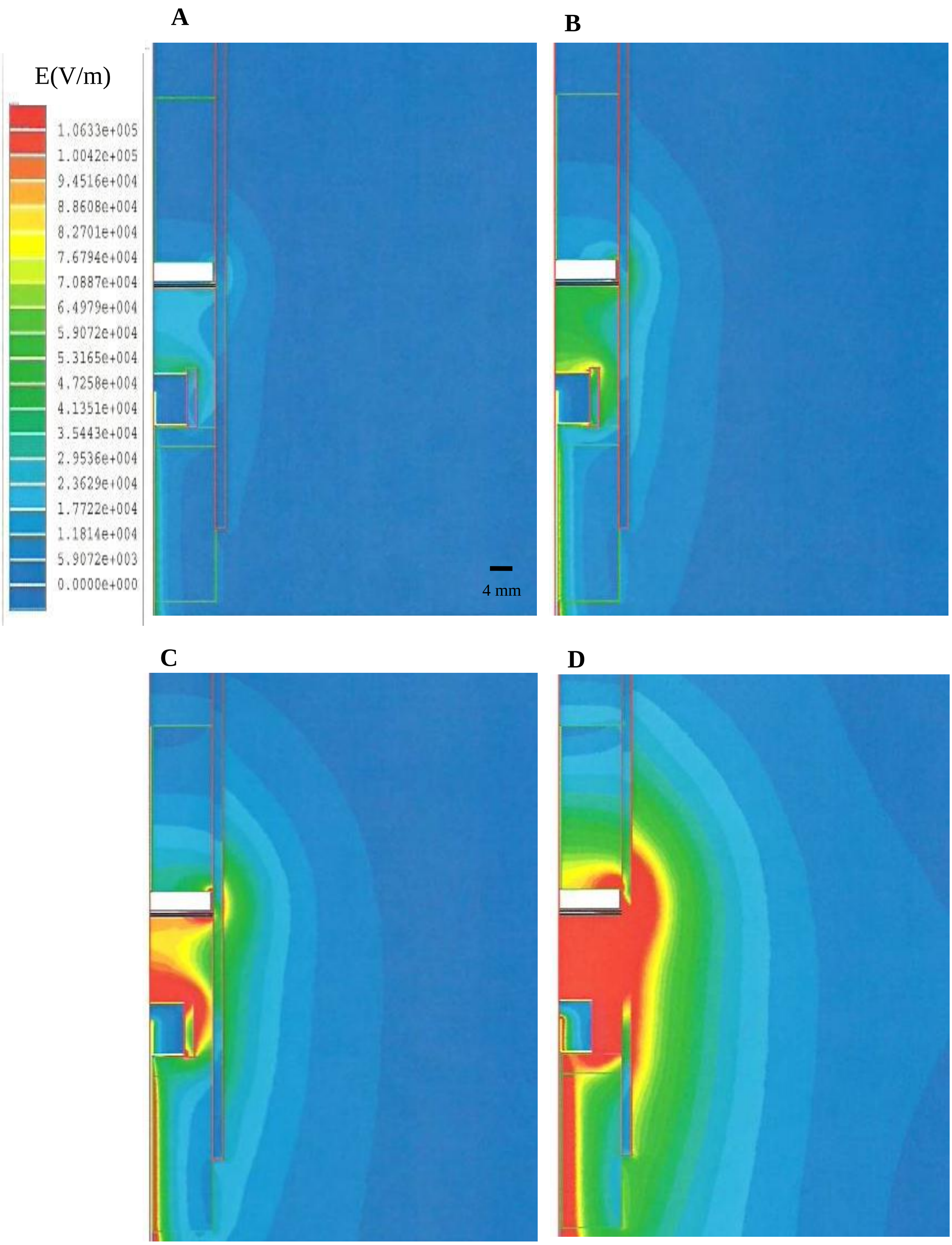

Figure 4.6 Electric field plots of a 'ZR' model of the Design 3 bioassay. (A) a 0.5 kV applied potential generated an electric field strength of 29 kV/m within the exposure area (EA), (B) at 1 kV (64 kV/m), (C) at 2 kV (76 kV/m) and (D) at 5 kV (100 kV/m).

4.4 Discussion

The bioassay designs revealed their suitability for keeping the flies alive for the whole period of the experiment with the absence of electric field (control experiment). However, the results showed knockdown or mortality when flies were exposed to static electric field for 3-186 hrs. Electric field modelling showed that the approximate threshold lower for knockdown and mortality was between 76 and 78 kV/m. Increasing the electric field to 94-100 kV/m, showed significantly higher knockdown and mortality. The results showed that males had higher mortality than females when exposed to electric field for 24 hr. LV_{50} and LT_{50} values reduced significantly with increasing voltage.

The study suggests that the electric charges on the fly's body are redistributed due to the electrostatic force that is induced by the presence of the electric field (Bhatnagar, 1993). This leads to polarisation of charges in the fly's body. Once the fly moves within the bioassay, the charges in its body redistribute, creating a temporary current which ultimately causes knockdown or mortality in Design 2. This notion is supported by Abdel-Salam (1995) and Liang (2011) who mentioned that static electric fields influence the charges on a conducting body and Mendis *et al*. (2000) found that the transmission of electrons through cells might cause physical disruption and the formation of pores in cell membranes.

Flies in Designs 1 and 3 also experienced electric fields, and when they touched the earth electrode, a rapid electrical discharge from the fly's body occurred creating an instantaneous current that caused an electrical shock, which eventually led to the death of the flies, as shown in Figure 4.7.

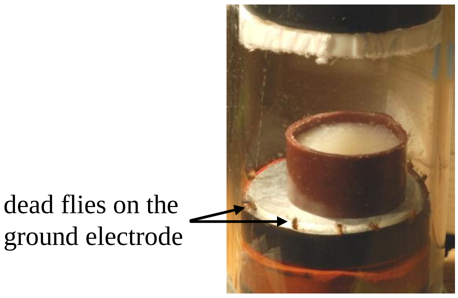

Figure 4.7 Exposure of flies to an electric field at 5kV (100 kV/m) using Design 1 bioassay. All flies were killed due to electrical shock within 12 hr of exposure.

Watson *et al*. (1983) previously showed using a methodology similar to that of Design 1 that exposure of *Drosophila* to electric fields of 352 kV/m caused mortality within 72 hr (Watson *et al*., 1983). In their experiment it was not clear whether knockdown or mortality was caused by electric fields or electric shock as both electrodes (charged and earth electrode) were not covered by insulating material. Electric field modelling was not used to understand the actual field that caused mortality. For example, modelling a similar design (Design 1) demonstrated *Drosophila* mortality with electric field strengths of 50-64 kV/m within 12 hr, suggesting that death occurred as a result of the electric field and electrical shock. This finding can be supported by Bindokas *et al*. (1988), who found that ELF electric field of 50-100 kV/m under wet conditions caused mortality in bees (*Apis mellifera*) that had landed on a conductive surface of the hive which might get electric shock.

Therefore, the bioassay designs in the present study provided further clarification of the lethal effect of static electric fields alone or associated with electrical shock to flies. For example, mortality was usually higher in Design 1 which had an exposed earth electrode. Design 2, with both electrodes covered, often showed the lowest mortality, and was the best design to assess the effects of electric fields without electrical discharge, showing significant lower mortality compared to the other designs after 36 hr and above of exposure. Design 3, with an earth connected to the food, resulted in intermediate mortality, in which electrical discharge would have been via contact with the food and slower than via direct contact with the earthed electrode in Design 1.

Additionally, it is known that other environmental stress cause mortality in *Drosophila* and the relationship of stress to mortality is clearly established e.g. LT_{50} at 38˚C appeared within 30 min (Berrigan, 2000). This research is the first to establish a reliable bioassay design that enable LT_{50} to be calculated of exposure to different voltage and static electric field strength e.g. LT_{50} at 4 kV (86 kV/m) observed within 9 hr in Design 2.

The results showed that knockdown and mortality in males were significantly higher than in females particularly when Design 2 was used. This may be because female flies spend more time around the medium laying eggs rather than flying close to the charged electrode, as male flies do. However, there was no significant difference between male and female flies in Design 1.This could be because the electric shock in Design 1 did not discriminate between genders, compared to the effect of electric fields only in Design 2.

Khazaeli (1995) noted that *Drosophila* mortality was different between gender when exposed to a desiccating air flow for 24 hr (males mortality was 74% compared to 30% in females). Exposure to an electric field often caused difference in gender mortality for the same time with a voltage of 4kV at Design 2 which caused the death of 76% of the males and 56% of the females.

The results of the analysis described here suggest that this new bioassay design could be also used to study the effect of static electric fields on *Drosophila* under various conditions including these stressors described above or to assess the effect of electric fields on other organisms, or this bioassay design could be used to study symptom expression and causation of any harmful effects of electric fields.

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Chapter 5

General discussion

5. General discussion

The results of this study clearly demonstrate that wild-type *Drosophila* avoided static electric fields. The results suggest that the physical movement of the wings by the electric fields may mediate avoidance behaviour. This became evident when wild-type flies with cut wings and vestigial wing mutants were exposed to static electric fields. They exhibited avoidance behaviour only at voltages higher than those that evoked avoidance of wild-type flies. Chronic exposure to static electric fields caused mortality. The bioassay designs clarified the precise contribution of the effect of static electric fields and electric shock on the mortality of flies. Mortality was critically dependent on the level of electric field applied to the flies. Modelling electric field within the experimental designs showed that mortality occurred at higher field strengths than those that induced avoidance behaviour.

This research is a comprehensive study integrating the influence of static electric fields on the behaviour of *Drosophila*, as well as its potential harmful effects. Previous studies have solely focused either on behaviour or health effects using a range of different organisms (Newland *et al*., 2008; Fawzia, 2002). *Drosophila* is commonly used as the model organism to evaluate the effect of environmental stressors such as temperature, drugs and starvation (Hung and chen, 2007). However, this project extends these studies in *Drosophila* by investigating the effect of static fields. In addition, further study might identify the possible detrimental effect of sub lethal electric field at the molecular level. The advantage of *Drosophila* is that it is a convenient and established model system widely used in genetic and developmental biology and can provide results applicable to higher organisms including humans. Many published bioassay designs reveal that the subjects would have also been exposed to electric shock discharge on contact with exposed electrodes; it is thus not possible to differentiate whether the effects were due to the exposure to electric fields or due to electric shocks (Watson *et al*., 1983). This research required the creation of novel experimental designs to accurately simulate exposure to electric field in the absence of electric shock.

In addition, the majority of experiments investigating the effects of electric fields tend to use fluctuating electric fields (McCann *et al*., 1998) which are additionally physically associated with magnetic fields. The results often claim that detrimental effects were due to the electromagnetic field component. However, the contribution of sub lethal electric fields in isolation has been comparatively poorly studied. The majority of studies exposing organisms to electric fields did not apply software modelling to measure the actual field strength within the experimental designs that were used (Bindokas *et al*. 1989) and exposure was often presented as applied voltage (Matsuda *et al*., 2011). Application the software modelling with Maxwell is commonly used in estimating electric fields emission output from electrical equipment such as transmission power lines but the application of this software modelling with the current research theme is novel.

5.1 Static electric fields and the possibility of use in pest control

Insects have been shown to exhibit a variety of behavioural responses to electric fields. A recent study found that static electric field exposure reduced movement in freely moving cockroaches (Jackson *et al*., 2011). The avoidance of static electric fields that were exhibited by *Drosophila* in this study might encourage researchers consider the use such static electric fields as a method of pest control. Traditional methods of control, such as chemical pesticides result in harmful effects on humans such as asthma and poisoning symptoms (Hoppin, 2008; Vander, 1998). Porrini *et al*. (2003) reported that using agrochemicals leads to a poisoning risk in bees and environmental pollution, while several studies have shown that the effectiveness of chemical pesticides may decline over successive uses, due to development of resistance to the chemical (Martin, 1997). Further, biological control methods have shown their ability to reduce pest populations (Charlet *et al*., 2002), however, some studies suggest that this method is not continually effective as pest insects can survive the control measures (Helyer *et al*., 2004). Insect trapping using hydrocarbon attractants such as Tetracosane and Pentacosane do not work effectively (Hanley *et al*., 2009).

Applying static electric fields as a novel technique for excluding pest insects is more environmentally friendly (Kakutani *et al*., 2012b) and effective in certain situations. Using static electric fields as a non-toxic pest control method was suggested previously, as a charged surface can affect insect movement (Jackson and McGonigle, 2005; Maw, 1962). Recent studies have shown that *Drosophila* and cigarette beetles are able to avoid static electric fields when they approach a charged screen indicating the potential to for use in fields to protect crops (Matsuda *et al*., 2011). Kakutani *et al* (2012a) designed a new physical device, termed an electric field screen (EF-screen), which was effective at capturing pest insects such as *Liriomyza sativae, Myzus persicae and Sitophilus oryzae* at different voltages. This EF-screen helped protect plants from insect attack and provided airy conditions when they used in an open-window greenhouse (Kakutani *et al*., 2012b). Little is known however of the field strengths that causes avoidance in these studies. The present study showed that the minimum electric field strength that caused avoidance was between 24-34 kV/m (0.5 kV). This field strength did not however show harmful effects (knockdown or mortality) when *Drosophila* were exposed to them for long term, and therefore this level of field strength could potentially be used in pest control. For example, using static electric fields for keeping insects out of buildings by fixing such charge screens around doors and windows has the potential to be used in insect control.

5.2 Artificial electric fields produced by high voltage power lines and public health.

The rapid increase in the use of electricity in many countries resulted in an increased number of transmission lines in urban areas (Bakhashwain *et al*., 2003). The AC power lines a commonly cause artificial electric and magnetic fields (EMFs) in the environment. The electric fields they generate can reach up to 11 kV/m at ground level (Dezelak, 2010; www.emfs.info). The existence of EMF in the environment has raised concerns in recent years, due to its potential to cause adverse effects on public health, such as breast cancer, brain tumours and cardiovascular diseases (Feychting *et al*., 2005; Draper *et al*., 2005). A number of epidemiological studies also suggest a relationship between long term exposure to EMF and childhood leukaemia (Ahlbom *et al*., 2001; Draper *et al*. 2005) in particular in those individuals living close to power lines (WHO, 2002). One EMF component, extremely low frequency (ELF) (3-300 Hz) which produces fluctuating electric and magnetic fields, has been studied widely and it has been suggested that it is responsible for causing the adverse effects on health rather than the static electric fields alone. Saunders *et al.* (1991) suggested that the adverse effects of the ELF electric field on humans resulted from an interaction between the electric field and the charge on the surface of the body which induce electrical potentials within the tissues and a weak current flow. This current may then cause an alteration in intracellular biochemical and physiological function leading to harmful effects

(Saunders *et al*., 1991; Tenforde, 1991; Hanafy, 2004). Therefore, this study aimed to investigate the effect of static electric fields in the absence of magnetic fields to evaluate the possible role in causing detrimental effects on organisms

The results showed that chronic exposure to relatively high static electric fields caused mortality in *Drosophila*. However, a further analysis of whether sublethal static electric fields, such as those fields found under transmission power lines or around electrical home appliances, might provide clarification of the effect of DC and AC power line in the environment and subsequently on human health, as the latter are associated with magnetic fields. The outcome might encourage electricity suppliers to develop new operating DC lines rather than AC power lines.

Drosophila is commonly used to analyse the effect of different environmental stress on health, such as temperature and starvation, heavy metals and they show a rapid induction of heat shock proteins (HSPs), such as hsp70 (Feder *et al*. 1997; Bourg, 2007b; Bournias-Vardiabasis, 1990). Goodman *et al*. (2009) demonstrated induction of heat shock protein in Planaria, *Dugesia dorotocethala* when exposed to extremely low frequency electromagnetic field of 0.008 mT (60 Hz) and human leukocytes at 0.01 mT (50 Hz) (Coulton *et al*., 2004).

Heat shock proteins are ubiquitous, present inside cells and have different functions in all organisms (Kregel, 2002). For example, they act as protection for other proteins by preventing aggregation of unfolded proteins when cells undergo several types of environmental stress, such as heat, temperature and oxygen deprivation. They also act under non-stress conditions; for example, transporting proteins from one compartment to another inside the cell, and protecting new proteins during their growth (Parsell and Lindquist, 1993; Morimoto *et al*., 1997; Feder *et al*., 1997).

The new bioassay design developed in this project should allow further analysis of whether long term exposure to low level of static electric fields could have the potential to cause heat shock proteins induction, or other changes in gene expression which could have adverse effects on health.

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