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Faculty of Medicine, Health and Life Sciences

School of Biological Sciences

INFLUENCES OF ELECTRIC FIELDS ON INSECTS

BY

EDMUND PETER HUNT
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ABSTRACT

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Forces generated by the Earth's natural electric fields, and electrical equipment, are known to evoke responses in some terrestrial and aquatic animals, yet how insects respond to such electrical forces remains to be analysed in detail. The investigations presented in this thesis have contributed to understanding how insects respond to friction charged surfaces and static electric fields, why these responses occur, and their impacts on insect fitness. Using the cockroach *Periplaneta americana* and the fruit fly *Drosophila melanogaster* as models, the results show that insects avoid friction charged surfaces and static electric fields, and avoidance is dependent on field strength. Computer field modelling software and high-speed video illustrated that electric fields exert forces on cockroach sensory appendages, notably the antennae, causing passive antennal movement. A combination of behavioural bioassays and electrophysiology studies identified exteroceptors on the base of the antennae, specifically on the scapal hair plate, as the primary means by which cockroaches detect static electric fields and evoke avoidance. The antennae as a whole, and mechanoreceptors located on other appendages, may be responsible for detecting static electric fields of higher magnitudes. Static electric fields also evoked behavioural changes in free-moving cockroaches, specifically klino- and orthokinesis, and a preference for regions containing no static electric fields was exhibited. By investigating these behavioural responses and examining life-history traits, it was concluded that long-term static electric field exposure may not impact the fitness of cockroaches or *Drosophila.* It remains possible, however, that field strengths higher than those used in this study may detrimentally affect insects. The findings presented here have furthered current understanding of the influence of electric fields on insects and provide the basis for further work to be carried out within pure and applied research remits.

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1. GENERAL INTRODUCTION

1.1 Electric fields within the environment

Natural electric fields exist between the Earth's surface and outer atmosphere and are generated by a variety of sources (Bering *et al.*, 1998; Israel, 1971; Roble, 1991). The primary source is a global electric circuit that is produced and maintained predominantly by thunderstorms (Bering *et al.*, 1998; Israel, 1971; Rycroft *et al.*, 2000). Thunderstorms can generate a difference in electrical energy between the Earth's surface and outer atmosphere of approximately 250,000 Volts (V), positive with respect to the Earth's surface (Adlerman and Williams, 1996; Bering *et al.*, 1998; Williams, 1994). This difference, or voltage potential, within the atmosphere results in an electric field at ground level of 100-300 Volts per metre (V/m) (Adlerman and Williams, 1996; Bering *et al.*, 1998; Israel, 1971; Reiter, 1993; Rycroft *et al.*, 2000). High conductivity at ground level causes this field to dissipate within minutes but it is maintained by charge generation in thunderclouds across the globe (Rycroft *et al.*, 2000). Positive charge above a thundercloud is conducted into the upper atmosphere and negative charge at the base of the cloud is transferred to Earth through the conductive lower atmosphere (Rycroft *et al.*, 2000); a mechanism that is dependent upon diurnal and seasonal variation (Adlerman and Williams, 1996; Rycroft *et al.*, 2000; Williams, 1994).

Atmospheric electric fields produced by the global electric circuit are not the only sources of electric fields within the environment. Interactions between materials generate charges and produce electrical forces many magnitudes greater than those within the atmosphere. A classic example of such forces is those that can be produced as humans walk across a carpet, during which the field strengths can reach up to 30kV/m (Chubb, 2003). Insects can also carry a net electric charge produced when walking on surfaces (Colin *et al.*, 1991; Edwards, 1962; Jackson and McGonigle, 2005; Yes'Kov and Sapozhnikov, 1976), and during flight by wings rubbing against the body or within the air (Gan-Mor *et al.*, 1995; Yes'Kov and Sapozhnikov, 1976).

High voltage power lines and electrical equipment also contribute to environmental electric fields by generating electromagnetic and electrostatic fields. Magnetic fields produced by high voltage power lines and electrical equipment can be up to five thousand times greater than the Earth's magnetic field (Aldrich and Easterly, 1987; Repacholi and Greenebaum, 1999). Static electric field measurements beneath power lines vary according to operating voltage, yet can reach up to 11kV/m (Bracken *et al.*, 2005), and fields surrounding electrical equipment up to 20kV/m (Repacholi and Greenebaum, 1999). The impact of high voltage power lines on atmospheric electric fields is variable and dependent on wind direction and surrounding landscape. Nevertheless, the fields generated can be over one hundred times greater than the Earth's natural electric field (Bracken *et al.*, 2005; Carter and Johnson, 1988; Fews *et al.*, 2002; Johnson, 1983; Sebo *et al.*, 1982).

Electric fields are not only present in the terrestrial atmosphere, but also in seawater and freshwater environments. Movement of ocean water through the Earth's geomagnetic field induces an electric field (Kalmijn, 1988; Sanford, 1971) and in freshwater electrical potentials are generated from electrochemical sources (Kalmijn, 1988). Oceanic electric fields have been recorded between 0.05nv/m and 5nv/m (Kalmijn, 1988), many orders of magnitude weaker than atmospheric electric fields due to the high conductivity of water (Manoj *et al.*, 2006). Nevertheless, the development of specific highly sensitive sensory structures in some fish species enables them to detect these low strength electric fields, and is an ability utilised during predation and orientation (Bullock, 1982; Kalmijn, 1988). Electroreception is further exploited in other fish species for intra- and interspecific communication by the active generation and detection of electric fields using specific sensory structures (Bullock, 1982; Heiligenberg and Bastian, 1984; Hopkins, 1974; Hopkins, 1988; Kalmijn, 1988).

The evolution of electro-sensory structures in aquatic organisms to detect electric fields at strengths lower than those experienced in the terrestrial environment raises questions about the possible existence of an electric sense in terrestrial invertebrates. To date, there is no evidence that terrestrial invertebrates possess specific mechanisms to detect electric fields. Reports demonstrate, however, that insects respond to a variety of electrical forces, including electric fields in the atmosphere, between materials and surrounding high voltage power lines.

1.2 Insects and electric fields

1.2.1 Insect responses to electric fields

Fabre (1918) first suggested that invertebrates respond to atmospheric electric fields, observing heightened activity of dung beetles before a thunderstorm (cited in Maw, 1962). Subsequent studies by Schùa (1952), among others, further demonstrated insect responses to fluctuating atmospheric electric fields (cited in Maw, 1961b). These reports did not, however, take into consideration the effects of atmospheric pressure changes on animals, a factor now known to influence insect behaviour (Wyatt, 1997). Laboratory studies exposing insects to electric fields of magnitudes similar to those within the atmosphere suggest that insects do respond to naturally occurring electric fields. Examples include aggregation of mosquitoes in regions of high atmospheric field strength (Maw, 1961b), and decreased locomotion of the fruit fly, *Drosophila melanogaster* and the blow fly, *Calliphora vicina*, exposed to ≈500V/m and above (Edwards, 1960a). Some ambiguity is apparent however, as reports indicate no changes in oviposition of the phantom hemlock looper, *Nepytia phanatasmaria,* or *D. melanogaster* exposed to electric fields ≈125V/m (Edwards, 1961). Self-generated electrical forces during insect locomotion and flight have been reported to influence insect behaviour: for example, net charge accumulated by flying and walking honey bees has been reported to alter behaviour of the bee parasite *Varroa jacobsoni*, and may ultimately assist attack by attraction of the parasite to the charged bee(Colin *et al.*, 1992). It has also been suggested that charge accumulation during honey bee flight could assist foraging and pollination by increasing the transfer of pollen grains onto plant stigmas (Colin *et al.*, 1991; Gan-Mor *et al.*, 1995).

A number of laboratory-based studies have investigated how electric field strengths higher than those in the atmosphere affect insects (Table 1.1), and have described influences on locomotion (Edwards, 1960c; Watson, 1984), preference (Perumpral *et al.*, 1978), and some developmental changes (Edwards, 1961; Levengood and Shinkle, 1960).

Electric field (V/m)	**Insect species**	**Responses**	**Author**
Unknown	*Ictoplectis conquisitor*	Decreased locomotion	Maw, 1961
1000 – 4000	*Drosophila melanogaster*	No difference in locomotion	Edwards, 1960
1500	*Aedes aegypti*	Aggregation	Riordan, 1973
12,000	*Drosophila melanogaster*	Increased locomotion	Edwards, 1960
18,000	*Nepytia phantasmaria*	Delayed eclosion and decreased fecundity	Edwards, 1961
20,000 – 50,000	*Musca domestica*	No location preference	Perumpral *et al.*, 1978
20,000 – 75,000	*Trichoplusia ni*	Decrease in wingbeat frequency	
100,000	*Musca domestica*	Location preference for untreated area	
100,000 – 150,000	*Trichoplusia ni*	No change in wingbeat frequency	
200,000	*Drosophila melanogaster* and *Calliphora vicina*	Agitation	Watson, 1984
300,000	*Calliphora vicina*	Paralysis	
400,000	*Drosophila melanogaster*	Paralysis	
480,000	*Drosophila melanogaster*	Increase in progeny yields	Levengood and Shinkle, 1960

Table 1.1 Previous studies investigating the effects of electrostatic fields higher than those encountered in the atmosphere on the behaviour of a variety of insect species. Most studies resulted in the majority of individuals responding to the treatment yet not all responses were detrimental.

Questions have also been raised as to whether electric fields associated with high voltage power lines affect invertebrate behaviour and populations. Studies have shown decreased flying activity and foraging of insects in the vicinity of power lines, in addition to lowered populations (Bindokas *et al.*, 1988; Orlov, 1990; Orlov and Babenko, 1987). Studies also demonstrate aberrant development of the honey bee, *Apis mellifera,* in hives positioned beneath 765kV power lines (Greenberg *et al.*, 1981a; Greenberg *et al.*, 1981b). Inconsistencies in the results are evident, however, as some invertebrates show raised activity or no behavioural changes in close proximity to power lines (reviewed in Orlov, 1990).

Nevertheless, ambiguity remains over the possible health effects of high voltage power lines on humans and interest in this continues to mount (Cheng *et al.*, 2000; Draper *et al.*, 2005; Olsen *et al.*, 1993). The causes of the apparent health effects are not conclusive, although electromagnetic fields and pollutant particles produced by high voltage power lines are thought to play a role (Fews *et al.*, 1999a; Fews *et al.*, 1999b; Hamza *et al.*, 2002). Investigating the influences of electric fields on invertebrates could reliably and quickly provide further information on the effects of power lines on biological systems.

Together, these studies described here demonstrate that insects are affected by electric fields, yet many questions remain unanswered and indicate the need for further work to be carried out clarifying the influences of electric fields on invertebrates.

1.2.2 The detection of electric fields by insects

Few studies have been aimed at understanding how electric fields are detected by insects. Involuntary movement of prominent sensory appendages exposed to electric fields, such as the antennae (Maw, 1961c; Yes'Kov and Sapozhnikov, 1976) and the wings (Bindokas *et al.*, 1989; Watson *et al.*, 1997) has been shown to occur (Table 1.2). Deflection of hairs on the legs of spiders has also been reported when individuals are exposed to electric fields similar to those beneath power lines (Orlov and Romanenko, 1989).

Movement of such sensory structures can initiate behavioural responses due to activation of mechanoreceptors present on and within them. For example, small hairs on cockroach cerci, the filiform hairs, are sensitive to air movement and initiate the escape response when deflected by oncoming predators (Camhi, 1984; Camhi and Tom, 1978a). The antennae are highly active and flexible appendages that are present on all insects (Okada and Toh, 2001; Schneider, 1964), and also contribute to the escape response of cockroaches via activation of mechanoreceptors during antennal movement (Comer *et al.*, 1994; Stierle *et al.*, 1994). Due to the sensitivity of these sensory structures to movement and the mechanical stimulation of them by electrostatic fields, these appendages could play a role in the detection of electrostatic fields.

Given the ability of some insects to detect magnetic fields by active deposition of iron particles within cells (Maher, 1998; Towne and Gould, 1985), there is some speculation that electric fields could be perceived by cellular means (Ishay *et al.*, 1991; Tenforde, 1991). Electric fields have been shown to alter cell membrane channel function (Liburdy, 1995; Tenforde, 1991), protein production (McLeod *et al.*, 1987), and DNA transcription (McCann *et al.*, 1993; Tenforde, 1991). Cellular effects such as these may not elicit behavioural changes, but nonetheless are a possible means by which electric fields could be perceived.

Genotoxic effects, notably DNA aberrations, have also been shown to occur in both invertebrates and vertebrates exposed to static electric fields (McCann *et al.*, 1993). Again, however, there are inconsistencies in the results and there is no evidence that such effects contribute to the detection of electrostatic fields (McCann *et al.*, 1998; McCann *et al.*, 1993). Nevertheless, these mutagenic changes do highlight the possible health issues associated with exposure to electric fields.

Electric field (V/m)	**Insect species**	**Sensory structure response**	**Author**
Unknown	*I. conquisitor*	Increased antennation	Maw, 1961
5000 (AC 30-50Hz)	*D. melanogaster*	Wing vibration	Orlov and Tolkacheva, 1989
20,000 – 25,000	*D. melanogaster*	Wing vibration	Watson, 1997
95,000	*A. mellifera*	Antennal vibration	Yes'Kov and Sapozhnikov, 1976
350,000	*A. mellifera*	Hair vibration	Bindokas *et al.*, 1989

Table 1.2 Responses of invertebrate sensory structures exposed to varying electric field strengths.

1.3 The biology of *Periplaneta americana*

The cockroach has been intensively used for scientific studies over many years for a number of reasons: their ease of use; hardiness; low cost; and high fecundity (Schal *et al.*, 1984). It is straightforward to culture cockroaches under controlled laboratory conditions, but in the natural environment they are regarded as a prolific pest.

1.3.1 Classification, distribution and habitat

The cockroach is a member of the order Orthoptera and the sub-order Blattaria. Differing morphology separates all species into five families (Schal *et al.*, 1984), the most common of which is the Blattoidea family that contains the abundant *Periplaneta* genus and the species used in this study, *Periplaneta americana* (Bell and Adiyodi, 1981). *P. americana* is a polyphagous insect and its diverse diet (Gier, 1947) contributes to its ability to survive in a variety of habitats, its success as a terrestrial organism and its pest status (Cornwell, 1968).

The potential for cockroaches to endanger human health and threaten resources has led to their prominence as a pest (Bell and Adiyodi, 1981). *P. americana* are prevalent in areas of food storage and handling, toilets and hospitals, thus providing disease carrying opportunities (Cornwell, 1968). Pathogens are present in the cockroach alimentary canal and on the cuticle, and the rapid movement of cockroaches between locations can facilitate disease transfer (Cornwell, 1968; Schal and Hamilton, 1990). Hypersensitivity to cockroach cuticular proteins has also been reported and allergic reactions are common in urban dwellings (Arruda *et al.*, 2001; Cornwell, 1968; Schal and Hamilton, 1990). These factors have led to a demand for cockroach control methods. To date insecticides are most commonly used due to their effectiveness against large populations, although this is an undesirable method in food preparation areas (Schal and Hamilton, 1990). As cockroaches impact humans primarily in the urban environment (Arruda *et al.*, 2001) non-toxic management methods are preferential, yet the success of pheromone-based control methods has been limited (Liang *et al.*, 1998; Nalyanya *et al.*, 2000).

1.3.2 Growth and development

P. americana pass through 6-14 life stages or nymphal moults, depending on factors such as humidity, temperature and food supply (Bell and Adiyodi, 1981). After the final moult reproduction can occur and the spermatophore, transferred from the male to female during copulation, is retained inside the female for up to a year during which an average of one egg case per week is released (Cornwell, 1968). Each egg case, containing approximately sixteen fertilised eggs, is commonly oviposited in a warm place in or on a variety of substrates: sand; soil; cans; food wrappers; and boxes (Bell and Adiyodi, 1981).

Incubation length and egg viability is correlated with ambient temperature and on average lasts twenty days (Gier, 1947). After emergence nymphal life-span duration lasts between 100 and 1000 days, depending on food availability and temperature (Gier, 1947). Adult longevity can be between 70 and 1500 days although it is commonly 150-200 days (Bell and Adiyodi, 1981). The long life-span, short incubation, high fecundity and nonspecificity for oviposition location contribute to the cockroach's success as a terrestrial invertebrate, abundant pest, and suitable model for research purposes.

1.3.3 Sensory structures

The role of the insect sensory system is to obtain and process information surrounding and within an individual (MacFarland, 1985). The central nervous system (CNS) of the cockroach is segmented into a number of bilaterally symmetrical ganglia (six in the abdomen, three in the thorax and one in the head); that are linked by paired connectives (Bell and Adiyodi, 1981; Cornwell, 1968). Receptors located on the periphery of the body are associated with the dendrites and cell bodies of sensory neurons, the axons of which project to their respective segmental ganglion (Camhi, 1984). Each ganglion is a collection of cell bodies, dendrites and axons, receiving information from peripheral sensory neurons and sending information via intersegmental axons in the connectives (Camhi, 1984). Synaptic connections between neurons enable the transfer of information across the CNS and ultimately initiate and mediate locomotor behaviour via motor neurons (Camhi, 1984; Delcomyn, 1998). For example, filiform hairs on the cerci, positioned on the posterior of the abdomen, are sensitive to air movement due to the dendrite of a sensory neuron located at the base of each hair that is activated by hair deflection (Camhi, 1984). Each sensory neuron arborises in the terminal ganglion and in turn may synapse with Giant Interneurons (GIs) (Camhi, 1984). The GIs send information to other ganglia and synapse with thoracic interneurons that are subsequently associated with motor neurons controlling leg muscle activity (Delcomyn, 1998). A complex network of interneurons between the input and output enable effective feedback mechanisms that regulate the escape behaviour of cockroaches in response to air movement to avoid predator attacks (Delcomyn, 1998).

The ganglion within the head, the supraoesophageal ganglion, is considered the brain of the cockroach, consisting of arborisations from many sensory neurons (Cornwell, 1968), including those from receptors on the antennae (Bell and Adiyodi, 1981; Schneider, 1964). The antennae are important sensory structures responsible for chemoreception, thermoreception, hyrgoreception and mechanoreception (Schneider, 1964). Being located rostrally and highly mobile, they provide detailed information on the external stimuli and a representation of the anterior environment (Toh, 1977). Only recently, however, have the antennae been found to play an important role in the escape response (Burdohan and Comer, 1996; Ye *et al.*, 2003), supplementing the function of the cerci.

1.4 Principles of electrical forces

1.4.1 Electromagnetism

All matter consists of atomic particles: neutrons, protons and electrons (Hammond, 1997). Attractive and repulsive forces act between protons and electrons, and this property constitutes electric charge - the fundamental factor in electromagnetism (Cross, 1987; Hammond, 1997). Electromagnetism incorporates two kinds of forces: those produced by stationary electric charges and those by charges in motion (Hammond, 1997). Stationary charges are 'electrostatic', and those that move generate magnetic fields and are hence 'electromagnetic' (Hammond, 1997). This study focuses on electrostatic forces, yet considering their relationship with electromagnetic forces, it is important to distinguish the two.

The key difference between electrostatic and electromagnetic forces is that the latter involves electric charges, specifically electrons, which have been set in motion resulting in an electric current (Hammond, 1997). An electric current can either be alternating or direct, and although both represent the flow of electric charge there are fundamental differences between them. Direct current (DC) is the continuous flow of charge in one direction (positive to negative) through a conductor (Hammond, 1997). Alternating current (AC) is not the continuous flow of current but a cyclic change in direction and magnitude of charge, the frequency of this being Hertz, Hz (Hammond, 1997). An example of AC charge is that supplied to transmission networks, commonly at frequencies between 50 – 60Hz (Tenforde, 1991). AC charges such as these, and any currents alternating at frequencies less than 300Hz, are known as Extremely Low Frequency (ELF) electric fields (Tenforde, 1991). ELF electric fields, and other AC electric fields give rise to one important characteristic, that being electromagnetic radiation. The alternating nature of the current leads to the production of an external current outside of the conductor it is passing through (electromagnetic radiation), and the current frequency is correlated with the magnitude of radiation (Hammond, 1997; Tenforde, 1991).

1.4.2 Electric charges and electrostatic forces

Static electric charges do not generate electromagnetic radiation, yet the principles behind the forces of both types of electromagnetism remain similar. True electrostatic forces occur on and around insulating materials due to atomic interactions (Cross, 1987; Montgomery, 1959). Negative electrons are distributed on energy levels within atoms and can move between these levels (Cross, 1987). If an atom contains one or more too many, or one or more too few electrons than required for a neutral state, it is termed a negative or positive ion respectively, and together electrons, positive, and negative ions are termed charges (Chubb, 2003).

When two or more charges are in close vicinity, forces occur between them (Cross, 1987). The force between two negative charges results in repulsion; attraction occurs between a negative and positive charge (Cross, 1987). Coulomb's Law states that these forces are proportional to the quantities of charge and log proportional to the distance between them (Chubb, 2003). Therefore the force at a given distance decreases if the quantity of charge decreases, and the force between two charges increases as the distance between them decreases.

The forces around and between charges are represented in two ways: field potentials and electric fields (Chubb, 2003; Cross, 1987). Field potentials represent the electric potentials of a charge in its surrounding environment (Hammond, 1997). The electric potential is the amount of work required (the energy) to move charge a certain distance against an electric field (equation 1.1) and is given the unit Volt (V) (Cross, 1987).

Equation 1.1 $V = Ed$

V = Volts

E = electric field

d = distance (in metres)

Equation 1.1 shows that the electric potential is relative to the distance of the charge source, and the potential supplied to a system has an effect on the electric field over a distance. The electric field is therefore a representation of the force within a system and is defined as a change of electric potential over distance, and given the units V/m (Chubb, 2003; Cross, 1987; Hockey, 1972). Electric fields cause the forces on, or between, charges at a given distance, and are represented as a series of lines or vectors extending from the positive charge (Fig. 1.1).

1.4.3 The triboelectric series and friction charging

Contact and separation of materials (tribocharging) releases energy due to changes in charge distribution at the atomic level (Chubb, 2003; Cross, 1987; Montgomery, 1959). During tribocharging, charges are transferred between atomic energy levels in and between the materials (Cross, 1987). This results in one material gaining a net charge and therefore an electric field is generated (Cross, 1987; Montgomery, 1959). The amount of charge transferred, and hence the strength of field, is dependent upon surface contamination, temperature, humidity and differences in the atomic properties of materials, among other factors (Chubb, 2003). In addition, charge transfer between materials varies according to the type of material, and this property forms the basis of the triboelectric series (Cross, 1987). This is a table of materials (Table 1.3) arranged according to the amount of positive charge that is transferred from one material to the other during contact (Cross, 1987). A material at the top of the series, such as nylon, will become highly positive if contacted with polyethylene, although the transfer of charge between nylon and cotton, for example, will not be so great due to their closer positions in the triboelectric series (Montgomery, 1959).

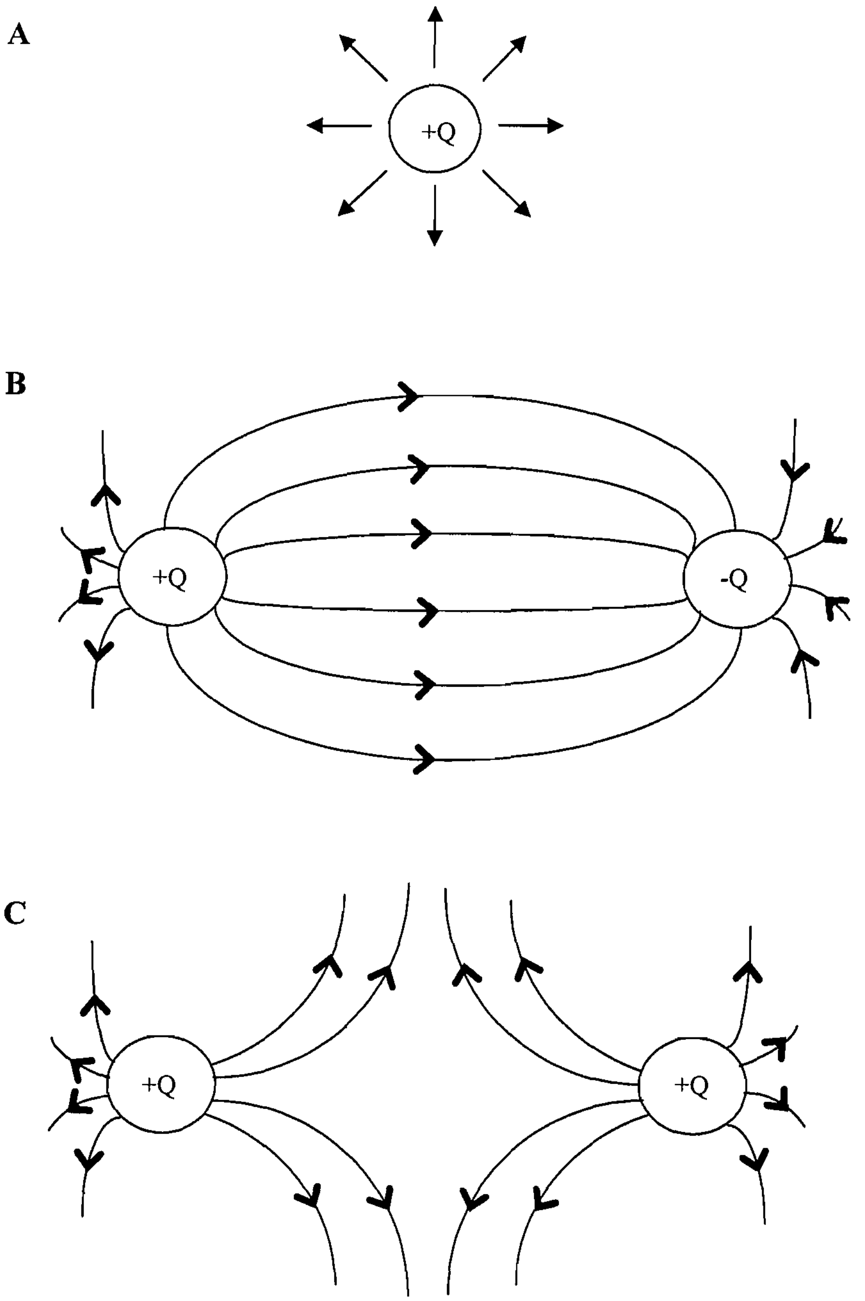


Figure 1.1 Electrical forces between point charges. (A) The electric field produced by a single charged body (+Q). (B) The forces between a positive (+Q) and negative (-Q) charge pass from the positive to the negative and are represented by electric field lines and arrows. (C) Forces generated by two charges of the same polarity are repelled.

	Material
+	Glass
	Human hair
	Wool
	Nylon
	Silk
	Aluminium
	Paper
	Cotton
	Copper
	Gold
	Perspex
	Polystyrene
	PVC
	Polyethylene
-	PTFE

Table 1.3 A selection of materials and their positions in the triboelectric series. Materials at the bottom of the table will be become highly negatively charged if contacted with a material from the top of the table. The amount of charge transfer between materials is relative to the distance between them in the series (Cross, 1987).

Rubbing materials together (friction charging) significantly increases charge transfer between materials in comparison to contact charging due to a rise in surface temperature of the materials during rubbing (Cross, 1987). Friction charging can therefore generate electric fields much greater than those produced by tribocharging. The reasons for improved charge transfer during friction charging are not fully understood, but it is accepted that the charge transferred is proportional to the energy applied during rubbing (Cross, 1987). It has been reported that applying additional energy to the charge transfer process can result in reversing the polarity of the materials that normally occurs during tribocharging (Cross, 1987).

Charge transfer at the atomic level by contact or friction charging is not the only process known to result in net charge gains. Interactions between charges and electric fields, known as induction charging (Fig. 1.2), also cause net changes in charge on conducting materials (Cross, 1987).

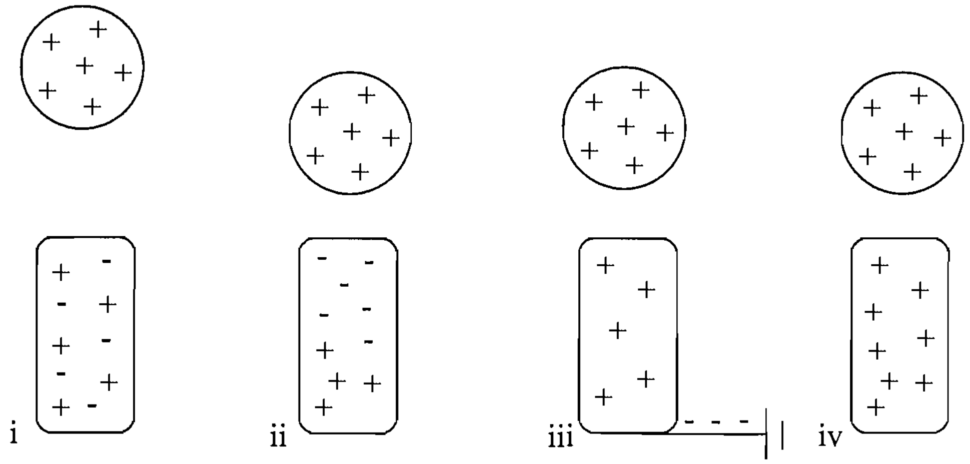


Figure 1.2 Schematic diagram of charge induction and conduction. (i) Charge can be induced on an insulated neutral conducting material (rectangular rod) when exposed to an earthed charged positive electrode (sphere). (ii) Separation of charge, or polarisation, occurs when the rod is earthed near the electric field produced by the charged sphere. (iii) Charge induction occurs when an earthed object is approached by a charged object. Negative charge flows to earth resulting (iv) in a net positive charge on the rod.

Once a material is charged, the charge does not remain indefinitely and will decay (Chubb, 2003). The rate of decay is dependent upon the ambient temperature, humidity, structural properties, and the voltage of the material (Cross, 1987). Charge decay is rapid when the voltage and charge are high, but decay decreases as the voltage and charge reduces over time (Chubb, 2003; Cross, 1987).

1.4.4 Charge flow

Applying an electric potential to a conductor results in the flow of charge and the forces involved are therefore no longer electrostatic (Cross, 1987). An electric potential can be produced using a high voltage power supply and once applied across a conductor, charge flows and a current is created (Cross, 1987). This, as explained in 1.4.1, generates a magnetic field (Hammond, 1997), yet forces between the charges remain indifferent to those produced by tribo- and friction charging, and can be controlled more effectively. For this reason, investigations carried out in this study used high voltage power supplies to generate minimal charge flow through a conductor and produce electric fields that could be regulated. The currents used, and hence any magnetic fields produced, were negligible. In addition, the currents were direct and not alternating; therefore no electromagnetic radiation was produced. Electric fields generated by the high voltage power supply in this study will be termed 'static electric fields' to distinguish them from the electrostatic forces produced by friction charging.

The production of charge can be also be regulated by corona charging (Chubb, 2003; Cross, 1987). Applying a high voltage between two electrodes, one having a fine end and the other flat, results in an electric field sufficient to cause air breakdown near the fine electrode (Chubb, 2003). Electrons within this region will be accelerated, detaching electrons due to collisions with atoms, and leaving positive and negative charges that migrate across the flat electrode depending on the polarity and magnitude of potential applied (Chubb, 2003; Cross, 1987). The electric field strengths required to induce air ionisation are greater than 250kV/m and are associated with light flashes and spark discharges (Chubb, 2003), including localised changes in air pressure (Phelps and Griffiths, 1976).

1.5 Aims and objectives

- Quantify the avoidance of *P. americana* exposed to friction charged surfaces and static electric fields;
- Examine the effect of field strength on cockroach avoidance of static electric fields;
- Define how free-moving cockroaches move when confronted with static electric fields;
- Calculate the electrical forces acting on *P. americana* and its sensory structures;
- Determine the mechanism responsible for static electric field detection;
- Investigate the neurological basis of static electric field avoidance;
- Assess the impact of long-term static electric field exposure on the fitness of *P. americana* and *Drosophila*.

2. THE AVOIDANCE OF ELECTRIC FIELDS

2.1 Introduction

Electric fields are reported to evoke behavioural changes in a number of organisms. To date, however, these responses have been characterised in few species. Observations indicate that insect locomotion is affected by transient exposure to electric fields (Edwards, 1960a; Maw, 1961b; Perumpral *et al.*, 1978; Watson *et al.*, 1997), yet it remains to be determined how insects respond when confronted by electric fields.

Of the animals studied, aquatic organisms, notably the Gymnotiform fish, are known to produce and exploit low strength pulsating electric fields for predation and communication (Bullock, 1982; Heiligenberg and Bastian, 1984; Kalmijn, 1988). The ability of electric fish to produce and detect electric fields is unique and no such mechanism has been reported in insects or any other animals. Laboratory and field observations have demonstrated changes in the behaviour of insects exposed to electric fields, commonly reporting decreases in locomotion (Edwards, 1960c; Maw, 1961c; Maw, 1962; Perumpral *et al.*, 1978; Watson *et al.*, 1997). Such responses have been evoked by a variety of electric field types: charged surfaces; low and high electric field strengths generated by high voltage power supplies; and fields produced by high voltage power lines.

Insect responses to charged surfaces have predominantly been shown to result in individuals avoiding, or being repelled by the charged region. For example, decreased insect catches have been reported to occur in plastic traps frictionally charged by surrounding foliage (Maw, 1964), and charged screens repel flying insects in their natural habitats (Maw, 1962). Movement of the parasitoid *Ictoplectis conquisitor* has also been shown to decrease when walking across a surface of increasing electrical potential (Maw, 1961c). Although such changes in behaviour have led to suggestions that charged surfaces could be exploited for pest control (Jackson and McGonigle, 2005; Maw, 1962; McGonigle *et al.*, 2002), they have not yet been quantified.

The ability to generate electric fields under laboratory conditions using high voltage power supplies has led to investigations concerning the influences of such fields on insects. The majority of studies have focussed on adverse effects, such as chromosome aberrations and paralysis, using electric field strengths from 10kV/m to 400kV/m (McCann *et al.*, 1998; McCann *et al.*, 1993; Watson, 1984). There are also indications that insect movement is influenced by high voltage electric fields far greater than those present in the atmosphere, >12kV/m (Edwards, 1960a; Edwards, 1961; Watson, 1984) and beneath high voltage power lines at field strengths no greater than 11kV/m (Bracken *et al.*, 2005; Orlov, 1990; Orlov and Babenko, 1987) . There are some indications that invertebrates exhibit preferences for regions containing no electric fields when given a choice between areas treated and untreated with high voltage electric fields greater than 100kV/m (Perumpral *et al.*, 1978). As a whole, results remain inconclusive and thorough investigations to quantify the behavioural responses of invertebrates to static electric fields have yet to be carried out.

Previous studies have contributed some information regarding the interactions between insects and electric fields, indicating the possible mechanisms behind insect responses to electric fields. For example, electrostatic forces have been shown to play a role in honey bee pollination (Colin *et al.*, 1991; Gan-Mor *et al.*, 1995) and during host-parasite interactions (Colin *et al.*, 1992). Charge measurements of insects during flight (Edwards, 1960b; Gan-Mor *et al.*, 1995) and walking (Edwards, 1962; Jackson and McGonigle, 2005; McGonigle *et al.*, 2002) have revealed an accumulation of charge during locomotion. How interactions between insects and electrical fields cause behavioural changes, however, has yet to be established.

The effect of light is a factor not previously examined in studies of insect responses to electric fields. Nevertheless, changes in insect behaviour, such as flight patterns (Cardé and Knols, 2000; Sherman and Dickinson, 2004), *Drosophila* larval movement (Hassan *et al.*, 2000) and ant aggregation (Depickère *et al.*, 2004), have been shown to occur when external light conditions are varied. Feedback mechanisms between motor outputs and visual inputs, evident in cockroaches, are thought to be responsible for these behavioural changes at different light levels (Mizunami, 1995a; Sherman and Dickinson, 2004), and are evident in cockroaches (Mizunami, 1995b; Okada and Toh, 1998) and other insects (Labhart and Meyer, 1999). Light sensitive ocelli positioned above cockroach compound eyes (Bell and Adiyodi, 1981; Mizunami, 1995a) provide information on external light levels and are associated with sensory inputs from olfactory, mechanosensory and visual centres, in addition to motor outputs (Mizunami, 1995a). In turn, the ocelli play an important role in a multi-modal sensory system, contributing to locomotory responses under varying light conditions (Labhart and Meyer, 1999; Mizunami, 1995b; Okada and Toh, 1998; Simmons, 2002). The integration of the visual system and motor outputs therefore raises the question whether insect responses to electric fields are affected by light intensity.

Although previous studies have demonstrated that electric fields influence insects, many questions remain unanswered. To quantify the responses, this study focuses on the preference behaviour of *P. americana* exposed to friction charged surfaces and static electric fields generated by a high voltage power supply. The bioassays carried out, in combination with electric field modelling using computer software, will provide a more detailed understanding of how insects are influenced by electric fields.

2.2 Materials and Methods

P. americana cultures were maintained in a licensed insectary at the School of Biological Sciences, University of Southampton, at 20-26°C under a 12L:12D light regime. Egg cases were collected from adult colonies and incubated at 28°C to optimise development and reduce hatching time (Gier, 1947). Newly hatched nymphs were regularly collected from egg cases, and maintained in sandwich boxes at 20-26°C under a 12L:12D light regime (7:00 on:19:00 off GMT). Nymph and adult cockroaches were fed on a diet of dog biscuits and water.

2.2.1 The avoidance of friction charged polytetrafluroethylene (PTFE)

2.2.1.1 Y-choice apparatus

Three identical Y-choice apparatus were constructed from 3mm thick square-cut glass and consisted of three open pathways, 100 x 15 x 20mm (Length [L] x Width [W] x Height [H]) in size, positioned 120° apart in a 'Y' configuration with one central pathway and two anterior pathways (Fig. 2.1). The open structure enabled additional base sections to be placed in, and removed from, the pathways. The central pathway housed a removable aluminium section (110 x 14 x 3mm), tapered at one end to ensure close contact with two removable Polytetrafluoroethylene (PTFE) sections (100 x 14 x 3mm, Amari Plastics PLC, Southampton) positioned in the two anterior pathways. The aluminium section was earthed by strand of uninsulated copper wire (0.33mm diameter, RS Electronics, UK) to localise the electric field at the junction of the pathways (the intersection). The walls of each pathway were coated with Fluon® to prevent cockroach escape and a glass sheet (205 x 205 x 3mm) was placed over the apparatus during each trial to eliminate air movement.

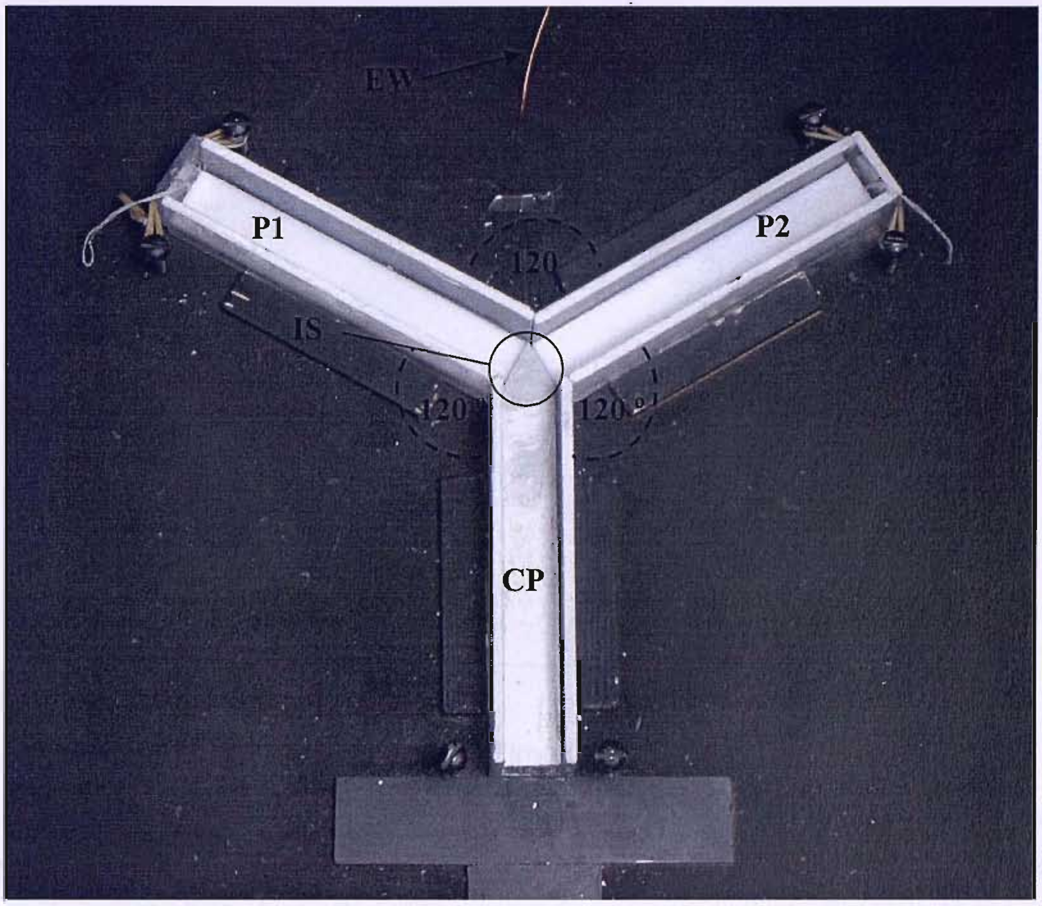


Figure 2.1 Photograph of the Y-choice apparatus. The central aluminium pathway (CP) was tapered at the intersection (IS), beneath which an uninsulated earthed copper earth wire was fixed (EW). The PTFE sections (P1/P2) could be removed from the two anterior pathways, positioned 120° apart, for friction charging.

Before each trial one PTFE section was frictionally charged by rubbing three times (up-down-up) using a small silk cloth (100 x 85mm) and then placed on a glass sheet (205 x 205mm) to stabilise for two minutes. The stabilisation period ensured consistency in the field strength during the trial. Preliminary investigations showed that the charge decay of PTFE stabilises after two minutes resulting in a stable electric field for over 6min (Fig. 2.2). After stabilisation, the field strength of the charged PTFE section was measured 10cm above the glass sheet (n = 37, -0.3±0.06kV/m) using a field meter (JCI 140, John Chubb Instruments, UK). The uncharged section was also measured to ensure that no charge accumulation had occurred.

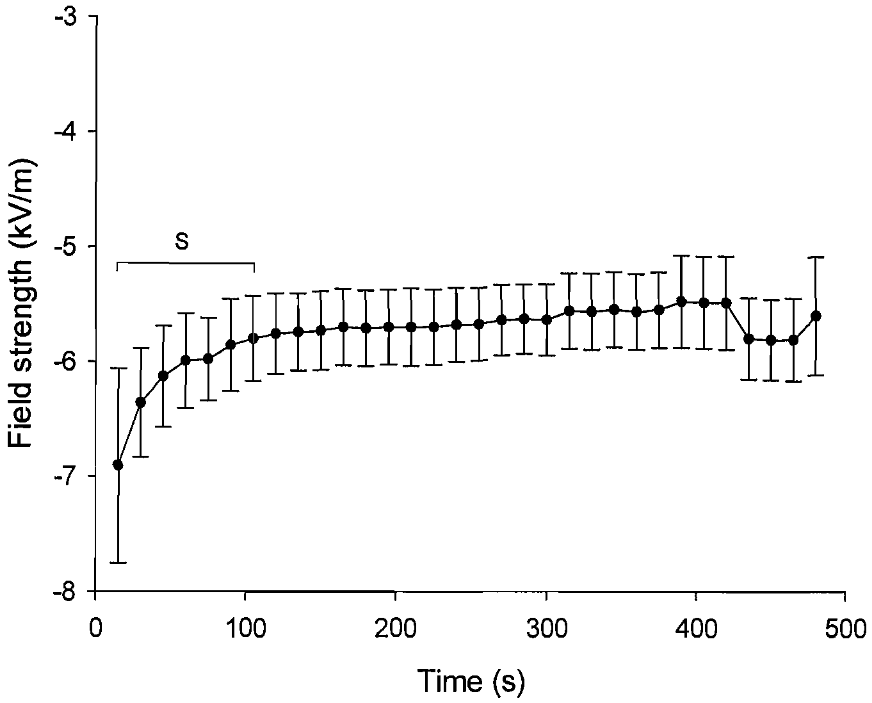


Figure 2.2 The charge decay properties of PTFE after friction charging, expressed as mean electric field strength (±SEM, $n = 5$) over time. The charge held on PTFE stabilises after 2min, the stabilisation period (S), and subsequently the field strength remains constant.

2.2.1.2 Behavioural bioassay

After charge stabilisation each section was placed into the pathways and the apparatus covered with glass. One third/fourth instar cockroach ($n = 37$, 7.2 ± 1mm) was gently placed into a release chamber (Fig. 2.3) using storkbill forceps and allowed to rest for approximately 2min. After release the pathway taken was noted ('avoid' or 'non avoid') once the cockroach had passed one body length along one of the anterior pathways. Cockroaches returning back down the central pathway were discounted. After each trial all PTFE sections were rinsed in 100% ethanol to remove charge and any pheromone traces from tested individuals, and left to dry for 5min. To control for natural bias the charged side was alternated between trials, and in addition a series of trials was carried out using two uncharged PTFE sections to test for any natural preference. The pathway taken by each cockroach was noted as 'left' or 'right' and cockroaches returning back down the central pathway discounted.

Y-choice bioassays were also carried out, based on the above protocol, using two charged PTFE sections ($n = 37$, -0.51±0.13kV/m), investigating the responses of cockroaches confronted by charged surfaces from both anterior directions. Cockroaches returning back down the central pathway were noted as 'avoid', and those taking either anterior pathway as 'non-avoid'.

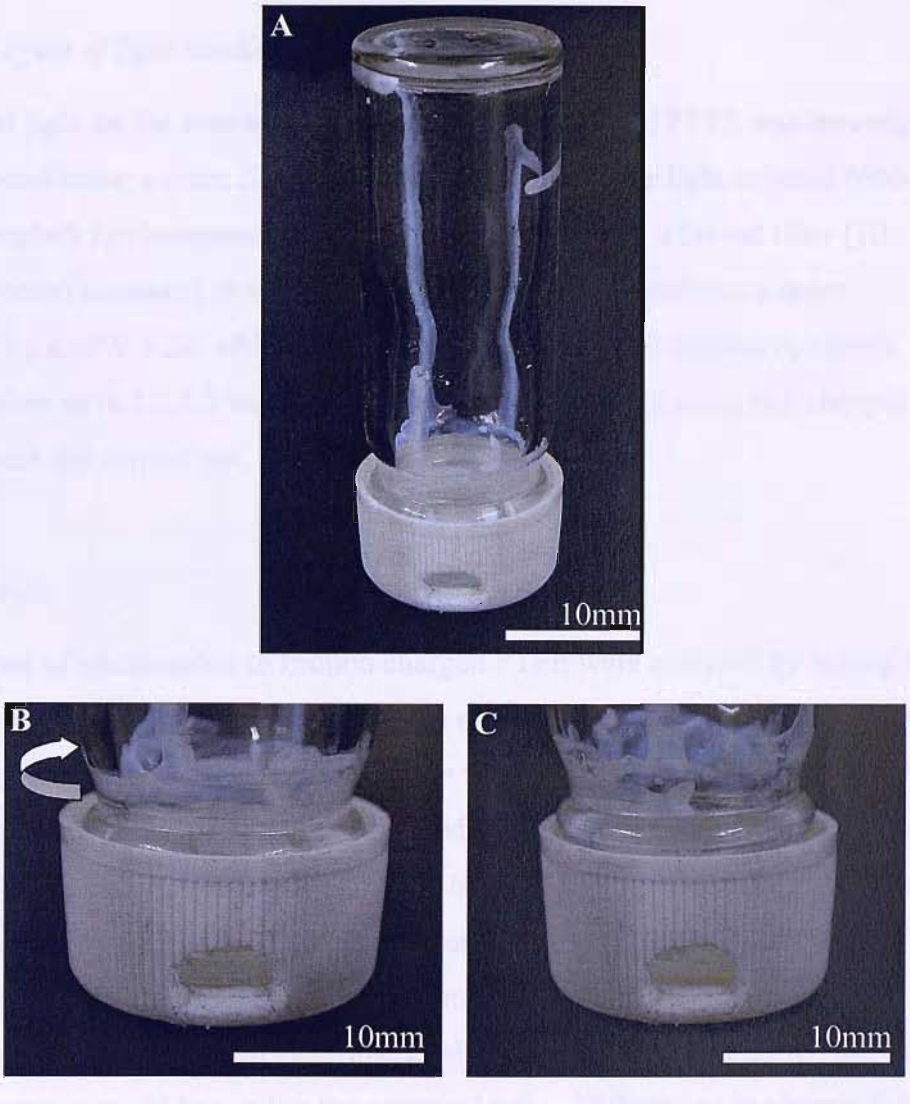


Figure 2.3 Photograph of the release mechanism used for entry of the cockroaches into the Y-choice apparatus. Each cockroach was released by turning (A) a universal glass bottle (83x25mm) anti clockwise (B), resulting in two adjacent 8-10mm holes cut out of the rim of the bottle and lid, (C) leaving a gap for the cockroach to walk into the central pathway of the Y-choice apparatus.

The Y-choice apparatus was enclosed in an earthed solid aluminium cage (385 x 380 x 550mm) with inside surfaces painted matt black to reduce visual input. To remove pheromone deposits, each apparatus was washed and soaked in 5% Decon90 solution with hot water (55°C) for 10min after every 5 trials. After rinsing with distilled water then washing with acetone, apparatus were dried in a drying chamber (110°C) for a minimum of 10min and left to cool. Experiments were carried out in a room illuminated by a 40W tube light covered with a far-red filter (Campbell Environmental Products, UK) at temperature 23.2±2°C and relative humidity (RH) 37±5%, between 10:00 and 19:00 GMT.

2.2.1.3 The effect of light condition on avoidance behaviour

The effect of light on the avoidance of cockroaches to charged PTFE was investigated under two conditions; a room illuminated by a 40W 1.2m tube light covered (600-700nm, Campbell Environmental Products, Preston UK) with a far red filter (10 lumens/sq meter) measured at the location of the Y-choice apparatus; a room illuminated by a 40W 1.2m white tube light with no filter (200 lumens/sq meter). The same procedure as in 2.2.1.2 was followed, although bioassays using two charged pathways were not carried out.

2.2.1.4 Analysis

The responses of cockroaches to friction charged PTFE were analysed by testing for differences in observed frequencies against an expected ratio. The observed frequencies were separated into: the number of individuals taking the left or right uncharged PTFE pathway; avoiding or non-avoiding the charged PTFE; avoiding or non-avoiding both charged PTFE pathways. Differences in the observed frequencies and the expected frequencies, based on a binomial distribution using the expected ratio of 50:50 or 0.5 (Van-Tol *et al.*, 2002) were tested using Binomial Tests of Proportions (S-Plus, Version 6.1 for Windows). If the control experiments showed bias for one pathway, the observed control proportion could be used as the expected ratio. Differences in electric field strengths of the friction charged PTFE sections were tested using Student's *t*-Test (Minitab, Version 13 for Windows). Test assumptions were met after testing data with Kolmogorov-Smirnov (normality) and Levene's Test (homogeneity).

Differences in cockroach preference behaviour to friction charged PTFE under varying light conditions were analysed using Chi-squared Tests of association after carrying out Yates' correction for continuity (Fowler *et al.*, 1998). Data were considered significant at the $P < 0.05$ level.

2.2.2 The avoidance of static electric fields at varying strengths

2.2.2.1 Y-tube apparatus

Three cylindrical 2mm thick silicon glass chambers, 150 x 30mm (L x D), were fused together in a 'Y' configuration 120° apart (Chemistry workshop, University of Southampton, Fig. 2.4). A small hole (7mm diameter) was cut out of the upper surface near the entrance to each anterior chamber in which a copper loop electrode, 5 x 28mm (W x D), was attached to an insulated socket (Fig. 2.5).

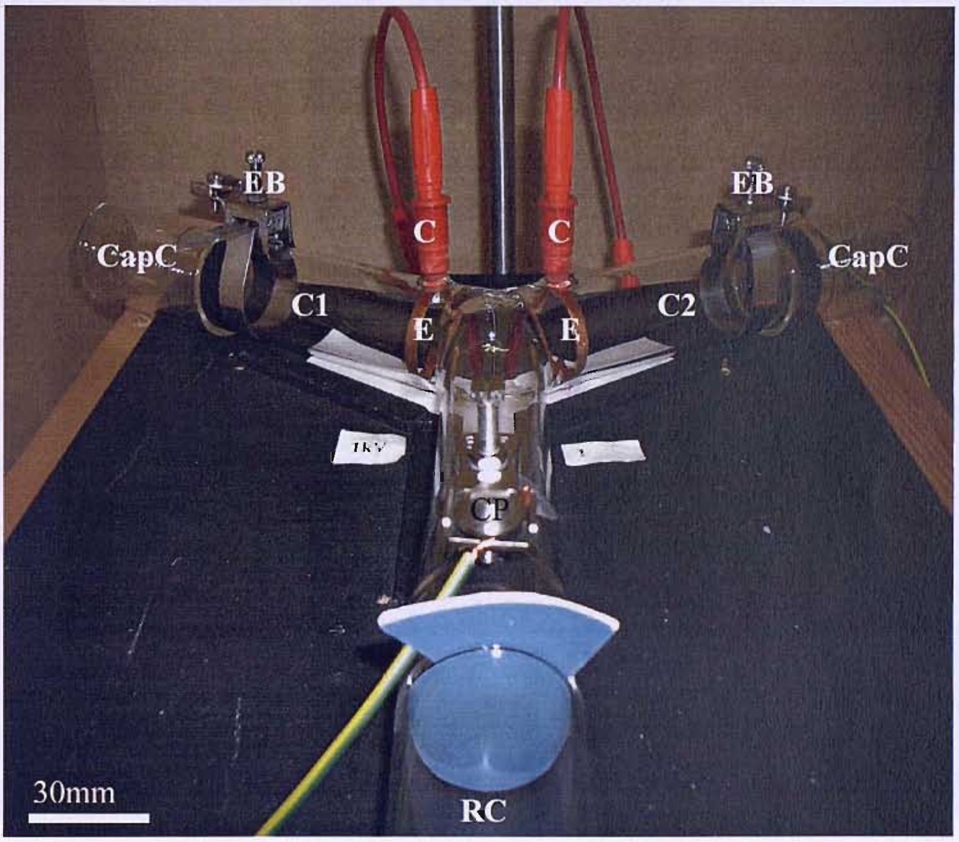


Figure 2.4 Photograph of the Y-tube apparatus showing the release chamber (RC) connected to the central chamber (CP). Copper loop electrodes (E), localised the field to one chamber (C1 or C2). At the end of each chamber a capture chamber (CapC) was attached to hold tested individuals. Aluminium domestic water pipe earth bands (EB) were used to localise the field within the treated chamber. The electrodes were connected to the Brandenburg Alpha III power source via high voltage insulated plugs (C).

One socket was connected to a high voltage power supply (Brandendurg Alpha III, Brandenburg, UK) with high voltage insulated wire. Aluminium earth rings were fixed 35mm from the end of each chamber to localise the electric field in the treated pathway. Two capture chambers (85 x 35mm, Chemistry workshop, University of Southampton) covered the ends of each anterior chamber to catch the cockroach after every trial.

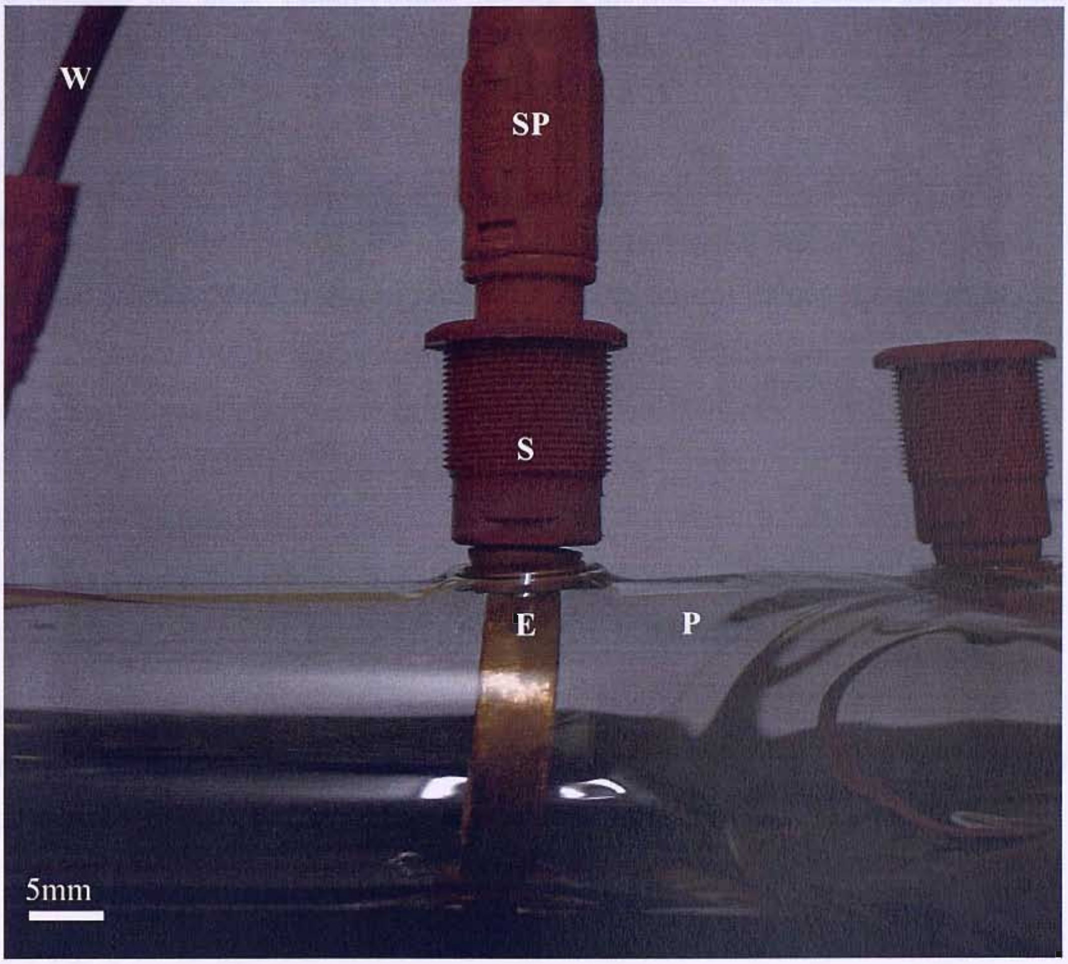


Figure 2.5 Photograph of the electrode and connectors in one chamber of the Y-tube (P). The copper electrode (E) is connected to an insulated socket (S) into which shrouded plugs (SP) were connected, and attached to a high voltage insulated wire (W) that lead to the Brandenburg power supply.

2.2.2.2 Behavioural bioassay

Using storkbill forceps a single cockroach was placed into a release chamber (85 x 35mm). A 3mm gap 2cm from the top of the chamber contained a cardboard 'trapdoor'. After sliding the release chamber onto the Y-tube, the trapdoor retained the individual within the chamber during a 2min rest period. Lifting the trapdoor allowed the individual to walk up the central chamber with minimum disturbance. The

Brandenburg high voltage power supply was adjusted to produce an electric field at 0V, 500V, 750V, 1kV, 2kV, 3kV or 4kV potentials in one pathway immediately as the cockroach entered the central chamber.

The route taken by each cockroach was noted as 'avoid' or 'non-avoid' if it took the untreated or treated chamber respectively, after it had reached one or more body length into a chamber entrance. Control trials, not using the high voltage power supply, were also carried out to test for natural preferences within the Y-tube apparatus. Cockroaches were therefore noted to take either the 'left' or 'right' chamber. Cockroaches that spent longer than 5min in the apparatus or returned back down the central chamber were discounted for treated and control trials.

The treated chamber was alternated after each trial to control for any natural bias that may have occurred due to external stimuli or pheromone deposition. In addition, to remove pheromone deposits, the equipment was washed and soaked in 5% Decon90 solution using hot water (55°C) for 10 min after every 5 trials. After rinsing with distilled water and washing with acetone, the apparatus was dried in a drying chamber (110°C) for a minimum of 10 min to remove solvent trace. Three apparatus were manufactured so one could be used for the trials, one washed, and one dried.

Experiments were carried out in a room illuminated only by a 40W 1.2m tube light covered with a far-red filter (Campbell Environmental Products, Preston UK) between 10:00 and 18:00 GMT at temperature 21.5±1.5°C and 35.7±3.7%RH.

2.2.2.3 The effect of light condition avoidance behaviour

To test the effect of light condition on the responses of cockroaches to electric fields at varying voltages, the above protocol was carried out under three light intensities; red illumination (10 lumens/sq meter) using a 40W 1.2m tube light covered with a far-red filter (Campbell Environmental Products, UK); medium-level illumination (35 lumens/sq meter) using a 60W 284mm white tube light; white illumination (200 lumens/sq meter) using a 40W 1.2m tube bulb with no filter. Cockroaches were tested under each light condition at three voltage potentials (1kV, 2kV and 4kV) in addition to control bioassays using no electric field to test for natural preferences. A randomised block design with voltage and light condition as factors controlled for day effects.

Experiments were carried out between 10:00 and 16:00, at temperature 22±1°C and 43±10%RH.

2.2.2.4 Analysis

Proportions of individuals taking the left or right chambers in control trials, and avoiding or non-avoiding the treated chamber in treated trials were analysed as in 2.2.1.4 using Binomial Tests of Proportions (S-Plus, Version 6.1 for Windows) for each field strength under each light condition. The effect of altering the field strength on the preference behaviour was tested using Chi-square Tests. The effects of light condition on cockroach responses to electric fields were also analysed at each field strength using Chi-square Tests. Data were considered significant at the $P < 0.05$ level.

2.2.3 Electric field modelling

Computer software (Maxwell SV, Version 7 for Windows) was used to calculate and illustrate the electric field distribution and strength within each Y-choice apparatus. The PTFE Y-choice apparatus was drawn as a simple 2D '*xy*' model representing a horizontal cross-section of the apparatus, the height of which was assumed infinite (Fig. 2.6A). A *xy* model was also drawn for the electric field Y-tube apparatus, in addition to an '*rz*' model that corresponded to a symmetrical, circular plane of view (Fig 2.6B). The *rz* model was a more accurate representation of the actual apparatus, yet only the treated pathway could be modelled using this method due to the symmetry of the model.

Two PTFE Y-choice models were created, incorporating either one or two charged PTFE sections, enabling comparisons of the electric field distributions and strengths to be made. Models were calculated using the mean charge on the surface of the PTFE section(s), determined from measurements during the experiments (see section 2.2.2.1).

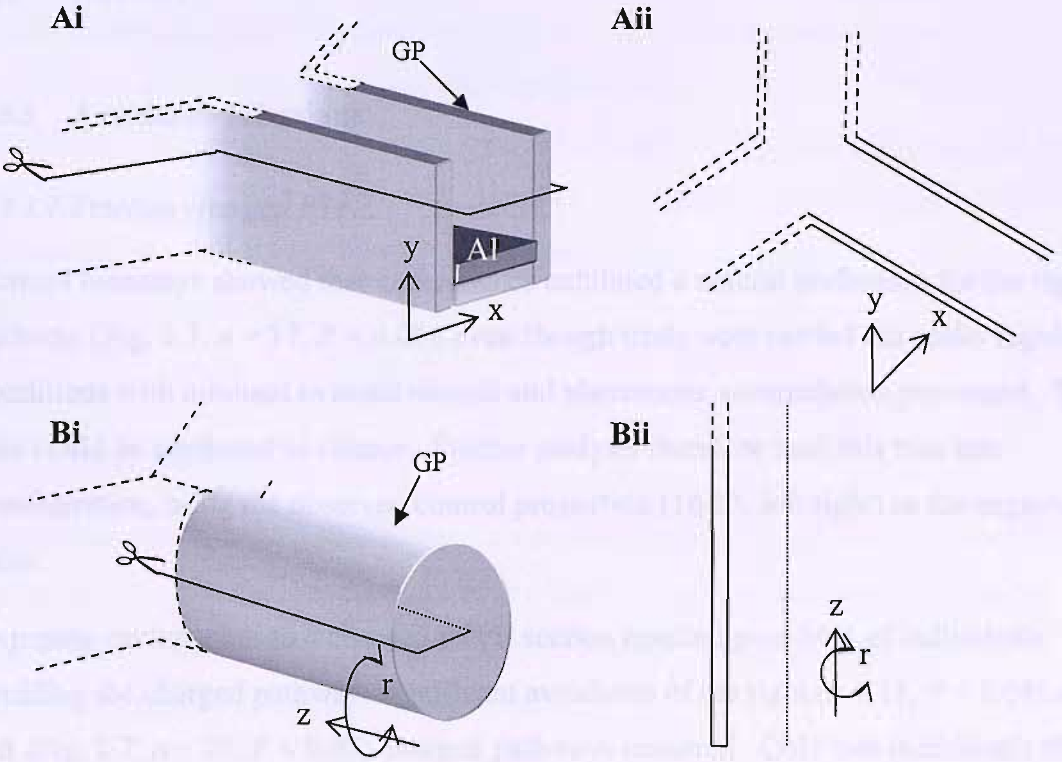


Figure 2.6 The *xy* and *rz* field modelling models. (Ai) A horizontal cross section of the PTFE Y-choice was taken to produce the *xy* model (Aii). (Bi) An *rz* model was also drawn for the electric field Y-tube from a symmetrical cross section taken from the centre of the tube, representing a circular model (Bii). *Al* aluminium central pathway; *GP* glass pathway

Maxwell SV default material properties (Appendix 8.1A) were assigned for all apparatus materials and the models were positioned within air to enable the closest possible representation to the actual apparatus. Maxwell SV bases calculations using a 'mesh' principle; the model is split into many small triangles, and calculations are made for each triangle and combined to give a complete model (Appendix 8.1B). The mesh complexity can be manually altered, and a more complex mesh (i.e. more triangles) results in a more accurate model. A mesh of increasing complexity, however, takes longer to calculate. Hence, there is a compromise between model accuracy and time to calculate. By using the default initial mesh, and not varying the mesh manually, calculations were achieved to an acceptable level of accuracy within reasonable time.

Three models of the Y-tube apparatus were drawn, assigning the copper ring in the treated chamber at voltage potentials of 500V, 1kV or 4kV. The electric field distribution and strength at these voltage potentials could therefore be compared.

2.3 Results

2.3.1 Avoidance behaviour

2.3.1.1 Friction charged PTFE

Control bioassays showed that cockroaches exhibited a natural preference for the right pathway (Fig. 2.7, $n = 37$, $P < 0.05$), even though trials were carried out under regulated conditions with minimal external stimuli and pheromone accumulation prevented. This bias could be attributed to chance. Further analysis therefore took this bias into consideration, using the observed control proportion (14:23, left:right) as the expected ratio.

Exposing cockroaches to a charged PTFE section resulted over 94% of individuals avoiding the charged pathway; significant avoidance of the right ($n = 18$, $P < 0.05$) and left (Fig. 2.7, $n = 20$, $P < 0.05$) charged pathways occurred. Only two individuals did not avoid the charged PTFE, and both took the right pathway. This is not likely to be a consequence of greater field strengths in the left pathway, given the field strengths did not differ between the left and right pathways ($t = -0.93$, d.f. = 36, $P > 0.05$).

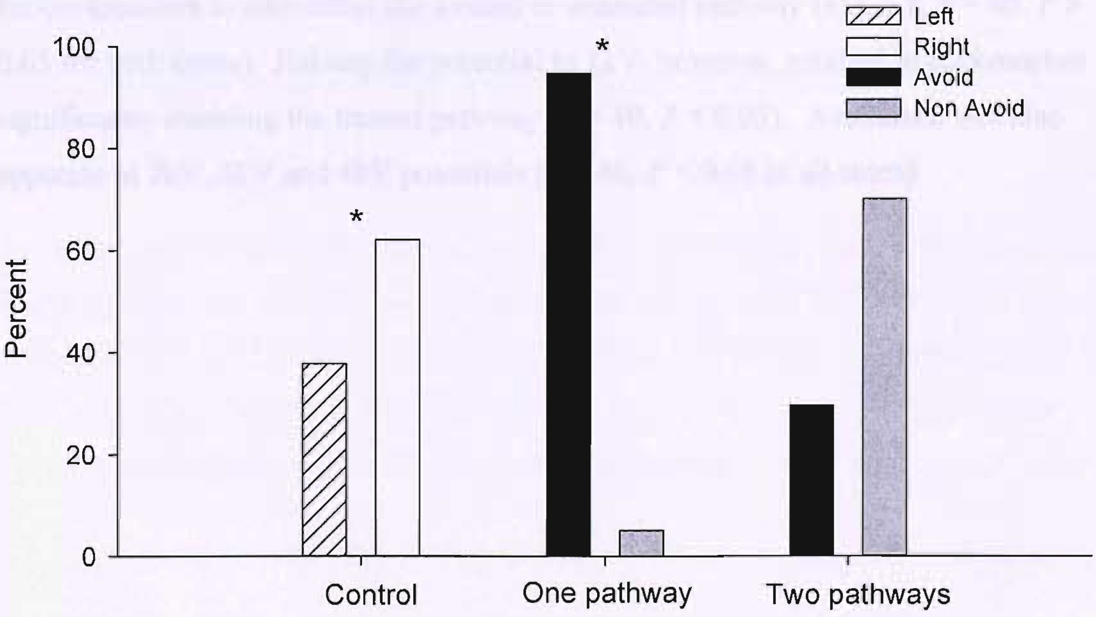


Figure 2.7 The avoidance of friction charged PTFE. Cockroaches exhibited a significant preference for the right uncharged pathway ($n = 37$, $P < 0.05$, represented by the asterisk). Considering this natural bias, significant avoidance occurred when one PTFE section was charged. No significant avoidance occurred when both anterior PTFE sections were charged ($n = 37$, $P > 0.05$).

The field strength produced when two PTFE sections were friction charged was significantly greater than when one was charged ($t = 8.65$, d.f = 49, $P < 0.05$). Regardless of the increased field strength, 70% of cockroaches took either the left or right charged pathway (Fig. 2.7). Cockroaches that approached two charged PTFE sections did not therefore exhibit significant avoidance by turning back down the central pathway ($n = 37$, $P > 0.05$).

2.3.1.2 Static electric fields

Although friction charged PTFE retains a consistent field strength that can be varied, it cannot be regulated with precision. Using a high voltage power supply, a paradigm was developed to accurately investigate avoidance of static electric fields at various strengths that could be used for further studies.

Cockroaches were first tested within an untreated Y-tube apparatus to determine if a bias for one pathway existed. No natural preference, however, for either the left or right untreated pathway was exhibited (Fig. 2.8, $n = 40$, $P > 0.05$).

Applying 500V and 750V potentials to one pathway caused no significant preference for cockroaches to take either the treated or untreated pathway (Fig. 2.8, $n = 40$, $P > 0.05$ for both cases). Raising the potential to 1kV, however, resulted in cockroaches significantly avoiding the treated pathway ($n = 40$, $P < 0.05$). Avoidance was also apparent at 2kV, 3kV and 4kV potentials ($n = 40$, $P < 0.05$ in all cases).

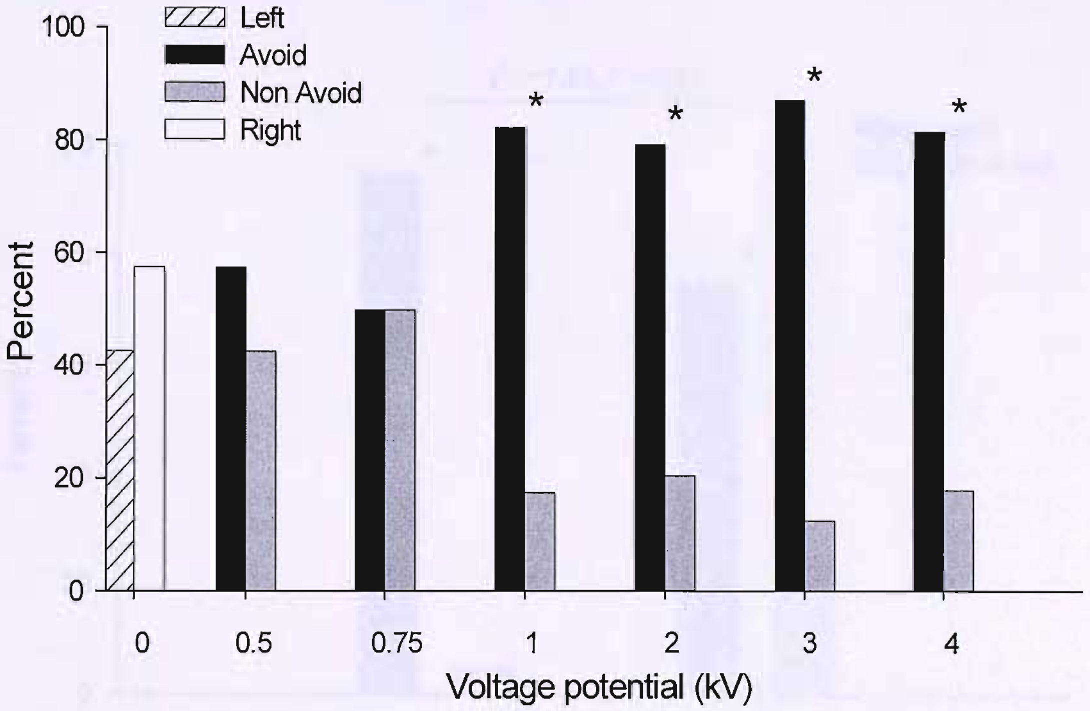


Figure 2.8 The avoidance static electric fields. Cockroaches exhibited no natural bias for the left or right pathway ($n = 40$, $P > 0.05$) within the Y-tube apparatus. Raising the voltage to 500V and 750V did not evoke significant avoidance ($n = 40$, $P > 0.05$ in both cases). Voltage potentials at 1kV and above did elicit significant avoidance of the treated pathway ($n = 40$, $P < 0.05$ in all cases, indicated by the asterisks).

2.3.2 The effect of light condition on avoidance behaviour

2.3.2.1 Friction charged PTFE

No significant bias for either side of the untreated Y-choice apparatus occurred under white light ($n = 20$, $P > 0.05$) but did exist under red light ($n = 37$, $P < 0.05$). Cockroaches exhibited significant avoidance under both red and white light (Fig. 2.9, $n = 37$, $P < 0.05$; $n = 20$, $P < 0.05$), although the proportion that avoided the treated pathway was significantly less at greater light intensities ($n = 60$, $\chi^2_1 = 5.62$, $P < 0.05$).

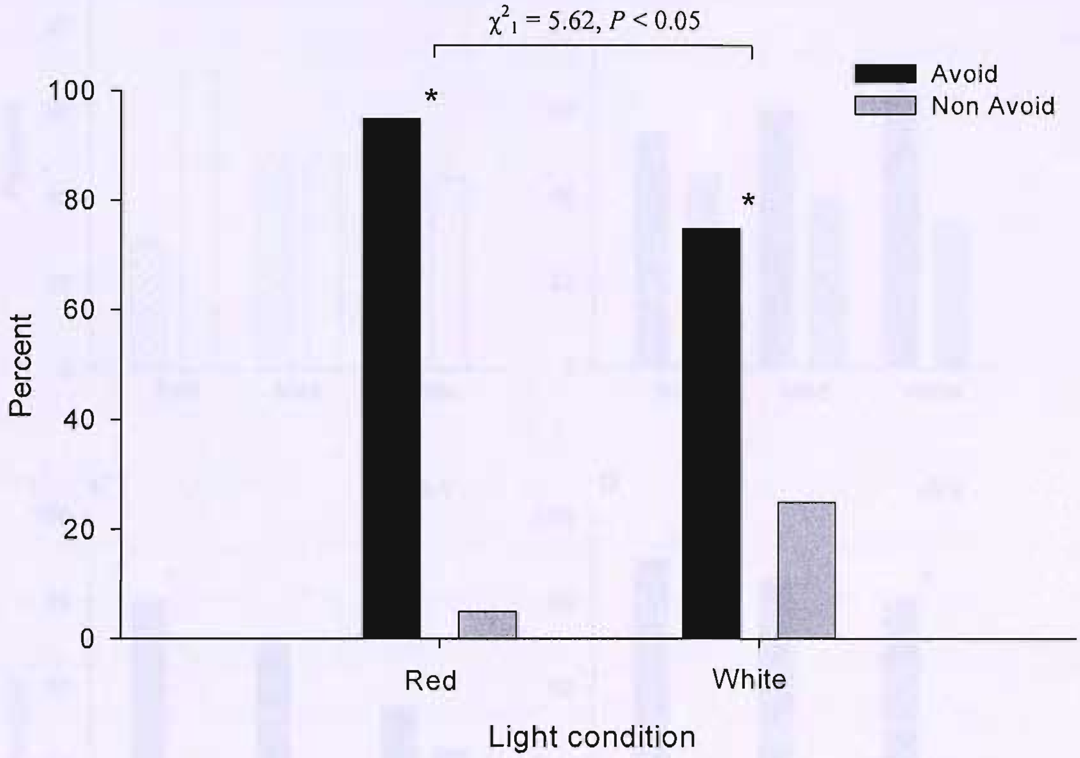


Figure 2.9 The effect of light on avoidance of friction charged PTFE. Cockroaches significantly avoided charged PTFE under both red and white light ($n = 37$, $P < 0.05$; $n = 20$, $P < 0.05$ respectively). There was, however, a significant effect of light on the proportion of cockroaches avoiding the charged PTFE ($n = 60$, $\chi^2_1 = 5.62$, $P < 0.05$).

2.3.2.2 Static electric fields

In an untreated Y-tube apparatus, cockroaches did not exhibit a natural preference for the left or right pathway under any light condition (Fig. 2.10A, $n = 20$, $P > 0.05$ for all cases). In addition there was no significant association in the choice of untreated pathway taken and the light level ($\chi^2_2 = 0.27$, $P > 0.05$), confirming that the light condition did not affect the natural preference of cockroaches.

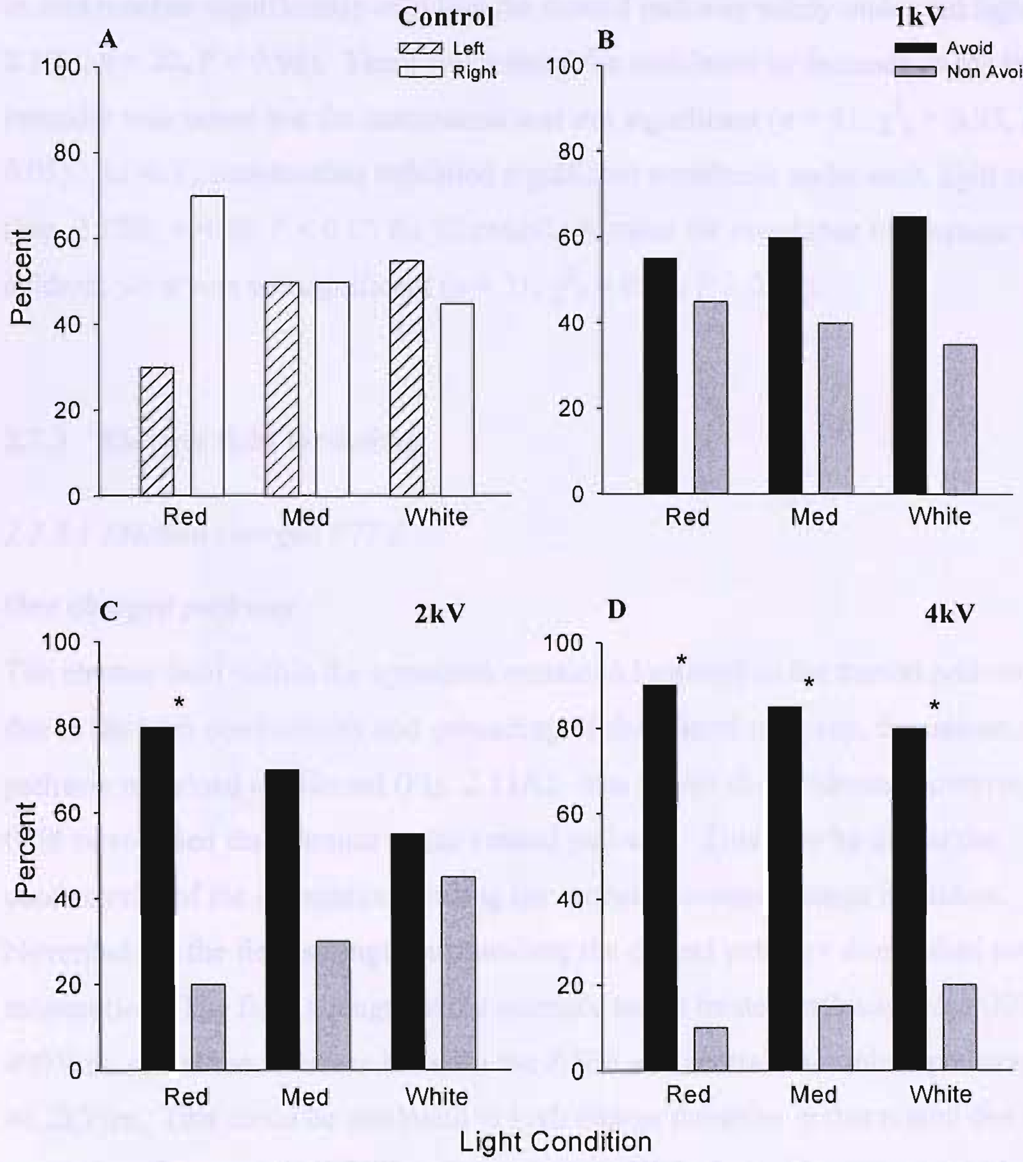


Figure 2.10 The effect of light on the avoidance of static electric fields. (A) No significant natural bias for the left or right pathway occurred under any light condition. (B) Avoidance did not occur at 1kV under any light condition ($n = 20$, $P > 0.05$ in all cases). (C) Raising the potential to 2kV evoked significant avoidance under red light ($n = 20$, $P < 0.05$), but not medium-level or white light conditions ($n = 20$, $P > 0.05$ for both cases). (D) A 4kV potential resulted in significant avoidance under each light condition ($n = 20$, $P < 0.05$ for all cases). There was no significant association between light condition and avoidance 1kV ($n = 36$, $\chi^2_2 = 0.17$, $P > 0.05$), 2kV ($n = 41$, $\chi^2_2 = 0.93$, $P > 0.05$) or 4kV ($n = 51$, $\chi^2_2 = 0.18$, $P > 0.05$).

Treating one pathway with a 1kV potential did not evoke significant avoidance under red, medium-level or white light (Fig. 2.10B, $n = 20$, $P > 0.05$ for all cases). In addition, avoidance increased as the light intensity was raised, although this association was not significant ($n = 36$, $\chi^2_2 = 0.17$, $P > 0.05$). Raising the potential to 2kV resulted in cockroaches significantly avoiding the treated pathway solely under red light (Fig. 2.10C, $n = 20$, $P < 0.05$). There was a trend for avoidance to decrease as the light intensity was raised but the association was not significant ($n = 41$, $\chi^2_2 = 0.93$, $P > 0.05$). At 4kV, cockroaches exhibited significant avoidance under each light condition (Fig. 2.10D, $n = 20$, $P < 0.05$ for all cases). A trend for avoidance to decrease was also evident, yet it was not significant ($n = 51$, $\chi^2_2 = 0.18$, $P > 0.05$).

2.3.3 Electric field modelling

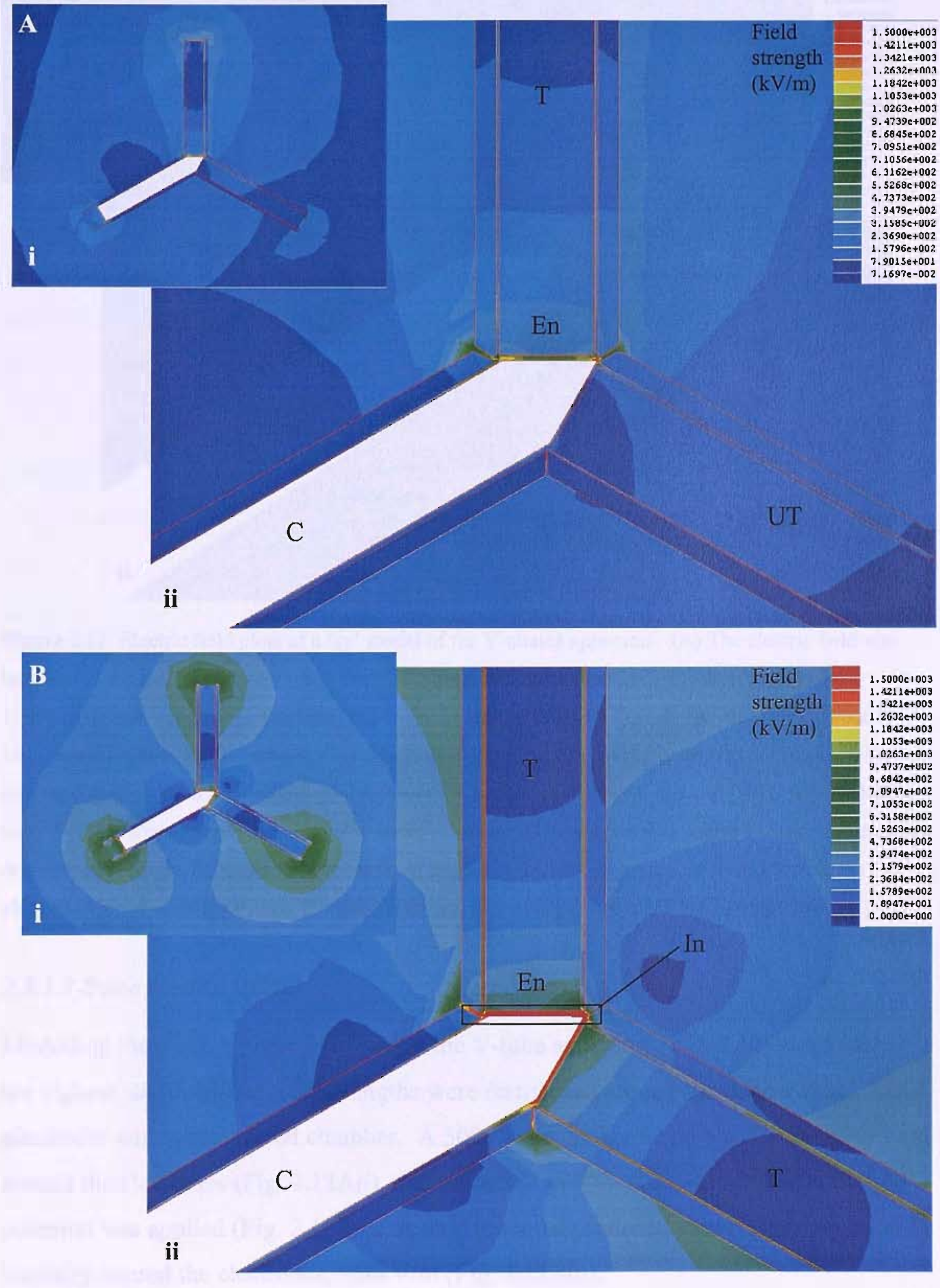
2.3.3.1 Friction charged PTFE

One charged pathway

The electric field within the apparatus remained localised to the treated pathway and, due to the high conductivity and grounding of the central pathway, the untreated pathway remained unaffected (Fig. 2.11A). The model does indicate, however, that a field surrounded the entrance to the central pathway. This may be due to the conductivity of the aluminium causing the model to assume charge induction. Nevertheless, the field strength surrounding the central pathway diminished towards the intersection. The field strength at the entrance to the treated pathway was ≈300 – 400V/m, and at the interface between the PTFE and central aluminium pathway ≈1.2kV/m. This could be attributed to high charge densities in this region due to the narrow gap between the PTFE and aluminium. High charge densities were also responsible for field strengths up to 1.5kV/m at the tapered edges of the central pathway.

Two charged pathways

Charging both PTFE sections resulted less localisation of the field than when one pathway was charged (Fig. 2.11B). At the entrances and interfaces of the pathways the field strengths were greater than when one pathway was charged (≈400 – 700 kV/m and 1.5kV/m respectively). Although the charge on the PTFE during the experiments was significantly greater when both sections were charged ($t = 8.65$, d.f. = 49, $P < 0.05$), further modelling shows the calculated differences in field strength were due to treating both pathways rather than the increased charge during the experiments; calculating a model using the same charge on both pathways measured when one PTFE section was charged revealed an increase in field strength within the apparatus (Fig. 2.11C).



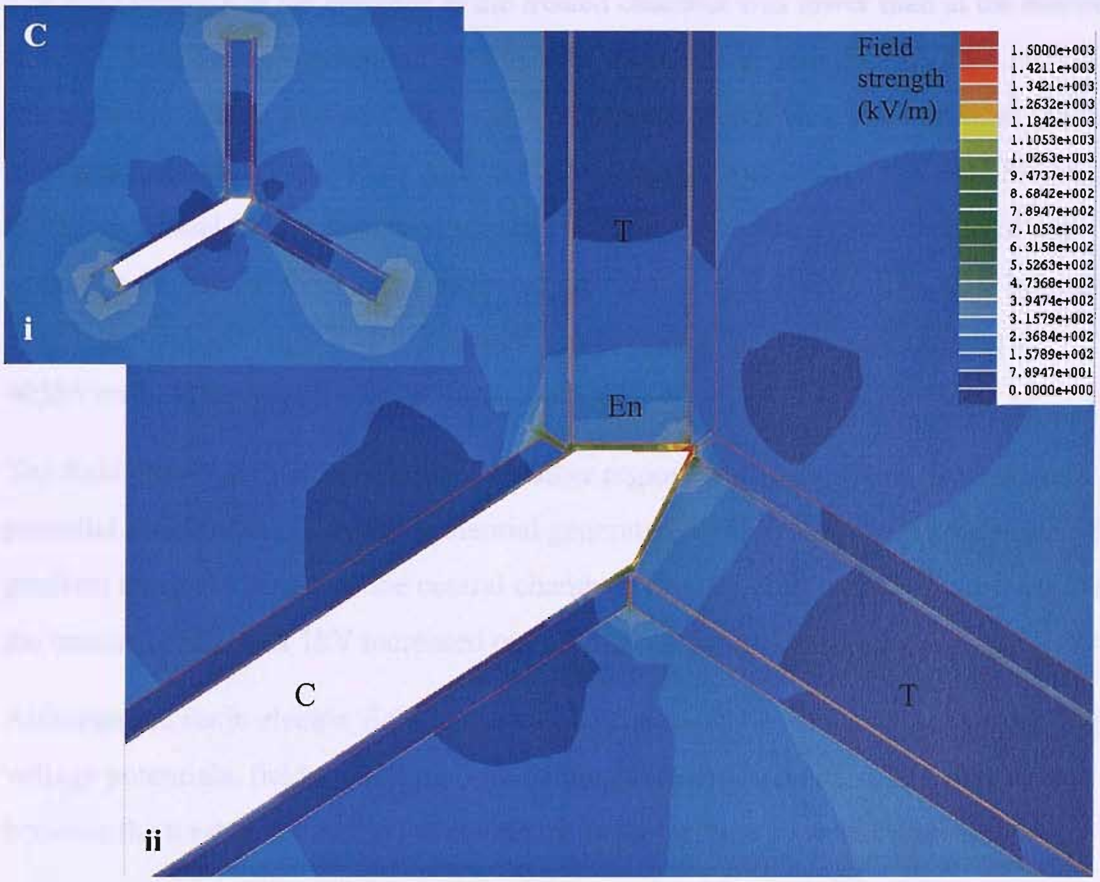


Figure 2.11 Electric field plots of a '*xy*' model of the Y-choice apparatus. (A) The electric field was localised to the treated pathway when one PTFE section was charged (Ai), resulting in a 1kV/m – 1.3kV/m field strength at the entrance (En) to the treated pathway (Aii). (B) The electric field was less localised when both PTFE sections were charged (Bi) and the field strength reached ≈1.5kV/m at the intersection (In) with a higher field gradient over the surface of the PTFE sections (Bii). (C) Modelling two PTFE sections charged to the same level as when one PTFE section was charged shows that charging two pathways results in higher field strengths at the decision zone, regardless of differences in the charged induced upon the PTFE. *T* treated pathway; *C* central pathway; *UT* untreated pathway.

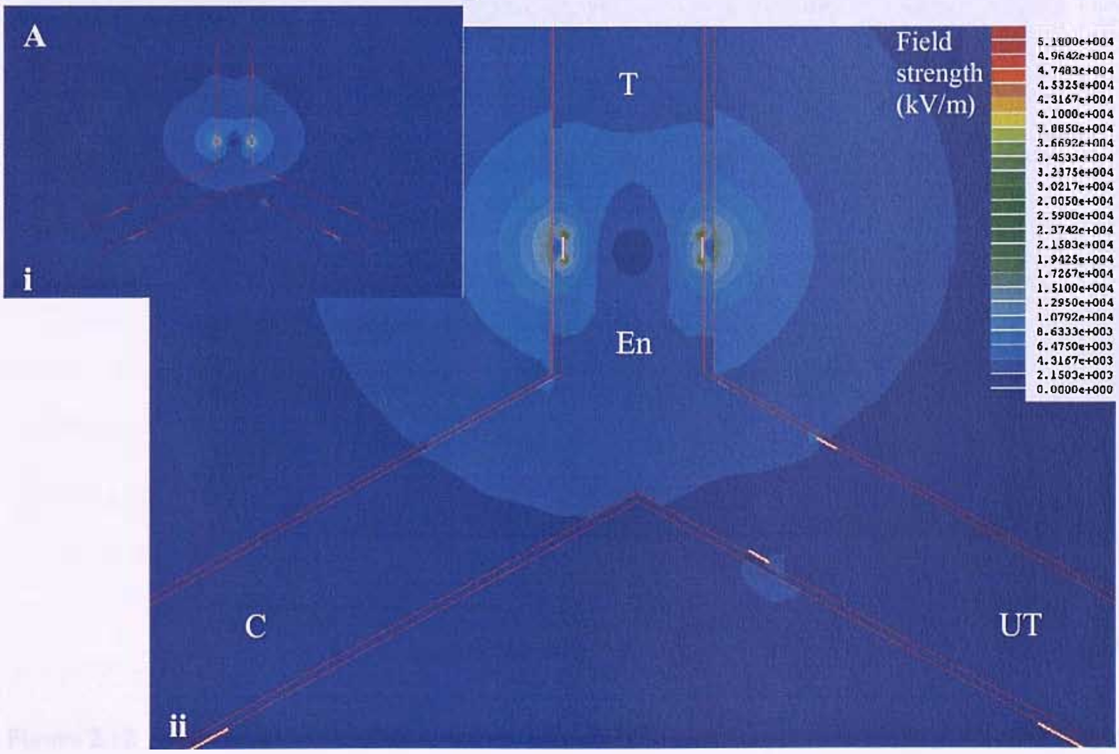
2.3.3.2 Static electric fields

Modelling the static electric field within the Y-tube apparatus (Fig. 2.12) showed that the highest static electric field strengths were distributed around the copper ring electrodes within the treated chamber. A 500V potential generated a 15 – 20kV/m field around the electrodes (Fig. 2.12Aii), that increased to 30 – 40kV/m when a 1kV potential was applied (Fig. 2.12Bii). A 4kV potential produced electric fields greater in intensity around the electrodes, >50kV/m (Fig. 2.12Biii).

The field strength at the entrance to the treated chamber was lower than at the electrodes at 500V, 1kV and 4kV potentials (4 – 6kV/m, 8 – 10kV/m and 25-30kV/m respectively). There was no difference in field strength between the entrances of the treated and untreated chambers when a 500V potential was used (≈4kV/m). Raising the voltage potential to 1kV however, resulted in the field becoming less localised and causing a 4 – 6kV/m field in the untreated chamber (Fig. 2.12Bii). The field became even less localised when a 4kV potential was applied (Fig. 2.12Biii), causing a ≈25kV/m field at the entrances to both chambers.

The field distribution also varied within other regions of the Y-tube as the voltage potential was increased. A 4kV potential generated a ≈4kV/m to ≈30kV/m electric field gradient along the length of the central chamber (Fig. 2.12Cii), yet the gradient within the central chamber at 1kV increased only threefold from ≈2kV/m to ≈6kV/m.

Although the static electric field in the Y-tube apparatus became less localised at higher voltage potentials, field modelling does demonstrate the differences in field strength between the treated and untreated chambers as the voltage potential was raised.



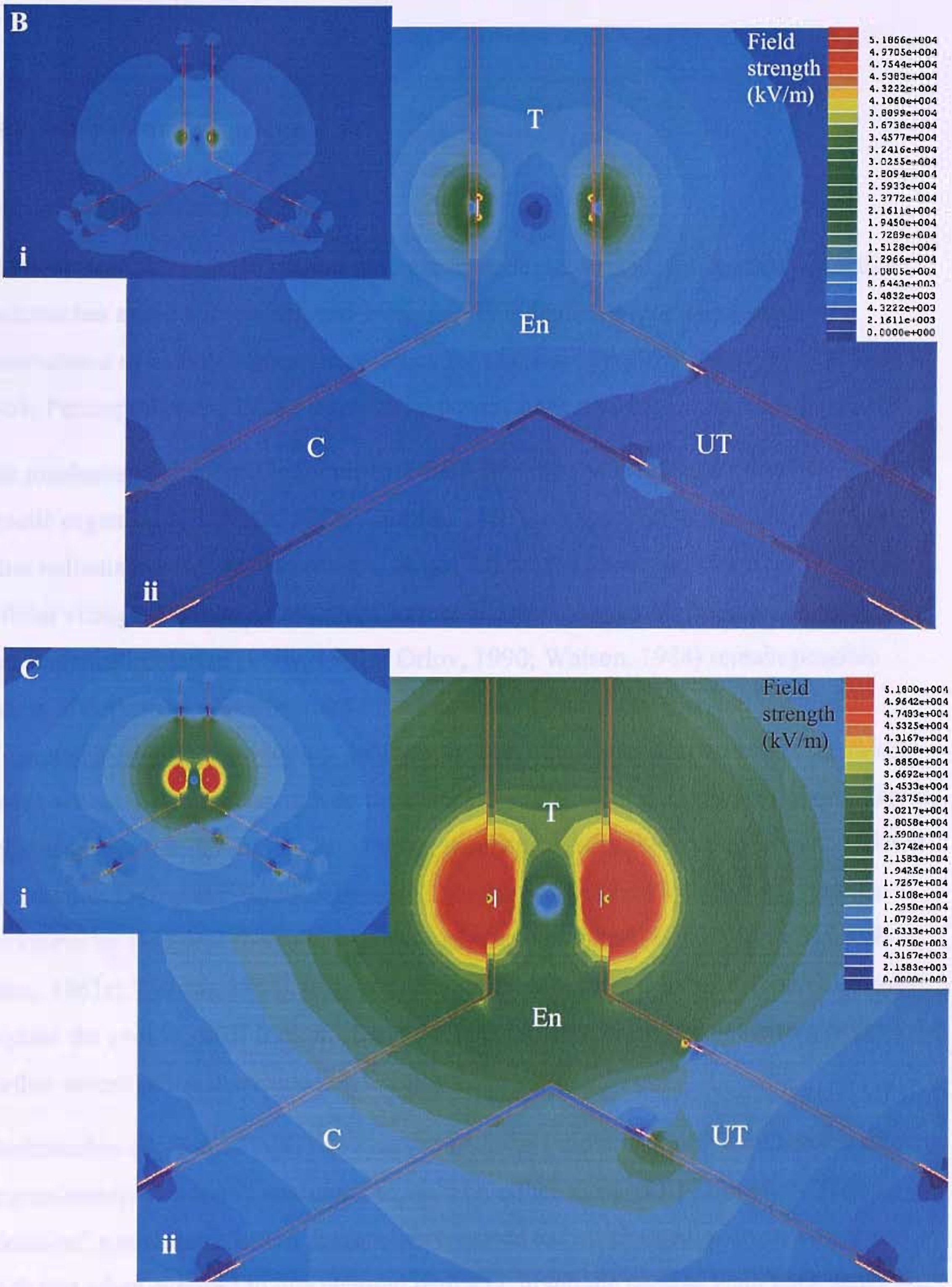


Figure 2.12 Static electric field plots of an '*xy*' model of the Y-tube apparatus at (A) 500V, (B) 1kV and (C) 4kV potentials. Static electric field distributions in the whole apparatus shows localisation of the fields at 500V (Ai) and 1kV (Bi) to the treated pathway. The electric field strength at the entrance (En) to the treated pathway was 4 – 6kV/m and 8 – 10kV/m at respective voltage potentials (Aii, Bii). Raising the potential to 4kV resulted in less field localisation (Ci) and the field strength was approximately 25 – 30kV/m. *T* treated pathway; *C* central pathway; *UT* untreated pathway.

2.4 Discussion

2.4.1 Avoidance behaviour

2.4.1.1 Friction charged PTFE

The behavioural bioassays carried out here have demonstrated, for the first time, that cockroaches avoid friction charged surfaces. This finding supports previous observations of insect location preferences for uncharged regions (Maw, 1962; Maw, 1964; Perumpral *et al.*, 1978), yet such responses have not, until now, been quantified.

The mechanisms responsible for electric field detection are well established in some aquatic organisms (Bullock, 1982; Kalmijn, 1988), yet speculation remains over how other animals may respond to electric fields. Of those hypotheses currently put forward, cellular changes (Liburdy, 1995; McCann *et al.*, 1993; Tenforde, 1991) and direct mechanical stimulation (Maw, 1961c; Orlov, 1990; Watson, 1984) remain possible means of detection. The electrical forces generated by friction charged surfaces, illustrated by the field modelling, indicate the magnitude and distribution of electrical forces within the apparatus. Given the transient exposure of cockroaches to such forces, and the apparent reflex responses to them, mechanical stimulation may remain a viable explanation for the avoidance behaviour. Mechanical stimulation of insect sensory structures by electrical forces have previously been reported (Bindokas *et al.*, 1989; Maw, 1961c; Watson, 1984), possibly accounting for changes in behaviour. This may explain the avoidance of friction charged PTFE presented here, yet remains a subject for further investigation in succeeding Chapters.

Cockroaches confronted with two charged pathways did not exhibit avoidance, and approximately two thirds continued to walk on either charged PTFE section. The ‘decision’ a cockroach has to make when exposed to two charged pathways contrasts with that when exposed to one charged pathway, given the need to walk back down the central pathway. This may conflict with the intrinsic wall-following, or thigmotactic, nature of cockroaches (Bell and Adiyodi, 1981; Cowan *et al.*, 2006; Okada and Toh, 2000). Space within the Y-choice apparatus was limited, and antennal contact with the pathway walls was observed to be maintained during a trial. Hence, thigmotaxis may have motivated cockroaches to walk on a charged PTFE section rather than turn back down the central pathway. A third of individuals tested did, however, walk back down the central pathway. This demonstrates that friction charged surfaces may present a stimulus sufficient in intensity to override the innate tendencies of cockroaches and evoke avoidance.

The avoidance of friction charge PTFE presented here provides an explanation for the reported low efficiency of friction charged plastic insect traps (Maw, 1964). Observations made by Maw (1964) showed that plastic traps became frictionally charged by rubbing against the surrounding foliage, and consequently significantly fewer insects were caught compared to traps that were not charged. The avoidance behaviour of cockroaches quantified in this investigation therefore highlights a need for the materials used and placement of insect traps to be taken into consideration.

2.4.1.2 Static electric fields

Cockroaches also elicited avoidance when confronted by static electric fields generated by a charged copper electrode. Field modelling illustrated a fundamental difference in the field distribution between the Y-tube and Y-choice apparatus; in contrast to the Y-choice, a gradient of decreasing field strength was apparent within the Y-tube as the distance from the electrode increased. Hence, unlike the Y-choice, cockroaches were not abruptly confronted by an electric field. This may therefore explain the higher field strength at the intersection required to elicit avoidance within the Y-tube apparatus (≈8 – 10kV/m) compared to the Y-choice apparatus (≈1 – 1.2kV/m).

By varying the voltage supplied to the electrode, the relationship between field strength and avoidance was investigated. The results presented here indicate that cockroaches exhibited a 'threshold' of avoidance at 8 – 10kV/m. These findings support previous observations, for example, Maw (1961c) reported a proportional decrease in locomotion of the parasitoid *I. conquisitor* walking over a surface of increasing charge. Watson (1984) indicated that *Drosophila* movement is also correlated with field strength. To date, however, no conclusions have been made as to why such relationships may exist. One possible explanation is the stimulus strength-dependent behavioural responses of insect (Delcomyn, 1998; MacFarland, 1985). For example, the likelihood of cockroach escape behaviour occurring in response to wind movement is conditional upon wind velocity and acceleration (Camhi, 1984). Hence, electric field strengths <8kV/m could not be sufficient to activate the system responsible for static electric field detection, resulting in no avoidance. It should be taken into account, however, that the threshold may lie between 750V and 1kV, given that voltages between 750V and 1kV were not tested. Further bioassays between 750V and 1kV could therefore be carried out to confirm the threshold. Nevertheless, this avoidance threshold will be used as a paradigm in the succeeding Chapters for further investigations.

2.4.2 The effect of light condition on avoidance behaviour

Increased light intensity elicited a trend for decreased avoidance of both friction charged PTFE and static electric fields. This association could be due to the integration of light and visual input with other modalities, notably touch and the detection of mechanical stimuli (Mizunami, 1995b). The ocelli are a highly sensitive collection of photoreceptors providing cockroaches and other insects, including dragonflies, locusts, bees and flies, information on light levels (Labhart and Meyer, 1999; Mizunami, 1995a; Simmons, 2002). Ultimately, they contribute to mediating behaviour given their sensitivity to different light conditions: for example, controlling flight in locusts and flies (Mizunami, 1995b), and modulating decreased locomotion of cockroaches in shaded areas (Okada and Toh, 1998). The role the ocelli play in the regulation of motor output under different light conditions could therefore be responsible for the decreased avoidance of charged PTFE and static electric fields as light intensity was increased. This trend also presents some evidence for the means by which cockroaches detect both friction charged surfaces and static electric fields. Given the integration of the ocelli with other sensory inputs, notably that from mechanoreceptors on the antennae (Mizunami, 1995b), and the change in avoidance at varying light intensities, the antennae could play a role in detection.

Until now, the responses of insects confronted with electric fields had not been quantified. The findings presented here have shown that cockroaches avoid both friction charged surfaces and static electric fields. Modelling the distribution of the fields experienced by cockroaches within the apparatus illustrated the forces acting on cockroaches. Although the system responsible for detecting electric fields cannot be identified, the interactions between cockroaches and electrical forces, and observations from previous studies (Bindokas *et al.*, 1989; Maw, 1961c; Watson *et al.*, 1997) indicate that mechanical stimulation may play a role. This study has therefore advanced our current understanding of the effects of electric fields on insects, but also raised the question of how insects detect electric fields and evoke avoidance.

3. THE DETECTION OF ELECTRIC FIELDS

3.1 Introduction

To date, few studies have examined how insects detect electric fields. Given the apparent influences of electric fields on insect sensory structures, speculation exists regarding the contribution of structures such as the wings and antennae in the perception and detection of electric fields (Bindokas *et al.*, 1989; Maw, 1961c; Watson *et al.*, 1997). Nonetheless, the means by which insect detect and subsequently respond to electric fields remains unknown.

Basic electrostatic laws dictate that directional forces are exerted between charged objects (Cross, 1987; Hammond, 1997). Due to the conductive and charging properties of insect cuticle (Ishay *et al.*, 1998; Machin *et al.*, 1994; Scheie and Smyth, 1967; Smith *et al.*, 1995), insect appendages are subject to electrical forces. Accordingly, they are reported to be influenced by both static electrical forces and forces produced by ELF electric fields. Wings of the honey bee, *A. mellifera*, for example, are deflected when positioned within a 150kV/m static electric field (Bindokas *et al.*, 1989). Similar observations have been made of *D. melanogaster* wings exposed to both static and ELF electric field strengths of approximately 20kV/m (Watson *et al.*, 1997). Honey bee antennae are also reported to move when exposed to >95kV/m static electric fields (Yes'Kov and Sapozhnikov, 1976), and involuntary antennal movements have been shown to occur as the parasitoid *I. conquisitor* walks over a charged surface (Maw, 1961c).

Not only, however, are invertebrate structures affected by electric fields, as electrical forces acting on body hairs are believed to contribute to human perception of electric fields (Chapman *et al.*, 2005; Shimizu and Shimizu, 2004). Body hair is commonly attracted towards the charged source, and the degree of displacement is proportional to the field strength (Shimizu and Shimizu, 2003; Shimizu and Shimizu, 2004). Removal of body hair when exposing human subjects to 60Hz, 8 - 35kV/m electric fields eliminates the ability of subjects to perceive such fields, indicating that body hair is responsible for detection (Chapman *et al.*, 2005).

The structures on insects reported to be affected by electric fields possess an array of sensory receptors that enable insects to respond to both internal and external cues (Dusenbery, 1992; Fraenkel and Gunn, 1961). Notably, the wings, antennae and cerci play an important role in mediating insect behaviour. Mechanoreceptive sensilla on, and surrounding, these sensory appendages can respond to mechanical stimulation, such as touch or wind movement (Camhi, 1984; Hiraguchi and Yamaguchi, 2000; Staudacher *et al.*, 2005). As a result, they activate neurological pathways that can ultimately lead to behaviour such as the escape response; a key survival response characterised by rapid evasive movement (Camhi, 1984; Delcomyn, 1998). The cerci, for example, are covered with fine filiform hairs that are directionally sensitive, in particular, to wind movement (Bell and Adiyodi, 1981). Deflection of these hairs plays a major role in the escape response, initiating turning and running away from posterior predator strikes (Bell and Adiyodi, 1981; Camhi, 1984).

Mechanoreceptors located in and on the antennae also mediate escape behaviour by contributing directional tactile information. Mechanoreceptors are abundant on and within the first and second proximal antennal segment (the scape and pedicel), specifically at the joint between the scape and pedicel (scape-pedicel joint, SP joint), and the joint between the head and scape (head-scape joint, HS joint) (Staudacher *et al.*, 2005). Proprioceptors are contained within the HS- and SP joint of most insects, for example, the Johnston Organ in the pedicel of cockroach (Bell and Adiyodi, 1981) and *Drosophila* antennae (Caldwell and Eberl, 2002). Exteroceptors are present on the antennal cuticle, including the hairless campaniform sensilla, and distinct regions of hairs on the scape and pedicel (Schneider, 1964; Toh, 1981). Stimulation of these receptors during antennal movement provides positional information of the antennae (Okada and Toh, 2000; Staudacher *et al.*, 2005), in addition to mediating the escape response (Comer *et al.*, 2003; Ye *et al.*, 2003).

Given the forces generated by electric fields, their possible effects on sensory appendages, and the role of the sensory structures in mediating behaviour, the antennae and cerci could contribute to the detection and subsequent avoidance of electric fields. To answer this question and further current understanding of insect and electric field interactions, the aims of the current study are twofold: to investigate what effects friction charged surfaces and static electric fields have on such structures, and to determine whether these structures are responsible for detection.

3.2 Materials and methods

3.2.1 The detection of friction charged PTFE

3.2.1.1 Electric field modelling

To investigate the influences of friction charged surfaces on cockroaches, the forces exerted on three body parts (the antennae, cerci, and whole body) of cockroaches within the Y-choice apparatus were calculated (Maxwell SV for Windows, Version 7). Two models were drawn; one with a single pathway charged, and one with both pathways charged. The models used were no different from those in Chapter 2, aside from including a representation of a cockroach at four positions within the apparatus at: the entrance; the junction between the PTFE pathways (the intersection); on a charged pathway; on an uncharged pathway. The cockroach was drawn as an oval object similar in size to cockroaches used during the bioassays (body length 7mm, width 3mm) with two triangular projections (length 9mm, distal width 1mm) acting as antennae (Gier, 1947). The electrical properties of these objects (Appendix 8.1A) were assigned according to measurements of the conductance and dielectric constant of *P. americana* nymph cuticle (Machin *et al.*, 1994; Smith *et al.*, 1995).

Force calculations are subject to a degree of error which can be manually altered, and the margins of error are associated with the accuracy of force calculation. Decreasing the margin of error meant increasing the number of times the model had to be calculated (number of requested passes). Increasing the number of requested passes was therefore associated with increased calculation time. A compromise between error and calculation time of the model was therefore made at 0.01% using fifteen requested passes, after plotting the percentage of error against the number of requested passes (Appendix 8.2A and B).

Results were tabulated and figures drawn to represent the forces acting on cockroaches. The total resultant forces (the sum of the forces acting in x and y directions) and the angles that the forces acted were calculated for each body part. Differences in the magnitudes and directions of forces were compared between locations, and models. In addition, the electric field within the apparatus was illustrated by drawing a vector plot showing the direction and magnitude of the electric field using arrows (vectors). This enabled the direction and strength of the electrical field exerted on the cockroach to be represented.

3.2.1.2 High speed video observations of the antennae

Given the forces calculated to act on the antennae, the influences of friction charged surfaces on the movement of antennae were investigated using high speed video equipment. Video footage of cockroaches (*n* = 4) approaching a treated pathway was recorded from horizontal and vertical views at 250frames/sec (MotionScope 1000S, Redlake Imaging Corp. CA, USA). Still images were captured and figures collated to illustrate the effects of charged PTFE on antennae.

3.2.1.3 The effect of sensory structure ablation on the avoidance of one charged PTFE pathway

Considering the effects of electric fields previously observed on prominent sensory appendages (Maw, 1961c; Watson, 1984), and calculated by field modelling, the contribution of the cerci, antennae and maxillary palps (Fig. 3.1A) in the avoidance of friction charged PTFE was investigated. Avoidance was examined using the Y-choice apparatus described in Chapter 2, after bilateral ablation of these appendages.

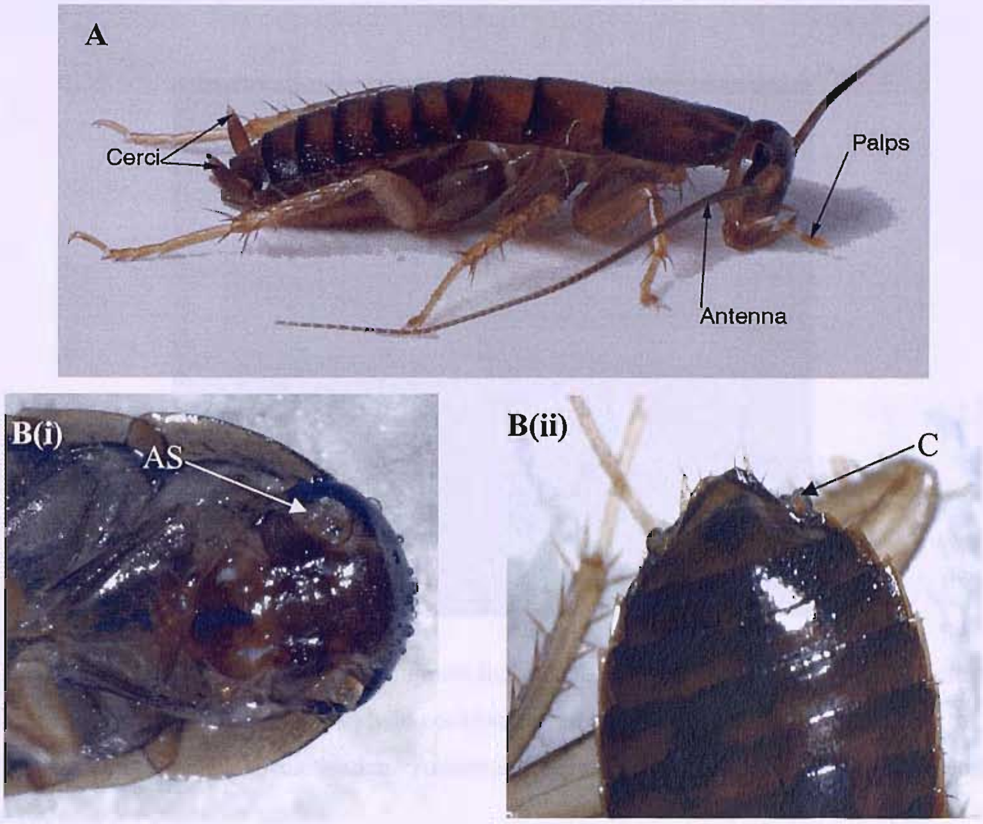


Figure 3.1 (A) Cockroach sensory appendages investigated in the avoidance of charged PTFE within the Y-choice apparatus. (Bi) The antennae were removed leaving a small part of the proximal antennal segment (AS) and (Bii) the majority of the cerci were removed (C) to investigate their role in the detection of charged PTFE.

Ablation of sensory structures

Bilateral ablations were carried out on third and fourth instar cockroaches (abdomen to head length, $n = 195$, 7.2±1mm) following anaesthetisation with CO_2 (Fig. 3.2). The sensory structures were ablated using fine iridectomy scissors (Fig. 3.1B). Sham experiments were also carried out on third and fourth instar cockroaches (abdomen to head length, $n = 44$, 9.1±0.7mm) by making a small incision in the ventral cuticle, sufficient to cause bleeding similar to that after sensory structure ablation.

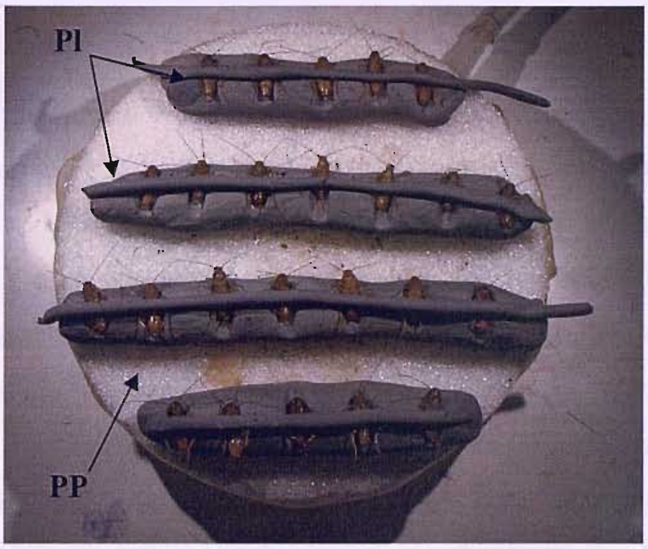


Figure 3.2 Set up used to anaesthetise and support individuals during ablation of sensory structures and antennal joint restriction. Plasticine (Pl) held cockroaches in place and a flow of CO_2 through the porous plate (PP) ensured constant anaesthetisation. Antennae were positioned at their typical resting position 60° from the anterior-posterior midline.

Behavioural bioassays

After surgery cockroaches were left to recover for 18-20h before performing bioassays. The same procedure for the bioassays and apparatus washing were carried out as described in Chapter 2, in a room illuminated by a 40W tube light covered with a far-red filter (Campbell Environmental Products, UK) at temperature 22.4±1.5°C and 35±5%RH, between 10:00 and 19:00 GMT. Responses to one ($n = 246$, -0.22±0.06kV/m) and two ($n = 444$, -0.21±0.12kV/m) charged pathways were examined.

The effect of ablation on preference within an untreated Y-choice apparatus was first analysed to determine whether a natural preference for the left or right pathway existed. The proportion of cockroaches taking the left or right pathway was therefore compared to the expected 50:50 ratio (Van-Tol *et al.*, 2002) for each ablation carried out using Binomial Tests of Proportions (S-Plus, Version 6.1 for Windows). The Binomial Test of Proportions also tested for avoidance for each ablation carried out, as well as differences in avoidance between ablations (S-Plus, Version 6.1 for Windows). Data were considered significant at the $P < 0.05$ level.

3.2.2 The detection of static electric fields

3.2.2.1 Electric field modelling

Force calculations were carried out to determine the influences of static electric fields on cockroaches within the Y-tube apparatus. The forces acting on two body parts, the antennae and whole body, were calculated at 1kV, 2kV and 4kV potentials at four locations within the Y-tube apparatus: the entrance; the intersection; the treated chamber, and the untreated chamber. The same models as detailed in Chapter 2 were used, appropriate materials assigned (Appendix 8.2), and percent error (0.01%) was determined as in 3.2.1.2. Differences in the magnitudes and directions of forces between the locations for a given potential, and between potentials, were compared. A vector plot was also drawn to represent the direction and magnitude of electric field within the apparatus.

3.2.2.2 High speed video observations of the antennae

High speed video observations of cockroaches within the Y-tube apparatus (as used in Chapter 2) were carried out to illustrate the effects of electrical forces on the antennae. Cockroaches were filmed at a horizontal angle through the apparatus using high-speed video equipment at 250frames/sec (MotionScope 1000S, Redlake Imaging Corp. CA, USA). Footage was taken of cockroaches (*n* = 4) exposed to electric fields produced by 1kV and 4kV potentials, in addition to controls using no electric fields, and still images were captured.

3.2.2.3 The effect of modifying antennal mechanoreceptor input on the avoidance of static electric fields

Given the influences of static electric fields calculated by field modelling and observed by high speed video footage, the role of the antennae in detecting static electric fields was investigated. To determine how the antennae contribute to static electric field detection, antennal sensory input was modified by: abolishing all antennal sensory input by bilateral ablation of the antennae, and restricting movement of the basal antennal joints (Fig. 3.3) to prevent mechanoreceptor stimulation at these locations.

To prevent basal joint movement, third and fourth instar cockroaches (abdomen to head length, n = 263, 8.06±0.56mm) were first anaesthetised and restricted on a porous plate (Fig. 3.3). The antennae were then positioned in their typical resting position, approximately 60° from the anterior-posterior midline (Comer *et al.*, 2003; Ye *et al.*, 2003). Movement of the HS joint ($n = 52$), and hence mechanoreceptor stimulation at this joint, was prevented by applying a non-toxic cyanoacrylate adhesive, VetBond® (WPI, Stevenage, UK), to the joint using a fine pulled microcapillary (Borosilicate standard wall, OD = 1mm, ID = 0.58mm, L = 10mm, Warner instruments Inc, USA) held in a micropipette holder (WPI, Stevenage, UK). The holder was connected to a 5ml syringe, and blowing into the open syringe enabled controlled adhesive application. Adhesion was subsequently aided by applying cyanoacrylate adhesive accelerator (RS Components, Corby, UK). The same procedure was carried out at the SP joint ($n = 57$) to prevent mechanoreceptor stimulation. Both the HS and SP joints ($n = 63$) were also fixed to eliminate mechanosensory stimulation from both regions.

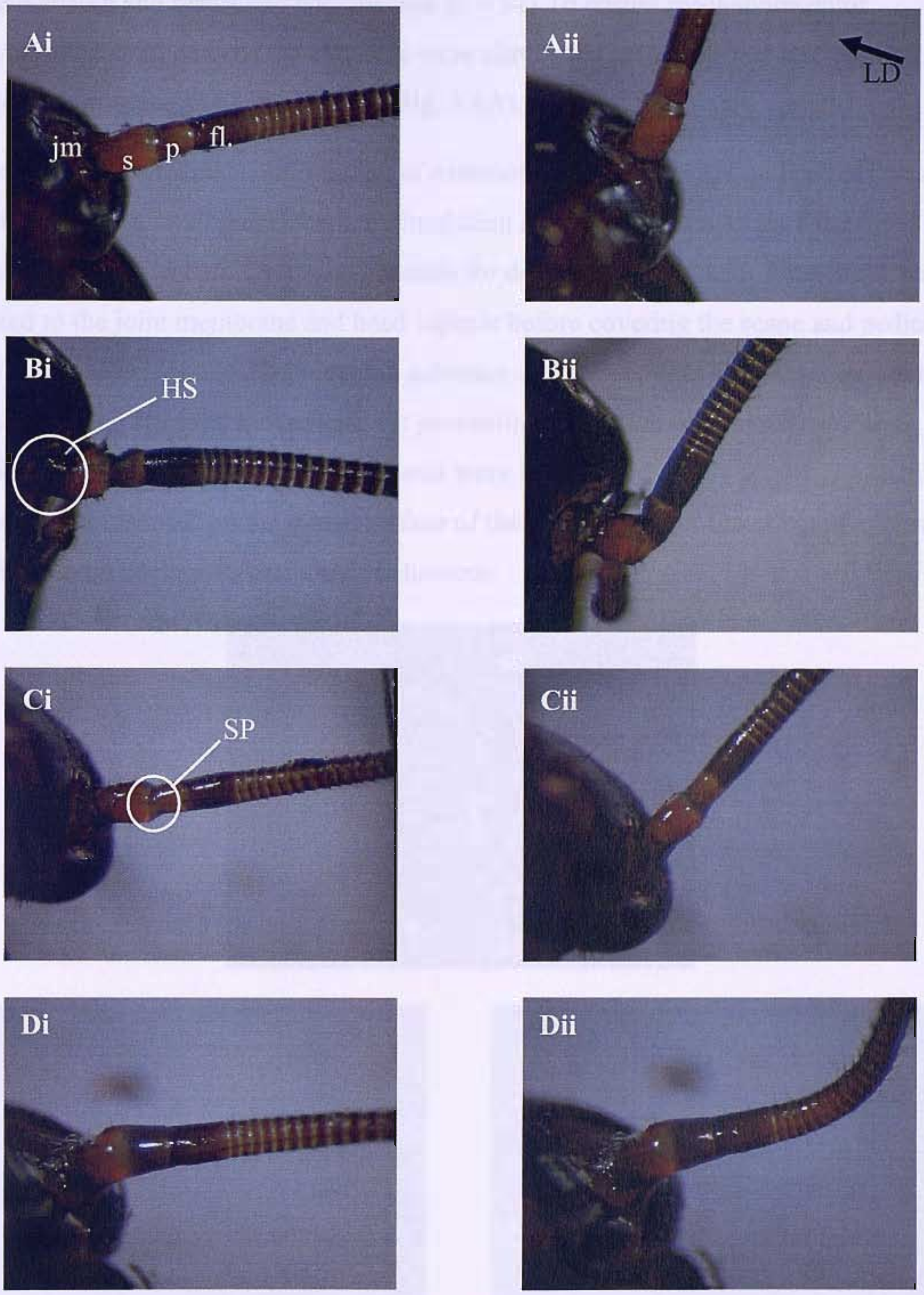


Figure 3.3 Antennal position (i) before and (ii) after a lateral displacement (LD) of the distal region of the flagellum when (A) no adhesive is present; (B) adhesive is present on the HS joint (HS); (C) adhesive has been applied to the SP joint (SP); (D) adhesive is present on both the HS and SP joints. Movement of the HS joint is restricted in B and D. The SP joint was immobile when adhesive was applied to that region, and only the flagellum moves when both joints have had adhesive applied. *jm* joint membrane; *s* scape; *p* pedicel; *fl.* flagellum.

Ablation of the antennae was carried out using a pair of fine iridectomy scissors after anaesthetising and restricting cockroaches (n = 91).To ensure mechanoreceptor activation from all parts of the antennae were eliminated, adhesive was also applied to the joint membrane and head capsule (Fig. 3.4A).

In an additional treatment, stimulation of exteroceptors on the scape and pedicel was prevented, but internal proprioceptor stimulation allowed to test whether external or internal mechanoreceptors were responsible for detection (Fig. 3.4B). Vaseline® was applied to the joint membrane and head capsule before covering the scape and pedicel with VetBond®. Vaseline® prevented adhesion of VetBond® to the joint membrane, hence allowing HS joint movement, but preventing deflection of external mechanoreceptor hairs. Sham experiments were also carried out by applying a small amount of VetBond® on the dorsal surface of the head to test for the effect of adhesive application on cockroach preference behaviour.

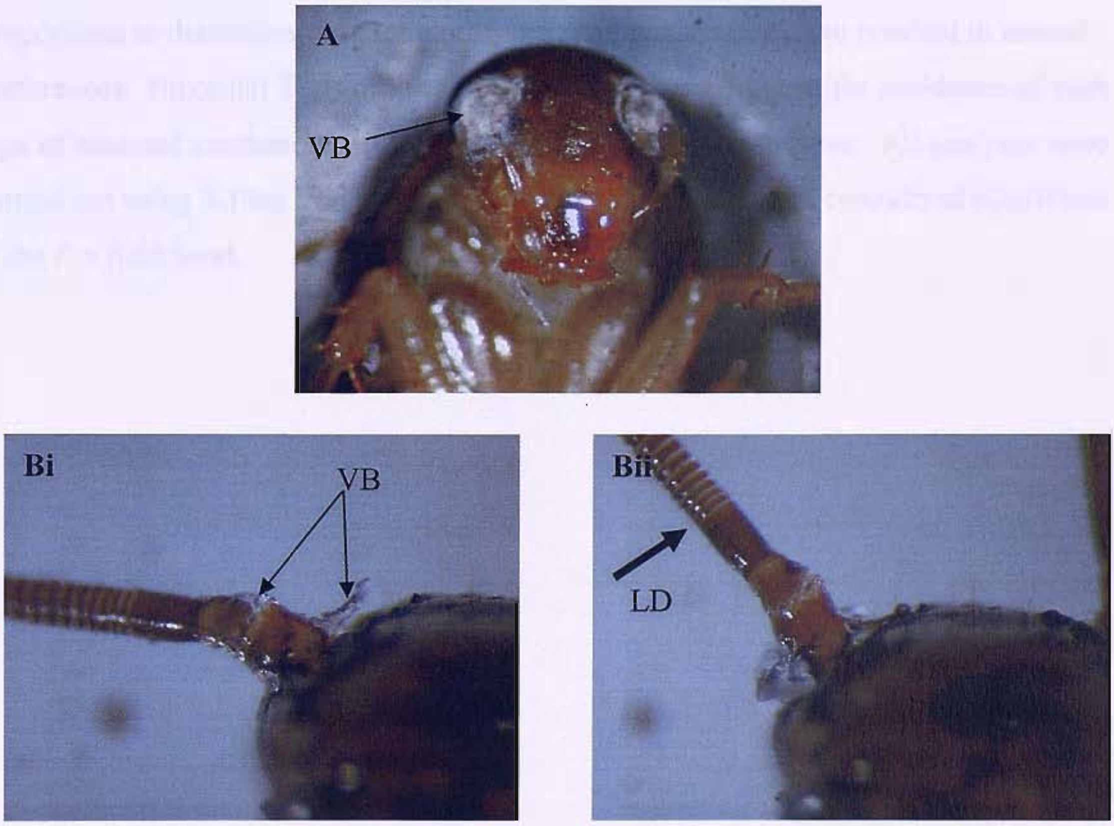


Figure 3.4 (A) Ablation of the antennae and application of VetBond® (VB) to the joint membrane and head capsule eliminating any mechanosensory input from the antennae. (Bi) Petrolium jelly was applied to the head capsule before covering the scape and pedicel with VetBond® to prevent external mechanoreceptor activation, yet (Bii) allow antennal movement at the HS joint and therefore internal proprioceptor stimulation. *LD* direction of lateral displacement

Behavioural bioassays

The same Y-tube apparatus and release chambers described in Chapter 2 were used to investigate the detection of static electric fields. A Brandenburg Alpha III high voltage power supply provided one pathway of the Y-tube apparatus with 1kV, 2kV and 4kV voltage potentials for each antennal modification carried out. Bioassays were carried out 18-20 hrs after surgery to allow for recovery. The antennae were observed under a dissecting microscope (Kyowa Optical SDZ-PL) preceding bioassays to ensure movement of the appropriate joint did not occur.

The bioassay procedure followed that described in Chapter 2, including apparatus washing. Bioassays were performed in a room illuminated by a 40W tube light covered with a far-red filter (Campbell Environmental Products, UK) at temperature 21.5±5.3°C and 33±6%RH, between 10:00 and 19:00.

Behaviour within the untreated Y-tube apparatus was analysed using Binomial Tests of Proportions to determine whether modifying antennal sensory input resulted in natural preferences. Binomial Tests of Proportions were also used to test for avoidance of each type of antennal mechanoreception modification, and between types. All analyses were carried out using S-Plus Version 6.1 for Windows, and data were considered significant at the $P < 0.05$ level.

3.3 Results

3.3.1 The detection of friction charged PTFE

3.3.1.1 Electric field modelling

One charged pathway

At the entrance to the Y-choice apparatus, opposing forces of similar magnitudes acted on the antennae and cerci (Fig. 3.5A), that could have been attributed to the field distribution within the apparatus. Notably, the central aluminium pathway was calculated to have generated an electric field (Chapter 2, Fig. 2.11), most likely due to charge induction due to its high conductivity (Cross, 1987).

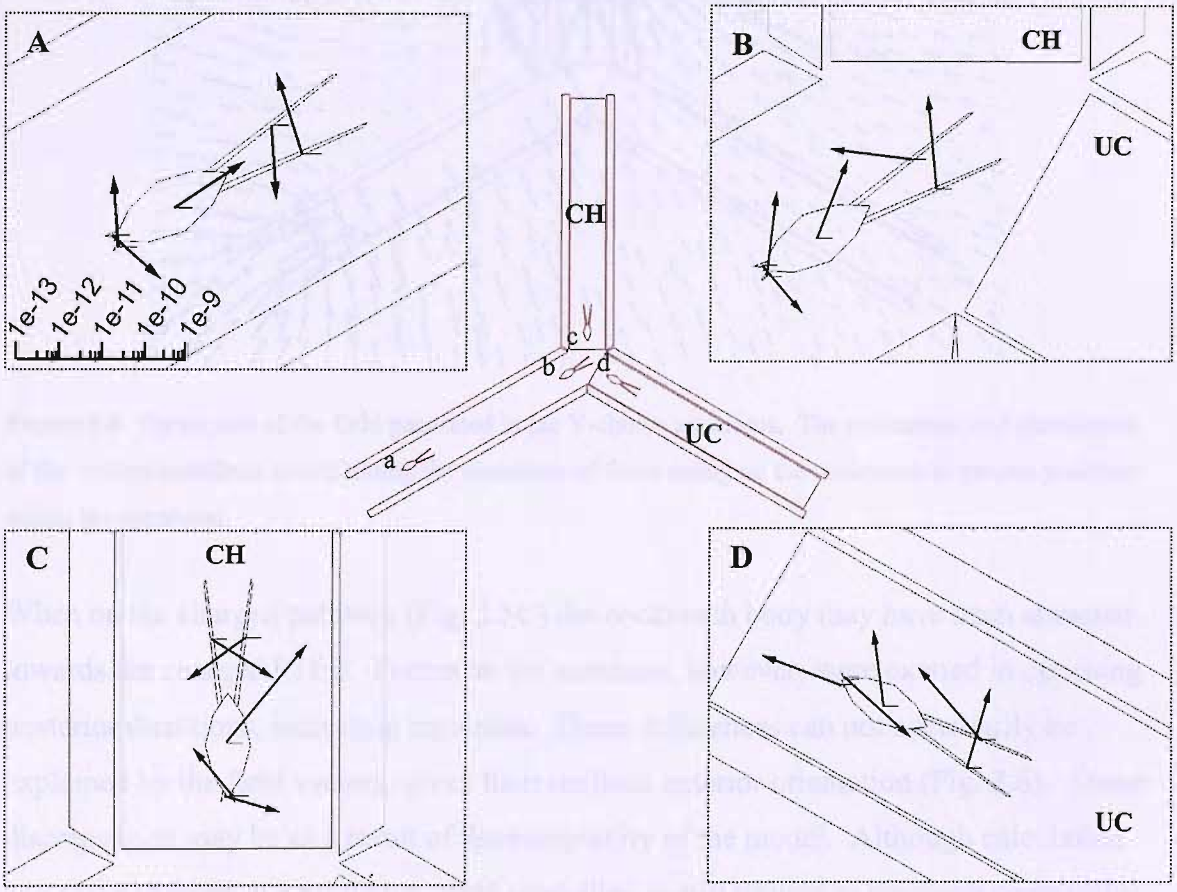


Figure 3.5 The forces acting on a cockroach within the Y-choice apparatus. Arrows represent direction and magnitude of the total resultant force on the cerci, antennae and whole body of the cockroach (A) at the entrance to the apparatus, (B) at the intersection between the charged (CH) and uncharged (UC) pathways, (C) on the treated pathway and (D) on the untreated pathway. Arrow length is representative of the force (in Newtons) as indicated by the log scale.

Electrical forces, or field vectors, are directed towards negative charges (Hammond, 1997), hence the field surrounding the central pathway was directed towards the negatively charged PTFE and aluminium (Fig. 3.6). Comparing the field vectors in the Y-choice and the forces acting on the cockroach, the forces exerted on the antennae were in accordance with the field vectors. This may therefore contribute to explaining opposing forces on the antennae.

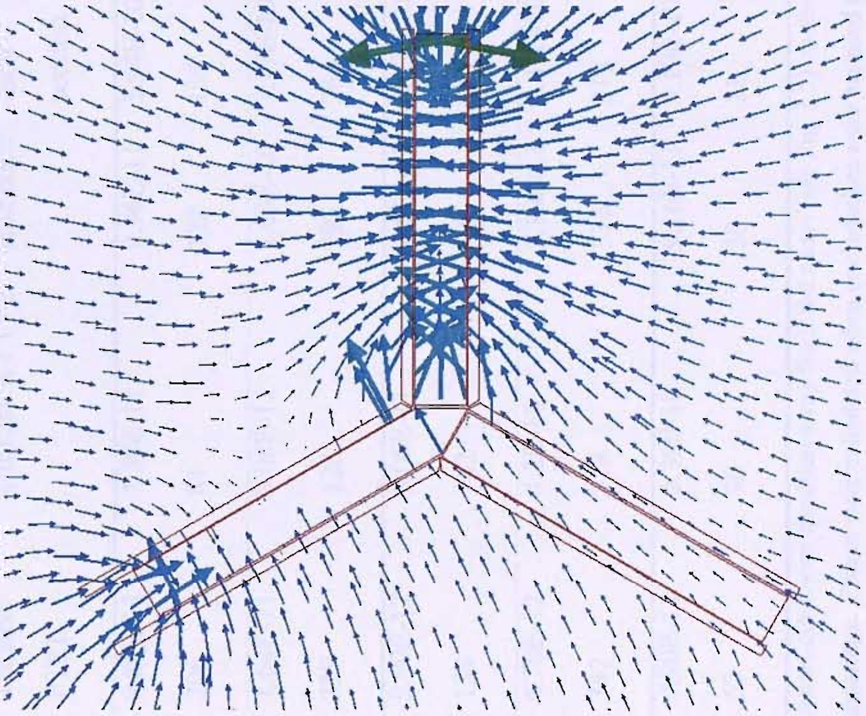


Figure 3.6 Vector plot of the field generated in the Y-choice apparatus. The orientation and distribution of the vectors contribute to explaining the directions of force acting on the cockroach at various positions within the apparatus.

When on the charged pathway (Fig. 3.5C) the cockroach body may have been attracted towards the charged PTFE. Forces on the antennae, however, were exerted in opposing posterior directions, indicating repulsion. These differences can not necessarily be explained by the field vectors, given their uniform anterior orientation (Fig. 3.6). These discrepancies may be as a result of the complexity of the model. Although calculation error was reduced to a minimum, field modelling is still limited to the mesh complexity and 2D model designs.

Structure	Resultant force (N) and angle (°)	One pathway charged				Two pathways charged		
		Position in apparatus				Position in apparatus		
		Entrance	Inter-section	Treated PTFE	Untreated PTFE	Entrance	Inter-section	PTFE
Right antenna	Total resultant	9.39E-12	7.9E-11	9.80E-12	1.39E-11	9.96E-11	3.94E-10	5.53E-11
	Angle	108	94	206	81	122	159	330
Left antenna	Total resultant	8.84E-12	4.30E-11	1.64E-11	3.08E-11	1.03E-10	3.19E-10	5.23E-11
	Angle	279	174	329	128	295	266	226
Right cercus	Total resultant	6.34E-12	3.29E-11	9.90E-12	3.08E-11	4.83E-11	1.62E-10	7.18E-11
	Angle	95	80	134	162	102	112	58
Left cercus	Total resultant	3.74E-12	1.19E-11	6.70E-12	4.2E-11	5.10E-11	1.45E-10	8.00E-11
	Angle	311	306	342	318	317	313	199
Whole body	Total resultant	7.70E-12	2.47E-10	9.90E-11	2.39E-10	9.16E-11	9.10E-10	6.68E-10
	Angle	30	66	57	96	30	21	250

Table 3.1 Total resultant forces and the angle the forces act in the counter-clockwise direction away from the *x* axis (see Fig. 3.5) on the sensory appendages and the whole cockroach body, at various positions within the Y-choice apparatus. Forces were calculated when cockroaches were exposed to one or two pathways, and demonstrate an increase in magnitude when two pathways were charged. Forces were also calculated when two pathways were charged at the same field strength produced when one pathway was charged and show the forces experienced by cockroaches under such conditions were still greater than when one pathway was charged

At the intersection the forces that acted upon the whole cockroach were greater in magnitude compared to the entrance: over thirty-fold on the whole body, and up to eight times greater on the antennae (Table 3.1, Fig. 3.5B). The antennae could have been attracted towards the charged pathway, the left antenna more so than the right. The forces on the cerci increased approximately three-fold, although no changes in direction occurred compared to the entrance.

Individuals that avoided the charged pathway (Fig. 3.5D) may have experienced forces lower in magnitude than at the intersection. The directions of force indicate that the antennae would have been repelled away from the charged surface, and the cerci and cockroach itself would have been attracted towards the pathway.

Two charged pathways

The directions of forces acting on cockroaches within the Y-choice apparatus when two pathways were charged were no different in direction to when one pathway was charged (Table 3.1 and Fig. 3.7), yet overall were of greater magnitude. These increases in force magnitudes could be explained by the greater field strength in the Y-choice apparatus, as described in Chapter 2.

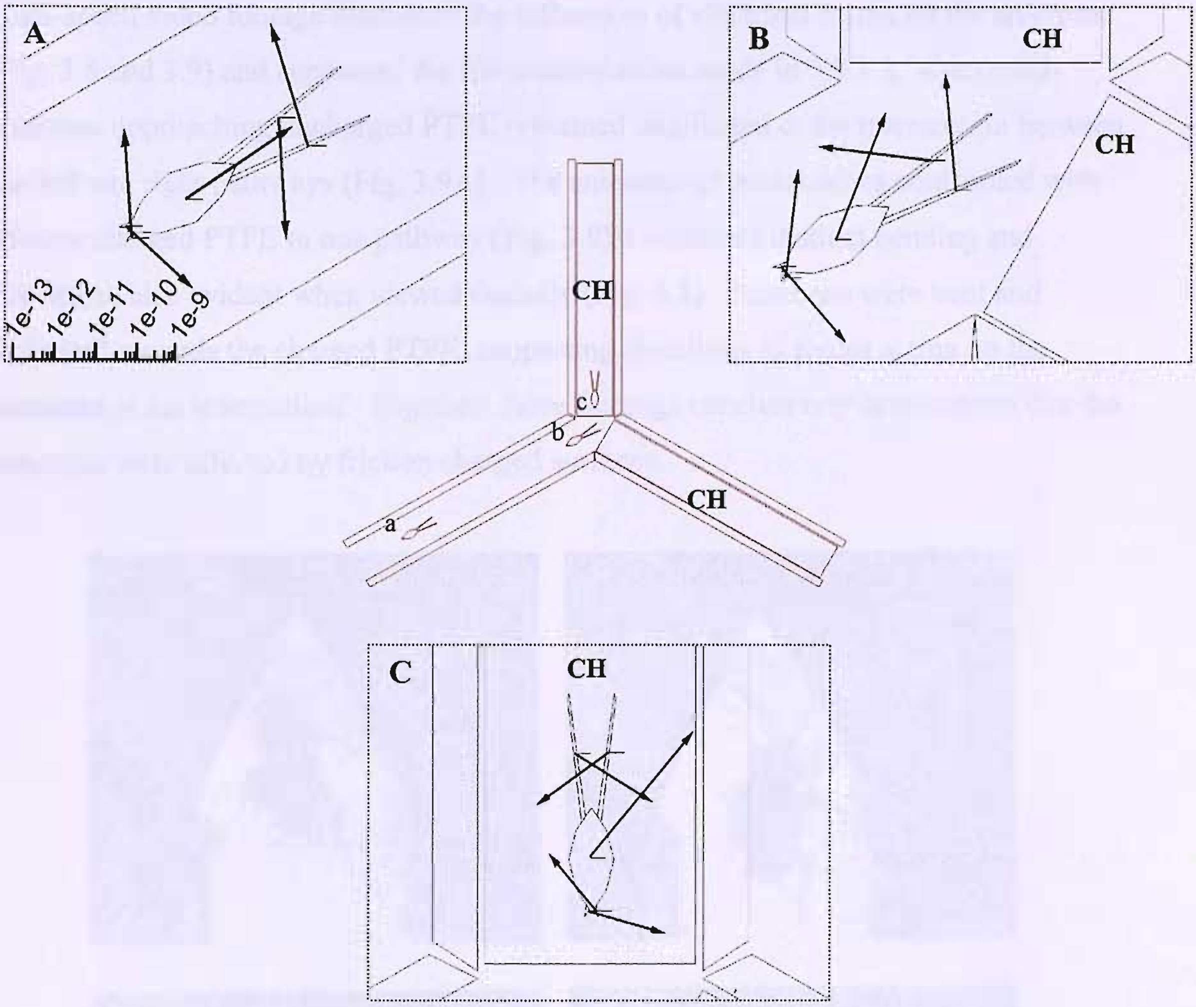


Figure 3.7 The forces acting on a cockroach exposed to two charged pathways within the Y-choice apparatus. The direction and magnitude of the total resultant forces on the cerci, antennae and whole body of the cockroach are represented by the arrows (A) at the entrance to the apparatus, (B) at the intersection between the treated pathways (CH), and (C) on the treated pathway. Arrow length is representative of the force (in Newtons) as indicated by the log scale.

3.3.1.2 High-speed video observations of antennal movements

High-speed video footage illustrated the influences of electrical forces on the antennae (Fig. 3.8 and 3.9) and supported the force calculations made in 3.3.1.2. Cockroach antennae approaching uncharged PTFE remained unaffected at the intersection between the left and right pathways (Fig. 3.9A). The antennae of cockroaches confronted with friction charged PTFE in one pathway (Fig. 3.9B) exhibited distinct bending and curvature, also evident when viewed dorsally (Fig. 3.8). Antennae were bent and deflected towards the charged PTFE, supporting directions of forces acting on the antennae at the intersection. Together, these findings conclusively demonstrate that the antennae were affected by friction charged surfaces.

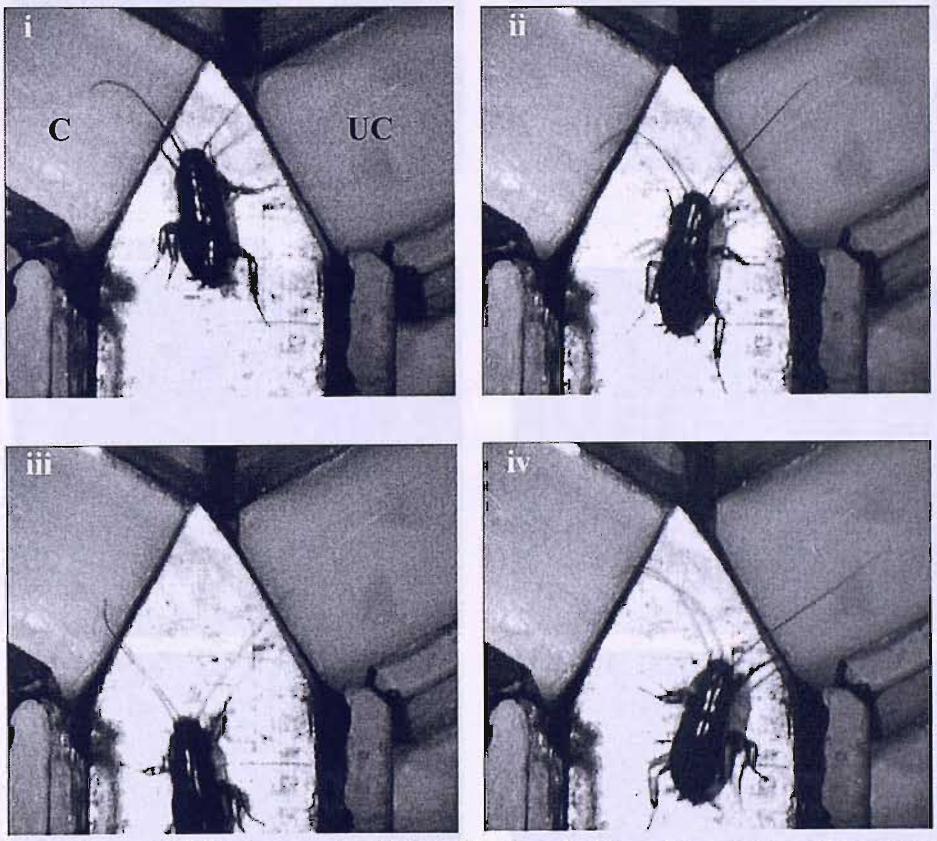


Figure 3.8 Sequence of high speed video images (i-iv) as a cockroach moves towards a charged (C) and uncharged (UC) PTFE section. The charged PTFE pathway only affects the antenna in proximity to it, ultimately ending in avoidance of the charged pathway (iv).

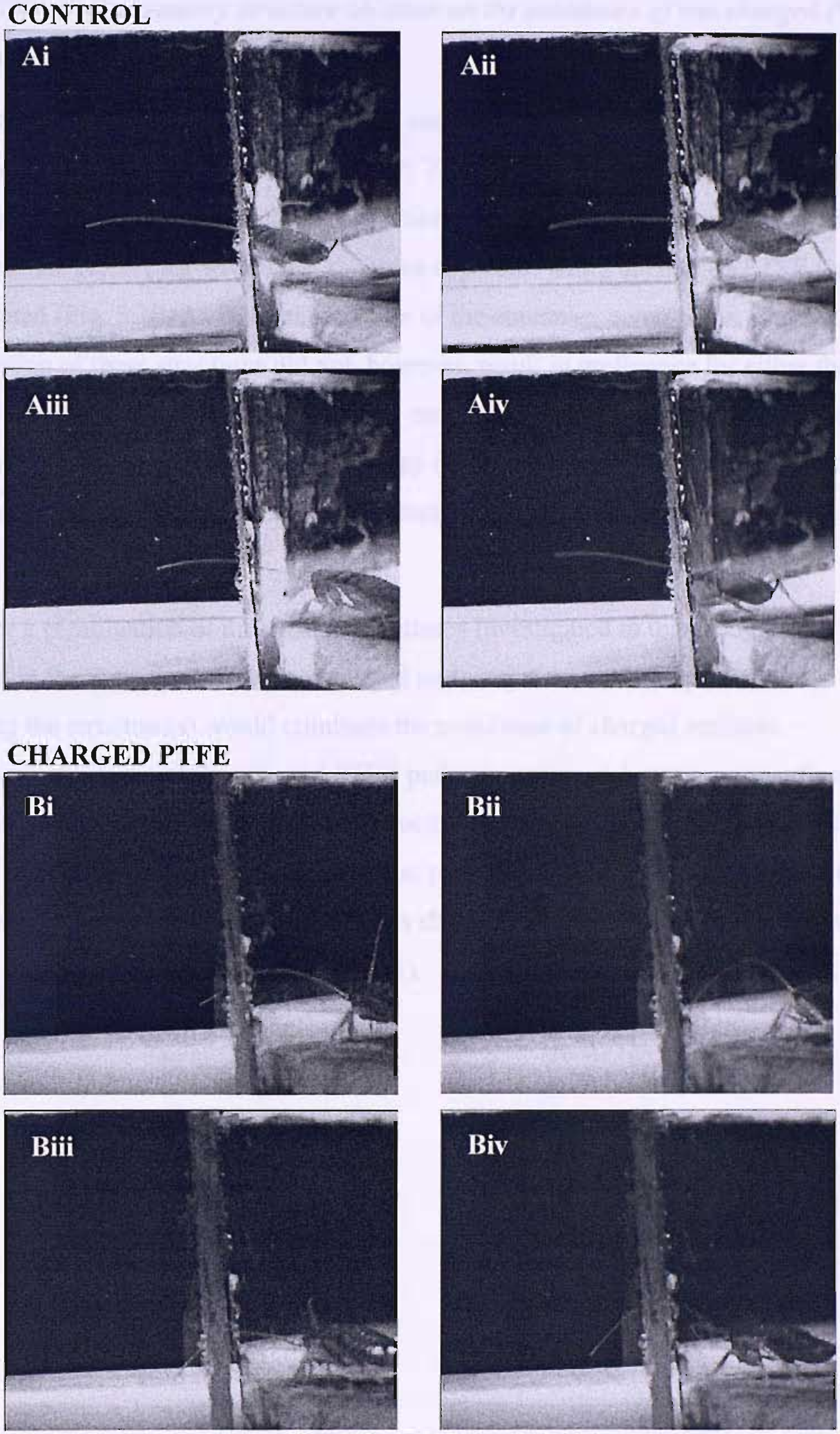


Figure 3.9 High speed video images of four different individuals (Ai-iv) exposed to uncharged PTFE and (Bi-iv) individuals exposed to friction charged PTFE. Bending of the antennae is evident when cockroaches were exposed to a charged PTFE pathway, but did not occur with uncharged PTFE.

3.3.1.3 The effect of sensory structure ablation on the avoidance of one charged PTFE pathway

The removal of sensory structures has been suggested to alter normal walking behaviour (Bell and Kramer, 1979; Comer *et al.*, 2003; Ye *et al.*, 2003). Therefore, before exposing cockroaches to charged PTFE sections, the effect of sensory structure ablation on directional preference within the Y-choice apparatus using uncharged PTFE was investigated (Fig. 3.10A). Bilateral ablation of the antennae, cerci, palps, or any combination of these structures did not, however, result in preference for either the left or right uncharged pathway ($P > 0.05$ in all cases). In addition, sham ablations did not evoke preference for the left or right pathway ($n = 40$, $P > 0.05$). Sensory structure ablation or surgery did not therefore affect natural preference within the Y-choice apparatus.

If one, or a combination of the sensory structures investigated in this study were involved in the detection of friction charged surfaces, it would be expected that removing the structure(s) would eliminate the avoidance of charged surfaces. Significant avoidance of the charged PTFE pathway occurred, however, regardless of the sensory structure, or combination or structures, removed (Fig. 3.10B, $P < 0.05$ in all cases). Sham experiments demonstrated that performing surgery on cockroaches did not affect the avoidance of electric fields, as sham individuals also significantly avoided friction charged pathways ($n = 40$, $P < 0.05$).

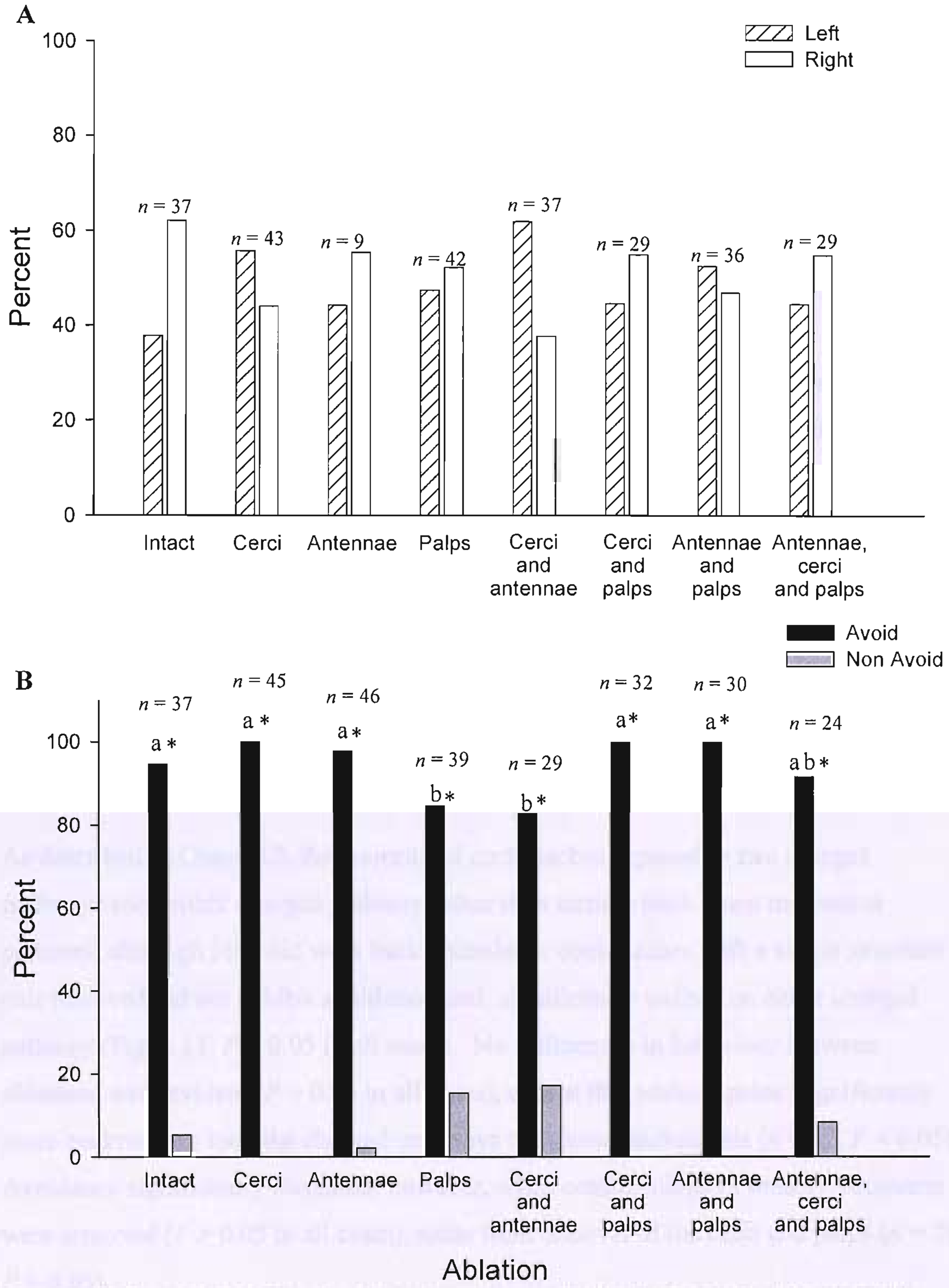


Figure 3.10 The avoidance of cockroaches exposed to uncharged PTFE sections and charged PTFE sections after sensory structure ablation. (A) No natural preference for the left or right pathway occurred after sensory structure ablations. (B) Cockroaches with any sensory appendage or combination of appendages removed significantly avoided the charged pathway ($P < 0.05$ in all cases, represented by asterisks). Differences in the avoidance behaviour are indicated by different lowercase letters, showing , for example, less avoidance occurred when the palps, and both the cerci and antennae were removed in comparison to intact individuals ($P < 0.05$ in both cases).

Nevertheless, differences in avoidance between the ablations may give an indication that the antennae could contribute to detection. Removing both the antennae and cerci resulted in less avoidance than when solely the cerci were removed ($n = 24$, $P < 0.05$). In addition, significantly less avoidance occurred without antennae and cerci than when the cerci and palps were removed ($n = 24$, $P < 0.05$). Absence of the antennae could therefore be responsible for these decreases in avoidance, indicating they may play a role in detection.

Variation in the results, however, needs to be taken into consideration. For example, removing both the antennae and palps resulted in 100% avoidance. In addition, cockroaches with no antennae exhibited no difference in avoidance compared to intact individuals ($n = 46$, $P > 0.05$). These discrepancies may be due to the ablation surgery, given the invasive nature of such surgery (Bell and Kramer, 1979; Comer *et al.*, 2003; Ye *et al.*, 2003).

3.3.1.4 The effect of sensory structure ablation on the avoidance of two charged PTFE pathways

As described in Chapter 2, the majority of cockroaches exposed to two charged pathways took either charged pathway rather than turning back down the central pathway, although 30% did walk back. Similarly, cockroaches with a single structure pair removed did not exhibit avoidance, and, significantly walked on either charged pathway (Fig. 3.11, $P < 0.05$ in all cases). No differences in behaviour between ablations were evident ($P > 0.05$ in all cases), except that without palps, significantly more cockroaches took the charged pathways than intact individuals ($n = 37$, $P < 0.05$). Avoidance significantly increased, however, when combinations of sensory structures were removed ($P > 0.05$ in all cases), aside from removal of the cerci and palps ($n = 29$, $P > 0.05$).

These results may not provide further evidence for the structure responsible for detecting friction charged surfaces, but do highlight that cockroaches exhibit a greater tendency to turn back down the central pathway when more sensory structures are removed.

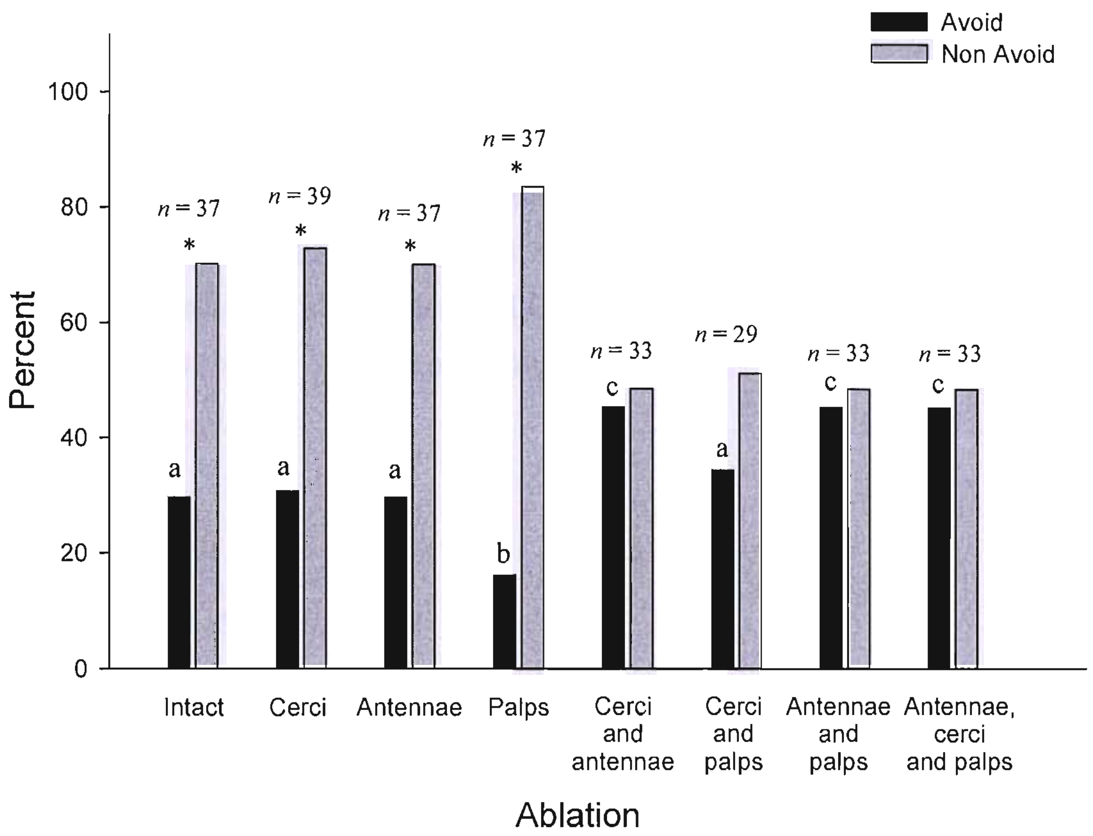


Figure 3.11 The avoidance of cockroaches exposed to two frictionally charged PTFE pathways after sensory structure ablation. Avoidance did not occur when the cerci, antennae, and palps were independently ablated ($P < 0.05$ in all cases, represented by asterisks). Even more cockroaches took the charged pathways when solely the palps were removed compared to intact individuals, and cockroaches with no cerci or antennae ($n = 37$, $P < 0.05$, indicated by lowercase letters). Removing combinations of appendages (aside from the cerci and palps) resulted in more individuals avoiding the charged pathways ($P < 0.05$ in all cases).

3.3.2 The detection of static electric fields

3.3.2.1 Electric field modelling

1kV

Field modelling indicates that at the entrance to the Y-tube apparatus opposing forces acted on the antennae (Table 3.2, Fig. 3.12A), similar to those exerted in the Y-choice apparatus. Unlike the forces calculated in the Y-choice, the directions of the field vectors do not account for these opposing forces (Fig. 3.13).

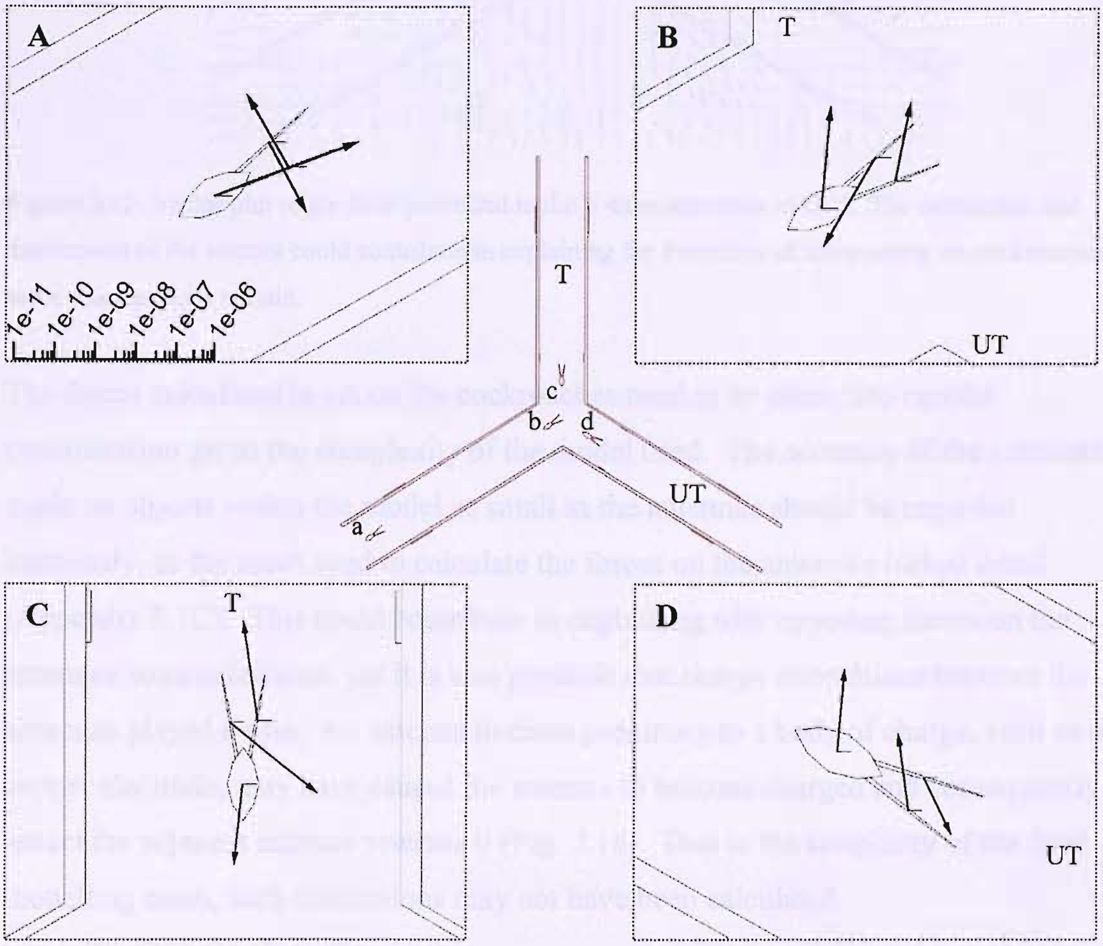


Figure 3.12 The forces acting on a cockroach within the Y-tube apparatus exposed to a pathway treated with a 1kV potential. Uppercase letters represent the positions of the cockroach, labelled with lowercase letters in the central diagram. Arrow length is representative of the force (in Newtons) as indicated by the log scale.

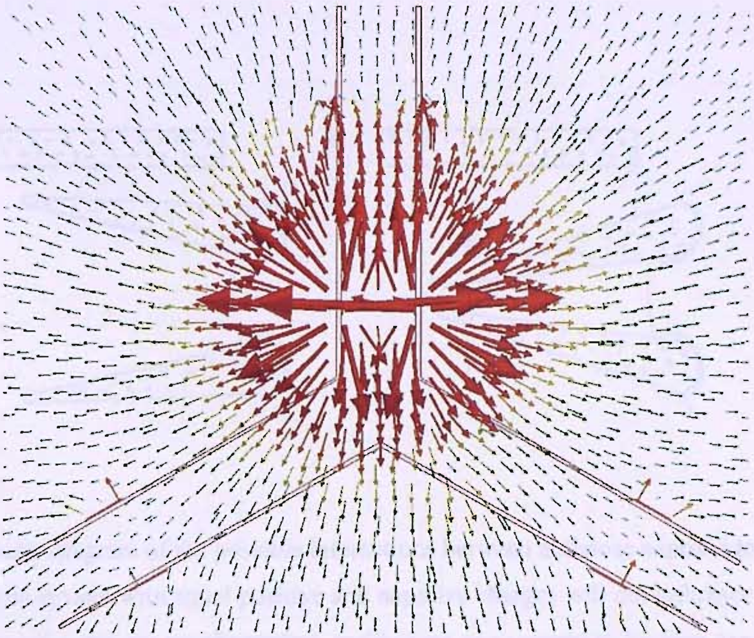


Figure 3.13 Vector plot of the field generated in the Y-tube apparatus at 1kV. The orientation and distribution of the vectors could contribute to explaining the directions of force acting on cockroaches, yet some discrepancies remain.

The forces calculated to act on the cockroaches need to be taken into careful consideration given the complexity of the model used. The accuracy of the calculations made on objects within the model as small as the antennae should be regarded cautiously, as the mesh used to calculate the forces on the antennae lacked detail (Appendix 8.1C). This could contribute to explaining why opposing forces on the antennae were calculated, yet it is also possible that charge interactions between the antennae played a role. An antenna in close proximity to a body of charge, such as the copper electrode, may have caused the antenna to become charged and consequently attract the adjacent antenna towards it (Fig. 3.14). Due to the simplicity of the field modelling mesh, such interactions may not have been calculated.

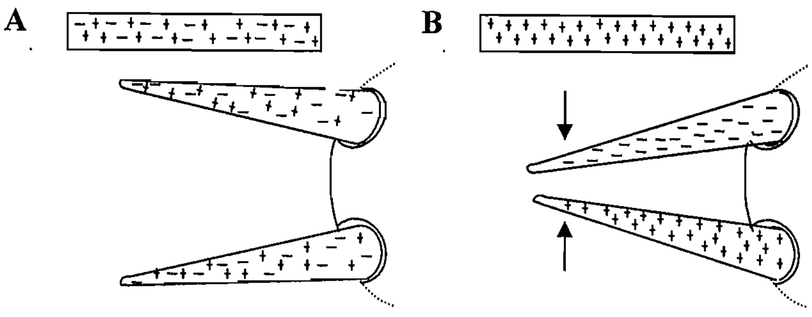


Figure 3.14 Schematic diagram of the possible interactions between antennae exposed to a charged surface. (A) A neutral system with equal positive and negative charges will not influence the antennae. (B) A charged surface (i.e. the copper electrodes) could cause one antenna to become negatively charged and therefore the other becomes positively charged. Subsequently, the antennae would be attracted towards each other.

Regardless of these differences, the calculations indicate that at the intersection (Fig. 3.12B) the forces acting upon the body of the cockroach increased over twenty-fold, attracting the cockroaches towards the treated chamber. In the treated chamber (3.12C), the right antenna may have been attracted towards the electrode and the left antennae repelled away from the electrode. Cockroaches that avoided the treated chamber (Fig. 3.12D) could have experienced forces of similar magnitude to those at the intersection. In the untreated chamber, cockroaches may have been repelled, although the magnitudes were little different to those within the treated chamber.

2kV and above

The directions of forces acting on cockroaches between locations within the Y-tube at 2kV and 4kV were little different to those calculated at 1kV. Overall, however, the magnitudes of force were greater (Table 3.2 and Fig. 3.15). The directions the forces acted did not differ between 2kV and 4kV, although the magnitudes were greater at 4kV than 2kV.

		Voltage potential (V)		
Position	**Angle and force (N) acting on**	1000	2000	4000
Entrance	Right antenna	6.95E-9	2.78E-8	1.11E-7
		121	122	121
	Left antenna	7.7E-9	3.08E-8	1.23E-7
		294	294	294
	Whole Body	3.43E-9	1.33E-8	5.46E-8
		22	22	22
Intersection	Right antenna	2.08E-8	8.34E-8	3.3E-7
		74	74	74
	Left antenna	4.19E-8	1.68E-7	6.7E-7
		237	237	237
	Whole Body	9.09E-8	3.64E-7	1.46E-6
		88	88	88
Treated chamber	Right antenna	7.92E-8	3.17E-7	1.27E-6
		98	98	98
	Left antenna	8.39E-8	3.36E-7	1.34E-6
		318	318	318
	Whole Body	4.92E-8	1.97E-7	7.87E-7
		273	273	273
Untreated	Right antenna	4.35E-8	1.74E-7	7E-7
		97	97	97
	Left antenna	9.86E-9	3.87E-8	1.54E-7
		285	285	285
	Whole Body	7.16E-8	2.86E-7	1.14E-6
		87	87	87

Table 3.2 The resultant forces acting on the antennae and whole body of a cockroach at various locations within the Y-tube apparatus as the voltage potential was altered. Direction of force is indicated by the angle, measured anti clockwise along the horizontal plane. The magnitude of force acting on cockroaches increased as the voltage potential was increased, in addition to increasing as the cockroach approached the treated pathway. The directions of force were dissimilar at each voltage potential.

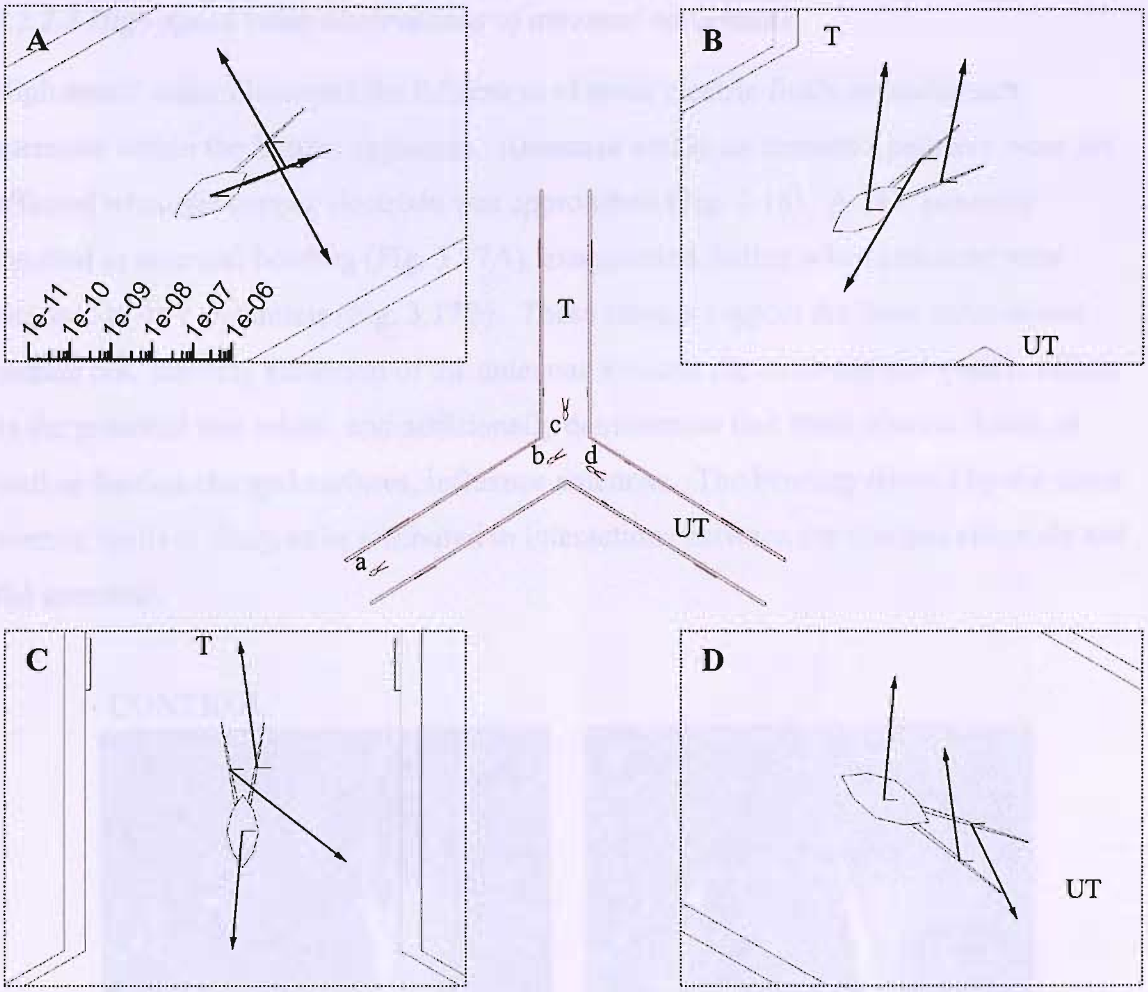


Figure 3.15 The forces acting on a cockroach within the Y-tube apparatus exposed to a pathway treated with a 4kV potential. Uppercase letters represent the positions of the cockroach, labelled with lowercase letters in the central diagram. Arrow length is representative of the force (in Newtons) as indicated by the log scale.

3.3.2.2 High-speed video observations of antennal movements

High-speed video illustrated the influences of static electric fields on cockroach antennae within the Y-tube apparatus. Antennae within an untreated pathway were not affected when the copper electrode was approached (Fig. 3.16). A 1kV potential resulted in antennal bending (Fig. 3.17A), exaggerated further when antennae were exposed to 4kV potentials (Fig. 3.17B). These images support the force calculations carried out, showing attraction of the antennae towards the electrode and greater effects as the potential was raised, and additionally demonstrate that static electric fields, as well as friction charged surfaces, influence antennae. The bending elicited by the static electric fields is likely to be attributed to interactions between the charged electrode and the antennae.

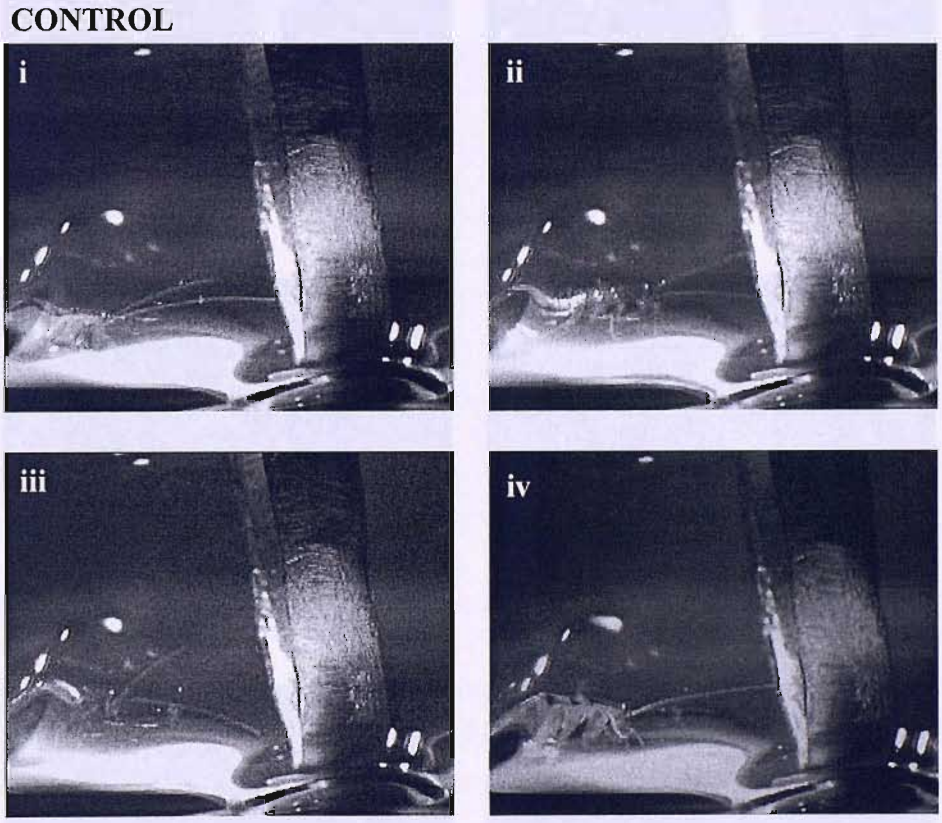


Figure 3.16 High speed video images of four different individuals (i-iv) approaching an untreated electrode within the Y-tube apparatus, demonstrating that the antennae are not affected by the untreated electrode.

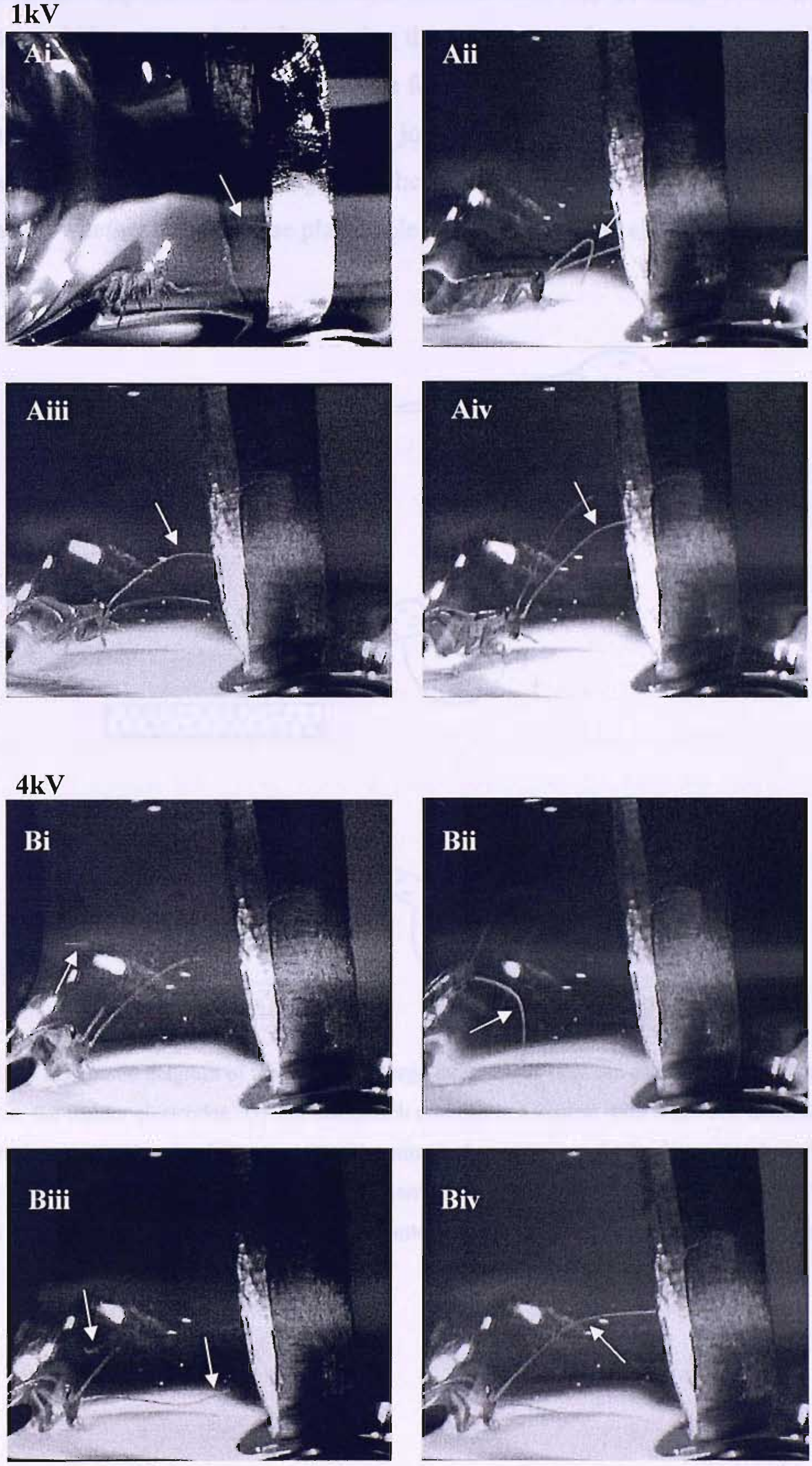


Figure 3.17 High-speed video images of four different individuals approaching electrodes (Ai-iv) at 1kV potential and (Bi-iv) 4kV potentials. Bending of the antennae is highlighted with arrows.

As a cockroach approaches the electrode, its neutral state may be affected and the antennae could become polarised attracting the antennae to the opposite charge (Fig. 3.18). This passive movement may then be followed by an active movement of the antennae by muscles within the SP and HS joint, causing the bending evident in the high speed video observations. Together, the force calculations and these effects raise the question whether the antennae play a role in detecting static electric fields.

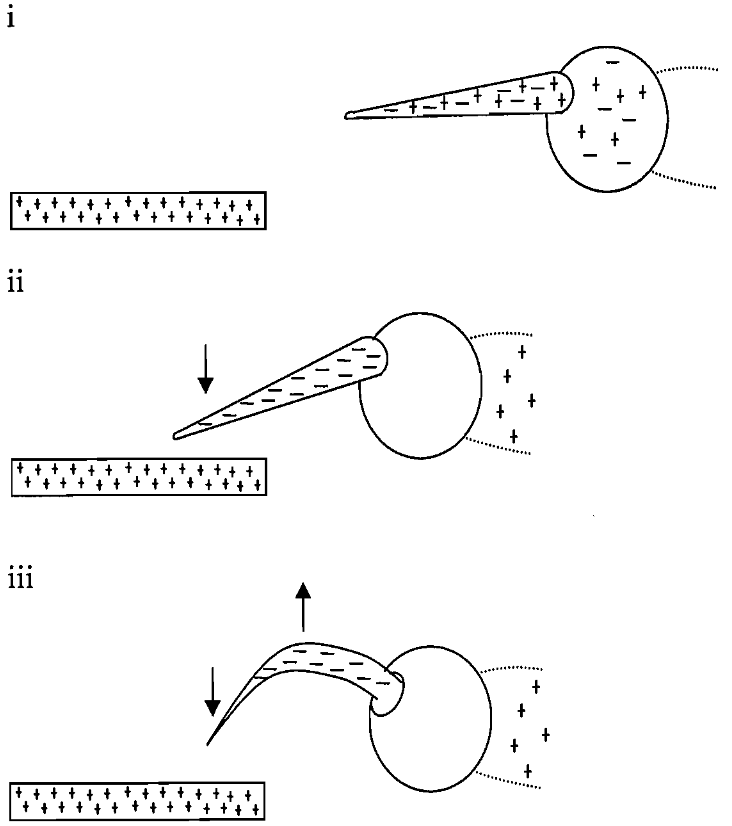


Figure 3.18 Schematic diagram of the possible charge interactions between an antenna and a charged surface, i.e. the copper electrodes. (i) The cockroach remains in a neutral state when at a distance from the electrode, yet (ii) when in close proximity, the antenna becomes negatively charged and passively attracted towards the electrode. (iii) Active movement of the antenna then causes upward movement, although attractive forces between the surface and antenna remain, hence bending of the antenna.

3.3.3.3 Location of external mechanoreceptors on nymph antennae

The distribution and location of mechanoreceptor hairs on the basal joints of a nymph antenna were identified and drawn (Fig. 3.19). In a similar morphology to adults (Comer *et al.*, 2003; Okada and Toh, 2000; Staudacher *et al.*, 2005), nymph antennae exhibit arrangements of sensory hairs that are in close proximity, located on the scape and pedicel segments. Distinct arrangements such as these are known as hair plates, fields or rows (Staudacher *et al.*, 2005) and each will hereafter be termed as a 'hair plate'.

On the pedicel, the hair plates existed solely at the proximal area of the segment close to the SP joint. Two hair plates were identified, one positioned dorsally (dorsal P-HP) and the other ventrally (ventral P-HP). These corresponded with the movement of the SP joint in the vertical plane (Toh, 1981). Hairs on the scape were more abundant yet exhibited distinct arrangements at the base of the segment, close to the HS joint, adjacent to the joint membrane. Hair plates were found on dorsal (dorsal S-HP), ventral (ventral S-HP), anterior (anterior S-HP) and posterior (posterior S-HP) locations. The HS joint can move in any direction (Okada and Toh, 2001; Staudacher *et al.*, 2005), hence contact of these hair plates with the joint membrane and head capsule will provide positional information of the antenna in most planes. Given the influences of electric fields on antennae and the contribution of the hair plates in providing information on antennal position, the possible role of these hair plates in the detection of static electric fields was investigated.

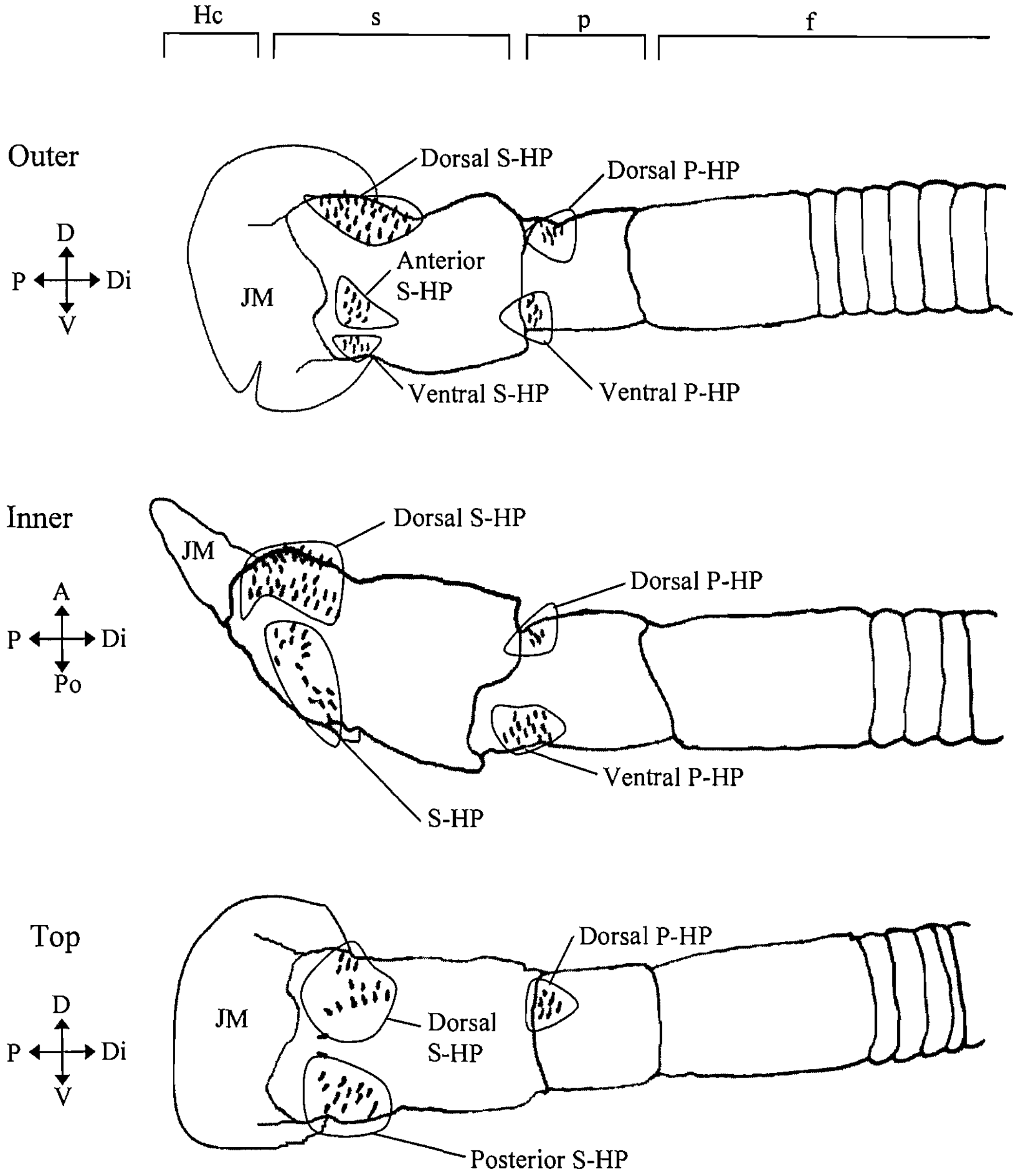


Figure 3.19 Nymph antennal mechanoreceptor hair plates located on the scape (s), and pedicel (p) of the dorsal, ventral anterior and posterior locations of a third to fourth instar nymph antenna viewed from the top, inner and outer positions. *f* flagellum, *Hc.* head capsule; *JM* joint membrane; *A* anterior; *Di* distal; *P* proximal; *Po* posterior; *V* ventral

3.3.3.4 The effect of modifying antennal mechanoreceptor input on the avoidance of static electric fields

Control bioassays were first carried out to test for any effect of restricting joint movement on natural preference within the Y-tube apparatus (Fig. 3.20). Ablating the antennae or restricting movement resulted in no significant preference for the left or right chamber ($n = 40$, $P > 0.05$ in all cases). In addition, sham experiments demonstrated no effect of VetBond® application on preference behaviour ($n = 40$, $P > 0.05$).

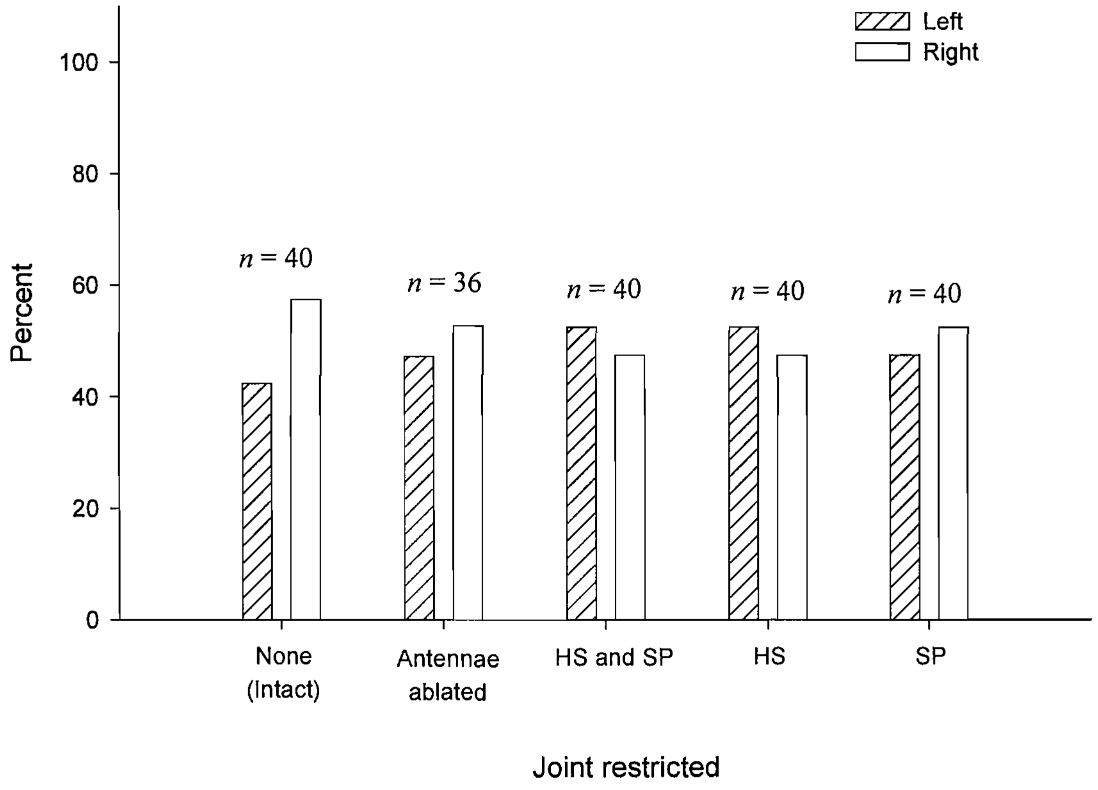


Figure 3.20 The effect of modifying mechanoreceptor input on the preference behaviour of cockroaches within an untreated Y-tube apparatus. No significant preference for either the left or right chamber occurred after surgery or restriction of mechanoreceptor input was performed. *HS* HS joint; *SP* SP joint

1kV

Modifying mechanoreceptor input by restricting movement of the both the HS- and SP joint, solely the HS joint, and by removing the antennae, resulted in no significant avoidance behaviour (Fig. 3.21, $P < 0.05$ in all cases). The proportions of these individuals that avoided the treated chamber were also significantly less than intact cockroaches ($P < 0.05$ in all cases). Avoidance did occur, however, when movement of the SP joint was prevented, and the proportion avoiding the treated chamber was not significantly different from that exhibited by intact cockroaches ($n = 40$, $P > 0.05$). Notably, the avoidance of cockroaches with HS joint movement prevented was significantly less than cockroaches with no SP joint movement ($n = 39$, $P < 0.05$). These findings therefore demonstrate that P-HP mechanoreceptors did not play a role in static electric field avoidance, yet S-HP mechanoreceptors could have contributed to electric field detection. Enabling movement of the HS joint yet preventing HS or SP joint exteroceptor stimulation did not result in avoidance ($n = 47$, $P > 0.05$), and the proportion that avoided the treated chamber was no different from cockroaches with the HS joint restricted ($n = 47$, $P > 0.05$). Proprioceptors within the scape therefore played no role in detecting static electric fields, demonstrating that S-HP exteroceptors were the likely candidate for detection.

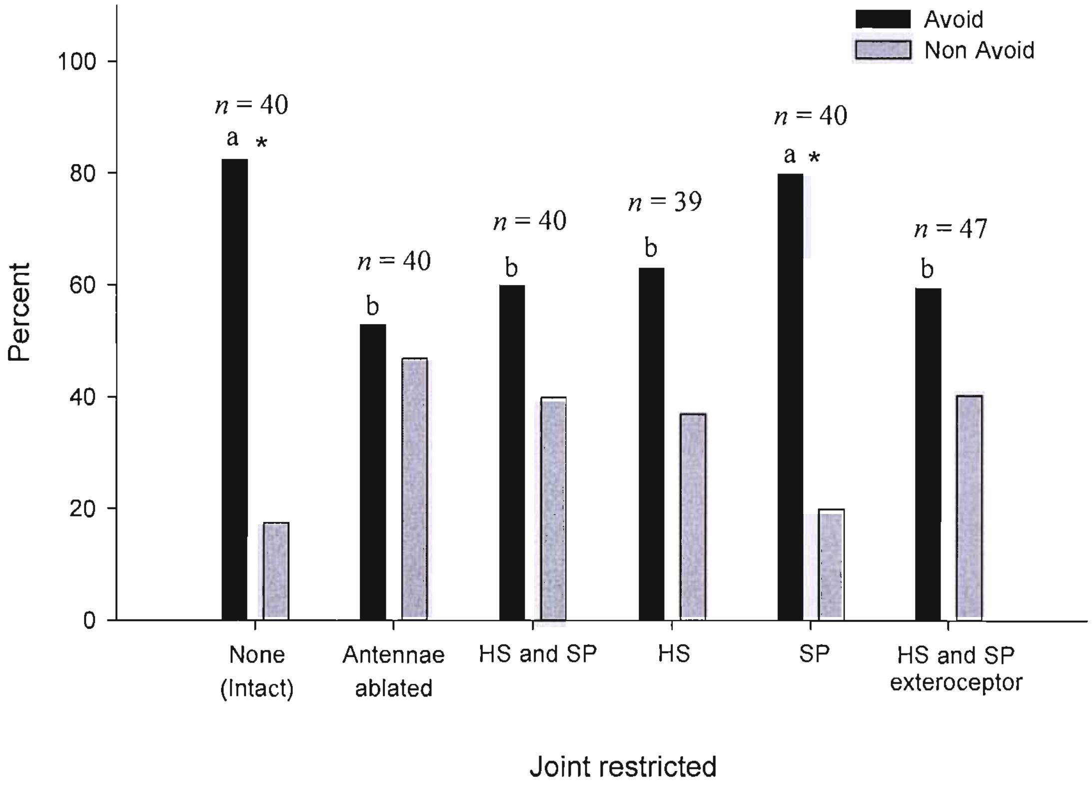


Figure 3.21 The effect of modifying mechanoreceptor input on the avoidance of electric fields at 1kV. Intact individuals and those with the SP joint fixed exhibited significant avoidance ($n = 40$, $P < 0.05$, represented by the asterisk). Avoidance significantly decreased when: the antennae were removed; both the HS- and SP joint, and the SP joint were restricted in comparison to intact cockroaches ($P < 0.05$ in all cases, represented by different lowercase letters). More cockroaches exhibited avoidance when the SP joint was fixed than when the HS joint was fixed ($n = 39$, $P < 0.05$). Preventing exteroceptor stimulation but allowing proprioceptor stimulation did not result in avoidance ($n = 47$, $P > 0.05$). The S-HP is therefore a likely candidate for static electric field detection.

2kV

Consistent with the results at 1kV, intact cockroaches, and those with the SP joint restricted, avoided static electric fields at 2kV potentials (Fig. 3.22, $n = 39$, $P < 0.05$; $n = 39$, $P < 0.05$ respectively). The proportion of cockroaches with the SP joint fixed that avoided the field was significantly greater than intact cockroaches ($n = 40$, $P < 0.05$). Contrary to 1kV, restricting the HS joint resulted in significant avoidance ($n = 39$, $P < 0.05$), yet fewer avoided the treated chamber than when the SP joint was fixed ($n = 39$, $P < 0.05$). Nonetheless, avoidance was no different between intact individuals and those with the HS joint restricted ($n = 39$, $P > 0.05$). This therefore indicates that mechanoreceptors aside from those of the S-HPs contributed to detecting static electric fields at 2kV potentials. Nevertheless, removing the antennae resulted in no avoidance (*n* = 40, $P > 0.05$), as did fixing both the HS- and SP joint (*n* = 40, $P > 0.05$). This suggests that at higher field strengths the additional mechanoreceptors, such as those on the flagellum, may play a role in static electric field detection.

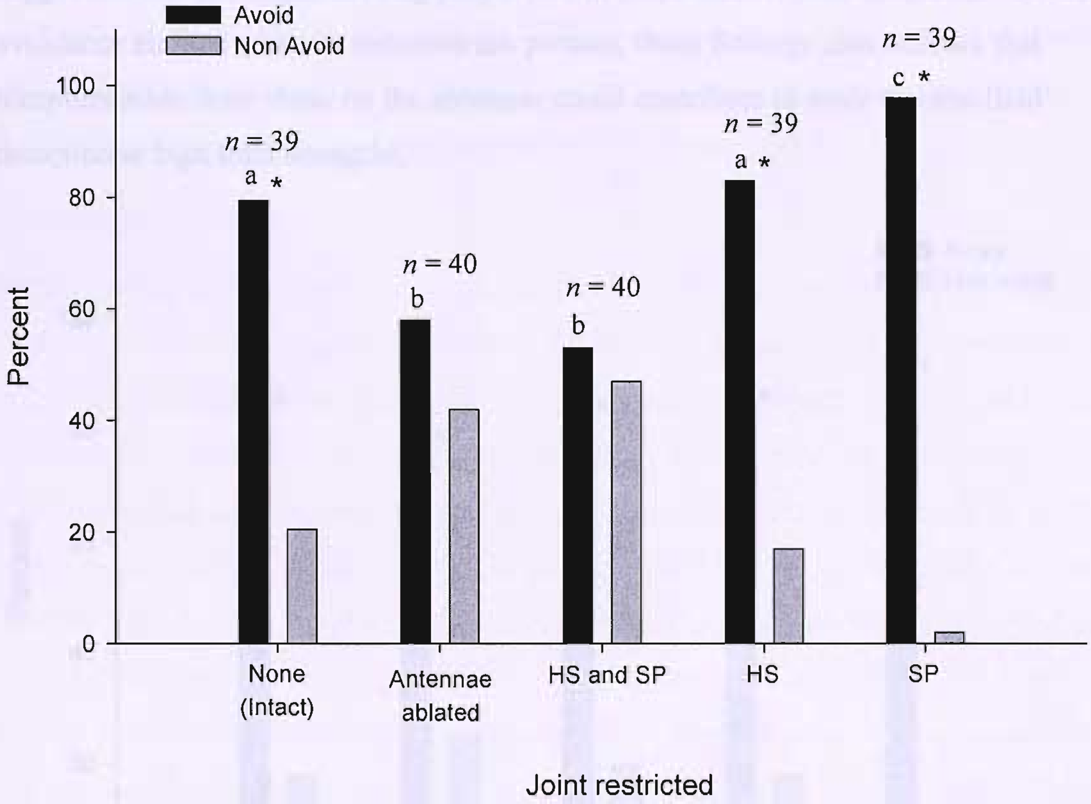


Figure 3.22 The effect of modifying mechanoreceptor input on the avoidance of electric fields at 2kV. Intact individuals and cockroaches with fixed HS, and SP joints significantly avoided the treated chamber ($P < 0.05$ in all cases, represented by the asterisks). Removing the antennae or restricting the HS and SP joints resulted in no avoidance. Avoidance between intact individuals and those with fixed HS joints was not significantly different, yet was different between HS joint fixed and SP joint fixed cockroaches (*n* = 39, $P < 0.05$, represented by different lowercase letters). Together the results indicate that S-HP mechanoreceptors may not contribute solely to avoiding higher strength electric fields, and the flagellum may play a role.

4kV

Raising the electric field strength resulted overall in increased avoidance (Fig. 3.23). Cockroaches significantly avoided the treated chamber regardless of the modification made ($P < 0.05$ in all cases). There were also no significant differences in avoidance between the joint fixed or when antennae were removed. This therefore supports the suggestion that the flagellum may play a role in static electric field detection. Given the avoidance elicited when no antennae are present, these findings also indicate that receptors aside from those on the antennae could contribute to static electric field detection at high field strengths.

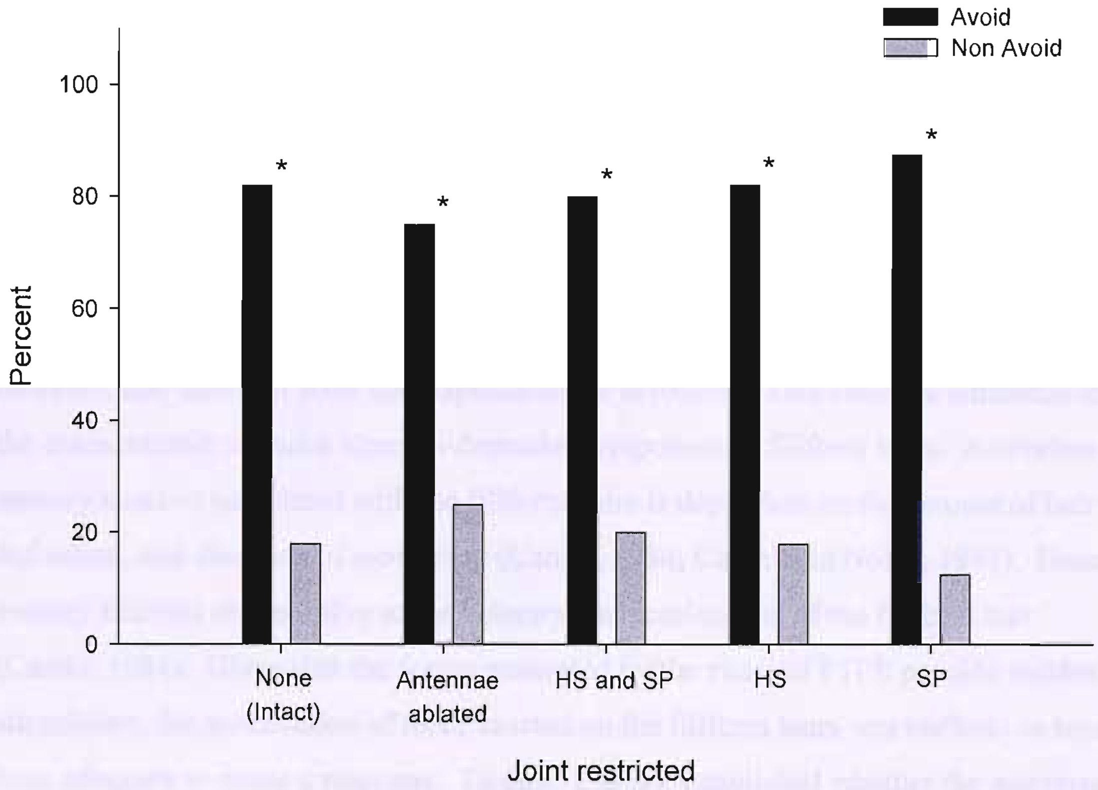


Figure 3.23 The effect of modifying mechanoreceptor input on the avoidance of electric fields at 4kV. Intact cockroaches and cockroaches with any modification of antennal sensory input significantly avoided the treated pathway ($P < 0.05$ in all cases, indicated by the asterisks). There were no significant differences in avoidance behaviour between the type of surgery carried out on cockroaches, indicating that the flagellum and, or, receptors not present on the antennae, play a role in static electric field detection.

3.4 Discussion

3.4.1 The detection of friction charged PTFE

Using a combination of field modelling and high speed video this study has quantified, and presented for the first time, the influences of electrostatic forces on cockroaches and their sensory structures. Although none of the structures examined could be identified as being uniquely responsible for the detection of friction charged surfaces, the results do indicate that stimulation of mechanoreceptors is fundamental.

One charged pathway

Friction charged PTFE exerted directional forces on the cerci, antennae, palps, and the cockroach itself. The magnitudes of these forces were dependent on their proximity to the charged surface. When a charged PTFE section was approached, high speed video illustrated the aberrant antennal postures elicited by the forces acting upon the antennae. The forces exerted were only two orders of magnitude greater than those on the cerci, hence the cercal filiform hairs may have been deflected. The ablation studies indicated, however, that the cerci were not responsible for detection. This could be attributed to the characteristic stimulus strength-dependent responses of filiform hairs. Activation of sensory neurons associated with the filiform hairs is dependent on the amount of hair deflection, and direction of movement (Camhi, 1984; Camhi and Nolen, 1981). Hence, sensory neurons are sensitive to the velocity and acceleration of the filiform hair (Camhi, 1984). Given that the forces generated by the charged PTFE provide sudden stimulation, the acceleration of force exerted on the filiform hairs was unlikely to have been adequate to cause a response. To date, it is not established whether the antennae exhibit such stimulus strength-dependent responses, although activation of specific antennal mechanoreceptor hairs is positively correlated with angle of hair deflection (Burdohan and Comer, 1996). Hence, this may also contribute to explaining why the antennae appeared not to play a role in the detection of friction charged surfaces.

The consistent avoidance exhibited by cockroaches may, however, have been due to sensory structures other than those examined. Friction charged PTFE was calculated to generate forces that acted on the whole body of the cockroach, essentially attracting it towards the treated pathway. Such forces may have exerted passive changes in body position that could have been perceived by mechanoreceptors on and within the legs.

Exteroceptive hairs cover the legs of cockroaches (Bell and Adiyodi, 1981) and other insect species, notably the locust (Newland and Burrows, 1997). Stimulation of these hairs, either individually or collectively during leg joint movement, can evoke an avoidance reflex (Newland and Burrows, 1997; Ridgel *et al.*, 2000). These sense organs, in addition to other exteroceptors, and proprioceptors within the leg, are known to monitor limb position and contribute to avoidance responses (Ebeling and Durr, 2006; Zill *et al.*, 2004). Additionally, mechanosensory hairs located between the neck and thorax, characteristic of many insects, regulate locomotion and flight (Bräunig *et al.*, 1983; Paulk and Gilbert, 2006). Together, these supplementary sense organs and the behaviours they mediate could therefore present an alternative means by which, in the event of stimulation by electrical forces, cockroaches avoid friction charged surfaces.

Two charged pathways

The consistency in avoidance between cockroaches with either the cerci, antennae, or no structures removed reiterates that the structures investigated may not be responsible for detecting friction charged surfaces. Nevertheless, the increase in avoidance when combinations of structures were removed demonstrates that these individuals exhibited a greater tendency to turn back down the central pathway. Given the multi-modal characteristics of many insect behaviours, notably the cockroach escape response, removing sensory input from multiple sets of sensory structures could heighten the sensitivity of other sensory organs (Comer *et al.*, 1994; Dickinson *et al.*, 2000; Ritzmann *et al.*, 1991). Hence, the increased avoidance could be explained by raised sensitivity to external stimuli due to elimination of multiple sensory inputs.

3.4.2 The detection of static electric fields

Cockroaches approaching a static electric field are subject to considerable electrical forces, indicated in this study by force calculations. Notably, as illustrated by high speed video observations, antennal position is influenced by these forces. The bioassays carried out here demonstrate, for the first time, that these influences are detected primarily by S-HP exteroceptors, ultimately mediating avoidance.

The involvement of the S-HPs in the detection of static electric fields supports current knowledge of their function on antennae. Cockroaches use antennae as multi-modal sensors to both passively and actively detect external environmental cues (Bell and Adiyodi, 1981). Active detection of objects, for example, involves stimulation of both extero- and proprioceptors, and similar properties apply to passive detection of high intensity stimuli (Okada and Toh, 2006). Displacement of the antennae by such stimuli in turn causes movement of the HS joint, deflecting S-HP sensilla during contact with the joint membrane (Okada and Toh, 2000). There are three S-HPs on adult cockroach antennae, located on the dorsal, medial and lateral surfaces (Staudacher *et al.*, 2005). The morphology of the S-HPs on nymph cockroach antennae, illustrated for the first time in this study, showed S-HPs present on all four surfaces. Regardless of these differences, the locations of the S-HP on both adult and nymph antennae enable a representation of antennal position in all directions (Okada and Toh, 2001). Hence, it is the displacement of the antennae by electrical forces that may be perceived by S-HP mechanoreceptors, enabling in the detection of static electric fields.

The perception of antennal movement and position by the S-HPs mediates a variety of behaviours, not only amongst cockroaches but a number of insects including locusts (Gewecke, 1974), stick insects (Durr *et al.*, 2001), bees (Kloppenburg, 1995) and crickets (Staudacher *et al.*, 2005). Such behaviours include locomotion and flight, antennal avoidance reflexes, and object orientation (Gewecke, 1974; Okada and Toh, 2000; Sherman and Dickinson, 2004; Staudacher *et al.*, 2005). Notably the basal joints play an important role in triggering cockroach escape away from the direction of an antennal stimulus (Comer *et al.*, 1994; Ye *et al.*, 2003). Together, the influences of electric fields on antennae, and the behaviour mediated by S-HPs may therefore explain the avoidance evoked by cockroaches confronted with static electric fields.

The S-HPs may not, however, have been responsible for static electric field detection when the voltage potential was raised, as preventing S-HP mechanoreceptor stimulation did not eliminate avoidance at 2kV. Information on antennal position is not solely provided by the S-HPs; input from the P-HPs and flagellae also contribute (Okada and Toh, 2000; Okada and Toh, 2006; Staudacher *et al.*, 2005). The SP joint moves only in the vertical plane, detected by the ventral and dorsal P-HPs (Okada and Toh, 2000; Toh, 1981). Although the S-HPs enable perception of antennal position in all directions, the P-HPs can provide additional information on vertical location and supplement input from the S-HPs (Okada and Toh, 2001; Staudacher *et al.*, 2005). Mechanoreceptors located on and within the flagellar segments are also reported be sensitive to displacement of the antennae (Comer *et al.*, 2003; Okada and Toh, 2006; Toh, 1977). Activation of proprio- and exteroceptors on the flagellum itself are reported to play a part in mediating escape, and other locomotor activities, in response to antennal stimulation (Camhi and Johnson, 1999; Comer *et al.*, 2003; Cowan *et al.*, 2006; Okada and Toh, 2006). Hence, sensory input from the pedicel and flagellum could be responsible for detecting the increased forces acting on the antennae at higher field strengths.

Mechanoreceptors isolated to the antennae were unlikely, however, to detect field strengths greater than those generated by 2kV potentials in the current study, given the avoidance when antennal sensory input was completely eliminated. In accordance with the possible detection of friction charged PTFE, electrical forces generated by 4kV potentials may act on the whole body causing changes in body posture. Such influences may increase load upon the legs that can be detected by campaniform sensilla located on the cuticle, and receptors associated with muscle tendons (adopemes) within the legs (Zill *et al.*, 2004). Stimulation of these receptors contribute not only to the coordination of insect locomotion (Ridgel *et al.*, 2000; Zill *et al.*, 2004), but also initiate excitatory responses mediating reflex behaviours, reported specifically in cockroaches (Noah *et al.*, 2001). These sensory abilities therefore indicate why avoidance is sustained when antennal sensory input is abolished.

For the first time, the findings presented here have shown that by influencing antennal position, the forces associated with static electric fields are detected primarily by the S-HPs. Hence, in accordance with current knowledge, S-HP mechanoreceptors could be fundamental in evoking avoidance of static electric fields. Exerting greater forces on cockroaches by raising the field strength may stimulate supplementary receptors responsible for perceiving body posture, ultimately sustaining the avoidance of static electric fields.

4. THE NEURAL BASIS OF ELECTRIC FIELD AVOIDANCE

4.1 Introduction

Current knowledge on how insects respond to electric fields is limited. No studies to date have investigated the neurological processes responsible for eliciting the behavioural changes of insects confronted by electric fields. Although electric fields have not before been considered as an external stimulus that insects can detect, the neuroethology of insects in response to many other stimuli, such as wind, touch and chemicals, is well established. Further understanding of the neural basis of static electric field avoidance could therefore be acquired based on what is known about other systems.

Escape behaviour in response to wind is common in many invertebrates including crickets (Gras and Horner, 1992), aphids (Braendle and Weisser, 2001), crayfish (Wine and Krasne, 1972) and cockroaches (reviewed in Camhi, 1984). The avoidance behaviour elicited by cockroaches to static electric fields could be considered similar to that of the turn-and-run escape response. Extensive studies of these invertebrates have revealed two pathways believed to be responsible for mediating the escape response: the ascending and descending mechanosensory pathways.

The ascending mechanosensory pathway originates from sensory neurons innervating fine filiform hairs on the cerci. Air movement deflects the filiform hairs causing conformational changes in the sensory neuron dendrites, thereby the neuron (Camhi, 1984). Sensory neurons synapse with a group of bilaterally paired giant interneurons (GIs) within the terminal abdominal ganglion, and activation of the sensory neurons excites the GIs (Westin *et al.*, 1977). The GIs run along the ventral nerve cord (VNC) from the terminal abdominal ganglion to the head, traversing through the thoracic ganglia (Burdohan and Comer, 1990; Camhi, 1984; Comer *et al.*, 1988). Within the thoracic ganglia specific thoracic local interneurons, type A thoracic interneurons (TI_As), receive input from the GIs, specifically the ventral GIs (vGIs). The TI_As make synaptic connections with motor neurons that evoke contraction leading to escape (Camhi, 1984; Ritzmann *et al.*, 1991).

The escape response is under multi-modal control in a variety of invertebrates, and believed not only to be mediated by the ascending pathway (Ritzmann *et al.*, 1991; Staudacher *et al.*, 2005). This is reported to be apparent in locusts (Bicker and Pearson, 1983), crayfish (Wine and Krasne, 1972), and alternative pathways to the GIs (non-GI pathways) are now thought to mediate the cockroach escape response (reviewed in Comer and Robertson, 2001; Staudacher *et al.*, 2005).

Cockroaches can also evoke an escape response to tactile stimulation of the antennae or antennal stimulation by high velocity air movement (Comer *et al.*, 1994; Stierle *et al.*, 1994). The descending neural pathway responsible for mediating this behaviour is composed of two bilateral pairs of interneurons, the descending mechanosensory interneurons (DMIs), originating in the proto- and deutocerebrum. Within these regions the DMIs receive input from basal antennal mechanoreceptors (Burdohan and Comer, 1996). The DMIs could therefore receive and mediate information from the scape and pedicel joints, both known to play an important role in the escape response (Comer *et al.*, 2003). The DMIs pass down the VNC (Comer and Robertson, 2001), have axonal arborisations in the thoracic ganglia and are in turn associated with the leg motor circuitry (Burdohan and Comer, 1996; Schaefer and Ritzmann, 2001). The precise pathway taken to reach the leg motor neurons has not yet been established, although the DMIs are reported to converge with the same TI_As that receive input from the ascending vGIs (Ritzmann and Pollack, 1994). The connections between the TI_As and leg motor neurons as well as local interneurons subsequently enable the initiation of escape behaviour (Ritzmann and Pollack, 1998; Schaefer and Ritzmann, 2001; Ye and Comer, 1996). The neural pathway described here may therefore be activated by the influences of static electric fields on cockroach antennae described in Chapter 3.

Cockroach escape behaviour is known to be dependent upon stimulus intensity. Essentially, increased passive antennal deflection is correlated with raised neural activity and behavioural responsiveness (Burdohan and Comer, 1996; Okada and Toh, 2001; Stierle *et al.*, 1994). Avoidance of electric fields was also shown to be dependent on the field strength and electrical forces experienced by cockroaches (see Chapter 2). Hence, studying the neural basis of avoidance could contribute to explaining why this association between field strength and avoidance occurred.

The motor output of a variety of insects is known to be under multisensory control (Mizunami, 1995a; Ritzmann *et al.*, 1991; Ye *et al.*, 2003). Notably, visual input and light level contribute to mediating behaviour in a variety of insects, for example, *Drosophila* and locust flight is stabilised by light input, in combination with mechanosensory feedback (Sherman and Dickinson, 2004; Simmons, 2002). Light intensity is also known to influence insect responses to external stimuli, such as a diminished sensitivity of locusts to crosswind during flight under low light levels (Riley *et al.*, 1988). Male gypsy moths, *Lymantria dispar*, also exhibit a reduction in flight speed and narrower search patterns in response to sex pheromone plumes under red light compared to white light (Cardé and Knols, 2000). Cockroaches are also highly sensitive to light intensity, and cessation of the escape response has been reported when cockroaches reach regions of low light (Okada and Toh, 1998). In addition, covering the eyes of adult cockroaches and applying a tactile stimulus to the antennae does not elicit a behavioural response, contrary to when eyes are uncovered (Ye *et al.*, 2003). These influences of light and visual input on behaviour are mediated by neural pathways from a combination of inputs, including the compound eyes and ocelli, that are ultimately associated with the thoracic ganglia (Mizunami, 1995a). Ritzmann (1991) reported that the TI_As are depolarised by a sudden increase in light, indicating that such a stimulus could evoke in itself an escape response. Repeated stimulation, however, ultimately results in no TI_A activity, inferring that light sensitive cells upstream of the TI_As habituate to continuous light stimulation (Ritzmann *et al.*, 1991). Current knowledge therefore indicates that motor activity is influenced by light intensity, and a decrease in static electric field avoidance as light intensity was increased was also exhibited in Chapter 2. This trend could be attributed to the influence of light level on neural pathways, and will be addressed in this Chapter.

To begin to understand the neural basis of static electric field avoidance, it is necessary to utilise extracellular electrophysiology. However, it is not possible to carry out electrophysiology in an environment containing high strength electric fields due to excessive electrical noise. An alternative method of stimulation utilising magnetic fields, based on a technique pioneered by Takahata *et al.* (1992), was implemented using equipment and expertise at the University of Hokkaido, Japan. Using this methodology it was possible to combine antennal stimulation similar to that of static electric fields with electrophysiology, providing further information on the role of the antennae and associated basal mechanoreceptors in the avoidance of static electric fields.

4.2 Materials and Methods

Cockroach colonies were maintained at the Graduate School of Science, University of Hokkaido, Japan, and at the School of Biological Sciences, University of Southampton regulated at 28°C and 30% relative humidity, 12L:12D light regime (8:00 on:20:00 off GMT+8hr). Egg cases and young nymphs were regularly isolated for instar determination.

4.2.1 Stimulation of the antennae

The antennae of nymph and adult cockroaches were stimulated using magnetic fields, produced by passing current through the energising coil of an electromagnet (180mm x 90mm, L x D), tapered at the end to localise field distribution. When fine iron powder was applied, the antennae could be stimulated by the magnetic field causing antennal movement. Preliminary experiments showed stimulation of nymph antennae was characterised by deflection and bending, whereas adult antennae primarily exhibited deflection. Stimulus strength was regulated by altering the voltage supplied to the magnet, and the differences in antennal movement using magnetic and electric fields were compared at different voltage potentials for both nymph and adults.

4.2.1.1 Stimulation of nymph antennae

Magnetic

3rd and 4th instar cockroaches (*n* = 5, 8.3mm ± 0.67) were restrained in Plasticine and positioned 20mm from the tip of the electromagnet (Fig. 4.1A). Spherical iron powder (< 10μm diameter, Alpha Aesar Ltd, Germany) was applied to each antenna (right antenna, *n* = 4, 0.0029g ± 8x10-4; left antenna, *n* = 4, 0.0025g ± 3x10-4) using a fine paint brush. Antennae were stimulated five times for 5s duration with 15s between each stimulus at 20V, 25V, 30V and 35V (termed ‘magnetic potentials’), controlled by an electronic stimulator (Nihon-Kohden SEN-3301). Experiments were video recorded (Sony DCR-TRV9) and individual frames digitised (Apple PowerBook G5) for further analysis.

Electric

The antennae of restrained 3rd and 4th instar cockroaches (*n* = 4, 8.5mm ± 0.62) were stimulated by static electric fields emitted from an aluminium electrode (30 x 7mm, D x W). The electrode was connected to a high voltage power supply (Brandenburg Alpha III), and the antennae exposed to electric fields generated at 1kV, 2kV, 3kV and 4kV (hereafter referred to as 'electric potentials'). Experiments were video recorded (Sanyo VCB-3372P) onto DVD (Panasonic DMR-E55EB).

Still frame images were captured from the video recordings (iMovie for Apple Macintosh, Version 4.1) and three parameters of antennal movement measured: the angle of the bend; the length to the bend; and the length of the bend (Fig. 4.1B, CorelDraw for Windows, Version 11 using CurveLength Calculator plug-in tool [www.clifton89.freeserve.co.uk]).

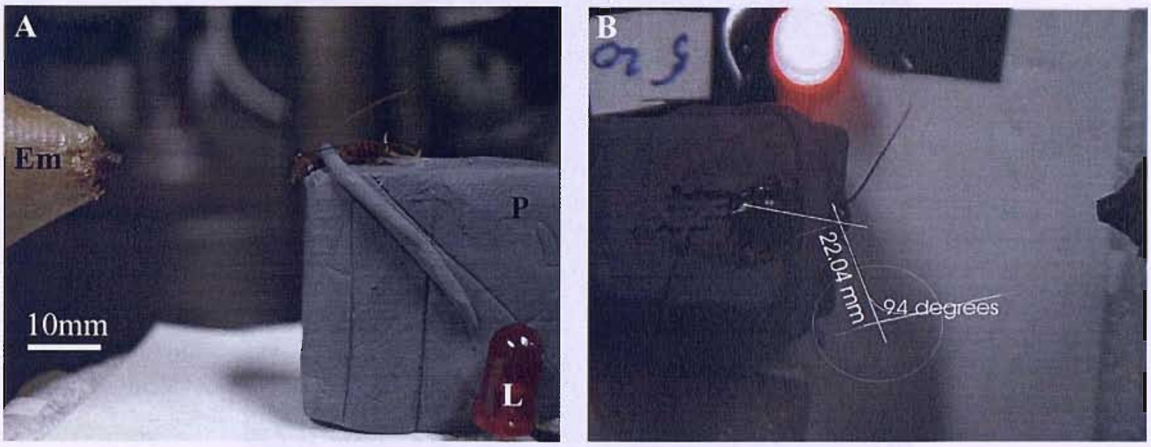


Figure 4.1 Photographs of the setup used to stimulate nymph cockroach antennae using magnetic fields. (A) Cockroaches were restrained in Plasticine (P), positioned 20mm opposite the tapered end of the electromagnet (Em) and maintained at the same height and longitudinal position. A single light emitting diode (L) signalled the onset and offset of the stimulus to assist video analysis. (B) Antennal movement was analysed by superimposing measurement lines on an antenna to measure the angle of bend, length to the bend and length of bend.

The effects of stimulation on each antennal movement parameter were compared between field types as the potentials were altered using a repeated measures General Linear Model (GLM). Potentials were paired (20V and 1kV, 25V and 2kV etc) and categorised into low, medium low, medium and high potentials. Preceding analyses, data assumptions were tested for normality using Kolmogorov-Smirnov Tests and homogeneity of variance was tested using Levene's Tests (SPSS for Windows, Version

14). Length to bend and length of bend data did not meet assumptions, therefore Box Cox transformations were carried out (Minitab for Windows, Version 12) to meet assumptions (Sokal and Rohlf, 1995). Data were considered significant at the $P < 0.05$ level.

4.2.1.2 Stimulation of adult antennae

Magnetic

Magnetic field stimulation of restrained adult antennae (Fig. 4.2) was carried out after one antenna was coated in iron powder ($n = 5$, 0.0153g ± 4×10^{-4}) using the same procedure as used with nymph cockroaches (4.2.1.1).

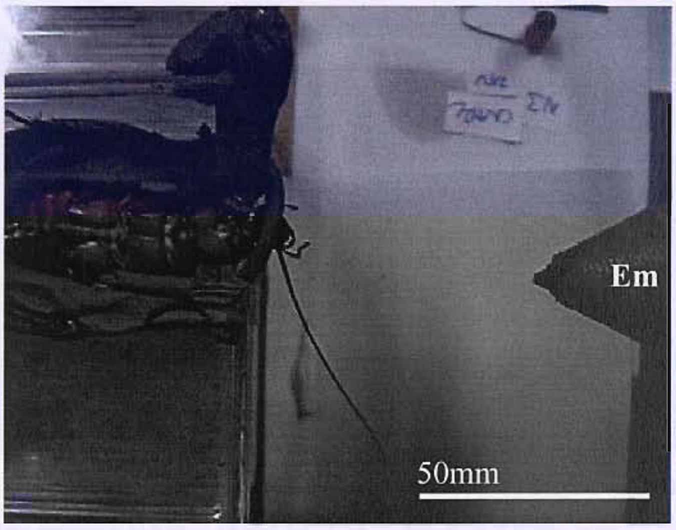


Figure 4.2 Photograph of the setup used to stimulate an antenna of a restrained adult cockroach using magnetic fields. The head of each individual was positioned 50mm opposite, and 10mm to one side of the electromagnet (Em) tip.

Electric

Adult cockroach antennae ($n = 5$) were also stimulated by static electric fields using restrained individuals positioned 50mm opposite a circular aluminium electrode (30 x 7mm, D x W). An identical earthed electrode was positioned posteriorly, and the anterior electrode was connected to a high voltage power supply (Brandenburg Alpha III). Antennae were stimulated using the same method given in 4.2.1.1 and at four potentials: 3kV, 4kV, 5kV and 6kV. All experiments were video recorded (Sanyo VCB-3372P) onto DVD (Panasonic DMR-E55EB).

After capturing still images, the angle of the proximal region of the flagellum position relative to the electromagnet was measured immediately before and 320ms after stimulus onset (Fig. 4.3, CorelDraw for Windows, Version 11). The difference in angle, hereafter termed 'antennal deflection', was measured once for every stimulus at each potential. Magnetic and electric potentials were grouped into potential pairs (20V and 3kV, 25V and 4kV etc). Differences in the mean antennal deflection for all individuals between field types at each potential pair were analysed using *t*-Tests (SPSS for Windows, Version 14) after assumptions of normal distributions and homogeneity of variances were met (Dytham, 2002). Differences in antennal deflection as the potential was varied were tested for each field type using regression analysis (Minitab for Windows, Version 12). A *t*-Test compared regression coefficients between the field types (Fowler *et al.*, 1998). Data were considered significant at the $P < 0.05$ level.

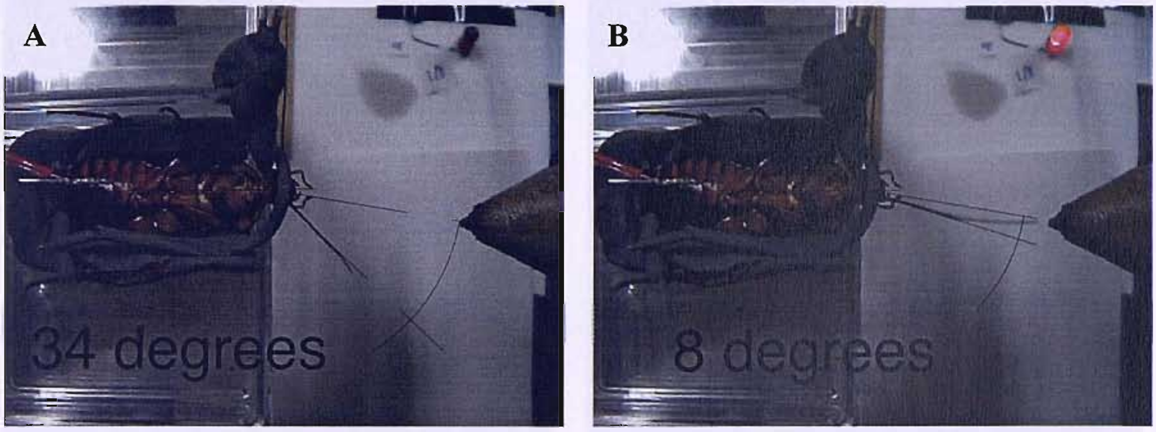


Figure 4.3 Measurement of adult antennal deflection (A) before and (B) after the magnetic field was applied. The angle of an antenna relative to the midline of the insect body was measured and the difference between the two angles was calculated to give the antennal deflection.

4.2.2 Extracellular VNC electrophysiology

4.2.2.1 Preparation of the VNC

The DMIs are bundled in the VNC (Burdohan and Comer, 1996) and can be readily accessed and recorded extracellularly from the neck connectives between the subesophageal ganglion and the prothoracic ganglion (Burdohan and Comer, 1996; Comer *et al.*, 2003). To reveal the neck connectives a small incision was made along the ventral edge of the neck using a micro-scalpel (Fig. 4.4A) and the remaining soft cuticle removed with fine iredectomy scissors. A single connective was isolated from the surrounding tissue and placed onto a bipolar hook electrode (125μm silver, teflon insulated except at the tips) using a dissecting hook (Fig. 4.4B).

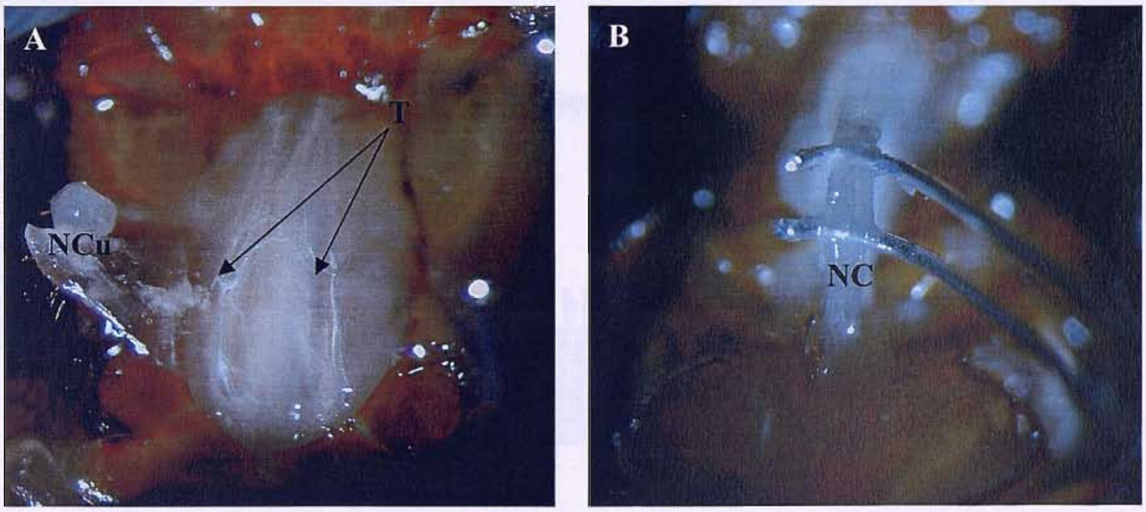


Figure 4.4 Photographs of VNC preparation. (A) The ventral neck cuticle (NCu) was incised and lifted to expose the neck connectives positioned beneath muscle tissue and the trachea (T). (B) After isolating one neck connective (NC), a bipolar hook electrode was positioned beneath the connective.

4.2.2.2 Extracellular VNC recordings

The DMIs receive the strongest input from stimulation of the antenna contralateral to the connective in which its axon is located (Burdohan and Comer, 1996; Ye and Comer, 1996). The antenna contralateral to the isolated neck connective was therefore coated in iron powder (0.0197g ± $4x10^{-4}$, $n = 5$) and positioned opposite the electromagnet as in 4.2.1.2 (Fig. 4.2). An earthed wire was inserted into the abdomen and petroleum jelly applied around the hook electrode to insulate the electrode and optimise recordings.

Electrical signals were amplified (Nihon-Kohden MEG-1100), displayed on an oscilloscope (Tektronix 5111A), and digitally recorded (Chart for Apple Macintosh, Version 5) during stimulation. The antenna of each individual was stimulated five times (5s duration) with a 15s rest period between each stimulus at 20V ('low') and 35V ('high') magnetic potentials. All experiments were video recorded (Sony DCR-TRV9) and carried out between 9:00 and 21:00 in a climate controlled laboratory at 20°C.

Antennal deflection commonly did not occur until the electromagnet reached full strength due to a 250ms transient increase in current (Fig. 4.5). DMI activity is characterised by large amplitude spike impulses that occur within 70ms of antennal deflection (Burdohan and Comer, 1996; Ye and Comer, 1996). Given the time taken for maximum field strength to be reached and antennal deflection to occur, recordings were analysed during a time window 320ms before and after stimulus onset.

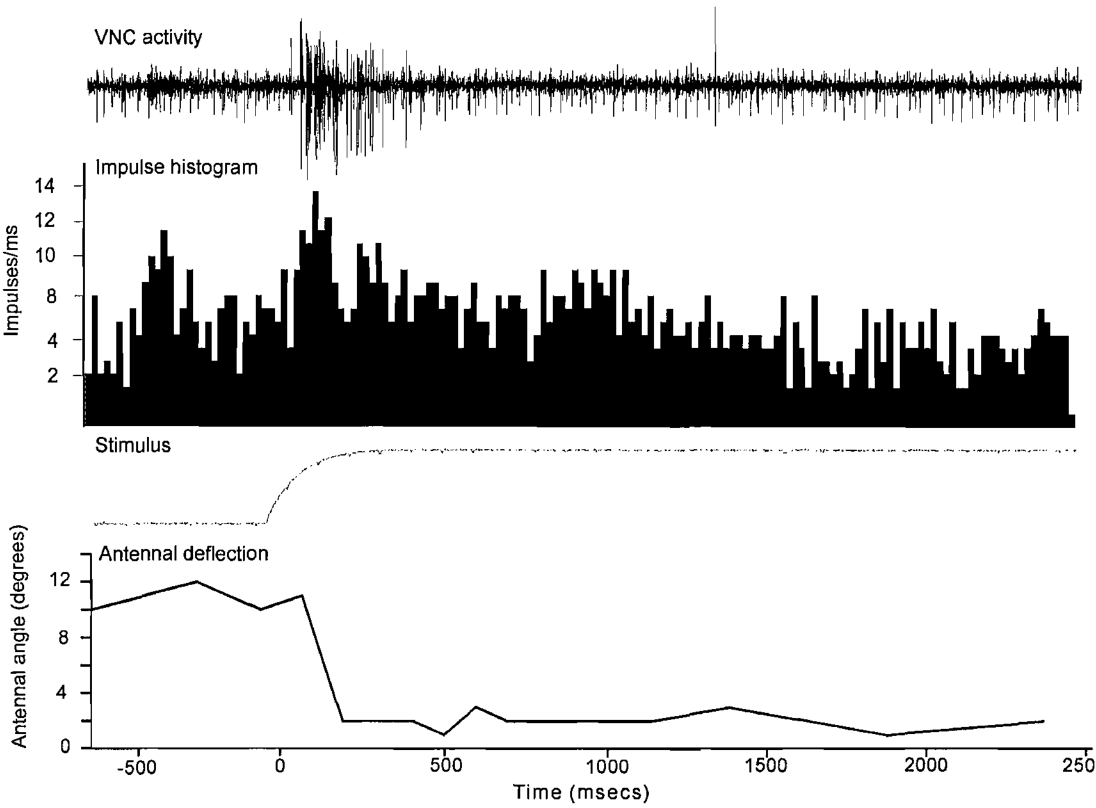


Figure 4.5 Antennal movement and associated VNC activity in cockroaches with coated antennae stimulated by magnetic fields. Impulse frequency increased after the stimulus onset as an antenna was deflected within 320ms after the stimulus onset. The delay in deflection can be explained by the time taken for the stimulus to reach full strength, as indicated by current strength (stimulus), and VNC activity did not increase until deflection occurred.

After determining the optimum noise threshold levels using a spike determinator (Spike Histogram for Chart 5), the large amplitude impulses evoked during the time window were analysed to measure DMI activity (Chart for Macintosh, Version 5.3, ADI Instruments). Neural activity from extracellular recordings of the VNC will hereafter be termed 'VNC activity'.

4.2.2.3 Prevention of S-HP stimulation

Stimulation of S-HP mechanoreceptors was prevented by restricting antennal movement at the HS joint. After anaesthetising and restraining an individual in Plasticine (n = 5), rigid adhesive (VetBond®, WPI, Stevenage, UK) was applied to the HS joint using a fine microcapillary (Borosilicate standard wall, Outside Diameter = 1mm, Inside Diameter = 0.58mm, L = 10mm, Warner instruments Inc, USA) held in a maipulator (WPI, Stevenage, UK). Blowing into an open-ended 5ml syringe connected to the manipulator enabled controlled adhesive application. Cyanoacrylate adhesive accelerator (RS Components, UK) was applied using a microcapillary to aid setting. After a short recovery period, VNC activity was recorded as in 4.2.2.2.

4.2.2.4 The effect of light on VNC activity

VNC activity during antennal deflection was investigated under different light conditions, white and red. Cockroaches ($n = 7$) were prepared for electrophysiology and an antenna was stimulated five times using a 35V magnetic potential, as in 4.2.2.2. After stimulation under white light, laboratory lights were turned off and a 25W desk lamp covered with a far red filter (Campbell Environmental Products, UK) was turned on. The antenna was stimulated a further five times and VNC activity recorded.

4.2.2.5 Analysis

Before analyses were made, Kolmogorov-Smirnov and Levene's Tests were used to test for normal distribution and equal variance of data, respectively. For each individual, the first stimulus to elicit deflection was chosen for analysis, as occasionally stimulation did not cause deflection. Differences in the mean impulse number before and after stimulus onset were tested at each magnetic potential using paired *t*-Tests. The mean angle of antennal deflection during the selected recordings was also analysed using paired *t*-Tests. This analysis was repeated for individuals with the HS joint fixed.

The effect of light condition on VNC activity was analysed by performing a paired *t*-Test on the difference in mean impulse number before and after stimulus onset between red and white light.

All analyses were carried out using SPSS for Windows, Version 14, and data were considered significant at the $P < 0.05$ level.

4.2.3 Behavioural bioassays

4.2.3.1 Japan and UK electromagnets

Behavioural bioassays were carried out in the UK, therefore electromagnets similar to the one used in Japan were constructed (School of Biological Sciences workshop, University of Southampton). Two electromagnets were manufactured, each composed of a bakelite bobbin (72mm x 40mm, L x D) around which insulated copper wire (D = 0.7mm) was wrapped to produce an energising coil 30mm in diameter. A soft iron core (110mm x 7mm, L x D) was inserted through the bobbin and held in place using a rubber O-ring. Two terminals leading from the energising coil could be connected to a low voltage power supply.

Nymph cockroach antennal movement elicited by the UK electromagnets was analysed using restrained 3rd and 4th instar cockroaches ($n = 7$, 8mm ± 0.3) with iron particles applied to the antennae (right antenna, $n = 7$, $0.0026\text{g} \pm 3\text{x}10^{-4}$; left antenna, $n = 7$, $0.0027\text{g} \pm 4\text{x}10^{-4}$). The head was positioned 20mm opposite the distal end of the electromagnet, and antennae were stimulated at 8V, 10V, 15V and 20V potentials five times for 5s duration at each potential, with 15s between each stimulus. Experiments were video recorded using a digital camera (Sanyo VCB-3372P) onto DVD (Panasonic DMR-E55EB). Images were captured and the same parameters as in 4.2.1.1 were measured.

4.2.3.2 Bioassays

The behavioural responses of 3rd and 4th instar nymph cockroaches ($n = 60$, 7mm ± 1) to magnetic fields were investigated using a Y-tube apparatus with an electromagnet held in each anterior pathway (Fig. 4.6). Responses to a combination of four conditions were investigated: after iron particle application onto the antennae ($n = 42$, right antenna 0.0025g ± $3\text{x}10^{-4}$; left antenna 0.0024g ± $4\text{x}10^{-4}$); no iron particle application; exposed to a magnetic field in one pathway; and not exposed to a magnetic field. The direction taken by each individual (right or left, avoid or non-avoid) was noted, in addition to the time taken to pass the electromagnet. The treated pathway was alternated after each trial and the apparatus was washed after ten trials to prevent following effects.

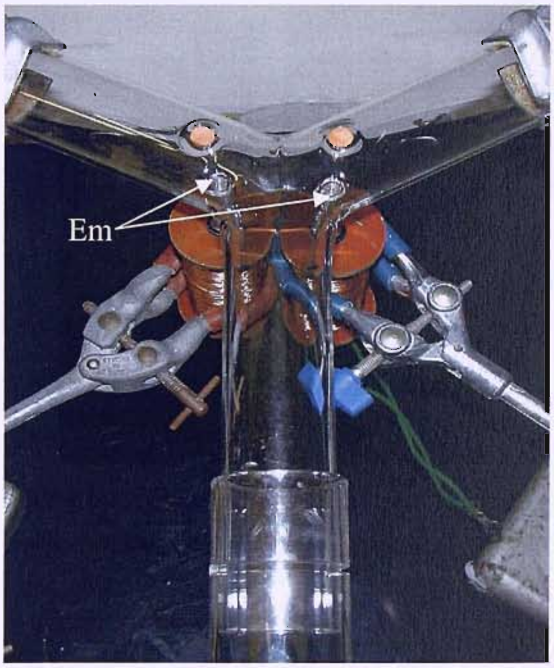


Figure 4.6 Photograph of the electromagnet Y-tube apparatus. The distal end of an electromagnet (Em) was positioned at the entrance to both the anterior pathways.

4.2.3.3 Analysis

To test for the effects of the magnetic field on antennal displacement using the electromagnet constructed in the UK, the same analysis as 4.2.1.1 was carried out.

Avoidance was analysed using Binomial Tests of Proportions with 50:50 as the expected ratio. The mean difference in the decision time (time to pass the electromagnet) of cockroaches exposed and not exposed to magnetic fields was tested using Mann-Whitney U Tests. Differences in the mean decision time of cockroaches exposed to magnetic fields were compared with the time taken for cockroaches to make a decision when confronted with electric fields. Data were analysed using Mann-Whitney U Tests and compared to data from previous video recordings taken from Chapter 2. The mean time for cockroaches to pass the electromagnet was compared between treatments (after iron particle application onto the antennae; no iron particle application; exposed and not exposed to magnetic fields) using Mann-Whitney U Tests (SPSS for Windows, Version 14). Data were considered significant at the $P < 0.05$ level.

4.3 Results

4.3.1 Stimulation of the antennae

4.3.1.1 Stimulation of nymph antennae

The antennal movement caused by antennal stimulation using magnetic fields was examined by investigating three movement parameters and comparisons with electric field stimulation were made.

Similar trends in antennal movement occurred between the field types at the selected potentials. Antennal bend became more acute as both the magnetic and electric potentials were increased (Fig. 4.7A), and there was no significant difference between the field types ($F_{3, 199} = 1.43$, $P > 0.05$). The length to the antennal bend or the length of the bend did not significantly differ between the field types as the potentials were raised (Fig. 4.7B, $F_{3, 199} = 0.19$, $P > 0.05$; Fig. 4.7C, $F_{3, 199} = 0.72$, $P > 0.05$ respectively). Disregarding the effect of potential, the angle of antennal bend did not differ between the field types ($F_{1, 199} = 0.04$ $P > 0.05$), although magnetic fields did evoke greater lengths to the bend and of the bend ($F_{1, 199} = 18.82$, $P < 0.05$; $F_{1, 199} = 16.62$, $P < 0.05$ respectively). This may be due in part to the differences in the field distribution of the two field types, given the source shapes were dissimilar.

Both field types therefore had similar effects on antennal movement, suggesting that magnetic field stimulation represents a suitable method to displace the antennae to mimic the effects of static electric fields on cockroach antennae.

4.3.1.2 Stimulation of adult antennae

Magnetic field stimulation without iron particles had no effect on the antennae ($F_{1, 15} = 2.29$, $P > 0.05$). Static electric field stimulation did not require antennae to be coated in iron powder application. Control tests with iron particles showed no significant difference in antennal deflection compared to uncoated antennae at a 5kV potential ($F_{1, 13} = 1.8$, $P > 0.05$). Iron particle application therefore had no effect on the movement of antennae exposed to static electric fields.

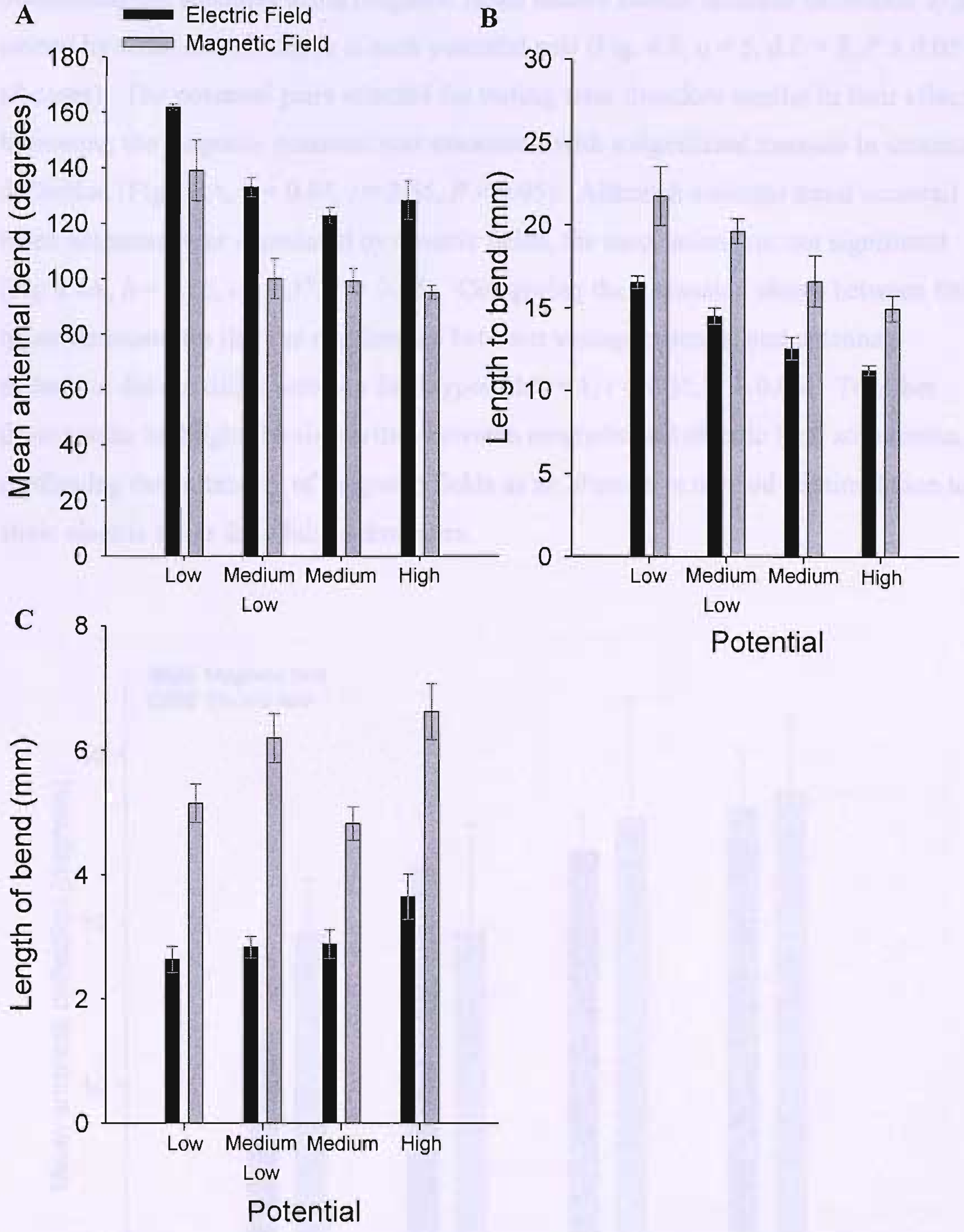


Figure 4.7 The effect of magnetic and electric fields on the mean angle of antennal bend (±SEM), the mean length to the bend (±SEM) and the mean length of the bend (±SEM) as the potentials were varied. (A) Mean antennal bend decreased as the selected magnetic and electric potentials were raised, and no significant differences between the field types occurred as the potential was altered ($F_{3,\ 199} = 1.43$, $P > 0.05$). (B) The mean length to the bend also decreased as the voltage was increased, and both field types equally affected the length to the bend ($F_{3,\ 199} = 0.19$, $P > 0.05$). (C) Effects on the length of the bend were not so pronounced, but similarities also existed between the field types at different potentials ($F_{3,\ 199} = 0.72$, $P > 0.05$). Omitting the effect of potential, magnetic fields evoked a greater length to the bend and bend length than electric fields ($F_{1,\ 199} = 18.82$, $P < 0.05$; $F_{1,\ 199} = 16.62$, $P < 0.05$ respectively).

Stimulating the antennae using magnetic fields caused similar antennal deflection to that caused by static electric fields at each potential pair (Fig. 4.8, $n = 5$, d.f. = 8, $P > 0.05$ in all cases). The potential pairs selected for testing were therefore similar in their effects. Increasing the magnetic potential was associated with a significant increase in antennal deflection (Fig 4.9A, $b = 0.84$, $t = 2.55$, $P < 0.05$). Although a similar trend occurred when antennae were stimulated by electric fields, the association was not significant (Fig 4.9A, $b = 3.26$, $t = 1.37$, $P > 0.05$). Comparing the regression slopes between field types demonstrates that the relationship between voltage potential and antennal deflection did not differ between field types (d.f. = 1, $t = 0.35$, $P > 0.05$). Together these results highlight the similarities between magnetic and electric field stimulation, confirming the suitability of magnetic fields as an alternative method of stimulation to static electric fields for adult cockroaches.

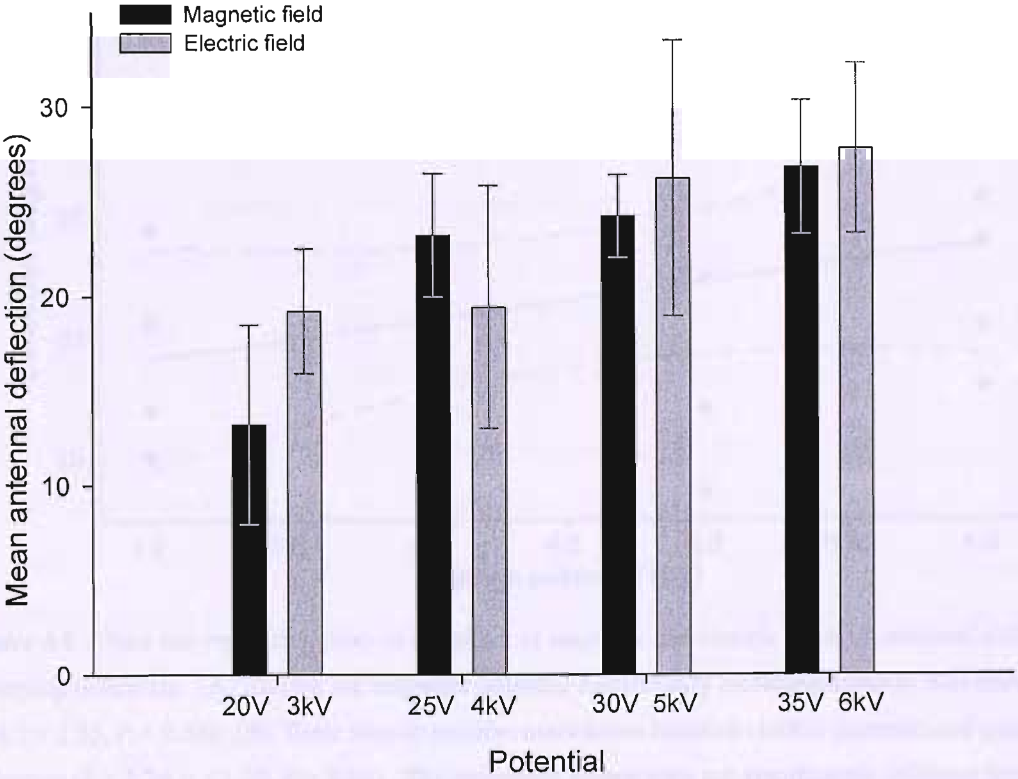


Figure 4.8 Mean deflection (±SEM) of a coated adult antenna exposed to magnetic and electric fields at different potentials. Increasing the electric or magnetic field strength caused greater antennal deflection. No significant differences occurred between each field type for a given pair of potentials ($n = 5$, d.f = 8, $P > 0.05$ in all cases).

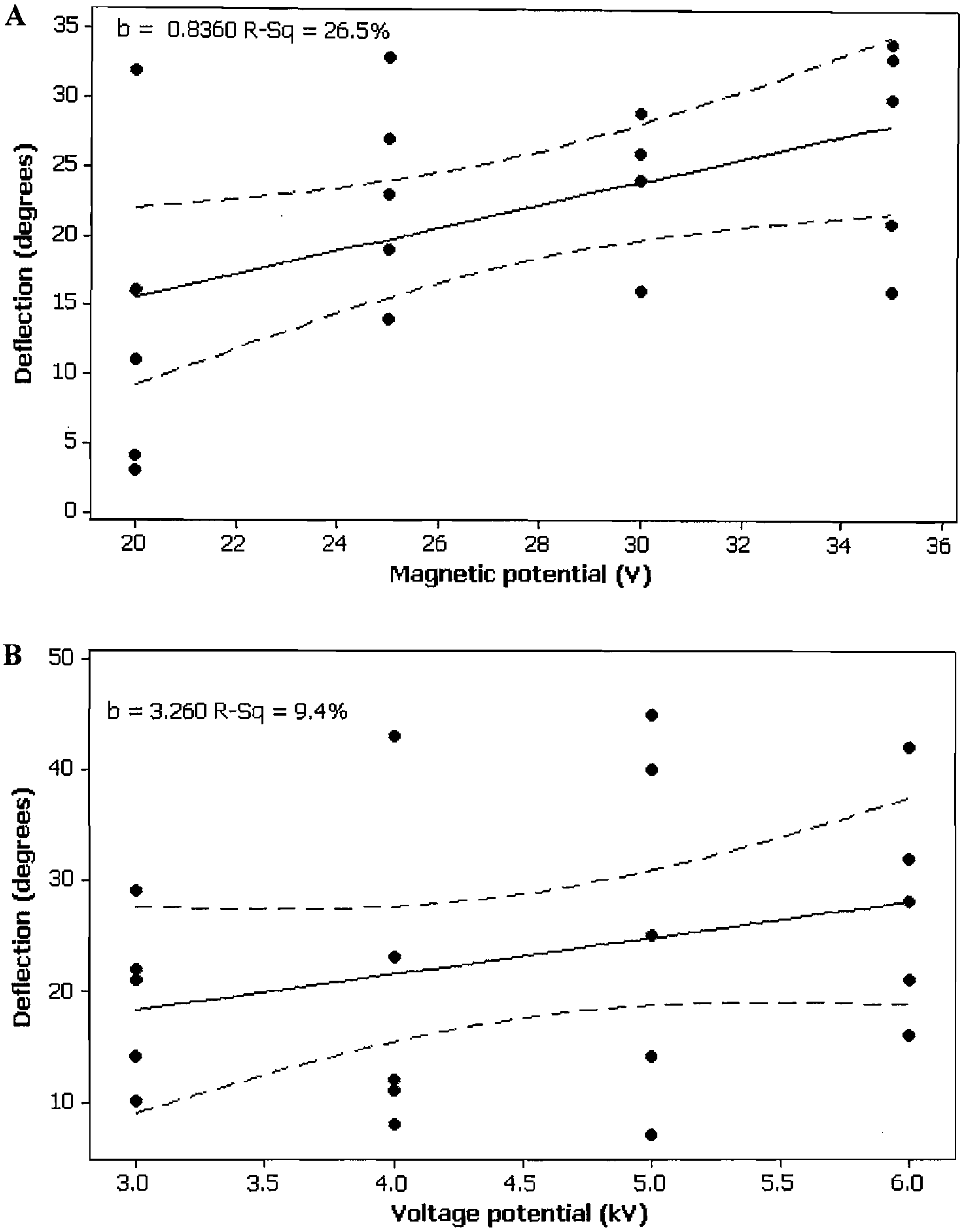


Figure 4.9 Fitted line regression plots of the effect of magnetic and electric fields on antennal deflection at varying potentials. (A) Raising the magnetic potential significantly increased antennal deflection ($b = 0.84$, $t = 2.55$, $P < 0.05$). (B) There was no positive association between electric potential and antennal deflection ($b = 3.26$, $t = 1.37$, $P > 0.05$). The regression slopes were not significantly different between field types (d.f. = 1, $t = 0.35$, $P > 0.05$), therefore the relationship between potential and antennal deflection was similar for magnetic and electric fields.

4.3.2 Extracellular VNC activity

4.3.2.1 The effect of antennal deflection on VNC activity

Control tests using uncoated antennae showed that magnetic field stimulation did not elicit a significant difference in mean VNC impulse number before and after stimulus onset when low ($n = 5, t = 0.18, P > 0.05$) or high ($n = 5, t = 0.63, P > 0.05$) magnetic potentials were applied (Fig. 4.10A). These results can be explained by the absence of antennal deflection (Fig. 4.11A and 4.12A), and demonstrates that magnetic fields *per se* did not evoke neural activity. Without iron powder application an antenna was not deflected by a magnetic field, and hence was associated with no change in VNC activity (Fig. 4.11A).

Coating an antenna with iron powder led to deflection when stimulated by a magnetic field, which was correlated with a burst of activity in the VNC (Fig. 4.11B). VNC activity increased only after post stimulus onset (within the 320ms time window) followed by low amplitude spike activity as the antenna remained deflected (Fig. 4.11B). Stimulating and deflecting coated antennae resulted in a significant increase in mean VNC activity at low potentials (Fig. 4.10B, $n = 5, t = 3.4, P < 0.05$). Raising the magnetic potential, thereby increasing antennal deflection in accordance with antennal stimulation data (see 4.3.1.2), also elicited an increase in VNC activity (Fig. 4.10B). The difference, however, was not significant ($n = 5, t = 2.4, P > 0.05$), which may in part be due to the deflection of antennae during electrophysiology.

Contrary to the data shown in 4.3.1.2, the antennal deflection during electrophysiology was not significant at low (Fig. 4.12A, $n = 4, t = -1.2, P > 0.05$) or high potentials ($n = 4, t = -0.3, P > 0.05$). In addition, the deflection that occurred at low potentials was greater than at high potentials (Fig. 4.12A). Hence, these differences in antennal deflection could have contributed to VNC activity not significantly increasing when antennae were stimulated by high magnetic potentials (Fig. 4.10B). This may be as a consequence of the electrophysiological equipment, or large variation.

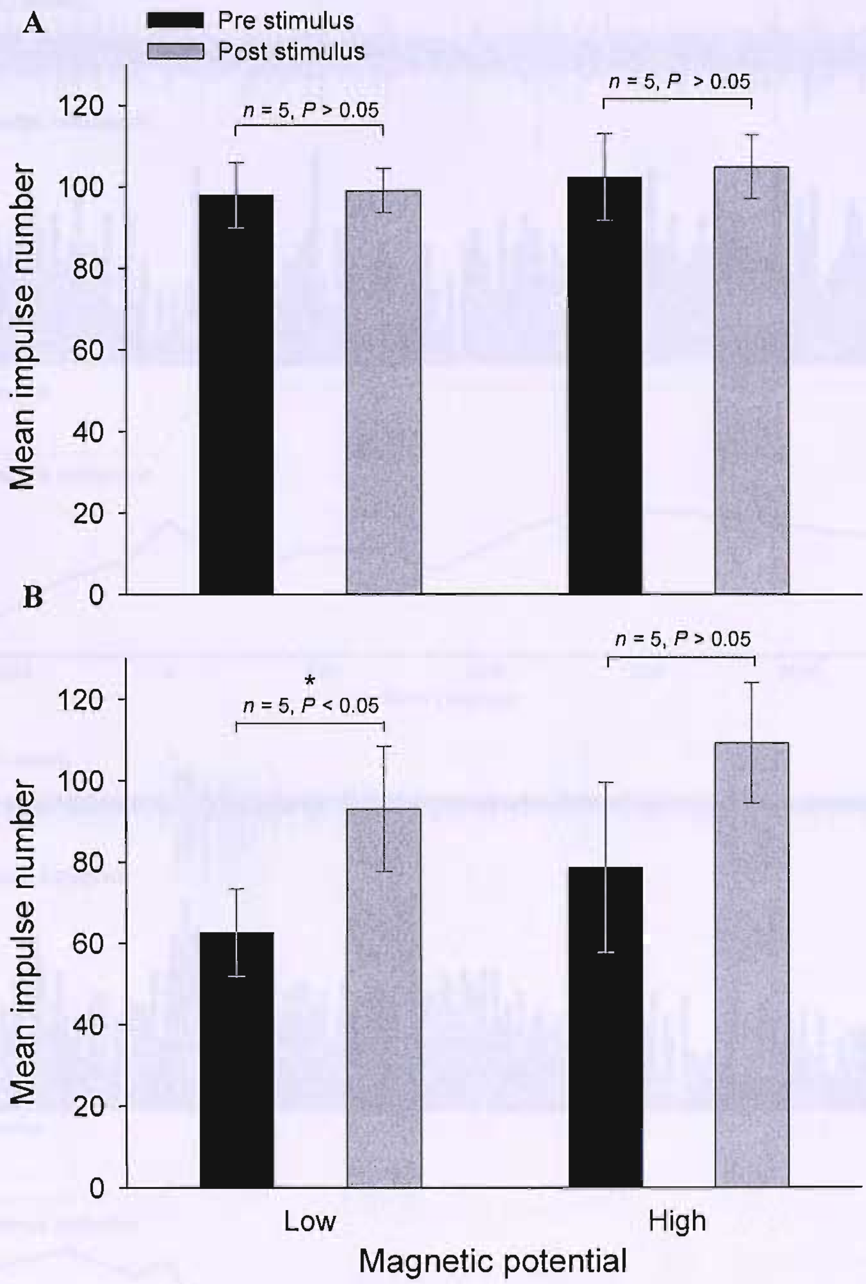


Figure 4.10 Mean impulse number (±SEM) recorded from the VNC 320msec before and after stimulus onset at low and high magnetic potentials. (A) No differences in VNC activity occurred when uncoated antennae were exposed to low or high magnetic potentials. (B) Stimulating coated antennae caused a significant increase in impulse number at low potentials ($n = 5$, $t = 3.4$, $P < 0.05$), but not at high potentials ($n = 5$, $t = 2.4$, $P > 0.05$), although a trend was evident.

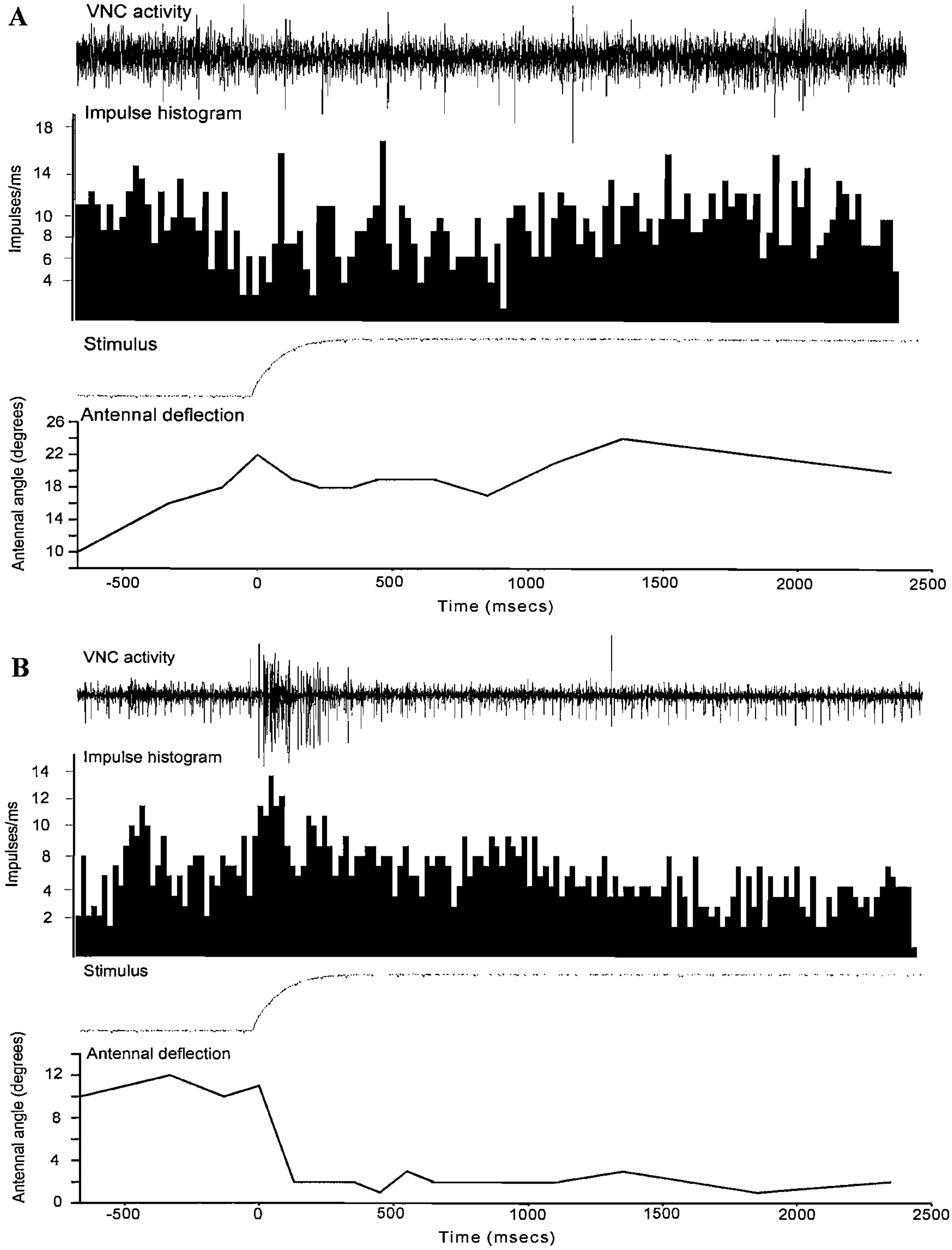


Figure 4.11 Antennal movement and associated VNC activity before and after stimulus onset in individuals with a coated and uncoated antenna. (A) Applying a magnetic field did not result in antennal deflection in control individuals and no change in VNC activity occurred after the stimulus onset. (B) In individuals with a coated antenna, antennal deflection and increased VNC activity commonly occurred after the stimulus onset within the 320ms time window.

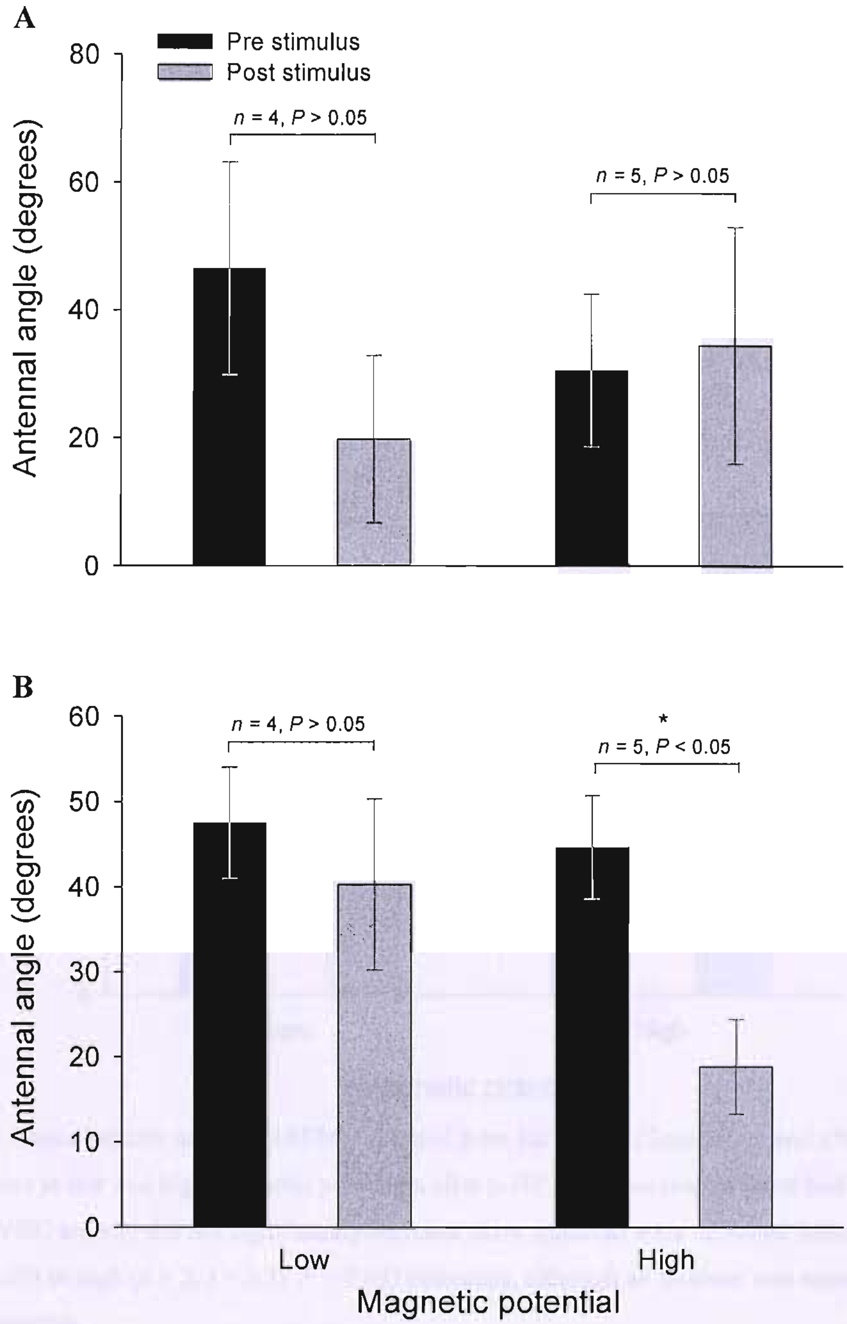


Figure 4.12 Mean antennal deflection (±SEM) of individuals during electrophysiology experiments at low and high magnetic potentials. (A) Mean antennal deflection was not significantly different at low ($n = 4$, $t = -1.9$, $P > 0.05$) or high ($n = 4$, $t = -0.3$, $P > 0.05$) magnetic potentials, although deflection was greater at low potentials. (B) Applying adhesive to the scape-head joint resulted in no significant deflection at low potentials ($n = 4$, $t = -0.76$, $P > 0.05$) although high potentials did evoke significant deflection ($n = 5$, $t = -4.93$, $P < 0.05$).

4.3.2.2 The effect of preventing S-HP mechanoreceptor stimulation on VNC activity

Preventing S-HP mechanoreceptor input by fixing the HS joint, and stimulating the antennae at low magnetic potentials did not elicit changes in VNC activity (Fig. 4.13, $n = 5$, $t = 0.5$, $P > 0.05$). Raising the magnetic potential, hence increasing antennal deflection, also evoked no significant change in VNC activity ($n = 5$, $t = 2.32$, $P > 0.05$), although an increase in mean impulse number did occur (Fig. 4.13).

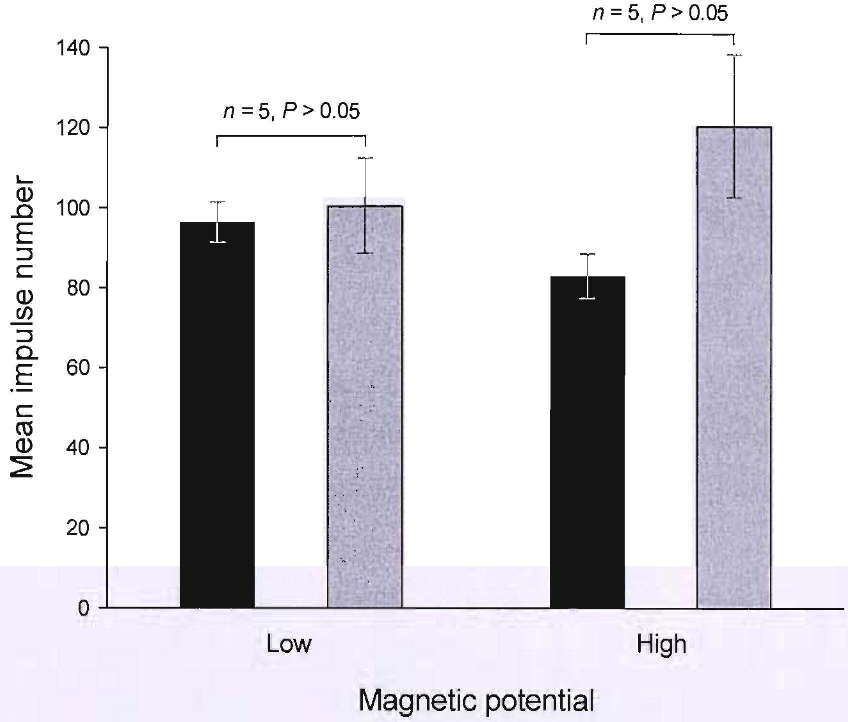
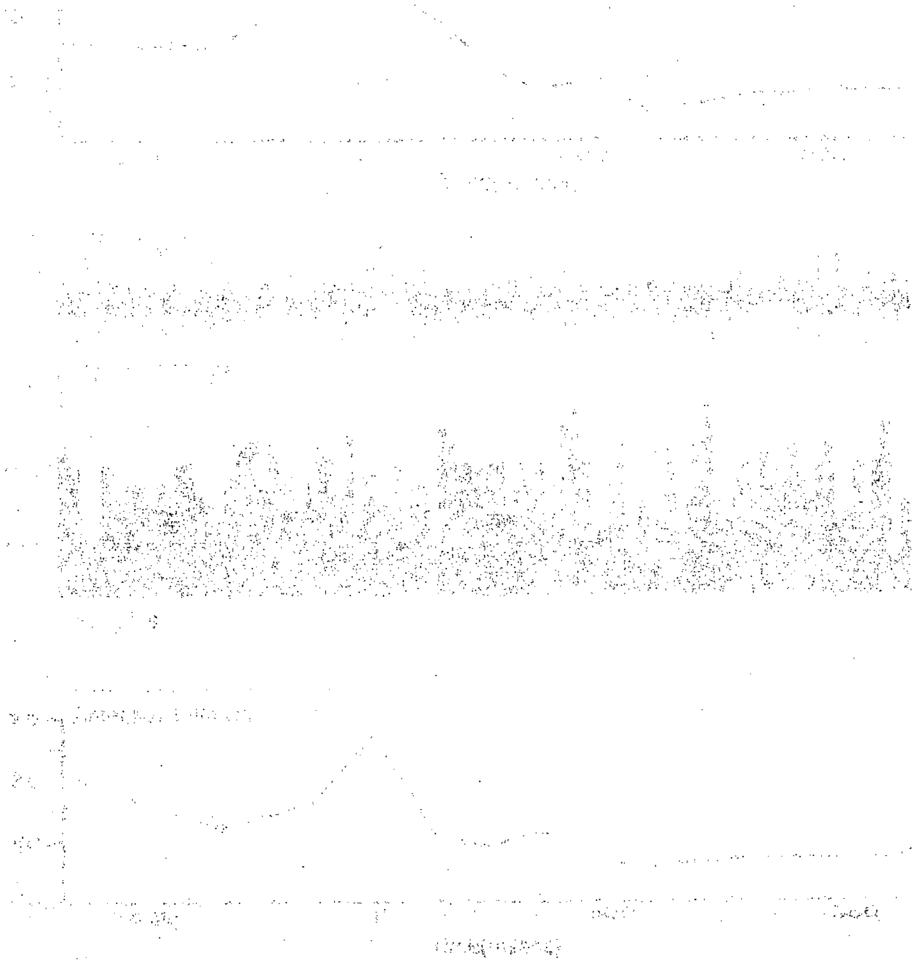


Figure 4.13 Mean impulse number (±SEM) recorded from the VNC 320ms before and after stimulation of the antennae at low and high magnetic potentials after S-HP mechanoreceptor input had been prevented. VNC activity did not significantly increase when antennae were deflected using low ($n = 5$, $t = 0.5$, $P > 0.05$) or high ($n = 5$, $t = 2.32$ $P > 0.05$) potentials, although an increase was apparent at high magnetic potentials.

Examining antennal deflection during electrophysiology reveals that at low potentials no significant antennal deflection occurred (Fig. 4.12B, $n = 4$, $t = -0.76$, $P > 0.05$). Antennal movement is fundamental to activate S-HP mechanoreceptors and cause DMI activity (Burdohan and Comer, 1996; Comer *et al.*, 2003). An absence of antennal deflection would therefore not be expected to elicit a change in VNC activity, and could explain absence of VNC activity at low potentials. Nevertheless, although there was no significant difference in mean antennal deflection at low magnetic potentials, preventing activation of mechanoreceptors on the S-HP did occasionally cause deflection when antennae were stimulated by low potentials, and this was correlated with no change in VNC activity (Fig. 4.14A).

High magnetic potentials were sufficient, however, to cause significant antennal deflection (Fig. 4.12B, $n = 4$, $t = -4.93$, $P < 0.05$), and regardless of this movement, VNC activity did not significantly increase (Fig 4.13B and 4.14B). Hence, this highlights the role of the S-HP in detecting antennal movements evoked by magnetic fields.



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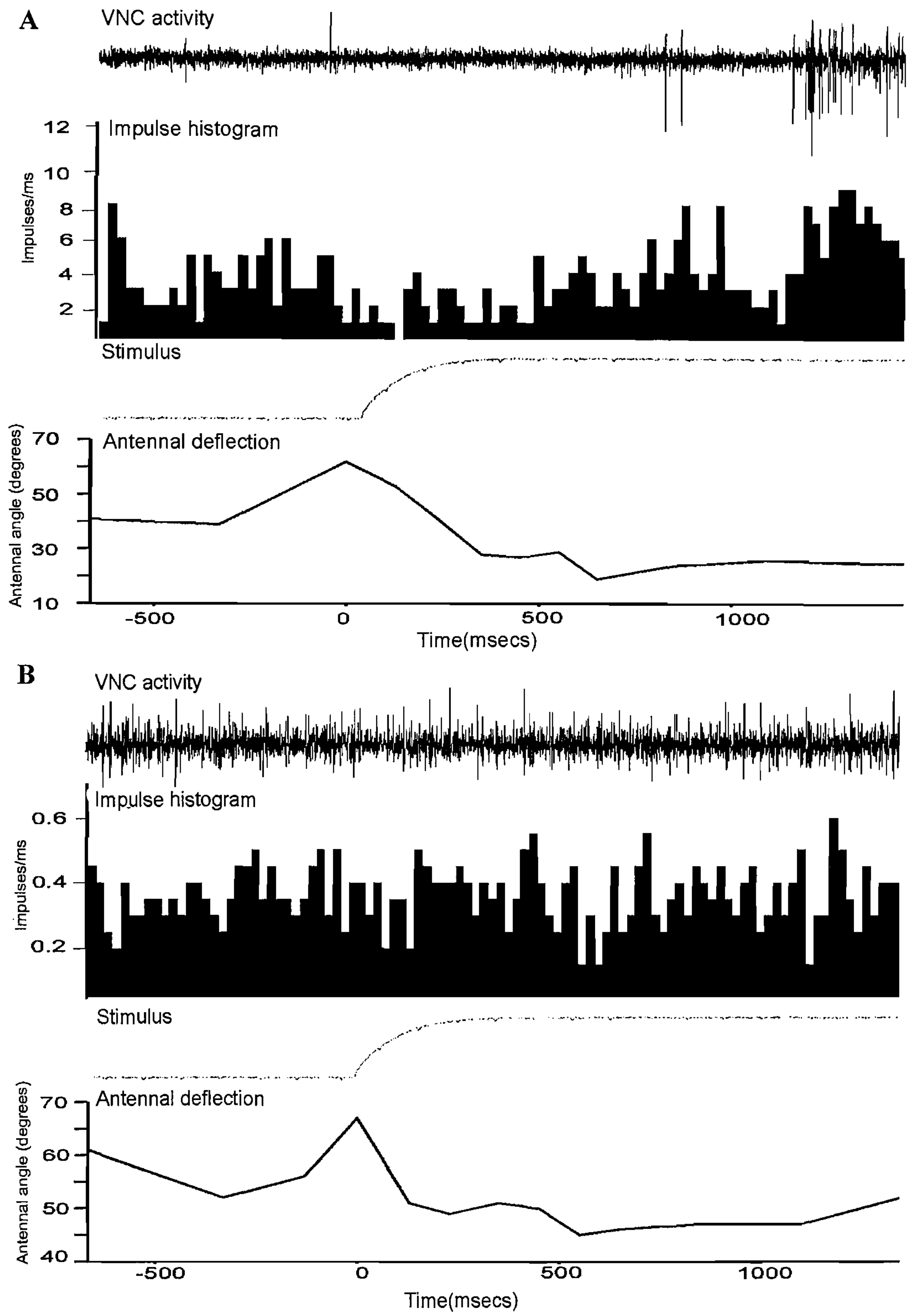


Figure 4.14 Antennal movement and associated VNC recordings before and after stimulus onset at low and high magnetic potentials in individuals with scapal mechanoreceptor stimulation prevented. (A) Low potentials occasionally caused antennal deflection, but stimulus onset was not associated with VNC activity. (B) Applying a high potential also caused antennal deflection and, likewise, was commonly associated with no increase in mean impulse number.

4.3.2.3 The effect of light on VNC activity

Stimulating antennae coated with iron powder using a high magnetic potential evoked a significant increase in VNC activity under white (Fig. 4.15, $n = 5$, $t = 5.01$, $P < 0.05$) and red light ($n = 5$, $t = 3.96$, $P < 0.05$). The change in VNC activity under white light following stimulation was not significantly different to VNC activity evoked under red light ($n = 5$, $t = 2.4$, $P > 0.05$). VNC activity elicited under red light was marginally greater than under white light (Fig. 4.15), yet this difference could be explained by variation and the effect of noise on recordings.

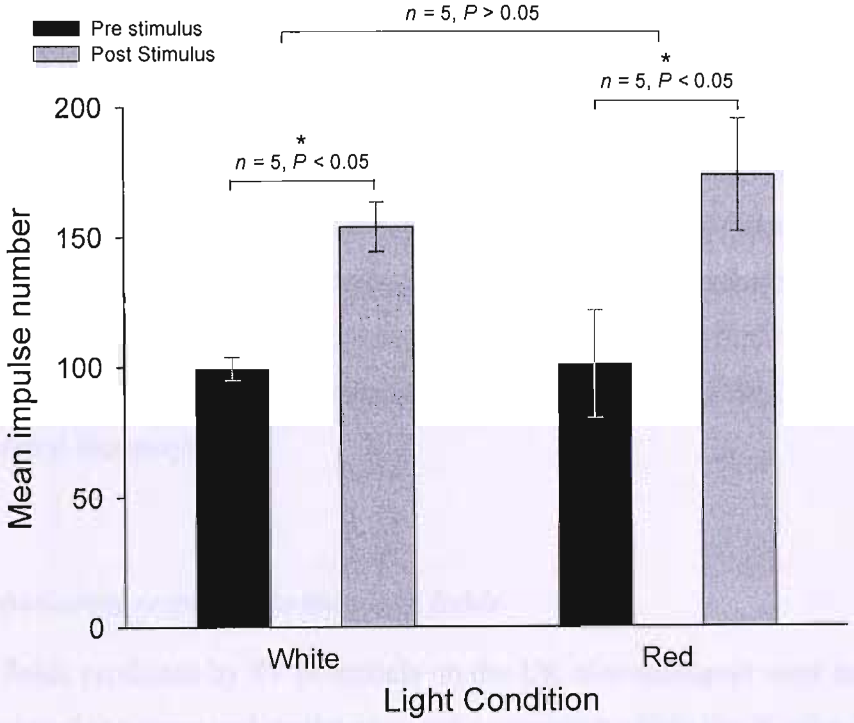


Figure 4.15 Mean impulse number (±SEM) recorded from the VNC under white and red light. VNC activity post stimulation at high magnetic potentials significantly increased under white ($n = 5$, $t = 5.01$, $P < 0.05$) and red light ($n = 5$, $t = 3.96$, $P < 0.05$). VNC activity post stimulation was marginally greater under red light conditions, yet the mean impulse number between light conditions was not significantly different ($n = 5$, $P > 0.05$).

4.3.3 Behavioural bioassays

4.3.3.1 Japanese and UK electromagnets

The voltage potentials applied to the UK electromagnet (8V, 10V, 15V and 20V) were selected to evoke similar nymph antennal movements as those caused by the potentials used with the Japanese electromagnet (20V, 25V, 30V and 35V). As the potentials of both magnets were increased the antennal bend became more acute (Fig. 4.16A), the length to the bend decreased (Fig. 4.16B), and there was a slight increase in the length of the bend (Fig. 4.16C). There was no significant effect of potential on the length to bend ($F_{1,\,175} = 0.04$, $P > 0.05$), length of bend ($F_{1,\,175} = 0.04$, $P > 0.05$) or angle of antennal bend ($F_{1,\,175} = 0.04$, $P > 0.05$) between the two magnet types. Regardless of the potential applied, the antennal bend did not differ (Fig. 4.16A, $F_{1,\,175} = 0.04$, $P > 0.05$). The length to the bend (Fig. 4.16B, $F_{1,\,175} = 18.82$, $P < 0.05$) and the length of the bend (Fig. 4.16C, $F_{1,\,175} = 16.62$, $P < 0.05$) was, however, significantly different between magnet types. This could be explained by differences in the electromagnet designs and structure. Nevertheless, these results indicate that nymph antennae were similarly influenced by both electromagnets, validating the use of the UK electromagnet for behavioural bioassays.

4.3.3.2 Behavioural responses to magnetic fields

Magnetic fields produced by 8V potentials on the UK electromagnet were sufficient to stimulate coated antennae and evoke antennal movement within the Y-tube, and were therefore used during behavioural bioassays. In addition, 8V potentials on the UK electromagnet had similar effects on nymph antennae to supplying 20V potentials on the Japanese electromagnet. As described in 4.3.2.1, 20V potentials on the Japanese electromagnet evoked increased VNC activity, and therefore stimulating antennae using the UK electromagnet with 8V potentials were assumed to elicit similar levels of VNC activity in nymph cockroaches.

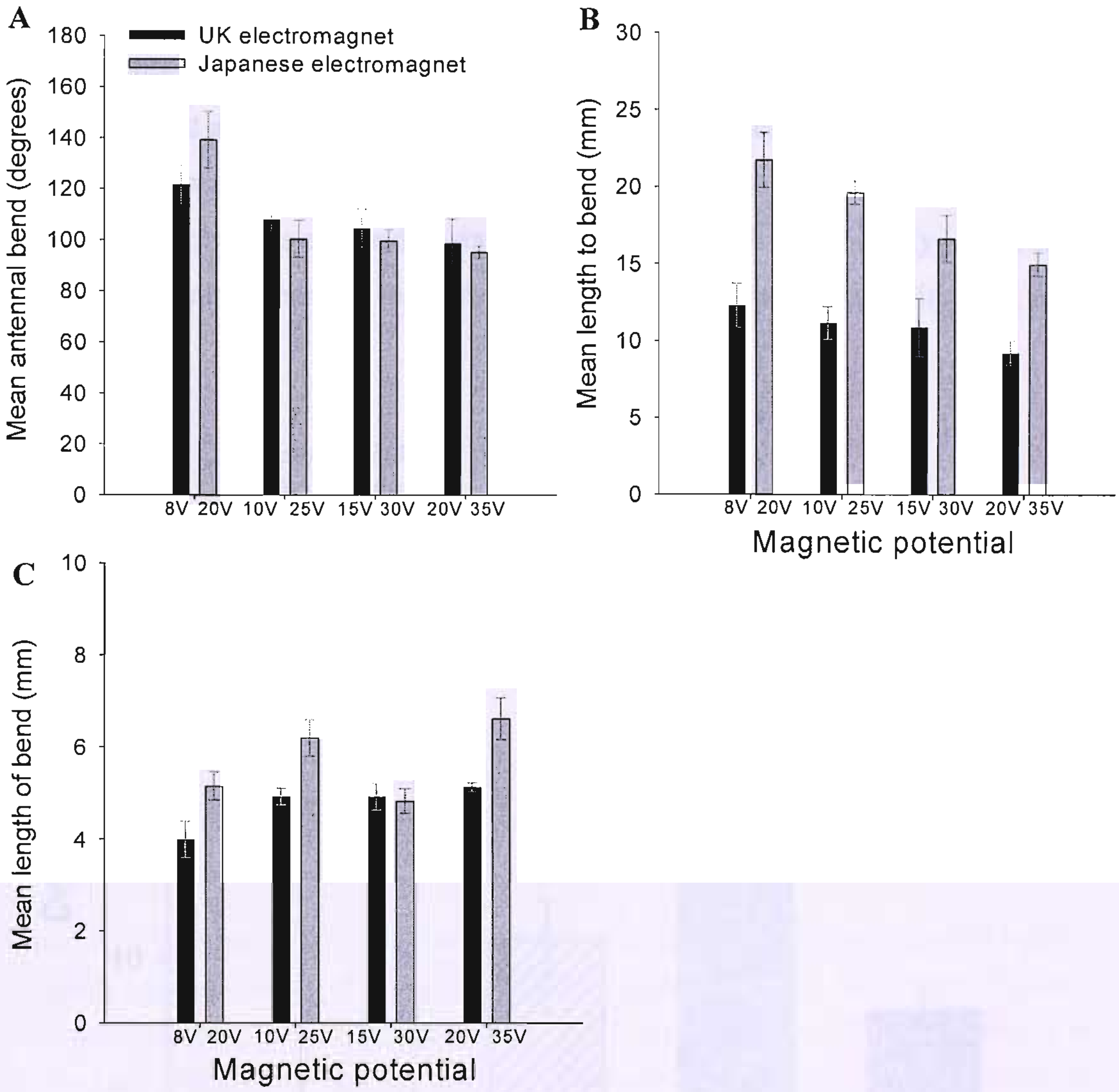


Figure 4.16 Mean antennal movement (±SEM) of nymph cockroaches exposed to the Japanese and UK electromagnet. (A) Both magnets evoked a greater antennal bend as potential was increased and there was no significant difference in effect between the magnets as potential was altered ($F_{1,\,175} = 0.04$, $P > 0.05$). (B) Raising the potential of both magnets also decreased the mean length to the bend. The Japanese electromagnet evoked greater length to the bend, yet there was no significant difference between the two magnets as potential was varied ($F_{1,\,175} = 0.04$, $P > 0.05$). (C) Both magnets evoked the same effect on bend length as potential was varied ($F_{1,\,175} = 0.04$, $P > 0.05$).

Cockroaches with antennae coated with iron powder which were exposed to no magnetic fields exhibited no natural side preference ($n = 32$, $P > 0.05$). Cockroaches did not, however, exhibit avoidance of magnetic fields ($n = 27$, $P > 0.05$). Nevertheless, individuals that approached the magnetic field took significantly longer to make a decision than when no magnetic fields were present (Fig. 4.17, $n = 44$, $P < 0.05$). Likewise, cockroaches exposed to static electric fields took significantly longer to take a treated or untreated chamber than when no electric field was present (Fig. 4.17, $n = 40$, $P < 0.05$).

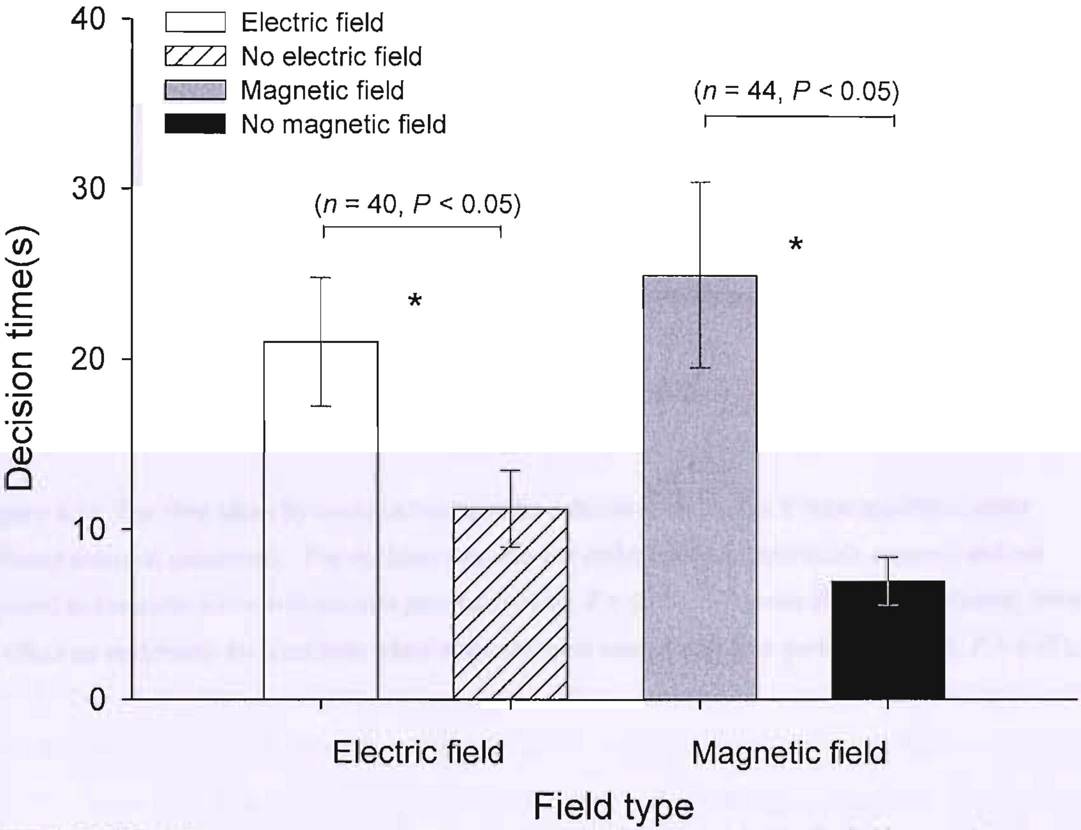


Figure 4.17 The time taken by cockroaches confronted by electric and magnetic fields to make a decision within the Y-tube apparatus. The time to take the treated or untreated pathway was greater when cockroaches were exposed to either magnetic or electric fields ($n = 44$, $P < 0.05$; $n = 40$, $P < 0.05$ respectively).

It was noted that coating the antennae with iron powder affected behaviour, as the time taken to make a decision for either untreated pathway was greater than cockroaches with uncoated antennae (Fig. 4.18, $n = 40$, $P < 0.05$). Magnetic fields alone, however, had no effect on behaviour as the time for cockroaches to take the right or left pathway did not differ from that of cockroaches taking the treated or untreated pathway (Fig. 4.18, $n = 40$, $P > 0.05$).

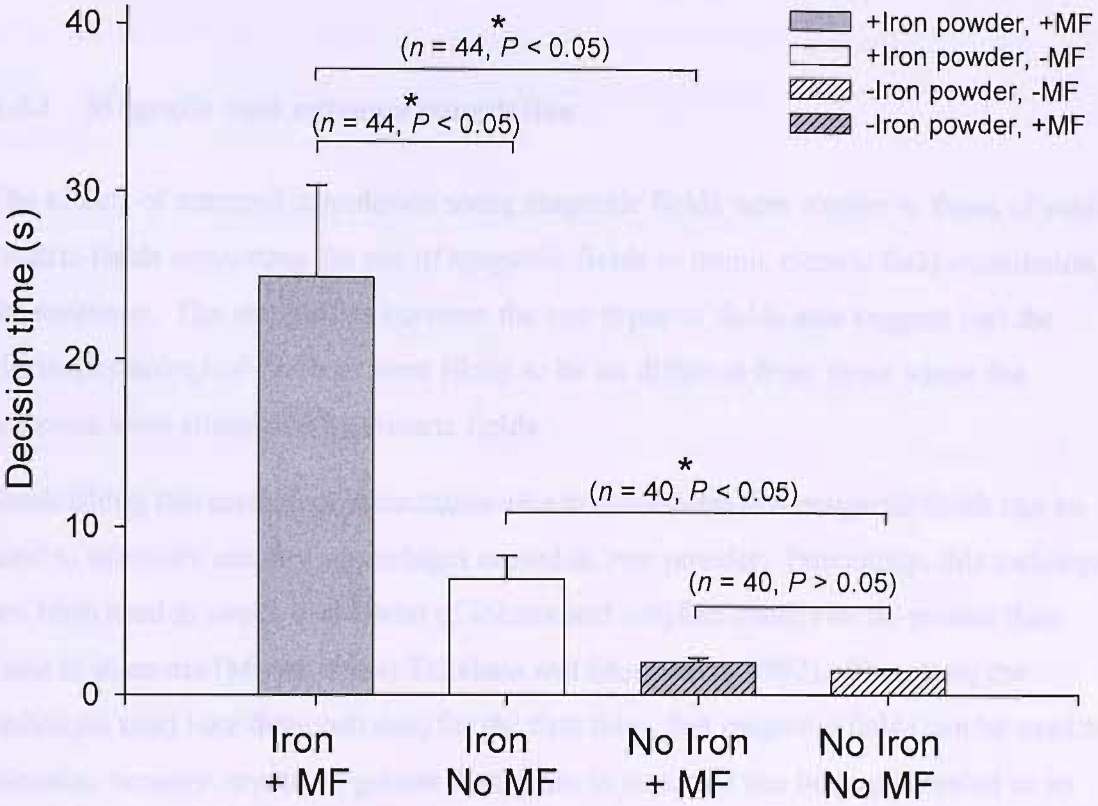


Figure 4.18 The time taken by cockroaches to make a decision within the Y-tube apparatus under different antennal conditions. The decision time did not differ between individuals exposed and not exposed to magnetic fields without iron powder ($n = 40$, $P > 0.05$). Magnetic fields did, however, have an effect on cockroach decision time when antennae were coated with iron particles ($n = 44$, $P < 0.05$).

4.4 Discussion

4.4.1 Magnetic field antennal stimulation

The effects of antennal stimulation using magnetic fields were similar to those of static electric fields supporting the use of magnetic fields to mimic electric field stimulation of the antennae. The similarities between the two types of fields also suggest that the electrophysiological findings were likely to be no different from those where the antennae were stimulated by electric fields.

Establishing this method of stimulation also demonstrates that magnetic fields can be used to stimulate sensory appendages coated in iron powder. Previously, this technique has been used to evoke movement of lobster and crayfish statocysts no greater than 1mm in diameter (Miyan, 1984; Takahata and Murayama, 1992). Therefore, the technique used here demonstrates, for the first time, that magnetic fields can be used to stimulate sensory structures greater than 1mm in size, and can be implemented as an alternative method of stimulation for a variety of sensory structures in future studies.

4.4.2 DMIs and the avoidance of static electric fields

The data shown here indicate that S-HP mechanosensory neurons and their association with the descending mechanosensory pathway could be the neural basis responsible for the avoidance of static electric fields. This advances the findings described in Chapter 3, further highlighting the role of the antennae and the significance of the S-HP in the avoidance of static electric fields.

Tactile stimulation of the cuticle or antennae has a substantial effect on thoracic motor output, believed to be controlled by connections between descending mechanosensory neurons and the TI_As (Ritzmann and Pollack, 1994; Ritzmann and Pollack, 1998). The experiments carried out in this study show that neural activity within the VNC increased when the antennae were stimulated, indicating that the DMIs responded to antennal deflection. Reports indicate that the DMIs converge on the TI_As and cause TI_A excitation and movement (Ritzmann *et al.*, 1991). The increased DMI activity in response to antennal stimulation in this study therefore suggests excitation of the TI_As and subsequent initiation of an escape response. Given the similarities in the influences of magnetic and electric fields on cockroach antennae, the results presented here indicate that the DMIs are at the very least partly responsible for mediating the avoidance of static electric fields.

Descending mechanosensory pathways, such as that described above, are not unique to cockroaches. DMIs homologous to those reported in cockroaches are known to exist in crickets, originating in the suboesophageal ganglion and extending to the first abdominal ganglion (Staudacher *et al.*, 2005). The supraoesophageal ganglion of locusts and other Orthoptera receives sensory input from a variety of modalities and coordinates behaviour. For example, the initiation of locust flight is modulated by tritocerebral commissure giant interneurone (TCG) activity that receives information from leg mechanoreceptors neurons (Bicker and Pearson, 1983). In addition, locust descending neural pathways process sensory information and activate selected motor circuitry (Heinrich, 2002). Descending interneurons are also reported to connect with both leg and wing motor neurons in Diptera (Burdohan and Comer, 1996) and grasshoppers (Rowell, 1989). Together, the similarities in function of descending neural pathways across insect suggest that the neural basis of static electric field avoidance described here could also apply to a variety of insects aside from cockroaches.

4.4.3 The relationship between stimulus strength and VNC activity

Chapter 3 demonstrated that the greater the field strength, the greater the force acting on the antennae. The apparent relationship between stimulus strength and the level of DMI activity could provide some explanation for the link between electric field strength and avoidance: first, the responsiveness of the descending mechanosensory pathway is known to be dependent on stimulus strength: for example, rostrally directed wind puffs < 1.2m/s do not evoke an escape response (Stierle *et al.*, 1994), and greater antennal deflection is associated with raised behavioural responsiveness (Okada and Toh, 2001); secondly, DMI impulse number has been shown to increase with greater antennal deflection (Burdohan and Comer, 1996); thirdly the TI_A responses are correlated with the level of antennal stimulation (Ritzmann and Pollack, 1994). Together, these relationships explain the association between stimulus strength and behavioural response characterised, for example, by the dependency of the cockroach escape response on intensity of antennal stimulation (Stierle *et al.*, 1994; Ye and Comer, 1996). Thus, the level of antennal stimulation by electrical forces could explain the increased avoidance as field strength was raised.

Such differences were not, however, shown in this study as no significant increase in VNC activity occurred during greater antennal deflection under high magnetic potentials. Nevertheless, examining the antennal deflection that occurred during electrophysiology revealed that high magnetic potentials did not cause antennal deflection, whereas low magnetic potentials did. VNC activity could therefore be said to be dependent upon the amount of antennal stimulation, and may therefore contribute to explaining why avoidance of static electric fields was dependent on field strength.

4.4.4 The mechanoreceptors responsible for static electric field detection

The data presented here highlight the importance of S-HP mechanoreceptors in the avoidance of electric fields, as preventing activation of S-HP exteroceptors elicited no significant increase in VNC activity at low or high magnetic potentials. The neuroanatomy of insect mechanosensory pathways leading from the HS joint is not currently well established (Staudacher *et al.*, 2005), but it is known that mechanosensory afferents from both the HS and SP joints primarily project to the deutocerebrum via the antennal lobe (Okada and Toh, 2000; Staudacher *et al.*, 2005). Mechanosensory neurons do not connect directly with DMIs in the antennal lobe (Burdohan and Comer, 1996) but do branch within the deutocerebrum (Staudacher *et al.*, 2005), passing down the VNC, ultimately activating leg motor neurons via the TI_As. Stimulation of exteroceptors on the HS, and SP joints, can therefore elicit motor output (Burdohan and Comer, 1996; Ritzmann and Pollack, 1994), and preventing activation will prevent motor output. Hence, the findings from this study, together with current knowledge, support the abolished avoidance of static electric fields when S-HP activation was prevented (Chapter 3) and emphasize the role of the S-HP in electric field detection.

Examining the antennal deflection of the individuals used during electrophysiology showed that high magnetic potentials caused greater antennal deflection than low magnetic potentials. Stimulation of mechanoreceptors on the SP joint and flagellum could have occurred at these higher magnetic potentials due to increased forces acting on the antennae. Associated with the greater antennal deflection was also a trend for VNC activity to increase. These results could therefore support the suggestion that the P-HP and flagellum may play a role in static electric field detection at higher field strengths (Chapter 3).

4.4.5 The effect of light on VNC activity

Motor output is ultimately regulated by a combination of sensory neuron inhibition and excitation and interactions between neuronal connections, amongst many other neuronal processes (Staudacher *et al.*, 2005). Notably, interneurons descending from the compound and simple eyes extend into the deutocerebrum and branch into regions containing antennal mechanosensory interneurons and ultimately influence behaviour. For example, the cricket antennal descending brain interneuron 1-2 (DBNi1-2), receives visual input from interneuron branches in the protocerebrum region of the brain descending from the compound eye (Gebhardt and Honegger, 2001). DBNi1-2 also receives sensory input from antennal mechanosensory neurons, which are modulated by visual inputs (Gebhardt and Honegger, 2001). Interactions such as these are abundant within the brain ganglia of many insects (Staudacher *et al.*, 2005) highlighting the importance of visual input in influencing antennal mechanosensory information.

Light level, as well as visual stimuli, is believed to play an important role in mediating motor output, represented by the extensive connections of neurons extending from the ocelli in brain regions containing antennal mechanosensory afferents (Mizunami, 1995a). These connections will ultimately regulate motor output (Mizunami, 1995a), such as the cessation of running when cockroaches reach areas of shade (Okada and Toh, 1998). The motor output of a number of insect species has been reported to be modified under varying light intensities (Cardé and Knols, 2000; Riley *et al.*, 1988; Sherman and Dickinson, 2004), and Chapter 2 presented the tendency for cockroaches to avoid static electric fields less when exposed to increased light intensities. This may not correlate with findings reported by Ye *et al.* (2003) who showed decreased escape responses when cockroach visual input was omitted, yet the contribution of the ocelli was not considered. Nevertheless, the previous studies described above show that visual input and light level do influence insect behaviour, and visual input is combined with antennal mechanosensory information influencing the final motor output. Such influences were not, however, evident from the electrophysiological experiments carried out in this Chapter; VNC activity was not significantly different when antennae were stimulated under red or white light, although activity was marginally greater under red light.

4.4.6 Behavioural bioassays

The behavioural bioassays carried out showed that nymph cockroaches did not significantly avoid the pathway containing magnetic fields. This indicates that the influences of magnetic fields on cockroach antennae within the Y-tube were not adequate to evoke avoidance. Although avoidance did not occur, the increase in time to take the treated or untreated pathway indicates that exposure of nymph cockroaches to magnetic fields did influence motor output. This is likely to have been as a result of antennal deflection within the Y-tube apparatus, and may have been due to S-HP mechanoreceptor activation detecting the influences of magnetic fields on the antennae.

The absence of avoidance and increase in time taken to make a decision could be explained by the stimulus strength-dependent responses of the descending mechanosensory pathway (Burdohan and Comer, 1990; Ritzmann and Pollack, 1994). This relationship contributes to explaining why mild stimulation of the antennae elicits only a freezing response whereby locomotion decreases (Okada and Toh, 2001), yet high intensity tactile stimulation and antennal deflection will evoke a turn-and-run response (Stierle *et al.*, 1994). Hence, magnetic fields within the Y-tube may not have been sufficient in strength to elicit adequate antennal deflection and evoke an avoidance response. Nevertheless, the influences of magnetic fields on antennae within the Y-tube did alter motor output.

Together, the findings presented here show, for the first time, that the DMI pathway, mediated by the S-HP, is responsible for evoking static electric field avoidance.

5. THE BEHAVIOUR OF FREE-MOVING COCKROACHES EXPOSED TO ELECTRIC FIELDS

5.1 Introduction

Few studies have investigated the movement of insects exposed to electric fields and even fewer have quantified the effects of electric fields on free-moving insects. Of those, observations have indicated that insect movement is correlated with electric field strength (Edwards, 1960a; Maw, 1961c; Maw, 1962; Watson *et al.*, 1997). The reports, however, have left many questions unanswered, and it remains to be understood how free-moving insects respond to electric fields.

Locomotor activity, the motor output of an organism, can change in response to directional or non-directional cues (Martin, 2003). Directional cues include stimuli such as light or chemical gradients (Fraenkel and Gunn, 1961). Animals may orient themselves towards or away from the source of these stimuli by changes in locomotor activity, termed taxis (Kennedy, 1977). For example, mayfly larvae, *Ephemeridae spp.*, exhibit increased turning (klinotaxis) and fly faster towards light (Jander, 1963). Female sex pheromone plumes also elicit klinotaxis in *P. americana* (Kennedy, 1977; Willis and Avondet, 2005). In contrast, responses to non-directional cues, termed kinesis (Kennedy, 1977), do not commonly involve orientation. The changes in locomotor activity to non-directional cues include, for example, the decreased velocity (orthokinesis) and increased turning (klinokinesis) of the mealworm beetle, *Tenebrio molitor*, within humid environments (Bell, 1990). Such changes in locomotor activity of free-moving insects in response to electric fields have yet, however, to be described.

Flying insects presented with the choice of a static electric field, or no field, exhibit a preference away from the field (Perumpral *et al.*, 1978), yet it is not known whether such preferences are exhibited by terrestrial walking insects confronted with static electric fields. Animals distribute themselves in different regions of a localised environment depending on the tactile cues they receive (Fraenkel and Gunn, 1961; Jeanson *et al.*, 2003). For example *D. melanogaster* and *Blatella germanica* exhibit a tendency to follow solid objects rather than walk in open spaces, behaviour known as thigmotaxis (Jeanson *et al.*, 2003; Martin, 2004). Thigmotaxis presents the ability to orient within an area by mechanical contact and contributes to the distribution of animals within a space (Fraenkel and Gunn, 1961; Jeanson *et al.*, 2003; Martin, 2004). Cockroaches are highly thigmotactic, and mechanosensory stimuli from the antennae play an important role in mediating this behaviour (Camhi and Johnson, 1999; Cowan *et al.*, 2006). Hair-like structures are thought to be influenced by electrical forces (Shimizu and Shimizu, 2003), and such effects were illustrated on the antennae of cockroaches in Chapter 3. The effects of electric fields on antennae, together with thigmotactic behaviour, suggest that the spatial distribution of free-moving cockroaches within an arena could be influenced by static electric fields.

Locomotor activity is a key behavioural trait that relies on complex physiological interactions (Martin, 2003; Martin, 2004). Hence, it is dependent on the internal state of an organism and is regarded as the basis of many behaviours (Martin, 2003). Exposing insects to electric fields may therefore not only elicit transient behavioural differences due to mechanosensory stimulation, but could also affect behaviour through intrinsic physiological changes. It is apparent that electric field exposure causes biological effects in a variety of organisms, and such effects can be induced within a short period of time. The majority of studies investigating these effects have focused on ELF electric fields: for example, exposure of human lymphocytes to high strength ELF electric fields > 250kV/m for 1min can cause chromosomal breakage (Nordenson *et al.*, 1984). In addition, elevated protein production, such as orthinine decarboxylase that is essential for polyamide synthesis, has been reported in mice cells exposed to 60Hz 1V/m ELF electric fields for 1hr (Tenforde, 1991). Some reports do show effects in organisms transiently exposed to static electric fields, such as DNA aberrations in *Escherichia coli* and *Drosophila* larvae (McCann *et al.*, 1993), yet results remain inconclusive.

Long-term electric field exposure has also been reported to elicit biological effects in a variety of organisms. In recent years, for example, concerns have arisen regarding the effects of electric fields produced by power lines on humans, stemming from the apparent increased incidence of cancer in individuals living in close proximity to high voltage power lines (Draper *et al.*, 2005; Fews *et al.*, 1999a; Fews *et al.*, 1999b). The majority of investigations into the biological effects of long-term electric field exposure have yielded results using ELF electric fields. For example, chromosomal aberrations in plants and invertebrates (McCann *et al.*, 1998; McCann *et al.*, 1993), and adverse effects on cell membrane transport (Funk and Monsees, 2006) have been reported in organisms exposed to ELF electric fields for >24hr. To date, however, little evidence has been presented indicating an effect of long-term static electric field exposure on organisms.

The regulation of locomotor activity is dependent on functional physiological processes (Dickinson *et al.*, 2000; Martin, 2004). Given the apparent detrimental cellular and physiological effects of ELF electric fields, long-term static electric field exposure could impact the locomotor activity of organisms. Such effects can be monitored by measuring the behavioural and locomotor responses of animals to stimuli, and ultimately give an indication of fitness (Fernandez *et al.*, 1999; Hebel *et al.*, 1997; Irschick and Garland, 2001).

The aim of the current study is to analyse the responses of free-moving cockroaches to static electric fields and, using these behavioural responses as a paradigm, to determine, for the first time, whether long-term static electric field exposure affects cockroach fitness.

5.2 Materials and Methods

5.2.1 The behavioural responses of free-moving cockroaches to static electric fields

5.2.1.1 The experimental arena

Cockroaches were exposed to a static electric field produced by an aluminium wire mesh semi-circle (130mm radius, mesh size 2.8mm, Locker Wire Weavers Ltd, Cheshire, UK) positioned on one side of a glass arena (Fig. 5.1, 190 x 30, D x H). The mesh was connected to the high voltage power supply (Brandenburg Alpha III). An identical earthed wire mesh semi-circle was placed on the opposite side, with a gap (30mm) between both meshes to localise the electric field to one half of the arena. The arena was placed on white paper (210mm diameter), beneath which an earthed wire mesh semi circle (130mm radius) was positioned, acting as a parallel earth plate. The arena, hereafter termed 'treated arena', was covered by a glass sheet (205 x 205 x 3mm) to hold the mesh sections in place and prevent air movement within the arena. The arena was positioned on a black medium density fibreboard (MDF) board (298mm x 298 x 15mm) on which reference points were marked to ensure consistent arena position for video analysis. The set up was contained within an earthed enclosure to reduce visual input, air movement and external electric fields.

Preliminary experiments demonstrated that analysing behaviour for less than 10min resulted in high variability, and temporal analysis indicated little change in behaviour beyond 10min. Cockroach behaviour within the arena was therefore filmed for 10min using a digital camera (Sanyo VCB-3372P) with lens (Computar® 03A) and recorded onto DVD (Panasonic DMR-E55EB).

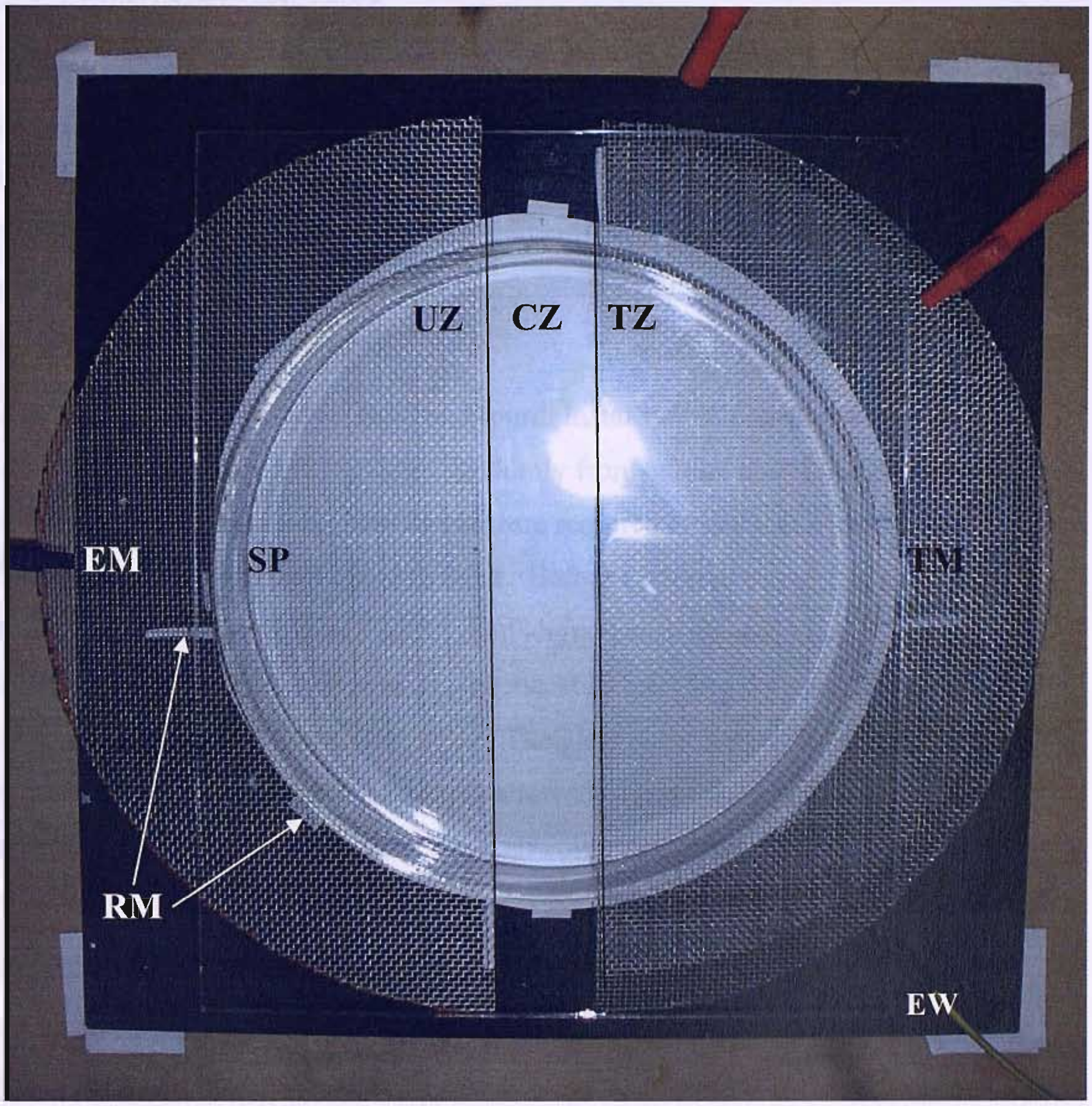


Figure 5.1 The circular arena apparatus. The earth mesh (EM) ensured the electric field emitted from the treated mesh (TM) was localised to the treated zone (Z) and central zone (CZ). The electric field was localised vertically using a semi-circle mesh positioned below the treated mesh and connected to earth via the earth wire (EW). The arena was positioned using the reference marks (RM). Each trial began when the cockroach passed the start point (SP) after a 2min rest period.

5.2.1.2 Experimental procedure

The protocol followed a randomised complete block design (RCBD) using five voltage levels of 1kV, 2kV, 3kV, 4kV and 5kV and the control (0kV). Twenty one repeats were carried out for each treatment, and three blocks of treatments were performed each day to control for any time or day effects (Wyatt, 1997). Control bioassays using an arena with no electric field (hereafter termed 'untreated arena') were also included in the RCBD.

At the beginning of each day, third and fourth instar individuals (body length, 8.13±0.7mm, $n = 126$) were selected randomly from culture and isolated in preparation for the bioassays. Three circular arenas were required as one was used for bioassay trials, one was washed, and one was dried. Before each trial began, the arena used for the bioassays was set up as in Fig. 5.1, and aligned with the reference marks. The glass sheet was temporarily removed and using storkbill forceps a cockroach was carefully placed into the central zone of the arena. The glass sheet was replaced, any mesh sections repositioned, and the cockroach allowed to rest for 2min. After the rest period the video recording was started and the high voltage power supply turned on at the appropriate voltage when the cockroach was positioned at the start point (Fig. 5.1).

After 10min the power supply was turned off and the cockroach removed. All mesh sections were rotated 90° clockwise to control for any room effects and two further trials were carried out with different cockroaches before washing the arena. The washing procedure involved soaking the arena in hot 5% Decon90® solution (55°C) for 15min and then rinsing in distilled water. Any potential remaining pheromone traces were removed by liberally washing the arena with 100% acetone. The arena was then placed in a drying chamber at 110°C for a minimum of 10min to remove the acetone. The arena was cooled to room temperature before subsequently being used for bioassay trials.

All experiments were carried out under red light between 09:00 and 19:00 at 22.9±1.8°C and 38.6±6%RH.

5.2.1.3 Analysis

The effects of varying field strength on cockroach locomotor activity and the distribution of cockroaches within the arena was analysed using video analysis software (EthoVision® 3.1, Noldus Information Technology, Netherlands). Comparisons in cockroach behaviour between each zone (untreated, central and treated) were made using five behavioural parameters measured in each zone: time spent (s); distance moved (cm); velocity (cm/s); absolute unsigned meander (°/cm); absolute unsigned angular velocity (°/s). EthoVision® automatically assigned and logged the x and y coordinates of the centre of each individual at 12 samples/s for the 10min trial duration. The application of input filters was deemed unnecessary after plotting and investigating sample data. Each trial was analysed at medium resolution video playback, and brightness and contrast were enhanced when necessary to optimise EthoVision® performance.

The effect of field strength on each behavioural parameter in each zone was tested using one way ANOVA after the assumptions of normal distribution and homogeneity of variance of the data had been met (Kolmogorov-Smirnov and Levene's Test respectively). Data that did not meet the assumptions were log transformed and re-tested (Sokal and Rohlf, 1995). Post hoc least significant difference (LSD) tests compared differences within parameters between voltage potentials for each zone. Differences between the zones were investigated using independent *t*-Tests for each voltage potential. All tests were carried out using SPSS (Windows, Version 12) and significance was determined at the $P < 0.05$ level.

5.2.2 The effect of long-term static electric field exposure on the behaviour of free-moving cockroaches

5.2.2.1 Apparatus

The apparatus used for long-term electric field exposure consisted of five glass dishes (190 x 10 x 3mm) positioned between two aluminium mesh sheets (450 x 550mm). The bottom aluminium mesh was connected to earth and a high voltage power supply (Brandenburg Alpha III) supplied the top aluminium mesh with a 4kV potential. The field strength within each dish produced by a 4kV potential was calculated to be comparable to that within the dishes used during bioassays. 4kV elicited the most apparent changes in free-moving cockroach behaviour within the treated arena, and hence was used to continuously treat cockroaches. A glass sheet (500 x 600 x 3mm) was placed over the dishes to prevent cockroach escape and hold the mesh in position. This setup is hereafter referred to as the 'treated setup'.

Each glass dish housed fourteen third and fourth instar cockroaches (body length, 9.78±0.7mm, $n = 168$) randomly selected from culture. Two 5mm Petri dish lids containing crushed dog biscuits ($0.025 \pm 1.7 \times 10^{-3}$g, $n = 100$) and two 5mm Petri dish lids containing moist cotton wool (0.15±0.014g, $n = 100$, dry wool weight with 5ml water) positioned opposite each other were also placed in each glass dish. Each glass dish and the cockroaches it housed was designated for use on one of the time intervals (see below).

An identical setup, not connected to the high voltage power supply (hereafter termed 'untreated setup'), was prepared and positioned adjacent to the treated setup and acted as the control. A grounded metal screen between each setup ensured electric fields were localised to the treated setup. The position of the setups was swapped daily to eliminate any room effects. Cockroaches within the treated setup are hereafter termed 'treated' and cockroaches enclosed within the untreated setup are hereafter termed 'untreated'.

5.2.2.2 Bioassays

The same experimental apparatus described in 5.2.1 was used to investigate the behaviour of free-moving treated cockroaches in a treated arena. Bioassays were carried out at six time intervals (0, 4, 8, 14, 18 and 22 days) to monitor the effect of continuous electric field exposure on behaviour over time. Bioassays using untreated cockroaches were carried out at the same intervals to test whether retaining cockroaches within the setup affected behaviour in a treated arena. The treated arena was divided into two zones (treated and untreated). Behaviour was examined solely at 4kV potentials, chosen due to the apparent changes of free-moving cockroach behaviour at this potential.

Simultaneous bioassays were carried out investigating the behaviour of treated and untreated cockroaches within an untreated arena. These bioassays acted as negative controls, testing for the effect of continuous electric field exposure on cockroach locomotion. The untreated arena was identical to that used in 5.2.1, and either half of the arena was separated into side A and B.

At each time interval the power supply of the treated setup was turned off allowing the glass sheet and top aluminium mesh to be removed. The glass sheet and mesh of the untreated setup was also removed, and the circular arenas prepared as detailed in 5.2.1.2. Using storkbill forceps a single cockroach was removed from each designated glass dish and carefully positioned in the centre of the appropriate arena. After a 2min rest period, filming of cockroach movement began when each individual reached the starting point (Fig. 5.1). Two digital cameras (Sanyo VCB-3372P) with lenses (Computar® 03A) were used for filming, and footage recorded onto DVD (2 x Panasonic DMR-E55EB). The high voltage power supply was turned on immediately after the appropriate individual had passed the starting point, providing one arena with a 4kV potential. Each bioassay lasted 10min and after all individuals had been tested (*n* = 7 in all cases at each time period) the setups were reassembled, food and water were replaced, and the power supply for the treated setup was turned on.

5.2.2.3 Analysis

Analysis was carried out using EthoVision® measuring the following behavioural parameters: time spent (s); distance moved (cm); velocity (cm/s); absolute unsigned meander (°/cm); and absolute unsigned angular velocity (°/s).

One-way ANOVA tested for differences in behaviour within each zone (or side) as the experiment progressed. Differences between the zones were analysed at each time interval using independent samples *t*-Tests. Analysis was repeated for each behavioural parameter for both treated and untreated cockroaches within the treated arena, in addition to treated and untreated cockroaches within the untreated arena.

The bioassays investigating the behaviour of free-moving cockroaches to electric fields were used as a paradigm to determine the effect of long-term electric field exposure on cockroach behaviour. Therefore, comparisons between the two studies were made. One way ANOVA tested for differences in each behavioural parameter exhibited by cockroaches between the two studies.

The distribution and variance of all data were tested according to the analysis performed, and if necessary, data were log or Box-Cox transformed to meet assumptions (Dytham, 2002; Sokal and Rohlf, 1995). All analyses were carried out using SPSS (Version 14 for Windows) and significance was determined at the $P < 0.05$ level.

5.3 Results

5.3.1 The behavioural responses of free-moving cockroaches to static electric fields

Cockroaches exhibited no natural side preference within an untreated arena, as the time spent or distance moved did not differ between either side of the arena (t = 0.173, d.f. = 40, $P > 0.05$; t = 0.166, d.f. = 40, $P > 0.05$ respectively). The mean velocity, meander and angular velocity, did also not differ between either side of an untreated arena (t = -0.125, d.f. = 40, $P > 0.05$; t = -0.267, d.f. = 40, $P > 0.05$; t = -0.176, d.f. = 40, $P > 0.05$ respectively). Together, these results demonstrate that cockroaches were not susceptible to following effects due to pheromone deposition (Miller and Koeiiler, 2000; Schal *et al.*, 1984) or external stimuli causing side bias.

5.3.1.1 Time spent

The region of the arena favoured by cockroaches differed as the voltage potential was raised (Fig. 5.2). This was indicated by a significant effect of voltage potential on the mean time spent in the treated and untreated zone ($F_{5,118} = 3.37$, $P < 0.05$; $F_{5,120} = 2.66$, $P < 0.05$). At intermediate voltage potentials (2kV and 3kV), cockroaches spent more time in the treated zone than the untreated zone (t = 4.188, d.f = 40, $P < 0.05$; t = 1.88, d.f = 40, $P < 0.05$ respectively). This behaviour was reversed at 4kV when cockroaches were repelled away from the electric field. Significantly less time was spent in the treated zone at 4kV in comparison to 1-3kV (d.f = 118, $P < 0.05$ for all cases), and more time was spent in the untreated zone (t = 2.746, d.f. = 40, $P < 0.05$). Cockroaches did not, however, continue to exhibit repulsion at 5kV (t = 0.9, d.f = 40, $P > 0.05$).

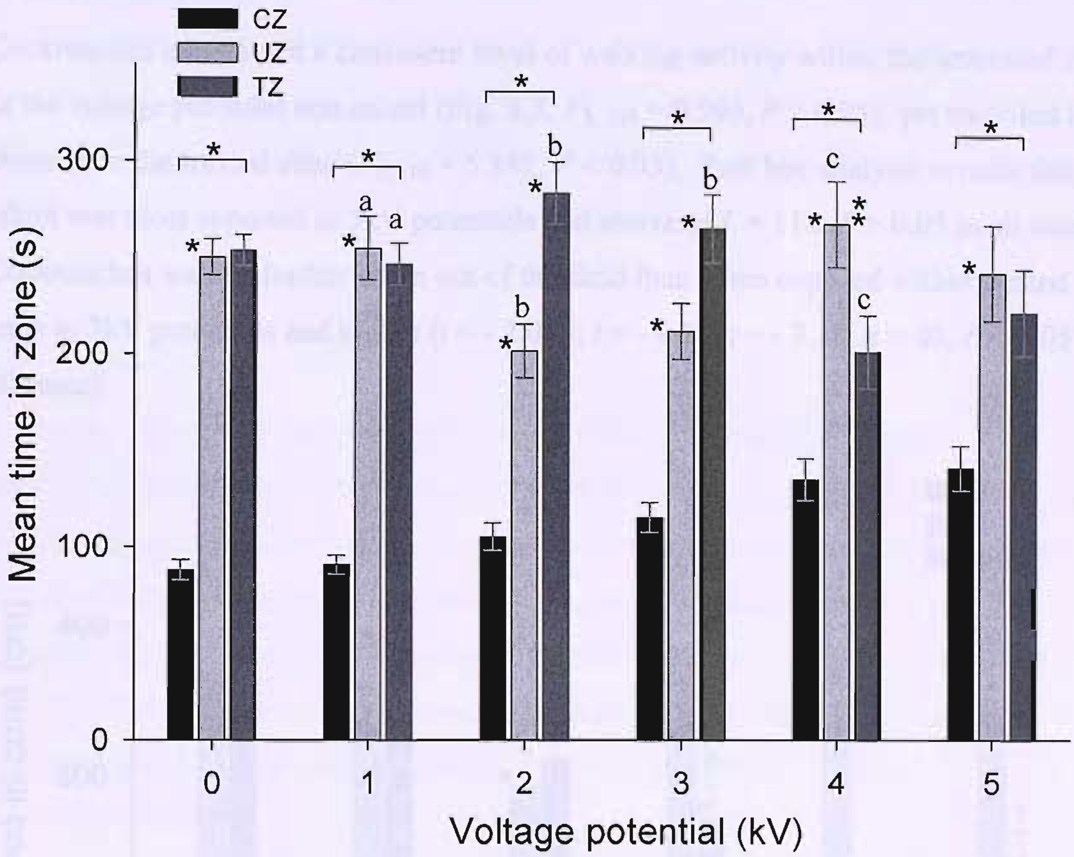


Figure 5.2 The effect of voltage potential on the mean time cockroaches (±SEM) spent in each zone of the arena. Voltage potential significantly affected the time spent in the central (CZ), treated (TZ) and untreated (UZ) zone ($F_{5,\,120} = 7.05$, $P < 0.05$; $F_{5,\,118} = 2.66$, $P < 0.05$; $F_{5,\,120} = 3.37$, $P < 0.05$ respectively). Different letters indicate significant differences within a zone between voltage potentials. Bracketed asterisks represent significant differences in the time spent between the treated and central zone, and asterisks positioned above bars indicate significant differences between the adjacent zones ($P < 0.05$ in all cases).

Cockroaches also spent significantly longer in the central zone as the voltage potential was raised ($F_{5,\,120} = 7.05$, $P < 0.05$). This could be attributed to decreased locomotion or higher turning rates – parameters that will be investigated in the succeeding sections. The significant differences in the time spent in the central zone compared to adjacent zones ($n = 40$, $P < 0.05$ in all cases) can be explained by the smaller area of the central zone.

5.3.1.2 Distance moved

Cockroaches maintained a consistent level of walking activity within the untreated zone as the voltage potential was raised (Fig. 5.3, $F_{5,\,120} = 0.996$, $P > 0.05$), yet travelled less distance in the treated zone ($F_{5,\,118} = 5.841$, $P < 0.05$). Post hoc analysis reveals this effect was most apparent at 3kV potentials and above (d.f. = 118, $P > 0.05$ in all cases). Cockroaches walked further when out of the field than when exposed within treated zone to 3kV potentials and higher ($t = -2.079$; $t = -4.17$; $t = -3.18$, $n = 40$, $P < 0.05$ in all cases).

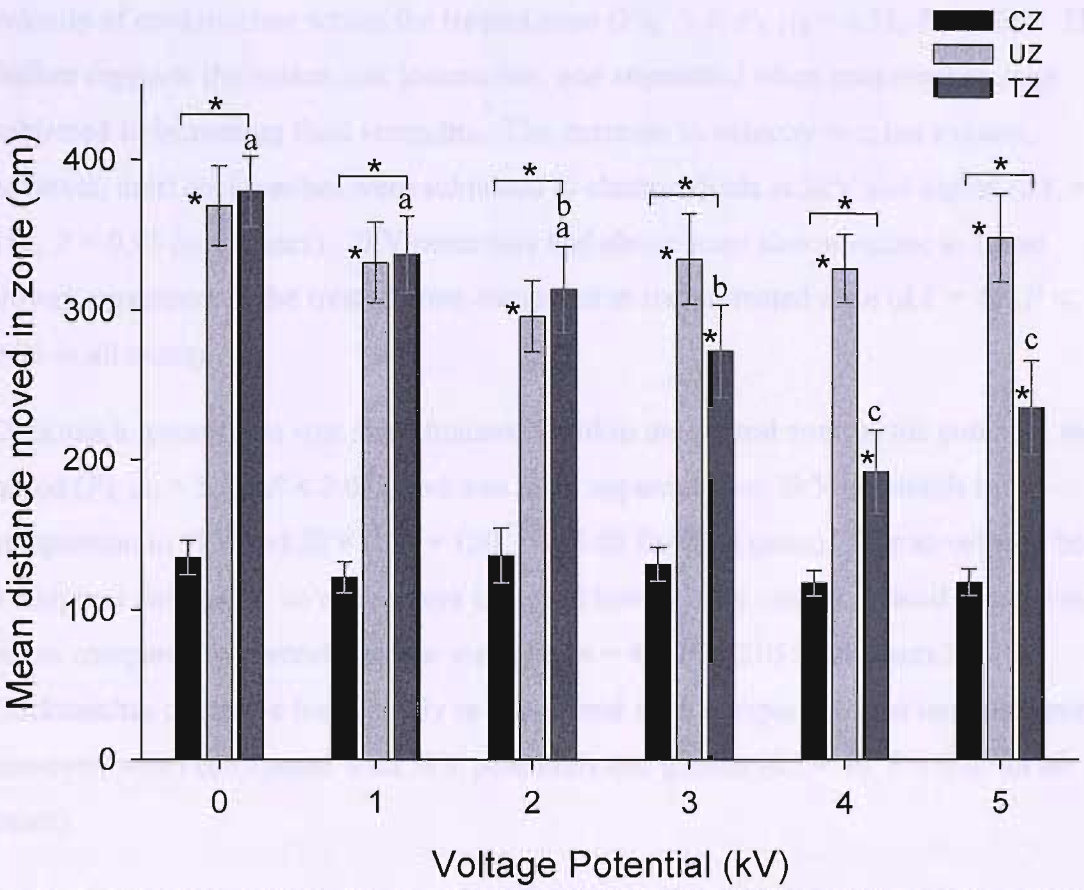


Figure 5.3 The effect of voltage potential on the mean distance cockroaches moved (±SEM) in each zone of the arena. Distance moved in the treated zone significantly decreased as the voltage potential was raised ($F_{5,\,118} = 5.84$, $P < 0.05$), apparent at 3kV potentials and above (d.f = 118, $P < 0.05$ in both cases). In addition, significantly less distance was moved in the treated zone at 3kV and above (represented by asterisks). The distance moved was significantly less in the central zone compared to the treated and untreated zones due to the differences in zone size.

The distance that cockroaches travelled was only affected when in the electric field, as the distance travelled by individuals confronted with an electric field in the central zone did not differ as the voltage potential was raised ($F_{5,\,120} = 0.77$, $P > 0.05$). Together, these results demonstrate that locomotion was attenuated in cockroaches subjected within, rather than confronted by, a static electric field.

5.3.1.3 *Velocity*

Increasing the voltage potential was correlated with a significant decrease in the velocity of cockroaches within the treated zone (Fig. 5.4, $F_{5,\,118} = 6.38$, $P < 0.05$). This further supports the notion that locomotion was attenuated when cockroaches were subjected to increasing field strengths. The decrease in velocity was not evident, however, until cockroaches were subjected to electric fields at 2kV and higher (d.f. = 118, $P < 0.05$ in all cases). 2kV potentials and above were also adequate to cause slower movement in the treated zone compared to the untreated zone (d.f. = 40, $P < 0.05$ in all cases).

Cockroach locomotion was also attenuated within the central zone as the potential was raised ($F_{5,\,120} = 5.82$, $P < 0.05$), and was most apparent from 3kV potentials in comparison to 1kV and 2kV (d.f. = 120, $P > 0.05$ for both cases). Due to velocity being a temporal parameter, no differences occurred between the central, treated or untreated zones compared to control and low voltages ($n = 40$, $P > 0.05$ in all cases.). Cockroaches did move less quickly in the central zone compared to the untreated zone, however, when confronted with 3kV potentials and greater (d.f = 40, $P < 0.05$ in all cases).

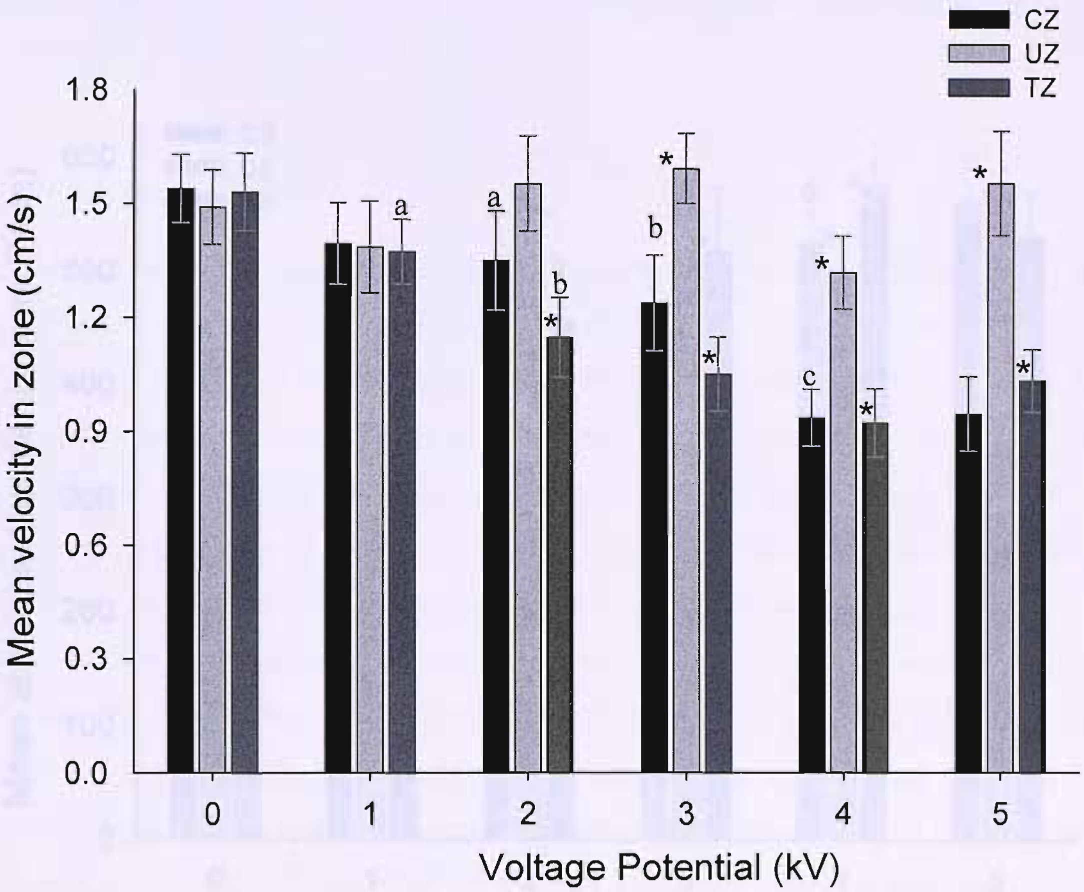


Figure 5.4 The effect of voltage potential on the mean velocity (±SEM) of cockroaches in each zone of the arena. A significant effect of voltage potential on velocity occurred in the central (CZ) and treated (TZ) zones ($F_{5,\,120} = 5.82$, $P < 0.05$; $F_{5,\,118} = 6.38$, $P < 0.05$ respectively). Cockroach velocity in the untreated (UZ) zone was not affected by field strength. Different lowercase letters represent significant decreases in the velocity within the central and treated zone as the voltage potential was altered. Asterisks represent significant differences between zones.

5.3.1.4 Absolute meander

Cockroaches exhibited greater turning, or sinuosity, when subject to electric fields within the treated zone as the field strength was raised (Fig. 5.5, $F_{5,\,120} = 11.8$, $P < 0.05$). Raising the field strength was also correlated with greater meander within the central zone ($F_{5,\,118} = 8.873$, $P < 0.05$). Hence, cockroach sinuosity increased when individuals were positioned both within and confronted by static electric fields.

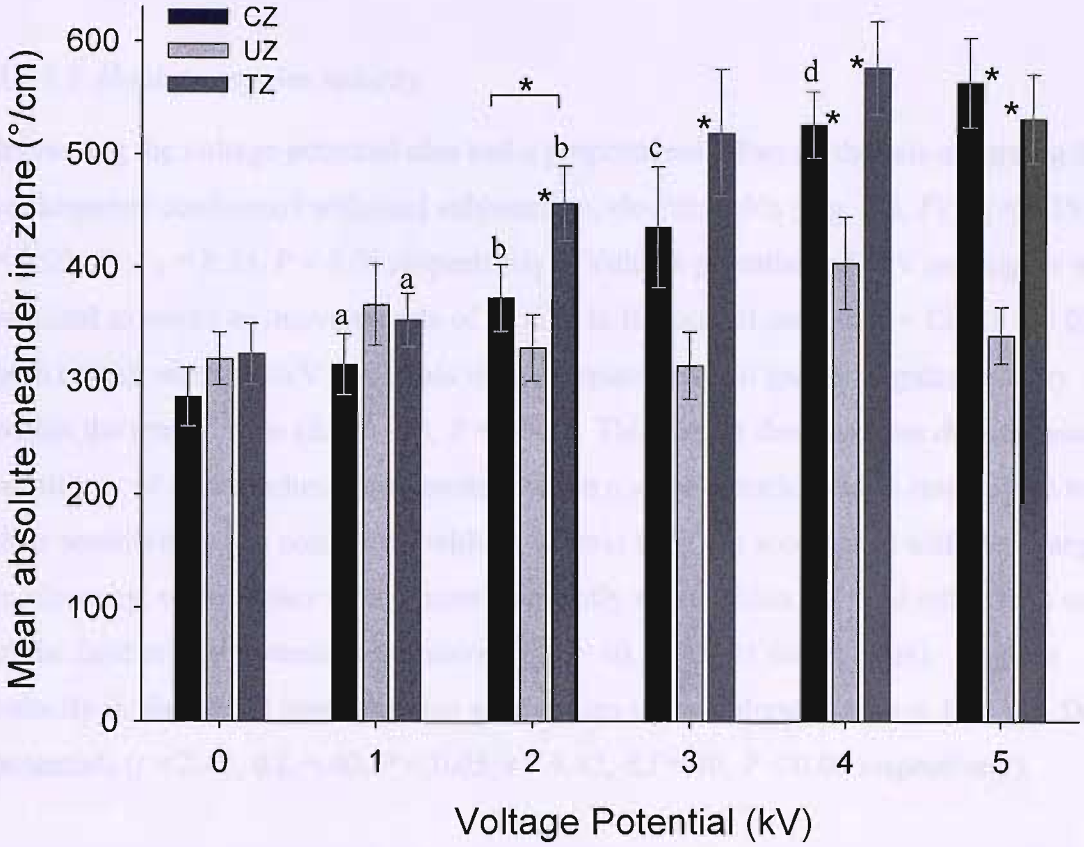


Figure 5.5 The effect of voltage potential on the mean absolute meander (±SEM) of cockroaches in each zone of the arena. Raising the voltage potential significantly increased meander in both the central (CZ) and treated (TZ) zones ($F_{5,120} = 11.8$, $P < 0.05$; $F_{5,118} = 8.87$, $P < 0.05$ respectively), yet meander in the untreated zone was not affected. 2kV potentials and above significantly increased cockroach meander within the central and treated zones (d.f. = 120, $P < 0.05$; d.f. = 118, $P < 0.05$ in all cases respectively), as represented by lower case letters. Differences between the zones, represented by asterisks, indicate increased sinuosity in the treated zone compared to the central or untreated zones when a 2kV potential was applied ($t = 2.39$, d.f. = 42, $P < 0.05$; $t = 3.17$, d.f. = 42, $P < 0.05$). Meander was greater in the central zone than the untreated zone at 4kV potentials and above ($t = 1.99$, d.f = 42, $P < 0.05$; $t = 4.04$, d.f = 42, $P < 0.05$).

The increased turning was apparent within both the treated zone and central zone when cockroaches were subjected to and confronted with 2kV potentials (d.f = 118, $P < 0.05$ in all cases). Meander was significantly greater in cockroaches subjected to the field than when out of the field at 2kV potentials and above (d.f. = 40, $P < 0.05$ for all cases). Higher voltage potentials (≥4kV), however, were required to elicit more turning in cockroaches positioned within the central zone than the untreated zone ($t = 1.98$, d.f. =

42, $P < 0.05$; $t = 4.04$, d.f. = 42, $P < 0.05$ respectively). This could be attributed to localisation of the field within the treated zone.

5.3.1.5 Absolute angular velocity

Increasing the voltage potential also had a proportional effect on the rate of turning in cockroaches confronted with, and subjected to, electric fields (Fig. 5.6, $F_{5,\,120} = 9.19$, $P < 0.05$; $F_{5,\,118} = 8.33$, $P < 0.05$ respectively). Voltage potentials of 4kV and higher were required to evoke an increased rate of turning in the central zone (d.f. = 120, $P < 0.05$ in both cases), whereas 2kV potentials were adequate to elicit greater angular velocity within the treated zone (d.f. = 118, $P < 0.05$). This further demonstrates the increased sensitivity of cockroaches when located within a static electric field in comparison to their sensitivity when confronted with an electric field. In accordance with the changes in sinuosity, cockroaches turned more frequently when within the field rather than out of the field at 2kV potentials and above (d.f. = 40, $P < 0.05$ for all cases). Angular velocity in the central zone was also greater than in the untreated zone at 4kV and 5kV potentials ($t = 2.43$, d.f. = 40, $P < 0.05$; $t = 4.42$, d.f = 40, $P < 0.05$ respectively).

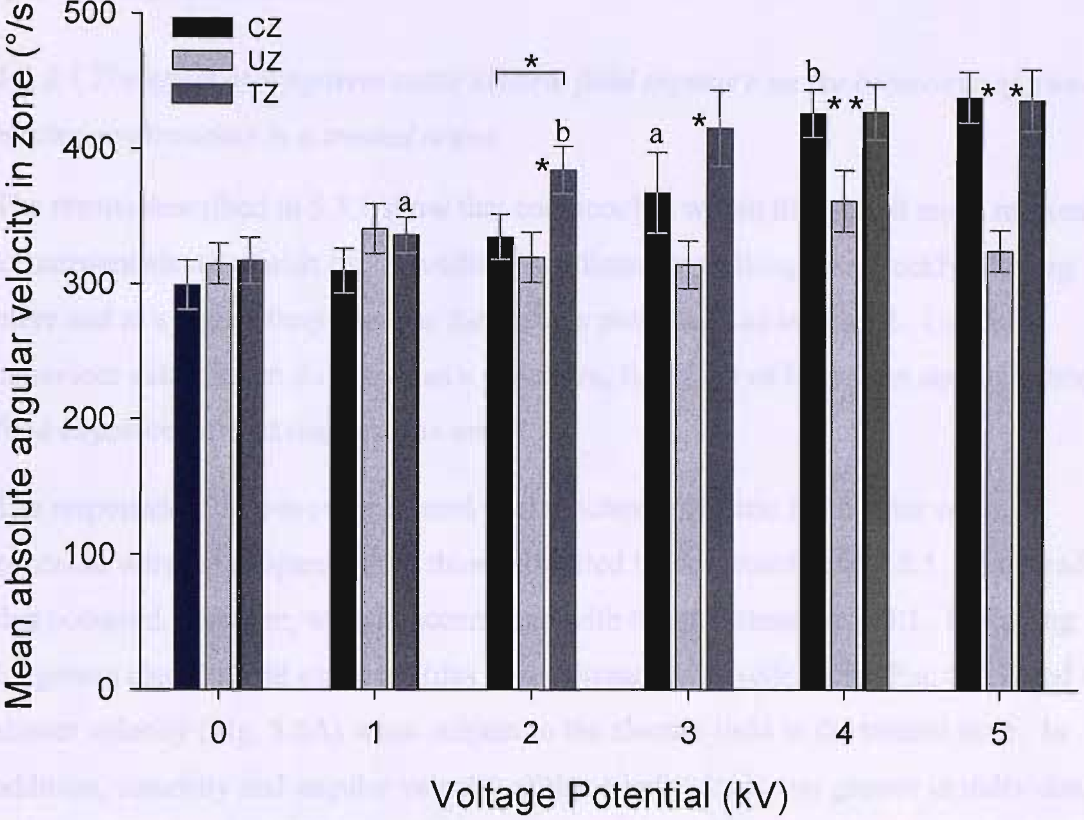


Figure 5.6 The effect of voltage potential on the mean angular velocity (±SEM) of cockroaches in each zone of the arena. Voltage potential significantly increased angular velocity in the central (CZ) and treated (TZ) zones ($F_{5,\,120} = 9.19$, $P < 0.05$; $F_{5,\,118} = 8.33$, $P < 0.05$ respectively), yet angular velocity in the untreated zone was not affected. Significant increases in angular velocity occurred in the central zone at 4kV and above (d.f = 120, $P < 0.05$, represented by different lowercase letters). Angular velocity significantly increased within the treated zone when a 2kV potential was applied (d.f = 118, $P < 0.05$). Angular velocity of cockroaches in the treated zone was greater than the central and untreated zone at 2kV ($t = 2.58$, d.f. = 40, $P < 0.05$; $t = -2.88$, d.f. = 40, $P < 0.05$ respectively, represented by asterisks). 4kV potentials were required to elicit greater angular velocity within the central zone compared to the untreated zone ($t = 2.43$, d.f. = 40, $P < 0.05$; $t = 4.42$, d.f = 40, $P < 0.05$ respectively).

5.3.2 The effect of long-term static electric field exposure on the behaviour of free-moving cockroaches

5.3.2.1 The effect of long-term static electric field exposure on the behaviour of free-moving cockroaches in a treated arena

The results described in 5.3.1 show that cockroaches within the treated arena responded to transient electric fields by: travelling less distance; walking less quickly; turning more and at a higher frequency, as the voltage potential was increased. Using the behaviour exhibited in this assay as a paradigm, the effect of long-term static electric field exposure on cockroaches was tested.

The responses of free-moving treated cockroaches to electric fields after constant exposure were less apparent than those exhibited by cockroaches in 5.3.1. The trends that occurred, however, were in accordance with those obtained in 5.3.1. Preceding long-term electric field exposure (day 0), cockroaches travelled less (Fig. 5.7B) and at a slower velocity (Fig. 5.8A) when subject to the electric field in the treated zone. In addition, sinuosity and angular velocity of these individuals was greater in individuals subjected to electric fields (Fig. 5.8B and C). Although there were contrasts in the behaviour between the treated and untreated zones, the differences were not significant (Appendix 8.3A and B). This lack of difference might be attributable to noise obscuring the results. Carrying out additional trials may have limited this effect and reduced variation, but was not feasible due to time restrictions. Nevertheless, these contrasts were maintained throughout the duration of the experiment. Differences between the zones of some behavioural parameters became more prominent after 18 days of continuous electric field exposure. Notably, the mean velocity in the treated zone was significantly less than in the untreated zone at days 18 and 22 (Fig. 5.8A, $t = -3.31$, d.f. = 10, $P < 0.05$; $t = -2.27$, d.f. = 10, $P < 0.05$ respectively). Additionally, meander significantly increased in the treated zone at day 18 (Fig. 5.8B, $t = 3.16$, d.f. = 10, $P < 0.05$). These effects were only short-lived, however, and were not maintained for the remaining duration of the experiment.

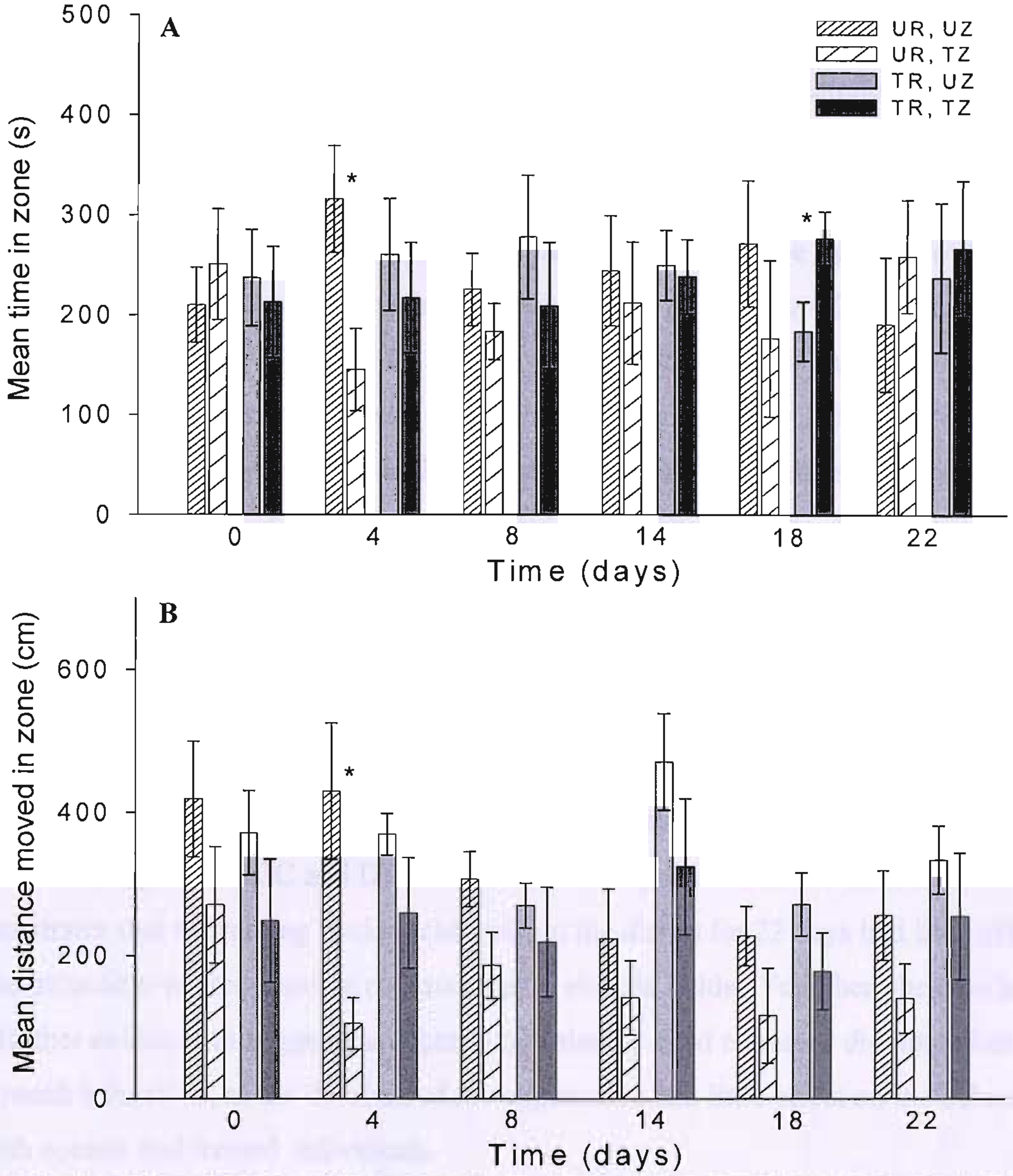
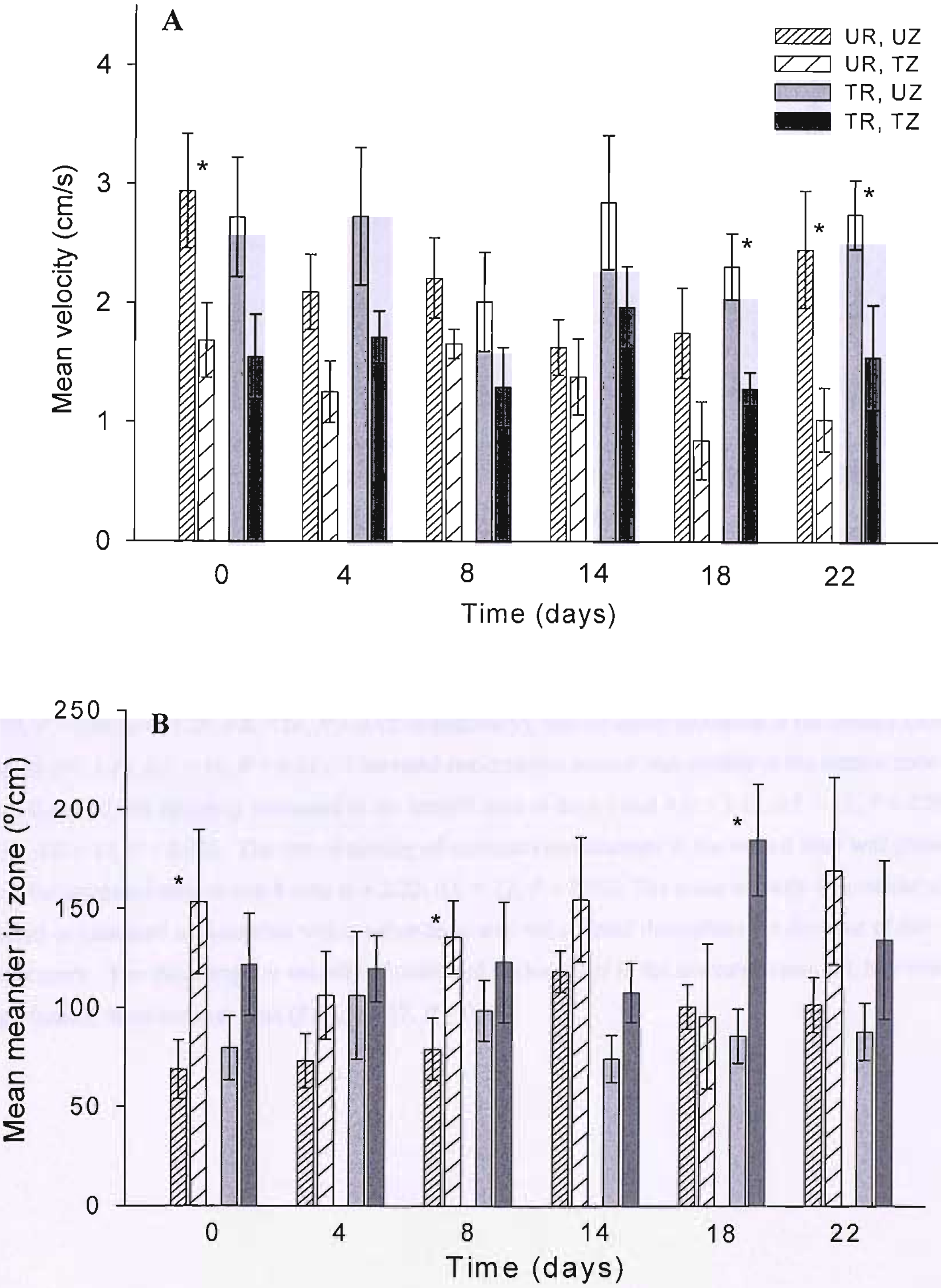


Figure 5.7 The mean time spent (±SEM) and distance moved (±SEM) of untreated (UR) and treated (TR) cockroaches in a treated arena at 4kV. A trend for both treated and untreated cockroaches to (A) spent less time and (B) travel less in the treated zone occurred throughout the experiment. Treated cockroaches did, however, spend significantly more time in the treated zone at day 18 ($t = 2.34$, d.f. = 10, $P < 0.05$, represented by asterisk). Untreated cockroaches spent significantly more time and travelled further in the untreated zone at day 4 ($t = 2.53$, d.f. = 12, $P < 0.05$; $t = -3.178$, d.f. = 12, $P < 0.05$ respectively). There was no relationship between (A) the mean time spent or (B) the mean distance moved in the treated (TZ) or untreated (UZ) zones as exposure time to electric fields increased. Retaining untreated cockroaches for the duration of the experiment also had no effect on the mean time spent and distance moved within the treated or untreated zones.

Untreated cockroaches (those not continuously exposed to electric fields) also exhibited contrasting behaviours between the zones during the experiment (Fig. 5.7 and 5.8) that were similar to those exhibited by treated cockroaches. This demonstrates that long-term electric field exposure did not affect cockroach behaviour. Untreated cockroaches did exhibit significantly different behaviours between the zones at earlier time periods than treated cockroaches. For example, the time spent and distance travelled out of the field was significantly less than within the field at day 4 (Fig. 5.7, $t = 2.53$, d.f. = 12, $P < 0.05$; $t = -3.178$, d.f. = 12, $P < 0.05$ respectively). Untreated cockroaches also turned significantly more in the treated zone at day 0 and 8 (Fig. 5.8B, $t = 2.22$, d.f. = 12, $P < 0.05$; $t = 2.33$, d.f. = 12, $P < 0.05$) and the angular velocity was greater in the treated zone at day 8 (Fig. 5.8C, $t = 2.33$, d.f. = 12, $P < 0.05$). Nevertheless, differences in the behavioural parameters between the zones did remain consistent throughout the duration of the experiment.

Time did, however, have a significant effect on the mean angular velocity of cockroaches within the untreated zone increased as the experiment progressed ($F_{5,\,33} = 2.57$, $P < 0.05$), yet no significant effect of time was seen in any other behavioural parameters (Appendix 8.3C and D). The absence of significant trends therefore demonstrates that restraining cockroaches within the dishes for 22 days had little effect on the behaviour of free-moving cockroaches to electric fields. Together, the data here add further evidence to suggest that continuous electric field exposure did not affect cockroach behaviour, as the duration of the experiment had little effect on the behaviour of both control and treated individuals.


A
UR, UZ
UR, TZ
TR, UZ
TR, TZ
Mean velocity (cm/s)
0
1
2
3
4
0
4
8
14
18
22
Time (days)
*
*
*
*
B
Mean meander in zone (º/cm)
0
50
100
150
200
250
0
4
8
14
18
22
Time (days)
*
*
*

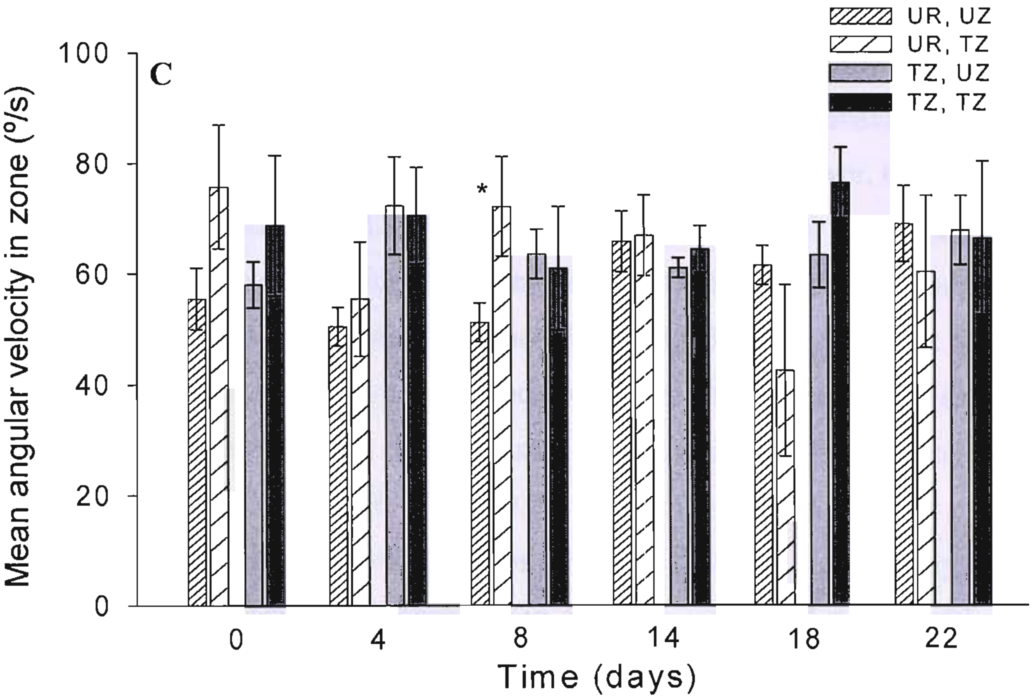


Figure 5.8 The mean velocity, meander and angular velocity (±SEM) of untreated (UR) and treated (TR) cockroaches in a treated arena. Both untreated and treated cockroaches commonly (A) moved slower, (B) turned more per cm and (C) turned more frequently in the treated zone throughout the experiment. The velocity of treated cockroaches was significantly less in the treated zone at days 18 and 22 ($t = -3.31$, d.f. = 10, $P < 0.05$; $t = -2.27$, d.f. = 10, $P < 0.05$ respectively), and sinuosity increased in the treated zone at day 18 ($t = 3.16$, d.f. = 10, $P < 0.05$). Untreated cockroaches moved less quickly in the treated zone at days 0 and 22 and sinuosity increased in the treated zone at days 0 and 8 ($t = 2.22$, d.f. = 12, $P < 0.05$; $t = 2.33$, d.f. = 12, $P < 0.05$). The rate of turning of untreated cockroaches in the treated zone was greater than the untreated zone at day 8 only ($t = 2.22$, d.f. = 12, $P < 0.05$). The mean velocity or meander of treated or untreated cockroaches within either zone was not affected throughout the duration of the experiment. The mean angular velocity of untreated cockroaches in the untreated zone did, however, significantly increase over time ($F_{5,33} = 2.57$, $P < 0.05$).

5.3.2.2 Comparisons between studies

As described, long-term electric field exposure did not manifest significant changes in the behaviour of free-moving cockroaches to electric fields. The behavioural responses of treated cockroaches to electric fields revealed similarities, however, to the responses established in the paradigm (5.3.1). The distance moved, meander and angular velocity of free-moving cockroaches before and after long-term electric field exposure was similar to naïve cockroaches tested in the paradigm (Fig. 5.9A-C, Appendix 8.3E). In addition, the mean time spent by treated cockroaches in either zone before (day 0) and after (day 22) long-term electric field exposure did not significantly differ to naïve cockroaches (Fig. 5.9D, $F_{2,32} = 0.74$, $P > 0.05$; $F_{2,33} = 0.34$, $P > 0.05$). The mean velocity of free-moving treated cockroaches out of the field was significantly greater than that exhibited by naïve cockroaches (Fig. 5.9E, $F_{2,31} = 4.6$, $P < 0.05$), although both exhibited increases.

Overall, the effects exhibited by treated cockroaches were not as apparent as those in naïve animals, presented in the paradigm. Nevertheless, the similarities in behaviour between studies highlight the consistency of the model used to investigate behavioural responses of free-moving cockroaches to electric fields. These comparisons also reiterate that continuous electric field exposure, at the levels used in this study, did not appear to affect cockroach behaviour.

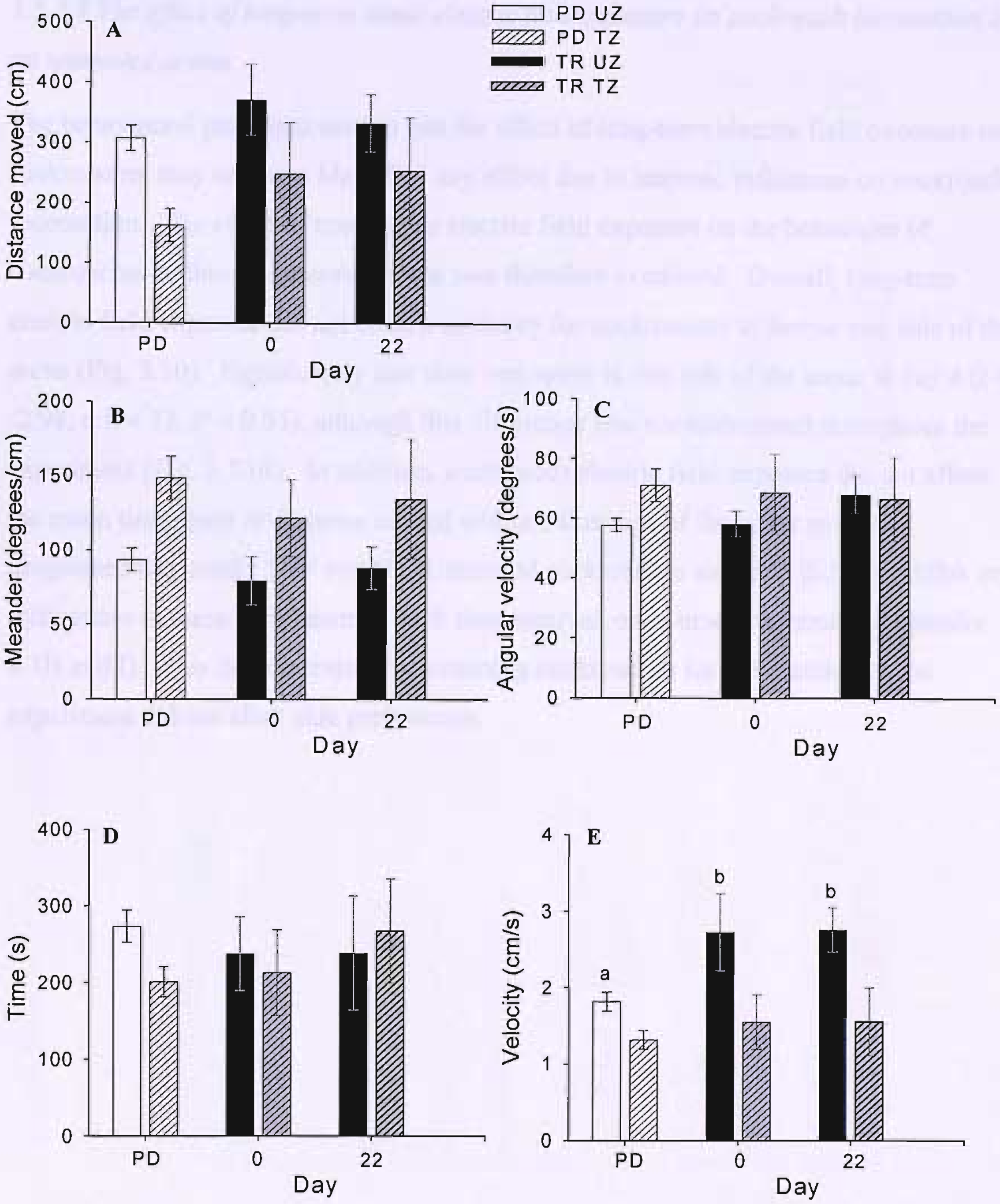


Figure 5.9 Comparisons of the behavioural responses of treated cockroaches (TR) before (day 0) and after (day 22) long term electric field exposure with those elicited by cockroaches in the paradigm (PD) described in 5.3.1. No differences in (A) the mean distance moved (±SEM), (B) meander, (C) angular velocity or (D) mean time spent in the treated (TZ) or untreated (UZ) zones of occurred between treated cockroaches and those tested in 5.3.1. (E) The mean velocity (±SEM) of treated cockroaches within the untreated zone was significantly greater than exhibited by cockroaches in 5.3.1, ($F_{2,31} = 4.6$, $P < 0.05$, represented by different lower case letters).

5.3.2.3 The effect of long-term static electric field exposure on cockroach locomotion in an untreated arena

The behavioural paradigm used to test the effect of long-term electric field exposure on cockroaches may not have identified any effect due to intrinsic influences on cockroach locomotion. The effect of continuous electric field exposure on the behaviour of cockroaches within an untreated arena was therefore examined. Overall, long-term electric field exposure did not elicit a tendency for cockroaches to favour one side of the arena (Fig. 5.10). Significantly less time was spent in one side of the arena at day 4 (t = -2.98, d.f. = 12, $P < 0.05$), although this difference was not maintained throughout the experiment (Fig. 5.10A). In addition, continuous electric field exposure did not affect the mean time spent or distance moved within either side of the arena as time progressed (Appendix 8.3F and G). Untreated cockroaches similarly did not exhibit any differences in these parameters at each time interval, or as time progressed (Appendix 8.3H and I). This demonstrated that retaining cockroaches for the duration of the experiment did not elicit side preferences.

[illegible]

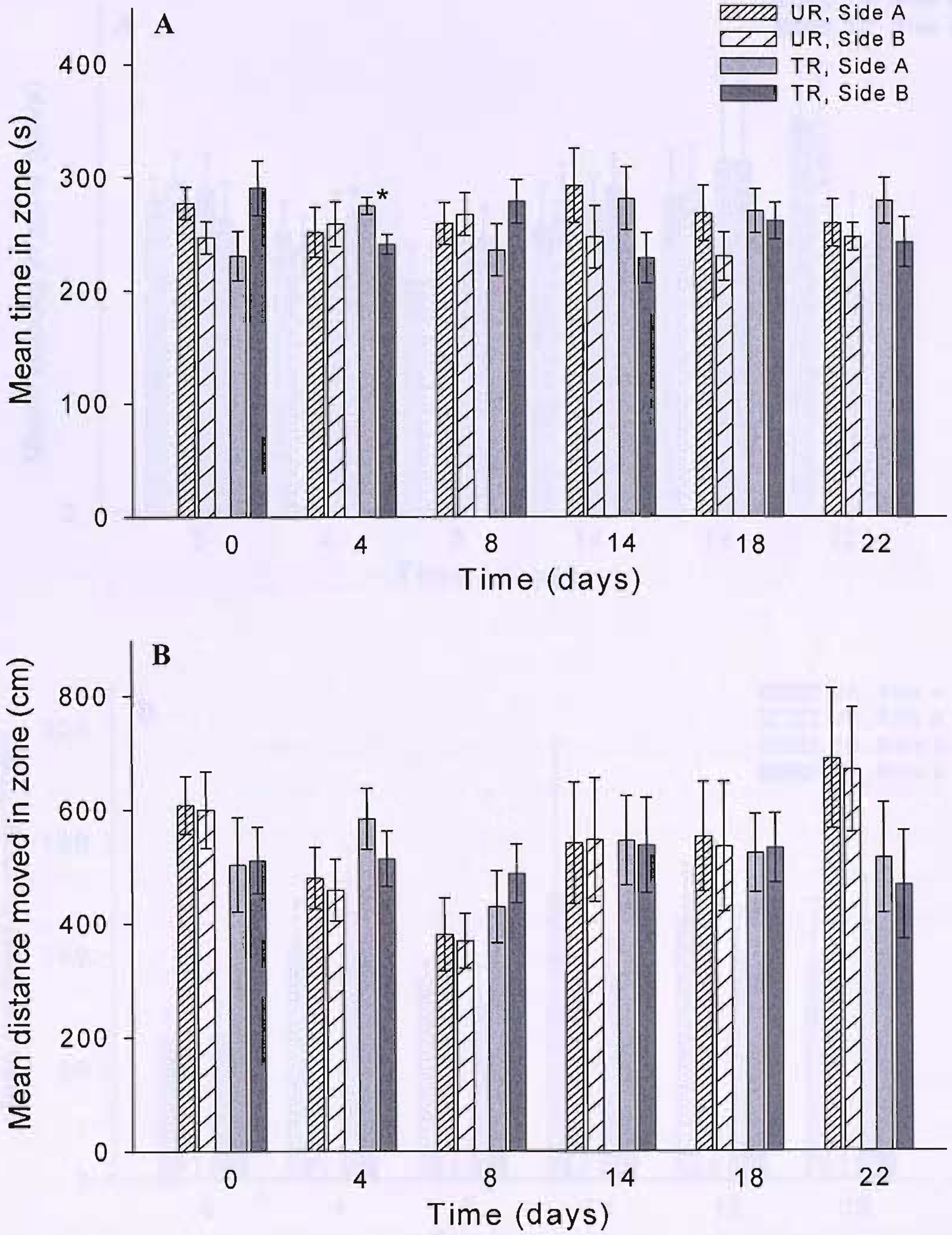
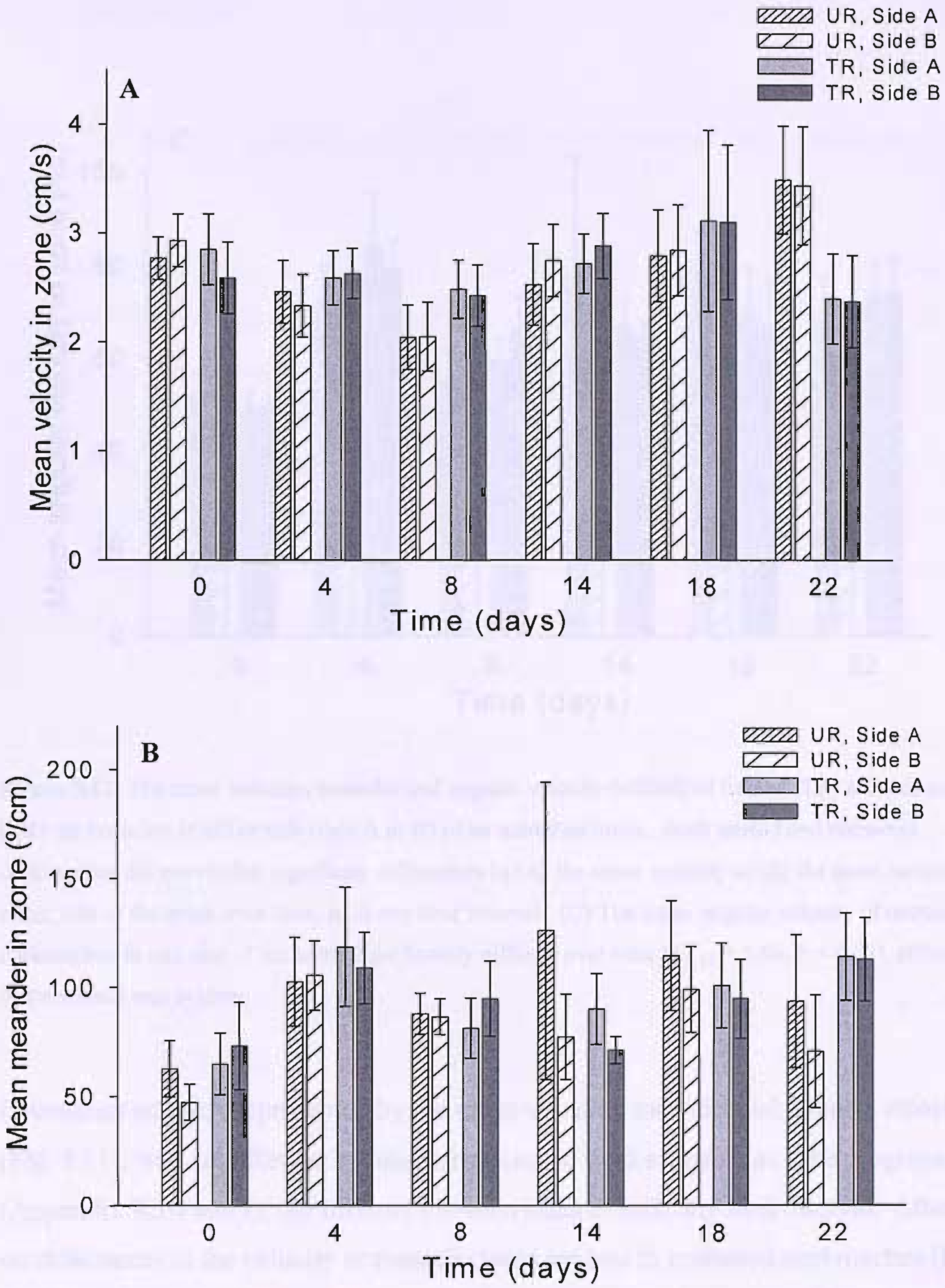


Figure 5.10 The mean time spent (±SEM) and distance moved (±SEM) by untreated (UR) and treated (TR) cockroaches within one side (A or B) of an untreated arena. Treated cockroaches spent significantly less time in side B at day 4 ($t = -2.98$, d.f. = 12, $P < 0.05$) yet this difference was not maintained throughout the experiment. Long-term electric field exposure did not affect the time spent or distance moved within either side of the arena as time progressed. Continuous exposure to electric fields did not therefore elicit a preference for either side of the untreated arena.


A
UR, Side A
UR, Side B
TR, Side A
TR, Side B
Mean velocity in zone (cm/s)
0
1
2
3
4
Time (days)
0
4
8
14
18
22
B
UR, Side A
UR, Side B
TR, Side A
TR, Side B
Mean meander in zone (°/cm)
0
50
100
150
200
Time (days)
0
4
8
14
18
22

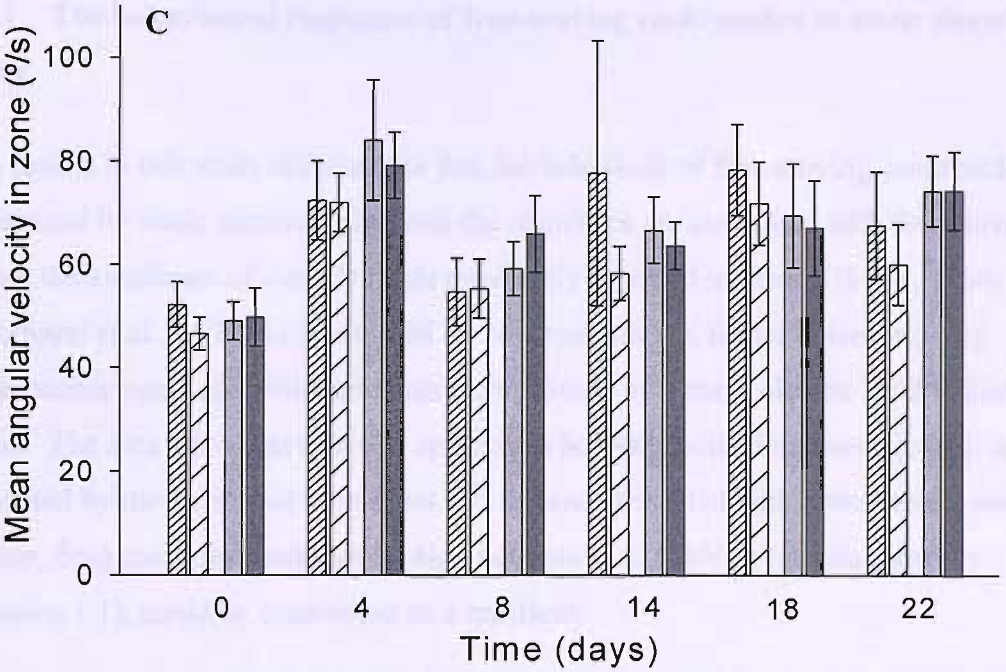


Figure 5.11 The mean velocity, meander and angular velocity (±SEM) of treated (TR) and untreated (UR) cockroaches in either side (side A or B) of an untreated arena. Both treated and untreated cockroaches did not exhibit significant differences in (A) the mean velocity or (B) the mean meander in either side of the arena over time, or at any time interval. (C) The mean angular velocity of untreated cockroaches in one side of the arena significantly differed over time ($F_{5,32} = 2.86$, $P < 0.05$), although no distinct trend was evident.

Locomotor activity, represented by the mean velocity, meander and angular velocity (Fig. 5.11), was not affected by long-term electric field exposure as time progressed (Appendix 8.3H and I), nor differed between sides at each any time interval. Likewise, no differences in the velocity or meander were evident in untreated cockroaches (Fig. 5.11A and B). Retaining cockroaches within the untreated apparatus did elicit a significant effect on the mean angular velocity within one side of the arena (Fig. 5.11C, $F_{5,32} = 2.86$, $P < 0.05$), yet the effect exhibited no clear trend. Together the data indicate that confinement to the apparatus or aging during the experiment did not affect cockroach behaviour.

5.4 Discussion

5.4.1 The behavioural responses of free-moving cockroaches to static electric fields

The results in this study demonstrate that the behaviour of free-moving cockroaches is influenced by static electric fields, and the responses are correlated with field strength. Given the avoidance of electric fields previously reported in insects (Maw, 1964; Perumpral *et al.*, 1978) and exhibited by cockroaches in Chapter 2, free-moving cockroaches could also elicit avoidance confronted by a static electric field within an arena. The data show that this was apparent when 4kV potentials were applied, as indicated by the decreased time spent and distance travelled within the treated zone. Hence, field strengths produced by 4kV potentials, ≈130kV/m (as calculated by equation 1.1), could be considered as a repellent.

At intermediate potentials (2kV and 3kV), cockroaches also travelled less within the treated zone than low potentials, however, this was associated with more time spent in the treated zone. Field strengths produced by intermediate potentials (≈66 – 100kV/m) may have caused momentary, frequent pausing that would have raised the time spent in the treated zone, and therefore not acted as a repellent.

The speed of cockroaches subjected to 66 – 100kV/m static electric fields within the treated zone decreased, supporting the possibility that pausing occurred and movement was intermittent. Velocity continued to decrease as the voltage was raised further, yet given the decreased time spent within the treated zone at > 130kV/m fields, such fields may have caused cockroaches to slow down, rather than elicit more pausing. Such a response is termed as inverse orthokinesis, and commonly occurs in insects in response to odours and mechanical stimuli (Kennedy, 1977). For example, the earwig, *Forficula spp.*, slows down in response to tactile stimulation when approaching a crevice or corner (Fraenkel and Gunn, 1961). In the current study, inverse orthokinesis in response to static electric fields > 66kV/m was also associated with an increase in turning rate within the treated zone, defined as direct klinokinesis (Budenberg, 1991; Fraenkel and Gunn, 1961; Kennedy, 1977). The ortho- and klinokinesis that occurred in the treated zone suggest that static electric fields act as an arrestant (Kennedy, 1978). An arrestant is commonly an olfactory stimulus, for example, the beetle *Teretrius* *nigrescens* slows down and turns more frequently in response to the dust and frass produced by its prey *Prostephanus truncatus* (Stewart-Jones *et al.*, 2005). The results presented in the current study indicate, for the first time, that a non-olfactory stimulus, namely static electric fields, act as an arrestant. Ortho- and klinokinesis can also occur in response to tactile stimulation. For example, earwigs and cockroaches have a tendency to remain in crevices, and do so by thigmotaxis; turning towards and slowing down in response to mechanical contact (Fraenkel and Gunn, 1961; Jeanson *et al.*, 2003). The antennae play an important role in detecting mechanical stimulation and mediating these thigmotactic responses (Camhi and Johnson, 1999; Cowan *et al.*, 2006). Given the influences of electrical forces on the antennae reported by Shimizu and Shimizu (2003, 2004), and described in Chapter 3, the antennae may therefore be responsible for the arrestment of free-moving cockroaches exposed to electric fields.

The responses of free-moving cockroaches within the central zone provide further evidence on how cockroaches orientate to electric fields. The field within the arena would not have been localised to the treated zone, therefore the central zone was a transitional region between no electric field in the untreated zone, and a field in the treated zone. Hence, in contrast to the non-directional effects within the treated zone, cockroaches within the central zone were exposed to directional cues from electric fields within the treated zone. Responses elicited by directional cues are termed taxes (Kennedy, 1977). The behavioural responses exhibited by cockroaches within the central zone included decreased velocity, and both increased turning and rate of turning as the voltage was increased. Although a change in velocity to directional stimuli is not classified as a taxis, changes in turning in response to directional cues are defined as klinotaxis (Budenberg, 1991; Kennedy, 1977). Orientated responses, such as klinotaxis, away from a stimulus source are commonly associated with repellents (Shorey, 1977). Given the increased turning in the central zone at high voltages, this therefore supports the suggestion that electric fields acted as a repellent at high voltages. Another characteristic behaviour in response to repellents, however, is the tendency for an animal to spend less time away from the stimulus source (Shorey, 1977), yet the time spent within the central zone increased as the field strength was raised. As the electric field was not restricted to the treated zone and was also present within the central, the responses within the central zone were not necessarily directed away from the treated zone suggesting that static electric fields cannot conclusively be defined as acting as a repellent.

Nevertheless, the data demonstrates that the locomotion of cockroaches confronted by an electric field was affected, and the behaviour was dependent upon field strength. Understanding the behavioural responses of cockroaches to static electric fields not only advances current knowledge of the influences of electric fields on insects, but is also important in developing pest control methods (Foster and Harris, 1997), and will be discussed further in Chapter 7.

5.4.2 The effect of long-term static electric field exposure on the behaviour of free-moving cockroaches

Intrinsic behavioural responses to stimuli can be affected by adverse environmental conditions or toxins, for example, the escape response of crabs is markedly affected after exposure to toxic metals (Hebel *et al.*, 1997). The detrimental effects of electric fields, such as chromosomal breakage (McCann *et al.*, 1993) and aberrant cell membrane function (Tenforde, 1991), indicate that the behaviour of free-moving cockroaches to electric fields could be influenced by long-term electric field exposure. The results presented in this study show, however, that the behaviour of free-moving cockroaches was not adversely affected during continuous electric field exposure.

From the onset and at any time interval during the experiment, the behavioural responses of free-moving treated cockroaches to electric fields were less apparent than the behaviour exhibited by naïve cockroaches in the paradigm. Increasing the number of individuals tested may have reduced variability, yet this was not feasible as all bioassays had to be carried out in a single day. Nevertheless, comparisons between naïve cockroaches, and those continuously exposed to electric fields, demonstrated behavioural similarities. Ultimately, the characteristic behaviours of free-moving cockroaches to static electric fields, such as decreased velocity and distance moved, in addition to increased sinuosity and turning rate in the treated zone, were apparent throughout the duration experiment. As this intrinsic behaviour was not affected by long-term electric field exposure, the results presented here indicate that static electric exposure does not impact the fitness of cockroaches.

The negative controls carried out in this study also provide further evidence that static electric fields do not detrimentally affect cockroaches. Measuring changes in locomotor activity can be used to monitor the environmental influences on an organism's fitness, as fitness is believed to be associated with the ability to respond to stimuli and the behaviour exhibited by animals (Irschick and Garland, 2001). Locomotion is dependent upon a cascade involving sensory, neural and muscular processes (Sherman and Dickinson, 2004). Reports show that such processes can be adversely affected by exposure to electric fields. At the cellular level, membrane transport proteins and cell-surface receptors are disrupted after ELF electric field exposure (Tenforde, 1991), and ELF electric fields also alter muscle function causing aberrant contractions in humans (Funk and Monsees, 2006). Long-term static electric field exposure did not, however, appear to significantly influence cockroach locomotion, and hence, fitness.

An explanation for these results could be provided by the physics within the system. As described, the majority of studies investigating biological effects of electric fields have used ELF electric fields. One fundamental difference between ELF and static electric fields is that the latter do not generate electromagnetic radiation (Hammond, 1997). Therefore, static electric fields do not induce internal electric fields within conductive material (Reiter, 1993), and hence, may explain why no effect was manifested. It is also believed that movement of a conductive object in a high strength static electric field, or air ionisation due corona discharge can give rise to electric fields within the object (McCann *et al.*, 1993; Reiter, 1993). During the experiment, however, it was observed that cockroaches retained within the setups exhibited little activity, nor was the field strength sufficient (> 300kV/m required) to cause air ionisation (Cross, 1987). The physical properties of the system may therefore explain why not effect was apparent. In addition, cockroaches are notably hardy organisms (Bell and Adiyodi, 1981), and possess resilient physiological processes that may not have been sensitive to the field strengths used in this investigation.

Although the data shown here demonstrate that cockroach fitness is not impacted by continuous static electric field exposure, it remains to be determined whether fields greater than those used in this study affect insect fitness, and if an alternative model is susceptible to such fields.

6. THE EFFECT OF ELECTRIC FIELD EXPOSURE ON *DROSOPHILA* FITNESS

6.1 Introduction

There are mounting concerns regarding the biological effects of electric fields on organisms (Repacholi and Greenebaum, 1999). A number of studies have been carried out investigating the impact of many field types on a variety of animals, yet the results remain unclear. Specifically, it is not known to date whether static electric fields are detrimental to animal fitness. Due to its affordability, high fecundity short life-cycle, and genetic tractability, *Drosophila* is widely accepted as a recognised model for laboratory-based fitness studies (Adams *et al.*, 2000; Grotewiel *et al.*, 2005; Le Bourg, 2004). Hence, the current study will, for the first time, use *Drosophila* as a model to examine the effect of continuous static electric field exposure on insect fitness.

The majority of studies investigating the biological effects of electric fields have focussed on two field types: ELF and static electric fields. Of those, the results reported show a combination of adverse and beneficial effects of electric field exposure. Evidence that ELF electric fields are detrimental to animals has been presented from studies into cellular, developmental and behavioural changes during ELF exposure. At the cellular level, chromosomal aberrations during metaphase of bovine lymphocyte division have been reported after exposure to 50Hz, 6.5kV/m electric fields (Dambrosio *et al.*, 1985). Similar effects have been reported in the bone marrow of mice subjected to 50Hz, 170kV/m fields for 24 hours (McCann *et al.*, 1993). Furthermore, the exposure of adult swine to a 60Hz, 30kV/m electric field adversely affects offspring, characterised by increased malformations (Juutilainen, 2005). Additionally, decreased egg production of the parasitoid *Scambus buoliana* continuously subjected to low strength ELF electric fields (80V/m, unknown frequency) has been reported (Maw, 1961a). In contrast, high strength 50Hz electric fields > 300kV/m cause paralysis and ultimately death of *Drosophila* and *C. vicina* (Watson, 1984). ELF electric fields have also elicited adverse behavioural effects on high order animals; primates exhibit stress symptoms, changes in social behaviour and deterioration of locomotion during and after exposure to 60Hz, 30kV/m electric fields (Easley *et al.*, 1991; Rogers *et al.*, 1995);

cattle preferentially move away from electric fields generated by high voltage power lines (Ganskopp *et al.*, 1991). Electric fields generated by high voltage power lines are also thought to impose health risks to humans, given the apparent correlation between human cancer incidence and proximity of living to high voltage power lines (Draper *et al.*, 2005). It has yet to be determined what elicits these effects, although ELF electric fields and air ionisation due to high strength static electric fields are believed to be contributing factors (Draper *et al.*, 2005; Fews *et al.*, 1999a; Fews *et al.*, 1999b).

ELF electric fields have also been reported to evoke "favourable" cellular changes, such as: raised protein synthesis in mammalian fibroblasts (McLeod *et al.*, 1987); increased DNA transcription (Goodman, 1983); and enhanced plant cell growth (Stenz *et al.*, 1998).

These results described above contrast additional studies, however, that show no adverse effects of ELF electric fields on animals, both at the cellular level, and during development using similar frequencies and field strengths (reviewed in Juutilainen, 2005; McCann *et al.*, 1993).

Studies investigating the effects of static electric fields on organisms have also presented mixed results. At the cellular level, static electric fields 150 – 330kV/m cause increased chromosome mutations in *Drosophila* (Portnov *et al.*, 1975). Male mice cells exposed to 15kV/m – 70kV/m fields also exhibit chromosomal aberrations during cell division (McCann *et al.*, 1993). There are also indications that static electric fields adversely affect invertebrate development, for example, larvae of the phantom hemlock looper, *N. phantasmaria*, take longer to emerge and adult female fecundity decreases during exposure to 18kV/m static electric fields (Edwards, 1961). There are also reports that static electric fields detrimentally affect honey bee colonies beneath high voltage power lines, exemplified by queen loss and decreased foraging (Greenberg *et al.*, 1981a; Greenberg *et al.*, 1981b). 30kV/m or 185kV/m static electric fields do not, however, elicit mutations in *Drosophila* larvae or adults (Diebolt, 1978; Hungate and Richardson, 1978), and behavioural contradictions have been observed in insects exposed to static electric fields varying from 1kV/m to 100kV/m (Edwards, 1960a; Perumpral *et al.*, 1978). Together these studies, measured by a variety of traits across a range of species, highlight the inconsistencies of static electric field effects on organisms reported to date and address the need for further conclusions to be made.

The reproductive success of an organism, and hence its fitness, can be negatively impacted by environmental influences. Inducing chromosomal mutations (Simmons and Crow, 1977), exposing individuals to toxins (Hebel *et al.*, 1997), and stress (Martin and Grotewiel, 2006) are all correlated with reductions in organism fitness. Given the cellular, developmental and behavioural effects of electric fields that have been reported, continuous static electric field exposure could affect the intrinsic physiology of insects, and ultimately impact fitness. There are many different parameters that can be used to measure fitness. Life history traits such as development time, body size, mating success and fecundity are all parameters commonly used to investigate insect fitness, notably that of *Drosophila* (Moller and Thornhill, 1997; Nunney, 1996; Partridge and Fowler, 1993; Simmons and Crow, 1977; Woods *et al.*, 2002). The current study will monitor the life history of *Drosophila* exposed to static electric fields from egg to adult life stage. Using well-established techniques, the investigations carried out will, for the first time, indicate whether continuous static electric field exposure has negative impacts on *Drosophila*, and ultimately insect populations.

6.2 Materials and methods

The *D. melanogaster* strain used, Avigliano, was originally collected from populations in Italy and had been kept under laboratory conditions for 2 ½ years in an outbred population. This strain provided a more robust model in comparison to inbred laboratory strains, hence giving a more accurate representation of wild-type populations. Larvae were cultured in 150ml bottles containing yeast/sugar medium and live baker's yeast. On reaching adulthood, flies were cultured in a 30 x 30 x 30cm Perspex® cage and maintained with water and honey for food, and incubated at 20±1°C in a 16L:8D light regime.

6.2.1 The avoidance of static electric fields at varying strengths

6.2.1.1 Y-tube apparatus

Y-tube bioassays were carried out to investigate whether *Drosophila* exhibited behavioural responses to electric fields. The same Y-tube apparatus described in Chapters 2 and 3 was used (Fig. 6.1), positioned vertically beneath a polarised 40W fluorescent light to encourage upward movement (Le Bourg, 2004). The central and release chamber was also sheathed in black card to further promote vertical movement. One copper ring electrode was connected to a power supply (Brandenburg Alpha II) using high voltage insulated wire.

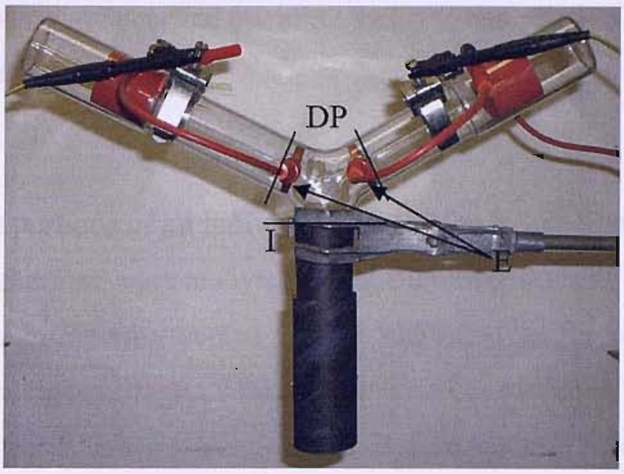


Figure 6.1 Photograph of Y-tube apparatus used to investigate the avoidance behaviour of *Drosophila* to electric fields of varying strengths. Movement towards the treated or untreated chamber was encouraged by covering the central and release chambers with black card. The chamber chosen by each individual was noted after passing the decision point (DP), one body length beyond an electrode (E), in addition to the time taken to travel from the intersection (I) to the decision point.

6.2.1.2 Y-tube bioassays

24hr prior to the bioassays, a random sample of *Drosophila* were taken from culture, sexed and isolated into individual sample tubes ($n = 80$) and their motivational state was raised using standard procedures by not providing food (Wyatt, 1997). Tubes were kept in humid sandwich boxes and incubated at 20±1°C in a 16L:8D light regime (6:00 on: 22:00 off).

Behavioural bioassays

Each individual was presented into the Y-tube apparatus by rapidly opening the sample tube, tapping the fly into the release chamber and attaching it to the central chamber. The power supply was then switched on at the necessary voltage potential: 500V, 1kV, 2kV. The time taken for the fly to make a decision was noted, starting when at the intersection between the two pathways, and finishing when an electrode was crossed (Fig. 6.1). The direction taken (left or right; avoid or non avoid) was also noted. After each trial, the electrode connected to the power supply was swapped to control for side effects. Ten trials were carried out before washing the apparatus, to limit pheromone residue and prevent following effects. Bioassays adhered to a randomised complete block design over two days, each day acting as one block to control for any day effect. Included in the design were trials using no electric fields to test for natural side preference. Experiments were carried out at 22.6±1.5°C and relative humidity 37±8% between 09:30 and 16:00 GMT.

6.2.1.3 Analysis

Differences in the proportions of all individuals taking the left or right pathway, or avoiding the treated chamber were analysed using Binomial Tests of Proportions for each voltage potential, using an expected ratio of 50:50 (S-Plus for Windows, Version 6.1). The effect of voltage on the time taken to make a decision was analysed using one way ANOVA (SPSS for Windows, Version 14). Before analysis data were tested to meet the assumptions of ANOVA using Kolmogorov-Smirnov Test (normality) and Levene's Test of homogeneity (variance). Data were considered significant at the $P < 0.05$ level.

6.2.2 The effect of continuous static electric field exposure on development time

6.2.2.1 Experimental apparatus

Before the experiment began, timed lays were set up to rapidly produce high egg quantities. Approximately 100 male and female adult flies from culture were retained on apple agar plates ($n = 5$). Each plate was smeared with a small amount of live bakers yeast to promote egg laying, and incubated at 20±1°C. After 3hr, 25 eggs were carefully removed from the apple agar plates and, under a dissecting microscope with graticule (Olympus SZX7, x25 magnification), placed into small Petri dishes (5cm diameter, $n = 10$) a 5 x 5 square, 5mm apart, each containing 15ml yeast/sugar medium.

Two experimental apparatus, each hereafter termed 'development setup', were prepared. Each development setup consisted of five glass dishes (190 x 30mm, D x H), and in the centre of each a Petri dish containing eggs was placed (Fig. 6.2). The dishes lay on an aluminium mesh sheet (450 x 550mm), and were covered by an identical mesh. A sheet of glass (400 x 500mm) ensured the aluminium mesh sheets were retained in position.

One development setup was connected to a high voltage power supply (Brandenburg Alpha III), and the other to earth. A 4kV potential was distributed across the top aluminium mesh of the treated setup, and the bottom mesh was earthed to localise the electric field. Field modelling indicated that a 4kV potential produced an electric field strength greater than that generated by 4kV within the Y-tube apparatus. Bioassays demonstrated that *Drosophila* exhibited avoidance at 1kV and above, hence 4kV potentials were expected to elicit effects on *Drosophila* retained within the development setup.

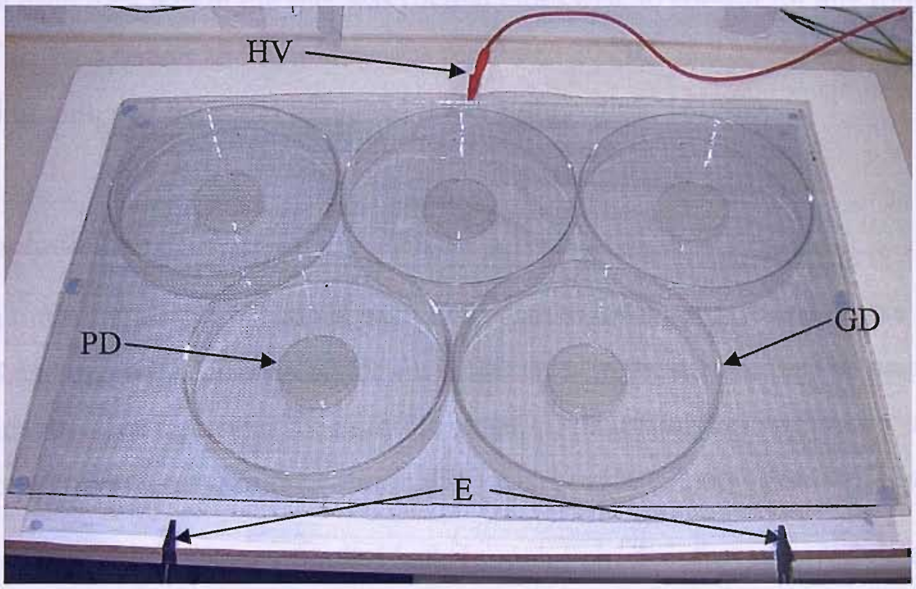


Figure 6.2 Photograph of the development setup used to test for the effect of continuous electric field exposure on development time. Each Petri dish (PD) containing the eggs was positioned within a glass dish (GD). Five glass dishes were placed on top of an aluminium mesh connected to earth (E) and another mesh placed on top connected to the high voltage power supply (HV). Alternatively, the top mesh was connected to earth for the control setup.

6.2.2.2 Experimental protocol

Petri dishes were checked under a dissecting microscope (Olympus SZX7, x25 magnification) every 24hr during egg and larval development. After turning the power supply off, the cumulative times for each individual to hatch and pupate were noted for every dish. Each individual was marked after hatching and pupation to prevent miscounting.

As eclosion approached, pupae were removed from the Petri dish and sexed in preparation for fecundity experiments. Under the microscope, males were identified by the presence of sex combs on the first leg pair, and separated from female pupae into two small glass vials (25 x 75mm). Each pair of vials was then placed into the corresponding glass dish and treatment continued until eclosion when time to eclose was noted and individuals were isolated for further experiments. The mortality at each life stage was also recorded by counting the number of larvae not to hatch, pupate or emerge.

Every 24hr the location of each development setup in the room was alternated and the position of each dish within the sets was rotated clockwise to control for any room effects. The temperature and humidity was regulated 20±1°C and 35±2%RH by an air-conditioning unit, and the room remained under light regime 16L:8D.

6.2.2.3 Analysis

The mean times for all individuals in each Petri dish to hatch, pupate and eclose were calculated and one way ANOVA tested for differences in the mean times between treated and untreated replicates for each life stage. Data assumptions were met after carrying out Kolmogorov-Smirnov (normality) and Levene's (homogeneity) Tests.

Differences in the proportions of individuals in each Petri dish that survived each life stage, termed 'survivorship', were tested using Mann-Whitney U Tests. SPSS for Windows (Version 14) was used and significance identified at the $P < 0.05$ level.

6.2.2.4 Electric field modelling

The electric field in a single glass dish and the field distribution within the enclosed Petri dish was calculated by drawing a '*rz*' symmetrical model, as described in Chapter 2, of the apparatus (Fig. 6.3).

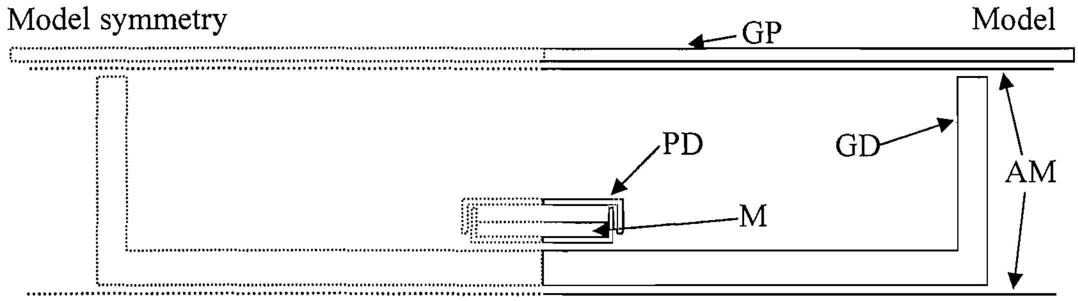


Figure 6.3 Diagrammatic representation of the model used for electric field modelling. The model was calculated using '*rz*' symmetry and therefore half the apparatus was drawn (solid lines). The opposite half of the apparatus (broken lines) was not drawn in the model, but assumed to be present in the calculations. *GP* Glass plate; *GD* Glass dish; *PD* Petri dish; *M* Medium; *AM* Aluminium mesh.

Each object of the apparatus was assigned its appropriate material, and permittivity and conductivity values (Appendix 8.1). The electric field was illustrated by plotting a vector field plot and percentage error of the model was calculated as no greater than 0.01%, as described in 3.2.1.1 (Maxwell SV, Version 7 for Windows).

6.2.3 Continuous electric field exposure and the effect of puparium size and adult body size

6.2.3.1 Method

Puparial case size and thickness is regarded as an accepted measure of fitness (Kraaijeveld and Godfray, 2003). The puparia containing the pupae held within the development setups were therefore kept and dried for several days for further measurements. The length of each puparium (not including pupal horns and anal papillae) and the maximum width was measured to the nearest 0.01mm under a microscope (Olympus SZX7) using an ocular micrometer at x20 magnification. Puparia were also weighed using a microbalance (OHAUS AP250D). Assuming pupae to be an ellipsoid (Fellowes *et al.*, 1998; Kraaijeveld and Godfray, 2003), volume (Equation 6.1) and thickness of each puparium (Equation 6.2) was calculated for pupae exposed (n = 55) and not exposed (n = 54) to electric fields.

Equation 6.1 $$4/3\cdot\pi\cdot(\tfrac{1}{2}W)\cdot(\tfrac{1}{2}L)$$ with W = width
L = length

Equation 6.2 $$\frac{\text{Puparium mass}}{\pi\cdot W\cdot(\tfrac{1}{2}W + (\tfrac{1}{2}L/F)\cdot\text{arcsine}(F/\tfrac{1}{2}L))}$$

with $F = \sqrt{((\tfrac{1}{2}L)^2 - (\tfrac{1}{2}W)^2)}$

Body size of adults that emerged after the development experiment was also recorded. The length from the wing tip to the major costal break (Fellowes *et al.*, 1999) to the nearest 0.01mm was measured under the microscope using an ocular micrometer at 20x magnification for adult flies exposed ($n = 57$) and not exposed ($n = 51$) to an electric field during development.

6.2.3.2 Analysis

Differences in the mean puparium volume and wall thickness of larvae exposed and not exposed to electric fields were analysed using *t*-Tests. Data assumptions were met after carrying out Kolmogorov-Smirnov Tests for normality and Levene's Tests of homogeneity. The effect of electric field exposure during development on the mean body size of adult flies, measured by the wing length, was analysed using a Mann-Whitney U-Test as transformation did not normalise the data. Analyses were carried out in SPSS for Windows, Version 14 and significance identified at the $P < 0.05$ level.

6.2.4 The effect of continuous static electric field exposure on female fecundity

To test whether continuous exposure to static electric fields affects *Drosophila* fitness, the fecundity of females continuously subjected to static electric fields was investigated. Two experiments were carried out examining the fecundity of females exposed to fields during both development and adulthood, and the fecundity of females exposed solely during adulthood. The experimental design therefore consisted of females exposed to electric fields during pre-adult development and adulthood; females exposed during development only; females exposed during adulthood only; and females not exposed during development or adulthood.

6.2.4.1 Experimental apparatus

Four experimental apparatus were used, each hereafter termed 'fecundity setup' (Fig. 6.4), consisting of four glass dishes (190 x 30mm, D x H) sandwiched between two aluminium meshes (500 x 400mm, L x H) and covered with a glass sheet (480 x 380mm, L x H). The bottom aluminium mesh was connected to earth in all four setups, and the top aluminium meshes of two setups were each connected to a high voltage power supply (Brandenburg Alpha II and III) supplying each mesh with a 4kV potential. This potential was used in accordance with 6.2.2 and the results obtained during behavioural bioassays.

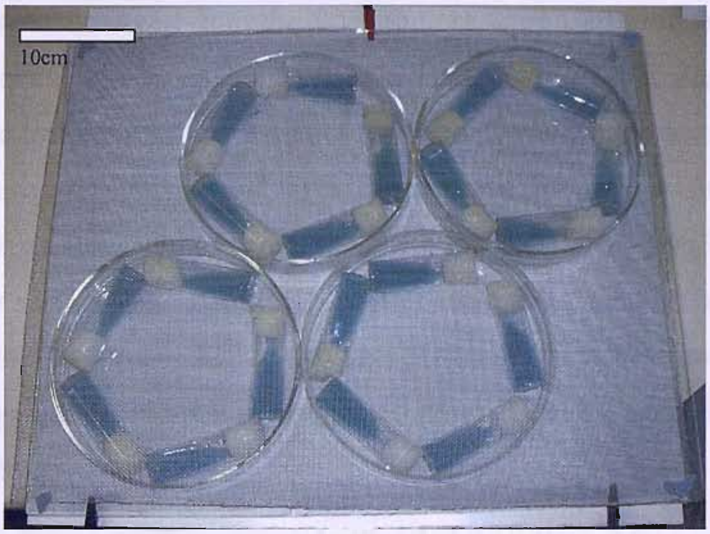


Figure 6.4 Photograph of fecundity setup. Each setup consisted of five vials within four glass dishes positioned on top of an earthed aluminium mesh. Another mesh, connected to the high voltage power supply, was placed on top of the dishes and covered with a glass sheet. Three identical setups tested the effect of electric field exposure during larval development on female fecundity, and exposure during adulthood on fecundity, in addition to controls.

Within each dish five small glass vials (25 x 75mm) containing a virgin female and male pair were equally arranged. Each vial contained 5ml blue coloured yeast/sugar medium (1ml blue food colouring per 500ml medium) set at an angle (Fig. 6.5), with a light smear of live bakers yeast to encourage egg laying.

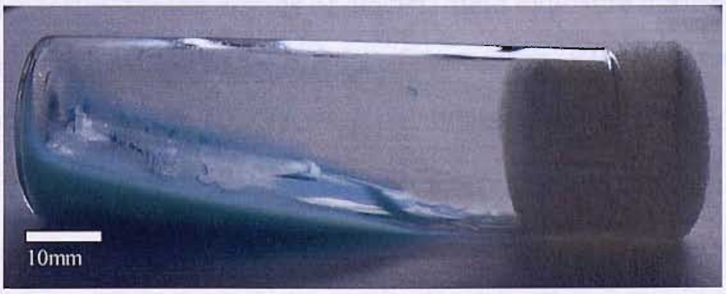


Figure 6.5 Photograph of vial containing yeast/sugar medium set at angle used to investigate effect of electric field exposure on female fecundity.

6.2.4.2 Experimental protocol

To test for the effect of electric field exposure during pre-adult development on fecundity, females that emerged from the development setup were isolated. When, on a single day, an adequate number of female flies had emerged ($n = 40$), flies were introduced into the fecundity setup. Females tested for the effect of electric field exposure solely during adulthood were taken from culture ($n = 40$). A single appropriate female was placed into one of the glass vials, alongside two fertile males taken from culture and labelled accordingly. After arranging vials within the glass dishes and preparing the setups, the high voltage power supplies were turned on. Setups were left in a temperature and humidity regulated room at 20±1°C and 35±2%RH under light regime 16L:8D.

Every 24hr the power supplies were turned off and vials collected for egg counting. Flies were transferred to new vials, dead males replaced from culture and any dead females noted. New vials were placed into the glass dishes, each dish rotated one position clockwise to control for any room effect, and the power supplies turned on. The number of eggs laid was then counted using a thumb counter.

6.2.4.3 Analysis

Female fecundity data were analysed using repeated measures GLM with day as the within-subjects factor, and exposure of electric fields during pre-adult development and exposure during adulthood as between-subjects factors (SPSS for Windows, Version 14). This therefore tested for the effects of electric field exposure during development on female fecundity, in addition to the effects of exposure solely during adulthood on fecundity. Interactions between factors were also analysed to determine whether differences in fecundity occurred between larval and adult exposure.

The mean proportion of female mortality per treatment was then analysed using a Mann-Whitney U-Test (SPSS for Windows, Version 14). Data were considered significant at the $P < 0.05$.

6.3 Results

6.3.1 The avoidance of static electric fields

Control bioassays demonstrated that neither the room nor the apparatus itself had an effect on *Drosophila* choice for the left or right chamber ($n = 20, P > 0.05$). Treating one chamber with a 500V potential resulted in no significant avoidance of the static electric field (Fig. 6.6A, $n = 20, P > 0.05$). Raising the potential to 1kV and 2kV did, however, cause significant avoidance ($n = 20, P < 0.05$ in both cases). *Drosophila* therefore exhibited avoidance of static electric fields, apparent within the Y-tube apparatus using field strengths produced by 1kV potentials and above.

Of the individuals tested, more females avoided the treated chamber than males, yet the proportions of avoidance at 1kV and 2kV potentials were not significantly different between male or female flies (Fig. 6.6B, $\chi^2 = 0.01$, d.f. = 1, $P > 0.05$; $\chi^2 = 2.22$, d.f. = 1, $P > 0.05$ respectively). More females also avoided 500V potentials than males, yet there was no significant difference in the proportions between sexes ($\chi^2 = 1.16$, d.f. = 1, $P > 0.05$). These results need to be considered with caution, however, as the number of individuals tested in this analysis was low. The random samples taken from culture used during the experiment resulted in considerably fewer males than females, possibly explaining the differences in the number of individuals avoiding the field.

Behavioural responses of *Drosophila* to static electric fields of varying strength were also monitored by measuring the time taken to make a decision. The time to pass either the treated or untreated electrode significantly increased as the voltage potential was raised (Fig. 6.7, $F_{2,54} = 3.2$, $P < 0.05$). The time taken to make a decision at 1kV potentials was significantly greater than at 500V (Post hoc LSD, d.f. = 38, $P < 0.05$), yet did not differ between 1kV and 2kV (Post hoc LSD, d.f = 38, $P > 0.05$). These differences support the trend in avoidance described above, and also show that static electric fields acted on *Drosophila,* manifesting changes in the ability of flies to choose one chamber. Taken together these results demonstrate that *Drosophila* exhibited avoidance of static electric fields, and the behavioural responses were dependent on field strength.

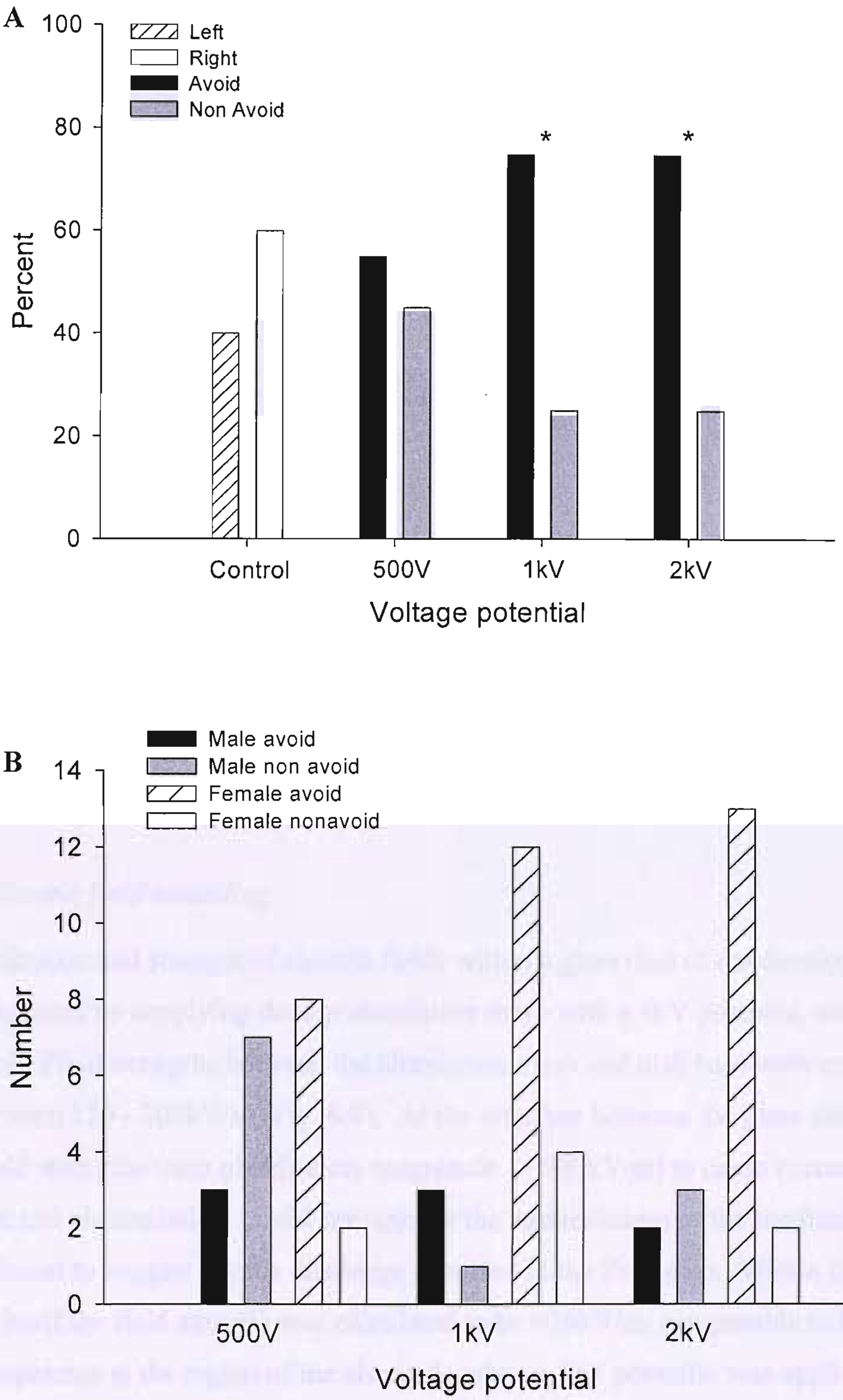


Figure 6.6 Avoidance behaviour of static electric fields exhibited by *Drosophila*. (A) *Drosophila* exhibited no natural side preference within an untreated Y-tube apparatus. A 500V potential did not evoke avoidance, but raising the potential to 1kV and 2kV resulted in significant avoidance ($n = 20$, $P < 0.05$ in both cases, represented by asterisks). (B) No significant differences in avoidance occurred between sexes. These differences could be attributed to the low total number of male flies tested.

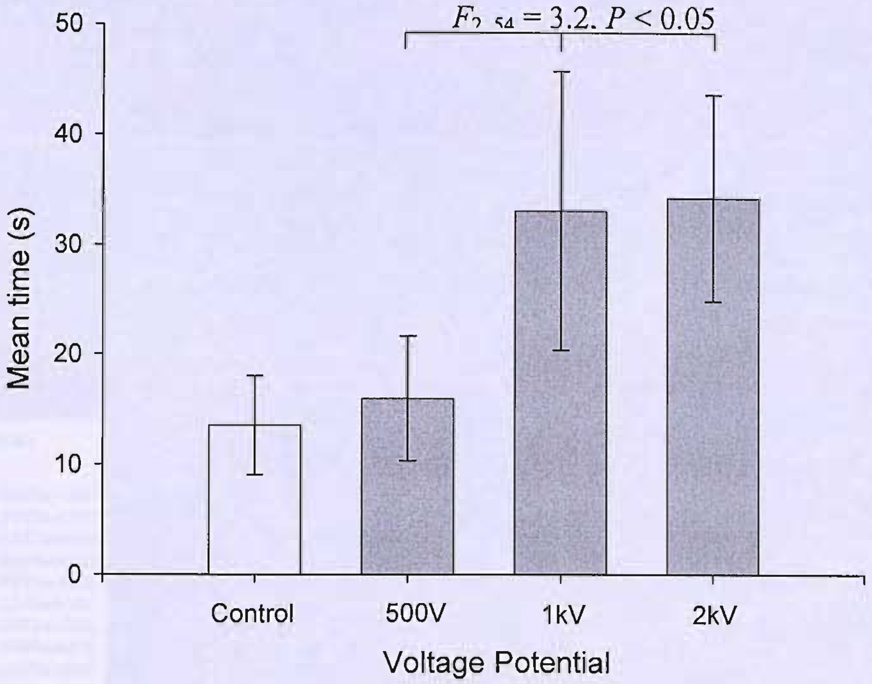


Figure 6.7 The mean time taken (±SEM) to make a decision within the Y-tube apparatus at varying field strengths. As the field strength was raised the mean time taken to make a decision significantly increased.

6.3.2 The effect of continuous static electric field exposure on development

6.3.2.1 Electric field modelling

The distribution and strength of electric fields within a glass dish of the development setup, produced by supplying the top aluminium mesh with a 4kV potential was calculated. Field strengths between the aluminium mesh and dish base were calculated to be between 150 - 200kV/m (Fig. 6.8). At the interface between the glass dish and mesh, field strengths were of sufficient magnitude (> 300kV/m) to cause corona discharge and air ionisation. Field strengths at the surface edges of the medium were also sufficient to suggest corona discharge occurred in the Petri dish. Within the medium itself the field strength was calculated to be ≈36kV/m, comparable to that in the Y-tube apparatus at the region of the electrode when a 4kV potential was applied (≈30kV/m). The field calculations within the medium should be considered with caution, however, as the field surrounding the apparatus was also calculated to be ≈36kV/m, and a static electric field should not be present within an object of high conductivity such as the medium (Appendix 8.1A). The limitations of the field modelling software described in Chapter 2 provide some explanation of this result.

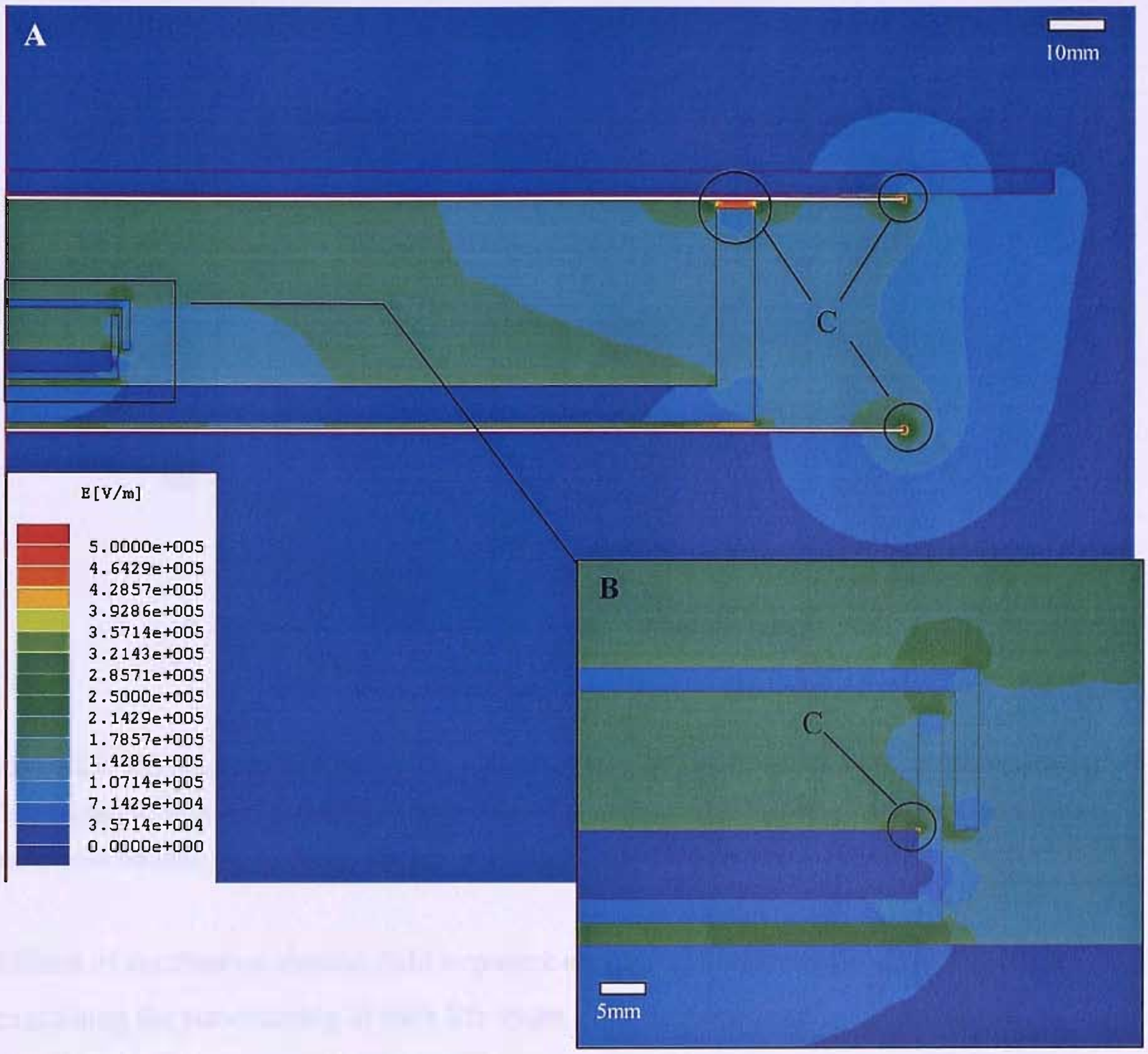


Figure 6.8 Electric field plots of the apparatus used during pre-adult exposure to electric fields. (A) Full model view showing regions of potential corona discharge (C) at the interface between mesh and glass dish. (B) Corona discharge could have also occurred at the surface edges of the medium and Petri dish.

6.3.2.2 Development traits

Continuous exposure of *Drosophila* to electric fields did not affect on the mean development time of eggs (Fig. 6.9, $F_{1,8} = 0.25, P > 0.05$), or larvae ($F_{1,8} = 1.1, P > 0.05$). In addition, there was no significant relationship between time to eclose and exposure to electric fields (Fig. 6.9, $F_{1,8} = 0.04, P > 0.05$). Pre-adult development time was therefore not affected by electric field exposure.

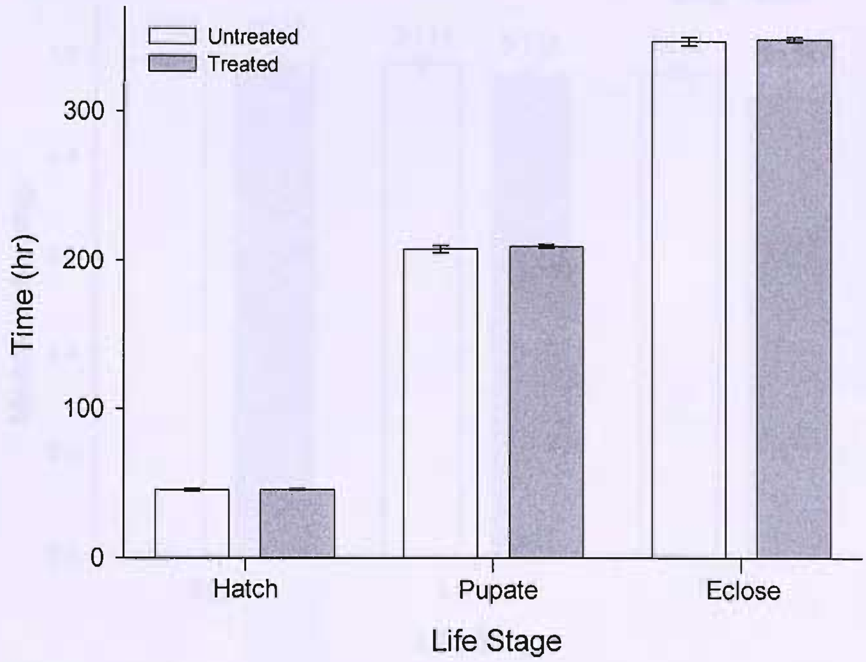


Figure 6.9 The mean time (±SEM) for *Drosophila* to hatch, pupate and eclose during long-term electric field exposure. No significant differences between control and treated individuals occurred, therefore no association between pre-adult development and static electric field exposure was evident.

Effects of continuous electric field exposure on *Drosophila* were also investigated by examining the survivorship at each life stage. The proportion of eggs exposed to electric fields that survived and hatched was not significantly different to unexposed eggs (Fig. 6.10, t = -0.24, d.f = 8, $P > 0.05$). The mean survivorship of larvae and pupae exposed to electric fields was marginally less than untreated individuals, although the differences were not significant (Fig. 6.10, t = -1.13, d.f = 8, $P > 0.05$; t = -0.99, d.f = 8, $P > 0.05$ respectively). Static electric field exposure throughout *Drosophila* development therefore did not affect fly mortality at any life stage.

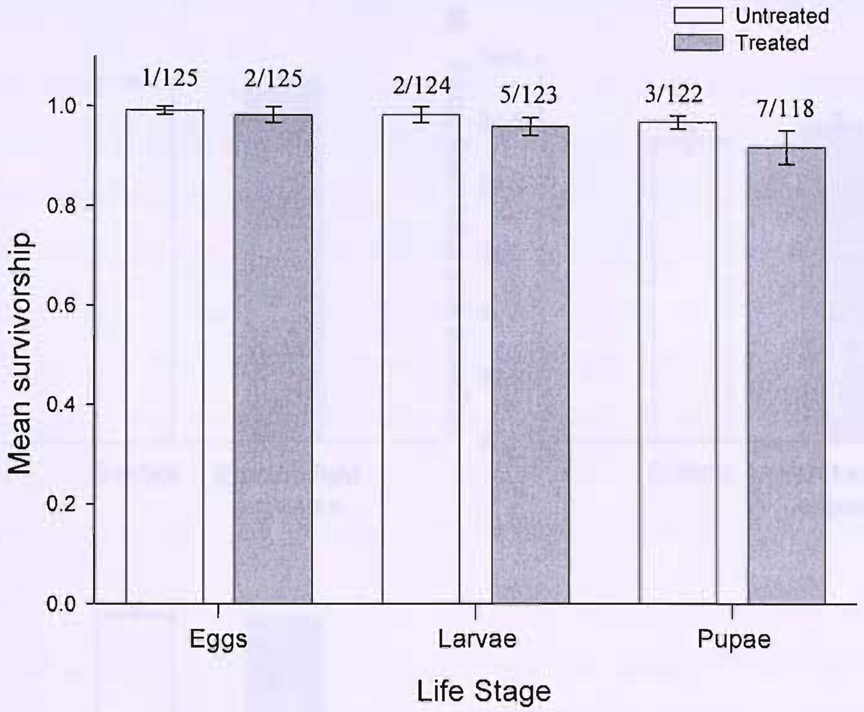


Figure 6.10 Mean survivorship (±SEM) at different life stages of *Drosophila* exposed and not exposed to electric fields. Exposure did not have a significant effect on mortality at the egg, larval or pupal life stages. The number of dead individuals and total sample size are noted above each bar.

6.3.2.3 Puparium and adult body size

The exposure of *Drosophila* to electric fields during pre-adult life stages did not have an effect on the development of pupal cases (Fig. 6.11). Neither the mean puparium size ($t = -0.9$, d.f = 107, $P > 0.05$) nor the mean wall thickness ($t = -0.99$, d.f = 107, $P > 0.05$) was affected by electric field exposure. Measurements of adult wing length also demonstrated no effect of continuous electric field exposure on *Drosophila* body size ($Z = -1.31$, d.f = 101, $P > 0.05$).

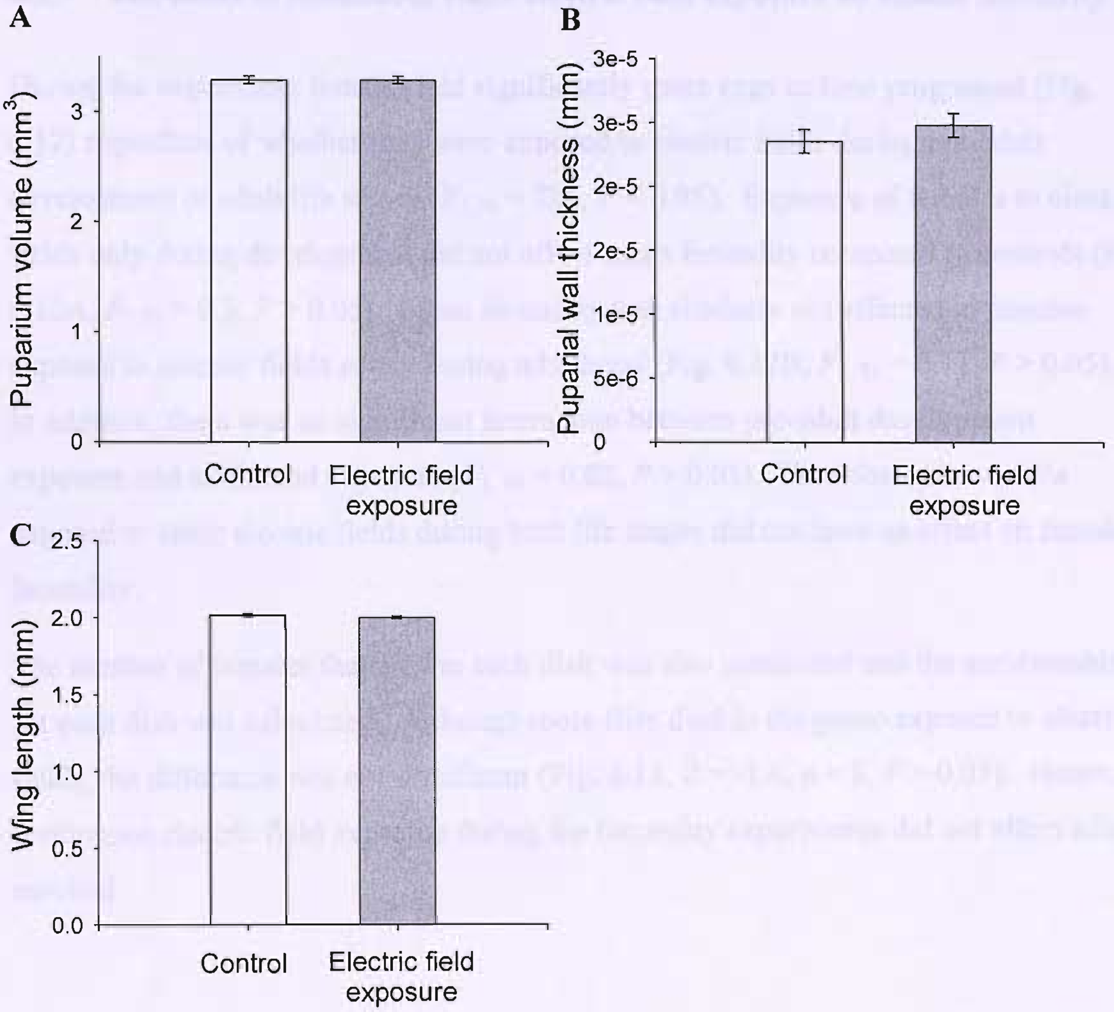


Figure 6.11 The effect of chronic electric field exposure on the development of pupal cases and adult body size. No significant difference in (A) the mean puparium volume (±SEM) or (B) the mean wall thickness (±SEM) occurred between treated and untreated individuals. (C) Adult body size, represented by the mean wing length (±SEM), was also not affected by long-term electric field exposure.

6.3.3 The effect of continuous static electric field exposure on female fecundity

During the experiment females laid significantly more eggs as time progressed (Fig. 6.12) regardless of whether they were exposed to electric fields during pre-adult development or adult life stages ($F_{1,76} = 726$, $P < 0.05$). Exposure of females to electric fields only during development did not affect mean fecundity compared to controls (Fig. 6.12A, $F_{1,76} = 0.3$, $P > 0.05$). Mean fecundity was similarly not affected in females exposed to electric fields solely during adulthood (Fig. 6.12B, $F_{1,76} = 0.11$, $P > 0.05$). In addition, there was no significant interaction between pre-adult development exposure and adulthood exposure ($F_{1,76} = 0.02$, $P > 0.05$). Therefore, *Drosophila* exposed to static electric fields during both life stages did not have an effect on female fecundity.

The number of females that died in each dish was also monitored and the survivorship for each dish was calculated. Although more flies died in the group exposed to electric fields, the difference was not significant (Fig. 6.13, $Z = -1.6$, $n = 8$, $P > 0.05$). Hence, continuous electric field exposure during the fecundity experiments did not affect adult survival.

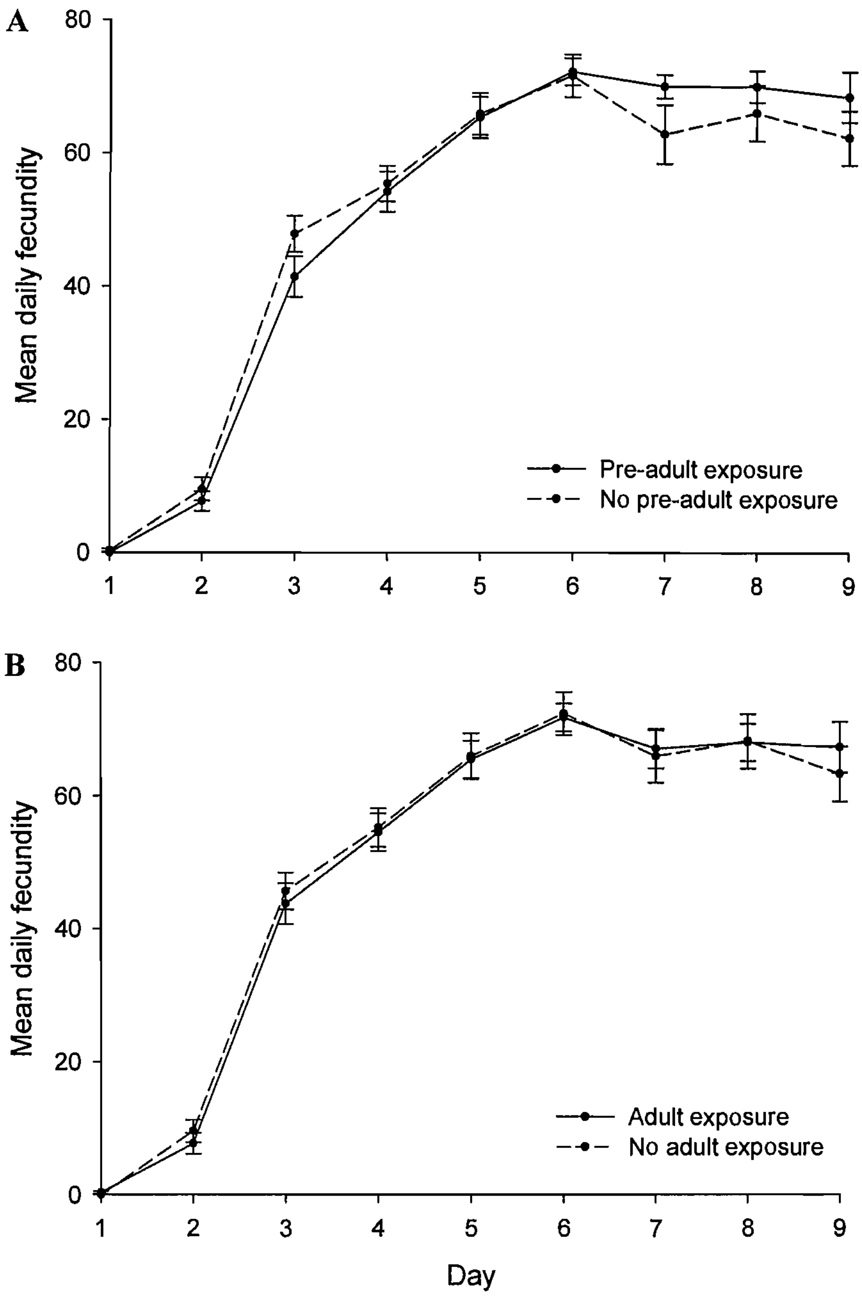


Figure 6.12 Mean number of eggs laid per day (±SEM) during 9 days. (A) Exposure of electric fields during pre-adult development did not affect the fecundity of females. (B) The fecundity of adult females exposed to electric fields was not affected by chronic electric field exposure and no interaction between the conditions was evident.

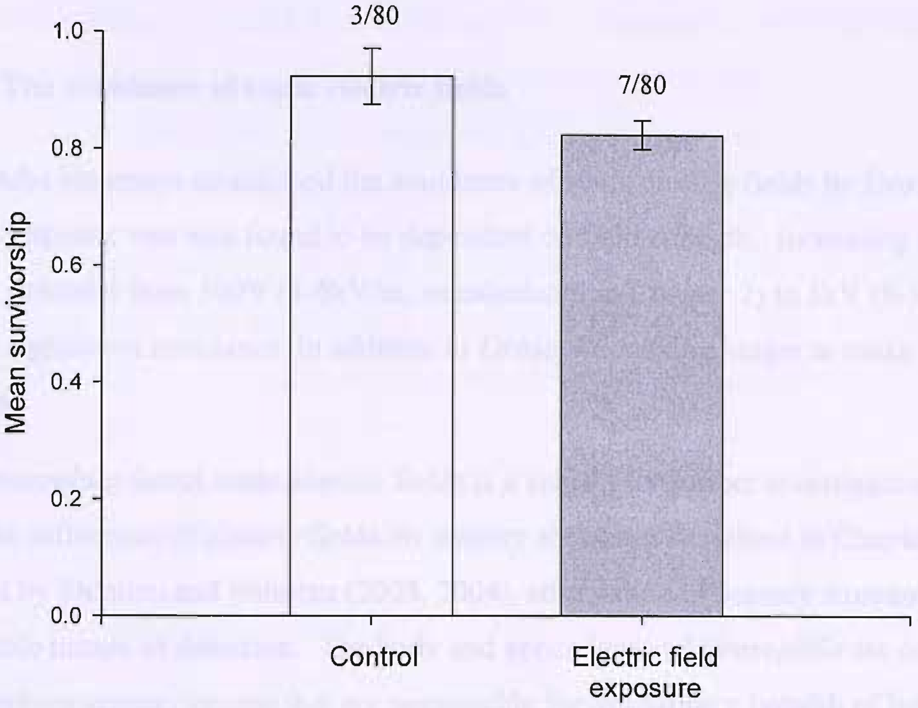


Figure 6.13 Mean survivorship (±SEM) of adult females exposed and not exposed to electric fields during the fecundity experiment. Exposure to electric fields did not have a significant effect on adult female fecundity. The total numbers of dead individuals and sample sizes are noted above the bars.

6.4 Discussion

6.4.1 The avoidance of static electric fields

The Y-tube bioassays established the avoidance of static electric fields by *Drosophila*, and the response was also found to be dependent on field strength. Increasing the voltage potential from 500V (4-6kV/m, as calculated in Chapter 2) to 1kV (8-10kV/m) elicited significant avoidance, in addition to *Drosophila* taking longer to make a decision.

How *Drosophila* detect static electric fields is a subject for further investigation, yet given the influences of electric fields on sensory structures described in Chapter 3 and reported by Shimizu and Shimizu (2003, 2004), stimulation of sensory structures could be a viable means of detection. The body and appendages of *Drosophila* are covered with mechanosensory organs that are responsible for mediating a breadth of behaviours and controlling locomotion (Kernan *et al.*, 1994; Walker *et al.*, 2000). The second proximal antennal segment contains a specialised type of mechanosensory organ, the Johnston Organ (JO), that acts as the auditory system of *Drosophila* and plays an important role during courtship (Caldwell and Eberl, 2002; Eberl *et al.*, 2000). Courtship songs vibrate the antennae activating chordotonal organ neurons within the JO, ultimately regulating locomotor responses and the courtship process (Eberl *et al.*, 1997). Such mechanosensory systems could be affected by electric fields, and thereby may contribute to the avoidance of electric fields.

Although male and female flies did not exhibit significant differences in avoidance, data should be considered with caution due to the low number of replicates carried out. Nevertheless, differences in avoidance between sexes could occur due to sexual dimorphisms, notably as female *Drosophila* are ≈10% larger than males (Reeve and Fairbairn, 1996). Based on Gauss' Law, the electrical forces acting on female flies may be larger than on males, given the positive relationship between object size and charge density (Chubb, 2003). Such increased forces acting on female flies could therefore be associated with heightened sensitivity to electric fields due to greater effects over the whole body, or increased mechanoreceptor stimulation. Contact chemosensory hairs such as sensilla chaetica, trichoidea and basiconica are also more abundant on female *Drosophila* antennae (Shanbhag *et al.*, 1999). These hairs, located primarily on the distal regions of the antennae are both mechanosensory and gustatory (Shanbhag *et al.*, 1999; Staudacher *et al.*, 2005), and hence could be stimulated by electrical forces. Although no differences in mechanoreceptor number between sexes have yet been reported in other appendages (Shanbhag *et al.*, 1992), the apparent antennal sexual dimorphism could contribute to differences in avoidance of static electric fields.

Even so, the avoidance of static electric fields presented here demonstrates that *Drosophila* are capable of perceiving and avoiding static electric fields, and highlights the similarities with the behavioural responses of *P. americana* described in Chapter 2.

6.4.2 The effects of continuous static electric field exposure on *Drosophila*

Nunney (1996) suggests that faster larval development is favourable to an individual to access greater available resources, hence, longer development is associated with decreased fitness (Nunney, 1996). No such effect was evident, however, in *Drosophila* exposed to static electric fields. Given the importance of puparium case thickness in the prevention of dessication and parasitoid attack, thinner puparial cases are correlated with decreased fitness (Fellowes *et al.*, 1998; Kraaijeveld and Godfray, 2003). Puparial cases were not, however, thinner after static electric field exposure. Mortality is also a clear indication whether aberrant environmental conditions affect organisms (Simmons and Crow, 1977), yet static electric fields did not elicit greater mortality at any life stage. Likewise fecundity, long regarded as a representation of fitness (Moller and Thornhill, 1997; Woods *et al.*, 2002), was not affected by static electric field exposure. Together, the results presented here demonstrate that static electric fields of the levels used in this study did not impact *Drosophila* fitness.

To date, the studies investigating the effects of electric fields on organisms focus mostly on one aspect of biology using select models: Genotoxic investigations have noted chromosomal aberrations in specific cell lines (McCann *et al.*, 1998; McCann *et al.*, 1993; Reese *et al.*, 1988); cellular studies report altered membrane binding of certain ions (Bawin and Adey, 1976; Blackman *et al.*, 1985), and reduced insulin release from pancreatic cells is also reported (Jolley *et al.*, 1983). Studies such as these do not directly demonstrate whether electric fields impact the ability for organisms to survive and reproduce. The life-history studies presented in this investigation, for the first time, answer this question and show that static electric fields do not affect insect fitness.

The absence of no apparent effect of electric field exposure on *Drosophila* fitness in this investigation could, however, be attributed to the electrical properties of the system. Although an electric field was calculated to be present in the medium, the complexity and limitations of the modelling need to be taken into consideration. Given its conductive properties, a static electric field may not have been generated within the medium, and *Drosophila* eggs and pupae may not have experienced the field. In addition, static electric fields, unlike ELF electric fields, are not classed as 'penetrative', and are believed not to induce electric fields or currents within biological tissue (Reiter, 1993). Current induction within tissue is believed to be the fundamental cause of biological ELF electric field effects (McCann *et al.*, 1993; Tenforde, 1991) and therefore may explain why *Drosophila* fitness was not impacted by long-term static electric field exposure. Any future investigations should therefore implement a method by which eggs and larvae can feed, but are not restrained within medium, for example, a thin layer of yeast paste. Any further work could also incorporate an assay to investigate whether adults within the fecundity setup are influenced by static electric field exposure, for example, examining locomotor activity within the vials.

It is reported that the generation of electric fields within tissue is secondary to corona discharge (McCann *et al.*, 1998). Corona discharge results in air ionisation and generation of an electric field within the air space (Cross, 1987) and is believed to be a contributor to adverse biological effects (Fews *et al.*, 1999a; Reiter, 1993). Although corona discharge was not calculated within the medium, field modelling did indicate some corona discharge could have occurred at the edges of the dish and medium (regions of > 300kV/m fields). This indicates that air ionisation could have occurred within the system, but may have not been sufficient to elicit effects. Hence, raising the field strength could generate more ionised air and may affect *Drosophila* fitness.

The behavioural bioassays carried out in this study show that *Drosophila* respond to static electric fields, and the response was dependent on field strength. The genotoxic and cellular effects reported to date are also commonly dose-dependent (McCann *et al.*, 1993; Reiter, 1993; Tenforde, 1991). Together these relationships indicate that increasing the field strength used in this study may impact *Drosophila* fitness; and any changes could be identified by monitoring life-history. Having introduced *Drosophila* as a model, this study has also opened new avenues that provide the opportunity to carry out genetic and molecular studies. Such investigations will therefore further current knowledge and highlight any possible fitness effects of static electric fields on insects.

7. GENERAL DISCUSSION

7.1 The behavioural responses of insects to electric fields

Most organisms are subjected to natural forces that exist within the environment. Such forces include those associated with the Earth's magnetic field (Skiles, 1985) and those coupled with static electric fields generated by the global electric circuit (Bering *et al.*, 1998). Some organisms have exploited these forces, exhibiting a repertoire of behaviours in response to them. This thesis has shown, using *P. americana* and *D. melanogaster* as models, that insects can detect and respond to static electric fields.

It is well established that birds, insects and fish are able to respond to the Earth's magnetic field due to the development of specialised detection mechanisms (reviewed in Vacha, 1997; Wiltschko and Wiltschko, 2006). Marine animals have also evolved specific sensory structures enabling the perception of aquatic electric fields, hence mediating behaviour during predation, communication and navigation (Bullock, 1982; Hopkins, 1988; Kalmijn, 1988). In addition, atmospheric electric fields have been reported to cause behavioural changes in insects (Edwards, 1960a; Maw, 1961b; Maw, 1962). Given the coexistence of organisms with natural forces and the behaviours associated with exposure to them, natural selection may have favoured the ability to respond to natural forces as a unique modality that could ultimately benefit an individual. The results shown in this thesis, however, provide some evidence contrary to this theory; avoidance of static electric fields and changes in locomotor activity occurred only at field strengths greater than those within the atmosphere. Hence, this indicates that the behaviour of insects to electric fields is unlikely to have evolved during coexistence with atmospheric electric fields.

Nevertheless, cockroaches and *Drosophila* did respond to electric fields strengths that are present within the environment, albeit as a circumstance of electrical equipment or interactions between synthetic materials. Static electric fields up to 20kV/m can be generated by equipment such as televisions and video display units (Repacholi and Greenebaum, 1999). In addition, static electric field strengths beneath high voltage power lines can reach 11kV/m at ground level, and far higher at close proximity to the power line (Fews *et al.*, 1999a; Fews *et al.*, 1999b). The results presented in this thesis showed that avoidance and behavioural changes occurred in free-moving cockroaches confronted with static electric fields >8kV/m. These findings therefore imply that household and office equipment could manifest changes in insect behaviour. In addition they also support, and could contribute to explaining, previous observations that report altered flying activity of insects near high voltage power lines (Orlov, 1990; Orlov and Babenko, 1987).

Having determined that insects avoid friction charged surfaces and, for the first time, quantified the response, the findings reported in this thesis may have important ramifications for experimental studies using insects. The susceptibility of dielectrics, notably polystyrene Petri dishes, to become charged should be carefully considered during entomological studies given the influences of charged PTFE on cockroach behaviour. It may be preferable, therefore, to refrain from carrying out insect behavioural bioassays in plastic apparatus. In addition, due to the influence of light on avoidance behaviour detailed in Chapter 2, any further studies investigating the influences of electric fields on insects should take light level into account. These findings also support current knowledge that light level affects insect behaviour (Cardé and Knols, 2000; Hassan *et al.*, 2000; Sherman and Dickinson, 2004; Simmons, 2002), and reiterates the importance of maintaining consistent light levels during behavioural investigations (Wyatt, 1997).

The behavioural changes of insects exposed to electric fields described in this thesis may not only be significant in the design of experiments, but could also have consequences for insect trapping methods. Maw (1964) noted that plastic insect traps become charged by rubbing against surrounding foliage, catching fewer specimens. To date, insect traps continue to be composed of plastics and other polymers vulnerable to charge transfer during friction charging. Not only does the avoidance behaviour established in the current study explain Maw's observation, but also presents the importance of taking into account the materials used for insect traps, and the locations they are positioned.

7.2 The detection of electric fields

The coexistence of organisms and natural forces has led to some animals evolving specialised means of detecting forces such as the Earth's magnetic and electric fields. For example, a specific form of iron oxide, magnetite (Fe_3O_4), is actively deposited in localised body regions of insects and birds, enabling the detection of varying magnetic field intensity (Maher, 1998; Wiltschko and Wiltschko, 2006). Likewise, some aquatic animals can perceive the Earth's magnetic field using specific structures; the ampullary organs (Kalmijn, 1971; Kalmijn, 1982). The ampullary organs are also used to generate and detect weak electric fields within sea- and freshwater; an ability utilised during predation, communication and navigation (Heiligenberg and Bastian, 1984; Hopkins, 1988; Kalmijn, 1988).

Given the evolution of specific structures to detect magnetic and electric fields in some organisms, one focus of this thesis was to determine whether insects may have also developed such a system. The investigations described in this thesis have presented that an established sensory modality, that being the antennae and its associated sensory systems, is likely to be involved in detecting static electric fields and evoking avoidance. The influences of electric fields on other slender, elongated structures, such as human hair, have previously been reported demonstrating bending and deflection (Chapman *et al.*, 2005; Shimizu and Shimizu, 2003; Shimizu and Shimizu, 2004). The current study supports these observations and demonstrates that insect detection of static electric fields is probably a result of the activation of S-HP exteroceptors. These sensory structures are established for purposes aside from the detection of static electric fields, notably the discrimination of tactile cues and implementation of escape behaviour (Comer *et al.*, 2003; Okada and Toh, 2000; Ye *et al.*, 2003). Hence, the ability to detect static electric fields is unlikely to have evolved as a unique modality for electric fields, but could be due to the activation of an already existing sensory system that has evolved due to its selective advantage of detecting and avoiding predators.

The studies presented here indicate that the S-HP exteroceptors are most possibly the primary sensory system responsible for static electric field detection, yet avoidance of friction charged PTFE and high strength static electric fields was sustained after removal of the antennae. This suggests that an alternative detection system could exist, possibly one that may have evolved specifically for detecting static electric fields. Rather than such a system evolving, however, the sustained avoidance after antennal and sensory structure removal could be explained by the physical charge interactions as cockroaches are confronted by an electric field. Observations during the bioassays showed that cockroaches without antennae were commonly in close contact with the charged electrode or friction charged PTFE. At such close proximity to a material of high charge density, corona discharge between cockroach cuticle and electrode or PTFE may have occurred (Chubb, 2003; Cross, 1987), possibly on the distal head region or legs. The result could have been a small shock and subsequently a reflex response.

Alternatively, or in addition, it is possible that mechanoreceptors located on body regions aside from the antennae contributed to the detection of higher strength static electric fields and friction charged PTFE. Not only does this support the suggestion that electric field detection is a consequence of the activation of established sensory systems, but also presents an interesting insight into understanding how insects respond to forces exerted upon them. Many questions remain over how insects, and other organisms, sense load applied upon them (Noah *et al.*, 2004; Zill *et al.*, 2004). Taking into account the findings from this thesis, electric fields could provide a means by which insects can be stimulated in order to investigate this subject, furthering current knowledge on how animals coordinate locomotion and control posture (Zill *et al.*, 2004).

The role of the antennae and whole-body mechanoreceptors in the detection of static electric fields described in this study is unlikely to be a characteristic unique to cockroaches. Not only does the avoidance evoked by *Drosophila* in Chapter 6 support this suggestion, but antennal and mechanoreceptor structure and function is remarkably similar across many insect species (Keil, 1997). Specifically the S-HP, the region of mechanosensory hairs on the scape now known to play a major role in detecting electric fields, is also apparent in insects other than cockroaches. Mechanosensory hair plates at the HS joint exist on many insects including locusts, beetles, moths, bees and stick insects (Durr *et al.*, 2001; Gewecke, 1974; Kloppenburg, 1995; Pelletier and McLeod, 1994; Staudacher *et al.*, 2005). These hair plates mediate learning (Scheiner *et al.*, 2005) as well as a variety of locomotor behaviours including flight (Kloppenburg *et al.*, 1997) and obstacle detection (Pelletier and McLeod, 1994), and provide information on antennal position (Staudacher *et al.*, 2005). Hence, given these morphological and behavioural similarities between insect species, mechanoreceptors on the scape could be responsible for detecting electric fields in other insects.

Nevertheless, the results presented here have not eliminated the possibility that alternative sensory modalities have evolved specifically for the detection of electric fields. Such systems, not focussed in this thesis, could be present internally such as the existence of iron particles in termites responsible for magnetic field detection (Maher, 1998). This therefore remains a question to be investigated further.

The results established in this thesis have not only advanced current understanding of the influence of electric fields on insects, but also have implications for applications outside pure research, notably in pest management. Previous studies have raised the possibility of using electric fields as a method of pest control, ranging from paralysis (Watson, 1984; Watson *et al.*, 1997), to gross changes in physiology (Edwards, 1961; McCann *et al.*, 1998; Tenforde, 1991), and manipulation of movement (Edwards, 1960a; Maw, 1962; Perumpral *et al.*, 1978). Often it is important to understand the behavioural responses of insects to a stimulus and the processes behind these responses, before instigating pest control methods (Foster and Harris, 1997). This thesis has contributed to understanding how insect behaviour could be manipulated by electric fields threefold: by quantifying the field strength dependent behavioural responses of free-moving cockroaches to static electric fields; establishing the avoidance behaviour; and determining the method of detection. Based on this knowledge, friction charging dielectrics or low current passed through discrete conductors could be used to repel walking pests. It may be possible to install such methods in areas of food preparation, around door frames, in horticultural growth centres, or in vending machines, acting as a non-toxic barrier mechanism to limit pest access. Nevertheless, additional factors including species specificity, safety to the user, and intensity of the electric field, amongst other attributes, need to be taken into consideration (Foster and Harris, 1997). Although the findings presented here need to be advanced to develop a viable pest control mechanism using static electric fields, they have re-established that such a method could be feasible.

7.3 The effects of static electric field exposure on insect fitness

The avoidance of static electric fields characterised in this thesis suggests that it may be selectively advantageous to avoid static electric fields as a mechanism of limiting exposure to an unfavourable stimulus. Testing for such selection pressures by consistently exposing *Drosophila* and cockroaches to static electric fields did not however, at the field strengths used in this study, affect insect fitness. Hence, there may be no fitness benefit to avoid static electric fields.

Although static electric fields of the magnitudes used in this study are not common within the environment (Repacholi and Greenebaum, 1999), they can be generated by high voltage power lines, notably between insulators and on corroded cables (Fews *et al.*, 1999a; Fews *et al.*, 1999b). The data shown in Chapters 5 and 6 therefore indicate that the fitness of insects inhabiting such environments may not be affected. The results also imply that static electric fields < 150-200kV/m, for example, beneath high voltage power lines, within substations, or surrounding electrical equipment (Repacholi and Greenebaum, 1999) may not manifest physiological changes and pose no long-term risk to insects. Preliminary experiments with *Drosophila* indicated, however, that courtship behaviour may be affected by the presence of static electric fields >8kV/m. Observations showed aberrant and ineffective courtship, possibly due to forces acting on wings, preventing correct courtship behaviour to take place. Due to difficulties with the apparatus and low control repeats, the data was not included in this thesis. Nevertheless these observations indicate that reproduction, and therefore ultimately insect fitness, could be affected by static electric field exposure.

The absence of an effect on ecological fitness could have been attributed to the physics within the systems. Unlike ELF electric fields, static electric fields do not generate electromagnetic radiation, therefore electric fields within materials are not induced (Hammond, 1997; Tenforde, 1991). Hence, they are not considered 'penetrative' (Reiter, 1993), and subsequently may not have manifested physiological changes within the animals. Nevertheless, static electric fields could affect the gross physiology of animals due to air ionisation. Static electric fields > 300kV/m cause the breakdown of air, releasing free electrons (Cross, 1987). Air ionisation has been reported to elicit decreased insect activity and fecundity (Edwards, 1960c; Edwards, 1961; Maw, 1962), as well as paralysis and DNA aberrations in *Drosophila* (McCann *et al.*, 1993; Watson *et al.*, 1997), yet no reports to date have investigated the life-history of insects exposed to ionised air. Further investigations based on those performed in this study could therefore be carried out using field strengths > 300kV/m. Having introduced *Drosophila*, life-history traits could be re-examined, and given its suitability as a laboratory model (Adams *et al.*, 2000; Le Bourg, 2004), proteomic and genomic analyses could also be carried out to establish whether subtle molecular changes occur due to continuous static electric field exposure. An additional explanation could be the forces exerted on both cockroaches and *Drosophila* retained in the setups. Although the field strengths within the setups (150-200kV/m) were greater than those required to evoke avoidance in the Y-tube (8-10kV/m), the forces exerted on individuals in the setups may not have been sufficient to stimulate mechanoreceptors and elicit behavioural changes. Any future investigation could use raised field strengths, monitoring behaviour within the setups to ensure responses are evoked, and examining whether adverse effects occur.

Increasing the field strength may not only affect mechanosensory structures, but could also influence cellular mechanotransduction mechanisms that are present in both prokaryotic and eukaryotic cells (Hamill and Martinac, 2001). Mechanotransduction mechanisms exist in *Drosophila* adults as well as larvae (Caldwell *et al.*, 2002; Kernan and Zuker, 1995; Walker *et al.*, 2000), and are known to modulate responses to mechanical stimuli and play a role in development and cell growth (Hamill and Martinac, 2001; Orr *et al.*, 2006). There are two means by which electrical forces could act on these systems to elicit adverse effects. First, it is possible that electrical forces could directly cause conformational changes in the cellular structure of, for example, mechanosensitive channels. This may result in modification of cell function that could ultimately affect normal development (Orr *et al.*, 2006). Second, repetitive activation of mechanotransduction systems by consistent stimulation from electrical forces could evoke cellular stress and subsequently aberrant cell function (Hamill and Martinac, 2001; Orr *et al.*, 2006). Hence, changes in mechanotransduction mechanisms of insects exposed to field strengths greater than those used in this study may be another means by which insects are adversely affected by static electric fields. Monitoring these changes could therefore provide an opportunity for further investigation.

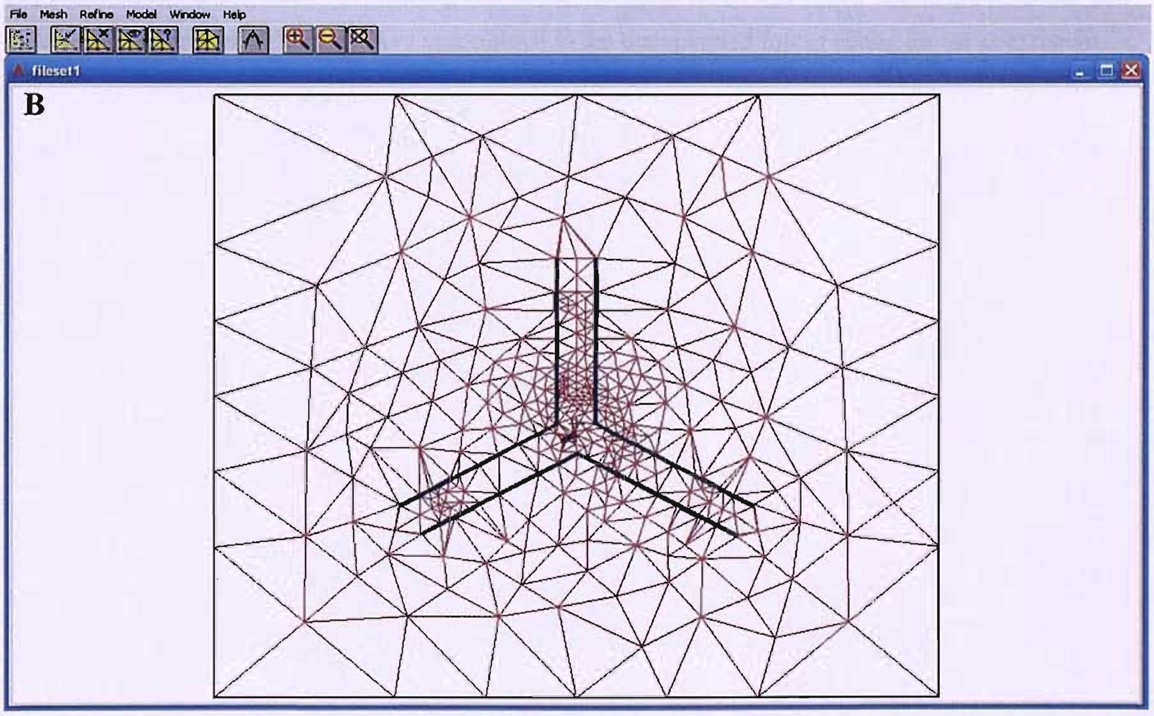
Together, the data presented in this thesis have established that insects avoid static electric fields, and how they do so using a sensory modality that has evolved for purposes aside from electric field detection. It remains possible however, that an additional system, perhaps one of cellular origin, contributes to the detection of electric fields. Nevertheless, given the absence of fitness effects after long-term static electric field exposure, the avoidance of electric fields is a response that most probably does not provide any selective advantage. Long-term static electric field exposure is therefore unlikely to pose a threat to insect individuals and whole populations.

8. APPENDICES

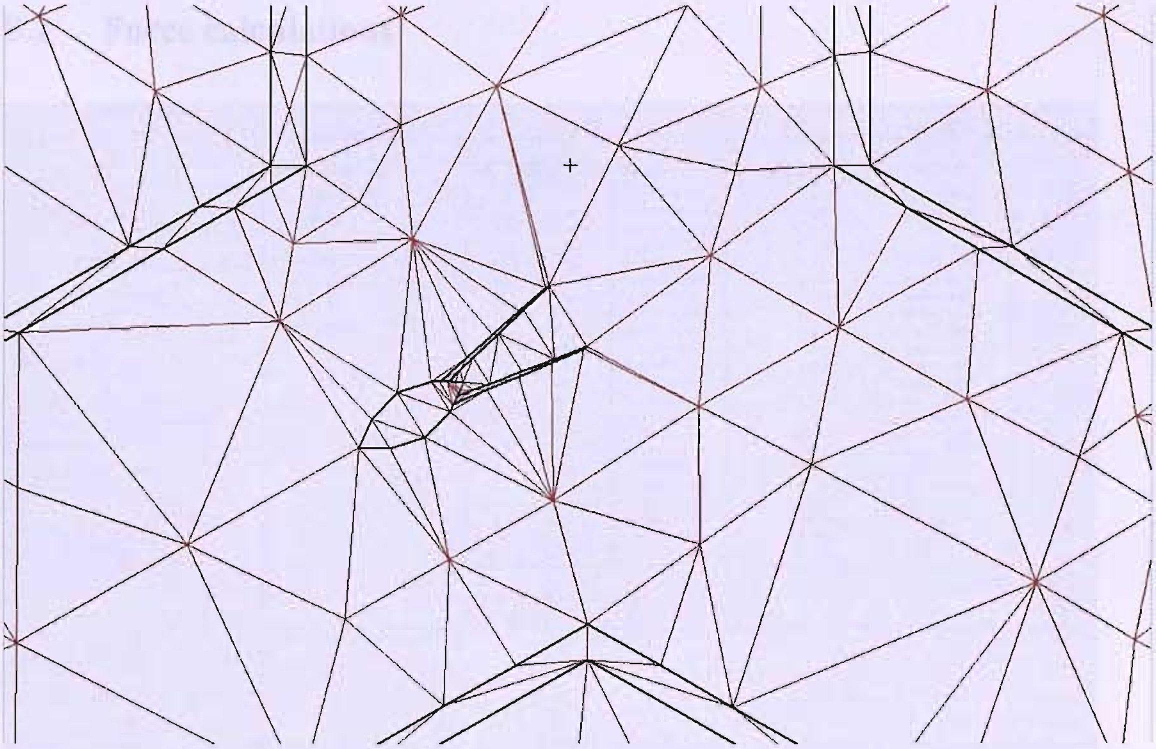
8.1 Field modelling

Material	Relative Permittivity	Conductivity
Aluminium	1	$3.8\text{x}10^{7}$
PTFE	2.08	0
Cockroach	2.5	7.5
Cerci and Antennae	2.5	7.5
Glass	5.5	$1\text{x}10^{-12}$
Copper	1	$5.8\text{x}10^{7}$
Background (Air)	1.0006	0
Drosophila medium	81	4
Petri Dish	2.25	0

(A) The electrical properties assigned to the materials within the Y-choice and Y-tube models.

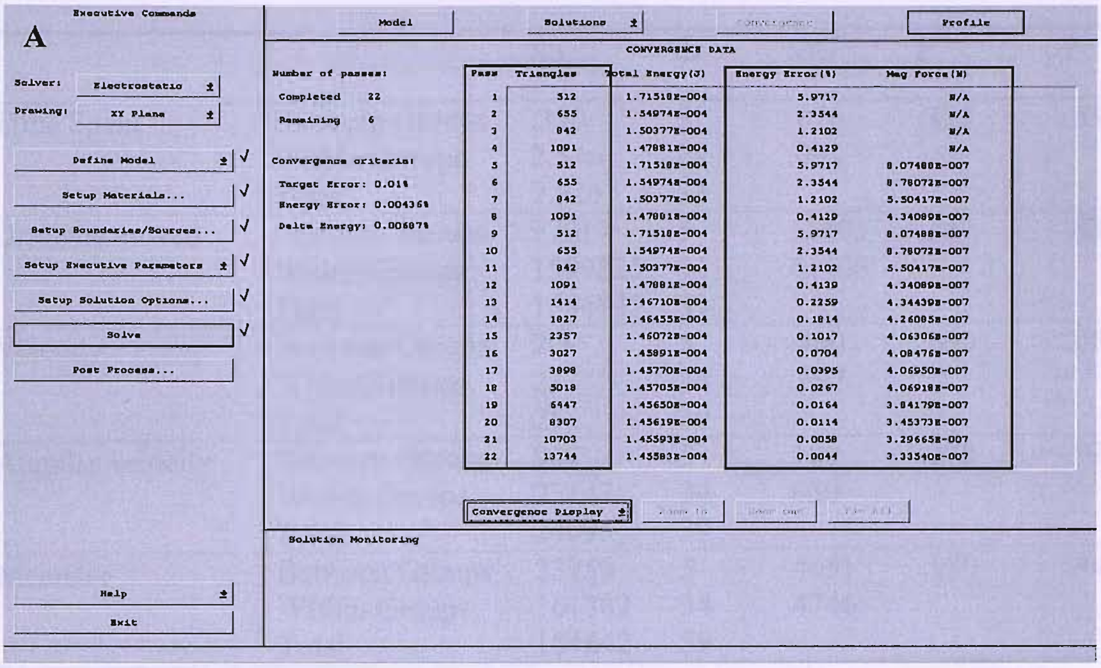


(B) The initial mesh was made up of a large number of triangles. The software calculates the requested parameters within each triangle and combines all calculations to create the final model.



(C) Zoom image of the mesh surrounding and within the cockroach. The small size of the antennae and comparatively few triangles may have contributed to the unexplained forces acting on the cockroach.

8.2 Force calculations



Solver: Electrostatic

Drawing: XY Plane

CONVERGENCE DATA

Number of passes:

Completed 22

Remaining 6

Convergence criteria:

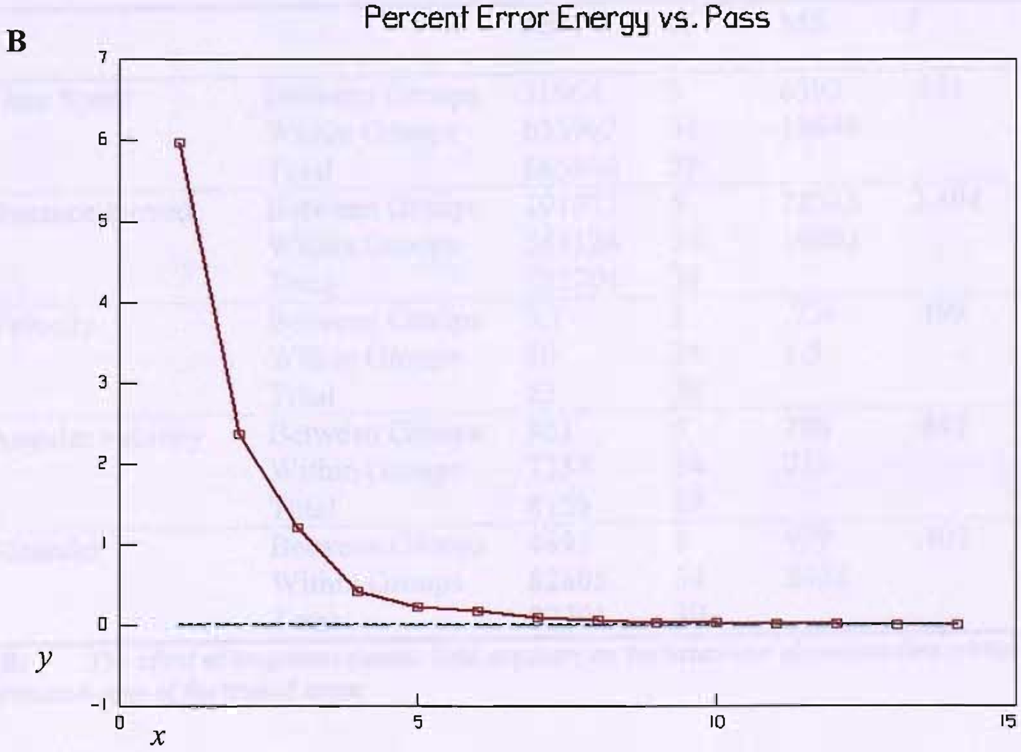
Target Error: 0.01%

Energy Error: 0.00436%

Delta Energy: 0.00687%

Pass	Triangles	Total Energy(J)	Energy Error(%)	Mag Force(N)
1	512	1.71518E-004	5.9717	N/A
2	655	1.54974E-004	2.3544	N/A
3	842	1.50377E-004	1.2102	N/A
4	1091	1.47881E-004	0.4129	N/A
5	512	1.71518E-004	5.9717	8.07488E-007
6	655	1.54974E-004	2.3544	8.78072E-007
7	842	1.50377E-004	1.2102	5.50417E-007
8	1091	1.47881E-004	0.4129	4.34089E-007
9	512	1.71518E-004	5.9717	8.07488E-007
10	655	1.54974E-004	2.3544	8.78072E-007
11	842	1.50377E-004	1.2102	5.50417E-007
12	1091	1.47881E-004	0.4139	4.34089E-007
13	1414	1.46710E-004	0.2259	4.34439E-007
14	1827	1.46455E-004	0.1814	4.26085E-007
15	2357	1.46072E-004	0.1017	4.06306E-007
16	3027	1.45891E-004	0.0704	4.08476E-007
17	3898	1.45770E-004	0.0395	4.06950E-007
18	5018	1.45705E-004	0.0267	4.06918E-007
19	6447	1.45650E-004	0.0164	3.84179E-007
20	8307	1.45619E-004	0.0114	3.45373E-007
21	10703	1.45593E-004	0.0058	3.29665E-007
22	13744	1.45583E-004	0.0044	3.33540E-007

(A) Each pass creates a more refined mesh with triangle number increasing. This is associated with a decrease in error and a more accurate representation of the resultant force.



(B) Plotting the percent error (y) v. pass (x) shows the decrease in error associated with the increase in pass number due to mesh refinement. The time taken for the model to be calculated increases proportionally with the number of passes. A compromise between percent error and time taken for model to be calculated therefore needs to be considered.

8.3 One –way ANOVA tables

		SS	df	MS	F	P
Time Spent	Between Groups	.380	5	.076	.835	.536
	Within Groups	2.546	28	.091		
	Total	2.926	33			
Distance moved	Between Groups	77017	5	15403	.347	.881
	Within Groups	1509825	34	44406		
	Total	1586842	39			
Velocity	Between Groups	2.3	5	.460	.660	.656
	Within Groups	23	34	.697		
	Total	25	39			
Angular velocity	Between Groups	947	5	189	.278	.922
	Within Groups	23147	34	680		
	Total	24095	39			
Meander	Between Groups	23259	5	4651	.980	.444
	Within Groups	161382	34	4746		
	Total	184642	39			

(A) The effect of long-term electric field exposure on the behaviour of cockroaches within the treated zone of the treated arena

		SS	df	MS	F	P
Time Spent	Between Groups	31964	5	6392	.343	.883
	Within Groups	633902	34	18644		
	Total	665866	39			
Distance moved	Between Groups	191577	5	38315	2.394	.058
	Within Groups	544124	34	16003		
	Total	735701	39			
Velocity	Between Groups	3.7	5	.736	.499	.774
	Within Groups	50	34	1.5		
	Total	53	39			
Angular velocity	Between Groups	901	5	180	.845	.528
	Within Groups	7257	34	213		
	Total	8159	39			
Meander	Between Groups	4895	5	979	.402	.844
	Within Groups	82805	34	2435		
	Total	87701	39			

(B) The effect of long-term electric field exposure on the behaviour of cockroaches within the untreated zone of the treated arena

		SS	df	MS	F	P
Time Spent	Between Groups	66229	5	13245	.659	.657
	Within Groups	663123	33	20094		
	Total	729353	38			
Distance moved	Between Groups	129394	5	25878	1.173	.343
	Within Groups	727746	33	22052		
	Total	857140	38			
Velocity	Between Groups	3.85	5	.771	1.564	.198
	Within Groups	16	33	.493		
	Total	20	38			
Angular velocity	Between Groups	5147	5	1029	1.124	.367
	Within Groups	30225	33	915		
	Total	35372	38			
Meander	Between Groups	27940	5	5588	.787	.567
	Within Groups	234437	33	7104		
	Total	262377	38			

(C) The effect of time on the behaviour of cockroaches within the treated zone of the treated arena

		SS	df	MS	F	P
Time Spent	Between Groups	69325	5	13865	.765	.582
	Within Groups	598448	33	18134		
	Total	667773	38			
Distance moved	Between Groups	284277	5	56855	1.856	.129
	Within Groups	1010939	33	30634		
	Total	1295216	38			
Velocity	Between Groups	7.4	5	1.47	1.467	.227
	Within Groups	33	33	1		
	Total	40	38			
Angular velocity	Between Groups	1919	5	383	2.571	.045
	Within Groups	4926	33	149		
	Total	6845	38			
Meander	Between Groups	11593	5	2318	1.862	.128
	Within Groups	41103	33	1245		
	Total	52697	38			

(D) The effect of time on the behaviour of cockroaches within the untreated zone of the treated arena

		SS	df	MS	F	P
Time Spent	Between Groups	9926	2	4963	.341	.714
	Within Groups	451784	31	14573		
	Total	461710	33			
Distance moved	Between Groups	22075	2	11037	.851	.437
	Within Groups	402284	31	12976		
	Total	424360	33			
Velocity	Between Groups	1.22	2	.61	4.6	.018
	Within Groups	4.1	31	.133		
	Total	5.3	33			
Angular velocity	Between Groups	536	2	268	2.05	.146
	Within Groups	4061	31	131		
	Total	4598	33			
Meander	Between Groups	1013	2	506	3.68	.695
	Within Groups	42663	31	1376		
	Total	43676	33			

(E) Differences in the behaviour of free-moving cockroaches in the treated arena of the paradigm compared to the behaviour of treated cockroaches

		SS	df	MS	F	P
Time Spent	Between Groups	18919	5	3783	1.496	.217
	Within Groups	86003	34	2529		
	Total	104922	39			
Distance moved	Between Groups	24466	5	4893	.151	.978
	Within Groups	1104634	34	32489		
	Total	1129101	39			
Velocity	Between Groups	2.5	5	.504	.486	.785
	Within Groups	35	34	1		
	Total	37	39			
Angular velocity	Between Groups	3299	5	659	2.068	.094
	Within Groups	10850	34	319		
	Total	14149	39			
Meander	Between Groups	10224	5	2044	1.102	.377
	Within Groups	63066	34	1854		
	Total	73291	39			

(F) The effect of long-term electric field exposure on the behaviour of cockroaches within side A of the untreated arena

		SS	df	MS	F	P
Time Spent	Between Groups	17134	5	3426	1.153	.352
	Within Groups	101096	34	2973		
	Total	118231	39			
Distance moved	Between Groups	92498	5	18499	.486	.784
	Within Groups	1294156	34	38063		
	Total	1386655	39			
Velocity	Between Groups	2.2	5	.437	.371	.865
	Within Groups	40	34	1.2		
	Total	42	39			
Angular velocity	Between Groups	4823	5	964	2.324	.064
	Within Groups	14109	34	414		
	Total	18932	39			
Meander	Between Groups	13554	5	2710	1.084	.387
	Within Groups	85064	34	2501		
	Total	98619	39			

(G) The effect of long-term electric field exposure on the behaviour of cockroaches within side B of the untreated arena

		SS	df	MS	F	P
Time Spent	Between Groups	5542	5	1108	.454	.807
	Within Groups	78189	32	2443		
	Total	83732	37			
Distance moved	Between Groups	347294	5	69458	1.545	.204
	Within Groups	1438209	32	44944		
	Total	1785503	37			
Velocity	Between Groups	7.2	5	1.437	1.790	.143
	Within Groups	25	32	.803		
	Total	32	37			
Angular velocity	Between Groups	3414	5	682	2.862	.030
	Within Groups	7635	32	238		
	Total	11049	37			
Meander	Between Groups	15192	5	3038	1.810	.139
	Within Groups	53718	32	1678		
	Total	68910	37			

(H) The effect of time on the behaviour of cockroaches within side A of the untreated arena

		SS	df	MS	F	P
Time Spent	Between Groups	6260	5	1252	.398	.846
	Within Groups	100633	32	3144		
	Total	106893	37			
Distance moved	Between Groups	346753	5	69350	1.670	.170
	Within Groups	1328662	32	41520		
	Total	1675415	37			
Velocity	Between Groups	6.7	5	1.3	1.778	.146
	Within Groups	24.2	32	.76		
	Total	31	37			
Angular velocity	Between Groups	4299	5	859	1.158	.351
	Within Groups	23756	32	742		
	Total	28056	37			
Meander	Between Groups	15408	5	3081	.591	.707
	Within Groups	166745	32	5210		
	Total	182154	37			

(I) The effect of time on the behaviour of cockroaches within side B of the untreated arena

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