THE NEUROETHOLOGY OF ACOUSTIC STARTLE/ESCAPE IN FLYING LOCUSTS

by

JEFFERY WAYNE DAWSON

A thesis submitted to the Department of Biology
in conformity with the requirements for the degree
Doctor of Philosophy

Queen's University
Kingston. Ontario. Canada
August. 2001

0-612-63417-5

Canada

ABSTRACT

The acoustic startle/escape response of the African migratory locust, *Locusta migratoria* L., is a short-latency stereotyped response to high-frequency sounds. The characteristic changes in wing kinematics and body posture are hypothesized to function in the avoidance of insectivorous bats. In this thesis, I present a detailed analysis of the changes in wing kinematics, flight motor pattern and free-flight behaviour accompanying this response. High speed cinematography and multi-channel electromyography were used to record wing kinematics and motor patterns of locusts responding to 30 kHz bat-like sounds. Within a few wingbeats of stimulus onset, wing stroke angles became asymmetric in which the forewing on the inside of the turn path occupied lower elevation angles than the contralateral wing throughout the downstroke. Forewing asymmetry was positively correlated with abdomen deflection; an index of attempted steering direction. Hindwing asymmetry was minimal or opposite in direction to forewing asymmetry. During steering attempts, depressor timing advanced in the wing beat cycle for both forewing and hindwing muscles and burst duration increased in all depressors on both sides of the animal. Depressor asymmetries were correlated with forewing, hindwing, and stroke reversal asymmetry for both wing pairs. These results support the model that asymmetric wing depression is effected by bulk shifts in depressor muscle activation that establish stroke reversal timing. Aerodynamically it is argued that asymmetric wing depression shifts the direction of lift generated by the forewings during downstroke translation creating roll torques that augment the effects of angle of attack and abdomen

deflection. Data also suggest that locusts control the timing of wing rotation for generating steering torques. Free-flying locusts in a flight room react to bat-like and other sounds with avoidance flight similar to responses of other insects reacting to bats and bat-like sound. Locust responses were independent of carrier frequency and pulse structure suggesting reactions were general startle reactions and not specifically bat-avoidance behaviours. Reactions of tethered locusts and wild-caught moths in the same flight room under similar conditions confirm the locust responses were not due to artefacts of the experimental set-up. These results suggest that tethering affects auditory processing in locusts.

This thesis is dedicated to my mother. Carole. for her love. support and encouragement.

STATEMENT OF CO-AUTHORSHIP

Dr. R. Meldrum Robertson is coauthor on all manuscripts that have come from this thesis due to his role as my graduate supervisor and mentor.

The high-speed kinematic data and videographic data of Chapter 2 were collected by Dr. Robertson and Dr. Daniel Robert (co-author) during 1993. Mr. Ken Dawson-Scully (co-author) digitized wing angles and abdomen positions from the high-speed films and videotapes. Motor pattern data appearing in figure 2.8A were collected by myself during 1995. Other motor pattern data were collected by Mr. Ken Dawson-Scully while completing his B.Sc. thesis during 1995 while under my supervision. All data analysis and writing of the manuscript was completed by myself but I must acknowledge substantial editorial assistance from Dr. Robertson with regard to the discussion section.

All data appearing in Chapter 3 were collected by myself. Mr. Fok-Han Leung (co-author) implanted the EMG electrodes in locusts that provided data during the summer of 2000 (not all of the EMG data appearing in the chapter). Mr. Ron Harding designed and built the 16 channel EMG amplifier used for EMG data collection. Mr. Harding also wrote software that enabled me to digitize the wing kinematic data collected with the high-speed digital camera. All data analysis and writing of the manuscript was completed by myself.

All data appearing in Chapter 4 were collected while working with Prof. Dr. Wolfram Kutsch (co-author) at the University of Konstanz, Germany during the summer of 1999. Moth collecting and identification and moth flight data collection was assisted

by Mr. Holger Martz (University of Konstanz). The behavioural audiogram (figure 4.4) of *Locusta migratoria* was determined by Mr. Craig C. K. Willis as part of his B.Sc. thesis during 1996 while under my supervision. Three-dimensional flight path data collection and reconstructions were completed by myself. All data analysis and writing of the manuscript was completed by myself.

vi

ACKNOWLEDGMENTS

I would like to thank my supervisor, Professor R. Meldrum Robertson, for his guidance, and support during completion of my degree at Queen's. I am especially thankful for the many opportunities he made possible for me over the years and I am indebted to him for his leniency and the latitude given to me to pursue my own ideas and interests.

I would also like to thank Professor James Fullard (University of Toronto) for the many discussions over the years about my thesis material. These discussions, in conjunction with advice offered by Mel and my supervisory committee, have significantly shaped the projects described herein. Over the years I have also enjoyed many collaborations with James that have not been directly related to my formal course of study. These side projects have enriched my graduate experience and have contributed significantly to my development as a scientist.

I thank my supervisory committee, Professors Barrie Frost, Chris Moyes, Heather Proctor, and Laurene Ratcliffe for valuable advice. In particular, I would like to thank Heather for being both a supervisor and friend. I would like to thank Chris for teaching me that biological significance and statistical significance are not the same and that "adaptive" means different things to different people. And I would like to thank Barrie for showing me that it is always important to think big (3-D) but that big projects do not necessarily require complicated devices (hockey sticks, light bulbs and coffee cans make wonderful visual stimulus generators). I would also like to thank Tice Post for his

doubt. this thesis would not have been completed without their help. I am especially grateful to Ron for building several pieces of equipment (in particular, a pair of dual channel position transducers and a 16 channel EMG amplifier) and writing software (to replace the lab's aged PEAK system) for my experiments. I also thank Ron and John for their generosities while writing my thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Definition
ANOVA	analysis of variance
CNS	central nervous system
CPG	central pattern generator
DA	depressor asymmetry
dB SPL	decibel sound pressure level (relative to 20 μPa reference pressure)
EMG	electromyography / electromyographic
EPSP	excitatory post synaptic potential
FWA	forewing asymmetry
HWA	hindwing asymmetry
IPSP	inhibitory post synaptic potential
LEV	leading edge vortex
MAV	micro-air vehicle
mVAC	medial ventral association centre
PSP	post synaptic potential

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

"Should the animal be free in its natural environment so that it can show "normal" behavior, or should it be placed in an experimental chamber where environmental factors can be controlled?"

Kenneth D. Roeder
from his text, *Nerve Cells and Insect Behaviour*, 1967.

One of the fundamental tasks of the nervous system of all organisms is the processing of incoming sensory information and integrating that information with ongoing motor activity to effect adaptive movements for navigation of the environment. Sound is used by many animals for communication and for orientation in the environment. Among terrestrial animals, it is only within the insects that ears are so diverse. Tympanal ears have evolved independently in numerous insect taxa strongly suggesting sound plays an important role in survival (Fullard and Yack, 1993; Hoy and Robert, 1996). Ears and hearing serve two primary purposes for all animals, first, ears are used for detection and recognition of sounds produced by conspecifics and/or heterospecifics, and second, ears are used for localizing sounds. Reacting appropriately to sound is of paramount importance for insects that fly at night and are at risk of predation by echolocating, insectivorous bats. In this thesis, I investigate the changes in wing kinematics, motor pattern, and free-flight behaviour of the African migratory locust, *Locusta migratoria* L., in response to acoustic stimulation with bat-like sounds.

It is now clear that tympanate ears protect many nocturnal flying insects from predation by insectivorous bats by evoking behaviours that aid in bat avoidance and escape (for reviews, see Hoy, 1992; Fullard and Yack, 1993; Hoy and Robert, 1996). An acoustic startle/escape response (ASR) is defined as a co-ordinated series of muscle contractions (i.e. a behaviour) of rapid onset that occurs in response to an abrupt, startling sound. Further, the sound that elicits the ASR must be meaningful to the insect and the reaction of the insect depends on the context of its ongoing behaviour at the time of the stimulus (Hoy, 1989; Hoy et al., 1989; Hoy, 1992).

Research that began as observations of moths evading capture by bats in the early 1960s has today culminated in similar observations from a diversity of insects that span five insect orders and numerous families and subfamilies. Acoustic startle responses have now been described for moths (see the classic volume by Roeder, 1967a), lacewings (Miller and Olesen, 1979), praying mantises (Yager et al., 1990), tiger beetles (Yager and Spangler, 1997), scarab beetles (Forrest et al., 1995), nocturnal butterflies (Yack and Fullard, 2000), crickets (Moiseff et al., 1978), katydids (Libersat and Hoy, 1991), and locusts (Robert, 1989). For members of certain of these insect orders, namely moths, praying mantises and lacewings, the connection between auditory sensitivity to frequencies above 20 kHz (bat detection) and behaviours that decrease the probability of predation (bat avoidance), is quite clear due to rigorous behavioural observation and electrophysiological investigation in both field and laboratory studies. Observation of the insects interacting with bats in their natural environment provides the best evidence for the function of the ASR but the technical challenges of field studies of nocturnal insects

and bats has often favoured less direct approaches. For example, in the field, playback of

recorded sounds and synthesized bat-like sounds evokes behaviours in scarab beetles and

nocturnal butterflies that are similar to those of the aforementioned insects. Less direct,

but still convincing evidence has also come from laboratory observations of the responses

of tethered insects stimulated with bat-like sounds. Tethered tiger beetles, crickets,

katydids, and locusts show elements of behaviours (e.g. abdomen and hindleg deflection

away from the stimulus, changes in wing kinematics) that are presumed to function in

changing the flight path of the insect if it were in free-flight.

The ASR of tethered flying *L. migratoria* was formally described by Robert

(1989). *Locusta migratoria* react with short latencies of 50 to 100 ms to high-frequency

bat-like sounds consisting of trains of short duration sound pulses with carrier frequencies

greater than 10 kHz presented at intensities greater than 45 dB SPL. Reactions consist of

abdomen deflection and dorsiflexion, hindleg extension and deflection, changes in wing

beat frequency, including flight cessation, changes in wing stroke kinematics and other

postural adjustments. Robert (1989) showed that abdomen and hindleg deflection in

response to stimulation with bat-like sounds was also accompanied by the production of

steering torques in directions opposite to the side of stimulation. Using the hearing

threshold of locusts for high-frequency sounds and assumptions about the flight speed of

a typical bat, and the intensity and frequency of its echolocation calls, Robert (1989)

estimated that locusts would be able to detect a bat at a distance of 20 m whereas a bat

would detect the locust only when it was within 5 m. These estimates suggest it is

possible that locusts possess an early warning system for detecting approaching, foraging

bats and that the ASR functions in bat avoidance by changing the flight path of the locust.

The question of whether bats eat locusts is still under debate although orthopterans, in

general, are palatable to gleaning bats (La Val and La Val, 1980; Belwood and Morris,

1987) and crickets and katydids have been found occupying 8% of the diet of *Taphozous*

perforatus, a large (20g), high-flying bat from Ethiopia (Rydell and Yalden, 1997).

Further, the lack of direct observation of free-flying locusts interacting with bats in the

field, or even reactions to bat-like ultrasound, leave an open question as to whether

locusts change their flight path in response to bat-like sounds.

The free-flight reactions of moths, lacewings and praying mantises seem to show

convergence in repertoire. Moths detecting the calls of nearby bats react with turns,

loops, active and passive dives, and increases in flight path erraticism (Roeder, 1962;

1964; 1974). Lacewings fold their wings and drop from the sky punctuating their descent

with occasional wing flips (Miller and Olesen, 1979). Praying mantises similarly respond

with turns loops and dives to the ground (Yager *et al.*, 1990); although unlike moths,

these reactions are not directional given the medial, thoracic position of the mantis ear

(Yager and Hoy, 1986; 1987). Tethered insects also show convergence in their responses

to bat-like sounds. Praying mantis (Yager and May, 1990), tiger beetle (Yager and

Spangler, 1997). cricket (Moiseff *et al.*, 1978; Pollack and Hoy, 1981; Pollack and

Plourde, 1982; Nolan and Hoy, 1986; Wyttenbach and Hoy, 1997). katydid (Libersat and

Hoy, 1991; Schulze and Schul, 2001), and locust (Robert, 1989; Robert and Rowell,

1992b) responses include abdomen and hindleg deflection, changes in wing beat

frequency and wing kinematics. flight cessation, and changes in body posture such as

head rotation and foreleg extension. These similarities suggest evolutionary convergence

due to common function and may reflect the aerodynamic constraints of generating a

rapid change in flight direction.

How is auditory information used to change ongoing flight behaviours to effect

changes in the flight path? How is auditory processing of bat-like sounds different than

processing of the sounds produced by conspecifics and why is it that only bat-like sounds

effect avoidance responses? Why does this diversity of animals show convergence on

similar behaviours in response to stimulation with bat-like sounds? Does this

convergence reflect a common motor strategy due to aerodynamic constraint? What are

the aerodynamic consequences of these kinematic changes? These are questions of

general interest to neuroethologists, and insect flight aerodynamicists. Answering these

questions requires an experimental animal sensitive to different auditory stimuli and that

will respond with quantifiable behaviours. Many insects have been chosen for

aerodynamic and kinematic studies of flight but, arguably, the most detailed analyses

have been completed for bumblebees (Dudley and Ellington, 1990a, b), the tobacco

hornworm moth, *Manduca sexta* (Willmott and Ellington, 1997a, b), dragonflies

(Wakeling and Ellington, 1997a-c), the fruitfly, *Drosophila* sp. (Dickinson and Götz,

1996; Lehmann and Dickinson, 1998;), and, of course, locusts. Of these insects, only

locusts are tympanate and possess a demonstrated ASR. Further, no studies have

examined, in detail, the changes in wing kinematics and motor patterns that effect rapid,

in-flight, escape responses. Locusts in general have been a model organism for insect

flight since the work of Weis-Fogh and Jensen in the 1950s (Jensen, 1956; Jensen and

Weis-Fogh, 1962; Weis-Fogh, 1956a, b; Weis-Fogh and Jensen, 1956). Since this time, considerable information has continued to amass concerning wing kinematics, aerodynamics, steering, muscle structure and function, central pattern generation of the flight rhythm, and more which is reviewed below. Further, the locust ear has been a model of insect audition since Gray (1960) described its structure and Michelsen (1971a-c) described the bioacoustic and physiological basis of direction and frequency discrimination. For these reasons more than any, locusts provide, at present, the best opportunity to answer questions about auditory sensorimotor integration of bat-like sounds for avoidance steering.

In this thesis, I present a detailed analysis of the changes in wing kinematics, motor pattern and free-flight behaviour that underlies the acoustic startle/escape reaction of flying locusts.

In the remaining pages of this chapter, I review the literature necessary for understanding the studies presented in this thesis. I begin by presenting the locust as a model system and describe certain aspects of its natural history and its amenability for studies of insect flight. I then describe the flight system of the locust in terms of wing kinematics, aerodynamics, motor control and steering. Following this, I explain how locusts hear, describe sounds of significance to the animal, and review our current understanding of how sounds are processed and integrated with the flight system of the locust. I end this chapter with a synopsis and overview of the remaining chapters of the thesis.

1.1 The Locust as a Model System

1.1.1 Locust Biology

The African migratory locust, *Locusta migratoria* L. (Order: Orthoptera, Family: Acrididae) and its subspecies are widely distributed and are found in equatorial regions of Africa south of the Sahara, parts of southern Europe, China, Asia and northern Australia (Barrass, 1964). The distinction between locusts and grasshoppers is primarily behavioural in that locusts tend to aggregate in large numbers for mass migration by swarming (Chapman, 1976). This distinction is not a taxonomic one as locust species occur in several subfamilies of the Acrididae (Uvarov, 1966). Ecologically locusts are found in areas of patchy vegetation due to their dual requirement of bare ground for oviposition and plants for food and shelter. *Locusta migratoria*, and locusts in general, are of great agricultural importance due to their potential for consuming and destroying crops. Concern continues today as human disturbance of natural areas and certain agricultural practices, such as clearing land for grazing and allowing land to be overgrazed, has inadvertently created habitat favourable for locusts. Also there is continued concern regarding the impact of pesticides on non-target species (Chapman, 1976). It is not surprising, therefore, that a rich literature spanning several decades has accumulated on many aspects of locust biology.

Locusts begin their life cycle as eggs deposited in soil within a protective matrix secreted by the female. The maximum number of eggs that can be deposited per pod depends on the number of ovarioles within the ovaries and in *L. migratoria* this is

between 80 and 105 depending on phase (see below). This potential is not realized and

typically a pod will contain approximately 60 eggs depending on the age and condition of

the female; approximately six pods will be deposited during the life of the locust. The

eggs develop in 8 to 12 days and first instar hoppers emerge from the egg chambers at the

soil surface. Locusts are hemimetabolous insects and as such the nymphs resemble the

adults. *Locusta migratoria* hoppers develop in five juvenile instars, requiring

approximately 20 to 45 days, depending primarily on temperature and food availability,

before they emerge as adults with fully formed wings. Reproductive maturation requires

approximately 28 to 33 days after adult emergence. In some species this period of

development is hastened by the presence of mature adults and it is believed that males

produce a pheromone that functions to bring the population into breeding synchrony

(Loher, 1960).

After adult emergence, a further developmental period of approximately two

weeks is required before the wings and flight apparatus is capable of sustaining normal,

powered, prolonged flight. During this period fat storage increases, muscles increase in

mass, the cuticle thickens, hardens and changes colour and both the peripheral and central

nervous system undergo substantial changes (Chapman, 1976; Wang *et al.*, 1993; Jensen

and Weis-Fogh, 1962; Burrows, 1996). These changes include growth and

reorganization of flight interneurons and sensory afferents (Gee and Robertson, 1994;

Gray and Robertson, 1996). Increases in afferent activity also influence the development

of the flight motor (Gray and Robertson, 1994; 1997). Overall, the period of adult

maturation is characterized by an exponential increase in wing beat frequency (Kutsch,

1973) and the ability of locusts to generate appropriate kinematics for generating lift and

thrust (Gewecke and Kutsch, 1979; Kutsch and Gewecke, 1979).

All locust species exhibit two behaviourally, and in some species morphologically

and physiologically, distinct phases (Uvarov, 1966). When locusts aggregate in large

numbers for migration by swarming, they are in the gregarious phase. At other times,

locusts tend toward isolation in their behaviour and are accordingly said to be in the

solitary phase. For several species of locusts, including *L. migratoria*, the phases can be

distinguished by morphological characteristics. Solitary phase *L. migratoria* possess a

pronotal crest and enlarged hind-femora relative to gregarious phase individuals. Also,

solitary phase females are larger than gregarious phase females, but solitary phase males

are typically smaller than gregarious phase males. The two phases are also

distinguishable by the colouration patterns of the nymphs. Gregarious phase *L.*

migratoria nymphs are black and orange but solitary phase nymphs are green or straw

coloured. The transformation between phases is believed to be primarily mediated by

frequent physical contact with conspecifics (i.e. the social environment) (Kennedy, 1956;

Uvarov, 1966).

Solitary and gregarious phase locusts differ in their flight tendencies with solitary

phase locusts having a greater tendency to fly at night (for dispersal) and gregarious

phase locusts tending to fly during the day (in swarms) (Kennedy, 1956). Mass

migrations allow the animals to exist in an environment where food supply is irregular

and seasonal. One trigger often cited for the initiation of swarms is the onset of rain

caused by the convergence of warm air masses; conditions that tend to favour growth of

new plants and creation of habitats suitable for breeding and hopper development. Swarm

sizes of desert locusts, *Schistocerca gregaria*, have been estimated to consist of many

million individuals spanning hundreds of square kilometers at altitudes reaching more

than 1000 meters (Uvarov, 1943). Swarm direction is primarily determined by wind

direction and swarms can travel 50 to 100 kilometers per day and several hundreds of

kilometers before dispersing. These distances are not travelled in single flights and

locusts set down during the night and during inclement weather; it is when the swarm

settles that it does the most damage to plants and crops. The mechanism underlying

swarm cohesion is still little understood but is believed to involve a combination of

sensory cues including an optical flow field, acoustic input, light cues, and detection of

wind generated by the beating wings of other locusts in the swarm (Chapman, 1976;

Kutsch *et al.*, 1994; Camhi *et al.*, 1995). Most impressive is the density of individuals

flying within swarms. Individuals have been observed traveling at speeds between three

and six meters per second with small inter-individual distances. Kutsch *et al.* (1994)

report that small pockets of locusts within a swarm may be aerodynamically coupled and

flying as close as 10 centimeters apart. Despite these high densities, and the fact that at

the edges of swarms locusts are observed changing direction to fly back into the swarm,

locusts have never been reported to have mid-air collisions. In other words, locusts are

agile fliers, able to change speed and direction very quickly.

For comprehensive information on the biology of locusts including ecology and

life-history, development, distribution and swarming behaviour, see the two volumes by

Sir Boris Uvarov (Uvarov, 1966; 1977) and reviews by Farrow (1990) and Gatehouse and

Zhang (1995).

1.1.2 Amenability for Flight and Sensorimotor Integration Studies

In locusts, flight is a robust behaviour and tethered locusts will beat their wings for long

periods of time, often several minutes and in some instances more than an hour,

facilitating data collection. The rhythmicity of the wing beat cycle means that large

amounts of cycle-to-cycle data can be collected quickly. A variety of recording methods

have been employed to record wing movement and muscle activity. Wing movements

have been recorded with various photographic techniques (strobe photography: Camhi,

1970; stereophotography: Zarnack, 1972; long exposure single frames: Thüring, 1986),

high-speed cinematography (Weis-Fogh and Jensen, 1956; Baker and Cooter, 1979a, b;

Baker et al., 1981), magnetic coils (Koch, 1977; Zarnack, 1978), and optoelectronic

methods (Hedwig and Becher, 1998). The accessibility of the flight muscles, coupled

with identifiable landmarks on the exoskeleton that aid in localization (e.g. sutures,

membranes, and spiracles), has facilitated multi-channel electromyography from tethered,

flying locusts (Wilson and Weis-Fogh, 1962; Zarnack and Möhl, 1977). Perhaps the

most significant advance facilitating neuroethological approaches to studying locust

flight has come from Wolfram Kutsch and colleagues who have recently developed

telemetric devices for recording flight muscle activity in free-flying locusts (Kutsch, et

al., 1993; Fischer, et al., 1996; Fischer, 1998). Although the size of available electronic

components has limited the device to two channels of information and the weight of the

battery necessary to power the device has restricted studies to primarily female

Schistocerca gregaria, considerable understanding is beginning to accumulate about the

relationship between wing kinematics and flight muscle activity during free-flight.

Locusts are capable of generating flight-like motor patterns in a restrained,

dissected condition making them amenable to studies using intracellular recording

techniques for determining the neuronal (cellular) basis of sensorimotor integration

(Robertson and Pearson, 1982). Like many invertebrates, locusts possess an accessible

CNS comprised of identifiable interneurons that are few and large in comparison with

the interneurons of vertebrate nervous systems (Comer and Robertson, 2001). This

means that the motor system controlling flight in locusts is more tractable than other

vertebrate or mammalian motor systems. It is becoming increasingly evident that many

similarities exist in the organizational principles governing CNS organization in

invertebrates and vertebrates. Because it is difficult, with few exceptions, to understand

the cellular and synaptic mechanisms of sensorimotor integration using vertebrate

models, locusts, and other invertebrate preparations, will prove valuable for future studies

(Murphey, 1986; Harris-Warrick, 1993; Pearson, 1993).

1.2 The Locust Flight System

1.2.1 Kinematics

Lift and thrust are produced by the coordinated movements of two pairs of wings. In

locusts, the wings move independently unlike other insects such as moths and bees in

which the forewings are structurally coupled to the hindwings. The forewings of locusts

are long, narrow and stiffer than the hindwings which are broader and more flexible. The

wings are comprised of thin cuticle and/or epicuticle within a lattice of veins and cross

veins. The veins, which are primarily structural, also carry tracheae, nerves for sensory

organs such as wind sensitive hairs, and haemolymph to maintain hydration of the cuticle

(Burrows, 1996). The shape of the wings is very important for generating aerodynamic

force. The forewings are each slightly twisted along their length and resemble the blade

of a propeller. The structure and arrangement of the veins work in concert with accessory

flight muscles and the sclerites at the base of the wings to control camber. A recent

biomechanical analysis of the hindwings has shown them to be particularly remarkable.

The camber of the hindwings during the downstroke is developed by the 'umbrella effect'

which is an automatic consequence of the mechanical properties of the cuticle, the

arrangement of the veins and cross veins, and the shape of the wing (Herbert et al., 2000;

Smith et al., 2000; Wootton, et al., 2000)

During straight, level flight, the body is positively pitched at an angle between 5

and 10 degrees relative to the flight path. The hindwing stroke leads the forewing stroke

and the left and right wing strokes are symmetrical about the dorsal-ventral axis of the

body. Forewing stroke amplitudes are smaller than hindwing stroke amplitudes by

approximately 30 degrees. Locusts typically beat their wings at rates between 16 and

26 Hz and flight speeds range from 3 to 6 meters per second depending on whether the

locust is in tethered or free-flight. During horizontal free-flight, flight speed is positively

correlated with wing beat frequency (Baker et al., 1981). As each wing is flapped, the

wing tip path typically follows a loop or figure-of-eight pattern and the major axis of this

looping pattern defines a stroke plane. The stroke plane of the forewings and hindwings

are not parallel and the forewing stroke plane tends to be inclined away from the vertical

more than the hindwing stroke plane (Baker and Cooter, 1979a; Fischer and Kutsch,

2000). The downstroke duration is normally longer than the upstroke duration but

depends on the ascent angle of the locusts flight path (Baker and Cooter, 1979a; Fischer

and Kutsch, 1999). At the end of the downstroke and upstroke the wings flip over to

establish the angle of attack for the next half stroke. Angle of attack, also called wing

twist or the amount of pronation or supination of the wing, is the angle formed between

the surface of the wing and the direction of air flow. In tethered flight, the wings are

normally pronated during the downstroke and supinated during the upstroke. Values for

kinematic parameters measured from tethered and free-flying *L. migratoria* are

summarized in table 1.1.

As noted above, many of these kinematic patterns depend on whether the locust is

in horizontal, climbing or descending flight. The downstroke is responsible for

producing most of the lift required for weight support and during climbing flight

downstroke duration increases while overall cycle length decreases (Fischer and Kutsch.

1999). Although this means that wing beat frequency increases, during ascending flight it

is not correlated with flight speed (Kutsch *et al.*, 1999; Fischer and Kutsch, 1999). Body

angle relative to the flight path decreases slightly as ascent angle increases (Kutsch *et al.*.

1999; Fischer and Kutsch, 1999). The angle of attack of the forewings increases

Table 1.1: Values of kinematic parameters surveyed from the literature* measured during horizontal flight in tethered and free-flying *Locusta migratoria*.

Parameter	Tethered		Free-flight	
	Value	Range	Value	Range
Wing beat frequency (Hz)[1]	19	16 to 24	23	18 to 26
Wing beat period (ms)	53	63 to 42	43	56 to 38
Flight Speed (m/sec)[1]	3.25	2.5 to 4.5	4.5	3.5 to 6
Upstroke to Downstroke Ratio[2]				
Forewings	1:1.5	1:1.3 to 1:1.6		1:1.1 to 1:1.5
Hindwings	1:1.4	1:1.3 to 1:1.6		1:1.1 to 1:1.8
Forewing stroke amplitude (degrees)[3]	80	75 to 140	100	– to 125
Hindwing stroke amplitude (degrees)[3]	110	95 to 135	135	– to 155
Body angle (degrees)[4]				
Relative to horizontal	not relevant	not relevant	7.4	-5.1 to 20.5
Relative to flight path	not relevant	not relevant	5.2	-5.3 to 18.4
Stroke plane (degrees)[5]				
Forewings	28	24 to 30	20	10 to 30
Hindwings	23		not measured	not measured

[1] Wing beat frequency and flight speed in tethered flight are affected by the speed of the windtunnel employed.

[2] Affected by body angle relative to horizontal (tethered) or ascent angle (free-flight): only range of values was cited for free-flight (Baker and Cooter, 1979a)

[3] Free flight wing stroke amplitude variation is as high as = 60 degrees (Baker and Cooter, 1979a)

[4] Body angle is fixed by experimenter during tethering therefore no values are presented. During free-flight, values measured from high-speed films of swarms are reported relative to horizontal and relative to the flight path of the individuals within the swarm.

[5] Measured relative to lateral view of body with 0 degrees directly above the animal and positive angles measured counter clockwise. Note: values depend on body angle (tethered) and ascent angle (free-flight). Values for tethered flight were invariant (Gewecke and Kutsch, 1979), values not measured for hindwings during free-flight (Baker and Cooter, 1979a).

* Data compiled from: Baker and Cooter (1979a, b); Baker et al. (1981); Dawson. et al. (1997); Gewecke (1970; 1975); Gewecke and Kutsch (1979); Kutsch and Gewecke (1979); Robertson and Reye (1992); Robertson et al. (1996); Schwenne and Zarnack (1987); Thüring (1986). Average values are cited where appropriate.

(becomes more pronated) and wing stroke amplitude of both the forewings and

hindwings increases (Fischer and Kutsch, 2000).

The movements of the wings do not solely depend on muscle activity. Kinetic

energy from wing movements is stored in the cuticle, muscle, and connective tissue of the

thorax which has a mechanical resonance near the wingbeat frequency of the animal.

This is energetically favourable at the ends of the half-strokes when the wings reverse

direction. In insects such as flies and bees. the mechanical properties of the thorax play a

more extensive role in generating wing movements. In these insects flight muscles are

myogenic and therefore contractions are stimulated by the movements of the flight

apparatus; neuronal input to the muscles acts to start. stop, and 'tune' the movements of

the wings (Dudley, 2000).

It would be incorrect to imply that the great diversity of insects that fly use the

same kinematic strategy. but observations from many insects. especially bees and

hawkmoths owing to their particularly well characterized kinematics (Dudley and

Ellington. 1990a. b; Willmott and Ellington. 1997a. b) indicate certain trends that are

useful for comparison with locusts. The following account describes the flight of a

typical insect (Ellington. 1999; Dudley, 2000). During horizontal forward flight, body

angle becomes more horizontal as the animal reaches its top flight speed. Wing stroke

amplitude tends to decrease with increasing speed, and as the animal accelerates forward.

the stroke plane tilts forward. For those insects capable of hovering, the stroke plane

approaches a horizontal level as forward velocity decreases to zero. Angle of attack

varies with flight speed and for some insects. a change in angle of attack alone is

sufficient for inducing forward acceleration. During slow forward flight and hovering, the body hangs below the wing bases and stability is aided by the pendular motion of the abdomen due to the relative positions of the centres of lift and mass. Similarly, during forward flight, the centre of mass, which is often at the thorax-abdomen junction, tends to produce an upward pitch of the head. For manoeuvrability, bilateral differences in stroke amplitudes and angle of attack produce roll torques whereas increases in drag on one side of the body, due to very high angles of attack in one wing, result in yaw torques. Pitching is controlled by fore-aft shifts in the centre of lift established by fore-aft differences in the angles of elevation of the wings relative to the stroke plane.

Unfortunately, the majority of research into locust flight has employed tethered locusts and many of the relationships between kinematic and flight variables during forward free-flight remain to be systematically quantified under controlled conditions. For example, for a free-flying locust in horizontal flight, the relationship between body angle, stroke plane angle of the fore- and hindwings, and wing stroke amplitude at different flight speeds is not known. Changes in angle of attack of the forewings and hindwings at different flight speeds has also not been determined for locusts in free-flight. The data in table 1.1 show that there is agreement between some kinematic variables in tethered and free-flying *L. migratoria* validating the use of tethered animals for many types of flight studies. However, certain variables such as wing beat frequency, flight speed and stroke amplitudes, as well as measurements of lift and drag, emphasize that the goal of future studies should be to employ free-flying animals. Knowing how

kinematic variables interact under identical conditions in free-flight is prerequisite for accurate mathematical modeling of the aerodynamic consequences of the kinematics.

1.2.2 Aerodynamics

To consider the flapping wings of insects to be no different than the wings of an aircraft would mean that steady-state aerodynamic mechanisms can account for the lift and thrust observed in flying insects. When insect wings are considered to be flat plates and the complex motions of the wings are ignored, then calculations of lift and drag will be insufficient to account for that produced by the insect in flight. This, of course, is the fallacy behind the claim that "an aerodynamicist proved that bumble bees can't fly" (McMasters, 1989). Steady-state aerodynamic mechanisms are those at play on a wing in constant velocity at constant angle of attack. The motion of insect wings, in contrast, are not steady. The wings are constantly changing direction as they move up and down and the angle of attack of the wings is constantly changing throughout the stroke. Further, the wing tips and wing bases travel through the air at different velocities. The unsteady movement of insect wings means the underlying aerodynamic mechanisms are different than those that keep airplanes and helicopters aloft.

Initial analyses of locust flight (Jensen, 1956; Weis-Fogh, 1956) treated the flapping wing as a time series of static states with the assumption that the instantaneous forces would be the same as those on a wing in steady motion at the same instantaneous velocity and angle of attack. This quasi-steady approach is most useful for modeling the fast forward flight of certain insects, bats, and birds where the flight velocity dominates

the air flow over the wings and the flapping motion minimally disturbs the steady flowing

air (Ellington, 1995). At slower flight speeds and larger flapping amplitudes, including

hovering, the quasi-steady approach breaks down, and for many insects, becomes totally

inadequate (Ellington, 1984a-f; Ennos, 1989; Dudley and Ellington, 1990a, b; Zanker and

Götz, 1990; Dudley, 1991; Wilkin and Williams, 1993). For locust fight, Cloupeau *et al.*,

(1979) measured lift in tethered flying locusts and showed that peak lift is approximately

twice the value calculated by Jensen (1956). Differences between measured and

calculated lift using quasi-steady approaches confirm that unsteady effects are dominant

in insect flight.

Lift is generated when air flow over the upper surface of a wing is faster than the

air flow beneath. The velocity difference depends on the camber and angle of attack of

the wing relative to the direction of the air meeting the leading edge of the wing. The

velocity difference can be represented by a vortex of air circulating around the wing

where the strength of the circulation of the vortex is directly proportional to the lift

generated by the wing (Ellington, 1984d). During flight, the wings must stop and reverse

direction at the end of each half stroke. In insects the leading edge of the wings are

particularly sharp and the rapid start of the stroke at high angles of attack creates an

additional vortex at the leading edge, called a leading edge vortex (LEV), within the

bound vortex of the wing. The circulation of the LEV creates additional lift that

augments the lift created by the bound vortex of the wing. Axial flow within the LEV

travelling toward the wing tip during translation stabilizes the LEV and delays separation

of the air flow from the wing surface in a process called delayed stall (Ellington, *et al.*, 1996).

One of the earliest unsteady effects to be suggested was the fling mechanism proposed by Weis-Fogh (1973) for the wasp *Encarsia formosa*. These wasps 'clap' their wings together dorsally then 'fling' them apart drawing air into the widening gap. The circulation of the air entering the gap generated by the rapid opening of the wings adds to the circulation bound to the wing thereby enhancing lift. Variations on the fling mechanism have been proposed by Ellington (1984d) and are employed by a variety of insects including locusts (Cooter and Baker, 1977).

At the ends of the translation phases of the wing stroke, the wings must rotate to establish the angle of attack for the next translation phase. As the wing nears the end of the downstroke, it supinates for the upstroke, and at the end of the upstroke, the wing pronates for the downstroke. Dickinson *et al.*, (1999) has shown that if the wing rotates just before the stroke reversal, rather than at or after the stroke reversal, then the rotation of the wing will augment the circulation of the vortex bound to the wing thereby creating additional lift. This effect is similar to the Magnus effect that explains why a baseball thrown with spin follows a curved trajectory.

When the wings reverse direction at the half strokes, the wings momentarily come to a stop and shed their bound vorticity as a stopping vortex. In some insects, like *Drosophila sp.*, the wingbeats are so rapid that the lingering vorticity from the previous stroke is 'captured' by the vorticity of the present stroke. The interaction of the new

bound vortex and the circulation in the 'captured' wake generates additional lift (Dickinson *et al.*, 1999).

The mechanisms described above are believed to be general principles underlying flight in many insects, however, the insect models used to determine these mechanisms (e.g. bees, hawkmoths, and fruitflies) are functionally two-winged insects. Locusts, and many other insects, are functionally four-winged and the aerodynamic interactions between the forewing pair and hindwing pair has not been studied thoroughly but are likely to be complex. One complexity is that both the forewings and hindwings produce lift but not in equal amounts; the hindwings produce approximately 75% of the lift required for weight balance in horizontal flight (Jensen, 1956). The high-lift mechanisms employed by insects could not have been predicted from quasi-steady analytical techniques and it is interesting engineers developing micro-air vehicles (MAVs) are turning to insects for kinematic strategies to maximize the payloads that these devices can carry. The mass supported by a MAV is proportional to n^2R^4 where n is wing beat frequency and R is wing length (Ellington, 1999). This means that increases in wing length are more effective at increasing payload than increases in wingbeat frequency. It follows that devices with four wings will be able to generate more lift than two-winged devices operating at the same wing lengths and wing beat frequencies and in the coming years, it is likely that focus will turn to functionally four-winged insects such as locusts.

1.2.3 Motor Control

During flight, the four wings are controlled by approximately 40 muscles (10 per wing)

which in turn are innervated by approximately 110 motorneurons with cell bodies in the

thoracic central nervous system (Burrows, 1996). The oscillation of down and up

movements of the wings are effected by depressor and elevator muscles respectively.

These muscles can be further classified as either direct muscles, indirect muscles or

accessory muscles depending on their insertion sites within the thorax relative to the

wing. Direct muscles control wing movement by inserting directly on the sclerites at the

base of the wing. Indirect muscles affect wing movement by distorting the shape of the

thorax; these muscles insert on the tergum and sternum of the thorax, or the coxa and

trochanter of the leg. Accessory muscles shape wing movements and affect angle of

attack. The arrangement of the flight muscles in the thorax is shown in figure 1.1 and the

nomenclature and kinematic effects of the major flight muscles are summarized in table

1.2. In locusts, muscle contraction is initiated neurogenically and therefore

depolarizations of the flight muscles are synchronous with motor neuron activity

originating at the central nervous system. The number of motorneurons innervating the

major flight muscles is indicated in table 1.2.

The basic flight rhythm in locusts is generated by a central pattern generator

(CPG) comprised of a pool of interneurons distributed within the three thoracic ganglia

and first three abdominal neuromeres (Wilson, 1961; Robertson and Pearson, 1984;

Robertson and Pearson, 1985a, b). The CPG is sufficient for generating the basic pattern

of alternating activity in elevators and depressors but interactions with proprioceptors

Figure 1.1. Diagram of the thoracic wing musculature of a locust with accompanying
videostills of a dissected *Locusta migratoria*. Dorsolongitudinal muscles and
central nervous system (A). Elevator muscles (B). Depressor muscles (C).
Anterior is to the left. dorsal is up. TAS, tympanic air sac: II, mesothoracic
ganglion: III, metathoracic ganglion: IICx, mesothoracic coxa; IIICx,
metathoracic coxa. Muscle numbers follow Snodgrass (1929). Diagrams
modified from Sandeman (1961) and Möhl and Zarnack (1977) as presented in
Burrows (1996). Scale bar is 10 mm for both diagrams and videostill images.
Videostill images taken by the author.

A

B

C
FW HW

85
114

IICx IIICx

IICx
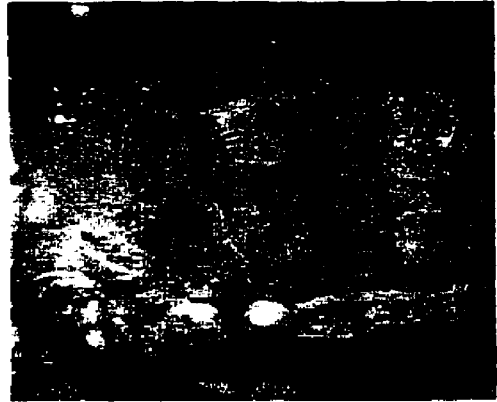
10 mm

Table 1.2: Major flight muscles and reported* kinematic effects.

Muscle[1]	Mesothorax	Metathorax	Kinematic effect	Insertion[2]	Motor Neurons[3]
First basalar	97	127	Depressor / Pronator	Direct	1
Second basalar	98	128	Depressor / Pronator	Direct	2
Subalar	99	129	Depressor /Supinator	Direct	2
Dorsolongitudinal	81	112	Depressor	Indirect	5
First tergosternal	83	113	Elevator	Indirect	1
Second tergosternal	84	--	Elevator	Indirect	1
Pleuro-alar (=pleuroaxillary)	85	114	Influences angle of attack	Accessory	2
Anterior tergocoxal	89	118	Elevator	Indirect	2
First posterior tergocoxal	90	119	Elevator	Indirect	3
Second posterior tergocoxal	91	120	Elevator	Indirect	4/6
Tergotrochanteral	103	133	Elevator	Indirect	3

[1]Muscle nomenclature according to Snodgrass (1929).

[2]Insertion refers to the site of muscle attachment: direct muscles insert onto the base of the wing. indirect muscles insert on locations within the thorax other than the wing bases (e.g. tergum. sternum. coxae and trochanters).

[3]The number of motor neurons innervating the muscle: for the second posterior tergocoxal. four motor neurons innervate the mesothoracic muscle and six innervate the metathoracic muscle. Burrows (1996) reports slightly different numbers of motor neurons innervating M127=2. M128=3 and M89=3.

*Wilson and Weis-Fogh. 1962: Bently. 1970: Elson and Pflüger. 1986: Pflüger et al.. 1986: Wolf. 1990: Burrows. 1996: Shoemaker and Robertson. 1998.

(e.g. tegulae, stretch receptors, and campaniform sensillae), exteroceptors (e.g. wind

sensitive hairs on the head and wings), and humoural factors (e.g. octopamine) confirm

that sensory feedback and neuromodulation is necessary to account for the emergent

properties of the flight system (Gewecke, 1970; Wendler, 1974; Gewecke, 1975; Bacon

and Tyrer, 1979; Altman, 1982; Horsmann, et al., 1983; Pearson, et al., 1983; Möhl,

1985a-c; Elson, 1987; Pearson and Wolf, 1987; Wolf and Pearson, 1987; Reye and

Pearson, 1988; Wolf and Pearson, 1989; Pearson and Ramirez, 1990; Ramirez and

Orchard, 1990; Ramirez and Pearson, 1991; Orchard et al., 1993; Wolf, 1993; Gee and

Robertson, 1998; Gee et al., 1998). Sensory input to the flight motor is critical for

making adaptive changes to the pattern of movement of the wings. A locust with a rigid,

uninterruptible flight pattern would not be able to make the continuous and numerous

adjustments necessary to cope with turbulence, motor errors in the flight apparatus, and

unexpected events occurring in the environment.

1.2.4 Motor Patterning

The function of the flight pattern generator is to produce a motor pattern that will evoke

appropriate wing movements that will result in the production of aerodynamic forces

sufficient to balance body weight during flight. A motor pattern is the coordinated series

of muscle activations that underlie a behaviour. In locusts, because contraction of the

flight muscles are under neurogenic control (i.e. the flight muscles are synchronous or

nonfibrillar), electromyograms (EMGs) are a good tool for studying aspects of the central

and motorneuronal events effecting flight. EMGs recorded from flight muscles provide

three types of information. The first is recruitment, which is the number of motor units

being activated within a cycle. When more than one motor unit is activated, the resulting

waveform in the EMG often appears larger in amplitude or with shoulders relative to the

waveforms of subsequent cycles. This is because different motor units of the same

muscle are activated synchronously or nearly so. The second piece of information is the

number of depolarizations of the muscle per activation cycle. This is a measure of the

force of contraction of the muscle (or depression of the wing). The last piece of

information is timing information. The timing of muscle activations (often called phase

shifts in the literature) are most important for flight and steering because asymmetries

between contralaterally homologous muscles and between different muscles on the same

side affect the timing of kinematic changes in the wings. The amplitude of the EMG

waveforms is often not useful because EMGs are extracellular recordings and susceptible

to physical parameters such as electrode position relative to the muscle of interest and

adjacent muscles, conductivity of the tissue and electrode material, and movement

artefacts. However, relative amplitude within a single recording can be used to discern

the activity of different motor units, and in some cases, antagonistic muscles (e.g.

elevator and depressors).

The force generated by contraction of the muscles is controlled by the number and

frequency of depolarizations. Typically, locust flight muscles are innervated by

relatively few (<5) motor neurons and recruitment probably plays a small role in

regulating contraction strength. Motor neuron terminals innervate the muscle fibres

along their length and in large muscles, such as the dorsal longitudinal muscles (M81,

M112), the innervation will be such that different motor units are formed. Because the

wing beat period is short (50 ms), twitches induced in depressor and elevator muscles can

overlap creating inefficiencies in flapping. Octopamine released during flight shortens

the duration of twitches and increases the force of muscle contractions (Whim and Evans,

1988).

As indicated by Schmidt and Zarnack (1987), when recording a flight motor

pattern it is important to record from as many muscles as possible because lift and thrust

are controlled by many kinematic parameters (e.g. wing stroke amplitude, angle of attack,

fore-hindwing interactions, etc.) which in turn are established by the concerted action of

all the muscles controlling each of the wings. Simultaneous multiple muscle recordings

from flight muscles during straight, tethered flight were first recorded by Wilson and

Weis-Fogh (1962) and later by others (Zarnack and Möhl, 1977; Möhl, 1985a;

Shoemaker, 1998; Kutsch, 1999). During horizontal tethered flight, the hindwings lead

the forewings and accordingly the hindwing depressor muscles are activated before the

forewing depressor muscles (figure 1.2). The lag between hindwing and forewing

depressor activation depends on wing beat frequency, but is typically in the range of 5 to

10 ms. All hindwing depressor muscles are essentially activated together and M127,

M128 and M129 intervals are typically less than 4 ms. In the forewings, M97 fires first,

followed by M98 and M99. M98 and M99 usually fire within approximately 2 ms of

each other. The downstroke is normally longer than the upstroke and elevator activity

begins just before or at the beginning of the upstroke.

28

Figure 1.2. The locust wingstroke and basic motor pattern. The larger amplitude hindwing stroke (open circles) leads the lower amplitude forewing stroke (closed circles) (Top). Approximate times of depressor and elevator activity are indicated in relation to the phases of the wingstroke (rectangles) (Bottom). Longitudinal depressors are the dorsolongitudinal (indirect depressor) muscles and vertical depressors are the direct depressor muscles of figure 1.1 and table 1.2. D-E, depressor-elevator interval; E-D, elevator-depressor interval. Modified from Weis-Fogh (1956b) as presented in Burrows (1996)*.

*Parts of the diagram presented in Burrows (1996) are also presumably from Wilson and Weis-Fogh (1962).

one wing beat cycle

150

120

90

Wing movement, degrees

front wing

hind wing

delay between
front and hind
wings

60

30

Front wing

longitudinal depressors

vertical depressors

elevators

D-E

E-D

Hind wing

longitudinal depressors

vertical depressors

elevators

0

20

40

60

Time, ms
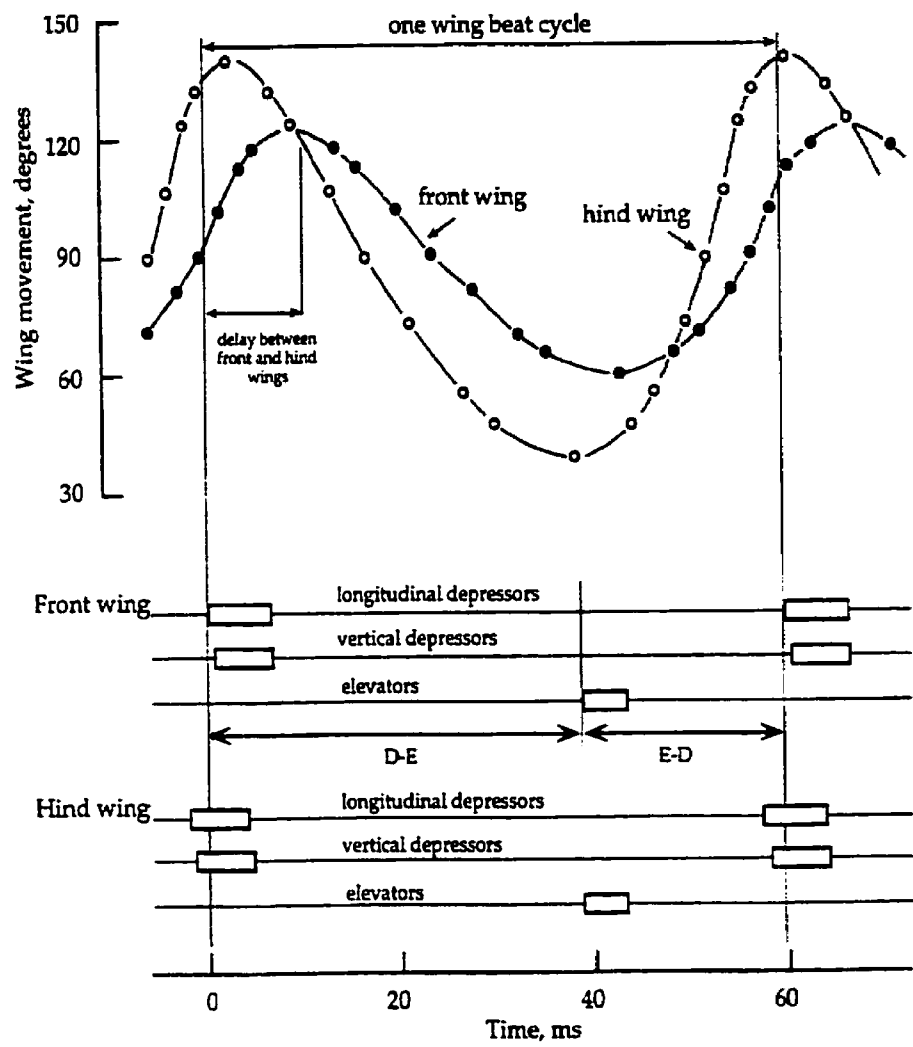
The motor pattern during straight, horizontal, free-flight is the same as during

tethered flight with the exception of the timing of M99 which occurs before M97 and

M98 (Kutsch, 1999). This difference in timing may be related to the requirement of free-

flying locusts to regulate lift. The timing of M99 (and M85) relative to M97 and M98

influences the angle of attack of the forewings (see below) which will affect the amount

of lift generated by the wings. Although only a trend, the activity of the motor units of

M99 may also depend on flight speed where the second motor unit increases spiking as

flight speed increases (Kutsch et al., 1999). During climbing flight, elevator timing is

delayed as ascent angle increases (Fischer and Kutsch, 1999). Both motor units of M99

are active during descending flight but only the first is active during climbing flight.

Similarly, M83 and M84 only have one motor unit that increases in number of spikes as

ascent angle increases (Kutsch et al., 1999). These results are consistent with

observations of tethered locusts at different body angles (Zarnack and Wortmann, 1989;

Wortmann and Zarnack, 1993).

1.2.5 Steering

Steering can be defined as behaviour that induces yaw, roll or pitch torques that change

the flight path of a flying locust (figure 1.3). Steering mechanisms are necessary to make

corrections to unexpected deviations from a straight flight path (correctional steering) and

to execute voluntary turns (intentional steering). Of these two types of steering, the

neuromuscular mechanisms underlying correctional steering to maintain a stable visual

field has been the most intensively studied.

Figure 1.3. Lift and thrust are the orthogonal components of the resultant force generated by the flapping motion of the wings during flight. Yaw, pitch and roll are rotations about the principle flight axes that result from torque forces generated during steering manoeuvres. Sideslip is lateral translation of the body without rotation about the flight axes. Pronation and supination are rotations of the wings about their longitudinal axes; pronation results when the leading edge tilts down relative to the direction of oncoming air, supination results when the leading edge tilts up. Taken from Möhl (1989).
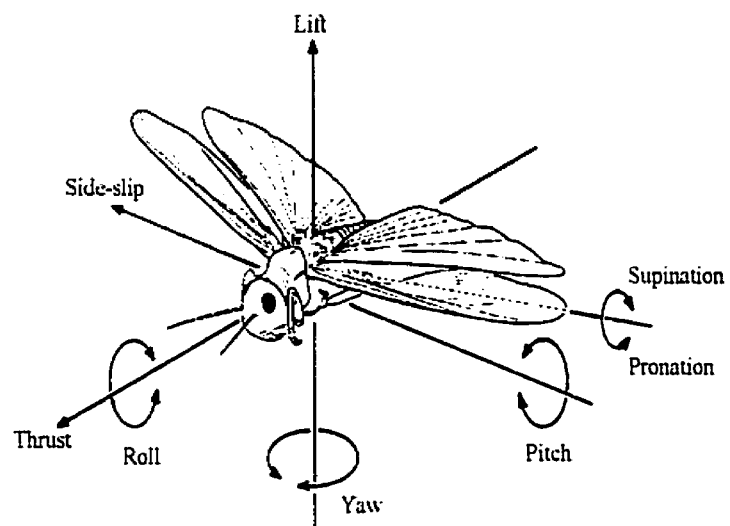
The locust optomotor response is a reaction to instabilities in the visual environment, detected by the compound eyes and ocelli, that cause a corrective steering response. The optomotor response of locusts can be demonstrated by placing a tethered flying locust on a torque meter in front of a textured pattern. When the pattern is moved, the locust will rotate its head and produce a steering torque in the direction of movement of the pattern (Robert, 1988). Head rotation is believed to provide visual reafference and a proprioceptive signal from mechanoreceptors at the neck that together improve the accuracy of the correctional steering response (Hensler and Robert, 1990; Robert and Rowell, 1992a). Unexpected deviations from straight flight can also produce momentary asymmetries in airflow over the locust body which are detected by the wind-sensitive mechanosensory hairs on the head and body (Weis-Fogh, 1949).

The correctional steering response, or 'autopilot', is mediated by deviation detector neurons which are large cells with somata in the supraoesophageal ganglion and descending axonal projections to the thoracic ganglia. They receive input from the compound eyes, ocelli and wind sensitive hairs and discharge tonically to specific stimuli. The morphology and response properties of three deviation detectors, DNI, DNM and DNC, have been characterized in detail (Griss and Rowell, 1986; Rowell and Reichert, 1986; Rowell, 1989). As an example, DNI responds best to an ipsilateral (with respect to the side of the descending axon) roll away from the horizon coupled with yaw and downward pitch, such as a banked, downward turn. Deviation detector neurons make connections with flight motor neurons but also with thoracic interneurons that, in turn,

make excitatory and inhibitory connections with specific flight motor neurons (Reichert and Rowell, 1985).

The thoracic interneurons are numerous and are divided into two classes based on response properties (Reichert and Rowell, 1985). The first class are all are multimodal and respond to wind, visual input from the compound eyes and ocelli, tactile input and/or auditory input. These neurons receive symmetric input from the ocelli, are not phasically modulated during flight, and therefore probably play a role in initiating flight or mediating startle responses but are not involved in correctional steering. The second class are more certainly involved in correctional steering and receive asymmetric ocellar input and respond to specific roll, and pitch conditions. These neurons are multimodal and are modulated during specific phases of the flight cycle (i.e. they are excited during either the depressor or elevator phase) (Reichert and Rowell, 1985). As an example, interneuron 016 is a candidate for definition as a thoracic interneuron: it is active during ipsilateral roll and is (possibly) post-synaptic to DNI and DNM, it is depolarized (and produces action potentials) during the depressor phase of the flight cycle and is inhibited during the elevator phase, and it receives input from interneurons of the flight pattern generator (e.g. IPSPs from interneurons 401 and 511 and EPSPs from interneuron 506) (Rowell and Reichert, 1991). The modulation of the thoracic interneurons by the flight pattern generator is important because is provides a mechanism for gating the descending information from the deviation detectors to the flight motorneurons during the appropriate phase of the wing beat cycle. Therefore, only when the flight pattern

generator is active, as is the case when the animal is flying, will corrective steering

responses be evoked (Reichert and Rowell, 1985; 1986; Reichert *et al.*, 1985).

Intentional steering occurs when an animal voluntarily changes its flight path.

This may be a response to an external cue such as impending collision, or an internal cue

such as finding a food source. Locusts must possess mechanisms for suppressing

correctional responses generated during intentional steering. For example, the visual

flow field will appear to move to the right both for an intentional turn to the left or an

unexpected deviation in flight path to the left. If the correctional steering response is not

suppressed then the motor command generated by the intentional steering response will

be in conflict. During intentional steering, head rotation is in the opposite direction to the

turn and current evidence suggests that optomotor interference is suppressed in such

instances (Robert and Rowell, 1992a).

Steering torques are generated by changes in wing kinematics and/or changes in

body posture that create asymmetric lift or thrust forces or increase drag on part of the

body. When locusts steer they deflect their abdomens and hindlegs toward the direction

of the intended turn (Camhi, 1970; Arbas, 1986; Baader, 1990; Lorez, 1995). Deflection

causes an increase in drag and shifts the centre of mass around which flight forces act

(Camhi, 1970; Zanker, 1988). Although these movements contribute to the steering

torques that are generated, it is changes in the wing kinematics that are most important

for steering. In fact, during correctional steering, often wing kinematic changes alone are

sufficient for restoring the flight path. The wing movements that are produced during

steering and their underlying motor patterns are not well understood, especially in free-

flight. Wing kinematic changes in tethered locusts that have been described as

contributing to steering torques include asymmetric changes in angle of attack of the

wings, asymmetric changes in wing stroke amplitude and phase shifts in the timing of the

wing beats either between forewing and hindwing pairs or between contralateral wing

pairs (for review see Rowell, 1988 and Dudley, 2000).

Studies of correctional and intentional steering most frequently cite changes in

angle of attack as being the most important kinematic parameter for generating steering

torques (Dugard, 1967; Baker 1979a; Zarnack, 1988; Waldmann and Zarnack, 1988).

During a turn, the wing on the inside of the turn path is more pronated than the wing on

the outside of the turn. Increasing the angle of attack (pitching the wing down relative to

the oncoming air) decreases the amount of lift produced by the wing. Angle of attack is

established by the relative timing of basalar, subalar and pleuro-alar muscles and motor

patterns recorded from the forewings during steering frequently show phase shifts in the

relative timing of these muscles (Zarnack and Möhl, 1977; Shoemaker, 1998). The

mechanism for establishing angle of attack was determined by Nachtigall (1981) and

Pfau and Nachtigall (1981) but is briefly reviewed in English by Nachtigall (1989).

Asymmetric forewing depression, coupled with asymmetries in angle of attack,

are commonly observed during avoidance reactions (intentional steering) to objects on

collision courses (Robertson and Reye, 1992) and intense radiant heat (Robertson et al.,

1996). During collision avoidance, forewing asymmetry is accompanied by the

production of yaw torques away from the oncoming object (Robertson and Johnson,

1993). Asymmetric changes in wingstroke amplitude are also observed during

correctional steering but aerodynamic explanations for their role are less clear (Thüring,

1986; Waldmann and Zarnack, 1988; Zarnack, 1988). Changes in the forewing stroke

relative to the hindwing stroke are also thought to play a role in steering (Schwenne and

Zarnack, 1987; Zarnack, 1988) but until the aerodynamics of these interactions are better

understood the significance of the observed kinematics will remain speculation.

Regardless, it is clear that the forewings play the dominant role in generating asymmetric

forces for steering and evidence of this is that hindwing kinematic changes during

steering, when they are observed, are small.

1.2.6 Motor Patterns During Steering

Left-right asymmetries in the timing of contralaterally homologous muscle activations are

most important for establishing asymmetric wing kinematics that will produce yaw and

roll torques. Zarnack and Möhl (1977) found that tethered locusts rotated independently

around their yaw, pitch and roll axes while flying in a wind stream shifted the activation

times of all the major depressor muscles relative to the mean time of the wing stroke

cycle (calculated as the mean time of all active spikes for a cycle). When locusts were

rotated in yaw, they found that muscles M97, M98, M127, and M128 (the first and

second basalars) shifted together and fired earlier on the ipsilateral side relative to the

direction of yaw rotation. Muscles M99 and M129 (the subalars) shifted together and

fired later on the ipsilateral side of the rotation. When locusts were rolled, they found

that all forewing muscles on the contralateral side relative to the roll direction shifted and

fired earlier whereas the muscles on the ipsilateral side were depolarized later. When

locusts were pitched, they found that the depressor muscles on left and right sides shifted

together with M97 and M98 advancing in the cycle and M99 and 129 firing later. For all

these stimuli, the amplitude of the rotations was 15 degrees and the magnitude of shifts in

the muscles was less than 6 ms. During these correctional steering responses, shifts in

muscle timing were accompanied by changes in the number of spikes per cycle in some,

but not all of the depressor muscles. During imposed yaw, M98 and M99 showed

increases in the number of spikes but M97 and M127 did not (Möhl and Zarnack, 1977).

Similar shifts in timing are observed when locusts are engaged in correctional

steering to re-stabilize a rolling visual horizon (Thüring, 1986; Schmidt and Zarnack,

1987; Waldmann and Zarnack, 1988). In these studies, the forewing depressors

ipsilateral to the turn direction fire earlier, by about 5 ms, than their contralateral

homologues but there is no accompanying change in the number of spikes per cycle. The

time shifts of the hindwing depressors, M127 (Schmidt and Zarnack, 1987) and M129

(Thüring, 1986) shift in opposite directions to the forewings.

Although intentional steering manoeuvres are not isolated in yaw, roll and pitch

as above, similar patterns of change in depressor timing are observed during thermal

avoidance steering (Shoemaker and Robertson, 1998). Forewing depressor muscles are

activated earlier on the side on the inside of the turn path but hindwing M127 are

activated later. The shifts in forewing muscle activation are larger than observed during

correctional steering and shifts as large as 12 ms have been observed (Baker, 1979b,

Robertson et al., 1996; Shoemaker and Robertson, 1998). Further, unlike correctional

steering, intentional steering manoeuvres are accompanied by increases in the number of

spikes per cycle. Hindwing motor patterns have received relatively little attention,

presumably because they are thought to contribute little to generating steering torques,

and shifts in depressor timing during intentional (and correctional steering) are small

relative to the shifts of seen in the forewings.

1.2.7 Tethering vs. Free-flight

Until recently, motor pattern studies have been restricted to studies with tethered animals.

Although tethering animals is convenient for detailed recordings of wing movement and

muscle activity and for employing other transducers during experimentation, this

convenience comes at a cost. Tethering affects the performance of the locust by opening

or interfering with sensory feedback loops that are normally closed during free-flight.

Even with sophisticated tethering equipment (Baader, et al., 1992; Spork and Preiss,

1993), restricting the movement of the animal results in either absent or inappropriate

exteroceptive and proprioceptive sensory information being processed by the animal.

The most commonly cited effect of tethering is that wing beat frequency of tethered

locusts is lower than that for free-flying locusts (Baker, et al., 1981; Kutsch and

Stevenson, 1981) and that tethered locusts frequently do not produce sufficient lift to

counter their body weight (Gewecke and Kutsch, 1979; Kutsch and Gewecke, 1979;

Zarnack and Wortmann, 1989). During steering studies, the direction of fictive turns is

often affected and this is apparent as biases in left-right asymmetries in the timing of

contralaterally homologous flight muscle activation (Robertson et al., 1996; Shoemaker

and Robertson, 1998). Although manipulating the tether can reverse the direction of

these biases, frequently it is not possible to eliminate the biases altogether (Dawson,

personal observation).

Despite the limitations of tethering locusts, it is clear that elements of the

behaviour are real. The responses of tethered locusts to bat-like sounds (Robert, 1989),

objects on collision course (Robertson and Reye, 1992; Robertson and Johnson, 1993)

and intense radiant heat sources (Robertson *et al.*, 1996; Shoemaker and Robertson,

1998) are coordinated responses that show agreement in several behaviours indicative of

attempted steering direction such as the direction of abdomen and hindleg deflection, the

direction of yaw torques, shifts in wing kinematic parameters such as angle of attack and

forewing asymmetry, and the sign of shifts in left-right M97 activity. Roeder (1967b;

1975) has offered enlightening discussion on the subject of exposing tethered insects to

sound and cautions that that certain aspects of the responses observed will be affected by

the effects of tethering more than others. This author agrees and notes that in the case of

startle/escape responses, which are characteristically short latency, abrupt reactions to a

stimulus, the initial events of the reaction are likely less affected by tethering than later

events. This is especially true in cases where reactions last several hundred wing beat

cycles (e.g. thermal avoidance).

1.3 Locust Bioacoustics

1.3.1 Hearing

In locusts, as with many members of the Acrididae, the ears are located on either side of

the first abdominal segment. The tympanic membrane of *Locusta migratoria* measures

approximately 1.5 mm wide and 2 mm long and is supported by a ring of rigid, heavily

scleritized cuticle recessed into the abdominal wall (figure 1.4). The tympanic membrane

is backed by several air sacs (labelled TAS in figure 1.1) derived from trachea. The air

sacs, which are open to the exterior of the animal by way of the spiracles of adjacent

segments, allow the tympanic membrane unimpeded vibration.

Sound localization occurs in two ways. At low frequencies (less than approximately 8

kHz), sounds are transmitted though the body, which acts as a low-pass filter, and the ear

acts as a mixed pressure and pressure gradient receiver. At high frequencies (greater than

approximately 10 kHz), sounds are diffracted and attenuated by the body and localization

is by inter-aural intensity differences (Römer, 1976; Miller, 1977). At these higher

frequencies the ear acts as a pure pressure receiver (Michelsen, 1971a-c). The sound

pressure gradient at the two ears of a locust anaesthetized in typical flight posture (mid

downstroke) exposed to 15 kHz and 30 kHz sounds is on average 8.2 ± 2.2 dB and is due

to a "sound shadow" cast by the body of the locust (Robert, 1989). The posture of the

wings, legs, and abdomen have a small effect on the ability of locusts to localize sounds

at these high frequencies (Adam, 1983; Miller, 1977; Robert, 1989).

40

Figure 1.4. Diagram of the locust tympanum (A). Video still image of a dissected *Locusta migratoria* tympanum with the tympanic air sac removed showing Müeller's Organ. View is from the inside animal looking out through the tympanic membrane. AN. auditory nerve; M. Müeller's Organ; Py. pyriform vesicle; Thick. region of thick membrane; Thin. region of thin membrane. Anterior is to the left. dorsal is up. Scale bar is 1.5 mm. Diagram taken from Gray (1960), videostill image taken by the author.

A
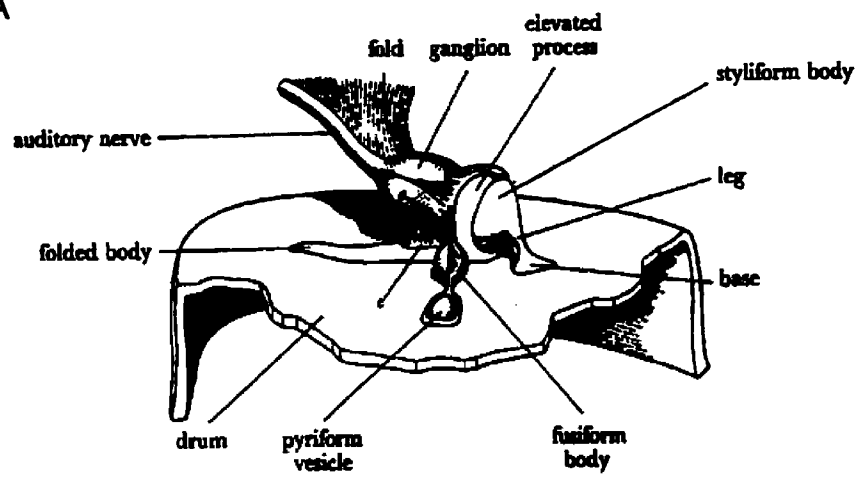
B

Frequency discrimination in locusts is due to the mechanical properties of the ear and the

spatial arrangement of the receptor cells within the auditory receptor organ, called

Müller's organ (Michelsen, 1971a-c; Stephen and Bennet-Clark, 1982; Breckow and

Sippel, 1985). The locust tympanum varies in thickness with anterior portions being

composed of relatively thick cuticle and posterior portions composed of relatively thin

cuticle (figure 1.4). Müller's organ is an assembly of sclerites attached to the interior

surface of the tympanic membrane at the transition of the thick and thin regions of

cuticle. It sits atop a tripod of three sclerites: the folded body and styliform body

forming two legs of the tripod positioned on the thick part of the membrane, and the

fusiform body forming the third leg which attaches at the pyriform vesicle on the thin

portion of the cuticle. The sclerites of Müller's organ contain approximately 60 to 80

scolopidial cells (Gray, 1960), the axons of which form the auditory nerve which enters

the metathoracic ganglion by way of nerve six. The scolopidial cells are organized into

four groups based on their location within Müller's organ (numbers in parentheses

indicate approximate number of scolopidial cells found): the a receptors (n=35) are found

near the elevated process, the b receptors (n=12) are near the tip of the styliform body,

the c receptors (n=10) are near the folded body and the d receptors (n=8) are near the

pyriform vesicle. The last group of receptors, the d group, are of particular importance in

high-frequency hearing because the pyriform vesicle is attached to the tympanum where

it is quite thin, although not the most thin region of the membrane (figure 1.4).

Pressure waves set the tympanic membrane into vibration but because it varies in

thickness and because of the presence (i.e. weight and location) of Müller's organ, the

vibrations of the system are complex. The system, as a whole, has two frequency ranges within which it resonates, one resonant peak is in a low frequency range of 1.5 to 9 kHz, and the other is in a higher frequency range of 10 to 30 kHz. Further, the membrane where the sclerites of Müller's organ attach resonate at different frequencies. The membrane at the folded body resonates at 3.5 kHz, at the styliform body it resonates at 5.6 kHz, and at the fusiform body it resonates at the highest frequency of 13 kHz. Depending on frequency, when the tympanum vibrates, a complex distribution of shearing forces are generated between the different sclerites of Müller's organ that generate a unique pattern of receptor potentials in the dendrites of the auditory receptor cells. Therefore, it is the relative activity of the different receptors, due to their spatial arrangement within Müller's organ and the mechanical properties of Müller's organ and the tympanic membrane that provides frequency specific information to the CNS.

Recently, Meyer and Elsner (1996) surveyed several acridid grasshopper species using laser-doper vibrometry to determine the relationship between membrane vibration characteristics, auditory nerve thresholds, and characteristics of the song spectra. This study surveyed 18 species from the subfamily Gomphocerinae, a group which uses elaborate acoustic signals in mate finding and recognition, and two species from the subfamily Oedipodinae including *L. migratoria*. They showed that for *L. migratoria* and the Gomphocerinae, the membrane is in maximum vibration around 8 kHz at the a cell attachment site and is in maximum vibration around both 8 kHz and 16 kHz at the d cell attachment site. Song spectra for *L. migratoria* and the majority of the Gomphocerinae indicate a low frequency peak around 8 kHz which agrees with the frequency of

maximum vibration at the a cell attachment site; however, the song spectra of the

Gomphocerinae also show a high-frequency peak around 30 kHz which is does not match

the frequency of vibration of the membrane at the d cell attachment site. Although Meyer

and Elsner (1996) only indicate a low frequency peak in the song spectra of *L.*

migratoria, this author's analysis of the stridulation sounds indicates a second peak in the

spectrum, albeit of lower energy relative to the peak frequency, between 30 and 40 kHz

(see below and figure 1.6). Therefore, just as in the Gomphocerinae, there appears to be a

discrepancy in the high-frequency tuning of the tympanum vibrations for *L. migratoria*.

Audiograms generated by recording compound action potentials from nerve six of

L. migratoria (figure 1.5) show greatest sensitivity to frequencies below 20 kHz which

are in register with the dominant frequency band contained in the song spectrum.

Audiograms from nerve six recordings of the Gomphocerinae, in contrast, are more

broadly tuned and typically show two best frequency ranges, one between 5 and 15 kHz

and the other around 30 kHz. This is also in agreement with the frequencies contained in

the song spectra but is not in agreement with the tuning of the tympanal vibrations at the

high-frequency receptor cell attachment locations. The explanation suggested by Meyer

and Elsner (1996) for this discrepancy is that the fusiform body has a resonant frequency

greater than 20 kHz. This is possible because the fusiform body is attached to the

membrane at the pyriform vesicle, a thin part of the membrane, and because it is

structurally separate from the rest of Müller's organ, it will therefore have unique

vibrational characteristics. Breckow and Sippel (1985) showed that the fusiform body

had a resonant frequency near 13 kHz but their tests only extended to a maximum of

Figure 1.5. Audiograms of *Locusta migratoria* derived from extracellular whole nerve
recordings from nerve six. Audiograms were determined with the hindwing
elevated to simulate the start of the downstroke (closed circles) and with the
hindwing depressed and covering the ear to simulate the bottom of the
downstroke (open circles). The audiogram shows that the locust ear is most
sensitive to sounds with carrier frequencies below 20 kHz (the frequency range of
the speaker used in the procedure did not allow testing below 5 kHz and therefore
determination of the best frequency). The number of low frequency receptors,
n≈57, originating in Müeller's Organ is much larger than the number of high-
frequency sensitive receptors, n≈8 (Gray, 1960). Therefore, in extracellular
whole nerve recordings, it is more difficult to detect the activity of the high-
frequency fibres against background activity and this results in an apparent
absence of high-frequency sensitivity. Despite this limitation, a slight increase in
sensitivity to frequencies between 30 and 45 kHz is apparent. Data are means
from five animals, error bars represent one standard deviation.

Audiograms were determined in a faraday cage lined with sound attenuating
foam. Animals were fastened to modeling clay ventrum up. A small window was
cut into the meso-metathoracic sterna to expose the metathoracic ganglion and
nerve six. Care was taken not to disturb the trachea or air sacs of the ear.
Extracellular recordings were made with stainless steel hook electrodes and
compound action potentials (CAPs) were amplified (Grass, model P15) and
displayed on the screen of a digital storage oscilloscope with sweep averaging
capabilities (Tektronix TekScope, model THS710A). A speaker (Technics, model
EAS10TH400B) was position 20 cm from the tympanum. Stimulus pulses
consisted of 5 ms shaped pulses (0.5 ms rise/fall time) presented at a 500 ms
period. During testing, the carrier frequency of the stimulus pulses was
incremented from 5 kHz to 75 kHz in 5 kHz steps. At each frequency, intensity
was gradually increased until threshold was determined. Threshold was detected
by triggering the oscilloscope with the stimulus pulses and averaging eight
consecutive sweeps. Threshold was reached when the amplitude of the CAP was
twice the amplitude of background noise. Sound intensity was calibrated by
broadcasting pure tones equal in peak to peak amplitude as the stimulus pulses
(Stapells *et al.*, 1982) to a ¼ inch Brüel and Kjær microphone (type 4135 without
protective grid) positioned in the faraday cage at the same position as the locust
tympanum during testing. Intensities were read directly from the output of a
Brüel and Kjær sound level meter (type 2610).
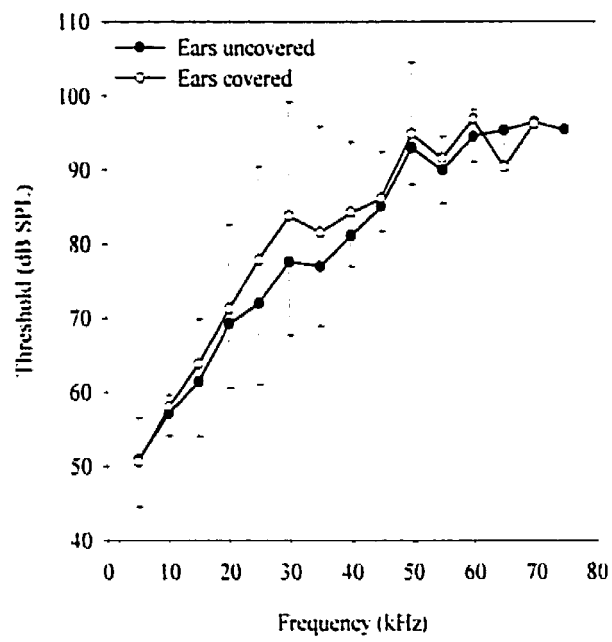
20 kHz. Therefore, as Meyer and Elsner (1996) suggest, it is an open question as to

whether there is a separate resonance peak in the ultrasonic region. This is an important

question to answer because if this is true for the Gomphocerinae then it would likely also

be true for *L. migratoria* and would provide a biophysical basis for explaining the high-

frequency tuning of the ASR of *L. migratoria* (Robert, 1989).

Extracellular recordings from nerve six are multiunit recordings and the amplitude

of the compound action potential depends on the number of receptor fibres active at the

site of the recording electrode. There are approximately 57 low frequency receptors and

8 high-frequency receptors in *L. migratoria* (Gray, 1960). Therefore it is more difficult

to detect the activity of the high-frequency receptors amongst background activity

occurring in the nerve than it is to detect the activity of the low frequency receptors. The

functional consequence of this is that threshold will be underestimated when based on

detecting the amplitude of the compound action potential, for example by considering

threshold when activity exceeds twice the amplitude of background activity (Yack *et al.*,

2000).

Another method of assessing auditory sensitivity, especially to high-frequency

sounds, is with audiograms derived from behavioural criteria for threshold detection.

This technique takes advantage of the fact that certain components of the acoustic

startle/escape response are relatively stereotyped behaviours and are easy to detect in

tethered, flying locusts. One parameter used by Robert (1989) and by this author (chapter

4, figure 4-4) is lateral deflection of the abdomen indicating an intended steering response

(Camhi, 1970). Behavioural audiograms show a clear best frequency between 25 and 30

kHz in tethered, flying locusts when they are stimulated with pulse trains of sound.

Further, this shows that although there are fewer high-frequency sensitive auditory

receptors, their connections with the flight system are quite consequential.

1.3.2 Sound Production

Locusta migratoria produce sounds with a tegmino-femoral mechanism in which the

metathoracic femur is moved up and down repeatedly against serrations found on the

intercalary vein of the forewing (Uvarov, 1966). The forewing vein is especially well

developed in males but both males and females are capable of sound production. The

fact that the forewings possess the 'file' is a departure from the typical situation found in

the Gomphocerinae, Truxalinae and Eremogryllinae in which the metathoracic femora

possesses a row of pegs which act as the 'file' (Uvarov, 1966). The sounds of *L.*

migratoria (figure 1.6) are broad-band with peak energies between 6 and 18 kHz and

little energy above 20 kHz (Kalmring, 1975b; Keuper, *et al.*, 1985; Meyer and Elsner,

1996). The sounds produced by acridids are generally not narrowly tuned (as is

frequently observed in crickets) but are broadband because the sounds are produced by a

stridulatory mechanism that is non-resonant. The acoustic signal typically contains one or

a few chirps which each consisting of 6 to 12 double pulses at a repetition rate of

approximately 10 to 12 Hz. The intensity of the sounds is approximately 60 dB SPL at

10 cm. The stridulatory sounds are primarily disturbance calls ('disabusing calls')

produced by interacting males and especially by males in copula when disturbed by other

47

Figure 1.6. Oscillograms and frequency spectra of the tegmino-femoral stridulatory
sounds of *Locusta migratoria*. Chirps from three different locusts were recorded
in a faraday cage lined with sound attenuating foam. Topmost oscillogram is two
chirps, lower oscillogram is an expanded view of the first chirp. Sounds are
produced when the metathoracic femur is moved against serrations present on
intercalary vein of the forewing. The first pulse in a chirp is the result of the leg
being elevated and subsequent double pulses are generated by the leg being
lowered and raised again against the 'file' on the forewing. The sounds are broad
band with peak energies between 8 and 20 kHz. Harmonics are also evident
between 30 and 50 kHz. Typically only a few chirps are produced at a time by
solitary males or males in copula when disturbed by other individuals. Each chirp
consists of 6 to 12 double pulses and the pulses within a double pulse are
produced at a rate of 10 to 12 Hz.

Sounds were recorded with a ¼ inch Brüel and Kjær microphone (type 4135 with
protective grid and with a type 2618 preamplifier) coupled to a Brüel and Kjær
sound level meter (type 2606) onto magnetic tape (BASF HiFi DP26) using a
RACAL instrumentation tape recorder (model Store-4) using a direct board with
the tape traveling at 30 ips. For analysis, signals on tape were replayed at 30 ips
and digitized at a sampling rate of 333 kHz (Axon Instruments Digidata, model
100B using Axoscope v7.0 software). Spectra were generated with ScopeDSP
v3.6a (Iowegian International Corp.)

2 V

100 ms

2 V

50 ms

| Locust 1 - double pulse | Locust 1 - first pulse | Locust 1 - second pulse |

| Locust 2 - double pulse | Locust 2 - first pulse | Locust 2 - second pulse |

| Locust 3 - double pulse | Locust 3 - first pulse | Locust 3 - second pulse |

individuals (Bar-Ilan *et al.*, 1969) but it is unlikely these sounds play a role in obtaining

mates (Haskell, 1957; Bar-Ilan *et al.*, 1969).

Locusta migratoria also produce sound by mandibular stridulation (Uvarov, 1966)

by way of an unknown mechanism involving the mandibles or other oral structures.

These sounds (figure 1.7) are produced most often immediately before and during

feeding but also by individuals interacting in a cage. They are short duration (1.4 ms),

broad band clicks or trains of clicks. Frequency spectra of the clicks often show two

peaks of energy; the first peak is approximately 8 kHz, and the second is approximately

30 kHz. Temporal-spectral parameters of the clicks are summarized in table 1.3.

1.3.3 Auditory Processing

Axons from the auditory receptors within Müller's organ enter the metathoracic ganglion

via nerve six and project ipsilaterally to the medial ventral association centre (mVAC) in

all three thoracic ganglia and the first abdominal neuromere of the metathoracic ganglion

(Tyrer and Gregory, 1982). The afferent terminations in mVAC are segregated

anatomically according to frequency and threshold intensity (Römer, 1985). Low

frequency fibres terminate ventral-medially with higher threshold afferents more

proximal than lower threshold afferents. High frequency fibres terminate ventrally more

distal to the low frequency fibres. Both frequency and intensity are important parameters

coded by many ascending auditory interneurons (Kalmring, 1975a).

Interneurons are found within all three thoracic ganglia that receive direct

monosynaptic input from afferents. Examples include interneuron 530 (=BSN1) and

Figure 1.7. Oscillograms and frequency spectra of clicks produced by mandibular
    stridulation in *Locusta migratoria*. Clicks were recorded while five males were
    interacting in a foam lined faraday cage. The topmost oscillogram shows seven
    clicks and the lower oscillogram shows an enlarged version of the first click.
    Although intensity was not measured, the clicks are very intense given the large
    amplitudes relative to background noise. The clicks are very short duration with
    durations less than two milliseconds. The clicks are broadband with two peaks of
    energy. The first peak typically occurs between 5 and 20 kHz. and the second
    between 20 and 45 kHz.

    Recording method as in figure 1.6.
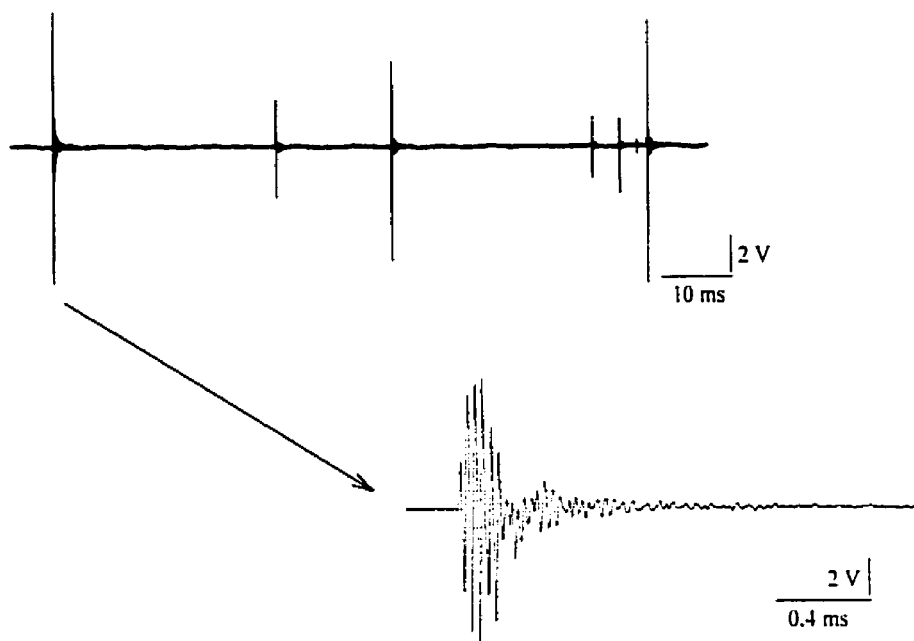
Frequency spectra of the first four clicks in the oscillogram:
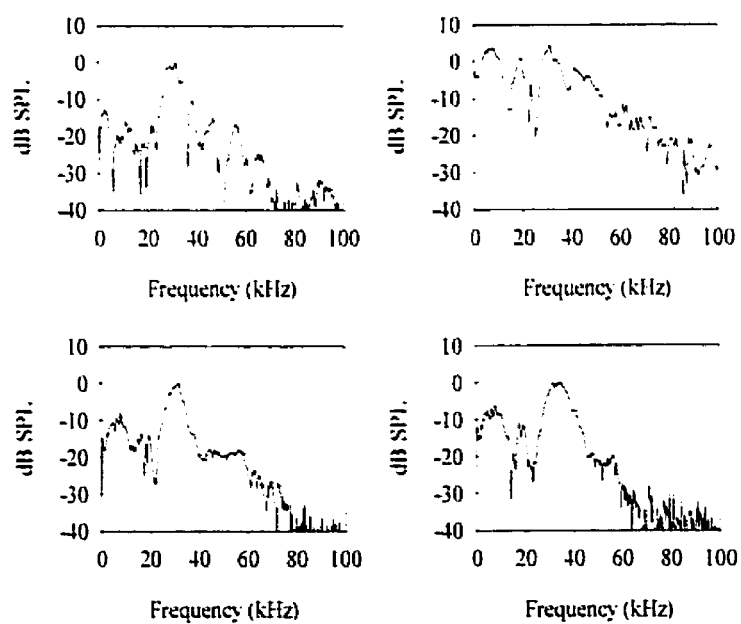
Table 1.3: Temporal and spectral characteristics of mandible clicks produced by *Locusta migratoria* males interacting in a cage. See figure 1.6 for recording and analysis method.

| Parameter | Train Duration (ms) | Clicks per Train | Click Duration (ms) | Inter-Click-Interval[1] | | Low Frequency[2] | | High Frequency[2] | |
				Clicks not in Bursts (ms)	Clicks in Bursts (ms)	Spectral Peak (kHz)	Bandwidth[3] Δf_{10dB} (kHz)	Spectral Peak (kHz)	Bandwidth[3] Δf_{10dB} (kHz)
Average	84.9	7.8	1.4	28.2	3.1	8.0	11.0	31.2	13.8
S.D.	18.5	1.5	0.4	12.9	1.5	1.2	2.1	3.3	4.1
Min	62.0	6	0.7	10.4	1.26	6.0	8.1	25.7	8.2
Max	114.0	10	2.4	56.9	8.5	9.9	14.3	35.8	21.7
N[4]	6	6	38	15	26	12	11	13	12

[1] Trains of clicks consisted of individual clicks and short bursts of clicks. Clicks within bursts of often increased in repetition rate (not evident given calculations above).

[2] Fourier transforms of the waveforms frequently. but not universally. showed two distinct peaks.

[3] Bandwidth was calculated as the difference in frequencies occurring 10 dB below the spectral peak.

[4] N refers to the number of trains or clicks analyzed. not the number of animals. Recordings were made from five male *Locusta migratoria* interacting in a cage.

interneuron 601 (=TN1). Synonomies for some auditory interneurons are listed in table

1.4. Some higher order thoracic auditory interneurons, such as interneuron SN5, have

tuning characteristics similar to the d-group (high-frequency) afferents (Marquart, 1985a

cited in Robert, 1989). Many of the higher-order thoracic auditory interneurons are

predominantly excited by high-frequency sounds. Interneuron 533, for example, is

inhibited by low frequency sounds but shows mixed excitatory and inhibitory responses

to conspecific sounds and is excited by high-frequency sounds (Römer and Seikowsky,

1985). This type of response pattern suggests it may play a role in detecting or

identifying predators such as echolocating bats. In general, the function of thoracic

auditory interneurons appears to be primarily extracting features of sounds and

localization of sounds rather than recognizing sounds. The thoracic auditory interneurons

vary in response properties with some interneurons simply responding phasically or

tonically to stimulation while others show preferred responses to certain frequencies and

intensities or to stimuli from particular directions (Kalmring, 1975a). The majority of the

auditory interneurons in the metathoracic ganglion possess this latter property and receive

binaural input from both ears suggesting they function in sound localization (Rowell and

McKay, 1969; Kalmaring et al., 1972; Römer et al., 1981; Römer and Dronse, 1982;

Römer and Seikowsky, 1985; von Helversen and Rheinländer, 1988).

The auditory interneurons in the suboesophageal ganglion and brain typically

possess long latencies suggesting they are several layers post-synaptic to the auditory

afferents (Kalmring, 1971). In contrast to the thoracic auditory interneurons, many of

these interneurons respond to the entire song (or stridulatory sequence) but not

Table 1.4: Some auditory interneurons responding to sound in the flight system of
Locusta migratoria as surveyed from the literature*.

Interneuron[1]	Synonomy
123	
138	SN1
139	SN2. UGN2
147	
314	C
406	
525	
526	
529	B2
530	BSN1
531	AN1. B1. TH3-AC1
532	
533	AN2
539	AN3
541	AN4
601	TN1
714	G
--	SN5. UGN5

[1] Nomenclature follows Robertson and Pearson (1983)

*Boyan. 1986: Hedwig. 1986: 1989: Kalmring. 1975a: Rehbein. 1976: Robert. 1989: Römer and Marquart. 1984: Römer *et al.*. 1988: Marqhart. 1985.

necessarily components thereof (Römer and Seikowsky, 1985) and may play a role in

recognition of sounds (Kalmring *et al.*, 1978). Further, several interneurons in the

suboesophageal ganglion also respond to visual stimuli suggesting they play a role in

multimodal integration. The idea that auditory information must make a loop through the

brain for recognition is supported by connective transection experiments that show that

the connections from the metathoracic ganglion to the brain (supraoesophageal ganglion)

must be intact on at least one side of the ventral nerve cord for *Chorthippus biguttulus*

(subfamily Gomphocerinae) to engage in response singing (Ronacher *et al.*, 1986).

There is increasing evidence that modularity of neural circuitry is a general

principle underlying central nervous system organization in both vertebrates and

invertebrates (Grillner, 1985; Leise, 1990; Comer and Robertson, 2001). Developmental

studies of the auditory system in locusts are also showing that there is modularity in

auditory processing circuitry and that this might be true of other tympanate insects as

well (Boyan, 1993). In the locust, the ear located on the first abdominal segment is

serially homologous with the metamerically repeating pleural chordotonal organs located

on the lateral walls of the abdominal and thoracic segments (Meier and Reichert, 1990).

The chordotonal organs in segments other than the first abdominal segment most likely

monitor stretching of the body wall (Hustert, 1978). The afferents of all the pleural

chordotonal organs project to the mVAC in their corresponding ganglia and make

connections with interneurons in each segment. Some of these interneurons have

common embryonic origin (i.e. they are homologues) creating the possibility that serially

repeating 'processing modules' exist throughout the length of the ventral nerve cord that have been co-opted for auditory processing.

Within the mesothoracic and metathoracic ganglia there is a set of serially and bilaterally homologous neurons that receive monosynaptic input from a single interneuron and therefore form a 'processing module' (Boyan, 1991; 1992). These neurons are all derived from a common neuroblast (NB 7-4) and are repeated along the length of the ventral nerve cord (Bastiani *et al.*, 1984; Pearson *et al.*, 1985). The serially homologous interneurons are organized into two 'arrays' (figure 1.8) (Boyan, 1992). The 'G' array consists of interneurons 529 (=B2) in the first abdominal neuromere of the metathoracic ganglion, 531 (=AN1. B1, TH3-AC1) of the metathoracic ganglion, and 714 (=G) of the mesothoracic ganglion. The second array, the 'C' array, consists of interneuron 319 of the first abdominal neuromere. 311 of the metathoracic ganglion. and 314 (=C) of the mesothoracic ganglion. The interneurons of the two arrays that are found in the same ganglion are each sibling cells. All of these interneurons receive monosynaptic input from interneuron 530 located in the mesothoracic ganglion which in turn receives monosynaptic input from low frequency (c. 4 kHz) and high-frequency (c. 12 kHz) auditory afferents (Boyan, 1992).

The interneurons in the two arrays all receive auditory input but in different ways. Interneurons 714 and 529 receive monosynaptic input from auditory afferents and excitatory input from other auditory interneurons (Pearson *et al.*, 1985; Römer and Marquart. 1984; 1985b). Interneuron 531 receives a mix of excitatory and inhibitory inputs to sound from other higher order interneurons. In addition to auditory input,

Figure 1.8. Summary of the synaptic connections between serially homologous cells in the G and C array. T-bars indicate excitatory monosynaptic connections. T2. mesothoracic ganglion; T3, metathoracic ganglion: A1, first abdominal neuromere of the metathoracic ganglion. Modified from Boyan (1992).

G-array
Serial homologs

C-array
Serial homologs

siblings

T2   714   314

T3   531   530   311

A1   529   319

Auditory afferents
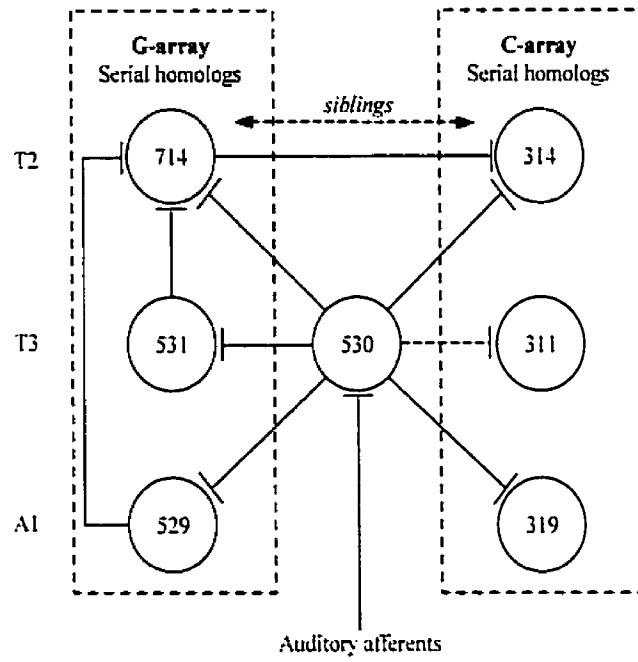
interneurons 714, 529, 314, 311 and 319 also receive input from the descending

contralateral movement detector DCMD (Pearson *et al.*, 1985); a motion sensitive visual

feature detector that may be involved in predator detection (Gray *et al.*, 2001).

The interneurons of the G array are synaptically coupled but the communication is

in an ascending direction only. This is to say that interneuron 529 makes a monosynaptic

excitatory connection with 714 but 714 does not make the reciprocal connection.

Similarly, interneuron 531 makes a monosynaptic connection with 714, but 715 does not

make the reciprocal connection, nor is there a connection from 531 to 529. Connectivity

experiments have not been completed with cells in the C array but the descending axonal

processes of each of the cells suggest that communication in this array is in the

descending direction. The connections between cells of the G array and sibling cells in

the C array are also unidirectional. In this case, interneuron 714 makes monosynaptic

excitatory connections with 314 but 314 does not make the reciprocal connection.

Finally, the cells in both the G and C arrays do not make connections with their bilateral

homologues, which is to say that, interneuron 714 in the left hemiganglion does not make

a direct connection across the midline to 714 in the right hemiganglion. These

connections are summarized in figure 1.8.

The properties of the post synaptic potentials (PSPs) of the neurons from the two

arrays, recorded from equivalent locations within the dendritic arborizations, are different

(Boyan, 1992). For example, the PSP recorded from interneuron 714 is a larger

amplitude, faster EPSP relative to an EPSP recorded from interneuron 531 or 314. This

suggests that information delivered to the cells is distributed differently within the

network due to the cable properties of the cells. The specificity of the direction of

information flow within the network may be related to the post-synaptic connections of

the cells. For example, interneurons 714 and 314 output to leg motorneurons and are

involved in jumping (Pearson *et al.*, 1985), whereas interneuron 529 outputs to flight

motor neurons and is also involved in bending the abdomen during flight steering

(Baader, 1991).

It is now well established that the evolutionary precursor to the tympanate ear, in

all insects, is the proprioceptive chordotonal organ (see reviews by Boyan, 1993; 1998;

Fullard and Yack, 1993 and Yager, 1999). This has been established by comparative

studies of tympanate and atympanate species (e.g. Yack and Fullard, 1990) and by

developmental studies (e.g. Meier and Reichert, 1990). The precursors to ears function in

proprioception by monitoring body movements (e.g. noctuoid moths, wing hinge stretch

receptor; locusts, abdominal body wall stretch), or function in exteroception, by

monitoring substrate vibrations transmitted through cuticle (e.g. crickets and katydids,

prothoracic tibial subgenual organs; praying mantis, vibration receptor). Despite the

diversity of locations on the body where ears are found (Yack and Fullard, 1993),

auditory afferents from all these insects, with the exception of praying mantises, project

ipsilaterally to the mVAC and make connections with local and intersegmental

interneurons. This strongly suggests that the auditory processing circuitry in all these

insects is derived from pre-existing mechanosensory processing circuitry.

If this is true it also suggests the origin of the mechanisms underlying in-flight

evasive responses and could explain why many insects show similarities in their

responses to bat-like sounds in free-flight and tethered flight. In the case of locusts, the

abdominal pleural chordotonal organs are serially homologous with the thoracic

chordotonal organs monitoring wing elevation. In the thoracic segments, these

proprioceptors have extensive influence over the flight pattern generator (Burrows, 1975;

Möhl, 1985b; Reye and Pearson, 1987). Therefore, it is quite possible that ears exert

their effects on the flight pattern generator *via* modules of circuitry homologous to the

circuitry that mediates proprioceptive influence on the wings. This is not surprising

given that during evolution pre-existing circuitry is more likely to be co-opted for new

purposes than the *de novo* creation of circuitry (Dumont and Robertson, 1986). Further,

as suggested by Yager (1999), the modularity of neural circuitry in the CNS suggests that

small changes may be all that is required for creating auditory evoked evasive responses

in insects. Simply disconnecting input to a proprioceptive circuit and connecting it to a

pre-existing escape circuit might contribute behavioural components to escape

behaviours. This means that circuitry used for wind-evoked escape (i.e. the cercal giant

interneuron system) and for terrestrial based protective displays such as wing flips and

fore-leg extension, may become part of in-flight auditory evoked evasive responses.

1.3.4 Auditory Input to the Flight System

It is clear that sound can affect the flight behaviour of locusts and it is common practice

in the laboratory when working with tethered locusts to initiate flight with hand clapping

and/or hissing. Haskell (1957) tested the idea that flight noise might influence the on-

mass take-off behaviour of gregarious locusts so as to initiate or maintain swarming

behaviour. He found that wing beat noise, as produced by the wings contacting the body

during flapping, when played back to settled and roosting locusts under some

circumstances initiated take-off. Yinon *et al.* (1971) completed a more comprehensive

behavioural analysis and found that pure tones and broadband white noise could initiate

flight in *Schistocerca gregaria*. More recently, Robert (1989) demonstrated that tethered

locusts attempt to steer away from high-frequency sounds by producing abdomen

deflections and yaw torques. Several investigations have now shown that auditory input

to the flight system of locusts is extensive (table 1.5) and is mediated by afferent

connections with several identifiable auditory interneurons or by direct connections to

flight interneurons (Boyan, 1985; 1986; Hedwig, 1989) but the neuronal mechanisms

underlying phonotactic steering in tethered flying locusts are still unknown.

The neuromuscular events occurring in the flight system of *Locusta migratoria*

following stimulation with sound have been summarized by Boyan (1985) and are

presented in figure 1.9. The first events recorded in the central nervous system is afferent

spikes at the metathoracic ganglion that make excitatory connections with several

auditory interneurons; these occur approximately 5 ms after stimulation. Following this,

activity is observed in flight interneurons and after approximately 20 ms, post synaptic

potentials are seen in flight motor neurons. Spiking in flight motor neurons and flight

muscles then follow with considerable variation in timing.

Boyan (1986) notes that it is often assumed in intracellular investigations that the

response of a neuron to a given stimulus recorded from an animal in a dissected,

restrained preparation will be the same as that that might be recorded, if it were possible,

Table 1.5: Some flight interneurons responding to sound in the flight system of *Locusta migratoria* as surveyed from the literature*.

Interneuron[1]	Influence[2]	Sound[3]	Notes[4]
122	Elevator	5 kHz	
201	Depressor	10 kHz	Makes direct connection with depressor motor neurons
204	Elevator	HF	Weak EPSPs to HF sound: Presynaptic to FMN 118/133.
207	Elevator	unspecified frequency	Response greatest during, or within two seconds of. rhythmic flight-like activity.
271	Elevator	HF	
302	Depressor	HF. 10 kHz. 16 kHz	APs to HF sound: EPSPs to 10 kHz sounds: Receives input from HF auditory receptors. forewing stretch receptor. cercal giant interneurons: Elicits IPSPs in elevator FMNs
401	Elevator	5 kHz	
501	Depressor	5 kHz	
513	Elevator	16 kHz	EPSPs to 16 kHz sound. responses potentiated by wind or prior flight activity
521	Depressor	5 kHz	EPSPs to 5 kHz sound
701	Depressor	16 kHz	IPSPs to 16 kHz sound
750	Elevator	HF	Weak EPSPs to HF sound: may be postsynaptic to DNC

[1] Nomenclature follows Robertson and Pearson (1983)

[2] The basic idea is that an interneuron is considered to influence the elevator phase of the flight pattern if it promotes wing elevation (e.g. makes an excitatory connection with elevator motorneurons) or contributes to inhibition of wing depression (e.g. makes inhibitory connections with depressor motorneurons). *Vice versa* for interneurons labelled depressors.

[3] Refers to the carrier frequency of the acoustic stimulus used during experiments. cited values are not meant to imply that these are the only frequencies to which these interneurons are responsive. HF refers to c. 8 kHz sounds generated by 'hissing'. 'making kissing sounds'. or 'clicking the fingernails' (Rowell and Reichert. 1991).

[4] AP. action potential: EPSP. excitatory post synaptic potential: IPSP. inhibitory post synaptic potential: FMN. flight motorneuron. DNC is a deviation detector neuron (see text).

* Boyan. 1984a. b: 1985: Rowell and Reichert. 1991.

Figure 1.9. Sequence of events occurring in the locust flight system of *Locusta*
migratoria resulting from auditory stimulation. The scale at the left is an
expansion of the first 20 ms of events. Acitivity is first recorded in auditory
receptors at the metathoracic ganglion, then auditory interneurons, then flight
interneurons and flight motorneurons and finally flight muscles. Activity first
occurs in elevator FIN 513, presumably for wing opening, followed by depressor
FINs. AIN, auditory interneuron; FIN, flight interneuron; FMN, flight
motorneuron; LF, low frequency; HF, high frequency; e. EPSP; i, IPSP; E,
elevator; D, depressor; Meta, metathoracic ganglion. Taken from Boyan (1985).

TIME (MS)

0 — Sound stimulus ▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪ 0 — Sound stimulus

2
                                              20 — 83.e (E) (16kHz)
                                                   — 89.e (E) (12kHz)
                                           — 61.ı (D) (14kHz) — psp in FMNs
4 — LF receptor spike in Meta[1]   40 — 119.e (E) (5kHz)
   — 601.e
   — 138.e
6 — G.e   (5kHz) ——— psp in AINs   60
   — 82.e
   — HF receptor spike in Meta[1]
8 — 513.e (E) (16kHz)   80

10 — 81.e (12kHz)   100 — Spikes in Elevator FMNs
   — 302.e(D) (10kHz)   psp in FINs   ($\bar{X}$=69.2 s.d. ± 31.8)
12 — 521.e(D) (5kHz)   120

14 — 701.ı (D) (14kHz)   140 — DL 81/112 activity
16 — spike in 513   160   ($\bar{X}$=147.0 s.d. ± 33.2)

18   180
   — 83.e (E) (16kHz) ——— psp in FMN
   — spike in 302
20 — 81 spike arrives in brain[2]   200

TIME (MS)

220

240

260

from an unrestrained animal engaged in its normal behaviour. However, it is clear that

the responses of neurons in sensory pathways can be affected by ongoing motor activity

and this is true of the auditory system in flying locusts. When a locust flies, the forward

motion of the animal generates air currents that flow over the body and tympana

providing a natural source of auditory stimulation. Similarly, as the hindwings beat they

contact the abdomen and hindlegs also creating sounds that are expected to be

particularly intense given the location of the ear on the first segment of the abdomen.

Therefore, wind and wing beat noise are two ethologically relevant sources of sound to a

flying locust.

Boyan (1986) found that the responsiveness of auditory interneurons to sounds.

measured as the number of spikes elicited to a given stimulus or the amplitude of post

synaptic potentials, changes with concomitant wind stimulation. He found that responses

of several auditory interneurons were suppressed during low frequency (5 kHz) acoustic

stimulation while others were unaffected or even enhanced. Boyan (1986) determined

that these effects were not due to activity in the flight pattern generator or were a result of

stimulation of the wind sensitive hairs on the head of the locust but were rather due to the

effects of wind on the tympanic membrane itself. Wind stimulation of the tympanic

membrane therefore affects the responsiveness of interneurons and effectively shifts the

sensitivity of the auditory system to higher frequencies.

Hedwig (1989) examined the responses of nerve six and thoracic auditory

interneurons to auditory stimulation in tethered flying and quiescent locusts. He found

that flight alone produced phasic and tonic activity that could be recorded from nerve six.

This activity was due to noise generated by the beating wings, as Boyan (1986) had

found, but was also due to flight induced vibrations of Müller's organ. When animals

were flying, activity in nerve six and in auditory receptor fibres elicited with a white

noise stimulus was only 19% of the value recorded in quiescent animals suggesting that

auditory sensitivity is greatly reduced during flight. Similarly, responses recorded in

interneurons to sound pulses were reduced during flight to between 26 and 35% of the

value recorded from resting animals. These findings suggest that vibrations generated by

the animal itself during flight influence auditory sensitivity. Further, it is not

unreasonable to expect that other motor acts such as walking, breathing and stridulation

will similarly affect auditory processing and mechanisms have been described for coping

with competing and/or interfering signals (Wolf and von Helversen, 1986; Lang and

Elsner, 1994; Lang, 1996).

Wind flowing over the locust body can also play a role in sensorimotor

integration by selectively gating information flow to motor outputs. Boyan (1989)

provides an excellent example. Interneuron 529 (=B2) receives monosynaptic input from

auditory afferents and the descending contralateral movement detector (DCMD).

Interneuron 529 also makes connections with elevator and depressor motor neurons in the

metathoracic ganglion. In the presence of wind, responses of interneuron 529 to 12 kHz

sound pulses are reduced 40% relative to values recorded in the absence of wind.

However, wind does not affect the response of 529 to visual stimuli. Therefore, in the

presence of simultaneous visual and auditory information, wind selectively gates the

visual information to the motor centres over the auditory information. This may have

important consequences during certain behavioural acts where visual information must take precedence over auditory information. During landing, for example, wind flowing over the body would suppress spurious activity from auditory afferents that might interfere with the visual-motor coordination necessary for landing. Conversely, when an animal is sitting on the ground and no wind is present flowing over the body, both auditory and visual information could be important in detecting approaching predators and triggering escape flight.

1.4 Summary and Overview of the Thesis

Like any insect that flies at night, locusts are susceptible to predation by insectivorous bats. Although there is no direct evidence that locusts form a regular or substantial portion of the diets of certain bat species, there is no reason to think they would not be a nutritional meal. This speculation is intriguing given that locusts possess an ASR that is similar to that of many other tympanate insects preyed upon by bats. Locusts possess ears that are sensitive to a broad range of frequencies that include both those contained in stridulatory sounds and those employed by large, high-altitude flying echolocating insectivorous bats (e.g. free-tailed bats, *Tadarida* sp. and emballonurid bats, *Taphozous* sp.). Social sounds, produced by tegmino-femoral stridulation and mandibular ʻclickingʼ, are unlikely to be heard by a locust in flight when produced by an individual on the ground and therefore likely play no role in orienting a flying individual. Diurnal animals, such as birds, are primarily visual predators and typically employ a tactic of stealth and not acoustic conspicuousness when hunting. Therefore, the only ethologically relevant

sources of sound, other than wing-beat noise, to flying locusts seem to be environmental

noise (leaves, wind) and that produced by echolocating bats.

The auditory system of the locust has been studied in considerable detail and the

emerging model of auditory processing seems to be one of parallel processing. Features

of sounds such as frequency, intensity and direction are extracted by thoracic auditory

interneurons. In the case of high-frequency sounds, there is evidence that direct

connections with flight pattern generator and flight motor neurons exist and it is

presumed that this facilitates rapid escape responses. Afferent auditory information also

simultaneously ascends to the suboesophageal ganglion and brain where recognition of

sounds takes place. Integration of auditory and visual information takes place in both the

cephalic and thoracic centres and there are mechanisms for resolving sensory conflict.

During tethered flight, sounds can influence the flight rhythm and evoke directional

responses. Further, during tethered flight it is possible that the ears are more sensitive to

high frequencies because of a masking effect mediated by wing beat noise and air

flowing over the locust body.

It is an exciting time for insect flight studies because recent advances in air-flow

visualization and the application of robotic approaches have facilitated the discovery and

characterization of several unsteady, high-lift mechanisms employed by insects for

manoeuvrability. Locusts have long been a model organism for insect flight studies and a

rich literature has accumulated on wing kinematics and motor patterns for straight flight

and steering. The majority of this work has been done with tethered locusts responding

to instabilities in artificial visual environments, or to imposed rotations about the flight

axes; however, recent advances in telemetry are beginning to determine the interplay

between wing kinematics and motor control. Comparisons of locusts engaged in

correctional steering with locusts engaged in intentional steering, such as collision or

thermal avoidance, show similarities in kinematics and motor patterning at only a basic

level. Wing kinematic and motor pattern changes observed during intentional steering

are larger in magnitude and perhaps interact more with changes in body posture (e.g.

hindwing-hindleg-abdomen interactions) than during correctional steering. As such, the

significance of one kinematic manoeuvre, asymmetric wing depression, has been

overlooked in the literature. Asymmetric wing depression is frequently observed during

intentional steering but only rarely during correctional steering and probably plays a

significant role in generating the large steering torques necessary for rapid escape

manoeuvres.

The ASR of locusts provides us with an ethologically relevant framework within

which studies of locust flight, intentional steering, and auditory sensorimotor integration

can be completed. The overall objective of this thesis is to determine how flying locusts

react to sound. Chapter two of this thesis documents asymmetric wing depression and

other changes in wing kinematics and body posture that accompany responses to

stimulation with bat-like sounds. The effect of asymmetric wing depression is believed to

generate a roll torque in the direction of the lower wing and the aerodynamic basis of this

is discussed. At the end of this chapter, a model is proposed to explain how asymmetric

wing depression is established. Chapter three determines the motor pattern for

intentional steering to bat-like sounds and tests the model presented in chapter two.

High-speed cinematography with concurrent multi-channel electromyography of depressor muscles show that bulk shifts in depressor timing establish the timing of stroke reversals which bring about asymmetric wing depression. Further, in this chapter it is discovered that asymmetries in onset of pronation may play a significant role in generating steering torques by affecting rotational circulation at the transition to the downstroke. In chapter four, I test Robert's (1989) hypothesis that the ASR in locusts functions in bat-avoidance by observing free-flying locusts presented with bat-like and other sounds while in flight in a large flight room. The results show that locusts do respond to sounds while in free flight and that their reactions are similar to those of other insects such as moth. lacewings and praying mantises reacting to bat and bat-like sounds. However, the locust responses do not show frequency dependency and I conclude that the reactions are a general startle reaction to sound and not specifically a bat-avoidance response. The last chapter of this thesis. chapter five. provides a general discussion of the significance of the findings of the studies comprising this thesis.

1.5 Literature Cited

Adam, L.-J. (1983). Hind femora influence directionality of the locust ear. *J. Comp. Physiol. A* **152**, 509-516.

Altman, J. (1982). The role of sensory inputs in insect flight motor pattern generation. *Trend. Neurosci.* **5**, 256-260.

Arbas, E. A. (1986). Control of hindlimb posture by wind-sensitive hairs and antennae during locust flight. *J. Comp. Physiol. A* **159**, 849-857.

Baader, A. (1990). The posture of the abdomen during locust flight: regulation by steering and ventilatory interneurones. *J. Exp. Biol.* **151**, 109-131.

Baader, A. (1991). Auditory interneurons in locusts produce directional head and abdomen movements. *J. Comp. Physiol. A* **169**, 87-100.

Baader, A., Schäfer, M., and Rowell, C. H. F. (1992). The perception of the visual flow field by flying locusts: A behavioural and neuronal analysis. *J. Exp. Biol.* **165**, 137-160.

Bacon, J. and Tyrer, M. (1979). Wind interneurone input to the flight motor neurons in the locust. *Schistocerca gregaria. Naturwissenschaften* **66**, 116.

Baker, P. S. (1979a). The wing movements of flying locusts during steering behaviour. *J. Comp. Physiol.* **131**, 49-58.

Baker, P.S. (1979b). The role of forewing muscles in the control of direction in flying locusts. *J. Comp. Physiol. A* **131**, 59-66.

Baker, P. S. and Cooter, R. J. (1979a). The natural flight of the migratory locust,

Locusta migratoria L. I. Wing Movements. J. Comp. Physiol. 131, 79-87.

Baker, P. S. and Cooter, R. J. (1979b). The natural flight of the migratory locust,

Locusta migratoria L. II. Gliding. J. Comp. Physiol. 131, 89-94.

Baker, P. S., Gewecke, M. and Cooter, R. J. (1981). The natural flight of the migratory

locust, Locusta migratoria L. III. Wing-beat frequency, flight speed and attitude.

J. Comp. Physiol. 141, 233-237.

Bar-Ilan, A. R., Shulov, A. and Pener, M. P. (1969). The stridulation of sexually

mature males of Locusta migratoria migratorioides (R. and F.) in relation to

temperature and some other factors. Physiol. Zool. 42, 417-428.

Barrass, R. (1964). The Locust: A Guide for Laboratory Practical Work. London:

Butterworth and Co. Ltd.

Bastiani, M., Pearson, K. G. and Goodman, C. S. (1984). From embryonic fascicles to

adult tracts: Organization of neuropile from a development perspective. J. Exp.

Biol. 112, 45-64.

Belwood. J. J. and Morris. G. K. (1987). Bat predation and its influence on calling

behaviour in neotropical katydids. Science. 238, 64-67.

Bentley, D. R. (1970). A topological map of the locust flight system motor neurons. J.

Insect Physiol. 16, 905-918.

Boyan, G. S. (1984a). Neural mechanisms of auditory information processing by

identified interneurones in orthoptera. J. Insect Physiol. 30(1), 27-41.

Boyan, G. S. (1984b). What is an "auditory" neurone? *Naturwissenschaften* 71, 482-484.

Boyan, G. S. (1985). Auditory input to the flight system of the locust. *J. Comp. Physiol.* *A* 156, 79-91.

Boyan, G. S. (1986). Modulation of auditory responsiveness in the locust. *J. Comp. Physiol. A* 158, 813-825.

Boyan, G. S. (1989). Synaptic input converging onto an identified premotor interneuron in the locust is gated according to modality. *Naturwissenschaften* 76, 431-433.

Boyan, G. S. (1991). A neuronal network defined by lineage in the locust CNS. *Naturwissenschaften* 78, 565-567.

Boyan, G. S. (1992). Common synaptic drive to segmentally homologous interneurons in the locust. *J. Comp. Neurol.* 321, 544-554.

Boyan, G. S. (1993). Another look at insect audition: The tympanic receptors as an evolutionary specialization of the chordotonal system. *J. Insect Physiol.* 39(3), 187-200

Boyan, G. S. (1998). Development of the insect auditory system. In: *Comparative hearing: Insects* (ed. R. Hoy, A. Popper and R. Fay). p. 97-138. Heidelberg and New York: Springer-Verlag.

Breckow, J. and Sippel, M. (1985). Mechanics of the transduction of sound in the tympanal organ of adults and larvae locusts. *J. Comp. Physiol. A* 157, 619-629.

Burrows, M. (1975). Monosynaptic connexions between wing stretch receptors and flight motoneurones of the locust. *J. Exp. Biol.* 62, 189-219.

Burrows, M. (1996). *The neurobiology of an insect brain.* Oxford: Oxford University Press.

Camhi, J. M. (1970). Yaw-correcting postural changes in locusts. *J. Exp. Biol.* **52**, 519-531.

Camhi, J. M., Sumbre, G., and Wendler, G. (1995). Wing-beat coupling between flying locust pairs: Preferred phase and lift enhancement. *J. Exp. Biol.* **198**, 1051-1063.

Chapman, R. F. (1976). *A Biology of Locusts.* London: Edward Arnold Ltd.

Cloupeau, M., Devilliers, J. F. and Devezeaux, D. (1979). Direct measurements of instantaneous lift in desert locust: comparision with Jensen's experiments on detached wings. *J. Exp. Biol.* **180**, 1-15.

Comer, C. M. and Robertson, R. M. (2001). Identified nerve cells and insect behaviour *Prog. Neurobiol.* **63**, 409-439.

Cooter, R. J. and Baker, P.S. (1977). Weis-Fogh clap and fling mechanism in *Locusta. Nature* **269**, 53-54.

Dawson, J. W., Dawson-Scully, K., Robert, D., and Robertson, R. M. (1997). Forewing asymmetries during auditory avoidance in flying locusts. *J. Exp. Biol.* **200**, 2323-2335.

Dickinson, M. H. and Götz, K. G. (1996). The wake dynamics and flight forces of the fruit fly *Drosophila melanogaster. J. Exp. Biol.* **199**, 2085-2104.

Dickinson, M. H., Lehmann, F., and Sane, S. P. (1999). Wing rotation and the aerodynamic basis of insect flight. *Science* **284**, 1954-1960.

Dudley, R. (1991). Biomechanics of flight in neotropical butterflies: aerodynamics and

mechanical power requirements. *J. Exp. Biol.* **159**, 335-357.

Dudley, R. (2000). *The Biomechanics of Insect Flight: Form, Function, Evolution.*

Princeton: University Press.

Dudley, R. and Ellington, C. P. (1990a). Mechanics of forward flight in bumblebees. I.

Kinematics and morphology. *J. Exp. Biol.* **148**, 19-52.

Dudley, R. and Ellington, C. P. (1990b). Mechanics of forward flight in bumblebees. II.

Quasi-steady lift and power requirements. *J. Exp. Biol.* **148**, 53-88.

Dugard, J. J. (1967). Directional change in flying locusts. *J. Insect Physiol.* **13**, 1055-

1063.

Dumont, J. P. C. and Robertson, R. M. (1986). Neuronal circuits: an evolutionary

perspective. *Science* **233**, 849-853.

Ellington, C. P. (1984a). The aerodynamics of hovering insect flight. I. The quasi-steady

analysis. *Phil. Trans. R. Soc. Lond. B* **305**, 1-15.

Ellington, C. P. (1984b). The aerodynamics of hovering insect flight. II. Morphological

parameters. *Phil. Trans. R. Soc. Lond. B* **305**, 17-40.

Ellington, C. P. (1984c). The aerodynamics of hovering insect flight. III. Kinematics.

Phil. Trans. R. Soc. Lond. B **305**, 41-78.

Ellington, C. P. (1984d). The aerodynamics of hovering insect flight. IV. Aerodynamic

mechanisms. *Phil. Trans. R. Soc. Lond. B* **305**, 79-113.

Ellington, C. P. (1984e). The aerodynamics of hovering insect flight. V. A vortex theory

Phil. Trans. R. Soc. Lond. B **305**, 115-144.

Ellington, C. P. (1984f). The aerodynamics of hovering insect flight. VI. Lift and power

requirements. *Phil. Trans. R. Soc. Lond. B* **305**, 145-181.

Ellington, C. P. (1995). Unsteady aerodynamics of insect flight. In: *Biological Fluid*

Dynamics (ed. C. P. Ellington and T. J. Pedley). Society for Experimental

Biology.

Ellington, C. P. (1999). The novel aerodynamics of insect flight: Applications to micro-

air vehicles. *J. Exp. Biol.* **202**, 3439-3448.

Ellington, C. P., van den Berg, C., Willmott, A. P. and Thomas, A. L. R. (1996).

Leading-edge vortices in insect flight. *Nature* **384**, 626-630.

Elson, R. (1987). Flight motor neuron reflexes driven by strain-sensitive wing

mechanoreceptors in the locust. *J. Comp. Physiol. A* **161**, 747-760.

Elson, R. and Pflüger, H-J. (1986). The activity of a steering muscle in flying locusts. *J.*

Exp. Biol. **120**, 421-441.

Ennos, A. R. (1989). The kinematics and aerodynamics of the free flight of some

Diptera. *J. Exp. Biol.* **142**, 49-85.

Farrow, R. A. (1990). Flight and migration in Acridoids. In *Biology of Grasshoppers*

(ed. R. F. Chapman and A. Joern). pp. 227-314. New York: John Wiley and Sons.

Fischer, H. (1998). *Untersuchungen zur Verhaltensphysiologie frei fliegender*

Heuschrecken unter Einsatz von Telemetrie: Grundlegende Beziehungen zwischen

Parametern eines nicht-stationären Systems. Ph.D. Thesis. University of

Konstanz. Germany.

Fischer, H. and Kutsch, W. (1999). Timing of elevator muscle activity during climbing in free locust flight. *J. Exp. Biol.* **202,** 3575-3586.

Fischer, H. and Kutsch, W. (2000). Relationships between body mass, motor output and flight variables during free flight of juvenile and mature adult locusts, *Schistocerca gregaria. J. Exp. Biol.* **203,** 2723-2735.

Fischer, H., Kautz, H., and Kutsch, W. (1996). A radiotelemetric 2-channel unit for transmission of muscle potentials during free flight of the desert locust, *Schistocerca gregaria. J. Neurosci. Methods* **64,** 39-45.

Forrest, T. G., Farris, H. E., and Hoy, R. R. (1995) Ultrasound acoustic startle response in scarab beetles. *J. Exp. Biol.* **198,** 2593-2598.

Fullard, J. H. and Yack, J. E. (1993). The evolutionary biology of insect hearing. *Trends Ecol. Evol.* **8(7),** 248-252.

Gatehouse, A. G. and Zhang, X. -X. (1995). Migratory potential in insects: variation in an uncertain environment. In *Insect migration: Tracking resources through space and time* (ed. V. A. Drake and A. G. Gatehouse). pp. 193-242. London: Cambridge University Press.

Gee, C. E. and Robertson, R. M. (1994). Effects of maturation on synaptic potentials in the locust flight system. *J. Comp. Physiol. A* **175,** 437-447.

Gee, C. E. and Robertson, R. M. (1998). Free-flight ability in locusts recovering from partial deafferentation. *Naturwissenschaften* **85,** 167-170.

Gee, C. E., Shoemaker, K. L., and Robertson, R. M. (1998). The forewing tegula: their significance in steering manoeuvres and free flight in *Locusta migratoria*. *Can. J. Zool.* **76**, 660-667.

Gewecke, M. (1970). Antennae: Another wing-sensitive receptor in locusts. *Nature* **255**:1263-1264.

Gewecke, M. (1975). The influence of the air-current sense organs on the flight behaviour of *Locusta migratoria*. *J. Comp. Physiol.* **103**:79-95.

Gewecke, M. and Kutsch, W. (1979). Development of flight behaviour in maturing adults of *Locusta migratoria*: I. Flight performance and wing-stroke parameters. *J. Insect Physiol.* **25**:249-253.

Gray, E. G. (1960). The fine structure of the insect ear. *Phil. Trans.R. Soc. Lond. B* **243**, 75-94.

Gray, J. R. and Robertson, R. M. (1994). Activity of the forewing stretch receptor in immature and mature adult locusts. *J. Comp. Physiol. A* **175**, 425-435.

Gray, J. R. and Robertson, R. M. (1996). Structure of the forewing stretch receptor axon in immature and mature adult locusts. *J. Comp Neurol.* **365**, 268-277.

Gray, J. R. and Robertson, R. M. (1997). Co-ordination of the flight motor pattern with forewing stretch receptor stimulation in immature and mature adult locusts. *Comp. Biochem. Physiol.* **118**, 125-130.

Gray, J. R., Lee, J. K. and Robertson, R. M. (2001). Activity of descending contralateral movement detector neurons and collision avoidance behaviour in response to head-on visual stimuli in locusts. *J. Comp. Physiol. A* **187**, 115-129.

Grillner, S. (1985). Neurobiological bases of rhythmic motor acts in vertebrates. *Science* **228**, 143-149.

Griss, C. and Rowell, C. H. F. (1986). Three descending interneurons reporting deviation from course in the locust. I. Anatomy. *J. Comp. Physiol.* **158**, 765-774.

Harris-Warrick, R. M. (1993). Pattern generation. *Curr. Opin. Neurobiol.* **3**, 982-988.

Haskell, P. T. (1957). The influence of flight noise on the behaviour in the desert locust *Schistocerca gregaria* (Forsk.). *J. Insect Physiol.* **1**, 52-75.

Hedwig, B. (1986). On the role in stridulation of plurisegmental interneurons of the acridid grasshopper *Omocestus viridulus* L. II. Anatomy and physiology of ascending and T-shaped interneurons. *J. Comp. Physiol. A* **158**, 429-444.

Hedwig, B. (1989). Modulation of auditory information processing in tethered flying locusts. *J. Comp. Physiol. A* **164**, 409-422.

Hedwig, B. and Becher. G. (1998). Forewing movements and intracellular motoneurone stimulation in tethered flying locusts. *J. Exp. Biol.* **201**, 731-744.

Helversen, von D. and Rheinländer, J. (1988). Interaural intensity and time discrimination in an unrestrained grasshopper: a tentative behavioural approach. *J. Comp. Physiol. A* **162**, 333-340.

Hensler, K. and Robert, D. (1990). Compensatory head rolling during corrective flight steering in locusts. *J. Comp. Physiol A* **166**, 685-693.

Herbert, R. C., Young, P. G., Smith, C. W., Wootton, R. J. and Evans, K. E. (2000). The hindwing of the desert locust (*Schistocerca gregaria* Forskål). III. A finite element analysis of a deployable structure. *J. Exp. Biol.* **203**, 2945-2955.

Horsmann, U., Heinzel, H-G, and Wendler, G. (1983). The phasic influence of self-generated air current modulation on the locust flight motor. *J. Comp. Physiol.* **150**, 427-438.

Hoy, R. R. (1989). Startle, categorical response, and attention in acoustic behavior of insects. *Ann. Rev. Neurosci.* **12**, 355-377.

Hoy, R. R. (1992). The evolution of hearing in insects as an adaptation to predation from bats. In *The Evolutionary Biology of Hearing* (ed. D. B. Webster, R. R. Fay, and A. N. Popper). pp115-129. New York: Springer-Verlag.

Hoy, R. R. and Robert, D. (1996). Tympanal hearing in insects. *Annu. Rev. Entomol.* **41**, 433-450.

Hoy, R., Nolen, T., and Brodfuehrer, P. (1989). The neuroethology of acoustic startle and escape in flying insects. *J. Exp. Biol.* **146**, 287-306.

Hustert, R. (1978). Segmental and interganglionic projections from primary fibres of insect mechanoreceptors. *Cell Tiss. Res.* **194**, 337-351.

Jensen, M. (1956). Biology and physics of locust flight. III. The aerodynamics of locust flight. *Phil. Trans. R. Soc. Lond. B* **239**, 511-552.

Jensen, M. and Weis-Fogh, T. (1962). Biology and physics of locust flight. V. Strength and elasticity of locust cuticle. *Phil. Trans. R. Soc. Lond. B* **245**, 137-169.

Kalmring, K. (1971). Acoustic neurons in the suboesophageal ganglion of the desert locust. *Locusta migratoria. Z. vergl. Physiologie* **72**, 95-110.

Kalmring, K. (1975a). The afferent auditory pathway in the ventral cord of *Locusta*

migratoria (Acrididae). I. Synaptic connectivity and information processing

among the auditory neurons of the ventral cord. *J. Comp. Physiol.* **104**, 103-141.

Kalmring, K. (1975b). The afferent auditory pathway in the ventral cord of *Locusta*

migratoria (Acrididae). II. Responses of the auditory ventral cord neurons to

natural sounds. *J. Comp. Physiol.* **104**, 143-159.

Kalmring, K., Rheinlaender, J. and Römer, H. (1972). Acoustic neurons in the ventral

nerve cord of *Locusta migratoria*: The influence of the direction of the sound

source on the response patterns. *J. Comp. Physiol.* **80**, 325-352.

Kalmring, K., Kuhne, R. and Moysich, R. (1978). The coding of sound signals in the

ventral-cord auditory system of the migratory locust. *Locusta migratoria. J.*

Comp. Physiol. **128**, 213-226.

Kennedy, J.S. (1956). Phase transformation in locust biology. *Biol. Rev.* **31**, 349-370.

Keuper, A., Otto, C., Latimer, W. and Schatral, A. (1985). Airborne sound and

vibration signals of bushcrickets and locusts: their importance for the behaviour in

the biotope. In: *Acoustic and Vibrational Communication in Insects*. (ed. K.

Kalmring and N. Elsner). pp. 135-142. Paul Parey.

Koch, U. (1977). A miniature movement detector applied to recording of wingbeat in

locusts. *Fortschr. Zool.* **24**(H2/3),327-332.

Kutsch, W. (1973). The influence of age and culture-temperature on the wing-beat

frequency of the migratory locust. *Locusta migratoria. J. Insect Physiol.* **19**, 763-

772.

Kutsch, W. (1999). Telemetry in insects: the "intact animal approach". *Theory Biosci.* 118, 29-53.

Kutsch, W. and Gewecke, M. (1979). Development of flight behaviour in maturing adults of *Locusta migratoria*: II. Aerodynamic parameters. *J. Insect Physiol.* 25:299-304.

Kutsch, W. and Stevenson, P. (1981). Time-correlated flight of juvenile and mature locusts: a comparison between free and tethered animals. *J. Insect Physiol.* 27, 455-459.

Kutsch, W., Camhi, J.. and Sumbre, G. (1994). Close encounters among flying locusts produce wing-beat coupling. *J. Comp. Physiol. A* 174, 643-649.

Kutsch, W., Van Der Wall, M., and Fischer, H. (1999). Analysis of free forward flight of *Schistocerca gregaria* employing telemetric transmission of muscle potentials. *J. Exp. Zoo.* 284, 119-129.

Kutsch, W., Schwarz, G., Fischer, H. and Kautz, H. (1993). Wireless transmission of muscle potentials during free flight of a locust. *J. Exp. Biol.* 185, 367-373.

La Val. R. K. and La Val, M. L. (1980). Prey selection by a neotropical foliage-gleaning bat, *Micronycteris megalotis. J. Mamm.* 61(2), 327-330.

Lang, F. (1996). Noise filtering in the auditory system of *Locusta migratoria* L. *J. Comp. Physiol. A* 179, 575-585.

Lang, F. and Elsner, N. (1994). Leg movement and hearing: biophysics and electrophysiology of the tympanal organ in *Locusta migratoria. J. Comp. Physiol. A* 175, 251-260.

Lehmann, F. and Dickinson, M. H. (1998). The control of wing kinematics and flight forces in fruitflies (*Drosophila* spp.). *J. Exp. Biol.* **201**, 385-401.

Leise, E. M. (1990). Modular construction of nervous systems: A basic principle of design for invertebrates and vertebrates. *Brain Res. Rev.* **15**, 1-23.

Libersat, F., and Hoy, R.R. (1991). Ultrasonic startle behavior in bushcrickets (Orthoptera; Tettigoniidae). *J. Comp. Physiol. A* **169**, 507-514.

Loher, W. (1960). The chemical acceleration of the maturation process and its hormonal control in the male of the desert locust. *Proc. R. Soc. Lond. B.* **153**, 380-397.

Lorez, M. (1995). Neural control of hindleg steering in flight in the locust. *J. Exp. Biol.* **198**, 869-875.

Marquart, V. (1985a). *Auditorische Interneurone in thorakalen Nervensystemen von Heuschecken: Morphologie, Physiologie und synaptische Verbindungen.* PhD thesis. Bochum.

Marquart, V. (1985b). Local interneurons mediating excitation and inhibition onto ascending neurons in the auditory pathway of grasshoppers. *Naturwissenschaften* **72**, 42.

McMasters, J. H. (1989). The flight of the bumblebee and related myths of entomological engineering. *Amer. Sci.* **77**, 164-169.

Meier. T. and Reichert, H. (1990). Embryonic development and evolutionary origin of the orthopterans auditory organs. *J. Neurobiol.* **21**, 592-610.

Meyer, J. and Elsner, N. (1996). How well are frequency sensitivities of grasshopper ears tuned to species-specific song spectra? *J. Exp. Biol.* **199**, 1631-1642.

Michelsen, A. (1971a). The physiology of the locust ear. I. Frequency sensitivity of single cells in the isolated ear. *Z. vergl. Physiologie* **71**, 49-62.

Michelsen, A. (1971b). The physiology of the locust ear. II. Frequency discrimination based upon resonances in the tympanum. *Z. vergl. Physiologie* **71**, 63-101.

Michelsen, A. (1971c). The physiology of the locust ear. III. Acoustical properties of the intact ear. *Z. vergl. Physiologie* **71**, 102-128.

Miller, L. A. (1977). Directional hearing in the locust *Schistocerca gregaria* Forskal. (Acrididae, Orthoptera). *J. Comp. Physiol.* **119**. 85-98.

Miller L. A. and Olesen, J. (1979). Avoidance behavior in green lacewings. I. Behavior of free flying green lacewings to hunting bats and ultrasound. *J. Comp. Physiol.* **131**, 112-120.

Möhl. B. (1985a). The role of proprioception in locust flight control. I. Asymmetry and coupling within the time patterns of motor units. *J. Comp. Physiol A* **156**. 93-101

Möhl. B. (1985b). The role of proprioception in locust flight control. II. Information signalled by forewing stretch receptors during flight. *J. Comp. Physiol A* **156**. 103-116.

Möhl. B. (1985c). The role of proprioception in locust flight control. III. The influence of afferent stimulation of the stretch receptor nerve. *J. Comp. Physiol A* **156**. 281-291.

Möhl, B. (1989). Sense organs and the control of flight. In: *Insect Flight* (ed. G. J. Goldsworthy and C. H. Wheeler). Boca Raton: CRC Press Inc.

Möhl, B., and Zarnack, W. (1977). Activity of the direct downstroke flight muscles of

    Locusta migratoria (L.) during steering behaviour in flight. II. Dynamics of the

    time shift and changes in the burst length. *J. Comp. Physiol A* **118**, 235-247.

Moiseff, A., Pollack, G.S., and Hoy, R.R. (1978). Steering responses of flying crickets

    to sound and ultrasound: mate attraction and predator avoidance. *Proc. Natn.*

    Acad. Sci. U.S.A. **75**, 4052-4056.

Murphey, R. K. (1986). The myth of the inflexible invertebrate: Competition and

    synaptic remodelling in the development of invertebrate nervous systems. *J.*

    Neurobiol. 17(6), 585-591.

Nachtigall, W. (1981). Der Vorderflügel grosser Heuschrecken als Luftkrafterzeuger. I.

    Modellmessungen zur aerodynamischen Wirkung unterschiedlicher Flügelprofile.

    J. Comp. Physiol. **142**, 127-134.

Nachtigall, W. (1989). Mechanics and aerodynamics of flight. In: *Insect Flight* (ed. G. J.

    Goldsworthy and C. H. Wheeler). Boca Raton: CRC Press Inc.

Nolen, T. G. and Hoy, R. R. (1986). Phonotaxis in flying crickets. I. Attraction to the

    calling song and avoidance of bat-like ultrasound are discrete behaviours. *J.*

    Comp. Physiol. A **159**, 423-439.

Orchard, I., Ramirez, J.-M. and Lange, A. B. (1993). A multifunctional role for

    octopamine in locust flight. *Annu. Rev. Entomol.* **38**, 227-249.

Pearson, K. G. (1993). Common principles of motor control in vertebrates and

    invertebrates. *Annu. Rev. Neurosci.* **16**, 265-297.

Pearson, K. G. and Ramirez, J. M. (1990). Influence of input from the forewing stretch

receptors on motoneurones in flying locusts. *J. Exp. Biol.* **151**, 317-340.

Pearson, K. G. and Wolf, H. (1987). Comparison of motor patterns in intact and

deafferented flight system of the locust. I. Electromyographic analysis. *J. Comp.*

Physiol. A **160**, 259-268.

Pearson, K. G., Reye, D. N., and Robertson, R. M. (1983). Phase-dependent influences

of wing stretch receptors on flight rhythm in the locust. *J. Neurophysiol.* **49**(5),

1168-1181.

Pearson, K. G., Boyan, G. S., Bastiani, M., and Goodman, C. S. (1985). Heterogenous

properties of segmentally homologous interneurons in the ventral nerve cord of

locusts. *J. Comp. Neurol.* **233**, 133-145.

Pfau, H. K. and Nachtigall, W. (1981). Der Vorderflügel grosser Heuschrecken als

Luftkrafterzeuger. II. Zusammenspiel von Muskeln und Gelenkmechanik bei der

Einstellung der Flügelgeometrie. *J. Comp. Physiol.* **142**, 135-.

Pflüger, H-J. Elson, R., Binkle, U. and Schneider, H. (1986). The central nervous

organization of the motor neurones to a steering muscle in locusts. *J. Exp. Biol.*

120, 403-420.

Pollack, G. S. and Hoy, R. R. (1981). Phonotaxis in flying crickets: Neural correlates. *J.*

Insect Physiol. **27**, 41-45.

Pollack, G. S. and Plourde, N. (1982). Directionality of acoustic orientation in flying

crickets. *J. Comp. Physiol. A* **146**, 207-215.

Ramirez, J.-M. and Orchard, I. (1990). Octopaminergic modulation of the forewing

stretch receptor in the locust. *J. Exp. Biol.* **149**, 255-279.

Ramirez, J.-M. and Pearson, K. G. (1991). Octopaminergic modulation of interneurons

in the flght system of the locust. *J. Neurophysiol.* **66**, 1522-1537.

Rehbein, H. (1976). Auditory neurons in the ventral nerve cord of the locust:

morphological and functional properties. *J. Comp. Physiol. A* **110**, 233-250.

Reichert, H. and Rowell, C. H. F. (1985). Integration of nonphaselocked exteroceptive

information in the control of rhythmic flight in the locust. *J. Neurophysiol.* **53**(5),

1201-1218.

Reichert, H. and Rowell, C. H. F. (1986). Neuronal circuits controlling flight in the

locust: how sensory information is processed for motor control. *Trends. Neurosci.*

9, 281-283.

Reichert, H., Rowell, C. H. F. and Griss, C. (1985). Course correction circuitry

translates feature detection into behavioural action in locusts. *Nature* **315**(6015),

142-144.

Reye, D. N. and Pearson, K. G. (1987). Projections of the wing stretch receptors to

central flight neurons in the locust. *J. Neurosci.* **7**, 2476-2487.

Reye D. N. and Pearson, K. G. (1988). Entrainment of the locust central flight oscillator

by wing stretch receptor stimulation. *J. Comp. Physiol. A* **162**, 77-89.

Robert, D. (1988). Visual steering under closed loop conditions by flying locusts:

flexibility of optomotor response and mechanisms of correctional steering. *J.*

Comp. Physiol. A **164**, 15-24.

Robert, D. (1989). The auditory behaviour of flying locusts. *J. Exp. Biol.* **147**, 279-301.

Robert, D. and Rowell, C.H.F. (1992). Locust flight steering. I. Head movements and

the organization of correctional manoeuvres. *J. Comp. Physiol. A* **171**, 41-51.

Robert, D. and Rowell, C.H.F. (1992). Locust flight steering. II. Acoustic avoidance

manoeuvres and associated head movements, compared with correctional

steering. *J. Comp. Physiol. A* **171**, 53-62.

Robertson, R.M. and Johnson, A.G. (1993). Collision avoidance of flying locusts:

Steering torques and behaviour. *J. Exp. Biol.* **183**, 35-60.

Robertson, R. M. and Pearson, K. G. (1982). A preparation for the intracellular

analysis of neuronal activity during flight in the locust. *J. Comp. Physiol.* **146**,

311-320.

Robertson, R. M. and Pearson, K. G. (1983). Interneurons in the flight system of the

locust: distribution, connections and resetting properties. *J. Comp. Neurol.* **215**,

33-50.

Robertson, R. M. and Pearson, K. G. (1984). Interneuronal organization in the flight

system of the locust. *J. Insect. Physiol.* **30**(1), 95-101.

Robertson, R. M. and Pearson, K. G. (1985a). Neural circuits in the flight system of the

locust. *J. Neurophysiol.* **53**(1), 110-128.

Robertson, R. M. and Pearson, K. G. (1985b). Neural networks controlling locomotion

in locusts. In: *Model Neural Networks and Behavior* (ed. A. I. Selverston).

Plenum Publishing.

Robertson, R. M. and Reye, D. N. (1992). Wing movements associated with collision-avoidance manoeuvres during flight in the locust *Locusta migratoria*. *J. Exp. Biol.* **163**, 231-258.

Robertson, R. M., Kuhnert, C. T., and Dawson, J. W. (1996). Thermal avoidance during flight in the locust *Locusta migratoria*. *J. Exp. Biol.* **199**, 1383-1393.

Roeder, K. D. (1962) The behavior of free flying moths in the presence of artificial ultrasonic pulses. *Anim. Behav.* **10**, 300-304.

Roeder, K. D. (1964). Aspects of the noctuid tympanic nerve response having significance in the avoidance of bats. *J. Insect Physiol.* **10**, 529-546.

Roeder. K. D. (1967a). *Nerve cells and insect behaviour.* Cambridge. MA: Harvard University Press.

Roeder, K. D. (1967b). Turning tendency of moths exposed to ultrasound while in stationary flight. *J. Insect Physiol.* **13**, 873-888

Roeder, K. D. (1974). Acoustic sensory responses and possible bat-evasion tactics of certain moths. *Proceedings of the Canadian Society of Zoologists Annual Meeting,* pp71-78.

Roeder, K. D. (1975). Neural factors and evitability in insect behaviour. *J. Exp. Zool..* **194**, 75-88.

Römer. H. (1976). Die Informationsverarbeitung tympanaler Rezeptorelemente von *Locusta migratoria* (Acrididae. Orthoptera). *J. Comp. Physiol. A* **109**, 101-122.

Römer, H. (1985). Anatomical representation of frequency in the auditory system of orthoptera. In: *Acoustic and Vibrational Communication in Insects* (ed. K. Kalmaring and N. Elsner). pp. 25-32. Paul Parey.

Römer, H. and Dronse, R. (1982). Synaptic mechanisms of monaural and binaural processing in the locust. *J. Insect Physiol.* **28**, 365-370.

Römer, H. and Seikowsky, U. (1985). Response to model songs of auditory neurons in the thoracic ganglia and brain of the locust. *J. Comp. Physiol. A* **156**, 845-860.

Römer, H. and Marquart, V. (1984). Morphology and physiology of auditory interneurons in the metathoracic ganglion of the locust. *J. Comp. Physiol. A* **155**, 249-262.

Romer, H., Rheinländer, J. and Dronse, R. (1981). Intracellular studies on auditory processing in the metathoracic ganglion of the locust. *J. Comp. Physiol. A* **144**, 305-312.

Römer, H., Marquart, V. and Hardt, M. (1988). Organization of a sensory neuropile in the auditory pathway of two groups of Orthoptera. *J. Comp. Neurol.* **275**, 201-215.

Ronacher, B., von Helversen, D., and von Helversen, O. (1986). Routes and stations in the processing of auditory directional information in the CNS of a grasshopper, as revealed by surgical experiments. *J. Comp. Physiol. A* **158**, 363-374.

Rowell, C. H. F. (1988). Mechanisms of flight steering in locusts. *Experientia* **44**, 389-395.

Rowell, C. H. F. (1989). Descending interneurons of the locust reporting deviation from flight course: What is their role in steering? *J. Exp. Biol.* **146**, 177-194.

Rowell, C. H. F. and McKay, J. M. (1969). An acridid auditory interneurone. I. Functional connexions and response to single sounds. *J. Exp. Biol.* **51**, 231-245.

Rowell, C. H. F. and Reichert, H. (1986). Three descending interneurons reporting deviation from course in the locust. II. Physiology. *J. Comp. Physiol. A* **158**, 775-794.

Rowell, C. H. F. and Reichert, H. (1991). Mesothoracic interneurons involved in flight steering in the locust. *Tissue and Cell* **23**(1), 75-139.

Rydell, J. and Yalden, D. W. (1997). The diets of two high-flying bats from Africa. *J. Zool. Lond.* **242**, 69-76.

Sandeman, D. C. (1961). Some aspects of the flight mechanism of the migratory locust. *Locusta migratoria migratoroides* R and F. Masters thesis. Department of Zoology. University of Natal. Pietermaritzburg. South Africa.

Schmidt, J. and Zarnack, W. (1987). The motor pattern of locusts during visually induced rolling in long-term flight. *Biol. Cybern.* **56**, 397-410.

Schulze, W. and Schul, J. (2001). Ultrasound avoidance behaviour in the bushcricket *Tettigonia viridissima* (Orthoptera: Tettigoniidae). *J. Exp. Biol.* **204**, 733-740.

Schwenne, T. and Zarnack, W. (1987). Movements of the hindwings of *Locusta migratoria*, measured with miniature coils. *J. Comp. Physiol. A* **160**, 657-666.

Shoemaker, K. L. and Robertson, R. M. (1998). Flight motor patterns of locusts responding to thermal stimuli. *J. Comp. Physiol. A* **183**, 477-488.

Smith, C. W., Herbert, R., Wootton, R. J. and Evans, K. E. (2000). The hindwing of the desert locust (*Schistocerca gregaria* Forskål). II. Mechanical properties and functioning of the membrane. *J. Exp. Biol.* **203**, 2933-2943.

Snodgrass, R.E. (1929). The thoracic mechanism of a grasshopper and its antecedents. *Smithson. Misc. Coll.* **82**(2), 1-111.

Spork, P. and Preiss, R. (1991). Control of flight by means of lateral visual stimuli in the desert locust. *Schistocerca gregaria*. In *Synapse-transmission-modulation*. Proc. 19th Göttingen Neurobiol. Conf. (ed. N. Elsner and H. Penzlin). Stuttgart. New York: Thieme Verlag.

Stapells, D.R., Picton, T.W. and Smith, A.D. (1982). Normal hearing thresholds for clicks. *J. Acoust. Soc. Am.* **72**, 74-79.

Steven, R. O. and Bennet-Clark, H. C. (1982). The anatomical and mechanical basis of stimulation and frequency analysis in the locust ear. *J. Exp. Biol.* **99**, 279-314.

Thüring, D.A. (1986). Variability of motor output during flight steering in locusts. *J. Comp. Physiol. A* **158**, 653-664.

Tyrer, N. M. and Gregory, G. E. (1982). A guide to the neuroanatomy of locust suboesophageal and thoracic ganglia. *Phil. Trans. R. Soc. Lond. B* **297**, 91-123.

Uvarov, B. P. (1943). The Locust Plague. *J. Roy. Soc. Arts* XCI(4631),109-118.

Uvarov. B. P. (1966). *Grasshoppers and Locusts: A Handbook of General Acridology. Volume 1. Anatomy, Physiology, Development. Phase Polymorphism. Introduction to Taxonomy.* Cambridge: Cambridge University Press (for the Anti Locust Research Centre).

Uvarov, B. P. (1977). *Grasshoppers and Locusts: A Handbook of General Acridology.*

Volume. 2. Behaviour, Ecology, Biogeography, Population Dynamics. London:

Centre for Overseas Pest Research.

Wakeling, J. M. and Ellington, C. P. (1997a). Dragonfly flight. I. Gliding flight and

steady-state aerodynamics. *J. Exp. Biol.* 200, 542-556.

Wakeling, J. M. and Ellington, C. P. (1997b). Dragonfly flight. II. Velocities.

accelerations and kinematics of flapping flight. *J. Exp. Biol.* 200, 557-582.

Wakeling, J. M. and Ellington, C. P. (1997c). Dragonfly flight. III. Lift and Power

Requirements. *J. Exp. Biol.* 200, 583-600.

Waldmann, B. and Zarnack, W. (1988). Forewing movements and motor activity

during roll manoeuvres in flying desert locusts. *Biol. Cybern.* 59, 325-335.

Wang, Z., Chen, X. and Haunerland, N. H. (1993). Flight muscle development in

juvenile and adult forms of the desert locust. *Schistocerca gregaria. J. Insect.*

Physiol. 39, 325-333.

Weis-Fogh, T. (1949). An aerodynamic sense organ stimulating and regulating flight in

locusts. *Nature* 164, 873-874.

Weis-Fogh, T. (1956a). Biology and physics of locust flight. II. Flight performance of te

desert locust (*Schistocerca gregaria*). *Phil. Trans. R. Soc. Lond. B* 239, 459-510

Weis-Fogh, T. (1956b). Biology and physics of locust flight. IV. Notes on sensory

mechanisms in locust flight. *Phil. Trans. R. Soc. Lond. B* 239, 553-584.

Weis-Fogh, T. (1973). Quick estimates of flight fitness in hovering animals. including

novel mechanisms for lift production. *J. Exp. Biol.* 59, 169-230.

Weis-Fogh, T. and Jensen, M. (1956). Biology and physics of locust flight. I. Basic

principles in insect flight. A critical review. *Phil. Trans. R. Soc. Lond. B* **239**,

415-458

Wendler, G. (1974). The influence of proprioceptive feedback on locust flight

coordination . *J. Comp. Physiol.* **88**, 173-200.

Whim, M. D., and Evans, P. D. (1988). Octopaminergic modulation of flight muscle in

the locust. *J. Exp. Biol.* **134**, 247-266.

Wilkin, P. J. and Williams, M. H. (1993). Comparison of the aerodynamic forces on a

flying sphingid moth with those predicted by quasi-steady theory. *Physiol. Zool.*

66. 1015-1044.

Willmott, A. P. and Ellington, C. P. (1997a). The mechanics of flight in the hawkmoth

Manduca sexta. I. Kinematics of hovering and forward flight. *J. Exp. Biol.* **200**.

2705-2722.

Willmott, A. P. and Ellington, C. P. (1997b). The mechanics of flight in the hawkmoth

Manduca sexta. II. Aerodynamic consequences of kinematic and morphological

variation. *J. Exp. Biol.* **200**. 2723-2745.

Wilson, D. M. (1961). The central nervous control of flight in a locust. *J. Exp. Biol.* **38**.

471-490.

Wilson, D. M. and Weis-Fogh, T. (1962). Patterned activity of co-ordinated motor units.

studied in flying locusts. *J. Exp. Biol.* **39**. 643-667.

Wolf, H. (1990). On the function of a locust flight steering muscle and its inhibitory

innervation. *J. Exp. Biol.* **150**. 55-80.

Wolf, H. (1993). The locust tegula: Significance for flight rhythm generation, wing

movement control and aerodynamic force production. *J. Exp. Biol.* **182**, 229-253.

Wolf, H. and Pearson, K. G. (1987). Comparison of motor patterns in intact and

deafferented flight system of the locust. II. Intracellular recordings from flight

motoneurons. *J. Comp. Physiol. A* **160**, 269-279.

Wolf, H. and Pearson, K. G. (1989). Comparison of motor patterns in intact and

deafferented flight system of the locust. III. Patterns of interneuronal activity. *J.*

Comp. Physiol. A **165**, 61-74.

Wolf, H. and von Helversen, O. (1986). 'Switching-off' an auditory interneuron during

stridulation in the acridid grasshopper *Chorthippus biguttulus* L. *J. Comp.*

Physiol. A **158**, 861-871.

Wootton, R. J., Evans, K. E., Herbert, R. and Smith, C. W. (2000). The hindwing of

the desert locust (*Schistocerca gregaria* Forskål). I. Functional morphology and

mode of operation. *J. Exp. Biol.* **203**, 2921-2931.

Wortmann, M. and Zarnack, W. (1993). Wing movements and lift regulation in the

flight of desert locusts. *J. Exp. Biol.* **182**, 57-69.

Wyttenbach, R. A. and Hoy, R. R. (1997). Spatial acuity of ultrasound hearing in

flying crickets. *J. Exp. Biol.* **200**, 1999-2006.

Yack, J. E. and Fullard, J. H. (1990). The mechanoreceptive origin of insect tympanal

organs: a comparative study of similar nerves in tympanate and atympanate

moths. *J. Comp. Neurol.* **300**, 523-534.

Yack, J. E. and Fullard, J. H. (1993). What is an insect ear? *Ann. Ent. Soc. Am.* **86**, 677-682.

Yack, J. E. and Fullard, J. H. (2000). Ultrasonic hearing in nocturnal butterflies. *Nature* **403**, 265-266.

Yack, J. E., Otero, L. D., Dawson, J. W., Surlykke, A. and Fullard, J. H. (2000). Sound production and hearing in the blue cracker butterfly, *Hamadryas feronia* (Lepidoptera, Nympahlidae) from Venezuela. *J. Exp. Biol.* **203**, 3689-3702.

Yager, D. D. (1999). Structure, development, and evolution of insect auditory systems. *Microsc. Res. Tech.* **47**, 380-400.

Yager, D. D. and Hoy, R. R. (1986). The cyclopean ear: A new sense for the praying mantis. *Science* **231**, 727-729.

Yager, D. D. and Hoy, R. R. (1987). The midline metathoracic ear of the praying mantis. *Mantisa religiosa. Cell Tissue Res.* **250**, 531-541.

Yager, D. D. and May, M. L. (1990). Ultrasound-triggered, flight-gated evasive maneuvers in the praying mantis *Parasphendale agrionina*. II. Tethered flight. *J. Exp. Biol.* **152**, 41-58.

Yager, D. D. and Spangler, H. G. (1997). Behavioral response to ultrasound by the tiger beetle *Cicindela marutha* Dow combines aerodynamic changes and sound production. *J. Exp. Biol.* **200**, 649-659.

Yager, D. D., May, M. L., and Fenton, M. B. (1990). Ultrasound-triggered, flight-gated evasive maneuvers in the praying mantis *Parasphendale agrionina*. I. Free flight. *J. Exp. Biol.* **152**, 17-39.

Yinon, U., Shulov, A. and Tsvilich, R. (1971). Audition in the desert locust: behavioural and neurophysiological studies. *J. Exp. Biol.* **55**, 713-725.

Zanker, J. M. (1988). How does lateral abdomen deflection contribute to flight control of *Drosophila melanogaster*. *J. Comp. Physiol. A* **162**, 581-588.

Zanker, J. M. and Götz, K. G. (1990). The wing beat of *Drosophila melanogaster*. II. Dynamics. *Phil. Trans. R. Soc. Lond. B* **327**, 19-44.

Zarnack, W. (1972). Flugbiophysic der Wanderheuschrecke (*Locusta migratoria* L.). I. Die Bewegungen der Vorderflügel. *J. Comp. Physiol. A* **78**, 356-395.

Zarnack, W. (1978). A transducer recording continuously 3-dimensional rotations of biological objects. *J. Comp. Physiol.* **126**, 161-168.

Zarnack, W. (1988). The effect of forewing depressor activity on wing movement during locust flight. *Biol. Cybern.* **59**, 55-70.

Zarnack, W. and Möhl, B. (1977). Activity of the direct downstroke flight muscles of *Locusta migratoria* (L.) during steering behavior in flight. I. Patterns of time shift. *J. Comp. Physiol. A* **118**, 215-233.

Zarnack, W. and Wortmann, M. (1989). On the so-called constant-lift reaction of migratory locusts. *J. Exp. Biol.* **147**, 111-124.

CHAPTER 2

FOREWING ASYMMETRIES DURING AUDITORY AVOIDANCE IN FLYING LOCUSTS[†]

2.1 Abstract

Flying locusts orient to sounds in their environment. Sounds similar to those produced by echolocating bats cause a flying locust to change its flight path. We used high-speed cinematography and videography to study changes in body posture and wing kinematics in response to stimulation with bat-like sounds. Locusts showed both negative and positive phonotaxis to this stimulus. Within a few wingbeats of stimulus onset (between 126 and 226 ms), locusts deflected their abdomens to one side and the angle of the left and right forewings with respect to the dorsal-ventral body axis became asymmetrical during the downstroke. This forewing asymmetry, in which the forewing on the inside of the turn became more depressed, ranged from 20 to 45 degrees (37 ± 9.7 degrees, mean $\pm$ S.D.) and was correlated with the direction and magnitude of abdomen deflection, a measure of steering in tethered, flying locusts. Hindwing stroke angle asymmetries were minimal or non-existent after stimulation. Coincident with changes in forewing asymmetry and abdomen deflection was a decrease in stroke amplitude (19 ± 6.5 degrees) of the forewing on the inside of the attempted turn. Motor patterns from forewing first

---

basalars (M97) showed an asymmetry in the timing of left and right depressor activation

that ranged from 10.4 to 1.6 ms (4.23 ± 2.85 ms). The number of spikes per depressor

burst increased to a maximum of three spikes in the muscle on the inside of the attempted

turn and depressor frequency (wingbeat frequency) increased by approximately 2 Hz

(2.17 ± 0.26 Hz). We suggest that the asymmetry in forewing first basalar activity is

causally related to the asymmetry in the timing of the initiation of the downstroke,

resulting in an asymmetry in the ranges of the stroke angles of the forewings, which

would impart a roll torque to the locust. This would augment the steering torques

generated by concurrent changes in the angle of attack of the fore- and hindwings and

changes in abdomen position to effect rapid avoidance manoeuvres.

2.2 Introduction

Many insects flying at night are susceptible to predation by insectivorous bats. The

classic example of this interaction is the in-flight behaviour of tympanate moths to the

echolocation calls of approaching bats (Roeder, 1962; 1974). An insect successfully

negotiating such an encounter must be able to detect and move itself away from the

source of the high-frequency sounds. Acoustic startle responses (ASRs) have been

described for many tympanate insects. most notably: crickets (Moiseff, *et al.*, 1978),

katydids (Libersat and Hoy, 1991), praying mantises (Yager, *et al.*, 1990; Yager and

May, 1990), and lacewings (Miller, 1975; Miller and Olesen, 1979). Ultrasound-

sensitive ears have been found in tiger beetles (Spangler, 1988; Yager and Spangler,

1995) and an ASR has been described in scarab beetles (Forrest, *et al.*, 1995). Acoustic

startle responses are typically of short-latency and show directionality to sounds of

behaviourally relevant frequencies and intensities occurring in a particular context (e.g.

flight) (Hoy, et al., 1989). Tethered flying Locusta migratoria also exhibit an ASR (Hoy,

et al., 1989; Robert, 1989; Robert and Rowell, 1992b). When stimulated with

short-duration sound pulses with carrier frequencies greater than 10 kHz and intensities

greater than 45 dB SPL, locusts increase their wing beat frequency and deflect their

abdomens and hindlegs away from the source of the sounds (Robert, 1989). Abdomen

and hindleg deflections increase drag on the side to which they are deflected (similar to

the action of a rudder) but they also shift the location of the centre of mass around which

the flight forces act (Zanker, 1988). These postural adjustments are accompanied by the

production of yaw torques suggesting that if the locust were free flying a change in flight

path would have resulted (Camhi, 1970; Arbas, 1986; Robert, 1989). Along with

postural adjustments, changes in wing kinematics also play a role in flight steering.

For a locust in level flight, lift must equal weight and thrust must equal drag. Lift

and thrust are controlled by wing kinematic parameters originating from the co-ordinated

activity of approximately 40 muscles and changes in wing movements that generate lift

may also, coincidentally, generate thrust. When a locust changes the direction of its

flight path, it must generate either yaw torques or roll torques or both. Torques are

generated when thrust and/or lift is changed asymmetrically on either side of the body.

Many of the data for wing kinematic changes during steering are derived from

studies of locusts engaged in correctional steering to maintain a straight course (e.g.

Thüring, 1986; Waldmann and Zarnack, 1988; Zarnack, 1988). Locusts also engage in

intentional steering to change their course (e.g. Baker, 1979a; Cooter, 1979; Robert,

1989; Robertson and Reye, 1992; Robertson, *et al.*, 1996). A comparison of correctional

steering (to an artificial horizon under closed loop conditions) and intentional steering

(elicited with high-frequency sounds) reveals that many of the mechanisms are similar; in

both types of steering, locusts modulate their wingbeat and use abdomen and hindleg

deflections to turn (Robert and Rowell, 1992a). However, differences are observed in the

two steering modes. In particular, the magnitude of the changes in wing kinematics and

body posture are different between correctional and intentional steering. During collision

(Robertson and Reye, 1992; Robertson and Johnson, 1993) and thermal avoidance

(Robertson, *et al.*, 1996), changes are of greater magnitude than during correctional

steering. Although abdomen and hindleg deflections, and the resulting yaw torques, in

response to high-frequency auditory stimuli have been documented (Robert, 1989), the

associated changes in wing kinematics are currently unknown.

During collision and thermal avoidance the forewings show profound

asymmetries during the downstroke and these are accompanied during thermal avoidance

by asymmetries in the timing of activation of the left and right forewing first basalar

muscles (M97, wing depressors). We show here that similar changes in wing kinematics

and motor patterns are employed by locusts in their steering responses to bat-like sounds

indicating that there may be a common motor strategy underlying rapid avoidance

manoeuvres in flight. Current ideas of flight steering in locusts (e.g. Schmidt and

Zarnack. 1987; Zarnack. 1988) explicitly discount a role in steering for phase shifts in the

timing of transitions to the downstroke. We argue here that such phase shifts are

critically important during intentional steering by producing an asymmetry in the range of stroke angles of the forewings, thereby imparting a roll torque that would augment the steering torques produced by other described kinematic asymmetries to increase the rapidity of the manoeuvre.

As with many wing kinematic studies employing stationary cameras and electromyographic recording equipment, it is an unfortunate necessity to tether the animal. The influences of tethering have been dealt with in detail in previous papers (Robertson and Reye, 1992; Robertson and Johnson, 1993; Robertson, et al., 1996) and they mainly relate to the removal of exteroceptive input that would normally be present during free flight. We therefore anticipated that biases would be present in our data in spite of our efforts to minimize the influences of the tether. Specifically, we predicted that all our locusts would show abdomen deflections away from the sound source indicating attempts to steer away from the sound source, however, we found that some locusts deflected their abdomens in one direction only despite the side of the applied stimulus and that others displayed a steering bias prior to stimulation. In our EMG recordings, this bias was manifest as a pre-stimulus asymmetry in the time of depressor activation and an asymmetry in the number of depressor spikes per burst in left and right M97 recordings. In spite of these observations we are confident that we investigated a real behaviour and not an artefact of the tether for the following reasons. First, abdominal deflections were elicited with behaviourally relevant sound intensities (thresholds between 45 and 55 dB) and deflections occurred within one or two wingbeats after stimulus onset. Second, co-ordinated, repeatable changes in wing kinematics (e.g.

forewing asymmetry) were observed that are similar to those of other avoidance

behaviours (collision avoidance - Robertson and Reye, 1992; thermal avoidance -

Robertson, et al., 1996). Finally, the direction of forewing asymmetry was predictable

from the direction of abdomen deflection even though the latter was not always away

from the sound source. The acoustic stimuli used in this study were adequate to induce

true steering manoeuvres whose direction and magnitude may have been affected by the

experimental set-up.

2.3 Materials and Methods

2.3 1 Animals

Locusta migratoria L. of either sex and at least two weeks past imaginal ecdysis were

selected from a crowded colony maintained at 31°C on a 16:8 light:dark cycle. All data

were collected at room temperature (approximately 22°C). Only apparently healthy

animals with intact wings were chosen for experiments.

2.3 2 High-speed Cinematography and Videography

Animals were dorsally tethered with wax by the pronotum to a rigid copper rod and

suspended 7 cm in front of a wind tunnel (mouth: 20 cm X 20 cm. length: 28 cm)

producing an air flow of 3 m·s^{-1} (verified with a hot-wire anemometer). Animals were

filmed from behind during sound presentations with either a high-speed 16 mm Locam

motion picture camera operating at 250 frames·sec^{-1} with a shutter speed of 1/1225 sec (5

animals) or a Hitachi camcorder (model VM5200A) operating at 60 frames·sec^{-1} with an

electronic shutter speed of 1/1000 sec (10 animals) (Fig. 2.1A).

Speakers (ACR Swiss, model FT17H) were placed equidistant (13.5 cm) from the

midline of the locust at an angle of 72 degrees relative to the long axis of the locusts

body. A light emitting diode was placed in the video frame to monitor the stimulus.

When animals were in stable flight, sounds were presented alternately from the left or

right with the side receiving the first presentation chosen arbitrarily. Sounds consisted of

10 ms, 30 kHz shaped pulses (2 ms rise/fall time) presented with a 20 ms period for a

duration of approximately 400 ms. The shaped pulses were synthesised by passing a 30

kHz sine wave from a frequency generator (Dynascan Corporation, model 3011) into a

custom-made pulse shaper that was gated by a pulse generator (World Precision

Instruments, model 831). The shaped pulses were then amplified (Harman-Kardon,

model HK 6100) and passed through a step attenuator (Hewlett Packard, model 350D).

Sound intensities ranged from 50 to 90 dB SPL. Sound pressure levels were measured

with a Brüel and Kjær sound level meter (model 2209) and a Brüel and Kjær 1/4 inch

condenser microphone (model 4135, with protecting grid). A Brüel and Kjær pistonphone

(model 4220) was used for calibrating the sound measuring equipment. Each sound

presentation at a particular intensity was considered a trial. Measurements (with the

102

Figure 2.1. For kinematic analyses, locusts were tethered 7 cm in front of a wind tunnel
equidistant from laterally placed speakers. Sounds were delivered from either the
left or right and responses were filmed from behind with either a high-speed
(Locam) camera or a Hitachi camcorder equipped with a 1/1000 s shutter (A)
For motor pattern analysis (B). EMG electrodes recorded activity in the left and
right forewing first basalar (M97) depressor muscles while locusts were flying in
front of a wind tunnel within a faraday cage lined with sound attenuating foam. A
single speaker was placed above the animal and one ear was deafened.

A

Wind tunnel
(3 m·s⁻¹)

Speaker

Tethered locust

Camera

B

EMG
wires

Tether

Speaker

Camera
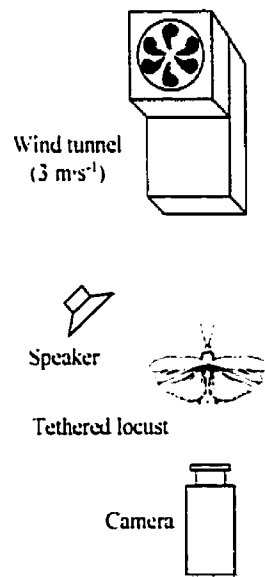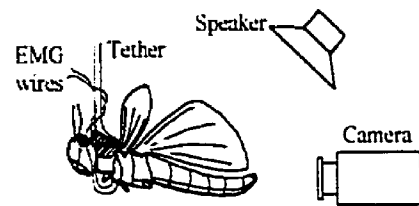
microphone described above) verified that sounds from each speaker produced sound

pressure level differences of 3 to 7 dB SPL across the body at the location of the ears.

Wing movements were analysed by methods previously described (Robertson and

Reye, 1992; Robertson and Johnson, 1993; Robertson, et al., 1996). Briefly, high speed

(Locam) films were analysed by projecting the image of each frame onto a piece of

tracing paper on a glass plate and measuring with a protractor the angles of each wing

and the horizontal and vertical positions of the abdomen. Care was taken to ensure the

image was not distorted after projection onto the glass plate. Angles were measured to

the nearest 5 degrees and abdomen position measurements were accurate to 0.5 mm.

Approximately 12 frames per wing beat cycle were captured using the Locam.

Videotaped trials were analysed with software and hardware from Peak

Performance Technologies (Englewood. CO. USA) which entailed encoding the

videotapes so that each frame could be reliably recalled and measured. Approximately 3

frames per wingbeat cycle were captured with the videocamera. For each video frame.

the top and bottom of the vertical tether. the tips of the wings. and the position of the tip

of the abdomen were marked. From the marked points. the computer calculated wing

angles and abdomen position relative to the tether. Angles from videotaped trials were

accurate to 5 degrees. abdomen positions were accurate to 0.5 mm. Forewing and

hindwing asymmetries (left wing angle minus right wing angle respectively for forewings

and hindwings) were then calculated from the wing angles measured when the animal

was executing downstrokes. Frames capturing downstrokes were identified when the

angular difference (the hindwing angle minus the forewing angle) of the wings on the

outside of the turn (direction of turn indicated by direction of abdomen deflection) was greater than 10 degrees.

For each trial, a pre- and post-stimulus measure of abdomen deflection, abdominal dorsiflexion. forewing asymmetry and hindwing asymmetry was obtained as follows. The pre-stimulus measurement was obtained by averaging 200 ms of data from the period immediately before the stimulus was applied and the post-stimulus measurement was obtained by averaging 200 ms of data 200 ms after the stimulus was applied (see boxed regions in Fig. 2.6). The changes in deflection, flexion, and asymmetries were then calculated by subtracting the pre-stimulus measurement from the post-stimulus measurement.

2.3.3 Motor Patterns

We examined motor patterns in response to acoustic stimulation in a large (65 cm deep. 125 cm wide. 75 cm tall) Faraday cage lined with sound-attenuating foam. Locusts were dorsally tethered as above and suspended 5 cm in front of a wind tunnel (mouth: 14 cm X 14 cm. length: 16 cm) producing an air flow of 3 m·s^{-1} (verified with a hot-wire anemometer). A single speaker (Technics leaf-tweeter. model EAS10TH400B. response flat (± 3 dB) to at least 40 kHz) was positioned 19.5 cm above the animal at an angle of 45 degrees (Fig. 2.1B). One ear (chosen arbitrarily) was destroyed with an insect pin by completely tearing the tympanic membrane. Care was taken not to disturb the underlying tracheae or to induce bleeding. The eyes and ocelli were painted with opaque nail polish

because of the lights required for filming and to eliminate the possibility that locusts were using visual cues for steering.

Sounds were presented when animals were in stable flight. Sounds consisted of 6 ms, 30 kHz shaped pulses (1 ms rise/fall time) presented with a 30 ms period. These stimulus conditions differ from those used for the kinematics above, however, both stimuli are sufficient for eliciting the reported wing movements. Sounds were generated by passing a 30 kHz sine wave generated by a function generator (BK Precision, model 3010) through a pulse shaper (Coulbourn Selectable Envelope Shaped Rise/Fall Gate, model S84-04) and amplifying with a custom built amplifier (using a National Semiconductor LM1875T chip). Sound intensity of the shaped pulses was calibrated by measuring the intensity of a pure tone of equivalent frequency and amplitude (Stapells, *et al.*, 1982) using a Brüel and Kjær (model 2206) sound level meter and a Brüel and Kjær 1/4 inch condenser microphone (model 4135, with protecting grid) placed next to a dead locust, with wings outstretched mimicking flight, tethered in the experimental position. We used 71, 76, 87, 97, and 100 dB SPL pulses to induce responses from the locusts. Trials consisted of a train of pulses at a particular intensity presented for approximately 500 ms. Each animal was left for at least two minutes between stimulus presentations.

Electromyographic (EMG) electrodes consisting of fine copper wire insulated except at the tip, were inserted just beneath the cuticle of the sternum into muscles M97 (forewing first basalars, depressors, numbering according to Snodgrass, 1929) and secured with a drop of wax. To prevent the animals from dislodging the electrodes during flight, all legs were removed at the coxae. Activity from left and right forewing

depressor muscles was referenced to a ground electrode inserted behind the head beneath

the pronotum. EMG signals were amplified using Grass Instruments (model P15)

differential AC preamplifiers and recorded onto videotape for later analysis using a

Panasonic videocassette recorder (model PV-4770-K) and Neuro-corder (model DR-

886). Peak times from left and right forewing depressor EMGs were obtained from

digitised recordings using software and hardware from Datawave Technologies

(Longmont, CO, USA).

2.3.4 Statistical Analysis

Statistical tests were performed using Jandel Scientific's SigmaStat software package

(Corte Madera, CA, USA). Data were tested for normality and heteroscedasticity and

appropriate parametric or non-parametric measures and tests were employed. All

statistical tests assumed significance when $P < 0.05$.

2.3.5 Definition of Terms

All wing angles were measured from behind the animal (i.e. viewing the animal along its

posterior-anterior body axis). For calculations of asymmetry and angular difference. zero

degrees is directly above the animal. *Forewing Asymmetry (FWA)* is defined as the

difference in the angle the left and right forewings make with the D-V axis. It is. by

convention, the right forewing angle minus the left forewing angle. A positive FWA

indicates the right wing is depressed more than the left wing. *Hindwing Asymmetry*

(HWA) is similarly defined except it is with reference to the left and right hindwings.

Angular Difference (AD) is the angle made between forewings and hindwings. It is defined as the hindwing angle (relative to the D-V axis) minus the forewing angle and a positive AD indicates the forewing is at a greater elevation than the hindwing. *Angular Difference Asymmetry (ADA)* is the right angular difference minus the left angular difference. Positive ADA values indicate a greater separation of the right fore- and hindwings. *Depressor Asymmetry (DA)* is defined as the difference in the time of activation of the left and right forewing depressor muscles. It is, by convention, the time of the left muscle activation minus the right. A positive DA indicates the right depressor muscle was activated before the left. *Abdominal deflection (rudclering)* is defined as the position of the tip of the abdomen in the right/left axis with zero as the midpoint (pre-stimulus position) and deflections to the right as positive. *Abdominal flexion* is defined as the position of the tip of the abdomen along the D-V axis with positive values indicating an elevation of the abdomen from its pre-stimulus position.

2.4 Results

In straight, stationary flight, locusts that were minimally affected by tethering flew with the hindwing stroke leading the forewing stroke and forewing stroke amplitude less than hindwing stroke amplitude. Locusts held their abdomens straight behind them, some with slight dorsiflexion, and upstrokes and downstrokes were symmetrical in left and right wing pairs. This posture changed after stimulation with ultrasound. One animal, stimulated from the right (Fig. 2.2) deflected its abdomen and left hindleg to the left (the inside of the presumed turn) and lifted its abdomen (dorsiflexion). The left and right

forewings had asymmetrical positions during the downstroke. In all animals tested, albeit

to varying degrees, we observed the characteristic changes in angle of attack with

increased pronation of the wings on the inside of the presumed turn. These changes are

well established as contributing to steering torques and we did not analyse their

occurrence or magnitude further. In this paper, we focus on observations of forewing

asymmetries and abdomen movements.

2.4.1 High-speed Cinematography

In the five animals filmed. stereotypical changes in wing kinematics and body posture

occurred shortly after the stimulus was applied. We chose one trial from each animal for

detailed analysis. Of those trials chosen. two were animals stimulated from the right and

three were animals stimulated from the left. Sound intensities ranged from 60 to 90 dB

SPL. Four of the five locusts filmed deflected their abdomens to the right (the remaining

locust showed no measurable abdomen deflection to the stimulus) and since direction of

abdomen deflection is correlated with steering direction in tethered. flying locusts (Arbas.

1986; Camhi. 1970; Robert, 1989). we assumed that abdomen deflection indicated an

attempt by the locust to steer in that direction. Thresholds for eliciting abdomen

ruddering were between 45 and 55 dB SPL as measured from two of the five animals.

Figure 2.2. Single high-speed cinematographic frame capturing a tethered flying locust during its response to a bat-like sound from the right (A) and corresponding measurements from the film of forewing and hindwing elevations (B). Large circles in B indicate the frame corresponding to the image above. Bat-like sounds were present during the entire sequence analysed. A consistent observation in the response to sound was the production of an asymmetry in the angle of the left and right forewings relative to the dorsal-ventral body axis during the downstroke. Although the forewings become strikingly asymmetrical, the hindwings do not. In many instances, forewing asymmetries were so extreme that the forewing on the inside of the turn was depressed to a point below the ipsilateral hindwing. Note the abdomen and hindlegs deflected away from the sound source with coincident abdominal dorsiflexion. We also observed changes in the angle of attack of the fore- and hindwings (i.e. the relative amounts of wing surface visible).

A

B

Right wing ········ Left wing

Forewing elevation (degrees)

150
120
90
60
30

Hindwing elevation (degrees)

150
120
90
60
30

0   50   100   150   200   250   300   350

Time (ms)
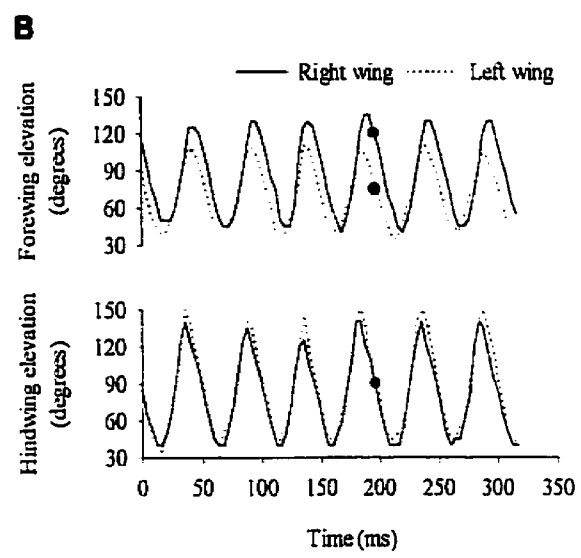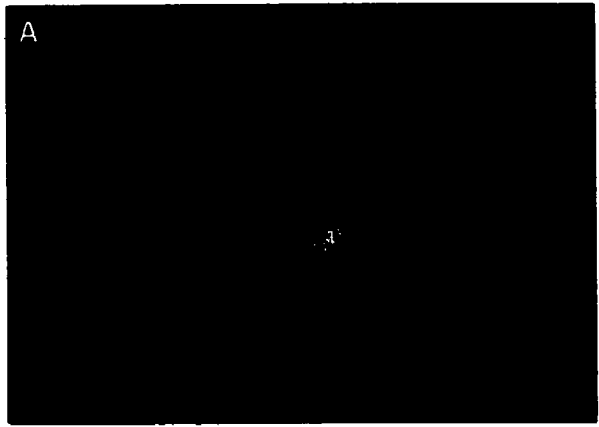
Positive forewing asymmetries during the downstroke were observed in all five animals. Two of the five animals filmed showed forewing asymmetries before the stimulus was applied; however, this bias was most likely an effect of tethering the animal (see introduction). Changes in forewing asymmetry (post-stimulus minus pre-stimulus) ranged from 20 to 45 degrees (37 ± 9.7 degrees, mean ± S.D.). Abdomen deflection (and flexion) preceded changes in forewing asymmetry by between 15 and 44 ms. Latency to abdomen deflection was between 100 and 210 ms. and latency to forewing asymmetry was between 126 and 226 ms. Unlike the forewings, appreciable asymmetries were not consistently observed in the hindwings in response to the stimulus. We observed abdominal dorsiflexion in four of the five animals filmed. Left and right forewing and hindwing elevation and calculated forewing and hindwing asymmetries with abdomen positions for one of the five animals filmed are presented in figure 2.3.

In four of the five animals filmed, forewing stroke amplitude on the inside of the turn path decreased between 15 and 30 degrees (19 ± 6.5 degrees) (Fig. 2.4). The time course of this decrease coincides with the time course of the change in forewing asymmetry and abdomen deflection. Differences in the period of elevation and depression were not observed between left and right forewings, however forewing depression was consistently longer than forewing elevation. Consistent changes in hindwing stroke amplitude were not observed.

Forewing asymmetry was most extreme at the start of the downstroke. We plotted the difference in time between maximum elevation of the left and right forewings (at the start of the stroke reversal) to compare it with the time course of changes in

111

Figure 2.3. Detailed description of the wing kinematics from one locust (different from
that in Fig. 2.2) stimulated with a bat-like sound from the left (stimulus bar)
filmed with the high-speed camera. Plots of hindwing and forewing elevation are
wing tip positions measured every 4 ms (zero degrees is directly below the
animal). Before the stimulus was applied, the right and left forewings began their
up- and downstrokes at the same time and the elevation angles of the right and left
wing pairs were equal throughout the stoke. After the stimulus was presented, the
abdomen lifted (dorsiflexion) and deflected away from the sound source to the
right indicating the animal was attempting to steer to the right. Further, the right
and left forewings developed an asymmetry in elevation angle during the
downstroke but not during the upstroke (boxed region). The wing on the inside of
the turn path, the right wing, began its downstroke before the wing on the outside
of the turn path, the left wing. This is not seen in the hindwings. Asymmetries
were quantified by plotting the difference in elevation angles between the left and
right wings. Hindwing and forewing asymmetries were calculated for every wing
angle measured during the response (solid line in respective asymmetry plots;
large circles are asymmetries calculated during the downstroke). Forewing
asymmetry during the downstroke was zero before the stimulus but increased to
approximately 40 degrees.

112
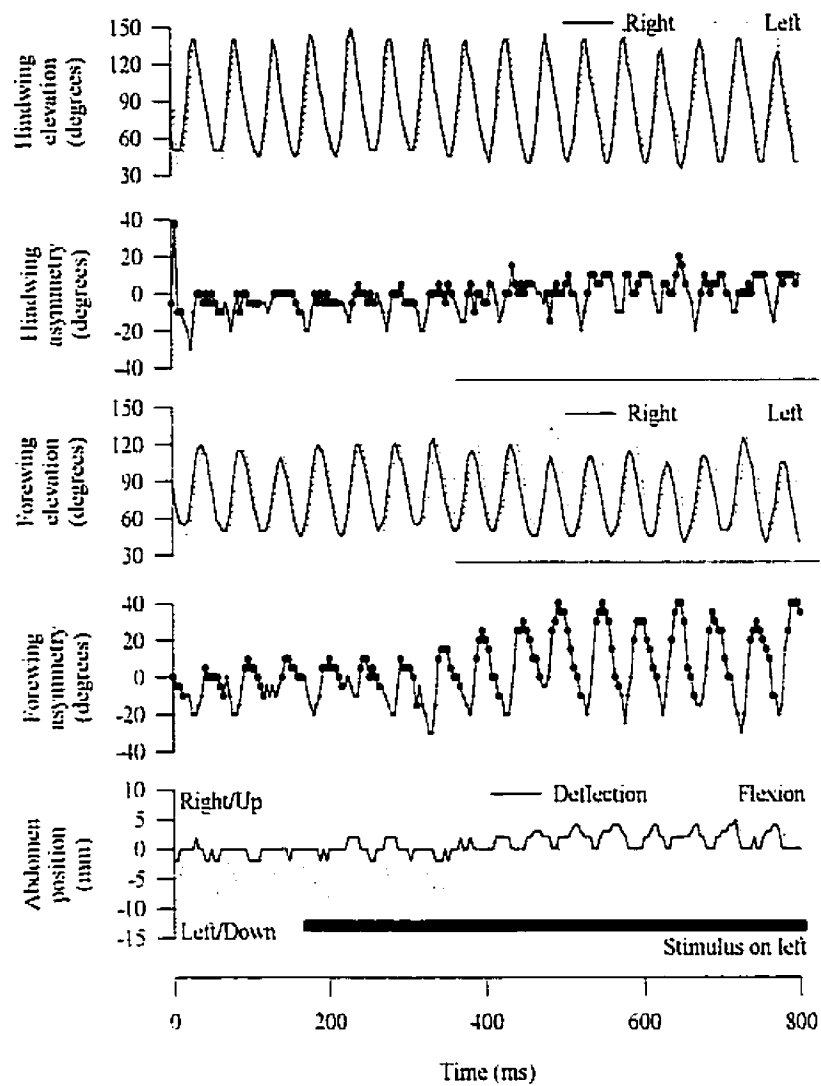
Figure 2.4. Wing stroke amplitude on the inside of the turn path (direction of turn
determined from abdomen deflection) was less than that on the outside of the turn
path. Changes in forewing asymmetry (see Fig. 2.3 for explanation of forewing
asymmetry) followed the same time course as changes in abdomen deflection. In
this trial, a 20 degree forewing asymmetry was present before the stimulus was
applied and is most likely an effect of the tether (see introduction). The data in
this figure are from a different animal than in figure 2.3.

Wing stroke amplitude (degrees)

—— Right   ⋯⋯ Left

120
100
80
60
40

Forewing asymmetry (degrees)

40
20
0
-20
-40

Abdomen deflection (mm)

20
10
0
-10
-20
-30
-40

Right

Left

Stimulus on left

0   100   200   300   400   500

Time (ms)
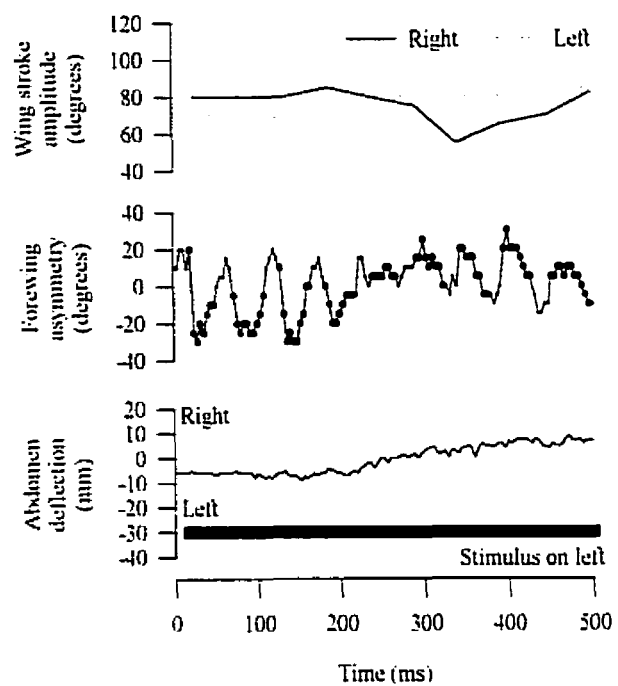
forewing asymmetry (Fig. 2.5). Even with the 4 ms time resolution of the high-speed

camera, a relationship between the difference in the time of initiation (left minus right) of

the downstroke and forewing asymmetry was observed in four of the five animals filmed.

The time course of this difference followed the time course of forewing asymmetry. Four

of the 5 animals filmed revealed significant (P<0.003) regressions with slopes ranging

from 2.26 to 3.28 (Fig. 2.5).

2.4.2 Videography

Frame by frame analysis of high-speed films limits us to detailed examinations of a small

number of animals (five in the case of this study). We used a Hitachi videocamera (with

a fast, 1/1000s shutter) to facilitate data collection and analysis of a larger sample of

locusts to verify the consistency of some of the observations made with the high-speed

camera. Specifically, we used the videotaped trials to determine how changes in the

magnitude and direction of forewing asymmetry were related to changes in abdomen and

to determine how the direction of the sound source affected forewing asymmetries.

Plots of forewing and hindwing asymmetry and horizontal and vertical positions

of the abdomen of one locust before and after stimulation are presented in figure 2.6 (see

Materials and Methods for explanation of boxed regions). This figure demonstrates that

the videographic method is sufficient for capturing changes in forewing and hindwing

asymmetries and abdomen deflection. Measurements of wing angles and abdomen

position were made from 85 sound presentations to 10 animals. Of the 10 animals

114

Figure 2.5. The time course of changes in downstroke initiation (Δt at maximum forewing elevation) followed the time course of changes in forewing asymmetry. The relationship between the time difference of downstroke initiation and forewing asymmetry was linear and predicts that forewing asymmetry increases by 2.26 to 3.28 degrees for each millisecond difference in the time of downstroke initiation. (Regression at right for data from animal presented at left.)

115
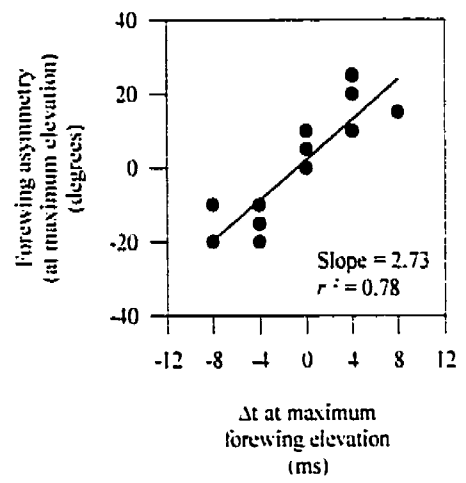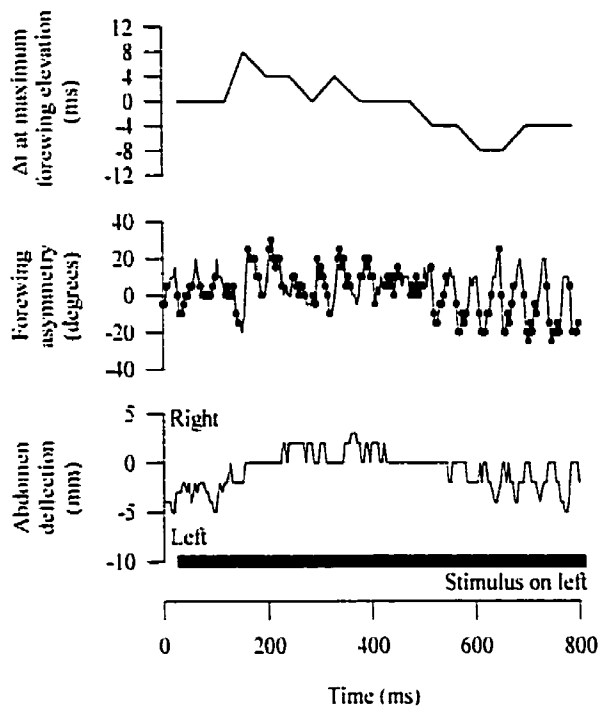
Figure 2.6. A sample of the data obtained from a videotaped trial from one locust. We selected frames capturing downstrokes (see *Materials and Methods* for explanation) and plotted forewing and hindwing asymmetry and horizontal and vertical positions of the abdomen before and after stimulation. Measurements of forewing and hindwing asymmetry and abdomen deflection and flexion were obtained by subtracting a 200 ms average of the pre-stimulus data from a 200 ms average of the post-stimulus data. The boxes indicate the data windows from which the pre- and post-stimulus measurements were taken.

Time (ms)
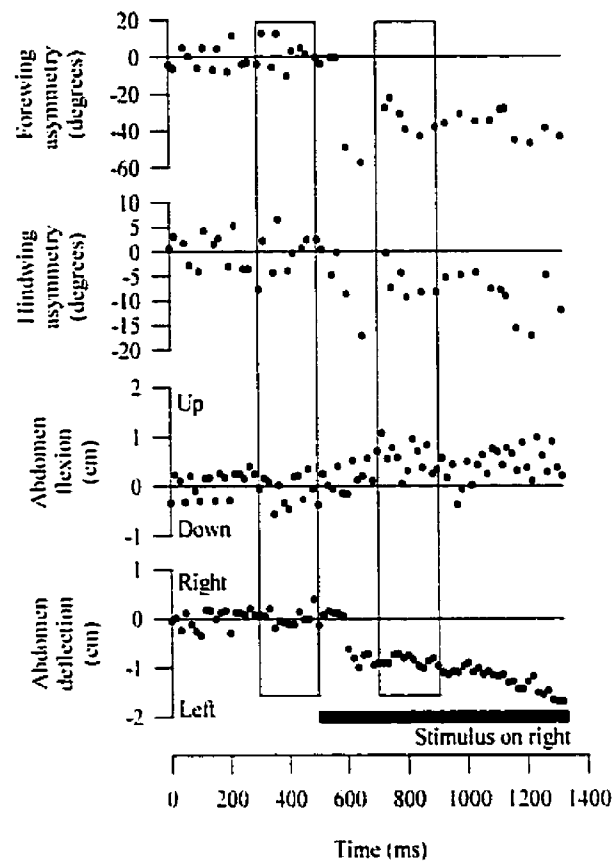
tethered, half (5) showed abdomen deflections in only one direction in spite of the side of the applied stimulus. Of the remaining five animals (which accounts for 39% (33/52) of the trials), 70% (23/33) exhibited negative phonotaxis.

Scatter plots of abdomen deflection (we discarded 14 trials from the initial 85 to use only trials with abdomen deflections greater than 0.1 cm) and forewing and hindwing asymmetries for all trials, irrespective of the direction of phonotaxis or stimulus intensity, revealed that only 7 of 71 trials (9.9%) show a discrepancy between the direction of abdomen deflection and the direction of forewing asymmetry (Fig. 2.7A). Similarly, 25 of 71 trials (35.2%) show a discrepancy between direction of abdomen deflection and hindwing asymmetry (Fig. 2.7B). Some animals yielded more trials than others and not all animals showed phonotaxis in one direction, therefore, we selected two subsets of data from the 71 trials from 10 animals. The first subset consisted of trials showing negative phonotaxis to 60 dB SPL sounds (Fig. 2.7 C. D). Where multiple trials from a single animal were chosen, the responses were averaged to avoid pseudoreplication. The magnitude and direction of forewing asymmetry was significantly correlated with the direction and magnitude of abdomen ruddering (Pearson product moment correlation, $R=0.794$, $N=7$, $P=0.033$) and hindwing asymmetry was neither correlated with abdomen ruddering nor forewing asymmetry (hindwing asymmetry vs abdomen ruddering: $R=0.138$, $N=7$, $P=0.769$; hindwing asymmetry vs forewing asymmetry: $R=0.337$, $N=7$, $P=0.459$). The second subset of data, consisting of trials from animals showing positive phonotaxis towards 60 dB SPL sounds, found the same relationships (Fig. 2.7 E, F). Magnitude and direction of forewing asymmetry was significantly correlated with

117

Figure 2.7. The magnitude and direction of forewing asymmetry was significantly correlated with the direction and magnitude of abdomen ruddering. Hindwing asymmetry was neither correlated with abdomen ruddering nor forewing asymmetry. Scatter plots of data from all trials combined (N=10 animals) shows measurements of abdomen deflection with corresponding forewing and hindwing asymmetries (abdomen deflections less than 0.1 cm omitted). Of 71 trials. only 7 (9.9%) showed disagreement between the direction of abdomen deflection and the direction of forewing asymmetry (A). Hindwing asymmetry does not show the same relationship, 25 of 71 trials (35.2%) show disagreement with direction of abdomen deflection (B). When examining responses to 60 dB sounds only (data from multiple trials per animal combined). animals turning away from the sound source (negative phonotaxis. C. D) and animals turning towards the sound source (positive phonotaxis. E. F) showed the same results.

All trials

Negative phonotaxis

Positive phonotaxis

Forewing asymmetry (degrees)

Hindwing asymmetry (degrees)

C
$r = 0.794$
$P = 0.033$
$N = 7$

D
$r = 0.138$
$P = 0.77$
$N = 7$

E
$r = 0.900$
$P = 0.037$
$N = 5$

F
$r = 0.803$
$P = 0.10$
$N = 5$

Abdomen deflection (cm)

Abdomen deflection (cm)
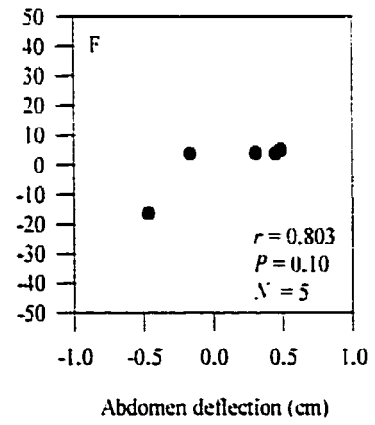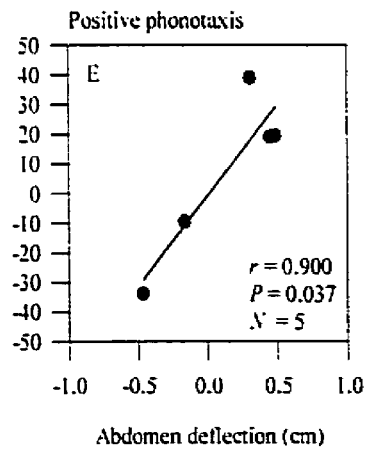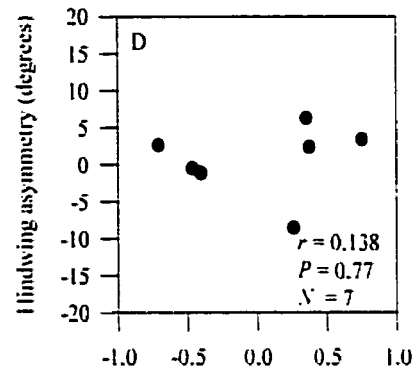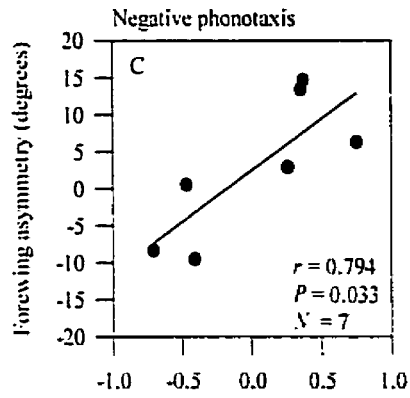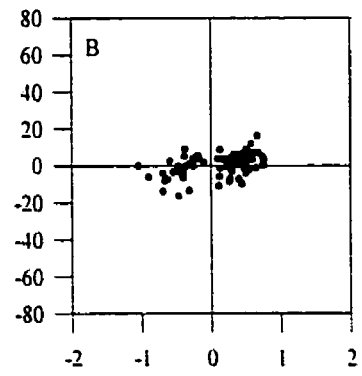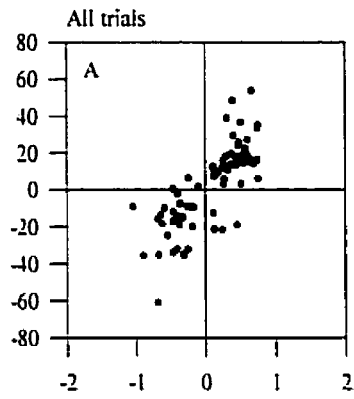
magnitude and direction of abdomen ruddering (R=0.9, N=5, P=0.0372) and hindwing

asymmetry was not correlated with either abdomen ruddering or forewing asymmetry

(hindwing asymmetry *vs* abdomen ruddering: R=0.803, N=5, P=0.102; hindwing

asymmetry *vs* forewing asymmetry: R=0.805, N=7, P=0.100).

2.4.3 Motor Patterns

Forewing asymmetries are most extreme at the initiation of the downstroke and a

relationship was found between the difference in the time of downstroke initiation of the

forewings and the magnitude of forewing asymmetry (Fig. 2.5). This prompted us to

record motor patterns from left and right forewing depressor muscles (forewing first

basalars, M97) to determine if similar asymmetries occur in response to bat-like sounds.

We recorded motor patterns from four locusts. In response to acoustic

stimulation, an asymmetry in the timing of left and right depressor muscle activation

occurred that ranged from 10.4 to 1.6 ms (4.23 ± 2.85 ms). The number of spikes per

depressor burst also increased in the muscle on the inside of the turn path (turn direction

assessed by the direction of abdomen deflection) and the number of spikes per burst in

the muscle on the outside of the turn path simultaneously decreased (Fig 2.8A). In all

animals, an asymmetry in the number of spikes per burst was present before the stimulus

was applied and is likely an artifact of tethering (see introduction). Wing beat frequency

(frequency of depressor activation) before the stimulus ranged from 15.4 to 18.4 Hz and

showed an increase of 2.17 ± 0.26 Hz following stimulus onset. The increases were

Figure 2.8. Motor patterns from left and right forewing first basalar muscles (larger
      spikes - M97. depressors: smaller spikes - crosstalk from an elevator muscle)
      show asymmetries of up to 10.4 ms after stimulation. In addition. more spikes per
      depressor burst (relative to the pre-stimulus condition) were observed in the
      muscle contralateral to the stimulus (inside of the turn path as assessed by
      direction of abdomen deflection) (A). Asymmetries in depressor muscle
      activation were accompanied by a 2.17 ± 0.26 Hz increase in wing beat frequency
      (measured from depressor bursts). The increase in depressor frequency was brief.
      lasting from 3 to 5 depressor cycles. but was coincident with the initiation of
      abdomen deflection and flexion (B). Note that depressor frequency ranged from
      15.4 to 18.4 Hz and in the short sequence of spikes selected for A. it appears that
      depressor frequency has decreased. The motor patterns (A) and changes in
      depressor frequency and depressor asymmetries (B) are from different animals.

A

No Stimulus

Left

Right

Sound on left

Left

Right

25 ms

B

Depressor frequency (Hz): 25, 20, 15, 10

Left

Depressor asymmetry (ms): 20, 10, 0, -10, -20

Abdomen position (cm): 10, 9, 8, 7, 6, 5

Right/Up

Deflection

Flexion

Left/Down

Sound on Left

Time (ms): -200, -100, 0, 100, 200, 300, 400, 500
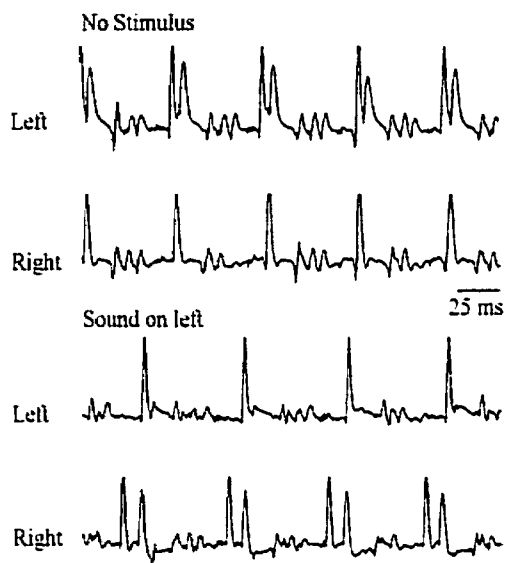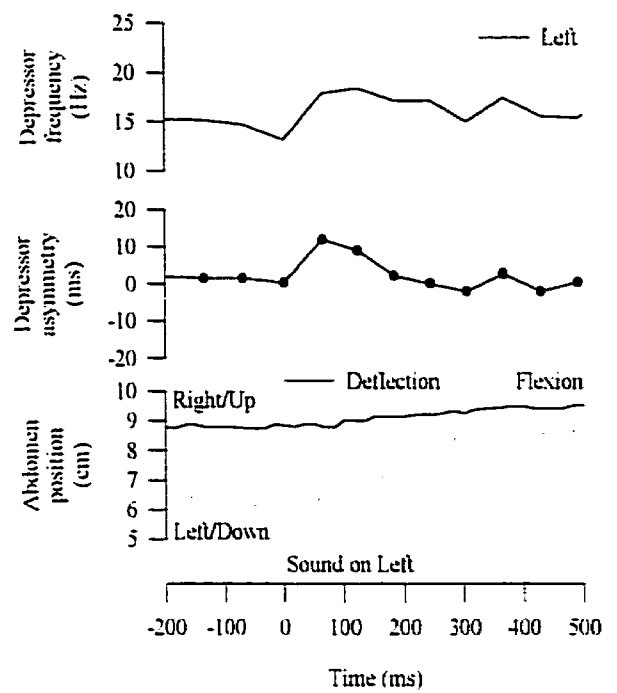
consistent. albeit brief, occurrences that lasted from 3 to 5 depressor cycles and were

coincident with the initiation of changes in abdomen deflection and flexion (Fig. 2.8B).

2.5 Discussion

Stationary, flying locusts attempt to avoid sounds that mimic the echolocation calls of

hunting bats (Robert, 1989; Robert and Rowell, 1992b). In this paper we describe our

investigations of asymmetries of forewing downstrokes of tethered flying locusts

attempting to steer in response to auditory stimulation. It is well established that abdomen

and hindleg deflections are correlated with the direction of turning in flying locusts

(Camhi, 1970; Arbas. 1986; Baader, 1990; Robert and Rowell, 1992a,b). and we used the

direction of abdomen deflection as an index of turning direction in our experiments. Our

principal findings can be summarised as follows: 1) attempted steering manoeuvres

evoked by ultrasound stimulation were accompanied by profound asymmetries in the

elevation angles of the forewings during the downstroke whereas the hindwings remained

relatively symmetrical during the downstroke; 2) the magnitude and direction of

abdomen deflection significantly correlate with those of forewing asymmetry; 3) the

forewing on the inside of the attempted turn was less elevated than the one on the outside;

4) the stroke transition from elevation to depression occurred earlier for the forewing on

the inside of the attempted turn; 5) the activation of M97 during a wingbeat cycle

occurred earlier. and there was an increase in the number of muscle potentials during

each cycle on the inside of the attempted turn. A similar motor strategy has been

described for collision (Robertson and Reye. 1992) and thermal avoidance (Robertson. *et*

al., 1996) during locust flight. We argue below that a phase shift in the timing of transition to the forewing downstroke has consequences for steering. This idea has previously been explicitly discounted for correctional steering in locusts (e.g. Zarnack, 1988).

2.5.1 Wing Kinematics

Numerous investigations of steering during locust flight (reviewed in Kammer, 1985; Rowell, 1988) have resulted in an emerging consensus of which wingbeat parameters can be altered to produce the required asymmetrical lift and thrust. Asymmetries in angle of attack (inside wings pronated; Dugard, 1967; Baker, 1979a; Waldmann and Zarnack, 1988, Zarnack, 1988), asymmetrical timing of stroke reversals (Thüring, 1986; Schwenne and Zarnack, 1987; Waldmann and Zarnack, 1988), and asymmetrical relative positions of the fore- and hindwings (Schmidt and Zarnack, 1987; Schwenne and Zarnack, 1987) have all been associated with steering manoeuvres and/or the production of steering torques. In this study and in previous investigations of intentional avoidance steering (Robertson and Reye, 1992; Robertson, *et al.*, 1996) the most striking observation was of the production of asymmetries in the forewing elevation angles during the downstroke. These asymmetries were primarily attained by changes in the relative timing of the initiation of the forewing downstroke on either side of the locust (earlier downstroke on the inside of the turn). We noted, but did not quantify, changes in the angle of attack of the wings (increased and early pronation inside the attempted turn) during the downstroke and we expect that such changes would have added to any aerodynamic effects of the

observed forewing asymmetry. The following discussion concentrates only on the forewing asymmetry as a kinematic mechanism *contributing* to the generation of steering torques.

An important question is whether the described forewing asymmetry on its own could have aerodynamic consequences for steering. It has been claimed (Zarnack, 1988) that such a phase shift in the timing of transitions to the downstroke could alter the generated forces only during the beginning and ending of shifting, or by virtue of the changed interaction with the hindwings (see below). However the forces generated by the forewings must interact continuously by virtue of the fact that each wing transfers these forces to the body of the locust at the wing hinge and the relative orientations of the forewings and the body will determine a resultant flight force vector. Assuming the two forewings are equal in their aerodynamic effect, the resultant lift vector of the forewing pair, considering the pair in isolation, must lie in a plane which bisects the angle between the two forewings. At different instances during the downstroke the angle between the forewings changes, and, if there is an asymmetry in the elevation angles of the forewings relative to the body, then throughout the downstroke the plane bisecting the angle between the forewings is inclined relative to the dorso-ventral plane of the animal. Thus, although thrust from the forewings may remain symmetrical, the resultant lift vector is directed away from the dorso-ventral plane and towards the side with the lower forewing, and this would occur throughout the force-generating downstroke and not just at the beginning and end of phase shifting. This is inescapably true whether or not locust flight is best considered from a standard quasi-steady perspective (Jensen, 1956) or a vortex-

shedding perspective (Brodsky, 1994). The effect of this would be to impart a roll to the

locust and this would allow the larger lift component of the hindwings to contribute to

changing the direction of flight . Assuming a symmetrical hindwing beat, hindwing lift

would be directed dorsally but, after the forewings impart a roll, dorsal hindwing lift

would be directed lateral to the original flight path and would no longer counteract the

weight. Clearly the forewings may not be equal in their aerodynamical effect during

steering due to relative differences in their angle of attacks (Zarnack, 1988; Robertson

and Reye, 1992), but this effect would add to, and not subtract from, any effect of the

forewing angular asymmetry. Given that the hindwings remain relatively symmetrical

during steering manoeuvres, a further consequence of a shift in the elevation ranges of

the forewings on the two sides could be a difference in the aerodynamic coupling

between the fore- and the hindwings of the two sides (Schmidt and Zarnack, 1987;

Schwenne and Zarnack, 1987; Wortmann and Zarnack, 1987, 1993), and this would also

act additively by reducing the lift on the side with the lower forewing. The complexity of

unsteady aerodynamic phenomena, if they are present in this situation, makes their effects

difficult to predict. In the absence of aerodynamic evidence to the contrary, we conclude

that the asymmetry in the elevation angles of the forewings during the downstroke,

caused by phase shifting of the transition to the downstroke, makes a significant

aerodynamic contribution to the generation of steering torques and that this is aided by

changes in the flight forces generated by single wings (angle of attack) and by changes in

the aerodynamic interference between the forewing and hindwing (separation angle). We

propose that the specific aerodynamic effect of the forewing asymmetry described here is

to roll the locust and allow the lift from all four wings to be directed lateral to the original

flight path. Simply redirecting the hindwings' forces relative to the flight path may

provide increased reaction speed compared with reconfiguring the hindwing beat to

redirect their forces relative to the animal.

2.5.2 Motor Patterns

As described above. the same manoeuvre has the effect of deflecting the resultant flight

force vector of the forewing pair towards the side of the lower forewing and increasing

the aerodynamic interference between the forewing and hindwings on the side where the

separation between the forewing and the hindwing is reduced. There are two motor

strategies which would lead to this configuration of the wings during the downstroke

during steering: a) co-ordinate the beats of right and left forewings so that they attain

asymmetrical elevations during the downstroke (coincidentally this would affect the

separation between the forewings and hindwings on either side): and b) co-ordinate the

beats of forewing and hindwing so as to minimise the separation between them (and thus

maximise interference) on the inside of the turn and maximise the separation on the other

side (coincidentally this would cause an asymmetry in the elevations of the forewings).

The important question is which motor strategy does the animal actually use to change

direction during flight? As noted above, a prominent viewpoint in the literature is that

asymmetry in the initiation of the downstroke to generate phase asymmetries does not

solve the aerodynamic problem of steering in locusts. except in that such an asymmetry

will cause the interactions between forewings and hindwings that are considered to be

more important for steering (Zarnack, 1988). However, in the context of avoidance

steering we favour the alternative interpretation of our data that it is the asymmetry in the

elevation angles of the forewings that is the parameter under control. This interpretation

is reasonable for the following reasons. First, the observation that the forewing on the

inside of the turn often appeared below the hindwing (e.g. Fig. 2.2; see also Fig. 3C in

Robertson and Reye, 1992) indicates that the strategy used cannot be described as to

minimise the separation between forewing and hindwing on the inside of the turn.

Second, when hindwing asymmetries were apparent they were in the same direction as

the forewing asymmetry (e.g. Figs. 2.6 & 2.7; see also Fig. 9 in Robertson and Reye,

1992 and Fig. 3 in Robertson, et al., 1996). This would have worked against the

generation of steering torques by an adjustment of the forewing-hindwing separations

whereas it would have contributed towards steering torques generated by inclination of

resultant force vectors of the forewing and hindwing pairs. Third, this interpretation helps

to resolve apparent anomalies in the literature on steering motor patterns. During visually

induced rolling the changes in the phase shift between corresponding contralateral

muscles were the largest observed in one particular study (Schmidt and Zarnack, 1987),

yet these authors could "offer no plausible interpretation of this finding", because the

degree of aerodynamic coupling between contralateral wings had not been investigated.

During the steering reaction of locusts passively yawed in a wind stream one of the

strongest effects was on the timing of the beginning of the forewing downstroke and on

the time intervals between contralateral homologous muscles (Zarnack, 1988), yet it was

concluded that although "these alterations of contralateral muscle activity are usually the

greatest" they are "unimportant for the generation of aerodynamic forces". It is likely

that large phase shifts between corresponding contralateral muscles (without changing the

relative timing of the activations of a single wing's muscles) would generate phase shifts

in the timing of stroke reversals between contralateral wings (without changing the form

of the beat of a single wing). One consequence of simply changing the relative timing of

stroke reversals of the two forewings by transiently increasing or decreasing the duration

of upstroke or downstroke in one cycle (e.g. Fig. 2.3; see also Fig. 9 of Robertson and

Reye, 1992) is that their stroke angles will occupy different ranges of elevation.

Moreover, if the initial change occurs with the beginning of the downstroke (i.e. to

change the relative duration of the upstroke during one cycle), then one forewing

becomes more elevated than the other and they remain asymmetrical during the

downstroke but return to symmetry for some part of the upstroke (Fig. 2.9). Thus, bulk

shifts in the phasing of contralateral muscles can be interpreted as the motor pattern

required to generate forewing asymmetries during the downstroke. Our experiments have

confirmed that large phase shifts between contralateral forewing depressor muscles

(M97) occur during attempted auditory steering manoeuvres. Such shifts may be

involved in generating differences in angle of attack but would also be required to shift

the timing of transition to the downstroke. Similar, though usually smaller, shifts in the

relative timing of M97 across the locust are well described in the steering literature

(Zarnack and Möhl, 1977; Baker, 1979b; Thüring, 1986; Schmidt and Zarnack, 1987;

Zarnack, 1988; Waldmann and Zarnack, 1988; Hensler and Rowell, 1990). Indeed, the

relationship between the relative timing of M97 and measured steering

Figure 2.9. Model of the effect of changing the relative timing of the forewing stroke reversal on forewing position. The difference in the time of left and right depressor activation (Δ Depressor asymmetry) results in a forewing asymmetry during the downstroke. Compare with Fig. 2.3, boxed region. An asymmetry in depressor activation only affects the downstroke, therefore, the forewings are elevated together.

Left wing
Right wing

Right depressor
Left depressor

Depressor asymmetry
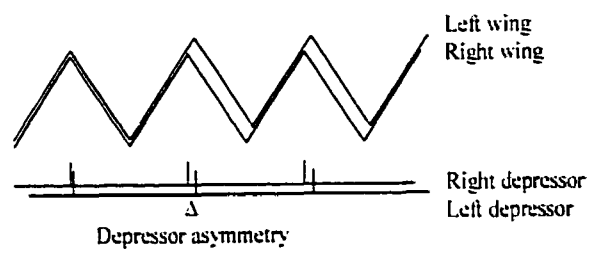
torque is so reliable that the timing shift can be used as a monitor of the direction and

magnitude of attempted turns to close the feedback loop in a flight simulator (Hensler and

Robert, 1990).

We also observed that the burst length of M97 increases on the inside of the

attempted turn. This is in accordance with other intentional steering manoeuvres (Baker,

1979b; Robertson, et al., 1996) though there is a poor or no correlation between M97

burst length and correctional steering (Thüring, 1986; Zarnack, 1988; Waldmann and

Zarnack, 1988). Similarly the increase in wingbeat frequency that we observed is likely

related to the fact that the locust is responding to an abrupt, startling stimulus in its

environment (Cooter, 1979; Wang and Robertson, 1988; Robert, 1989).

2.5 3General Conclusion

Our results suggest that ultrasound information is processed by flight circuitry (see e.g.

Boyan. 1985; 1989) to produce asymmetries in the flight motor pattern that would elicit

turns. One component of the motor pattern change is a shift in the relative timing of M97

(forewing depressor muscles) and this contributes to generating shifts in the timing of

transitions to the downstroke and consequently asymmetry in the elevation of the

forewings during the downstroke. The alteration in forewing kinematics results in

asymmetrical lift to impart a roll to the locust which allows the combined lift of all four

wings to be directed lateral to the original flight path. This acts in combination with

asymmetries in angle of attack, asymmetrical interference between the forewings and

hindwings. postural alterations and an increase in wingbeat frequency to generate rapid

banked downward turns. The motor strategy employed by the locust to steer in response to bat-like sounds is very similar to the strategy observed in collision and thermal avoidance. We suggest that these three sensory modalities (visual, thermal and auditory) converge and elicit a common avoidance behaviour mediated by a common neuronal circuit.

2.6 Acknowledgments

2.7 Literature Cited

Arbas, E.A. (1986). Control of hindlimb posture by wind-sensitive hairs and antennae

during locust flight. *J. Comp. Physiol.* A **159**, 849-857.

Baader, A. (1990). The posture of the abdomen during locust flight: Regulation by

steering and ventilatory interneurones. *J. Exp. Biol.* **151**,109-131.

Baker, P.S. (1979a). The wing movements of flying locusts during steering behavior. *J.*

Comp. Physiol. A **131**,49-58.

Baker. P.S. (1979b). The role of forewing muscles in the control of direction in flying

locusts. *J. Comp. Physiol.* A **131**,59-66.

Boyan, G.S. (1985). Auditory input to the flight system of the locust. *J. Comp. Physiol.*

A **156**,79-91.

Boyan, G.S. (1989). Synaptic input converging onto an identified premotor interneuron

in the locust is gated according to modality. *Naturwissenschaften* **76**.431-433.

Brodsky, A.K. (1994) *The Evolution of Insect Flight*. Oxford University Press: Oxford.

229pp.

Camhi, J.M. (1970). Yaw-correcting postural changes in locusts. *J. Exp. Biol.* **52**, 519-

531.

Cooter, R.J. (1979). Visually induced yaw movements in the flying locust, *Schistocerca*

gregaria (Forsk.). *J. Comp. Physiol.* A **131**.67-78.

Dugard, J.J. (1967). Directional change in flying locusts. *J. Insect Physiol.* **13**.1055-

1063.

Forrest, T. G., Farris, H. E., and Hoy, R. R. (1995) Ultrasound acoustic startle response in scarab beetles. *J. Exp. Biol.* **198**, 2593-2598.

Hensler, K. and Robert, D. (1990). Compensatory head rolling during corrective flight steering in locusts. *J. Comp. Physiol.* A **166**,685-693.

Hensler, K. and Rowell, C.H.F. (1990). Control of optomotor responses by descending deviation detector neurones in intact flying locusts. *J. Exp. Biol.* **149**,191-205.

Hoy, R., Nolen, T., and Brodfuehrer, P. (1989). The neuroethology of acoustic startle and escape in flying insects. *J. Exp. Biol.* **146**, 287-306.

Jensen, M. (1956). Biology and physics of locust flight. III. The aerodynamics of flight. *Philos. Trans. R. Soc. Lond. (Biol).* **239**, 511-532.

Kammer, A.E. (1985). Flying. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology.* (ed. G. A. Kerkut and L. I. Gilbert). New York: Pergamon Press. pp. 491-552.

Libersat, F., and Hoy, R.R. (1991). Ultrasonic startle behavior in bushcrickets (Orthoptera: Tettigoniidae). *J. Comp. Physiol.* A **169**, 507-514.

Miller, L.A. (1975). The behavior of flying green lacewings, *Chrysopa carnea,* in the presence of ultrasound. *J. Insect Physiol.* **21**, 205-219.

Miller, L.A., and Olesen, J. (1979). Avoidance behavior in green lacewings. I. Behavior of free flying green lacewings to hunting bats and ultrasound. *J. Comp. Physiol.* **131**, 112-120.

Moiseff, A., Pollack, G.S., and Hoy, R.R. (1978). Steering responses of flying crickets

to sound and ultrasound: mate attraction and predator avoidance. *Proc. Natn.*

Acad. Sci. U.S.A. **75**, 4052-4056.

Robert, D. (1989). The auditory behaviour of flying locusts. *J. Exp. Biol.* **147**, 279-301.

Robert, D. and Rowell, C.H.F. (1992a). Locust flight steering I. Head movements and

the organization of correctional manoeuvres. *J. Comp. Physiol.* A **171**, 41-51.

Robert. D. and Rowell, C.H.F. (1992b). Locust flight steering II. Acoustic avoidance

manoeuvres and associated head movements, compared with correctional

steering. *J. Comp. Physiol.* A **171**, 53-62.

Robertson, R.M. and Johnson, A.G. (1993). Collision avoidance of flying locusts:

Steering torques and behaviour. *J. Exp. Biol.* **183**, 35-60.

Robertson, R.M. and Reye, D.N. (1992). Wing movements associated with collision-

avoidance manoeuvres during flight in the locust *Locusta migratoria*. *J. Exp. Biol.*

163, 231-258.

Robertson, R.M.. Kuhnert, C.T.. and Dawson, J.W. (1996). Thermal avoidance during

flight in the locust *Locusta migratoria*. *J. Exp. Biol.* **199**, 1383-1393.

Roeder, K.D. (1962). The behavior of free flying moths in the presence of artificial

ultrasonic pulses. *Anim. Behav.* **10**, 300-304.

Roeder, K.D. (1974) *Nerve cells and insect behavior*. Harvard University Press:

Cambridge.

Rowell, C.H.F. (1988). Mechanisms of flight steering in locusts. *Experientia* **44**,389-395.

Schmidt, J. and Zarnack, W. (1987). The motor pattern of locusts during visually

induced rolling in long-term flight. *Biol. Cybern.* **56**,397-410.

Schwenne, T. and Zarnack, W. (1987). Movements of the hindwings of Locusta

migratoria, measured with miniature coils. *J. Comp. Physiol.* A **160**,657-666.

Snodgrass, R.E. (1929). The thoracic mechanism of a grasshopper and its antecedents.

Smithson. Misc. Coll. **82**(2), 1-111.

Spangler, H.G. (1988). Hearing in tiger beetles (Cicindelidae). *Physiol. Ent.* **13**, 447-

452.

Stapells, D.R., Picton, T.W. and Smith, A.D. (1982). Normal hearing thresholds for

clicks. *J. Acoust. Soc. Am.* **72**, 74-79.

Thüring, D.A. (1986). Variability of motor output during flight steering in locusts. *J.

Comp. Physiol.* A **158**, 653-664.

Waldmann, B. and Zarnack, W. (1988). Forewing movements and motor activity

during roll manoeuvers in flying desert locusts. *Biol. Cybern.* **59**.325-335.

Wang, H.-Y.S. and Robertson, R.M. (1988). Changes of the hindwing motor pattern

associated with phonotactic steering during flight in the cricket. *Teleogryllus. J.

Comp. Physiol.* A **164**.219-229.

Wortmann, M. and Zarnack, W. (1987). The influence of several parameters of wing

movement on the lift of *Schistocerca gregaria* during descending, horizontal or

ascending flight. In *New frontiers in brain research* (ed. N. Elsner and O.

Creutzfeldt). Poster 53. Stuttgart: Thieme.

Wortmann, M. and Zarnack, W. (1993). Wing movements and lift regulation in the flight of desert locusts. *J. Exp. Biol.* **182**,57-69.

Yager, D.D. and May, M.L. (1990). Ultrasound-triggered, flight-gated evasive maneuvers in the praying mantis *Parasphendale agrionina*. II. Tethered flight. *J. Exp. Biol.* **152**, 41-58.

Yager, D.D., May, M.L. and Fenton, M.B. (1990). Ultrasound-triggered, flight-gated evasive maneuvers in the praying mantis *Parasphendale agrionina*. I. Free flight. *J. Exp. Biol.* **152**, 17-39.

Yager, D.D. and Spangler, H.G. (1995). Characterization of auditory afferents in the tiger beetle, *Cicindela marutha* Dow. *J. Comp. Physiol.* A **176**, 587-599.

Zanker, J.M. (1988). How does lateral abdomen deflection contribute to flight control of *Drosophila melanogaster. J. Comp. Physiol.* A **162**,581-588.

Zarnack. W. (1988). The effect of forewing depressor activity on wing movement during locust flight. *Biol. Cybern.* **59**. 55-70.

Zarnack, W. and Möhl, B. (1977). Activity of the direct downstroke flight muscles of *Locusta migratoria* (L.) during steering behavior in flight. I. Patterns. *J. Comp. Physiol.* A **118**,215-233.

CHAPTER 3

ACOUSTIC STARTLE/ESCAPE REACTIONS IN TETHERED FLYING LOCUSTS: MOTOR PATTERNS AND WING KINEMATICS UNDERLYING INTENTIONAL STEERING.[†]

3.1 Abstract

By simultaneously recording flight muscle activity and wing kinematics in tethered, flying *Locusta migratoria* L., we have investigated relationships between asymmetric muscle activations and the kinematics of the stroke reversals at the onset of wing depression during intentional steering manoeuvres elicited with bat-like sounds. Bilateral asymmetries in all forewing direct depressor muscles (M97, 98, 99) were positively correlated with asymmetric forewing depression and asymmetries in the timing of forewing stroke reversals. Similarly, bilateral asymmetries in hindwing depressor muscles (M127 and M128 but not M129) were positively correlated with asymmetric hindwing depression and asymmetries in the timing of the hindwing stroke reversal; M129 was negatively correlated with these shifts. Hindwing asymmetries in muscle timing and wing kinematic parameters were smaller and occurred in the opposite direction than corresponding kinematic parameters of the forewings. These findings support the hypothesis that intentional steering manoeuvres employ bulk shifts in

---

depressor muscle timing that affect the timing of the stroke reversals thereby establishing

asymmetric wing depression. We also found correlations between forewing depressor

muscle asymmetries and asymmetries in the timing of the onset of forewing pronation

before the downstroke (dorsal wing flip). Pronation asymmetries were established by

changes in the latency of the wing flip relative to the time of the forewing stroke reversal.

This suggests that locusts may actively control the timing of wing rotation as a

mechanism for generating steering torques. These effects may act in concert with forces

generated by fore-hindwing interactions to establish rapid escape manoeuvres.

3.2 Introduction

There is a growing list of insects with descriptions of acoustic startle/escape responses

(ASRs) that can be elicited by high frequency ($\geq$20 kHz) sounds similar to the

echolocation calls of hunting, insectivorous bats. The list to date includes moths (Roeder.

1962; 1967), butterflies (Yack and Fullard. 2000), lacewings (Miller and Olesen. 1979),

praying mantises (Yager et al.. 1990), crickets (Moiseff. et al.. 1978; Nolen and Hoy.

1986), katydids (Libersat and Hoy. 1991), beetles (Forrest. et al.. 1995. Yager and

Spangler, 1997), and locusts (Robert. 1989; Robert and Rowell. 1992). This list is not

surprising given the intense selection pressure bats place on nocturnal flying insects: a

fact aptly summarized in the statement "... any insect that flies at night is vulnerable to

predation by bats" (Hoy et al.. 1989). For many of the insects above, ASRs have been

described that consist of postural adjustments such as head rolling. leg extension and

abdomen movement. and changes in wing kinematics. For an in-flight ASR to be

adaptive in the context of bat avoidance, the insect must be able to change its course of

flight away from the source of the sounds. Although body posture adjustments such as

leg and abdomen deflections may contribute to steering manoeuvres, it is the wings that

bring about the substantial changes in aerodynamic forces necessary to move the insect

away from the stimulus. The ASR of the locust, *Locusta migratoria* L., provides an

excellent opportunity to study changes in wing kinematics and underlying motor patterns

that bring about these critical changes in direction. In this paper, we show that elements

of the motor pattern recorded from forewing and hindwing direct depressor muscles

correlate with key changes in wing kinematic parameters that are believed to generate

steering torques for intentional steering manoeuvres.

The ASR of tethered flying locusts, first described by Robert (1989), can reliably

be elicited with trains of short duration sound pulses with carrier frequencies greater than

10 kHz and intensities greater than 45 dB SPL. The reaction consists of head rolling

away from the stimulus side, hindleg deflection, abdomen deflection and dorsiflexion,

changes in wing beat frequency (including flight cessation) and changes in wing stroke

kinematics. In tethered flying animals these changes are accompanied by the production

of yaw torques away from the stimulus and it is presumed that these changes are capable

of changing the flight path of free-flying individuals (Robert, 1989; Robert and Rowell,

1992). Free-flying locusts have recently been shown to react to synthesized sounds with

turns, loops and spirals, drops to the ground, and rapid ascents: manoeuvres consistent

with those described for other insects (e.g. moths, lacewings and praying mantises) tested

in free-flight with real bats (Dawson *et al.*, 2001). Dawson *et al.* (1997) extended the

description of the ASR for tethered locusts and showed that it is accompanied by the

production of profound asymmetries in the elevation angles of the forewings during the

downstroke. Forewing asymmetries are accompanied by changes in angle of attack of the

wings and increases in wing beat frequency and are believed to contribute to the forces

generated by these kinematic changes. Forewing asymmetries are also seen when locusts

are avoiding collision with oncoming objects (Robertson and Reye, 1992; Robertson and

Johnson, 1993) and when locusts are avoiding intense radiant heat sources (Robertson *et

al*, 1996; Shoemaker and Robertson, 1998) suggesting that the production of forewing

asymmetry is a common strategy employed by locusts trying to evade hazards.

There is a rich and copious literature on the neuromuscular mechanisms

underlying steering in locusts. It is fair also to say that nearly all mechanisms described

to date relate to how locusts adjust their flight system to maintain a straight course of

flight in the face of environmental perturbation and non-linearities in the flight machinery

(Burrows, 1996). Recently consensus has begun to emerge that intentional steering (e.g.

to avoid a predator) is a different behaviour than steering to maintain a straight course of

flight (correctional steering). These two types of steering are superficially similar but

differ in many important regards. Wing kinematic changes and postural adjustments

during intentional steering are frequently larger relative to those seen during correctional

steering (Robertson and Reye, 1992; Robertson *et al*, 1996; Dawson *et al*, 1997).

Abdomen deflection is accomplished with different segments during intentional steering

(Camhi, 1970). And, during intentional steering to bat-like sounds, the direction of head

roll is opposite to that during optomotor steering (Robert and Rowell, 1992).

The neuromuscular mechanisms underlying steering have mostly been studied

using locusts engaged in correctional steering (for reviews see Rowell, 1988; Burrows,

1996). Recordings from flight muscles show that the changes that occur during steering

are in recruiment. burst length (spikes per burst) and phase (time of depolarization).

Changes in recruitment of motor fibres and in spikes per burst affect the force of muscle

contraction and do not directly affect the timing of wing movements. It is shifts in the

timing of contralaterally homologous muscle depolarizations that is most sensitive to

changes in yaw, pitch and roll (Zarnack and Möhl, 1977). The timing of activation of

contralateral muscles will bring about asymmetries in the behaviours of left-right wing

pairs. and. depending on the effects the muscles exert on the wings, this can have

aerodynamic consequences. Zarnack and Möhl (1977) recorded motor patterns of locusts

flying in a laminar wind stream while being rolled. pitched and yawed; the three flight

axes being manipulated in isolation. They recorded from the six direct depressor muscles

of the fore- and hindwings on each side of the locust and found that many of the muscles

shift together. Thüring (1986) found similar shifts in depressor muscles in response to

movement of an artificial horizon and noted that the shifts were correlated with the

production of roll torques. Phase shifts in the time of activation of forewing and

hindwing muscles on one side of the insect and in the time of activation of muscles

within a single wing also occur and do have effects on wing kinematics. Wilson (1968)

suggested the forewings can act as leading-edge flaps for the hindwings thus the time of

forewing depression and elevation relative to the hindwing will affect the flow of air

across the wing pairs. The angle of attack of a wing, which is critically important in the

amount of lift it is able to generate, is established by the relative timing of muscles within

the wing (Nachtigall, 1989).

The neuromuscular mechanisms underlying intentional steering have only

recently begun to be addressed. Examination of wing kinematics during collision,

thermal, and auditory avoidance show similar wing kinematic strategies consisting of

asymmetric forewing depression during steering responses suggesting that a common

motor strategy may be employed by locusts for intentional steering (Robertson and Reye.

1992; Robertson and Johnson, 1993; Robertson *et al.*, 1996; Shoemaker and Robertson.

1998; Dawson *et al.*, 1997). Dawson *et al.*, 1997 examined wing kinematics with high-

speed cinematography and found that FWA was correlated with asymmetries in the

timing of the stroke reversal and that forewing asymmetry was accompanied by large

shifts (larger than typically observed during correctional steering) in forewing first

basalar activity. This study proposed a model relating depressor muscle timing and

asymmetric wing depression (Fig. 3.1). This model predicts that wing asymmetry is

established by the relative timing of the stroke reversals of the left and right wings. In the

model. the left and right wings are elevated symmetrically but differences in the timing of

the left and right depressor muscles cause one wing to reverse direction before the other

thereby generating asymmetric wing depression. Since stroke reversal timing is

determined by depressor muscle activity. the prediction from this model is that bulk shifts

in several direct depressor muscles would be observed during intentional steering

responses. Coordinated shifts in left and right M97. M98. M99, M85 and M127 (names

after Snodgrass, 1929) have been recorded during thermal avoidance steering

141

Figure 3.1. A model predicting that asymmetric depression of the wings results from bulk shifts in the timing of depressor muscles which bring about changes in the timing of stroke reversals. In the schematic above. left direct downstroke depressor muscles fire before the right depressor muscles bringing about an earlier stroke reversal of the left wing relative to the right. The result is that the left wing is depressed more than the right wing throughout the downstroke but not during the upstroke. Modified from Dawson *et al.*. (1997).

Stroke reversal ──→ asymmetry

Wing asymmetry

Right wing
Left wing

Right depressor
Left depressor

Depressor ──→ ←──
asymmetry
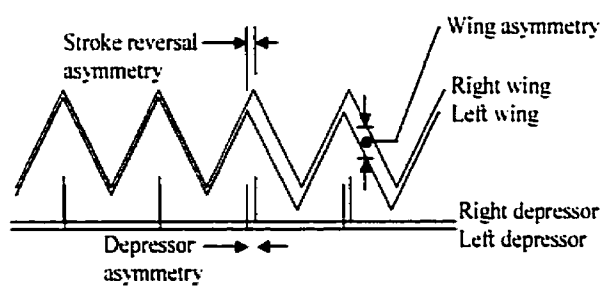
(Shoemaker and Robertson, 1998) and were found to be correlated with asymmetric wing

depression supporting this model. However, this study did not employ high speed

cinematography and detailed kinematics of the stroke reversal could not be obtained. The

purpose of this study was to test the hypothesis that asymmetric wing depression is

established by asymmetries in the stroke reversal brought about by bulk shifts in

depressor muscle activity. We have addressed this question with simultaneous recordings

from direct depressor muscles and high speed digital cinematography.

3.3 Materials and Methods

3.3.1 Animals

Adult male and female *Locusta migratoria* L. aged 15 to 20 days post imaginal moult

were used in these experiments. All animals were reared under crowded conditions in a

continuously breeding culture maintained at $30^{\circ}C$ with 60% relative humidity under a

16h:8h light:dark cycle.

Animals were tested in a large (65 cm deep, 125 cm wide, 75 cm tall) foam-lined

Faraday cage (Fig. 3.2) with a wind tunnel (circular mouth, diameter 15 cm) providing an

air stream of 3 $m \cdot s^{-1}$ as measured with a hot-wire anemometer (TSI, model 67-7 with

sensor model 1610-12). Animals were tethered dorsally by one of two methods. In the

first method, animals were attached directly to a rigid copper rod with a small drop of

beeswax on the pronotum. In the second method, a small aluminium saddle was fastened

across the pronotum with beeswax. The aluminium saddle could be held in the

Figure 3.2. Diagram of experimental apparatus. Locusts were tethered inside a large faraday cage in front of a wind tunnel producing an air stream of 3 m/s. Bat-like sounds were presented from a speaker that could be positioned along an arc equidistant from the locust at any angle lateral and posterior to the locust. The faraday cage was lined with contoured foam to minimize reflection of high frequency sounds. EMG signals were amplified by a custom built 16 channel EMG amplifier and digitized at 25 kHz per channel. A 500 fps high speed digital camera positioned directly behind the locust captured changes in body posture and wing kinematics. All recording equipment was synchronized by a common trigger allowing us to reconstruct the motor patterns underlying changes in body posture resulting from the auditory stimulus.

Wind tunnel
3 m·s⁻¹

Faraday cage with
sound-attenuating foam

90°   270°

EMG amplifier
and digitizer

500 fps high speed
digital camera

Trigger

Bat-like sound
generator
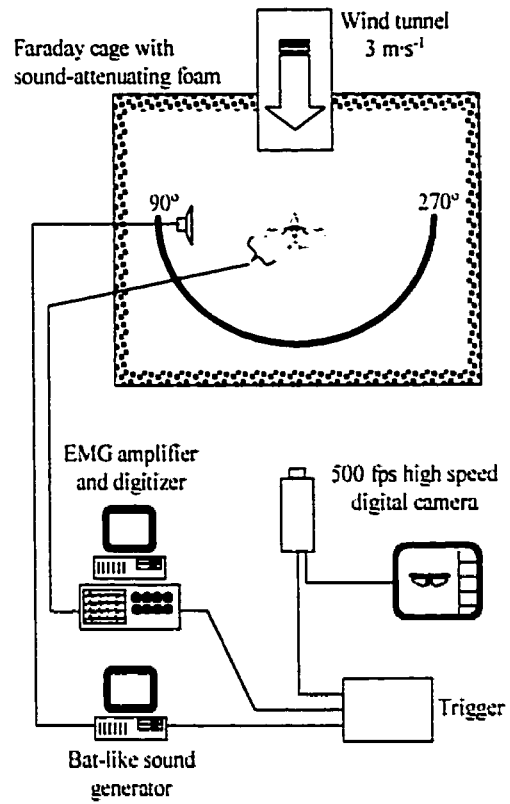
jaws of an alligator clip to allow easy removal of the animal from the experimental

chamber between experiments. For all experimental observations animals, were

suspended 10 cm in front of the wind tunnel and adjusted for trueness with the wind

stream. Adjustments were aided by examining the wing strokes with our high-speed

camera (see below) for symmetrical wing strokes on the left and right sides of the body

and limb alignment with the body. Animals were flown until they assumed a tucked

flight posture after which time stimuli were presented.

3.3.2 Acoustic Stimuli

A pattern that mimics the feeding buzz (Simmons *et al.*, 1979) of an echolocating bat was

chosen as our acoustic stimulus. A train of 10 shaped pulses each with a 1 ms

exponential rise/fall time and 8 ms sustain time repeated with a 20 ms inter-pulse-interval

(producing a period of 30 ms or pulse frequency of 33.3 pulses per second) were

presented from a two-inch cone tweeter (Motorola, model KSN1078A). The pulses were

synthesized with sine waves with a carrier frequency of 30 kHz. The speaker was

positioned 20 cm from the locust with the centre of the speaker aligned vertically with the

locust tympanum. Sounds were always presented at least two minutes apart to avoid

habituating the animal with repeated stimuli.

Our sounds were digitally synthesized with an arbitrary waveform generator (PC

Instruments, model PCI-311, Akron OH, USA) controlled by a microcomputer with

software supplied by the manufacturer (BenchTop Lite, v3.3J). Sound presentation was

initiated by way of a TTL trigger pulse generated by a Grass Instruments electronic

stimulator (model S8, Grass Instruments, Quincy MA, USA) applied to the arbitrary

waveform generator.

Sound intensity of the pulses was determined by matching pure tones of

equivalent amplitude and frequency (dB peSPL; Stapells, et al., 1982) to the stimulus

pulses and measuring the SPL from a Brüel and Kjær sound level meter (type 2610) with

a Brüel and Kjær ¼ inch microphone (type 4135, without protective grid) positioned

20 cm in front of the speaker (where the locust tympanum was during experiments). The

sound level meter was calibrated with a Brüel and Kjær pistonphone (type 4228).

3.3.3 Electromyography

Electrodes consisted of 80 μm diameter (50 μm core) copper wire insulated except at the

tip. Unanaesthetized locusts were placed right side down in a large wax filled Petri-dish

and secured with adhesive tape. Electrodes were then successively implanted into left

M97, M98, M99, M127, M128 and M129 muscles (names after Snodgrass, 1929)

through small holes, made with a minuten pin, in the pterothoracic episterna and epimera.

Electrodes were secured with a minimum of wax applied to the pleura and wires were

guided and secured to the aluminium saddle at the pronotum. The locust was then turned

left side down, re-secured with tape, and six more electrodes were implanted into the

contralateral muscles with wires being similarly secured. Last, a common ground

electrode was inserted through the distal region of the second abdominal tergite and

secured with wax. The position of the ground electrode and wax did not obscure the ear.

Care was taken in locating the ground electrode so that internal tympanal tracheal airsacs

were not compromised. All twelve electrode wires and the ground wire were bundled

together at the aluminium saddle and encased along their length to the recording

amplifier in a small amount of flexible plastic adhesive (LePage 5F, Brampton ON,

Canada). After electrodes were implanted locusts were returned to their holding cages,

with food, for 12 to 24 hours before testing. The EMG electrodes had little effect on the

ability of the locusts to flap their wings and generate flight and steering forces as we

readily observed locusts performing climbing flights, turns, take-offs and landings while

tethered loosely by only the EMG wires.

Electrode signals were amplified with a custom built 16 channel differential

amplifier and digitized at 25 kHz per channel using hardware and software from Axon

Instruments (Digidata 1200B with Axoscope v8.0, Axon instruments, Foster City CA,

USA). The digitized traces included a record of the trigger pulse for presenting the bat-

like sounds. From the recordings, we measured the time of each muscle activation (as the

time of the positive going peak of the waveform for the first spike in spike-bursts) and the

number of spikes per burst for at least 15 cycles before and 15 cycles after the stimulus.

Depending on depressor frequency, this window was between 1200 and 1600 ms.

We confirmed electrode position after experiments by dissection. Animals were

injected with a 1:4 mixture of 95% ethanol to glacial acetic acid and left at room

temperature for 24 to 48 hours. For each animal, the wings and legs were removed, the

animal was decapitated and the abdomen was removed distal to the 3rd abdominal

segment. The thorax and remaining portions of the abdomen were then cut dorsally and

ventrally along the midline and each hemisection was pinned in a water filled petri dish

with a cork bottom. The dorsal longitudinal and elevator muscles were then carefully

removed to reveal the underlying depressor muscles. Each depressor muscle was then

carefully teased apart until the tip of the electromyographic electrode was found. Results

of electrode placement and inspection of the electromyographic recordings were used to

determine which muscles were included in subsequent analyses.

3.3.4 High-speed Digital Cinematography

Animals were filmed from behind using a Motion Scope 500 frame per second high-

speed digital camera (model HR500. Optikon, Kitchner ON, Canada). We used a 6 mm

1:1.4 TV lens and shuttered the camera at 1/2500 s. We used a 250 W halogen lamp

positioned above the camera and a 300 W halogen lamp positioned below the camera.

Both lamps were directed onto the posterior of the locust from an angle of approximately

30 ° above and below respectively. The light intensity at the locust was 135 Watts/m^2.

This light, which caused the temperature of the faraday cage to rise above ambient room

temperature, enabled us to complete our experiments at temperatures between 27° and

32°C.

To facilitate observation of the wings in the high speed images, we applied marks

to the wings with white correction fluid (Liquid Paper ®, Gillette Company. Boston MA.

USA). We placed a dot at the tip of each of the four wings. a dot on the forewing where

the costal vein meets the leading edge, and a narrow stripe to mark a wing chord on the

forewing that began at the leading edge and ended where the 2nd cubital vein meets the

anal edge of the wing. These markings were placed on both upper and lower surfaces of the wing.

The high speed camera was triggered with the same signal that initiated the sound presentations and therefore we were able to obtain simultaneous records of postural and wing kinematic changes with electromyographic recordings from flight muscles. After the camera was triggered, we copied a 1.5 second segment (750 frames) of the camera memory to videocassette at a rate of one frame per second for later analysis. The video tapes were then digitized using video digitizing hardware (All in Wonder 128, ATI Technologies Inc. Thornhill ON, Canada) and saved as AVI files. Custom software was then used to measure wing elevation angles, the time of stroke reversals, the onset of wing rotation at the stroke reversals (the timing of the wing flip), and abdomen position from the AVI files.

The exact frame rate of our camera was determined by filming a light emitting diode that flashed at precisely measured intervals of time. The flash was generated by a Grass instruments stimulator (model S8, Grass Instruments, Quincy MA, USA) and the interval between flashes was measured with a digital storage oscilloscope (Gould, model DSO630). Frame rate calculations were repeated several times using different intervals of time and with the camera cold (right after booting) and warm (on for at least three hours). The average time between frames was 2.0598 ± 0.0003 ms (mean $\pm$ S.D.) and was not affected by camera temperature (operating time).

3.3.5 Data Analysis and Statistical Treatment of the Data

Spike times and elevation angles were used to calculate measures of left-right motor and

wing asymmetry. These variables, and their calculations, are summarized in Table 3.1.

Calculations follow conventions established in Dawson *et al.* (1997). These variables are

defined such that positive values always indicate an intended (fictive) turn to the right

and negative values always indicate a fictive turn to the left. The data presented in figure

3.3, 3.7, 3.10, and 3.11 is from the same locust (L33R1) and is presented to illustrate the

typical time course and cycle by cycle changes observed when a locust reacted to our

stimulus.

Statistical treatment of the data followed procedures in Zar (1984) and Sokal and

Rohlf (1981). Statistical tests were performed using JMP v3.2.1 (SAS Institute Inc.).

Significance was assumed when P<0.05.

3.4 Results

Locusts began to fly as soon as they were placed in the wind stream. Most locusts

required slight adjustment after securing the tether in front of the wind stream to achieve

symmetrical wing beats and a proper flight posture although it was not possible to

achieve perfect symmetry. When locusts were stimulated with 30 kHz bat-like sounds.

they reacted with abdomen and hindleg deflection. abdomen dorsiflexion. changes in

wing kinematics, and increases in depressor muscle activation (wing beat) frequency

(Fig. 3.3). Gender based differences in tethered flight responses were not observed in

Table 3.1: Definition and explanation of variables. These terms follow definitions in Dawson *et al.*, (1997).

Variable	Description (units)	Calculation[1] / Notes[2]
DA	Depressor asymmetry (ms)	Calculated as the time of the left depolarization minus the time of the right depolarization. A positive value indicates the right muscle is activated before the left.
FWA-DS HWA-DS	Fore- or Hindwing asymmetry during the downstroke (°)	The asymmetry in elevation angles[1] of the wings at one third of the downstroke. Calculated as the angle of the right wing minus the angle of the left wing. Positive values mean the right wing is more elevated than the left wing.
FW-SRA HW-SRA	Fore- or Hindwing stroke reversal asymmetry (°)	Difference in time between the left and right stroke reversal. Calculated as the time of the left stroke reversal minus the time of the right stroke reversal. Positive values mean the right wing began the downstroke before the left wing.
FWA-SR HWA-SR	Fore- or Hindwing asymmetry at the stroke reversal (°)	The asymmetry in elevation angles· of the wings at the moment of the stroke reversal (beginning of the downstroke of the respective wings). Calculated as the angle of the right wing minus the angle of the left wing. Positive values mean the right wing is more depressed than the left.
FW-PrA HW-PrA	Fore- or Hindwing pronation asymmetry (ms)	Refers to asymmetries in the time of the onset of pronation (the beginning of the wing flip). See Fig. 3.12. Calculated as the time of the left wing flip minus the time of the right wing flip. Positive values mean the right wing pronated (flipped) before the left.
ADA	Angular difference asymmetry (°)	Refers to asymmetries in the "gap" between left and right fore- hindwing pairs. Angular difference is calculated by subtracting the elevation angle[1] of the forewing from the elevation angle of the hindwing. Asymmetries are calculated by subtracting the above difference from the left fore-hindwing pair from the difference calculated for the right fore- hindwing pair. A positive value indicates a smaller "gap" on the right side of the locust.

[1] Wing angles are measured with 0 ° directly above the locust.
[2] The order of the terms in the calculations have been chosen such that positive results (° or ms) reflect right "turns" and negative results reflect left "turns".

151

Figure 3.3. Locusts responded to stimulation with bat like sounds with lateral abdomen
deflection. dorsiflexion, and an increase in depressor (wing beat) frequency. This
locust (L33R1) responded with abdomen deflection to the left (A), abdominal
dorsiflexion (B), and a 3 Hz increase in depressor frequency (C). Yellow regions
indicate the post-stimulus period. The images above are single frames from the
high speed camera taken at the times indicated. Arrows indicate the position of
the wing tips. the tip of the abdomen and the left hindleg (which deflected into the
turn after stimulation). The poor quality of the images is a result of the
experimental procedure wherein we use a minimum of light during filming. The
oscillations in (B) are due to the beating wings.

Abdomen deflection (arbitrary units)

A

↓=Left

Abdomen flexion (arbitrary units)

B

↑=Up

Depressor frequency (Hz)

C

24
23
22
21
20
19

-1000 -800 -600 -400 -200 0 200 400 600 800

Time (ms)
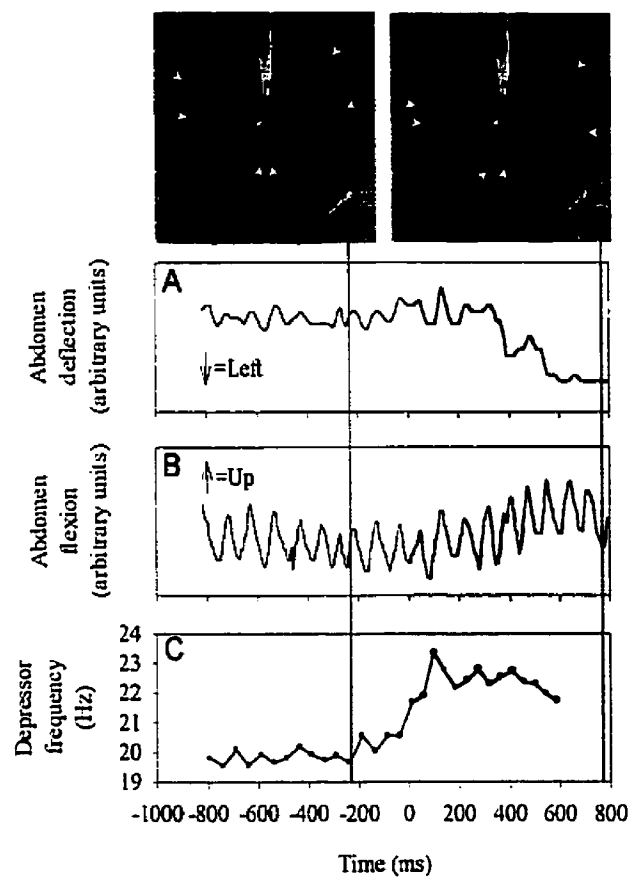
any of the experiments in this study. From EMG recordings, we also saw shifts in

depressor muscle timing coincident with changes in wing kinematics. A plot of wing

elevation angle as a function of time, for one locust, shows asymmetric depression of the

forewings and hindwings following stimulation (Fig. 3.4). Asymmetric wing depression

was more pronounced in the forewings (particularly evident in the last six cycles of the

forewings in the figure) than the hindwings. In this trace, stroke amplitude did not

change substantially for either wing but the range of angles through which the wings

swept changed. The right forewing was more depressed than the left forewing after

stimulation and we interpret this as a fictive turn to the right. The change in forewing

asymmetry was accompanied by asymmetries in the times of left and right depressor

muscle activation in M97, the forewing first basalar. The forewing second basalar, M98,

did not show any appreciable changes in this trace.

In this study, we recorded responses to bat-like sounds presented from 90 ° left at

88 dB from 24 locusts. From those 24, 19 showed fictive turns to the left, three showed

fictive turns to the right, and for the remaining two locusts, turn direction could not be

determined with certainty (Table 3.2). Turn direction was determined by looking at a

number of behaviours: direction of abdomen deflection, forewing asymmetry measured

at one third downstroke, depressor asymmetry and angular difference asymmetry. In all

but one locust there was consensus between all behaviours.

153

Figure 3.4. Plot of forewing and hindwing angles with accompanying spike times of M97 and M98 from one locust (#D17L1R1). The left and right forewing stroke angles become asymmetric during the downstroke approximately 200 ms after stimulus onset. This is accompanied by asymmetric activation of the forewing first basalar, M97 but not the forewing second basalar. M98. Asymmetries in hindwing angles during the downstroke are not as pronounced as for the forewings. Note the increase in wing beat frequency after the stimulus. Blue traces are the left wing, red traces are the right wing.

Forewing angle (°)

Hindwing angle (°)

M97
M98

90° left, 80 dB

Time (ms)
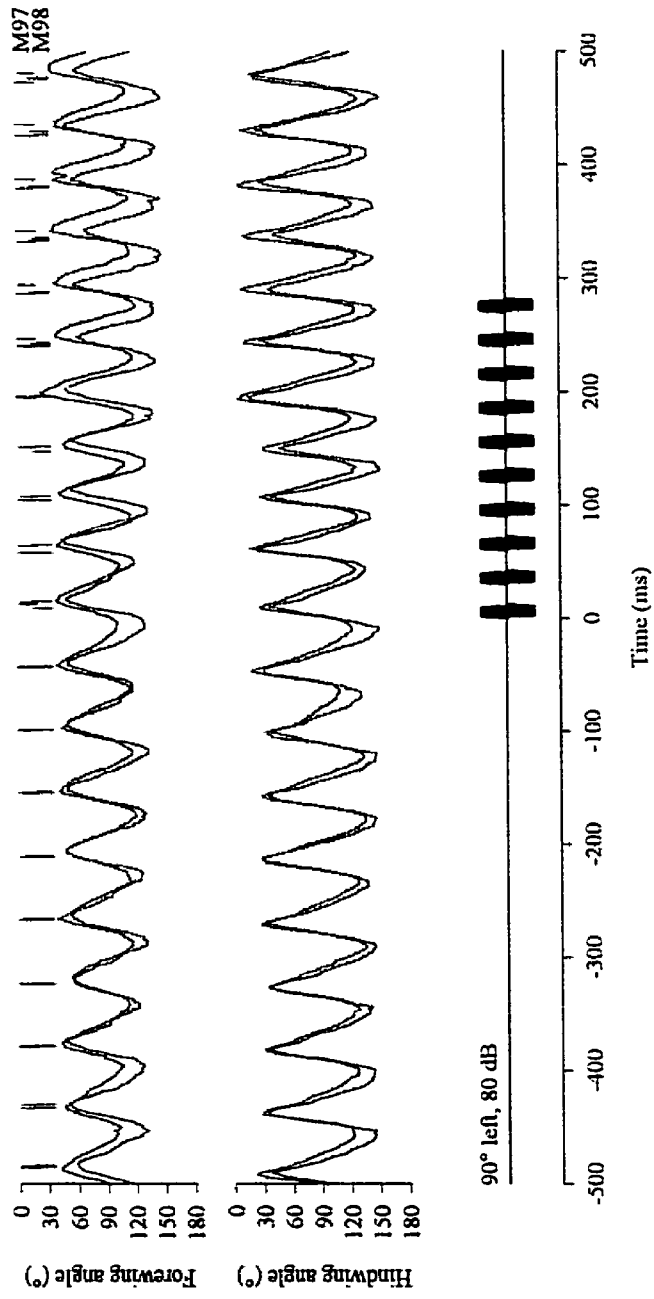
Table 3.2: Summary of correspondence between direction of abdomen deflection, sign of forewing asymmetry during the downstroke, sign of forewing depressor asymmetry, and sign of angular difference asymmetry. All locusts were stimulated with 30 kHz bat-like sounds (see text) at 88 dB from 90 ° to the left. Of 24 locusts included in the data set, 19 locusts fictively turned left, three fictively turned right (yellow), and two could not be determined (blue). The latter two were included in the data set because other aspects of the motor pattern, (e.g. abdomen flexion, increases in depressor frequency, changes in depressor spiking), suggested that they reacted to the stimulus. Only one locust (22), produced contradictory information (abdomen deflection to the right with positive depressor asymmetry but negative forewing asymmetry during the downstroke and negative angular difference asymmetry). (+) indicates a positive shift in the variable, (-) indicates a negative shift in the variable (see Table 3.1 for further explanation).

Locust	Abdomen Deflection	Abdomen Flexion	FWA-DS	DA[1]	ADA
9	Left	Up	-	-	-
10	Left	Up	-		-
11	Left	Up	-	-	-
12	N/C	Up	N/C	N/C	N/C
13	Left	Up	-	-	-
15	Right	Up	+	+	+
16	Left	Up	N/C	-	N/C
17	Left	Up	-	-	-
18	Left	Down	-	-	-
19	Left	Up	-		-
20	N/C	N/C	-	-	-
21	N/C	Up	-	-	-
22	Right	Up			
24	N/C	Up	-	-	-
25	Left	Up	-	-	-
26	Left	Up	-		-
27	Left	Up	-	-	-
28	Left	Up	-		-
29	Right	Up	+	N/C	+
30	Left	Up	-	-	-
31	N/C	Up	+	+	N/C
32	Left	Up	-	-	-
33	Left	Up	-	-	-
34	Left	Up	-	-	-

[1] DA is measured from forewing depressors, usually M97; empty cells indicate no forewing depressor activity was recorded for that locust.

[2] N/C refers to situations where we could not discern a clear change in the post-stimulus response from the pre-stimulus condition.

155

Figure 3.5. Latencies of components of the ASR elicited with 30 kHz bat-like sounds
presented at 88 dB from 90 ° left. The first evidence of steering occurred as shifts
in depressor asymmetry (and spikes per burst) (median = 80.6 ms) and
asymmetric forewing depression (median = 82.4 ms), which were followed by
abdomen dorsiflexion (median = 278.1 ms) and abdomen deflection (median =
316.2 ms).
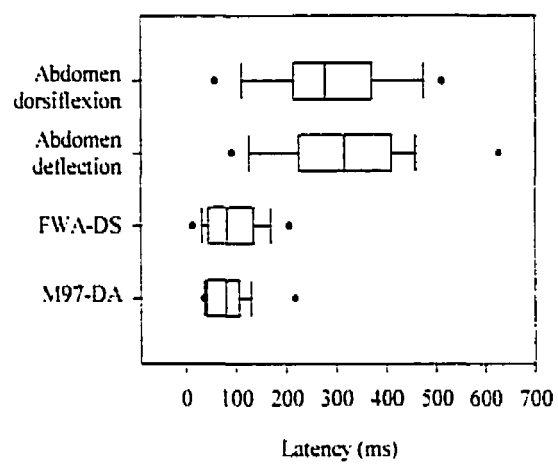
Latency (ms)

3.4.1 Stimulus Angle, Intensity and Latency to Response

We determined latencies to key elements of the ASR described above (Fig. 3.5). From 15 locusts that provided forewing first basalar (M97) records, we found that shifts in M97 (median = 80.6 ms, 1st & 3rd quartiles = 46.0 ms, 104.8 ms respectively) were coincident with shifts in forewing asymmetry (median = 82.4 ms, 1st & 3rd quartiles = 45.3 ms, 134.9 ms), but substantially preceded abdominal dorsiflexion (median = 278.1 ms, 1st & 3rd quartiles = 220.4 ms, 365.6 ms), and abdomen deflection (median = 316.2 ms, 1st & 3rd quartiles = 226.6 ms, 406.8 ms).

From a sample of 19 locusts (different animals from the 24 mentioned above), we tested the effect of stimulus intensity (N=6) and direction (N=13) on the magnitude and direction of forewing M97 activity. The magnitude of depressor shifts increased with increasing intensity of the stimulus (Fig. 3.6A). Depressor asymmetry shifts, without regard to direction, elicited with 60 and 65 dB sounds were 3.1 ± 1.1 ms (mean $\pm$ S.E.M.) and were significantly different than shifts elicited with 70 dB sounds and higher which were 7.5 ± 1.1 ms (ANOVA of treatments by subjects, F=5.75, df=5,5, P=0.006; means compared post-hoc with multiple Student t-tests, P<0.05). Depressor frequency (Fig. 3.6B) increased for all locusts tested by 2.0 ± 0.4 Hz from 17.1 ± 0.7 Hz after stimulation but the magnitude of the increase was not dependent on stimulus intensity (ANOVA of treatments by subjects, F=1.02, df=5,5, P=0.431). The magnitude of M97 depressor shifts, elicited with an 80 dB stimulus, did not depend on stimulus angle (Fig. 3.6C) (ANOVA of treatments by subjects, F=0.51, df=12,3 P=0.683) nor was the number of

Figure 3.6. (A) Forewing first basalar (M97) depressor asymmetry shifts (post-stimulus
peak minus pre-stimulus median value, calculated irrespective of direction)
increased with increasing stimulus intensity. Data are means ± standard error.
Horizontal bars indicate significant differences (N=6 for all intensities except 65
and 70 dB where N=5). (B) Depressor frequency increased between 2 Hz after
stimulation but the increase was not dependent on stimulus intensity (data from
the same locusts as above: sample sizes as above). (C) M97 depressor asymmetry
shifts, a measure of steering direction, were not dependent on the position of the
speaker when placed at different angles beside and behind the locust. Zero
degrees is directly in front of the locust, 90 degrees is left of the locust. Data from
10 locusts at 90 °, 8 locusts at 135 °, 7 locusts at 225 °, and 10 locusts at 270 °.
Values are means ± standard error. (D) The number of locusts showing positive
or negative depressor asymmetries (i.e. making fictive turns to the right or left
respectively) was not contingent on speaker position (data from the same locusts
in C).
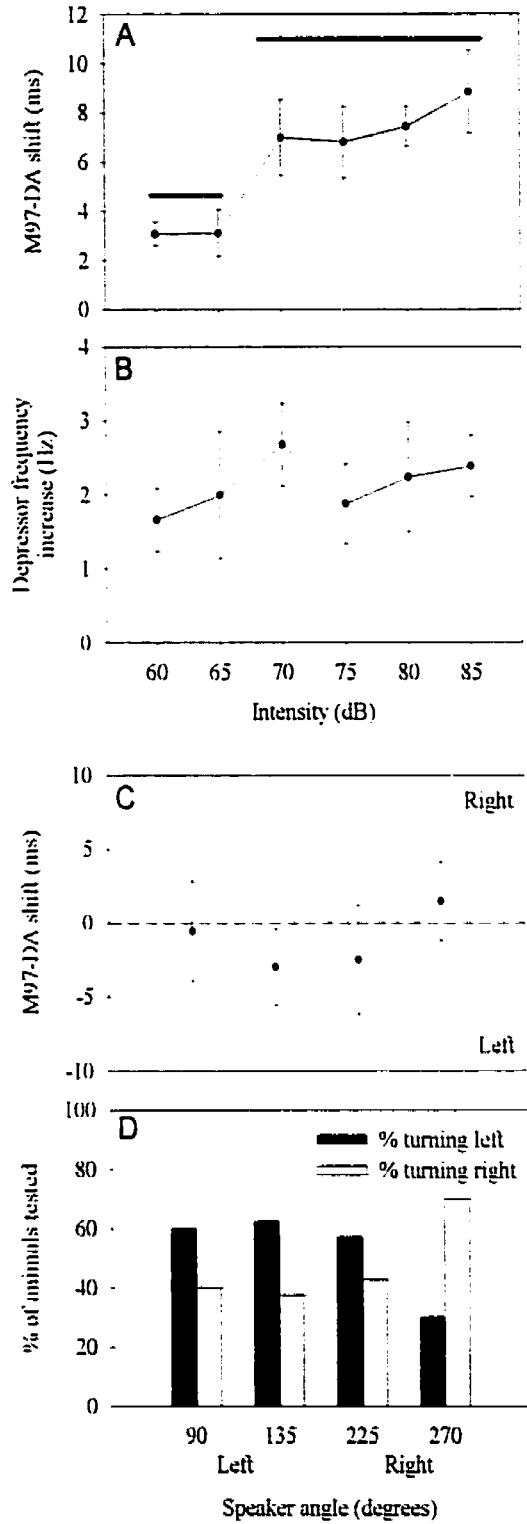
animals that turned left or right (Fig. 3.6D) (Contingency table G-test, G=2.668, df=3, P=0.431)

.3.4.2 Motor Pattern

We implanted EMG electrodes into the forewing first and second basalars, M97 and M98 respectively, and the subalars, M99, and the hindwing first and second basalars, M127 and M128 respectively, and the subalars, M129. From the 24 locusts stimulated with bat-like sounds. we obtained successful EMG recordings from 15 M97 left- right pairs, 6 M98 pairs. 17 M99 pairs, 21 M127 pairs. 15 M128 pairs, and 22 M129 pairs. Only two animals yielded data for all six muscle pairs. We typically obtained data from at least four muscle pairs for a given locust. A recording from one of the two locusts in which all twelve direct downstroke muscles were successfully penetrated is presented in figure 3.7.

A conspicuous feature of many of our recorded motor patterns was that spiking was often absent for many cycles in forewing M99, and to a lesser extent M98. and hindwing M128. Spiking in all muscles would resume immediately after the stimulus was presented. This is seen in figure 3.7 for M98. M99 and M128. Following presentation of the stimulus. several changes occurred in the motor pattern. Muscles that were not being rhythmically depolarized began to fire with every wing beat cycle. There was an increase in the number of depolarizations seen with each cycle (hereafter referred to as spikes per burst). The frequency of depressor muscle activation increased and the timing of depolarizations advanced in the cycle relative to the hindwing stroke reversal.

159

Figure 3.7. EMG recordings from one locust (L33R1) from 12 direct downstroke
muscles. six in the forewings and six in the hindwings, three per side for each
wing. Before the stimulus M98 and M99 fire intermittently but when firing the
spikes are in time with the wing cycle. After the stimulus, all muscles are active
and there is an increase in number of depolarizations per cycle (spikes per burst)
for all of the muscles that last for several cycles post-stimulus. Yellow area is
presented on an expanded time scale at the right to show asymmetries in
contralateral depressor muscle pairs. Forewing depressor muscles typically show
larger shifts than hindwing depressors. The stimulus is a 30 kHz bat-like sound
presented from 90 ° left of the locust at 88 dB SPL (additional details in text).

M97   L   R
M98   L   R
M99   L   R
M127   L   R
M128   L   R
M129   L   R

Stimulus

100 ms
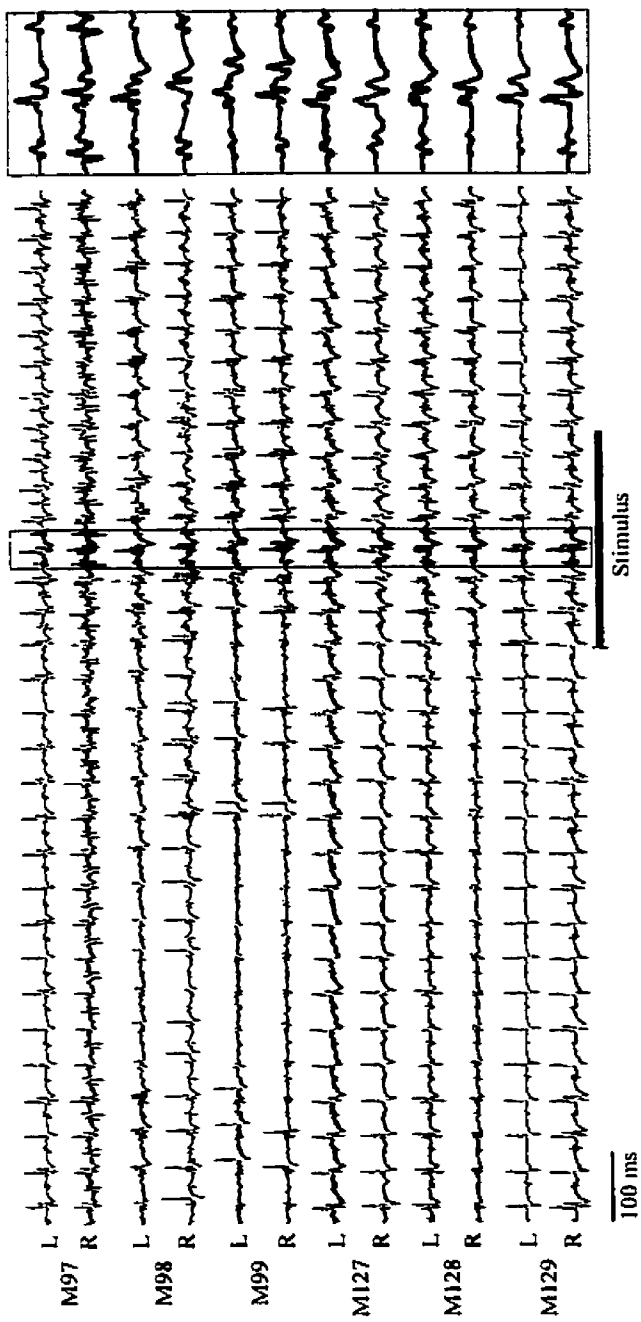
Lastly, depolarization of contralateral muscle pairs became asymmetric (more asymmetric) (boxed region of Fig. 3.7).

3.4.3 Spikes Per Burst

Before a stimulus was applied, depolarizations of the muscles occurred as singlet or doublet waveforms (spikes). We observed an increase in the number of spikes per burst in all depressor muscles on both the left and right side of the locust. The increase occurred irrespective of the direction of the fictive turn. Close examination of locusts that fictively turned to the left confirms this (Fig. 3.8). Significant increases were found in all depressor muscles (paired t-tests. $P<0.05$) on both the left and right side of the locust. The increase in spikes per burst was seen even when abdomen deflection and forewing asymmetry were not apparent (e.g. Locust 12, Table 3.2).

3.4.4 Shifts Relative to the Hindwing Stroke Reversal

From the 19 animals that fictively turned left, we calculated the time of activation of each muscle relative to the hindwing stroke reversal (Fig. 3.9). The hindwing stroke reversal time was calculated by taking the average of the left and right wing stroke reversal times. We chose the hindwing stroke reversal as a reference of cycle time because asymmetries in the hindwing stroke reversals are not as pronounced as they are for the forewings during steering. There was considerable variability in activation times of all the muscles before the stimulus was presented and depolarizations of contralateral muscle pairs often did not fire synchronously as might be expected for straight flight. From the medians of

Figure 3.8. The number of depolarizations per cycle (spikes per burst) increased after stimulation on both the left and right sides in locusts. Data above are for locusts turning left only. For each locust, the median number of spikes per burst was calculated for the pre- stimulus and post-stimulus period and the mean $\pm$ S.D. of the medians are plotted. Sample sizes are indicated in parentheses. Asterisks (*) indicate significant differences (paired t-tests, P<0.05). Diagram in the centre shows the relative location of the forewing and hindwing direct downstroke muscles within the left side of the pterothorax of the locust (dorsal view) (modified from Möhl and Zarnack, 1977).

Left

Right

Spikes Per Burst

M97 * (N=13)

M98 * (N=5)

M99 * (N=10)

Pre- Post-

Spikes Per Burst

M127 * (N=17)

M128 * (N=10)

M129 * (N=17)

Pre- Post-

M97 * (N=13)

M98 * (N=3)

M99 * (N=10)

Pre- Post-

Spikes Per Burst

M127 * (N=17)

M128 * (N=9)

M129 * (N=17)

Pre- Post-

Spikes Per Burst

162
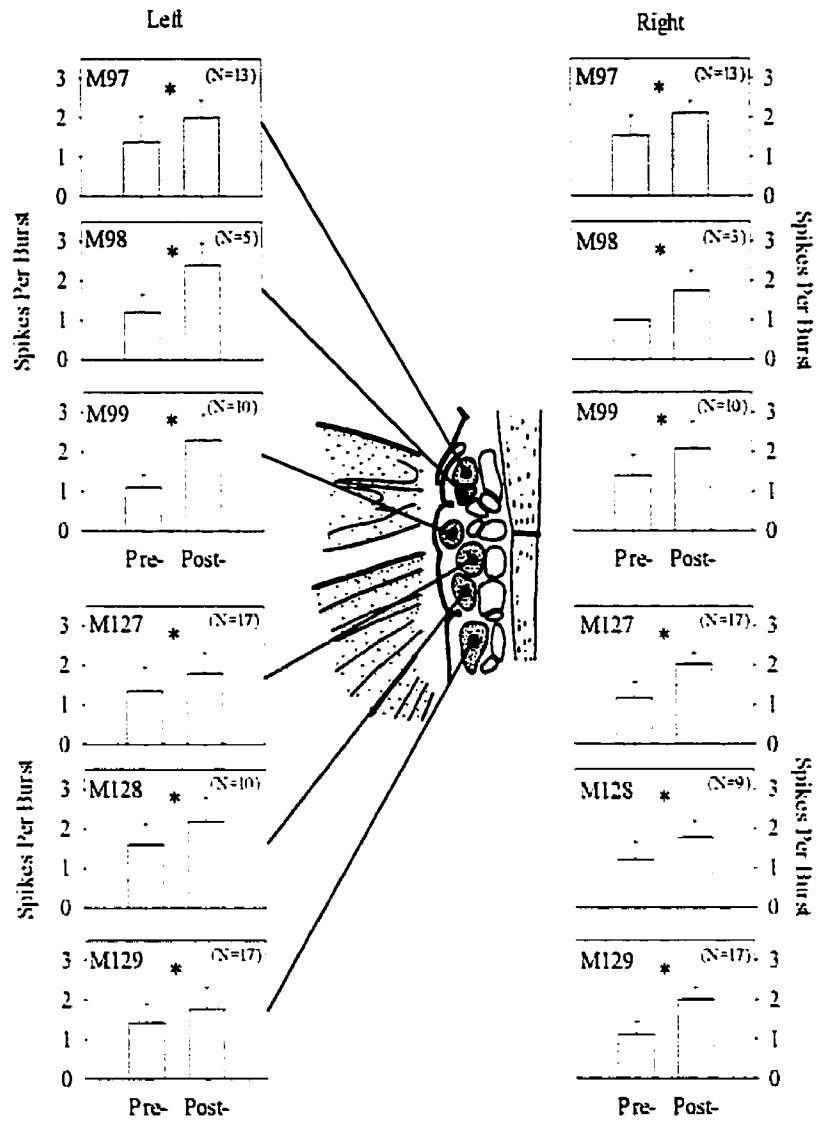
Figure 3.9. Spike times of the forewing and hindwing direct downstroke muscles calculated relative to the time of the hindwing stroke reversal (normalized to a 50 ms wing beat period). Data are taken from locusts turning to the left only. All muscles fired earlier in the stroke when presented with a 30 kHz bat-like sound from 90 ° to the left at 88 dB SPL. For each muscle, we calculated the latency of each depolarization (on a cycle by cycle basis) to the hindwing stroke reversal (median of left and right stroke reversal). These latencies were then normalized to a 50 ms cycle period. The median of all the pre-stimulus latencies was then calculated as was the median of all the post-stimulus latencies. These values were determined for all 19 locusts tested that turned left (as determined by consensus of direction of abdomen deflection. wing asymmetry. and depressor asymmetry) and were plotted as separate boxplots for the pre-stimulus (white boxes) and post-stimulus (shaded boxes) period. We chose the hindwing stroke reversal because the hindwings show smaller shifts during steering than the forewings and therefore represented a stable marker of the time of each wing beat cycle. Boxplots show the first and third quartiles (box) with the median indicated by the line within the box. Whiskers show 10th and 90th percentiles and dots show data outside of the aforementioned percentiles.

R M129 ·
L M129 ·
R M128 ·
L M128 ·
R M127 ·
L M127 ·
R M99 ·
L M99 ·
R M98 ·
L M98 ·
R M97 ·
L M97 ·

Pre-
Post-

-20 -15 -10 -5   0   5   10   15   20

Latency to hindwing stroke reversal (ms)
(data normalized to 50 ms cycle period)
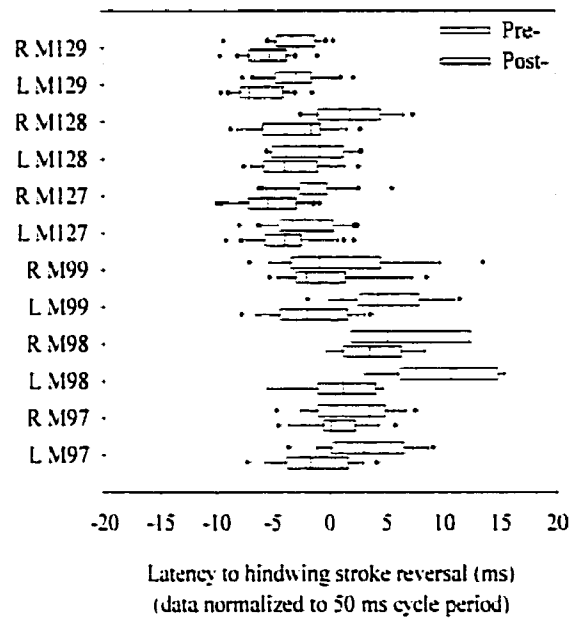
the spike times, the first basalars and subalars were depolarized before the second

basalars for the forewings and hindwings. Following stimulation, this pattern persisted

but all muscles shifted and fired earlier in the stroke. We also noted that all muscles

except M127 showed a greater shift of the left muscle relative to the right; this is

explored in more detail below.

3.4.5 Shifts of Fore- and Hindwing Contralateral Muscle Pairs

Asymmetries in contralateral depressor muscle activations were abrupt with the onset of

the stimulus and often appeared within the first three post-stimulus wing beat cycles.

Depressor asymmetries in all forewing muscles, M97, M98, and M99, shifted together

whereas hindwing M127 and M128 depressor asymmetry shifted opposite to M129.

Figure 3.10A, F show the time course of shifts in forewing and hindwing muscles for one

locust, L33R1. Some locusts showed an initial shift in one direction followed by a

prolonged shift in the opposite direction (see Fig. 3.10A and note the initial positive shift

in M98 followed by a prolonged negative shift). From the 19 locusts that turned left, we

calculated the magnitude of depressor shifts by taking the average (across locusts) of the

differences between median post-stimulus and median pre-stimulus depressor

asymmetries (for each locust) calculated on a cycle by cycle basis. The magnitude of

shifts in forewing M97 was smallest at 6.62 ± 0.54 ms (mean $\pm$ S.E.M.) in comparison to

the other forewing muscles and was the least variable. M98 shifts were 9.26 ± 2.08 ms

and shifts in M99 were 12.05 ± 2.05 ms. Shifts in hindwing depressor asymmetry were

164

Figure 3.10. Time-course of forewing and hindwing motor pattern and kinematic
changes for one locust (L33R1) following stimulation with a 30 kHz bat-like
sound at 88 dB SPL from 90 ° to the left (Yellow region). Depressor
asymmetries for all three forewing muscles shift to the left (negative) (A). The
onset of depressor shifts are coincident with shifts in all the forewing kinematic
variables measured. Forewing asymmetry measured at one third of the
downstroke (B) showed an initial shift to the right (positive) followed by a large
and prolonged shift to the left. This is also seen in C, E and K. Hindwing
depressor asymmetries are less pronounced than for the forewings (note the
different ordinant scale in F). M127 and M128 depressor muscles shift to the
right (positive) while M129 shifts to the left (negative). The coincident changes
in hindwing kinematic variables is less obvious: hindwing shifts are small in
comparison to forewing shifts. Angular difference asymmetry (K): a measure of
the relative separation between forewings and hindwings on the left and right
shows that a greater fore- hindwing gap forms on left side of the locust. Because
the hindwing shifts are small, this gap is mostly created by asymmetric forewing
depression.

Forewings

Hindwings
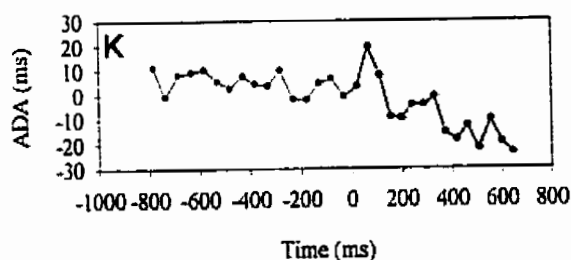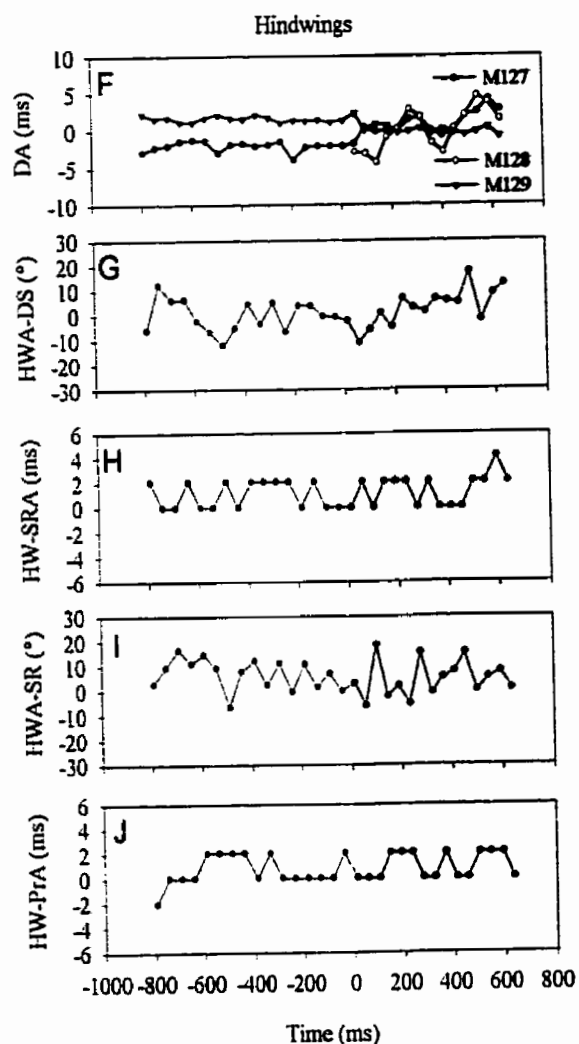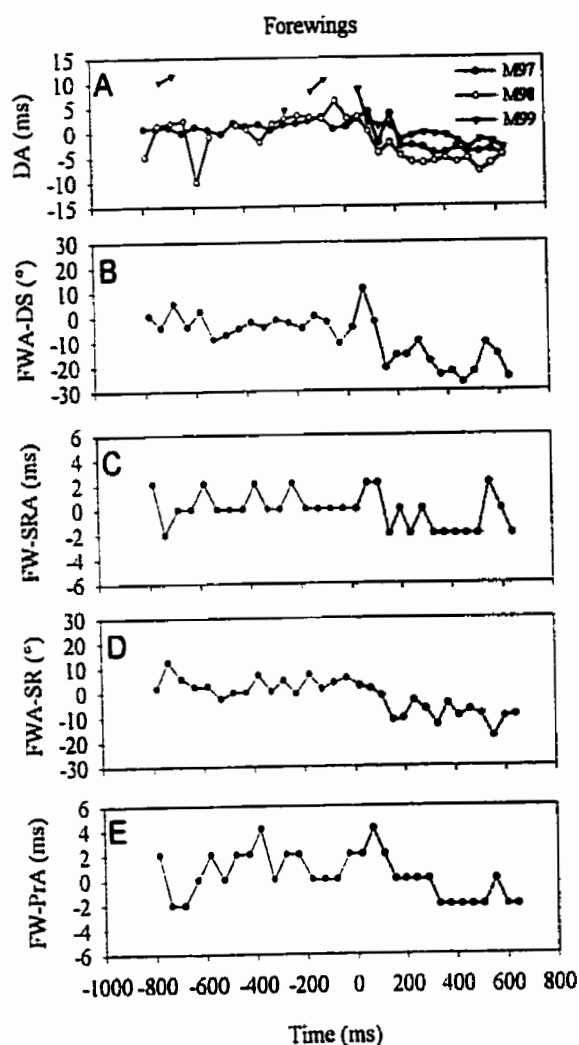
smaller than shifts in forewing depressor asymmetry. M127 shifts were 3.79 ± 0.52 ms,

shifts in M128 were 5.46 ± 0.91 ms and shifts in M129 were 2.15 ± 0.54 ms.

3.4.6 Correlation of Motor Activity and Wing Kinematics

Asymmetries in all forewing kinematic variables shifted in time with and in the same

direction as asymmetries in the forewing muscles at the onset of the stimulus (Fig. 3.10B-

E). Corresponding shifts in hindwing kinematic variables either did not occur or were

not as pronounced as those in the forewings (Fig. 3.10G-J). When hindwing shifts were

present they occurred in the opposite direction than those of the forewings (e.g. hindwing

asymmetry measured at one-third downstroke, Fig. 3.10G compared with Fig3.10B).

Shifts in angular difference asymmetry are plotted in Fig. 3.10K.

Due to high cycle to cycle variability in depressor activity and wing kinematic

measurements, we performed pairwise correlations between motor and kinematic

variables for each locust on a cycle-by-cycle basis. This is in contrast to a method that

would take averages of shifts over a number of cycles and then perform correlations on

the average shifts across all locusts. We feel the former method gives a more accurate

impression of steering because the cycle-to-cycle variability, which may be an important

aspect of motor control, is preserved in the analysis. A scatter plot matrix of all pairwise

combinations of depressor muscle activity and wing kinematic measurements is shown

for the forewings (Fig. 3.11A) and the hindwings (Fig. 3.11B) for one locust, L33R1.

The strongest correlations were found between forewing first basalar activity (M97) and

Figure 3.11. Scattergrams of all pairwise correlations for one animal (L33R1) of depressor asymmetry and kinematic changes for the forewings (A) and hindwings (B). Each X-Y pair in the scatterplots is from a single wingstroke cycle. Blue dots are from cycles before the stimulus, red dots are from cycles after the stimulus (note in some scatterplots the post-stimulus data is plotted on top of the pre-stimulus data). Ellipses are 95% density regions of the correlation (enclosing 95% of the data within the scatter plot). The greater the ratio of the major to minor axis lengths, the stronger the correlation. Asterisks (*) indicate significant correlations (Pearson Product Moment, $P<0.05$).

A
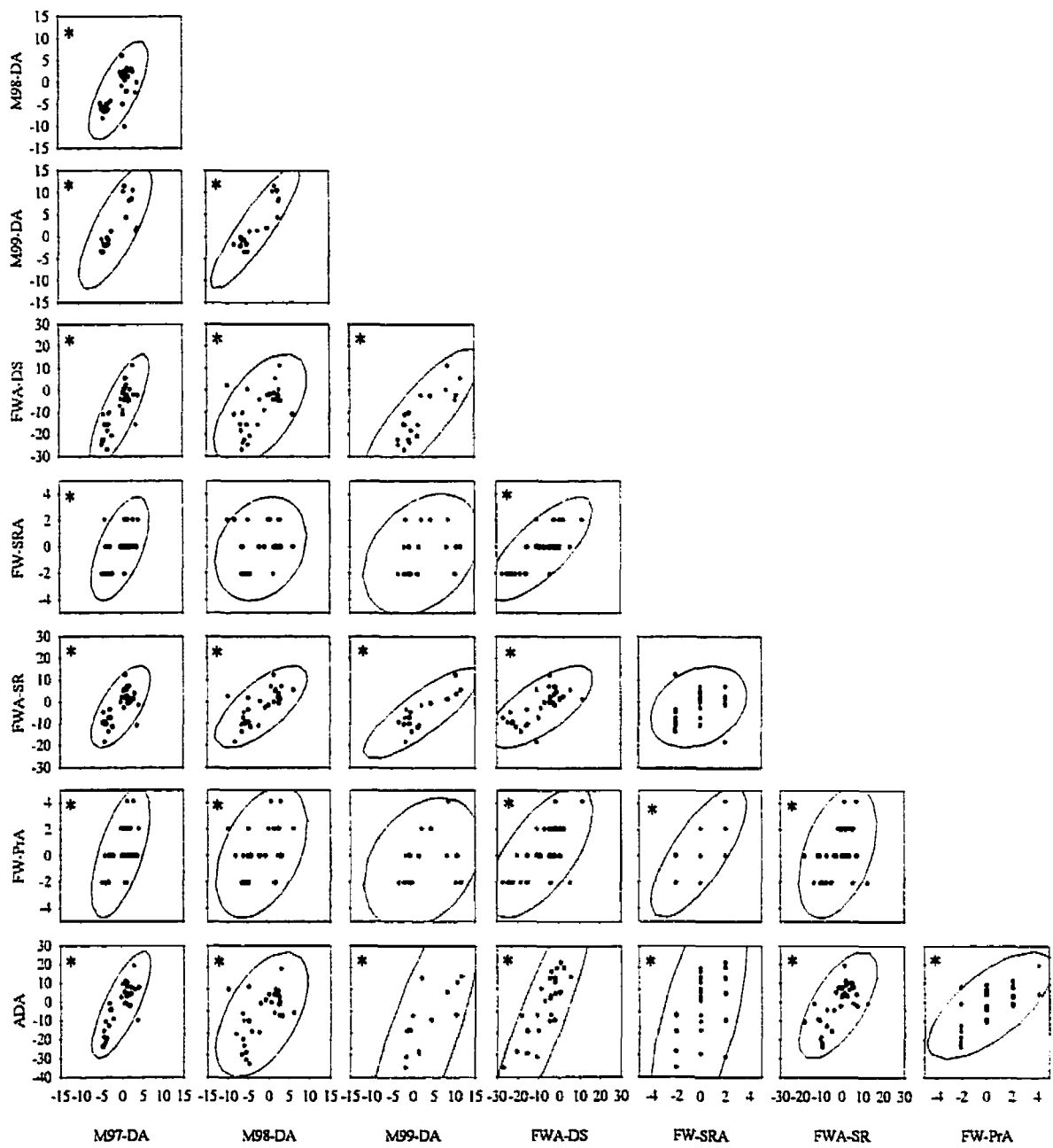
B
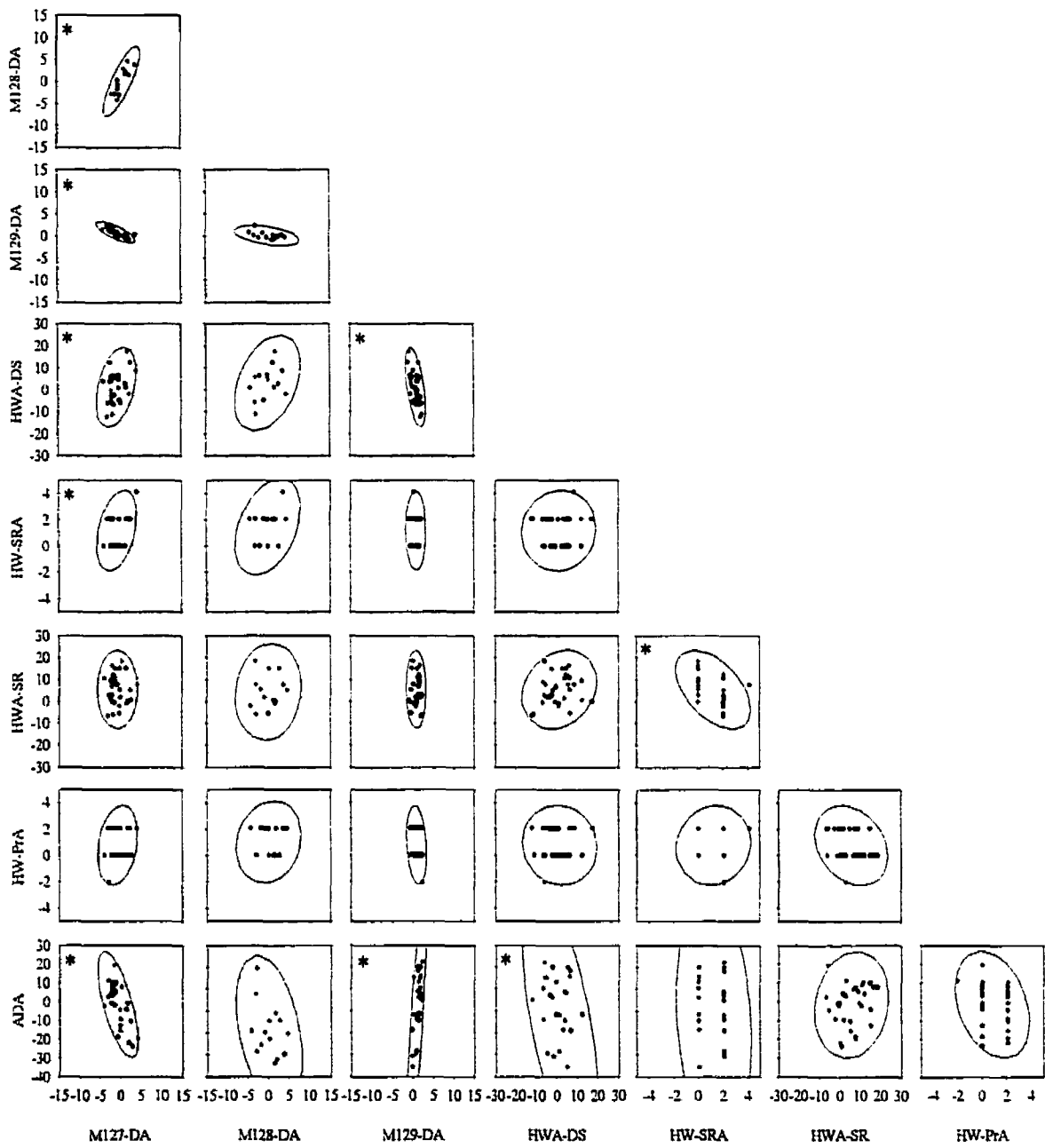
the other variables and also between angular difference asymmetry and the other

variables. This also applies to hindwing first basalar activity and angular difference

asymmetry; however, correlations of the other hindwing variables were, as expected, less

strong and were frequently not-significant.

Individual correlations were generated for all combinations of depressor and wing

kinematic measurements for each of the 24 locusts tested (regardless of steering direction

and magnitude of response). For each variable pair, we then tallied the number of

significant positive and significant negative correlations. From only the significant

correlations, we determined which variables (muscle shifts and wing kinematic shifts)

tended to shift together (positive correlations) or oppositely (negative correlations). The

data from all 24 locusts are summarized in Table 3.3 including the number of locusts that

yielded data for each pairwise combination and the number of non-significant

correlations. By generating all pairwise correlations for motor and wing kinematic

parameters for each locust individually and then summing, across locusts, the number of

significant and non-significant correlations, we have controlled for spurious significant

correlations (see Rice, 1989) that might affect our overall conclusions. Further, the

tallied correlations in Table 3.3 were tested with a Binomial test to determine which

correlations are significant more often than by chance alone.

When examining this table it is important to look at the relative numbers of

positive and negative correlations and not the number of significant correlations relative

to non-significant correlations. This is because non-significant correlations could have

resulted from two factors: that there was no biological relationship (i.e. no shift in the

Table 3.3: Summary of multiple pairwise correlations (Pearson Product Moment) between muscle shifts and measured wing kinematic variables. Values in the cells are the number of significant (P<0.05) positive (+) correlations, significant negative (-) correlations, non-significant (N/C) correlations and the number of animals examined. Asterisks (*) in the cells indicate significantly more positive than negative or significantly more negative than positive correlations than expected by chance (Binomial test. P<0.05). The colour of the cells reflects situations (not statistical significance) where there are more positive than negative correlations (green) or more negative than positive correlations (red); only cells with two or more significant correlations are coloured.

	M97-DA	M98-DA	M99-DA	FWA-DS	FW-SRA	FWA-SR	FW-PrA	M127-DA	M128-DA	M129-DA	HWA-DS	HW-SRA	HWA-SR	HW-PrA
M96-DA														* N/C N
M99-DA														* N/C N
FWA-DS														* N/C N
FW-SRA														* N/C N
FWA-SR														* N/C N
FW-PrA														* N/C N
M127-DA														* N/C N
M128-DA		* 3 4												* N/C N
M129-DA		* 5 6						11 12						* N/C N
HWA-DS		* 5 6												* N/C N
HW-SRA	14 15	* 6 6	14 16	1 23 24	18 24	21 24	24 24							* N/C N
HWA-SR		* 6 6												* N/C N
HW-PrA	* 14 15	* 5 6	14 16		18 24	21 24	24 24		21 22			24 24		* N/C N
ADA									15 15			22 24		

variables), or that the steering response was small (i.e. that the shift in one or both

variables was small). For example, the correlation between forewing asymmetry at one

third downstroke and M99 depressor shifts was calculated for 16 animals. Of the 16

correlations, 7 were positive, none were negative and 9 were non significant. We believe

that it would be wrong to conclude that M99 does not shift with forewing asymmetry

because there were more non-significant correlations than significant correlations, or that

less than half the correlations were significant. Some of the non-significant correlations

resulted from small shifts that were not statistically detectable. To be conservative, we

decided not to try to distinguish between non-significant correlations resulting from small

shifts (which are biologically significant) and non-significant correlations resulting from

a lack of biological relationship. In our example, the meaningful interpretation comes in

noting that 7 out of 7 significant correlations were positive (i.e. that forewing asymmetry

and M99 depressor asymmetry shifts in the same direction)

The forewing muscles, M97, M98, and M99 were all positively correlated (shifted

together) but were negatively correlated with shifts in M127 and M128 of the hindwings.

Shifts in hindwing M129 were positively correlated with shifts in the forewing muscles.

All forewing kinematic variables shifted with the forewing depressor muscles and shifted

with angular difference asymmetry. The exception is that forewing asymmetry at the

stroke reversal and forewing asymmetry sometimes shifted together and sometimes not.

Shifts in hindwing muscles and hindwing kinematic variables were less clear

because there was a higher proportion of non-significant correlations. However,

hindwing M127 and M128 shifted together but opposite to M129. Hindwing kinematic

variables shifted with M128 and M128 but opposite to angular difference asymmetry.

The frequent observation that the hindwings shift opposite to the forewings is

reflected in the correlation between hindwing asymmetry and forewing asymmetry

measured at one-third downstroke; 12 of 13 significant correlations were negative.

3.4.7 Timing of the (Dorsal) Wing Flip

At the end of each half-stroke, the wing must rapidly rotate to establish an appropriate

angle of attack for translation. We measured the time of the onset of rotation at the

initiation of the downstroke, which we call the dorsal wing flip following terminology

from Dickinson *et al.* (1993), and calculated the latency to the stroke reversal for each

wing (Fig. 3.12). In 11 of the 19 locusts that turned left, we found changes in the

latencies of the left and/or right forewings that were coincident with the onset of the

stimulus. For some locusts, changes occurred in both left and right forewings while in

others, changes occurred in only one wing. Four of seven locusts showed an increase in

latency in the right wing, and six of eight locusts showed a decrease in latency in the left

wing. Changes in latency were between 2 and 6 ms. We did not see changes in hindwing

latencies. Asymmetries in the timing of the forewing wing flip were significantly

correlated with forewing first basalar (M97) depressor asymmetry (11 of 12 significant

correlations were positive), forewing asymmetry measured at one-third downstroke (14

of 14 significant correlations were positive) and with forewing stroke reversal asymmetry

(22 of 22 significant correlations were positive) (Table 3.3).

171

Figure 3.12. (A) Just before the beginning of the downstroke, the forewing flips
(pronates) to establish the angle of attack for the downstroke (yellow region
illustrating period of left forewing flip). Throughout the downstroke the left wing
is more depressed than the right showing this locust (L13R1) fictively turned left
(segment starts 90 ms after stimulus onset). We note, however, this locust is odd
in that the right stroke reversal occurs before the left but add that the right wing
retards the initial moments of its descent (most obvious in the first stroke reversal
shown). Because of this, the right wing flip occurs before the left when normally,
for left turns, the left wing flips before the right. Regardless, the left wing is
pronated more than the right which is consistent with a turn to the left. The angle
of attack was approximately −4.4 ° during the downstroke and was between 40 °
and 50 ° before the flip began. The peak of the instantaneous velocity of rotation,
which is between 100 and 250 °/s, occurs just before the downstroke beings. (B)
The wing on the outside of the turn flips earlier relative to its stroke reversal than
the wing on the inside of the turn path. This data, from a different locust (L34R1)
than above, fictively turned to the left and shows that the right wing flip is
advanced in the cycle relative to the left. The changes in latency to the stroke
reversal for the left and right forewings is coincident with the development of
forewing asymmetry.

A
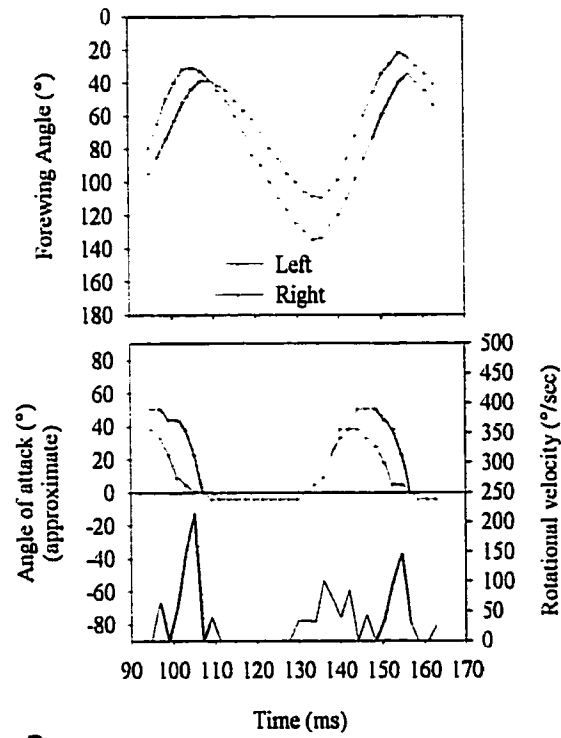
B
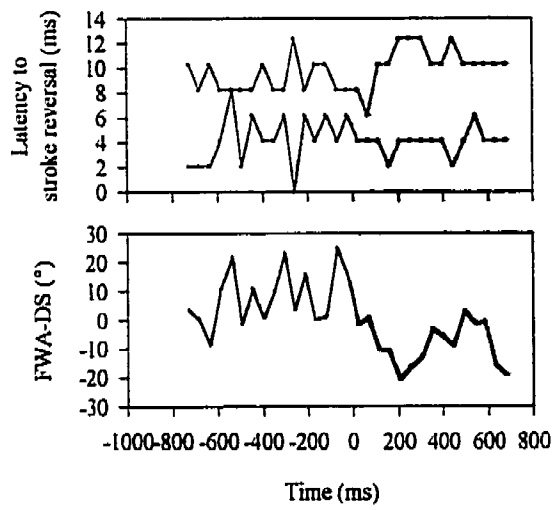
3.5 Discussion

The function of the locust acoustic startle response is to move the animal away from the

source of sound that elicited the reaction. The reactions of our locusts are consistent with

observations from Robert (1989) and Dawson et al. (1997). In response to stimulation

with bat-like sounds, we saw abdomen deflection and dorsiflexion, increases in depressor

(wing beat) frequency, asymmetric wing depression in the forewings and hindwings, and

changes in depressor muscle activity. Changes in abdomen deflection (Camhi, 1970) are

correlated with steering (both intentional and corrective) and their role, with abdomen

dorsiflexion and changes in depressor (wing beat) frequency, have been addressed in a

previous paper (Dawson et al.,1997). We will therefore limit our discussion to the topic

of forewing asymmetry and its underlying motor control as well as the possible

significance of our observations of the dorsal wing flip. The dorsal wing flip has not

previously been described in locusts.

3.5.1 Stimulus Angle, Intensity, and Latency to Response

Acoustic startle/escape responses in insects elicited with bat-like sounds are evoked with

behaviourally relevant sound intensities and consist of short latency, stereotyped

behaviours that are often directional (Hoy, et al., 1989). Reactions in our locusts, first

evident as shifts in depressor asymmetry, occurred with a median latency of 80.6 ms

which corresponds to as few as two wing beat cycles after stimulus onset. The magnitude

of depressor shifts was intensity dependent and seemed to show a discrete (step-wise)

increase for intensities 70 dB and greater. Robert (1989), looking at abdomen deflection,

found similar results wherein the magnitude of abdomen deflection increased with

increasing sound intensity to 65 dB but plateaued at stimuli 70 dB and higher. We found

that responses elicited with 80 dB sounds presented from different speaker positions were

non-directional. In moths, responses to ultrasound in free-flight are affected by intensity.

When stimulated with intense ultrasound, as would be produced when a bat is close,

moths show non-directional responses such as passive falling to the ground with closed

wings, power dives to the ground, spiralling flights to the ground, loops in the air, and

abrupt climbs. When the ultrasound stimulus is less intense. as would be the case if a bat

were farther away, responses consist more of turns to change the flight path away from

the source of the sounds (Roeder, 1962; 1974). Roeder suggested that the diversity of

manoeuvres observed to intense ultrasound. which he found difficult to categorize in any

systematic way, might be adaptive in that it would increase the difficulty a bat has in

tracking the target. Although the idea that the ASR in *Locusta migratoria* functions in

bat-avoidance is under debate (Dawson. *et al.*. 2001). short latency. intensity dependent.

steering reactions would be adaptive to any unexpected sound encountered while in

flight.

The fact that the locusts used for motor patterns and kinematics turned

significantly more often to the left than to the right when stimulated with 88 dB sounds

from 90 ° to the left (Binomial test. P=0.05) is likely not a biological preference for

direction based solely on speaker position. In this part of our study it was unavoidably

necessary to use bright lights during filming and locusts strongly orient to light (Uvarov.

1977; Dawson *et al.*, 2001), therefore, shadows and other visual asymmetries present in

the flight chamber most likely have affected the direction of turns. This is not, however,

a limitation in our attempts to correlate motor patterns and wing kinematics during

intentional steering. The responses we observed were coordinated, stereotyped,

reactions involving wing movements, leg and abdomen movement, and muscle activity

and, as Table 3.2 clearly shows, there was agreement between these different indexes of

steering direction.

3.5.2 Motor Pattern and Wing Kinematics

To understand how flight muscle activity effects the changes in wing kinematics,

and presumably free-flight responses of acoustically startled locusts, we have returned to

the methods employed by numerous researchers nearly 25 years ago namely,

simultaneous multiple channel electromyography and wing position monitoring. During

this intensive period of research, large advances were made in understanding steering

responses by inducing changes in the three principle flight axes (yaw, roll, and pitch) and

recording corresponding changes in muscle activity and wing kinematics. The idea,

presumably, was that the isolated responses to yaw, roll and pitch represented the "parts"

of a system and the "sum" of these parts, in various proportions, would represent the

behaviour of the intact system operating in free-flight under different conditions. These

original studies isolated the principle flight axes by encouraging locusts to correctively

steer to resume a straight course of flight using a variety of stimuli (both visual and

mechanosensory) and a significant benefit of this approach is that locusts will sustain

corrective postures for many (tens to hundreds) of cycles allowing motor and kinematic

parameters to be averaged over a large number of cycles. Except perhaps for thermal

avoidance steering (Shoemaker and Robertson, 1998), intentional steering manoeuvres

such as those involving auditory (and visual-collision course) stimuli are very abrupt,

short duration dynamic responses lasting typically less than a dozen wing beat cycles.

Further, a major difference between intentional and corrective steering is in the extent of

involvement of postural adjustments. During correctional steering wing kinematic

changes alone are often adequate to bring about sufficient changes in flight forces to

restore straight flight; however, because leg and abdomen movements are routinely

observed during intentional steering, it is not unreasonable to suspect motor patterns and

wing movements to differ in magnitude or components from those seen during

correctional steering. With this said, however, parsimony would suggest similarities at a

most basic level.

The purpose of this paper was to determine if asymmetric depression of the

forewings was established by asymmetric timing of the stroke reversals at the start of the

downstroke. This model predicts that there should be strong correlations between

forewing asymmetry measured during the downstroke, stroke reversal asymmetry, and

forewing asymmetry at the stroke reversal. For our correlation analysis, we chose to

examine responses on a cycle by cycle basis as opposed to correlating values derived by

averaging a number of post- or pre-stimulus cycles. There is ample evidence that locusts

can adjust their wing movements on a cycle by cycle basis (Wendler, 1974; Burrows,

1975; Reye and Pearson, 1987; 1988) and for startle/escape responses it is not

unreasonable to suspect that flight forces change rapidly. Our responses were abrupt and

short duration and these effects were apparent in both wing kinematics and motor

patterns. This is consistent with observations of Möhl and Zarnack (1977) who noted

that responses to imposed roll could be generated within 1 to 2 cycles of stimulus onset.

Forewing asymmetry measured at one third downstroke was positively correlated

with forewing stroke reversal asymmetry (19 of 19 significant correlations were positive)

and forewing asymmetry at the stroke reversal (16 of 16 significant correlations were

positive). Further, we found strong correlations between these kinematics and depressor

muscle timing asymmetries for all forewing muscles (M97, M98, and M99). Similar

results were observed in the hindwing but we found fewer significant correlations.

Hindwing asymmetry measured at one third downstroke was positively correlated with

hindwing stroke reversal asymmetry (2 of 2 significant correlations were positive) and

hindwing asymmetry measured at the stroke reversal (2 of three significant correlations

were positive). Hindwing asymmetries were also positively correlated with M127 and

M128 depressor asymmetries. These results support our model that bulk shifts in

depressor timing is the motor pattern underlying asymmetric wing depression for both the

forewings and hindwings.

Asymmetries in the hindwings were less pronounced than in the forewings and

were opposite in direction (12 of 13 significant correlations were negative), so during a

left turn, the right hindwing was lower than the left hindwing. This is reflected in

correlations of hindwing first and second basalar activity with forewing motor or

kinematic parameters. This occurrence is supported by observations of correctional

steering to an artificial horizon (Thüring, 1986). Hindwing subalar activity (M129) is

positively correlated with forewing kinematics and muscle shifts suggesting its role is not

to affect the timing of the stroke reversal. The hindwing kinematics and negative

correlations between M129 shifts and M127 shifts are supported by observations of

Schmidt and Zarnack (1987). The fact M129 is advanced in the cycle on the inside of the

turn path, however, suggests that it might play a role in establishing or maintaining

hindwing angle of attack.

Although not empirically tested, forewing asymmetry could generate steering

torques by redirecting the balance of forces generated during forewing translation. A

potential mechanism for this has been adequately described in Dawson *et al.* (1997).

Asymmetric forewing depression is a prominent manoeuvre observed in other types of

intentional steering such as collision avoidance (Robertson and Reye, 1992) and thermal

avoidance (Robertson *et al.*, 1996) and has also been observed during correctional

steering (Baker, 1979; Thüring, 1986; Zarnack, 1988; Waldmann and Zarnack, 1988.)

Therefore, characterizing the underlying motor pattern is important for understanding

locust flight in general.

The most complete reconstruction of a motor pattern for intentional steering was

completed for thermal avoidance steering in locusts by recording from M97, M98, M99,

M127, and M85 (Shoemaker and Robertson, 1998). The motor pattern during straight

flight agreed with observations from Wilson and Weis-Fogh (1962) and consists of

hindwing M127 activity leading forewing M97, M99, and M98 activity in that order

(Shoemaker and Robertson, 1998). This sequence of muscles contractions is the same

order that we observed but we can add that the hindwing M129 activity follows M127 but

precedes M128 activity; all of which preceded forewing muscle depolarizations. After

stimulation, Shoemaker and Robertson (1998) found that the order of M97, 99, 98 stays

the same but that they fire earlier in the cycle (advance). Hindwing M127 shifts opposite

to M97 and recedes in the cycle. We found the same pattern of shift but add that M127

recedes and can become coincident with M97 timing. Further, the order of activation of

the hindwing muscles also stays the same so M129 activity follows M127 activity but

precedes M128 activity.

The depressor shifts that we observed share many similarities with observations

from Zarnack and Möhl (1977) for correctional steering. They found that locusts

oscillated in yaw, pitch and roll in a wind stream shifted the times of spiking relative to

the wing stroke cycle (calculated as the mean time of all active spikes for a cycle).

When locusts were rotated in yaw, they found that M97, M98, M127, and M128 shift

together and fire earlier on side on the inside of turn with M99 and M129 shifting

together and firing later on the side on the inside of the turn. When locusts were pitched,

they found that muscles on left and right side shift together with M97 and 98 advancing

in the cycle and M99 and 129 firing later. When locusts were rolled, they found that all

forewing muscles on the side opposite the roll direction shifted to fire earlier; the muscles

on the side opposite roll direction fired later. This was confirmed in a later study that

calculated timing relative to M129 as a marker of the hindwing stroke (Waldmann and

Zarnack, 1988). In our locusts, we found that all forewing muscles on the inside of the

turn fired earlier than their contralateral homologues which is similar to the above

observations of locusts in yaw and roll but not pitch.

During straight flight (pre-stimulus), when depressors were depolarized they

generally produced one or two spikes. After the stimulus, the number of spikes per burst

increased in all depressors. Because the different depressors are innervated by different

numbers of motor neurones (two neurones innervate M97, M99, M127, M128; one

neurone innervates M98, M129) it is difficult to determine if the increase is due to

increases in recruitment or increases in the number of activations of each unit or a

combination of both. Regardless, the number of spikes per burst is a measure of the

strength of muscle contraction. The increase occurred on both sides of the locust

regardless of turn direction and this is in contrast to observations by Shoemaker and

Robertson (1998) for thermal avoidance steering who described a "push-pull"

relationship between contralateral depressors with the depressors on the inside of the turn

increasing in spikes per burst and the depressors on the outside of the turn path

decreasing in spikes per burst. Other intentional steering studies have shown similar

results for M97 to what we found (Baker, 1979, Dugard, 1967, Waldron, 1967). Studies

of correctional steering have produced mixed results with respect to spikes per burst.

Möhl and Zarnack (1977) observed an increased burst length with the muscle that fires

earlier (on inside of turn). Others found that during roll, there was no change in burst

length (Waldmann and Zarnack, 1988; Schmidt and Zarnack, 1987; Thüring, 1986).

We believe that the functional consequences of the changes in wing movements

effected by the motor pattern we describe above is to produce roll torques in the direction

of the more depressed forewing. The accompanying changes in abdomen deflection and abdomen dorsiflexion would affect the direction (and magnitude) of these torques. Abdomen deflection, by increasing drag on the side to which it is deflected (Camhi, 1970) and by shifting the centre of mass of the locust (Zanker, 1988), would contribute a yaw torque to the roll produced by the wings. Further, abdomen dorsiflexion, which would shift the centre of mass anterior would produce a downward pitching torque. The net result of all these contributions would be to produce a banked downward turn or spiral. Locusts in free flight have been observed making such manoeuvres in response to startling sounds (Dawson, et al., 2001).

3.5.3 Timing of the (Dorsal) Wing Flip

We found strong, significant correlations between forewing depressor muscle asymmetries and asymmetries in the timing of the onset of forewing pronation before the downstroke (dorsal wing flip). We also found that 11 of the 19 locusts that turned left showed coincident changes in the latency of the wing flip to the forewing stroke reversal with the onset of the stimulus. Therefore, locusts may actively control the timing of wing rotation and we believe this may be an additional mechanism for generating steering torques.

Dickinson et al., (1999) built a scaled model of Drosophila melanogaster with sensors capable of measuring the aerodynamic forces generated by the wings during kinematic manoeuvres. From kinematics based on observations of real flies, transient peaks in aerodynamic forces were observed at the start and end of each upstroke and

downstroke that result from the timing of the wing flip relative to the stroke reversal.

These aerodynamic forces were generated by rotational circulation as the wing flipped

over for translation of the next half-stroke. Dickinson's experiments showed that a peak

in lift is produced when the flip is completed before the stroke reversal. Our observations

are consistent with the results of his model. In our experiment, the forewing wing flip

preceded downstroke translation (by approximately 8% to 15% of the cycle length) and

the velocity of rotation of the wings was between 100°/s and 250°/s: values in the same

range as measurements for *Drosophila*. If for our locusts the wing flip is generating lift

by rotational circulation, then during straight flight these forces would add to the forces

generated during translation.

Dickinson *et al.*, (1999) found that advancing the timing of rotation relative to the

stroke reversal affects the magnitude of the wake generated by the wing. For *Drosophila*,

because of wake capture, in which the wing interacts with the shed vortex from the

previous stroke, the timing of wing rotation represents a very sensitive mechanism of

regulating lift and a potential means for generating steering forces. During visually

induced steering, *Drosophila* advance the timing of supination on the outside of the turn

(Dickinson, *et al.*, 1993).

If rotation of the wing generates lift then three possibilities exist that might

contribute to the generation of steering torques for locusts. First, without changing the

amount of lift generated by rotation of the left and right wings, asymmetries in the

elevation angles at the moment of the stroke reversal could affect the direction of the

rotational lift vector. Second, asymmetries in the magnitude of rotational lift can be

generated by asymmetrically advancing the time of rotation relative to the stroke reversal.

Dickinson *et al.*, (1999) found that advancing the time of rotation increased lift whereas

delaying the time of rotation produced negative lift at the start of the upstroke. Of the 11

locusts that showed changes in wing flip latency relative to the stroke reversal, four of

seven locusts showed an increase in latency in the right wing (the wing on the outside of

the turn), and six of eight locusts showed a decrease in latency in the left wing (Fig.

3.12B). These observations are consistent with a strategy to either increase lift on the

outside of the turn path or to decrease lift on the inside of the turn path. Third,

asymmetries in the time that rotational lift is produced may affect steering torques. Our

correlations show that the wing flip occurs earlier in the wing on the inside of the turn

and further that angular difference asymmetries, which are a measure of the relative gap

between fore- and hindwings on the left and right side of the locust, are significantly

correlated with forewing depressor timing and kinematics. There is likely to be

considerable interaction between the forces generated by the forewings and hindwings

owing to the pattern of airflow between the two wings during forward flight (Schmidt and

Zarnack, 1987; Schwenne and Zarnack, 1987). Because of fore-hindwing interactions,

lift produced by rotation may interact with the aerodynamic effects of the relative gaps

between forewing and hindwings. In a manner analogous to wake capture in *Drosophila*,

the hindwings could be interacting with the shed vortices from the forewings (the

hindwing stroke leads the forewing stroke). This may be the reason why hindwing

asymmetries are less pronounced than forewing asymmetries.

3.5.4 Conclusions

In this paper we have recorded from the direct downstroke flight muscles of the forewings and hindwings and measured differences in timing between contralaterally homologous muscles. Relative shifts in the timing of contralateral homologues are correlated with bilateral asymmetries in the timing of the stroke reversal, which in turn, are correlated with asymmetric wing depression. These results support previous predictions that forewing asymmetry results from bulk shifts in depressor timing that affect the timing of stroke reversals. As we explained at great length in a previous paper (Dawson, et al., 1997), asymmetric wing depression may contribute to the production of steering torques by affecting the left-right balance of forces generated by each wing; an imbalance that would add to any aerodynamic effects generated by angle of attack of the wings and changes in body posture. Finally, this study presents evidence that the timing of the wing flip may be important for steering. Timing of the wing flip may generate asymmetric forces directly, or indirectly *via* interactions with the hindwings. The time course of changes in wing asymmetry and depressor asymmetry in this study is the same as reported in Dawson *et al.* (1997) and in previous studies of other intentional steering manoeuvres, namely collision avoidance (Robertson and Reye, 1992; Robertson and Johnson, 1993) and thermal avoidance (Robertson *et al.*, 1996; Shoemaker and Robertson, 1998) suggesting that locusts employ a common motor program for effecting rapid avoidance/escape movements.

3.6 Acknowledgements

3.7 Literature Cited

Baker, P.S. (1979). The role of forewing muscles in the control of direction in flying

locusts. *J. Comp. Physiol. A* **131**, 59-66.

Burrows, M. (1975). Monosynaptic connexions between wing stretch receptors and

flight motoneurones of the locust. *J. Exp. Biol.* **62**, 189-219.

Burrows, M. (1996). *The neurobiology of an insect brain.* Oxford: Oxford University

Press.

Camhi, J. M. (1970). Yaw-correcting postural changes in locusts. *J. Exp. Biol.* **52**, 519-

531.

Dawson, J. W., Dawson-Scully, K., Robert, D., and Robertson, R. M. (1997).

Forewing asymmetries during auditory avoidance in flying locusts. *J. Exp. Biol.*

200, 2323-2335.

Dawson, J. W., Kutsch, W. and Robertson, R. M. (2001). Auditory evoked evasive

manoeuvres in free-flying locusts and moths. Submitted to JEB. March. 2001.

Dickinson, M. H., Lehmann, F., and Götz, K. (1993). The active control of wing

rotation by *Drosophila. J. Exp. Biol.* **182**, 173-189.

Dickinson, M. H., Lehmann, F., and Sane, S. P. (1999). Wing rotation and the

aerodynamic basis of insect flight. *Science* **284**, 1954-1960.

Dugard, J. J. (1967). Directional change in flying locusts. *J. Insect Physiol.* **13**, 1055-

1063.

Forrest. T. G., Farris, H. E., and Hoy, R. R. (1995) Ultrasound acoustic startle

response in scarab beetles. *J. Exp. Biol.* **198**, 2593-2598.

Hoy, R., Nolen, T., and Brodfuehrer, P. (1989). The neuroethology of acoustic startle

and escape in flying insects. *J. Exp. Biol.* **146**, 287-306.

Libersat, F., and Hoy, R.R. (1991). Ultrasonic startle behavior in bushcrickets

(Orthoptera; Tettigoniidae). *J. Comp. Physiol. A* **169**, 507-514.

Miller. L.A., and Olesen, J. (1979). Avoidance behavior in green lacewings. I. Behavior

of free flying green lacewings to hunting bats and ultrasound. *J. Comp. Physiol.*

131. 112-120.

Möhl. B., and Zarnack, W. (1977). Activity of the direct downstroke flight muscles of

Locusta migratoria (L.) during steering behaviour in flight. II. Dynamics of the

time shift and changes in the burst length. *J. Comp. Physiol A.* **118.** 235-247

Moiseff. A., Pollack, G.S., and Hoy, R.R. (1978). Steering responses of flying crickets

to sound and ultrasound: mate attraction and predator avoidance. *Proc. Nam.*

Acad. Sci. U.S.A. **75.** 4052-4056.

Nachtigall, W. (1989). Mechanics and aerodynamics of flight. In: *Insect Flight* (Eds: G.

J. Goldsworthy and C. H. Wheeler). Boca Raton: CRC Press Inc.

Nolen, T. G. and Hoy, R. R. (1986). Phonotaxis in flying crickets. I. Attraction to the

calling song and avoidance of bat-like ultrasound are discrete behaviours. *J.*

Comp. Physiol. A. **159.** 423-439.

Reye, D. N. and Pearson. K. G. (1987). Projections of the wing stretch receptors to

central flight neurons in the locust. *J. Neurosci.* **7.** 2476-2487.

Reye D. N. and Pearson, K. G. (1988). Entrainment of the locust central flight oscillator

by wing stretch receptor stimulation. *J. Comp. Physiol. A.* **162**, 77-89.

Rice, W. R. (1989). Anayzing tables of statistical tests. *Evolution* 43(1), 223-225.

Robert, D. (1989). The auditory behaviour of flying locusts. *J. Exp. Biol.* **147**, 279-301.

Robert, D. and Rowell, C.H.F. (1992). Locust flight steering II. Acoustic avoidance

manoeuvres and associated head movements, compared with correctional

steering. *J. Comp. Physiol. A* **171**, 53-62.

Robertson, R.M. and Johnson, A.G. (1993). Collision avoidance of flying locusts:

Steering torques and behaviour. *J. Exp. Biol.* **183**, 35-60.

Robertson, R.M. and Reye, D.N. (1992). Wing movements associated with collision-

avoidance manoeuvres during flight in the locust *Locusta migratoria*. *J. Exp. Biol.*

163, 231-258.

Robertson, R.M., Kuhnert, C.T., and Dawson, J.W. (1996). Thermal avoidance during

flight in the locust *Locusta migratoria*. *J. Exp. Biol.* **199**, 1383-1393.

Roeder, K.D. (1962) The behavior of free flying moths in the presence of artificial

ultrasonic pulses. *Anim. Behav.* **10**, 300-304.

Roeder, K.D. (1967) *Nerve cells and insect behavior*. Cambridge: Harvard University

Press.

Roeder, K. D. (1974). Acoustic sensory responses and possible bat-evasion tactics of

certain moths. *Proceedings of the Canadian Society of Zoologists Annual*

Meeting, pp71-78.

Rowell, C. H. F. (1988). Mechanisms of flight steering in locusts. *Experientia* **44**, 389-395.

Schmidt, J. and Zarnack, W. (1987). The motor pattern of locusts during visually induced rolling in long-term flight. *Biol. Cybern.* **56**, 397-410.

Schwenne, T. and Zarnack, W. (1987). Movements of the hindwings of *Locusta migratoria*, measured with miniature coils. *J. Comp. Physiol. A* **160**, 657-666.

Shoemaker, K. L. and Robertson, R. M. (1998). Flight motor patterns of locusts responding to thermal stimuli. *J. Comp. Physiol. A.* **183**, 477-488.

Simmons, J. A., Fenton, M. B., O'Farrell, M. J. (1979). Echolocation and pursuit of prey by bats. *Science* **203**, 16-21.

Snodgrass, R.E. (1929). The thoracic mechanism of a grasshopper and its antecedents. *Smithson. Misc. Coll.* **82**(2), 1-111.

Sokal. R. L., and Rohlf. F. J. (1981). *Biometry: The principles and practice of statistics in biological research.* Second Edition. New York: W. H. Freeman and Co.

Stapells, D.R.. Picton. T.W. and Smith. A.D. (1982). Normal hearing thresholds for clicks. *J. Acoust. Soc. Am.* **72**, 74-79.

Thüring, D.A. (1986). Variability of motor output during flight steering in locusts. *J. Comp. Physiol. A* **158**, 653-664.

Uvarov, B. P. (1977). *Grasshoppers and Locusts: A Handbook of General Acridology. Volume. 2. Behaviour. Ecology. Biogeography. Population Dynamics.* Cambridge: Cambridge University Press. and London: Centre for Overseas Pest Research.

Waldmann, B. and Zarnack, W. (1988). Forewing movements and motor activity during roll manoeuvres in flying desert locusts. *Biol. Cybern.* **59**, 325-335.

Waldron, I. (1967). Neural mechanism by which controlled inputs influence motor output in the flying locust. *J. Exp. Biol.* **47**, 213-228.

Wendler, G. (1974). The influence of proprioceptive feedback on locust flight coordination . *J. Comp. Physiol.* **88**, 173-200.

Wilson, D. M. (1968). The nervous control of flight and related behaviour. *Adv. Insect Physiol.* **5**, 289-338.

Wilson, D. M. and Weis-Fogh, T. (1962). Patterned activity of co-ordinated motor units. studied in flying locusts. *J. Exp. Biol.* **39**, 643-667.

Yack, J. E. and Fullard, J. H. (2000). Ultrasonic hearing in nocturnal butterflies. *Nature* **403**, 265-266.

Yager, D. D. and Spangler, H. G. (1997). Behavioral response to ultrasound by the tiger beetle *Cicindela marutha* Dow combines aerodynamic changes and sound production. *J. Exp. Biol.* **200**, 649-659.

Yager, D.D., May, M.L, and Fenton, M.B. (1990). Ultrasound-triggered. flight-gated evasive maneuvers in the praying mantis *Parasphendale agrionina*. I. Free flight. *J. Exp. Biol.* **152**, 17-39.

Zanker, J.M. (1988). How does lateral abdomen deflection contribute to flight control of *Drosophila melanogaster*. *J. Comp. Physiol. A* **162**, 581-588.

Zar. J. H. (1984). *Biostatistical Analysis.* 2nd Edition. Englewood Cliffs. New Jersey Prentice-Hall.

Zarnack, W. (1988). The effect of forewing depressor activity on wing movement

during locust flight. *Biol. Cybern.* **59**, 55-70.

Zarnack, W. and Möhl, B. (1977). Activity of the direct downstroke flight muscles of

Locusta migratoria (L.) during steering behavior in flight. I. Patterns of time shift.

J. Comp. Physiol. A **118**, 215-233.

CHAPTER 4

AUDITORY-EVOKED EVASIVE MANOEUVRES IN FREE-FLYING

LOCUSTS AND MOTHS[†]

4.1 Abstract

Locusts are tympanate insects sensitive to sounds from 2 to more than 40 kHz. Previous

studies have shown that tethered locusts possess an ultrasound-sensitive acoustic startle

response in which animals respond more frequently to trains of pulses with carrier

frequencies of 30 kHz than to pulse trains below 10 kHz. These studies speculate that

locusts in free-flight will react with avoidance manoeuvres to the echolocation calls of

aerially-foraging insectivorous bats. We tested the hypothesis that locusts (*Locusta*

migratoria L.) would react specifically to bat-like sounds with avoidance manoeuvres in

free-flight in a flight room under controlled temperature and light conditions using a

variety of synthesized sounds. We presented free-flying locusts with sounds that varied

in temporal structure ranging from a stridulatory-like envelope to a bat-like (feeding-

buzz) envelope and varied in frequency from low (5 kHz) to high frequency (30 kHz).

Locusts responded indiscriminately to all sounds with turns. loops. and dives. Responses

were between 6% and 26% and were independent of carrier frequency and pulse

structure: locusts were as likely to respond to 5 kHz stridulation-like sounds as they were

---

to 30 kHz, bat-like sounds. Since these results did not support previous findings and speculation from studies of tethered locusts, we tested wild-caught moths and tethered locusts in the flight room under the same temperature and light conditions and with the same auditory stimulus patterns to confirm that the free-flight results were not an artefact of conditions in the flight room. Moths responded with loops, dives, and turns with low response rates (between 5% and 10%) to 5 kHz bat-like sounds but with high response rates (greater than 80%) to 30 kHz bat-like sounds. Similarly, tethered locusts, positioned in the same locations as the free-flying locusts, responded more frequently (between 60 and 80%) to 30 kHz than to 5 or 12 kHz (20% to 40%) bat-like sounds. No differences were found in response rates between full (ambient light greater than 100 lux) and reduced light (attenuated 90%) for locusts (free-flying or tethered) or moths. We conclude that locusts react to sounds while in free flight but that free-flying locusts do not show the same discrimination based on frequency that is observed in tethered locusts. These results support other studies concluding that tethering affects auditory processing of sounds which affects motor output and therefore emphasizes the need for testing insects in free-flight. Although the turns, loops, and dives observed are typical of the in-flight evasive manoeuvres of moths, lacewings and praying mantises interacting with foraging insectivorous bats, the low response rates and lack of frequency dependency suggests that locust responses may not be specifically for bat-avoidance or that locusts may possess additional, non-acoustic, defences from bats.

4.2 Introduction

Tympanate ears protect many nocturnal flying insects from predation by insectivorous

bats (for reviews see Hoy, 1992; Fullard and Yack, 1993; Hoy and Robert, 1996).

Typically, an insect detecting an approaching bat will react with an acoustic

startle/escape response (ASR) which results in movement away from the predator (Hoy,

1989; Hoy, *et al.*, 1989: Hoy, 1992). The most extensively studied ASR is observed in

free-flying moths interacting with bats. Moths detecting a distant bat will turn away from

the sound whereas moths detecting a close bat will respond with loops, dives and

increases in flight path erraticism (Roeder, 1962; 1964; 1967a; 1974). Similar last ditch

responses are seen in praying mantises (Dictyoptera) and lacewings (Neuroptera). Yager

et al. (1990) showed that the praying mantis *Parasphendale agrionina* avoided capture

by *Lasiurus borealis* and *L. cinereus* with steep, spiralling power dives and Miller and

Olesen (1979) showed that several species of green lacewing, *Chrysopa* sp., fold their

wings and dive, punctuating their descent with wing flips, when interacting with

Pipistrellis pipistrellis and *Myotis brandtii* in a dimly lit cage. Similar behaviours are

observed in other insects using synthesised bat-like sounds. A group of nocturnal

butterflies, the Hedyloidea, showed responses to intense ultrasound when in flight that

included steep dives, climbs, loops, and turns (Yack and Fullard, 2000), and, free-flying

scarab beetles, *Euetheola humilis*, were collected in the field in large funnel traps with

bat-like sounds being broadcast from speakers atop the traps: the insects falling into the

traps presumably having executed bat avoidance responses to the sounds (Forrest *et al.*,

1995). Thus, for this diversity of free-flying insects, spanning four insect orders, the

behavioural reactions to the echolocation calls of bats seem to show convergence on a

fixed behavioural repertoire consisting of turns, loops, and active and passive dives

during flight.

Observing unrestrained animals is ideal (Kutsch, 1999), however, collecting data

from nocturnally active insects interacting with flying bats in their natural environment

poses certain technical difficulties that are not easily overcome. This fact, coupled with

the obvious benefit of restraining an animal for recording behavioural elements with

transducers and presenting controlled acoustic stimuli, has resulted in many biologists

opting to work with tethered flying insects stimulated with synthesized bat-like sounds.

By far the most extensively studied in this regard are members of the Orthoptera. Field

crickets (Gryllidae) and katydids (Tettigoniidae) respond to bat-like sounds with an ASR

that consists of abdomen and hindleg deflection, head rolling, flexion of the antennae,

wing twisting (inferred from basalar muscle activity), and changes in wing beat

frequency and flight cessation (Moiseff *et al.*, 1978; Pollack and Hoy, 1981; Moiseff and

Hoy, 1983; Pollack *et al.*, 1984; Nolan and Hoy, 1986; Libersat and Hoy, 1991; and see

reviews by Doherty and Hoy, 1985; Pollack and Hoy, 1989; Hoy, 1989; Hoy *et al.*, 1989;

and Hoy, 1992). Other tethered flying insects react to bat-like sounds in a similar way, for

example, tiger beetles, *Cicindela marutha*, respond to bat-like sounds with head rolling,

leg extension, and changes in wing kinematics (Yager and Spangler, 1997). Rare are

studies that have compared responses from free-flying and tethered animals but results

from praying mantises (Yager and May, 1990), lacewings (Olesen and Miller, 1979) and

moths (Roeder, 1967b) suggests that the convergence in free-flight responses is reflected in the responses of tethered insects and that elements of these postural adjustments have aerodynamic significance in flight control (Camhi, 1970; Arbas, 1986; Zanker, 1988; Dawson *et al.*, 1997).

The acridid grasshopper, *Locusta migratoria* L. also possesses an ASR with similar characteristics to those described above and by association with crickets and katydids, the ASR has been hypothesized to function in bat avoidance (Robert, 1989). Tethered flying locusts react with short latencies of 50 to 100 ms to high-frequency bat-like sounds consisting of trains of short duration sound pulses with carrier frequencies greater than 10 kHz presented at intensities greater than 45 dB SPL with abdomen deflection and dorsiflexion, hindleg extension and deflection, changes in wing beat frequency, including flight cessation, changes in wing stroke kinematics and other postural adjustments (Hoy *et al.*, 1989; Robert, 1989; Robert and Rowell, 1992; Dawson *et al.*, 1997). Robert (1989) showed that abdomen and hindleg deflection in response to stimulation with bat-like sounds was accompanied by the production of steering torques in directions opposite to the side of stimulation. Further, Dawson *et al.* (1997) showed that bat-like sounds produce asymmetric wing depression coincident with abdominal deflection and dorsiflection and suggested that free-flying locusts would react with banked, downward turns. The purpose of this paper was to test the hypothesis that locusts in *free-flight* react to sounds and to determine if these reactions are specifically to bat-like sounds, which would be in contrast to a general startle reaction to any high-intensity sound.

To test this hypothesis, we presented all combinations of stimuli that varied among three different carrier frequencies (5 kHz, 12 kHz, and 30 kHz) and three different pulse structures (stridulation-like, intermediate, and bat-like). Since flight tendencies in *Locusta migratoria* are affected by light intensity (Uvarov, 1977) we also tested animals under light levels comparable to daylight (full light) and dusk (reduced light). We chose the carrier frequencies and temporal patterns of the pulses based on sounds that might have significance in the natural behaviour of locusts. For example. recordings of wing-beat noise show spectral peaks between 3.5 and 5 kHz (Haskell, 1957) and stridulation sounds show peaks at 12 kHz (Kalmring, 1975b). We predicted that we would not see avoidance to 5 kHz or 12 kHz stridulation-like sounds. however, from published observations of tethered locusts (Hoy *et al.*, 1989; Robert. 1989; Robert and Rowell. 1992; Dawson *et al.*. 1997) including Robert's (1989) behavioural audiogram. we predicted we would see a high proportion of responses to 30 kHz bat-like sounds. Further. following from the findings of Dawson *et al.* (1997) and the convergence of behavioural responses seen in other insects in free-flight. we predicted that if we saw avoidance reactions they would consist of banked. downward turns away from the sound source.

4.3 Materials and Methods

4.3.1 Animals

Adult male and female *Locusta migratoria* L. aged 15 to 20 days post imaginal moult

were used in these experiments. All animals, except those for the behavioural audiogram,

were reared in a crowded culture maintained at the Universität Konstanz at 36 °C (day)

and 26 °C (night) with 75% relative humidity under a 12h:12h light:dark cycle. Animals

for the behavioural audiogram were reared in a crowded colony maintained at Queen's

University in a 16h:8h light:dark cycle at 30 °C (day and night) with 60% relative

humidity. All locusts used in this study were inspected for defects in wing structure,

unusually large amounts of wear on the wing tips, and missing appendages such as

hindlegs or antennae. Only locusts in apparent excellent condition were used in this

study.

Moths were collected from a deciduous woodland area that bordered on unused

agricultural fields. This area, called Hockgraben, is located 47°41'18" N, 9°11'27" E and

surrounds the grounds of the Universität Konstanz. Moths were collected from mercury-

vapour lamps illuminating paths through Hockgraben between 22:30 and 0:00 hr during

July and August 1999 using hand nets. Moths were kept in a screen cage approximately

30 cm long by 20 cm deep by 30 cm tall in a refrigerator at 4 °C to prevent self inflicted

damage to their wings until tested in the flight room. All moths were tested within two

days of capture. To ensure correct identification of the moths, we prepared a reference

collection of moths from the area and only tested those that could be positively identified.

Moths were identified using Forster and Wohlfahrt, (1960, 1971, and 1981), and

collection records from 1992-93 as cited in Bauer (1993). Our reference collection

presently resides in the care of Mr. Holger Martz and WK at the Universität Konstanz.

4.3.2 Behavioural Audiogram

Behavioural audiograms were collected by monitoring abdomen deflections (Robert,

1989) from tethered, flying locusts stimulated with incrementing intensities of a bat-like

sound stimulus of randomly chosen carrier frequencies between 10 and 70 kHz. Animals

were tested in a foam-lined Faraday cage with a variable speed fan providing an air

stream of approximately 3 m·s⁻¹; measured with a hot-wire anemometer (TSI model 67-7

with sensor model 1610-12). Animals were attached to a rigid tether by a small amount

of beeswax on the pronotum and suspended from a retort stand in the air stream.

Animals were allowed to fly until they assumed a tucked flight posture after which time

the audiogram procedure began.

The direction of abdomen deflection was monitored with a photosensitive position

transducer. The transducer was positioned below the distal portion of the abdomen and a

light was placed above the animal. During flight, lateral movement of the abdomen

would differentially shade two adjacently placed photocells. The photocells were

connected in a circuit such that unequal illumination of the photocells, when the abdomen

was off centre, would produce either a positive or negative potential. We monitored this

voltage and a marker of stimulus onset, on a 4-channel, 100 MHz digital storage

oscilloscope (Gould, model DSO630) and recorded the direction and latency of the response.

Shaped sound pulses with carrier frequencies between 10 and 70 kHz at intervals of 5 kHz were presented from a loudspeaker (Technics, model EAS10TH400B) located 17 cm from the locust and positioned at a 45° angle to the longitudinal axis of the animal. The centre of the speaker was aligned in the vertical plane with the locust tympanum. Shaped, 5 ms, sound pulses (0.5 ms rise/fall, 4 ms sustain) presented with a 20 ms period were generated by passing a sine wave from a waveform generator (BK Precision, model 3010) through an envelope shaper (Coulbourn, model S84-04) and amplifier (National Semiconductor, amplifier module model LM1875T). The continuous pulse trains were gated to the speaker by a hand-held switch. At each frequency tested, the sound intensity of the hand-triggered pulse trains was increased from silence until a noticeable deflection of the abdomen away from the speaker was observed. The hand-triggered pulse trains were typically 500 ms in duration and care was taken to avoid habituating the animal with repeated stimuli. The amplitude of the sound pulses at threshold was recorded from the oscilloscope screen and later converted into decibel peak equivalent sound pressure levels (dB peSPL; Stapells et al., 1982). Sound intensity of the pulses was determined by matching pure tones of equivalent amplitude and frequency to the stimulus pulses and measuring the SPL from a Brüel and Kjær sound level meter (type 2610) with a Brüel and Kjær ¼ inch microphone (type 4135, without protective grid) positioned where the locust tympanum was during testing. During calibration it was necessary to band-pass

filter certain frequencies (Krohn-Hite, model 3500) input to the sound level meter. The

sound level meter was calibrated with a Brüel and Kjær pistonphone (type 4228).

4.3.3 Flight Room

Observations of free-flying locusts, moths and tethered, flying locusts were made in a

large room with an observation area measuring 8 m long, 5.7 m wide, and 2.2 m tall. The

flight room walls and ceiling were white and the walls and floor were marked with a grid

in red and black tape (1 m intervals along floor. 0.5 m intervals along walls). We used

these markings to define a co-ordinate system for referring to the location of locusts and

equipment in the room (Fig. 4.1). Temperature was maintained at $32 \pm 3°C$ with four

Ismet 2000 Watt forced-air heaters (Ako-Ismet Elektrogeräte. Villingen-Schwennigen);

two located at the front of the room and two at the back of the room. The air streams

from the heaters were directed away from the centre of the room to reduce the possibility

of thermal eddies in the flight path of the locusts. Relative humidity of the room at 30°C

is approximately 65 to 75 %. This flight room has been used in the past for several

studies of free-flight in locusts (Kutsch et al., 1999; Fischer and Ebert, 1999; Fischer and

Kutsch. 1999).

We positioned a DC-powered photographer's studio lamp (Profilux. model 200.

Hedler Systemlicht GmbH, Runkel/Lahn. with an Osram metal halide bulb. model HMI)

in one corner of the flight room (co-ordinates 2.7 at a height of 1.1 m). Additional

lighting was provided by fluorescent tube fixtures on the walls close to the ceiling with

one located on the right wall (relative to the front of the room at 0,0) above the

Figure 4.1. The flight room. (A) Photograph and (B) diagram of the room showing location of equipment and the flight path (dashed line) of locusts past the speakers. The operational area of the flight room measured 8 m long by 5.7 m wide by 2.2 m tall and was separated from equipment in the rest of the room by two large heavy cloth curtain partitions (heavy lines). To facilitate observations, the room was marked in 1 m intervals along the floor and 0.5 m intervals along the walls which define the co-ordinate system used in this study (X=width, Y=length, Z=height). The room was illuminated by a photographer's studio lamp located at (2, 7, 1.1), fluorescent tube fixtures on the wall, one above the photographer's lamp located at (3, 7, 2.1), and one on the opposite wall at (-2.7, 4.5, 2.1), and a single 60 Watt incandescent light bulb at (0, 1.5, 2.1). Note that the fluorescent tube fixture on the ceiling in the centre of the room in the photograph (A) was not illuminated during experiments. The room was heated by four 2000 Watt forced air heaters (rectangles), two at the front of the room and two at the rear (arrows indicate the direction of air flow away from the centre of the room). Locusts were launched by dropping them into flight from a height of approximately 2 m at co-ordinates (0, 1, 2). As locusts fell they opened their wings and then flew forward (dashed line), under their own power, toward the photographer's lamp in the corner of the flight room past two speakers located at (2.5, 5.6, 1.15 and 2.5, 6, 1.15); direction of speakers indicated by arrows. Sounds were presented when the locust flew past the 4 m mark.

A

B
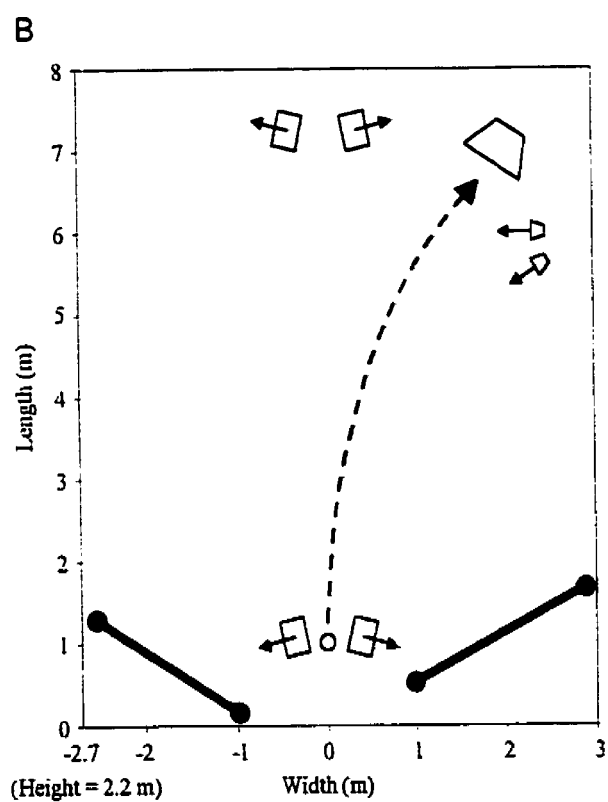
photographer's studio lamp, and one located in the centre of the left wall. A single 60 Watt incandescent light bulb was also located 0.5 m in front of the point where locusts were launched into flight. For observations under reduced light intensity, we placed 90% attenuation neutral density filters (#211, Lee Filters, Andover Hampshire, UK) on all the lights except the incandescent bulb. We measured the distribution of light in the room by measuring light intensity at 1 m intervals along the length and width of the entire flight room. For these measurements a light meter (Panlux electronic Luxmeter. Gossen GmbH) was fixed to a tripod at a height of 1 m and was rotated 360 degrees at each measurement location. The direction, read from a protractor fixed to the tripod. and magnitude of maximum intensity, was recorded. For all locations in the room, at both illumination levels, maximum light intensity was in the direction of the photographer's studio lamp in the corner of the flight room.

4.3.4 Acoustic Stimuli

A pair of two inch cone tweeters (Motorola. model KSN1078A) were positioned on the right side of the flight room. The speakers were 1.15 m above the floor, 0.5 m from the right wall and 0.4 m apart from each other. One speaker was directed toward a point just in front of the launch location and the other was directed toward the centre of the room. Using two speakers was necessary to create a sufficiently broad. distributed sound field given the size of the room and the directional properties of each speaker. The flight room. light positions. speaker positions. and launch position are shown in figure 4.1.

An arbitrary waveform generator (PC Instruments, model PCI-311, Akron, Ohio)

controlled by a microcomputer with software supplied by the manufacturer (BenchTop

Lite, v3.3J) generated all waveforms used in the flight room. Sound presentation was

controlled by way of keyboard entry (space-bar tap), or by a TTL trigger pulse generated

by a Grass Instruments electronic stimulator (model S88) applied to the arbitrary

waveform generator.

We used three temporal patterns for our acoustic stimuli (Fig. 4.2). The first

pattern was based on the stridulation sounds produced by *Locusta migratoria* as

described by Kalmring (1975b). This pattern consisted of pairs of shaped pulses each

with a 6 ms rise time, 9 ms sustain time, and 2 ms fall time. The pairs of pulses

(measuring a total of 34 ms) were presented with an inter-pulse-interval of 42 ms

producing a stimulus period of 76 ms (13.2 pulses per second). We presented 10 pairs of

pulses as a train for a total stimulus time of 718 ms. The second pattern was taken from

Robert (1989). This pattern consists of single pulses with a 1 ms rise/fall time and 18 ms

sustain time. These pulses are presented with an 80 ms inter-pulse-interval yielding a

stimulus period of 100 ms (10 pulses per second). We presented trains of 10 pulses for a

total stimulus time of 920 ms. Our last pattern, designed to mimic the temporal pattern of

a feeding buzz of an echolocating bat (Simmons *et al.*, 1979), consists of pulses with a 1

ms rise/fall time and 8 ms sustain time presented with a 20 ms inter-pulse-interval

producing a period of 30 ms (33.3 pulses per second). We presented trains of 10 pulses

for a total stimulus time of 280 ms. Each of the patterns was synthesised with sine waves

with carrier frequencies of 5, 12, and 30 kHz.

204

Figure 4.2. The three temporal patterns of pulse trains used for testing locusts in the
flight room. The three patterns follow a gradient from stridulation-like to bat-like.
The stridulation-like pattern is derived from acoustic recordings published in
Kalmring (1975). The intermediate pattern is that used in Robert (1989). The
bat-like pattern, used by Dawson *et al.* (1997), was chosen to mimic the feeding
buzz (after Simmons, 1979) of an echolocating bat that is in the final stages of
pursuit of a prey item. All three patterns consist of shaped pulses (parameters as
indicated) with carrier frequencies of 5, 12 or 30 kHz.

Stridulation-like

42 ms inter-pulse-interval, 13.2 pps   ... 10 pulses in train
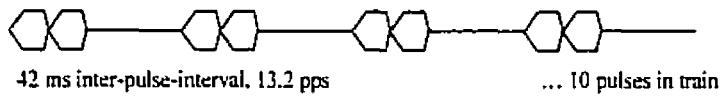
6 ms rise, 2 ms fall
9 ms sustain, paired pulses

Intermediate

80 ms inter-pulse-interval, 10 pps   ... 10 pulses in train
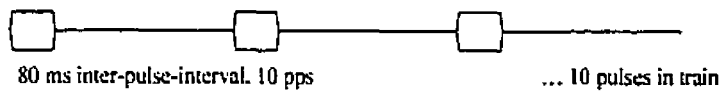
1 ms rise/fall
18 ms sustain

Bat-like

20 ms inter-pulse-interval, 33.3 pps   ... 10 pulses in train
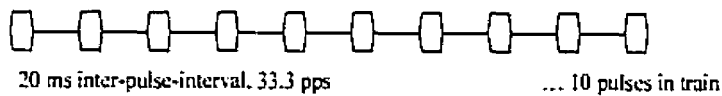
1 ms rise/fall
8 ms sustain

We measured the sound field in the flight room for all three stimulus frequencies (5, 12, and 30 kHz) and at three different heights (0.5 m, 1.15 m (speaker height), and 1.5 m). Sounds were measured at 0.5 m intervals along a rectangular region of the flight room in front of the speakers (the flight zone). At each measurement interval, sound intensity was determined by measuring the SPL of pure (sinusoidal) tones with carrier frequencies equivalent to the stimulus pulses. Sound pressure levels were measured with a Brüel and Kjær ½ inch microphone (type 4133) coupled to a Brüel and Kjær measuring amplifier (type 2606). For measurements, the microphone was pointed directly at the pair of speakers except when in close proximity to the two speakers where we adjusted the direction of the microphone so as to record the maximum sound intensity encountered. The ½ inch microphone has a flat response to 40 kHz.

To verify the quality of the acoustic stimuli in the flight room, we examined recordings of the stimuli broadcast from the speakers recorded at speaker height (1.15m) from the centre of the "flight zone" at co-ordinates (1.5). The broadcast sounds were recorded with a Brüel and Kjær ½ inch microphone (configuration as above) and separately with a Brüel and Kjær ¼ inch microphone (type 4135) with a Brüel and Kjær preamplifier (type 2618). Both microphones were coupled to the measuring amplifier described above with the 22.4 Hz high-pass filter engaged. Signals were recorded onto 7" reels of magnetic tape (BASF HiFi DP26) using a RACAL instrumentation tape recorder (model Store-4) using a direct board with the tape travelling at 30 ips. Signals on tape were replayed at 30 ips (i.e record speed) and digitised (Axon Instruments Digidata, model 1200B using Axoscope v7.0, Axon Instruments, Foster City, CA) with a

333.3 kHz sampling rate. Spectral analysis of the sounds was performed with ScopeDSP

(v3.6a, Iowegian International Corp., Shawnee Mission KS, USA).

The size of the room, the composition of the walls (cement floor and walls, wood

tile ceiling), and the fact that we broadcast from two speakers simultaneously, resulted in

a complex sound field in the locust flight zone. The intensity of the 30 kHz stimuli

attenuated over shorter distances than the 12 and 5 kHz stimuli as expected and we

adjusted the amplitude of the waveforms input to the speakers so that the intensity of the

stimulus pulses at the 4.5 m mark (the point along the locust's flight path at the moment

of stimulation) was between 75 dB and 78 dB for all stimulus frequencies. Sound

intensities were greatest from measurements at speaker height (1.15 m) relative to

measurements 0.5 m above the floor or 0.7 m below the ceiling. Recordings of the

stimuli showed that some echo was present in the 5 kHz stimuli but it was not sufficient

to significantly distort the envelope of the stimuli. Spectral analysis of the recorded

stimulus pulses showed that they were narrowly tuned at their broadcast frequency and

that the stimulus pulses were the dominant sound in the room.

4.3.5 Observations of Locusts in Free-flight

On each day of observation, we collected 10 to 20 locusts from the colony and arbitrarily

(but with regard to sex) assigned them to individual numbered clear plastic containers

kept under a 150 Watt heat lamp (Osram Siccatherm) at the rear of the flight room. The

containers measured approximately 15 cm deep with a 10 cm square mouth and provided

ample room for movement. The rear area of the flight room, containing the locusts and

the stimulus generating equipment, is out of the sound field of the speakers and was

separated from the observation area by heavy black cotton curtain partitions. Each locust

was launched into flight and allowed to fly within the flight room at least three times

before formal observations began. During formal observations, we cycled through the

locusts for each sound pattern, light intensity, and sound intensity regime. We consider

each flight of each locust to be independent as an individual locust was never tested

under the same conditions more than once (except for controls, see below).

All locusts were launched from the same position at the centre of one end of the

flight room (co-ordinates 0,0). Locust flight was initiated by holding the locust at a

height of approximately 2 meters (± 5 cm) and releasing it. The locust was held between

the thumb and first and second fingers, dorsum up, by pinching the metathoracic tibia-

femur joint and folded wings. The descent of the locust induced wing opening and

locusts normally began forward flight at a distance of between 1 m and 0.3 m from the

floor. No forward velocity was intentionally added to the locust during launch.

Locusts typically flew from the launch position toward the photographer's studio

lamp in the corner of the flight room (dashed line in Fig. 4.1B). All flights were observed

by two people (WK and JWD) in the flight room with vantage points at approximate right

angles to this flight path. JWD launched and observed the locusts as they flew the length

of the room while WK made observations while sitting along the left wall of the room (at

approximate co-ordinates –2, 2). Locusts were stimulated with sound as they approached

the speakers. Stimuli were manually triggered when locusts were within 2.5 meters (i.e.

on the right side of the room) of the speaker and at least 20 cm above the floor and 50 cm

below the ceiling (to avoid wall effects). Locusts that did not fly through this "flight zone" were immediately flown again. If after five flights the locust did not enter the flight zone, they were omitted from the observations for that experiment. From the calibrations of the sound field, sound intensity was typically between 73 and 78 dB (Fig. 4.3A-C) at the location of stimulus onset.

We made detailed notes about the in-flight reaction of each locust as it flew toward the lamp, past the speakers. Notes included details such as whether or not a reaction was observed, changes in flight speed, direction, altitude, and the location of the reaction relative to the speakers (for intensity estimates). We also scored the landing positions of locusts on the walls, floor and ceiling of the flight room on a three dimensional diagram of the flight room. For the purpose of scoring the landing position, some locusts flew on a straight flight path then, within 0.5 m of the wall (or lamp) would veer into a new direction which we presume was to avoid collision. Where locusts veered away from the walls and lamps, we scored the location before veering (i.e. the landing/collision site extrapolated from the flight path immediately before veering).

Locusts varied in their flight paths in that some locusts flew with lots of zigzags, turns, and changes in altitude on their path toward the lamp. To be sure we were not mistaking coincident erratic manoeuvres with reactions to sounds, we observed trials in which locusts were launched into flight but were not presented with sound. Animals that flew in such a way that we could not distinguish reactions to sounds from their flight tendencies were discarded from the analysis. Similarly, after all data were collected, we

209

Figure 4.3. Calibration of the sound field and light intensity in the flight room. (A, B, C) Isointensity plots of broadcast sounds (in dB SPL), at speaker height, in relation to the location of the speakers in the flight room for the three carrier frequencies used in experiments. For all frequencies, sound intensity in the room near the point in the flight path where the locusts are stimulated (1, 4 to 1,5; see Fig. 4.1), was app. 75 to 78 dB SPL. The complex distribution of intensities was a result of using two speakers app. 40 cm apart broadcasting in offset directions (see Fig. 4.1) and the acoustics of the flight room (cement walls and floor with a particle board ceiling). Background noise in the flight room was typically between 61 and 64 dB SPL. See methods for other measurements of the acoustics of the flight room. (D, E) Isointensity plots of light intensity measurements (lux) for the flight room in reduced and full light. Light intensity was uniformly decreased by placing neutral density film (0.9 attenuation) over all light fixtures with the exception of the incandescent bulb near the launch position. The direction of maximum light intensity at all measurement points (1 m intervals along the length and width of the room) was in the direction of the photographer's lamp in the corner of the flight room.

A 5 kHz   **B** 12 kHz   **C** 30 kHz

D Reduced Light   **E** Full Light
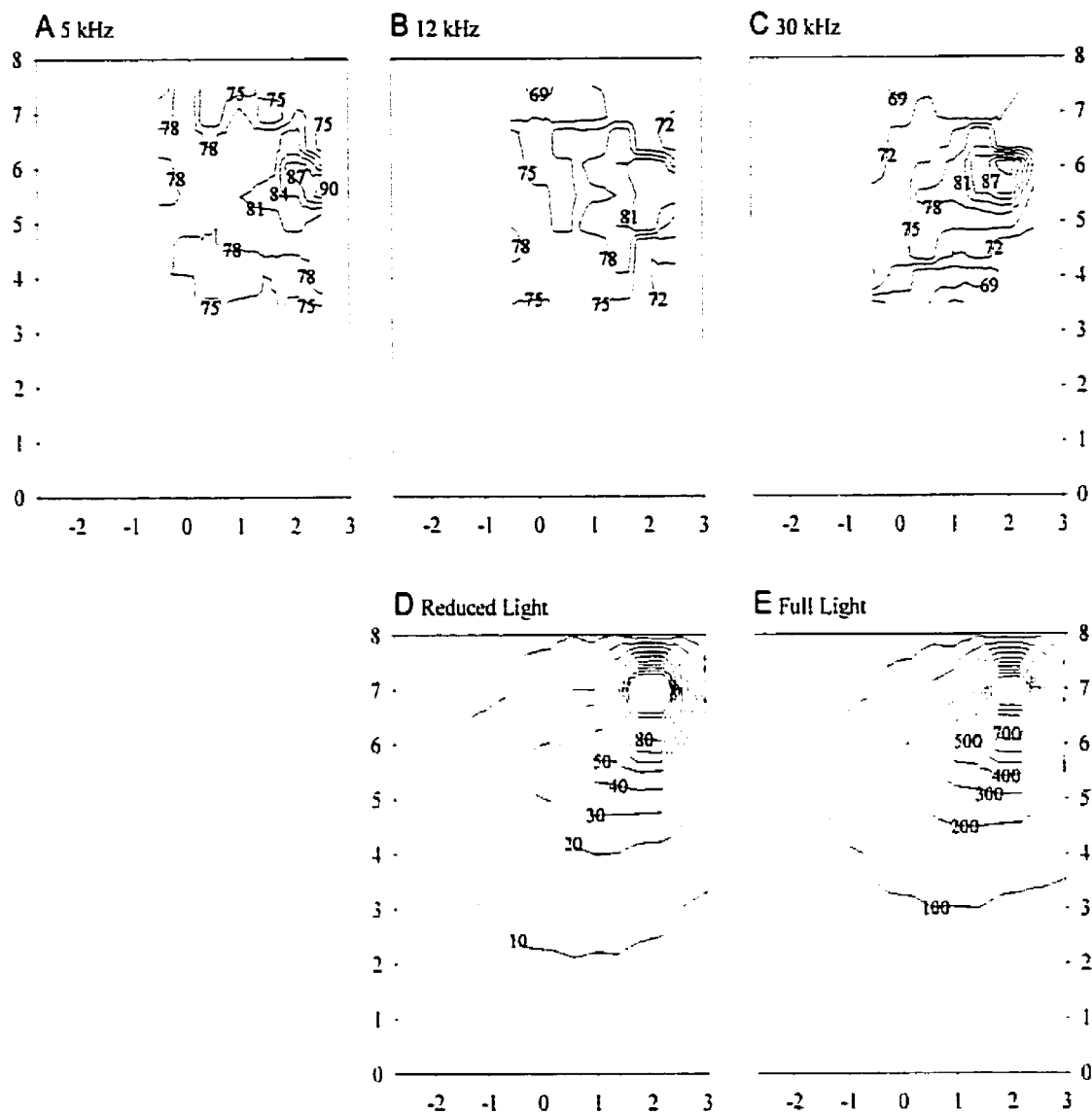
discarded all data from animals that could not be tested for 3 or more trials of the nine

sound pattern-frequency combinations.

4.3.6 Observations of Moths in Free-flight

Moths were tested under identical conditions as the locusts. After moths were identified,

they were separated into individual, labelled containers (the same used for holding the

locusts, see above). All moth flights were observed from two different vantage points in

the flight room by two individuals. It was not possible to launch moths into flight the

same way as locusts given the fragile nature of these insects. To initiate flight, the

container was held so that the moth could climb out and enter flight under its own

volition. For some individuals, a gentle flick of the container was necessary to coax the

moth off of the rim of the container. When moths were within a few meters of the

speakers (in the same location as the locusts at stimulation), and were not within 50 cm of

the ceiling or 20 cm of the floor, they were presented with sound. We tested moths with

the bat-like sound pattern with carrier frequencies of 5, 12, and 30 kHz in both full light

and reduced light. Notes describing the flight path before and after stimulation indicating

any reactions to sound were recorded in a manner identical to the locust flight trials.

4.3.7 Observations of Locusts Tethered in the Flight Room

On each day of testing, several locusts were retrieved from the colony and a copper

saddle was affixed to the pronotum of each with melted beeswax. Locusts were then

separated into labelled plastic containers until testing. A 1.15 m tall retort stand was

positioned in the flight room at co-ordinates (1,5), the "far" location or (2,6) the "near"

location relative to the speakers. The "far" tether was 1.7 m from the speakers and the

"near" tether was 0.5 m from the speakers. A desk fan (Braun, model HL1) was placed

25 cm in front of the tether and produced a wind stream of 2.5 m/sec (measured with a

Lambrecht Type 641 bN anemometer). We recorded the sounds produced by the fan

(recording method as above for stimulus pulses) at the same distance as the tethered

locusts to verify that it was not a source of high-frequency sounds. The sound intensity

of the noise produced by the fan was 60 dB (at 25 cm) and spectral analysis showed that

most of the sound energy was below 10 kHz: the fan did not produce any high-frequency

(greater than 20 kHz) sound.

All aspects of the flight room were identical to those during free-flight tests.

During testing, individual locusts were retrieved from their containers, affixed to the

tether and were stimulated with sound. When tethered, the locusts faced the lamp in an

alignment consistent with the observed flight path of free-flying locusts. When necessary,

locusts were prodded into flight by touching their abdomen or head or by suddenly

removing tarsal contact with a finger momentarily presented to the locust. Locusts were

stimulated with sound only after they had assumed a tucked flight posture, which

occurred after 10 to 30 seconds of flight. Tethered locusts were tested with the bat-like

sound pattern with carrier frequencies of 5, 12 and 30 kHz. Sound intensity, depending

on frequency, was between 78 dB and 80 dB at the far location and between 82 dB and

90 dB at the near location. Locusts were tested at both locations in full light and reduced

light.

Locusts were videotaped from behind using a Panasonic MS1 SVHS video

camera with a 1:1.4 TV zoom lens shuttered at $1/250^{th}$ of a second. A second video

camera (Panasonic VN-SX50 also with high-speed shutter) filmed the stimulus monitor

lamp of the Grass S88 stimulator used to gate the computer synthesised stimulus pulses

broadcast from the speakers and an information card noting the locust being tested, its

location in the flight room and the stimulus being broadcast. The video signals from the

two cameras were mixed (Panasonic WJ-AVE5 mixing board) and recorded onto a single

3M SE180 colour SVHS videotape using a Philips VR960 SVHS VCR. For analysis, the

videotape (which was converted from European PAL format to North American NTSC

format) was reviewed on a Panasonic editing VCR (model AG7300) with single-frame

shuttle job capability and a Sony Trinitron video monitor (model PVM1341). During

review of the videotape, detailed notes were made of any reactions to the stimulus. These

notes included things such as abdomen deflections, abdomen dorsiflections, hindleg

extensions, changes in wing-beat frequency, wing kinematics and flight cessation.

4.3.8 Three-dimensional Re-construction of Locust Flight Paths

We used two cameras (a Panasonic MS1 SVHS video camera, model NV-MS1 HQ

(Camera A) and a Bauer (=Bosch) SVHS video camera, model VCC550 AF (Camera B))

positioned at equal heights and such that their video fields overlapped at 90^c. Both

cameras had a 1:1.4 TV zoom lens shuttered at $1/250^{th}$ of a second. As locusts flew

through the overlapping video fields, the relative position of the images of the locust in

the two cameras was used to reconstruct the location of the locust relative to the co-

ordinate system of the flight room (see below).

Two additional cameras were used to monitor stimulus presentation. A

Universum model VKR-4650 and a Panasonic model NV-SX50, each with a high-speed

shutter, filmed the stimulus monitor lamp of the Grass S88 stimulator which gated the

output of the computer synthesised sound pulses. We also placed a card indicating the

stimulus presented, the locust being flown and the flight number for that locust next to

the stimulus monitor. The images from each of these cameras were mixed with images

from the flight room cameras. Images from Camera A were mixed with images from the

Panasonic camera using a Videonics mixing board, and images from Camera B were

mixed with images from the Universum camera using a Panasonic WJ-AVE5 mixing

board. The images, linked in time by the flash of the stimulus monitor, were recorded

onto separate VHS tapes. Images from Camera A were recorded using a Philips VR960

SVHS VCR recording onto 3M-SE180 SVHS colour video tape and images from Camera

B were recorded using a Blaupunkt RTV256 VCR recording onto Sony Super DXE-240

VHS videotape. The end result was two videotapes, each containing images of the same

locust but filmed at right angles and each tape had a record of the stimulus monitor (for

synchronising the sequences during analysis) and a record of the locust being filmed and

the stimulus presented.

For reconstruction of the flight paths the videotapes (which were converted from

European PAL to North American NTSC format) were reviewed on a Panasonic AG7300

editing VCR. The horizontal and vertical location of the locust relative to the flight room

walls and floor (i.e. its projected location on the walls) was recorded for each video

frame. For each camera, the distance from the camera lens to the wall in view was

known and we could calculate the angle, relative to the optical axis of each camera, of a

line from the camera lens to the projected locust location on the flight room wall. The

actual location of the locust is the point where these two lines intersect. Because the

cameras were at the same height, we could find this point relative to one of the cameras

by solving a system of three equations in two unknowns. Finally, because the location of

that camera in the flight room was known, we used a linear transformation to find the

locust location relative to the room co-ordinates.

4.3.9 Statistical Treatment of the Data

All statistical tests assumed significance with $P \leq 0.05$. Unless otherwise indicated. N

represents the number of individual locusts observed. Contingency table G tests and P-

values were calculated using procedures in Zar (1984). Two-by-two contingency table G

tests and associated William's corrected G values were calculated with Windows 2X2

Tables (v2.0 by Kelvin F. Conrad).

4.4 Results

4.4.1 Behavioural Audiogram

The frequency sensitivity of the ASR in tethered flying locusts, in a wind tunnel. is

shown in figure 4.4. Sound pulses greater than 25 kHz elicited avoidance responses with

lower stimulus intensities than sound pulses of lower frequencies. The best frequency for eliciting abdomen deflections was 30 kHz with a mean threshold of 54 dB SPL. The median latency to abdomen deflection with 30 kHz sound pulses was 120 ms (35ms, 175ms. 1st and 3rd quartiles respectively; N=7).

4.4.2 Observations of Locusts in Free-flight

Locusts had little difficulty manoeuvring in the flight room. Under bright light, locusts were observed circling the lamps and speakers, landing on the walls, floor, and ceiling and jumping into flight without apparent difficulty. Gender based differences in free-flight ability were not observed except that females, owing to their larger mass, were more difficult to launch into flight given our method of flight initiation. We spent three weeks systematically trying different lighting conditions, speaker locations and stimulus parameters in the flight room. From this period of preliminary observation we found that locusts would not fly or enter into sustained flight from our launch technique in the dark or under very low light (less than approximately 2 lux). We also found that locusts strongly orient to sources of light in the flight room. We used this to our advantage and found that by varying the amount of ambient light in the room, and the location of the lights, we could launch locusts at one end of the flight room and they would fly on a direct flight path.

Figure 4.4. Behavioural audiogram based on the steering responses of seven tethered, flying locusts. At each frequency tested, locusts were exposed to shaped sound pulses which gradually increased in intensity (see methods). The lowest intensity required to elicit a noticeable abdominal deflection at each frequency was recorded as threshold. The best-frequency was found to be 30 kHz with a mean threshold intensity of 54 dB SPL. The median latency for abdomen deflection was 120 ms (35 ms, 175 ms, 1st and 3rd quartiles, respectively). Thin lines are audiograms from individual locusts. The thick line is the mean audiogram.
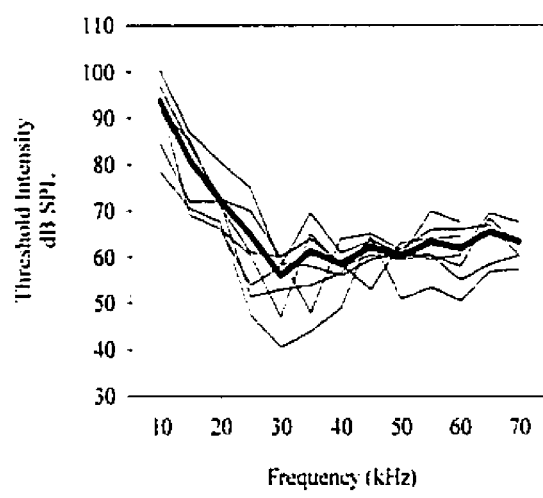
past our speakers, to the photographer's studio lamp positioned in the corner of the room (dashed line, Fig. 4.1). Under the same conditions, repeated flights of the same locust yielded consistent flight paths and landing positions (Fig. 4.5). After this period of trial-and-error we were satisfied that locusts responded to sounds while in free-flight, that our equipment was arranged in a manner sufficient for eliciting responses, and that we were making observations from good vantage points in the flight room.

We saw a diversity of behaviours in response to sounds including left and right turns, upward and downward turns, loops, spirals, ditches/drops to the ground and zigzagging (wobbling) in the flight path. Unlike tethered flight, locusts in free-flight are not confined to a single plane of movement and left and right turns were often combined with changes in elevation producing, for example, an upward left turn or a downward right turn. We categorized the locusts responses according to the most pronounced component of these manoeuvres. For example, a large left turn with slight upward motion was categorized as a left turn. Downward turns, when the locust did not contact or land on the ground, and ditches/drops to the ground were combined into the same category. Upward turns, due to the relatively low ceiling in the flight room (2.2 m), often yielded collisions with the ceiling. Spirals or loops were frequently observed and consisted of locusts following a cork-screw type flight path with an initial upward component and being completed without the locust contacting the ground. We observed both large and small diameter cylinders in the spirals. Zigzag and wobbling flight paths were observed when the locusts repeatedly changed either elevation or azimuth in an up-down-up-down-up-down or a left-right-left-right-left-right fashion respectively.

218

Figure 4.5. Diagram of the flight room showing the landing positions of locusts tested in a series. Each locust was exposed to all combinations of sound pattern and frequency (in a randomly chosen order). The landing positions are concentrated on the right side of the room illustrating the orientation of the locusts to the photographer's studio lamp. One locust (number 9. male) is indicated in each frame (blue triangles). In response to 5 kHz stridulation sounds. locust 9 flew in a spiral with increasing speed during its ascent. and in response to the 30 kHz intermediate pattern and 30 kHz bat-like pattern. locust 9 ditched to the ground (circled blue triangles). For many responses. reactions to the stimulus did not affect the landing position where in a manoeuvre would be executed and the locust would recover and continue toward the lamp. The lower most diagram (control) shows the landing positions of locusts flown without sounds being presented.

30 kHz   12 kHz   5 kHz

Stridulation-like

Intermediate

Bat-like

Control
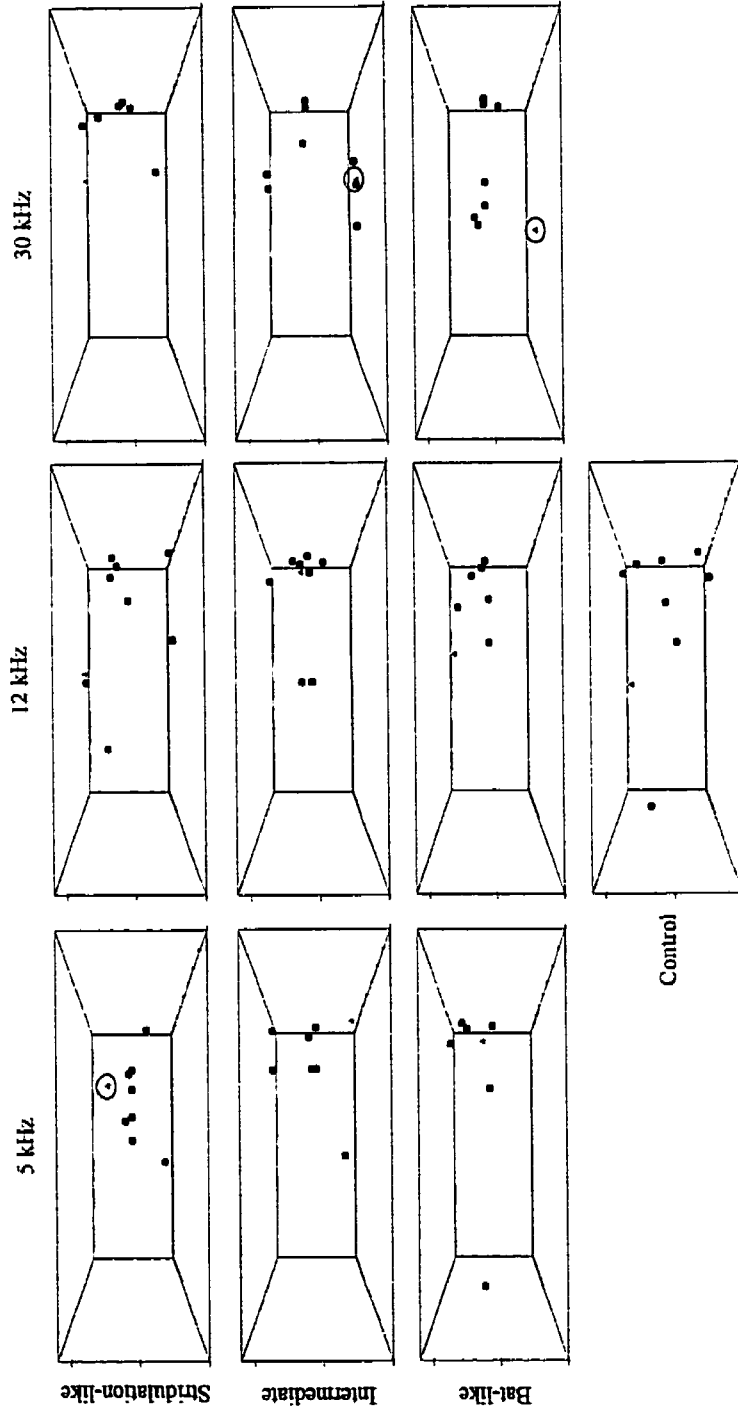
Three dimensional re-constructions of the flight paths of four locusts responding to sound in the flight room are shown in figure 4.6. This figure shows typical reactions to sound such as loops (Fig. 4.6A), turns away from the speaker (Fig. 4.6B, C). and drops to the ground (Fig. 4.6D). Figure 4.6C shows a clear increase in flight speed away from the speaker as the turn is completed. We frequently observed changes in flight speed as part of the reactions to sound. Also, many locusts would remain motionless for several seconds on the ground or walls, apparently frozen, after certain reactions, e.g. ditches to the ground.

The frequency of the different manoeuvres (e.g. left turn. right turn. loop. etc.) varied between 0 and 11% in response to the different sound patterns with net reactions to sounds occurring between 6 and 26% of flights (Fig. 4.7). The type of manoeuvre observed did not depend on the stimulus given in either full light (Fig. 4.7A) or reduced light (Fig. 4.7B) (full light: Contingency table G test. G=40.404. df=40. P=0.452; reduced light: G=24.859, df=40. P=0.971). Since the speakers were positioned on the right side of the flight path. we expected more left turns (i.e. away from the speaker) than right turns. Of all the manoeuvres that were turns. all except one were left turns in flights in full light, and all were left turns in flights in reduced light (all sound patterns combined, responses tested against an expected 1:1 ratio of left:right turns. full light: χ^2 Goodness of fit test. χ^2_c=11.529, df=1. P=0.0007; reduced light: χ^2_c=5.143. df=1. P=0.023).

When all manoeuvres were collapsed into response or no response. response rate was not contingent on the sound pattern presented. In other words, no one sound pattern elicited more responses than any other sound pattern (Fig. 4.8A) (full light: Contingency

220

Figure 4.6. Three-dimensional reconstructions of segments of the flight paths of four
different locusts responding to sounds in the flight room. A bat-like sound with
30 kHz carrier frequency elicited a loop (A) and a turn away from the speakers
(B). The locust in (C) was stimulated when flying close to the speakers and
responded with a turn and a clear increase in flight speed away from the source of
the sounds. Bat-like sounds at 12 kHz elicited a quick drop to the ground in front
of the lamp (D). Arrows indicate the time of stimulus onset and the asterisk (*)
indicates the beginning of reconstructed sequence. The length of the flight path
segments are defined by the overlapping field of views of the two cameras used to
film the responses (see methods). Sequences were typically 16 to 19 frames or
app. 260ms to 320 ms total time. Time between points is 16.7 ms

A 30 kHz, Bat-like sound
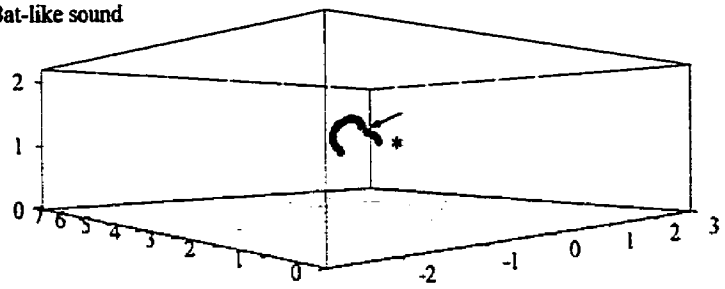
B 30 kHz, Bat-like sound
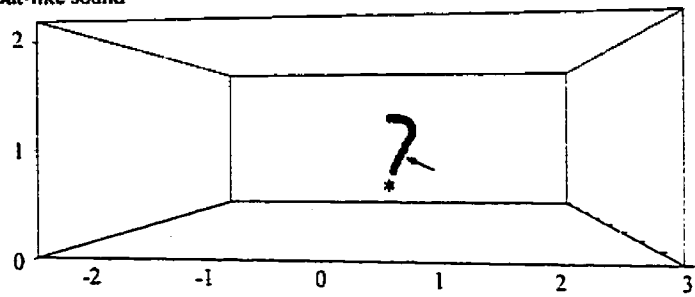
C 12 kHz, Intermediate sound
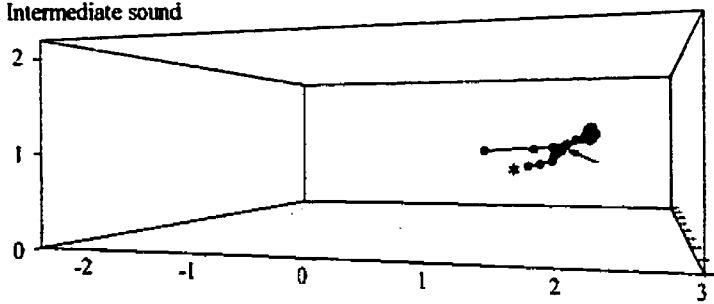
D 12 kHz, Bat-like sound
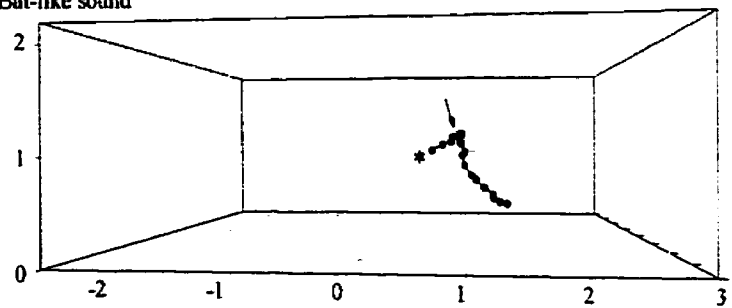
Figure 4.7. The distribution of locust responses to the nine combinations of sound patterns and frequencies in (A) full light and (B) in reduced light. The sound patterns and frequencies were chosen to establish a gradient from presumably non-aversive (stridulatory-like, 5 kHz) to aversive (bat-like, 30 kHz) stimuli. Locusts responded to all sound patterns with steering manoeuvres and the distribution of manoeuvres observed were not contingent on the stimulus presented. Response rates were low to all stimuli. Left turns (i.e. turns away from the speaker) were more frequent than right turns. Locusts were observed by two people in the flight room and the reactions of the locusts were categorised as turns (left or right), changes in altitude (up or down), loops (sometimes spiralling flight) or other. The proportion of locusts showing any of the indicated responses (R) and no responses (NR) is summed at the right of each histogram. N represents number of individuals observed.

A Full light

Stridulation-like 5 kHz · Stridulation-like 12 kHz · Stridulation-like 30 kHz

Intermediate 5 kHz · Intermediate 12 kHz · Intermediate 30 kHz

Bat-like 5 kHz · Bat-like 12 kHz · Bat-like 30 kHz

N=28 · N=27 · N=27 · N=24 · N=30 · N=30 · N=23 · N=27 · N=29

B Reduced light

Stridulation-like
5 kHz

Stridulation-like
12 kHz

Stridulation-like
30 kHz

Intermediate
5 kHz

Intermediate
12 kHz

Intermediate
30 kHz

Bat-like
5 kHz

Bat-like
12 kHz

Bat-like
30 kHz

N=15

N=15

N=10

N=16

N=15

N=8

N=16

N=15

N=10

Response (%)

table G test, G=7.895, df=8, P=0.444; reduced light: G=11.091, df=8, P=0.197). When

responses to each sound pattern were considered individually, the number of locusts

responding did not depend on the light intensity under which they were tested except for

the intermediate sound pattern presented at 12 kHz (Contingency table G test, G_c=4.270,

df=1, P=0.039; all other patterns, P=0.116 to P=0.959). When data were collapsed into

the three sound patterns, the number of locusts responding to each pattern was not

contingent on light intensity (Stridulatory-like: Contingency table G test, G_c=2.889, df=1,

P=0.089; Intermediate: G_c=2.829, df=1, P=0.093; Bat-like: G_c=0.332, df=1, P=.565).

Similarly, when data were collapsed into the three carrier frequencies of the sound

patterns, the number of locusts responding at each frequency was not contingent on light

intensity except at 5 kHz (5 kHz: Contingency Table G test, G_c=4.087, df=1, P=0.043, 12

kHz: G_c=1.154, df=1, P=0.283; 30 kHz: G_c=0.033, df=1, P=0.856) (Fig. 4.8B).

In summary, locusts in free-flight reacted to sounds while they flew past the

speakers toward the photographer's studio lamp in the corner of the flight room. The

frequency of responses did not depend on the temporal pattern of the stimulus or on

carrier frequency. A variety of reactions were observed including turns, loops and spirals

but the type of reaction also did not depend on the temporal pattern of the stimulus or

carrier frequency. Responses were also independent of light intensity. These findings

are in contrast to predictions, from previous studies using tethered locusts, that locusts in

free-flight would react to high-frequency bat-like sounds more frequently than low-

frequency sounds. To verify that our results were not artefacts of conditions in the flight

Figure 4.8. The proportion of locusts responding in full light and reduced light was not contingent on the sound pattern presented (A) or the carrier frequency of the sound pattern (B).
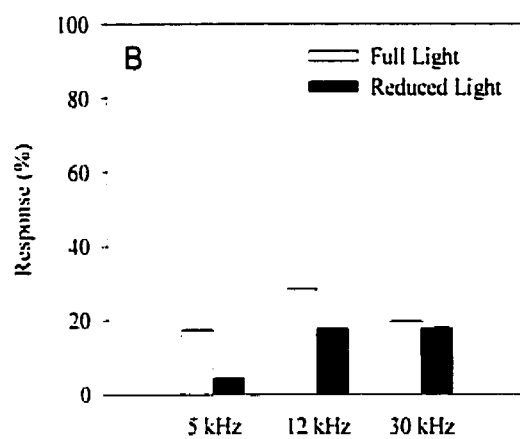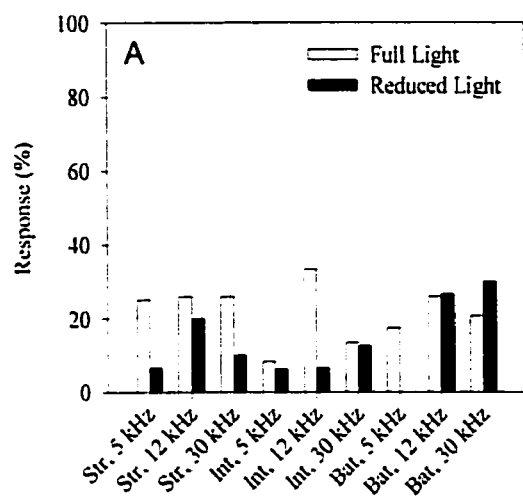
room, we examined responses of wild-caught moths and tethered locusts tested in the

same flight room under the same conditions as the free-flight tests.

4.4.3 Observations of Moths in Free-flight

The moths that we tested in the flight room are listed in Table 4.1. We tested a total of 74

individuals derived from three superfamilies and seven families. The majority of moths

tested did not fly in a straight flight path from the point of launch to the lamp in the

corner of the flight room. There was considerable variation both within and between

moth species in their flight tendencies. Typically, moths flew with intermittent bouts of

fast and slow flight sequences punctuated with frequent turns and changes in elevation.

Notable examples of this type of flight behaviour come from *Ochropleura plecta*

(Noctuidae) and *Ochrostigma melagona* (Notodontidae). However, other species such as

Craniophora ligustri (Nocuidae), *Diacrisia sannio* (Arctiidae), and *Phoesia tremula*

(Notodontidae) flew with slower (flight speeds estimated to be less than 1 m/s), mostly

straight, smooth flights from the point of release to the walls or lights (not necessarily the

photographer's lamp). Because the moths did not fly in a straight path from launch to the

light past the speakers, we did not distinguish between left and right turns.

The proportion of moths responding to bat-like sounds at 5, 12, and 30 kHz with

the different manoeuvres above are presented in figure 4.9A. The manoeuvres with which

the moths responded (turn, loop, etc.) were not contingent on the sound presented in

either full light or reduced light (full light: Contingency table G test, $G=9.6751$, $df=8$,

$P=0.289$; reduced light: $G=5.4258$, $df=8$, $P=0.711$). More moths responded to 30 kHz

Table 4.1. Moth species tested with bat-like sounds in free-flight in the flight room.

Super Family	Family	Genus & Species[1] Author	N[2]
Drepanoidea	Drepanidae	*Drepana falcataria* L.	1
Geometroidea	Geometridae	*Cabera exanthemata* Scopoli	1
	Geometridae	*Opisthograptis luteolata* L.	4
	Geometridae	*Peribatodes (=Boarmia) rhomboidaria* Schiff.	3
	Geometridae	*Plagodis dolabraria* L.	1
	Thyatiridae	*Thyatira batis* L.	1
Noctuoidea	Arctiidae	*Diacrisia sannio* L.	3
	Arctiidae	*Phragmatobia fuliginosa* L.	11
	Lymantriidae	*Porthesia similis* Fuessly	1
	Noctuidae	*Autographa (=Phytometra) gamma* L.	3
	Noctuidae	*Cosmia (=Calymnia) trapezina* L.	2
	Noctuidae	*Craniophora ligustri* Schiff.	9
	Noctuidae	*Ipimorpha subtusa* Schiff.	1
	Noctuidae	*Mythimna ferrago* Fabricius	2
	Noctuidae	*Mythimna (=Sideridis) impura* Hbn.	2
	Noctuidae	*Ochropleura plecta* L.	11
	Noctuidae	*Pharetra rumicis* L.	2
	Noctuidae	*Rhyacia (=Amathes, =Xestia) c-nigrum* L.	2
	Noctuidae	*Talpophila matura* Hufn.	3
	Notodontidae	*Notodonta dromedarius* L.	1
	Notodontidae	*Ochrostigma melagona* Brkh.	8
	Notodontidae	*Pheosia tremula* Clerk.	2

Total number of individual moths tested: 74

[1]Genus & species names follow Forster and Wohlfart (1960, 1971, 1981); genus names in parentheses indicate synonyms.
[2]Number of individuals observed.

bat-like sounds than to 5 or 12 kHz sounds in both full light and reduced light (full light:

Contingency table G test, G=62.3654, df=2, P<<0.0001; reduced light: G=108.4610,

df=2, P<<0.0001) (Fig. 4.9B). The number of moths reacting to bat-like sounds at each

of the three frequencies was not contingent on light intensity (5 kHz, Contingency table

G test, G_c=0.037, df=1, P=0.848; 12 kHz, G_c=0.112, df=1, P=0.738; 30 kHz, G_c=3.190,

df=1, P=0.074).

4.4.4 Observations of Locusts Tethered in the Flight Room

Locusts were tethered at speaker height at two locations in the flight room: a location

near to the speaker (co-ordinates 2,6) and one farther from the speaker (co-ordinates 1,5)

At both locations, locusts responded to sound with: flight cessation, deflection of the

abdomen and hindlegs either toward or away from the speaker, extending a hindleg by

opening the femoral-tibial leg joint, dorsiflection of the abdomen, skipping a stroke in the

normal wing beat cycle, and increasing wing beat frequency. From our videotapes, we

carefully noted the presence of these and other clear responses to the onset of the sound

stimulus. Typically responses occurred within three wingbeats (approximately 150-

200 ms) of the stimulus and behaviours observed outside of this window were deemed

not to be a reaction to the sound. Similarly, locusts that were exhibiting aspects of the

above behaviours before the stimulus was applied were discarded from analysis. Since

the above responses to bat-like sounds of tethered locusts have been amply documented

in the literature (Robert, 1989; Robert and Rowell, 1992; Dawson et al., 1997), we

Figure 4.9. The reaction of moths to bat-like sounds at 5, 12, and 30 kHz in the flight. The type of reaction (turns, changes in elevation, loops) observed in response to the stimulus did not depend on the frequency of the sound (A), but moths responded more often to 30 kHz sounds than to 5 or 12 kHz sounds (B). The number of moths responding in full light was not different than the number responding in reduced light.

A   Free-flying moths tested in the flight room

Bat-like, 5 kHz Full Light — N=47

Bat-like, 12 kHz Full Light — N=42

Bat-like, 30 kHz Full Light — N=56

Bat-like, 5 kHz Reduced Light — N=59

Bat-like, 12 kHz Reduced Light — N=49

Bat-like, 30 kHz Reduced Light — N=66

B Free-flying moths tested in the flight room

Legend: Full Light, Reduced Light

Y-axis: Response (%) — 0, 20, 40, 60, 80, 100

X-axis: 5 kHz, 12 kHz, 30 kHz
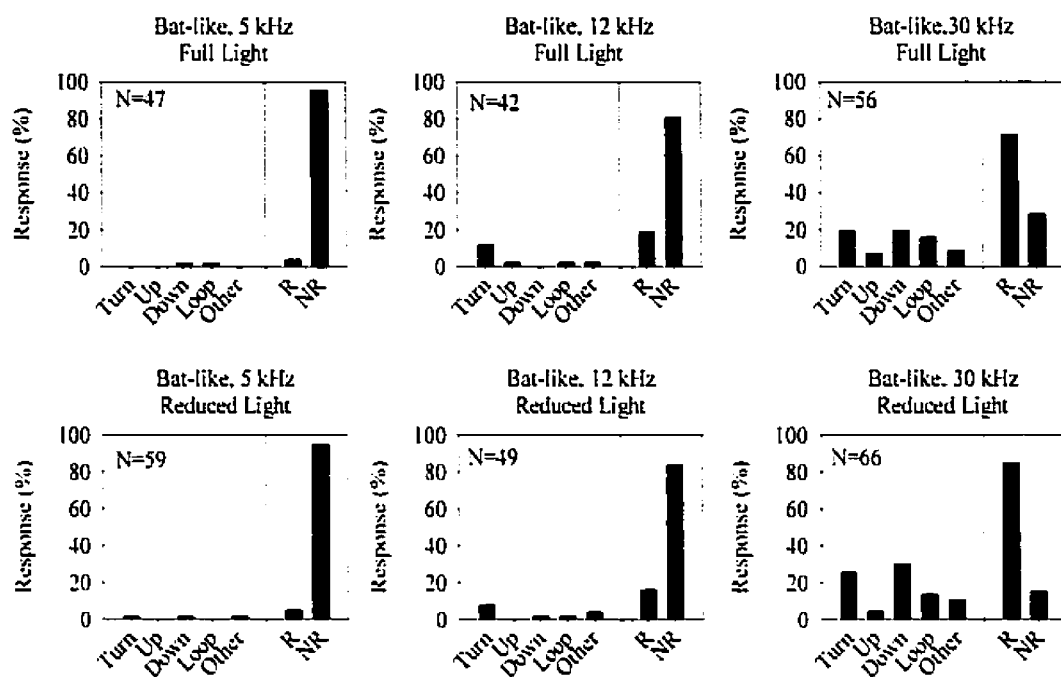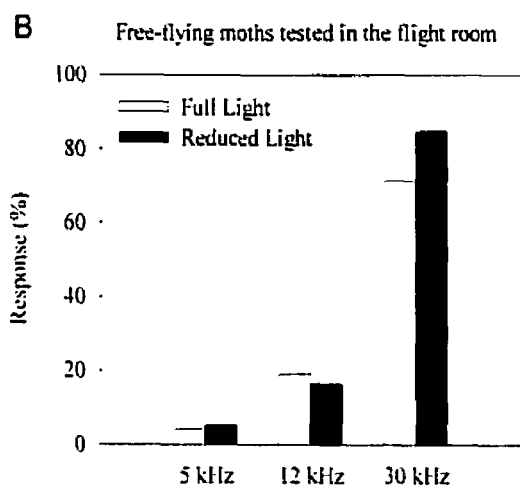
Figure 4.10. Tethered locusts responded to bat-like sounds with flight cessation, abdomen deflection and dorsiflexion. deflections or extensions of the legs and changes in the wing beat including increases in wing beat frequency. The number of locusts responding was contingent on the frequency of the bat-like sounds with more locusts responding to the high frequencies at both near and far (relative to the speaker) tether locations. More locusts responded at the near tether than to the far tether. Response rates were not different in full light and reduced light.

Tethered locusts in the flight room
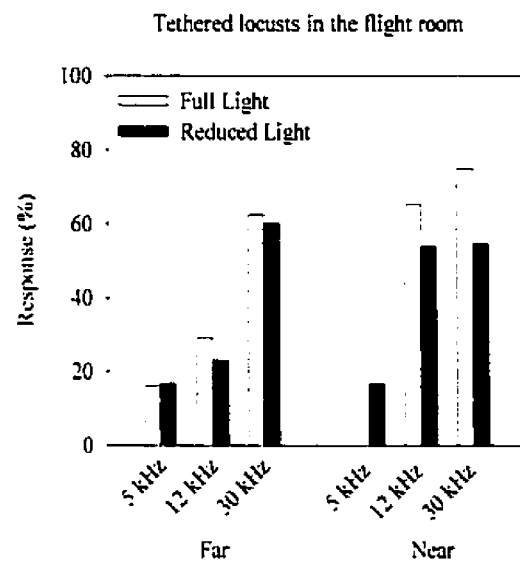
simply counted the number of locusts responding and not responding from our detailed notes.

The proportion of locusts responding to bat-like sounds with carrier frequencies of 5, 12 and 30 kHz, in full light and reduced light, at the two tether locations, are presented in figure 4.10. Sound intensity was greater at the near location than at the far location for all frequencies tested (12 dB louder at 30 kHz, 2 dB louder at 12 kHz, and 7 dB louder at 5 kHz). Overall, more locusts responded to high-frequency than to low frequency stimuli and responses were more frequent to 12 and 30 kHz stimuli at the near tether than at the far tether. In full light, the proportion of locusts responding was contingent upon the carrier frequency of the bat-like sounds with more locusts responding to 30 kHz (near: Contingency table G test, G=18.096, df=2, P=0.0001; far: G=12.359, df=2, P=0.002); the same trend is present in reduced light, but is not statistically significant (near: Contingency table G test, G=4.985, df=2, P=0.082: far: G=5.255, df=2, P=0.072) most likely because fewer locusts were tested in reduced light. When each frequency tested is considered independently, the number of locusts responding in full light and reduced light are not different (Contingency table G test, P values range 0.294 to 0.961).

4.5 Discussion

The purpose of this study was to determine if locusts would react to sounds while in free-flight and to determine if the reactions were bat-avoidance responses. From previously published observations of tethered locusts responding to bat-like sounds, including

behavioural audiograms (Robert, 1989; Dawson *et al.*, 1997), we predicted that locusts in

free-flight would show banked, downward turns away from our sound source depending

on the frequency and the pulse structure of the auditory stimulus presented. We found

locusts responded to sounds with a diversity of manoeuvres consisting of turns, loops and

dives. Although these manoeuvres are typical of other insect reactions to bats, what was

unexpected was that response rates of free-flying locusts were independent of carrier

frequency and pulse structure of the stimulus, and further, that response rates to high

frequency sound (30 kHz) were lower than expected from observations of tethered

locusts.

4.5.1 Behavioural Audiogram

With few exceptions (e.g. Popov, 1981; Mason, 1991, Fonseca *et al.*, 2000; Yack *et al.*,

2000), the tuning of an insect's ear is matched to the range of sounds of biological

relevance to the insect (Fullard and Yack, 1993). Extracellular tympanal nerve (N6)

recordings, intracellular receptor recordings and tympanal membrane vibration

recordings have shown that locust ears are sensitive to sounds from 2 kHz to greater than

40 kHz (Michelsen, 1971a, Kalmring, 1975a, Meyer and Elsner, 1996). Our behavioural

audiogram, which measured the threshold for eliciting abdomen deflection away from the

sound source (i.e. an avoidance response of the whole animal) found a best frequency of

30 kHz at a threshold of 54 dB SPL and agrees with Robert (1989) who found a best

frequency of 25 kHz at 45 dB threshold. The slight disagreement is most likely due to

differences in our criteria for threshold (Michelsen, 1971b) and in the stimulus used for

eliciting responses; Robert (1989) used longer duration stimulus pulses (20 ms) presented

in trains with longer periods (10 pps). Our behavioural audiogram, recorded from

tethered, flying locusts, suggests that although locusts can detect a wide frequency range,

it is the high frequency sounds that are important for a locust to detect and avoid while in

flight.

4.5.2 Observations of Locusts in Free-flight

If the ASR elicited in free-flying locusts was a bat-avoidance response then response

rates of free-flying locusts would have been frequency dependent with higher response

rates being elicited with high-frequency bat-like sounds than low-frequency sounds.

Further, if responses elicited in tethered locusts were an indication of free-flight

performance, as is widely believed, then response rates of free-flying locusts to the three

bat-like sound patterns should have been similar to those observed in tethered flying

locusts. We found response rates were independent of both carrier frequency and pulse

structure of the stimulus and that response rates were low (between 13% and 26%) to

sounds at all frequencies. Had our observations of free-flying locusts shown high

response rates to high-frequency bat-like sounds, a likely conclusion would have been

that the selection pressure exerted by echolocating bats, presumably the source of high-

frequency sounds encountered by locusts flying at night, would have been responsible for

the tuning of the ASR. Since this was not the case, we must determine if our discrepancy

is due to technical problems, such as observer error, poor stimuli, or artefacts in the flight

room, or due to biological factors.

All flight tests were observed by two people from different vantage points in the room and both observers had to agree on what was seen before it was tallied. Disagreements resulted in individual locusts being re-flown or in flights being discarded from the data set. We also flew animals without presenting sound to verify that responses were reactions to the stimulus and not to other factors and locusts that flew erratically or responded in the middle of the flight room in the absence of sound were discarded from the data set. Thus we reduced our sample size while retaining locusts that showed definite reactions. This increased the percentage of responding locusts and thus our estimates of response rate are arguably generous. With this said, however, we realize that the relatively small numbers of locusts tested in the flight room under the different conditions means that certain of our negative findings should be interpreted cautiously.

We are confident that the quality of the stimuli presented in the flight room, which were spectrally verified (see methods), were sufficient to have elicited responses from free-flying locusts because we saw steering responses from free-flying moths and locusts tethered at two different locations in the sound field. Although we adjusted the intensities of 5 and 12 kHz stimuli to match the intensity of 30 kHz stimuli at the point of stimulation as locusts flew though the flight zone, the maximum intensity that could be broadcast from our speakers was 98 dB at 5 kHz and 84 dB at 12 kHz also measured at the location where locusts were stimulated. We ran trials at these maximum intensities and response rates (data not shown) were higher (between 12% and 48%) for all the frequencies and sound patterns tested but response rates were still not statistically dependent on the frequency or sound pattern tested. Thus sound intensity alone can not

explain why free-flying locusts exposed to 30 kHz sound patterns did not respond as

often as tethered locusts. Further, the intensity of the stimulus pulses at 5 and 12 kHz (78

to 81 dB) may have been subthreshold for the ASR measured in our behavioural

audiogram, but these intensities were well above threshold for the auditory abilities of the

ear in this frequency range. Locusts reacted to all of the sounds irrespective of pattern

and carrier frequency as aversive because all turns (except one) observed in free-flying

locusts were away from the speakers not just responses to the bat-like, high-frequency

pattern as was expected.

Robert (1989) showed that pure tone stimuli (mimicking a hunting bat) and two-

tone stimuli, consisting of a 30 kHz tone presented concurrently with a 5 kHz tone 10 to

15 dB more intense than the first, have different behavioural effects with the former

eliciting negative phonotaxis and the latter suppressing it in *L. migratoria*. Robert

suggested that the biological significance of the difference in behavioural effects would

be to suppress an ASR that would otherwise be elicited by high-frequency components in

the sounds produced by beating wings. We agree with this interpretation, however, we

do not believe it is the reason for our low response rates to 30 kHz stimuli.

Measurements from *Schistocerca gregaria* show that wingbeat noise is composed

primarily of frequencies between 3.2 and 5 kHz and has an intensity between 62 and 71

dB (Haskell, 1957). Locusts flying in the sound field (Fig. 4.3A, B, C) in the flight room

would always be receiving stimuli that are, at a minimum, 5 dB greater than the

intensities of wing beat noise and therefore suppression of the ASR is not a likely

explanation. Further, Boyan (1986) found that auditory sensitivity in *L. migratoria* may

be modulated by wind (as might be produced during flight) in a frequency dependent

fashion.   He found responses of certain auditory interneurones are suppressed by low

frequencies (5 kHz) while responses of other interneurons (e.g. 714) are potentiated by

high frequency (12 kHz) sounds in the presence of a wind stimulus. The speculation is

that the shift in sensitivity might prevent confusion between low frequency sounds in the

environment with wing beat noise and that increased sensitivity to high frequency sounds

might facilitate the detection of conspecifics and/or predators.

One possible explanation for the discrepancy in response rates to 30 kHz sounds

might be that locusts flying past a stationary speaker emitting a pattern of calls

mimicking a feeding buzz or searching calls of a bat, at constant intensity, may not be a

good simulation of an insect-bat encounter. Inspection of strobe photographs of moths

(Roeder, 1962) and lacewings (Miller and Oleson, 1979) interacting with bats show that

the movements of the predator and prey through three dimensional space are complex

and that the intensity of the emitted calls, as perceived by the insect, would be more

variable than those perceived by locusts in our flight room. A similar situation would be

true when tethering an insect at a fixed distance to a speaker: an equally unrealistic

situation. Tethering ensures that the intensity of the sounds perceived by the insect is

constant during a stimulus and thus response rates of tethered locusts (and other insects)

may be generous.

After all is considered above, we remain trying to explain why the response rates

of free-flying locusts to high frequency sounds (30 kHz) were lower than response rates

of tethered locusts in the same flight room, under the same lighting conditions, and in the

same locations relative to the speakers as the free-flying locusts. Because there are

numerous interacting factors that could account for our discrepancy, we turned to

observations of moths in the flight room as a "biological control". There is now ample

evidence in the literature that bats are the predominant selection pressure shaping the

acoustic startle response of moths and, our belief was that if locusts are also preyed on by

bats, then their response rates to 30 kHz bat-like sounds should be similar to those of

moths tested in the same flight room, under the same light conditions.

4.5.3 Observations of Moths in Free-flight

Moths caught from wild populations around the University of Konstanz tested in the

flight room showed high response rates (75% to 85%) to high frequency (30 kHz) bat-

like sounds and few reactions to low frequency (5 kHz) bat-like sounds. Moths did not

have difficulty flying in the flight room under either light intensity and our observations

are consistent with both field and flight room observations by other researchers. Roeder

(1962) observed 87.5% of wild moths responding to bat-like sounds being broadcast.

These responses were composed of 49% dives, 30% turns and 8.5% loops. Similarly,

Rydell et al., (1997) observed behavioural reactions of geometrid winter moths (Agriopis

spp., Erannis spp. and Alsophila aescularia) to 26 kHz pure tones presented in pulses

controlled by hand (5-10 pulses over 1-2 seconds) in the field and in a flight room. In the

field, 96% of moths responded to bat-like sounds with zigzags (at distances greater than 5

m), spiralling and diving flight (at distances less than 5 m), and in a flight room, 100% of

trials showed responses.

Unlike locusts. the frequency dependant nature of the responses of moths can be explained by the tuning characteristics of the moth tympanic organ as noctuoid moths are. at present, believed not able to discriminate frequency (further, we acknowledge that frequency discrimination in geometrid moths is an untested hypothesis (Surlykke and Filskov, 1997)). For the moth families tested, the sensitivity of the ears, as revealed by audiograms recorded from extracellular tympanic nerve recordings, is greatest for frequencies from 25 to 70 kHz (Fullard, 1988; Surlykke and Filskov, 1997; Fullard and Dawson, 1999) and is likely a reflection of the frequencies in the calls of echolocating bats in the area around Konstanz (Roeder, 1970; Fullard, 1982).

Moths responded with turns. loops, dives. and a number of other manoeuvres that were clear responses to the stimulus (grouped as ‘other’ in Fig. 4.9A). These manoeuvres are similar to those observed by Roeder (1962). Roeder observed a variety of responses of moths responding to bat-like sounds (70 kHz pulse trains) broadcast from a speaker atop a tall pole in the field. He described some responses as sharp dives. where the moth kept its wings motionless during descent. as power dives. where the moth flew toward the ground at a rapid rate. and passive dives. where the descent was interrupted by wing movements. Responses also consisted of deviations from a horizontal flight path (turns). as well as series of loops and tight turns. Roeder admitted having difficulty categorizing the behaviours (as we did for locusts and moths) because in many instances one type of response would be followed by another. for example, a dive preceded by a tight climb. loop or turn. Responses also involved increases in wing beat frequency of 10 to 20 %. Response latencies in free-flying moths were between 0.2 and 1 second. Roeder also

noticed that moths that were stimulated when far from the speaker tended to react with

turns whereas moths that were closer to the speaker at the time of stimulation would react

with dives, spirals and loops suggesting that intensity might be a cue for the behavioural

reaction. We neither observed, nor looked for effects of sound intensity given the size of

our flight room and the intensities of sounds we presented. We did notice that moths that

turned away from the speakers relative to moths that dived, looped and spiralled, did so

when close to the speaker or when far from the speaker. This does not discount Roeder's

hypothesis but shows that moth responses are quite variable, the variability perhaps

supporting his hypothesis that it adds to the survival value of the moth escape response

by making it more difficult for a predator in pursuit (Roeder, 1975). When stimulation

resulted in moths landing, often they remained motionless for several seconds; an

observation we also made of many free-flying locusts immediately after a response was

observed.

4.5.4 Observations of Locusts Tethered in The Flight Room

We tethered the locusts in positions in the flight room that corresponded with locations

along the flight path of free-flying locusts. We saw higher response rates to both 12 and

30 kHz bat-like sounds when the tether was closer to the speaker. The reactions were not

different from observations previously published for locusts responding to high-

frequency sounds, namely, abdomen deflection and dorsiflexion, hindleg extension,

changes in wing beat frequency including flight cessation and, changes in wing

kinematics (Hoy *et al.*, 1989; Robert, 1989; Robert and Rowell, 1992; Dawson *et al.*,

1997). Our response rates at 5, 12, and 30 kHz are consistent with our data for thresholds of the ASR at these frequencies. When locusts were tethered at the far location (co-ordinates 1,5), the sound intensity for 5 kHz and 12 kHz stimulation was 80 dB and for 30 kHz was 78 dB. The thresholds for the behavioural audiogram are above 80 dB for 5 and 12 kHz but is 54 dB for 30 kHz. Therefore at 30 kHz, the stimulus was approximately 24 dB above threshold. Similarly, at the near tether, the sound intensity for 5 kHz was 87 dB. for 12 kHz, was 82 dB and at 30 kHz, was 90 dB. These intensities were above threshold for the ASR at both 12 and 30 kHz but not 5 kHz and we saw high response rates to both 12 and 30 kHz stimuli (Fig. 4.10). As stated above, the locust ear is broadly tuned to frequencies from 2 kHz to more than 40 kHz and it is unlikely that locusts could not hear the 5 kHz or 12 kHz sounds. Our data shows that locusts simply did not respond to those sounds with ASR-typical behaviours.

Another factor likely to contribute to higher response rates, in general, of tethered locusts is that it is easier to see and score responses from a tethered animal than from one in free-flight. We videotaped the tethered locusts and scored responses after watching the tapes, sometimes using a frame-by-frame shuttle jog. Using this technology it is possible to see very subtle movements of the locust in response to the stimuli. This might partially account for why response rates for tethered locusts at 30 kHz are higher than for free-flying locusts but does not account for why they are the same at 5 and 12 kHz. While observing the tapes we took detailed notes of all behaviours (postural adjustments) occurring in response to all the sounds. If we were able to see reactions in tethered locusts but not in free-flying locusts, it suggests that some of the postural adjustments

that are elicited are either not elicited in free-flight or do not have behavioural

consequences (i.e. aerodynamic significance) during free-flight.

4.5.5 Effects of Light Intensity

The locusts we used in this study were all in the gregarious phase and reports of locusts

flying at night are typically in the solitary phase (Kennedy, 1956; numerous citations in

Uvarov, 1977; see also Farrow, 1990; Gatehouse and Zhang, 1995). To our knowledge

nobody has examined differences in auditory sensitivity between gregarious and solitary

L. migratoria. We tested locusts and moths under full and reduced light and our logic for

this was simple: that animals flying in light conditions comparable to dusk (less than 100

lux) might be more sensitive to high-frequency sounds because ambient light intensity

may be a cue signalling potential threat from bats. *Locusta migratoria* flight can be

stimulated or inhibited by abrupt changes in light intensity (Uvarov, 1977). We did not

find a systematic effect of light intensity on the reactions of the locust to sounds except

that locusts were more reluctant to fly under our reduced light conditions (note smaller

sample sizes in Fig. 4.7B). When ambient light levels were less than approximately 2

lux, we had difficulty launching locusts into flight wherein they would immediately land

on the ground and remain still.

We found that locusts strongly orient toward sources of bright light (relative to

background light intensity). Our light intensity calibrations confirm the brightest point in

the room was the photographer's lamp and the direction of maximum light intensity was

always in the direction of the lamp. Boyan (1989) showed that a flight interneuron, 529,

which receives both auditory and visual input, is gated by the presence of wind input to

the locust. In instances where visual and auditory information are present during a wind

stimulus (i.e. during flight) visual information takes precedence over auditory input. It is

possible, therefore, that visual orientation by the locust to the light we used to establish a

flight path past the speakers overrode the ASR. With this noted, however, tethered

locusts were tested while facing the photographer's studio lamp and because their

responses to 30 kHz bat-like sounds were higher than our free-flight responses, we can

not conclude this is the sole reason for our discrepancy.

4.5.6 Tethering Effects and Auditory Sensitivity

Free-flying locusts respond to sound with avoidance manoeuvres that are similar to

avoidance manoeuvres observed in moths, lacewings, and praying mantises. This is in

contrast to tethered locusts that react more often to high-frequency sounds. We have

considered many technical reasons (lighting, speaker position, room effects, etc.) for this

disagreement: however, observations of free-flying moths in the flight room under

identical conditions as locusts strongly suggests that biological factors underlie the

differences between free-flying and tethered locusts.

    We feel the most plausible biological explanation for our results is that tethering

affects the auditory system of locusts. During tethered flight, movement of the wings and

mechanical deformations of the thorax produce phasic and tonic activity in the auditory

nerve (N6) that arises from stimulation of the receptors at the tympanum (Hedwig, 1988).

This activity results in the auditory system being approximately 20 dB less sensitive to

certain (low frequency) sounds during flight relative to when an animal is stationary. It is therefore possible that tethering artifactually accentuates high-frequency responses.

Tethering may also affect the processing of auditory information within the locust CNS. Tethering removes sensory input normally present during flight such as an optical flow field (Baader, 1991; Baader, *et al.*, 1992; Spork and Preiss, 1991), input to wind sensitive hairs, and proprioceptive feedback from cuticular receptors. These differences may result in tethered locusts behaving acoustically as locusts on a substrate. When a locust is on the ground sound plays an important role in its biology and like other orthopterans, the ability to distinguish different types of sounds in the environment is adaptive. For crickets, pitch has been shown to be one cue for this categorical discrimination (Wyttenbach *et al.*, 1996). For locusts, low-frequency sounds such as stridulation, mandible clicking, wing-beat noise, and even wind are not aversive stimuli whereas higher-frequency sounds, such as might be produced by gleaning bats and rodents, or coincident sounds such as rustling vegetation produced by terrestrial vertebrate predators, signal potential dangers (Sales and Pye, 1974; Fullard, 1988). The eastern sword-bearer conehead, *Neoconocephalus ensiger*, possesses an ASR in which it ceases or pauses calling when stimulated with high-frequency, but not low frequency, sounds (Faure and Hoy, 2000). Tethered locusts may categorically discriminate sounds and produce avoidance reactions preferentially to high frequency stimuli. Free-flying locusts, lacking the mechanical interference of a tether, might not gate their responses according to frequency. In this case, a free-flying locust may openly gate all auditory responses to an avoidance reaction. This would be a conservative response to all sounds.

both alluring and aversive, but would be adaptive when the source of sounds is a predator such as an insectivorous bat.

The results of this study raise an important question: *Do aerially-hawking bats eat locusts?* To our knowledge, the remains of *L. migratoria*, or other locust species, have not been positively identified in stomach or faecal pellet contents of bats. This result is surprising given that locusts would be a meal of nutritional value to a bat given their high protein and carbohydrate content (Uvarov, 1966). Locusts are palatable to birds (Uvarov, 1966) and orthopterans in general, are palatable to gleaning bats (La Val and La Val, 1980; Belwood and Morris, 1987). Locusts also are known to fly in open environments at altitudes as high as 100m to 1000m during migratory flights and during dispersal flights of solitary-phase individuals (Chapman, 1976; Riley and Reynolds, 1997). Many large, high-flying, aerial hawking, bats (i.e. predicted characteristics of bats that might feed on locusts [see Freeman, 1981; Barclay and Brigham, 1991; 1994; Waters *et al.*, 1995]) e.g. *Tadarida* spp. (Kingdon, 1974) are found in the same geographic areas as locusts and use echolocation calls with peak frequencies around 30 kHz (e.g. *Taphozous mauritianus*, 25 kHz [Fenton *et al.*, 1980], larger *Tarida* spp., ~25 kHz and *Scotophilus nigrita*, 30 kHz [Fenton and Bell, 1981]). These frequencies are in agreement with the best frequency of the behavioural audiogram for the ASR recorded for tethered flight; however, as we have found in this study, locusts will react with avoidance manoeuvres to *many* sounds while in free-flight, not specifically bat-like, high-frequency sounds.

Free-flying locusts may have low response rates relative to moths because they possess additional defences against bat predation. One defence may be in the tendencies

of locusts to aggregate in large numbers and fly in large swarms (Uvarov, 1943).

Swamping is a strategy used by many animals, such as mayflies (Sweeney and Vannote,

1982), monarch butterflies (Calvert et al., 1979; Brower and Calvert, 1985), and

minnows (Hager and Helfman, 1991) for protection from predators. Detecting the

echolocation calls of bats is not the only way of avoiding predation by bats. Many moth

species (both eared and earless) avoid bats by flying at times that bats are not active,

flying at altitudes different than bats, or flying erratically (Roeder, 1974; Morrill and

Fullard, 1992; Lewis et al., 1993). Others (e.g. Saturniidae, Sphingidae) enjoy protection

by their size and agility in flight (Roeder, 1974). It is not unreasonable to think that the

large size of locusts, and their agile flight and flight heights offers them additional

mechanisms of protection from bats. It must be noted, however, that possessing

alternative defensives to auditory detection of bats is not in itself sufficient to cause a

decrease in sensitivity of the ear to high-frequency sounds (Fullard and Dawson, 1999)

4.7 Literature Cited

Arbas, E. A. (1986). Control of hindlimb posture by wind-sensitive hairs and antennae during locust flight. *J. Comp. Physiol. A* **159**, 849-857.

Baader, A. (1991). Auditory interneurons in locusts produce directional head and abdomen movements. *J. Comp. Physiol. A*, **169**, 87-100.

Baader, A., Schäfer, M., and Rowell, C. H. F. (1992). The perception of the visual flow field by flying locusts: A behavioural and neuronal analysis. *J. Exp. Biol.*, **165**, 137-160.

Barclay, R. M. R., and Brigham, R. M. (1991). Prey detection, dietary niche breadth, and body size in bats: why are aerial insectivorous bats so small? *Am. Nat.* **137**(5), 693-703.

Barclay, R. M. R., and Brigham, R. M. (1994). Constraints on optimal foraging: a field test of prey discrimination by echolocating insectivorous bats. *Anim. Behav.* **48**, 1013-1021.

Bauer, R. (1993). Untersuchung zur Anlockung von nachtaktiven Insekten durch Beleuchtungseinrichtungen. Diploma thesis, University of Konstanz.

Belwood, J. J. and Morris, G. K. (1987). Bat predation and its influence on calling behaviour in neotropical katydids. *Science*, **238**, 64-67.

Boyan, G. S. (1986). Modulation of auditory responsiveness in the locust. *J. Comp. Physiol. A*. **158**, 813-825.

Boyan, G. S. (1989). Synaptic input converging onto an identified premotor interneuron

in the locust is gated according to modality. *Naturwissenschaften* **76**, 431-433.

Brower, L. P. and Calvert, W. H. (1985). Foraging dynamics of bird predators on

overwintering monarch butterflies in Mexico. *Evolution* **39**(4), 852-868.

Calvert, W. H., Hedrick, L. E., and Brower, L. P. (1979). Mortality of the monarch

butterfly (*Danaus plexippus* L.): Avian predation at five overwintering sites in

Mexico. *Science* **204**, 847-851.

Camhi, J. M. (1970). Yaw correcting postural changes in locusts. *J. Exp. Biol.*, **52**, 519-

31.

Chapman, R. F. (1976). *A Biology of Locusts*. London: Edward Arnold Ltd.

Dawson, J.W., Dawson-Scully, K., Robert, D. and Robertson, R. M. (1997). Forewing

asymmetries during auditory avoidance in flying locusts. *J. Exp. Biol.* **200**, 2323-

2335.

Doherty, J. and Hoy, R. (1985). Communication in insects. III. The auditory behaviour

of crickets: some views of genetic coupling, song recognition, and predator

detection. *Q. Rev. Biol.* **60**, 457-472.

Farrow, R. A. (1990). Flight and migration in Acridoids. In *Biology of Grasshoppers*

(ed. R. F. Chapman and A. Joern). pp. 227-314. New York: John Wiley and Sons.

Faure. P. A. and Hoy, R. R. (2000). The sounds of silence: cessation of singing and

song pausing are ultrasound-induced acoustic startle behaviors in the katydid

Neoconocephalus ensiger (Orthoptera; Tettigoniidae). *J. Comp. Physiol A* **186**,

129-142.

Fenton, M. B. and Bell G. P. (1981). Recognition of species of insectivorous bats by

their echolocation calls. *J. Mamm.* **62**(2), 233-243.

Fenton, M. B., Bell, G. P., and Thomas, D. W. (1980). Echolocation and feeding

behaviour of *Taphozous mauritianus* (Chiroptera: Emballonuridae). *Can. J. Zool.,*

58, 1774-1777.

Fischer, H. and Ebert, E. (1999). Tegula function during free locust flight in relation to

motor pattern, flight speed and aerodynamic output. *J. Exp. Biol.* 202, 711-721.

Fischer, H. and Kutsch, W. (1999). Timing of elevator muscle activity during climbing

in free locust flight. *J. Exp. Biol.* **202**, 3575-3586.

Fonseca. P. J., Münch, D., and Hennig, R. M. (2000). How cicadas interpret acoustic

signals. *Nature* **405**, 297-298.

Forrest, T. G., Farris, H. E. and Hoy, R. R. (1995). Ultrasound acoustic startle

response in scarab beetles. *J. Exp. Biol.* **198**, 2593-2598.

Forster, W. and Wohlfahrt, T. A. (1960). *Die Schmetterlinge Mitteleuropas. Band III:*

Spinner und Schwärmer (Bombyces und Sphinges). Franckh'sche

Verlagshandlung. Stuttgart: W. Keller & Co.

Forster, W. and Wohlfahrt, T. A. (1971). *Die Schmetterlinge Mitteleuropas. Band IV:*

Eulen (Noctuidae). Franckh'sche Verlagshandlung. Stuttgart: W. Keller & Co.

Forster, W. and Wohlfahrt, T. A. (1981). *Die Schmetterlinge Mitteleuropas. Band V:*

Spanner (Geometridae). Franckh'sche Verlagshandlung. Stuttgart: W. Keller &

Co.

Freeman, P. W. (1981). Correspondence of food habits and morphology in insectivorous

bats. *J. Mamm.* **62**(10), 166-173.

Fullard, J. H. (1982). Echolocation assemblages and their effects on moth auditory

systems. *Can. J. Zool.* **60**, 2572-2576.

Fullard, J. H. (1988). The tuning of moth ears. *Experientia* **44**, 423-428.

Fullard, J. H. and Dawson, J. W. (1999). Why do diurnal moths have ears?

Naturwissenschaften **86**, 276-279.

Fullard, J. H. and Yack, J. E. (1993). The evolutionary biology of insect hearing.

Trends Ecol. Evol. **8**(7), 248-252.

Gatehouse. A. G. and Zhang, X. –X. (1995). Migratory potential in insects: variation in

an uncertain environment. In *Insect migration: Tracking resources through space

and time* (ed. V. A. Drake and A. G. Gatehouse). pp. 193-242. London:

Cambridge University Press.

Hager. M. C. and Helfman, G. S. (1991). Safety in numbers: shoal size choice by

minnows under predatory threat. *Behav. Ecol. Sociobiol.* **29**, 271-276

Haskell. P. T. (1957). The influence of flight noise on the behaviour in the desert locust

Schistocerca gregaria (Forsk.). *J. Insect Physiol.* **1**, 52-75.

Hedwig, B. (1988). Modulation of auditory information processing in tethered flying

locusts. *J. Comp. Physiol.* A. **164**, 409-422.

Hoy, R. R. (1989). Startle, categorical response. and attention in acoustic behavior of

insects. *Ann. Rev. Neurosci.* **12**, 355-377.

Hoy, R. R. (1992). The evolution of hearing in insects as an adaptation to predation from

bats. In *The Evolutionary Biology of Hearing* (ed. D. B. Webster, R. R. Fay, and

A. N. Popper). pp115-129. New York: Springer-Verlag.

Hoy, R. R. and Robert, D. (1996). Tympanal hearing in insects. *Annu. Rev. Entomol.*

41, 433-450.

Hoy, R. R., Nolan, T. and Brodfuehrer, P. (1989). The neuroethology of acoustic

startle and escape in flying insects. *J. Exp. Biol.* **146**, 287-306.

Kalmring, K. (1975a). The afferent auditory pathway in the ventral cord of *Locusta*

migratoria (Acrididae). I. Synaptic connectivity and information processing

among the auditory neurons of the ventral cord. *J. Comp. Physiol.* **104**, 103-141.

Kalmring, K. (1975b). The afferent auditory pathway in the ventral cord of *Locusta*

migratoria (Acrididae). II. Responses of the auditory ventral cord neurons to

natural sounds. *J. Comp. Physiol.* **104**, 143-159.

Kennedy, J.S. (1956). Phase transformation in locust biology. *Biol. Rev.* **31**, 349-370.

Kingdon, J. (1974). *East African Mammals: An atlas of evolution in Africa. Vol. II. Part*

A. (Insectivores and Bats). Chicago: University of Chicago Press.

Kutsch, W. (1999). Telemetry in insects: the "intact animal approach". *Theory Biosci.*

118, 29-53.

Kutsch, W., Van Der Wall, M., and Fischer, H. (1999). Analysis of free forward flight

of *Schistocerca gregaria* employing telemetric transmission of muscle potentials.

J. Exp. Zoo. **284**, 119-129.

La Val, R. K. and La Val, M. L. (1980). Prey selection by a neotropical foliage-

gleaning bat, *Micronycteris megalotis. J. Mamm.* **61(2)**, 327-330.

Lewis, F. P., Fullard, J. H., and Morrill, S. B. (1993). Auditory influences on the flight

behaviour of moths in a Nearctic site. II. Flight times, heights, and erraticism.

Can. J. Zool. **71**, 1562-1568.

Libersat, F. and Hoy, R. R. (1991). Ultrasonic startle behavior in bushcrickets

(Orthoptera; Tettigoniidae). *J. Comp. Physiol. A* **169**, 507-514.

Mason, A. C. (1991) Hearing in a primitive ensiferan: The auditory system of

Cyphoderris monstrosa (Orthoptera: Haglidae) *J. Comp. Physiol. A.* **168**. 351-

363.

Meyer, J. and Elsner, N. (1996). How well are frequency sensitivities of grasshopper

ears tuned to species-specific song spectra? *J. Exp. Biol.* **199**. 1631-1642.

Michelsen, A. (1971a). The physiology of the locust ear. I. Frequency sensitivity of

single cells in the isolated ear. *Z. vergl. Physiologie* **71**. 49-62..

Michelsen, A. (1971b). The physiology of the locust ear. III. Acoustical properties of the

intact ear. *Z. vergl. Physiologie* **71**, 102-128.

Miller L. A. and Olesen, J. (1979). Avoidance behavior in green lacewings. I. Behavior

of free flying green lacewings to hunting bats and ultrasound. *J. Comp. Physiol.*

131. 112-120.

Moiseff, A. and Hoy, R. R. (1983). Sensitivity to ultrasound in an identified auditory

interneuron in the cricket: a possible neural link to phonotactic behavior. *J. Comp.*

Physiol. **152**, 155-167.

Moiseff, A., Pollack, G. S. and Hoy, R. R. (1978). Steering responses of flying crickets to sound and ultrasound: mate attraction and predator avoidance. *Proc. Natn. Acad. Sci. U.S.A.*, 75, 4052-4056.

Morrill, S. B., and Fullard, J. H. (1992). Auditory influences on the flight behaviour of moths in a Nearctic site. I. Flight tendency. *Can. J. Zool.* 70, 1097-1101.

Nolen, T. G. and Hoy, R. R. (1986). Phonotaxis in flying crickets. I. Attraction to the calling song and avoidance of bat-like ultrasound are discrete behaviours. *J. Comp. Physiol. A*, 159, 423-439.

Olesen, J. and Miller, L. A. (1979). Avoidance behaviour in green lacewings. II. Flight muscle activity. *J. Comp. Physiol.* 131, 121-128.

Pollack, G. S. and Hoy, R. R. (1981). Phonotaxis in flying crickets: Neural correlates. *J. Insect Physiol.* 27, 41-45.

Pollack, G. S. and Hoy, R. R. (1989). Evasive acoustic behavior and its neurobiological basis. In *Cricket behaviour and neurobiology.* (ed. F. Huber, T. Moore and W. Loher). pp. 340-363. Ithaca, New York: Cornell University Press.

Pollack, G. S., Huber, F. and Weber, T. (1984). Frequency and temporal patter-dependent phonotaxis of crickets (Teleogryllus oceanicus) during tethered flight and compenssated walking. *J. comp. Physiol* A. 154, 13-26.

Popov, A. V. (1981). Sound production and hearing in the Cicada. *Cicadetta sinuatipennis* Osh. (Homoptera, Cicadidae). *J. Comp. Physiol. A.* 142, 271-280.

Riley, J. R. and Reynolds, D. R. (1997). Vertical-looking radar as a means to improve forcasting and control of desert locusts. In *New Strategies in Locust Control* (ed.

S. Krall. R. Peveling, and D. Ba Diallo), pp47-54. Basel, Switzerland: Birkhäuser

Verlag.

Robert, D. (1989). The auditory behaviour of flying locusts. *J. Exp. Biol.* **147**, 279-301.

Robert, D. and Rowell, C. H. F. (1992). Locust flight steering II. Acoustic avoidance

manoeuvres and associated head movements, compared with correctional

steering. *J. Comp. Physiol. A*, **171**, 53-62.

Roeder, K. D. (1962). The behaviour of free flying moths in the presence of artificial

ultrasonic pulses. *Anim. Behav.* **10**, 300-304.

Roeder, K. D. (1964). Aspects of the noctuid tympanic nerve response having

significance in the avoidance of bats. *J. Insect Physiol.* **10**, 529-546.

Roeder, K. D. (1967a). *Nerve cells and insect behaviour*. Cambridge, MA: Harvard

University Press.

Roeder. K. D. (1967b). Turning tendency of moths exposed to ultrasound while in

stationary flight. *J. Insect Physiol.* **13**, 873-888

Roeder, K. D. (1970). Episodes in insect brains. *Am. Sci.* **58**, 378-389

Roeder, K. D. (1974). Acoustic sensory responses and possible bat-evasion tactics of

certain moths. *Proceedings of the Canadian Society of Zoologists Annual

Meeting.* pp71-78.

Roeder, K. D. (1975). Neural factors and evitability in insect behaviour. *J. Exp. Zool.*,

194, 75-88.

Rydell, J. Skals, N., Surlykke, A., and Svensson, M. (1997). Hearing and bat defence

in geometrid winter moths. *Proc. R. Soc. Lond. B* **264**, 83-88.

Sales, G, and Peye, D. (1974). *Ultrasonic communication by animals*. London: Chapman & Hall

Simmons, J. A., Fenton, M. B., O'Farrell, M. J. (1979). Echolocation and pursuit of prey by bats. *Science* **203**, 16-21.

Spork, P. and Preiss, R. (1991). Control of flight by means of lateral visual stimuli in the desert locust, *Schistocerca gregaria*. In *Synapse-transmission-modulation*. Proc. 19th Göttingen Neurobiol. Conf. (ed. N. Elsner and H. Penzlin). Stuttgart, New York: Thieme Verlag.

Stapells, D. R., Picton, T. W. and Smith, A. D. (1982). Normal hearing thresholds for clicks. *J. acoust. Soc. Am.* **72**, 74-79.

Surlykke, A. and Filskov, M. (1997). Hearing in geometrid moths. *Naturwissenschaften* **84**, 356-359.

Sweeney, B. W. and Vannote, R. L. (1982). Population synchrony in mayflies: A predator satiation hypothesis. *Evolution* **36**(4), 810-821.

Uvarov, B. P. (1943). The Locust Plague. *J. Roy. Soc. Arts* **XCI**(4631), 109-118

Uvarov, B. P. (1966). *Grasshoppers and Locusts: A Handbook of General Acridology*. Volume I. Anatomy, Physiology, Development, Phase Polymorphism, Introduction to Taxonomy. Cambridge: Cambridge University Press. and London: Centre for Overseas Pest Research.

Uvarov, B. P. (1977). *Grasshoppers and Locusts: A Handbook of General Acridology*, Volume. 2. Behaviour, Ecology, Biogeography, Population Dynamics.

Cambridge: Cambridge University Press, and London: Centre for Overseas Pest

Research.

Waters, D. A., Rydell, J.. and Jones, G. (1995). Echolocation call design and limits on

prey size: a case study using the aerial-hawking bat *Nyctalus leisleri*. *Behav. Ecol.*

Sociobiol., **37**, 321-328.

Wyttenbach, R. A., May, M. L., and Hoy, R. R. (1996). Categorical perception of

sound frequency by crickets. *Science* **273**, 1542-1544.

Yack, J. E. and Fullard, J. H. (2000). Ultrasonic hearing in nocturnal butterflies. *Nature*

403, 265-266.

Yack, J. E., Otero, L. D., Dawson, J. W., Surlykke, A. and Fullard, J. H. (2000).

Sound production and hearing in the blue cracker butterfly, *Hamadryas feronia*

(Lepidoptera, Nympahlidae) from Venezuela. *J. Exp. Biol.* **203**, 3689-3702.

Yager, D. D. and May, M. L. (1990). Ultrasound-triggered. flight-gated evasive

maneuvers in the praying mantis *Parasphendale agrionina*. II. Tethered flight. *J.*

Exp. Biol. **152**, 41-58.

Yager, D. D. and Spangler, H. G. (1997). Behavioral response to ultrasound by the tiger

beetle *Cicindela marutha* Dow combines aerodynamic changes and sound

production. *J. Exp. Biol.* **200**, 649-659.

Yager, D. D., May, M. L. and Fenton, M. B. (1990). Ultrasound-triggered, flight-gated

evasive maneuvers in the praying mantis *Parasphendale agrionina*. I. Free flight.

J. Exp. Biol. **152**, 17-39.

Zanker, J. M. (1988). How does lateral abdomen deflection contribute to flight control

   of *Drosophila melanogaster*. *J. Comp. Physiol.* **162**, 581-588.

Zar, J. H. (1984). *Biostatistical Analysis*, 2nd Edition. Englewood Cliffs, New Jersey:

   Prentice-Hall.

CHAPTER 5

GENERAL DISCUSSION

Robert (1989) provided the first formal description of the acoustic startle/escape response

(ASR) in tethered flying locusts and speculated that the reactions observed function in bat

avoidance. This speculation was based on similarities in postural adjustments and yaw

torque production observed between crickets and locusts when similarly presented with

bat-like sounds. Many nocturnally active tympanate insects possess ASRs and this list

now spans several orders and numerous families (Fullard and Yack, 1993; Hoy, 1992).

For an ASR to be adaptive, it must facilitate movement away from the source of the

sounds or render the animal less conspicuous. For an insect in flight, changes in body

posture may contribute to movement away from the source of the sounds, but changes in

wing kinematics effect the substantial forces necessary for steering torque production. Of

the diversity of tympanate insects with ASRs, locusts provide the best opportunity, at

present, for understanding the neuromuscular events underlying avoidance steering to

bat-like and other sounds. This thesis has attempted to determine some of these events

and their neuroethological basis. The studies presented in this thesis were undertaken

with three primary objectives: 1) to describe the wing kinematic changes, in particular

forewing asymmetry, accompanying auditory evoked steering responses, 2) to determine

the underlying motor pattern for forewing asymmetry and intentional steering in general,

and 3) to test the hypothesis that locusts react to bat-like sounds with avoidance

manoeuvres when in free-flight.

Tethered locusts flying in front of a wind stream stimulated with bat-like sounds attempt to turn by deflecting their abdomens and hindlegs to one side. These steering attempts are accompanied by asymmetric forewing depression in which the forewing on the inside of the attempted turn path occupies lower elevation angles than the contralateral wing throughout the downstroke. Forewing asymmetries are large in comparison with hindwing asymmetries and are also accompanied by bilateral asymmetries in the timing of forewing first basalar muscle activation. The number of depolarizations per cycle in first basalar muscles increases on the side of the attempted turn and there is an overall increase in depressor frequency (wing beat frequency) as a result of stimulation.

Asymmetric wing depression is seen in locusts reacting to objects on collision course (Robertson and Reye, 1992; Robertson and Johnson, 1993) and in locusts reacting to laterally placed sources of intense radiant heat (Robertson et al.. 1996; Shoemaker and Robertson, 1998). Prior to these publications it was believed that bilateral asymmetries in wing angles during the downstroke would contribute little to the aerodynamic forces required for generating steering torques (Schmidt and Zarnack. 1987; Zarnack. 1988). This was perhaps because previous studies of locust flight steering employed locusts engaged in correctional steering where wing asymmetries are typically small. relative to those observed in intentional steering. and therefore were considered secondary to the effects of changes in angle of attack. Here, it is argued that asymmetric wing depression is important for generating steering torques and that the aerodynamic effects complement the effects of differences in angle of attack to generate roll torques.

During asymmetric depression of the forewings, the lift generated by the wing

pair is predicted to be directed at an angle inclined towards the side of the locust with the

lower wing relative to the dorsal-ventral axis of the body. These effects would add to

effects of pronation and supination and would not affect the thrust produced by the wing

pair. One appealing aspect of this idea is that it is potentially effected by steady

aerodynamic mechanisms. Further, recent discoveries of unsteady, high-lift aerodynamic

mechanisms operating during the translational phase of the wing stroke, such as the

leading edge vortex and delayed stall (Ellington *et al.*, 1996; Willmott *et al.*, 1997), also

supports the possibility that forewing asymmetry has an aerodynamic basis. However, an

unavoidable truth is that little is known about the aerodynamic basis of forward flight in

insects and even less is known about the aerodynamics of functionally four-wing insects

such as locusts.

What is needed now is a quasi-steady aerodynamic model of wing depression that

estimates the translational forces generated by the forewings and hindwings during

symmetrical and asymmetrical wing strokes. Comparison of quasi-steady estimates of

the forces with values of lift, thrust, and rotational torque measured from locusts

executing avoidance steering on a flight balance would confirm whether the underlying

mechanism is a steady or unsteady high-lift mechanism (Ellington, 1984). Unfortunately,

little is presently known about the aerodynamic interactions between forewings and

hindwings and this poses a significant difficulty in implementing a quasi-steady model.

These interactions are likely quite complex and significant in locust flight (Schmidt and

Zarnack, 1987; Schwenne and Zarnack, 1987; Wortmann and Zarnack, 1987; 1993).

Observations of asymmetric wing depression have only been made from tethered

locusts reacting to various stimuli, and as such, it is an open question as to whether

asymmetric wing depression is a kinematic strategy employed by free-flying animals.

One technical difficulty in working with free-flying locusts is that it is hard to use high-

speed cinematography to measure wing kinematics because of the flight speeds and

distances travelled during flight. Locusts simply do not hover in front of a camera lens

facilitating data collection. Locusts will, however, fly on reasonably straight flight paths

toward sources of bright light and will fly around objects, such as lamps, to avoid

collision. Similarly, interrupting these flights is possible by presenting startling sounds.

This suggests that it might be possible to collect high-speed cinematographic data on

wing kinematics during intentional steering of free-flying locusts. The analysis of this

data would, however, be quite complicated but would yield valuable data on wing angles,

stroke plane, wing stroke angles and angle of attack. Incorporating data from free-flying

locusts would greatly improve the accuracy of any quasi-steady modelling attempt.

If asymmetric wing depression is important for intentional steering, then how are

the asymmetries in wing depression established? It was suggested that asymmetries in

the timing of depressor muscles might establish asymmetries in the timing of stroke

reversals for the left and right wings which, in turn, would bring about asymmetric

depression of the wings during the downstroke. Because downstroke translation of each

wing is controlled by the coordinated action of many depressor muscles, the prediction

from this model was that bulk shifts in depressor muscles would be observed during

intentional steering and that bilateral asymmetries would be correlated with several

kinematic parameters of the stroke reversal and downstroke.

High-speed (digital) cinematography with concurrent multiple muscle

electromyographic recording show that asymmetries in the timing of the stroke reversal

and bilateral shifts in depressor muscle timing are correlated with forewing asymmetry;

observations entirely consistent with the above model. Clearly the most significant

limitation of this method is that correlation can not substantiate a causative role of

depressor activation in establishing the timing of the stroke reversal. Intracellular

recording and stimulation of depressor motor neurons needs to be completed to determine

the role of each muscle in establishing the wing kinematics seen during steering. Hedwig

and Becher (1998) have completed one such study during their development of a new

optoelectronic method of recording wing movement. They found that electrical

stimulation of the motorneuron that innervates the forewing first basalar muscle (MN97)

reduces the amplitude of the downstroke; an observation entirely consistent with the

results of this study. A related methodological approach might be to use a computer to

generate models of motor patterns that could be used to stimulate multiply implanted

EMG electrodes. High-speed cinematography could then be used to compare the effects

of adjusting the relative timing of muscle activations on the resulting wing movements in

deafferented. tethered flying locusts.

High-speed cinematography of the stroke reversals also revealed a second.

unexpected. result. The timing of the left and right wing flip (supination to pronation

transition at the end of the upstroke) was asymmetric and relative to the onset of the

stroke reversal in more than half of the locusts filmed. The timing of the stroke reversal

is a potent mechanism for establishing asymmetric flight forces by way of increasing

rotational circulation at the end of wing translation (Dickinson *et al.*, 1999). These

observations provide evidence, for the first time, that locusts may actively control

asymmetries in the timing of the wing flip and use the resulting asymmetries in lift during

steering.

The coordination between postural adjustments and wing kinematic changes, with

plausible aerodynamic consequences, coupled with a defined motor pattern strongly

suggest that locusts in free-flight will react to bat-like sounds with banked downward

turns. This is predicted because it is the logical outcome of a predicted roll torque

generated by asymmetric wing depression, a yaw torque generated by abdomen

deflection and asymmetries in angle of attack, and a pitching torque generated by

abdominal dorsiflexion. Robert (1989) suggested that the reactions of tethered locusts

function in bat-avoidance and also predicted that locusts in free-flight would show

avoidance reactions to bat-like sounds. His audiogram of the ASR showed locusts

responded most frequently to pulsed sounds with 25 kHz carrier frequencies and suggests

that reactions of free-flying locusts will be frequency dependent. Further, the sounds

produced by locusts during stridulation are not likely to be heard by individuals in flight.

In other words, locusts are predicted to respond more frequently to bat-like sounds than

non-bat-like sounds.

To test Robert's (1989) hypothesis, sounds that varied on a continuum in two

dimensions from conspecific-like (low frequency, stridulatory sounds) to predator-like

(high frequency, bat-like) were presented to locusts as they flew in free-flight towards a bright light source. Locusts responded to all of the sounds regardless of pulse structure and frequency. These results did not show the frequency-sensitivity that behavioural audiograms derived from tethered flying locusts predicted. Moths and tethered locusts tested under the same conditions showed that the lack of frequency dependency in the responses of free-flying locusts was not due to conditions in the flight room. These results suggest that tethering may affect auditory processing and we conclude that the locust ASR is a general startle reaction to any sound.

The conclusion that locusts react to any sound, not just bat-like sounds is an attractive one that is consistent with the behaviour of gregarious phase locusts (Uvarov, 1977). Gregarious phase locusts are typically diurnal in their habits, flying during the day in swarms and settling during the night (Kennedy, 1956). The large number of locusts in a swarm is also consistent with a predator swamping strategy. However, there is no reason to suspect that auditory physiology would be different between solitary phase locusts, that fly at night for dispersal, and gregarious phase locusts. Further, what remains enigmatic is that the responses of locusts in free-flight are in many ways similar to those reported for other insects responding to bat and bat-like sounds (turns, loops, dives) except in their frequency dependency. If the ASR is not specifically a bat-avoidance response, then why are the manoeuvres observed in free-flight so similar to manoeuvres observed in insects that use their ears exclusively for bat-detection (e.g. many moths, lacewings, and praying mantises)? The answer to this question may be found in understanding how insect ears have evolved.

The emerging picture of the evolution of insect ears is that they are all derived from proprioceptive chordotonal organs and that this is true regardless of the form of the ear or where the ear is located on the insect body (e.g. abdomen, thorax, wing, leg, or mouth) (Boyan, 1993; Yager, 1999). The auditory afferents of all insects project to the same neuropile, the medial ventral association centre, in the CNS, with the possible exception of praying mantises in which the afferents project to nearby associated structures (Boyan, 1993; Yager and Hoy, 1987). There seems to be a common 'bauplan' for insect audition based on pre-existing mechanosensory pathways in the CNS that are being repeatedly co-opted for audition (Boyan, 1993). Support for this is that a prominent auditory interneuron in the locust, interneuron 531 (=B1) is homologous with interneuron MR-501-T3 in the praying mantis (Yager and Hoy, 1989). Similarly, within locusts there is evidence of serially repeating processing modules derived from developmentally related neurons, and this is quite possibly true of other insects (Boyan, 1992). Therefore, connections of auditory processing neurons (and modules) with the flight pattern generator and flight motor neurons are likely to be similar across insect taxa. It is not unreasonable, therefore, to suspect that the kinematic and aerodynamic mechanisms employed by locusts are not fundamentally different from those employed by moths or other insects given both the constraints of biomechanics and limits in the types of aerodynamic mechanisms (steady and unsteady) that insects can use for flight control and steering.

5.1 Literature Cited

Boyan, G. S. (1992). Common synaptic drive to segmentally homologous interneurons in

the locust. *J. Comp. Neurol.* **321**, 544-554.

Boyan, G. S. (1993). Another look at insect audition: the tympanic receptors as an

evolutionary specialization of the chordotonal system. *J. Insect Physiol.* **39**(3),

187-200.

Dickinson, M. H., Lehmann, F. and Sane, S. P. (1999). Wing rotation and the

aerodynamic basis of insect flight. *Science* **284**, 1954-1960.

Ellington, C. P. (1984). The aerodynamics of insect flight. IV. Aerodynamic

mechanisms. *Phil. Trans. R. Soc. Lond. B* **305**, 79-113.

Ellington, C. P., van den Berg, C., Willmott, A. P., and Thomas, A. L. T. (1996).

Leading-edge vortices in insect flight. *Nature* **384**, 626-630.

Fullard, J. H. and Yack, J. E. (1993). The evolutionary biology of insect hearing.

Trends Ecol. Evol. **8**(7), 248-252.

Hedwig, B. and Becher, G. (1998). Forewing movements and intracellular motoneurone

stimulation in tethered flying locusts. *J. Exp. Biol.* **201**, 731-744

Hoy, R. R. (1992). The evolution of hearing in insects as an adaptation to predation from

bats. In *The Evolutionary Biology of Hearing* (ed. D. B. Webster, R. R. Fay and

A. N. Popper). pp115-129. New York: Springer-Verlag.

Kennedy, J.S. (1956). Phase transformation in locust biology. *Biol. Rev.* **31**, 349-370.

Robert, D. (1989). The auditory behaviour of flying locusts. *J. Exp. Biol.* **147**, 279-301.

Robertson, R. M. and Johnson, A. G. (1993). Collision avoidance of flying locusts:

steering torques and behaviour. *J. Exp. Biol.*, **183**, 35-60.

Robertson, R. M., Kuhnert, C. T. and Dawson, J. W. (1996). Thermal avoidance

during flight in the locust *Locusta migratoria. J. Exp. Biol.*, **199**, 1383-1393.

Robertson, R. M. and Reye, D. N. (1992). Wing movements associated with collision

avoidance manoeuvres during flight in the locust *Locusta migratoria. J. Exp.*

Biol., **163**, 231-258.

Schmidt, J. and Zarnack, W. (1987). The motor pattern of locusts during visually

induced rolling in long-term flight. *Biol. Cybern.* **56**,397-410

Schwenne. T. and Zarnack, W. (1987). Movements of the hindwings of Locusta

migratoria, measured with miniature coils. *J. Comp. Physiol. A* **160**,657-666.

Shoemaker, K. L. and Robertson, R. M. (1998). Flight motor patterns of locusts

responding to thermal stimuli. *J. Comp. Physiol. A* **183**, 477-488.

Uvarov, B. P. (1977). *Grasshoppers and Locusts: A Handbook of General Acridology,*

Volume. 2. Behaviour, Ecology, Biogeography, Population Dynamics.

Cambridge: Cambridge University Press. and London: Centre for Overseas Pest

Research.

Willmott, A. P., Ellington, C. P. and Thomas, A. L. R. (1997). Flow visualization and

unsteady aerodynamics in the flight of the hawkmoth *Manduca sexta. Phil. Trans.*

R. Soc. Lond. B **352**, 303-316

Wortmann, M. and Zarnack, W. (1987). The influence of several parameters of wing

movement on the lift of Schistocerca gregaria during descending, horizontal or

ascending flight. In *New frontiers in brain research* (ed. N. Elsner and O. Creutzfeldt), Poster 53. Stuttgart: Thieme.

Wortmann, M. and Zarnack, W. (1993). Wing movements and lift regulation in the flight of desert locusts. *J. Exp. Biol.* **182**,57-69.

Yager, D. D. (1999). Structure, development, and evolution of insect auditory systems. *Microsc. Res. Tech.* **47**, 380-400.

Yager, D. D. and Hoy, R. R. (1987). The midline metathoracic ear of the praying mantis, *Mantis religiosa. Cell Tissue Res.* **250**, 531-541.

Yager, D. D. and Hoy, R. R. (1989). Audition in the praying mantis, *Mantis religiosa* L.: identification of an interneuron mediating ultrasonic hearing. *J. Comp. Physiol* *A* **165**, 471-493.

Zarnack, W. (1988). The effect of forewing depressor activity on wing movement during locust flight. *Biol. Cybern.* **59**, 55-70.

CURRICULUM VITAE

April . 2001

Biographical Information

Name:	Jeffery Wayne DAWSON
Date of Birth:	November 18. 1967. Hamilton. Ontario CANADA
Citizenship:	Canadian

Education

Jan. 1995 to
Present
Ph.D. candidate
Queen's University. Department of Biology
Thesis Supervisor: Professor R. Meldrum Robertson
Ph.D. Thesis: "Neuroethology of acoustic startle/escape in flying locusts"

Sept. 1992 to
Jan. 1995
M.Sc.
University of Toronto. Department of Zoology
Thesis Supervisor: Professor James H. Fullard
M.Sc. Thesis: "A Neurophysiological description of the central pattern generator underlying sound production in two species of tiger moths (Lepidoptera: Arctiidae)"

Sept. 1987 to
Apr. 1992
B.Sc.
Erindale College. University of Toronto
Completed Specialist Programs (Honors) in Biology and Animal Behaviour
Thesis Supervisor: Professor James H. Fullard
B.Sc. Thesis: "Thoracic control of tymbal activation in the dogbane tiger moth. *Cycnia tenera* (Hübner) (Lepidoptera:Arctiidae)."

Sept. 1982 to
June 1987
Secondary School Honours Graduation Diploma
Scott Park Secondary School
1055 King Street East. Hamilton. Ontario L8L 3P3

Honours

1994	Erindale College Teaching Assistant Excellence Award (University of Toronto)
2000	Christopher Knapper Award for Excellence in Teaching Assistance (Queen's University)
2000	Departmental Student Council Teaching Assistant Award (Department of Biology. Queen's University)

Memberships in Professional Societies and Organizations

Since 1993   International Society for Neuroethology
Since 1994   Canadian Society of Zoologists
Since 1996   Society for Neuroscience

Scholarships, Fellowships and Awards

1998	Queen's Graduate Award	$ 2,000.00
1998-99	Ontario Graduate Scholarship	$ 11,859.00*
1998	Queen's Graduate Award	$ 400.00
1997-98	Ontario Graduate Scholarship	$ 11,859.00*
1996-97	Queen's Graduate Fellowship (declined)	$ 5,400.00*
1995-97	Natural Sciences and Engineering Research Council of Canada (NSERC) Postgraduate Scholarship (PGSB)	$ 34,800.00*
1993	University of Toronto Open Master's Fellowship	$ 3,400.00
1990	Alan F. Coventry Memorial Scholarship	$ 700.00*

Travel Awards:

Apr. 1999	School of Graduate Studies and Research, Queen's University (to attend 25th Annual Meeting of the East Coast Nerve Net)	$ 300.00
Feb. 1998	Graduate Dean's Travel Grant, Queen's University (to study aerodynamic mechanisms of locust flight at Cambridge University, UK with Prof. Charlie P. Ellington)	$ 2,500.00*
Nov. 1995	Neurons, Networks, and Motor Behavior Symposium Organizing Committee Scholarship (to attend conference of same name)	$(US) 198.00
Apr. 1995	School of Graduate Studies and Research, Queen's University (to attend 21st Annual meeting of the East Coast Nerve Net)	$ 300.00
Apr. 1994	Department of Zoology, University of Toronto (to attend 20th Annual Meeting of the East Coast Nerve Net)	$ 500.00
Apr. 1993	Department of Zoology, University of Toronto and Department of Biology, Erindale College, University of Toronto (to attend 19th Annual Meeting of the East Coast Nerve Net)	$ 370.00 $ 200.00

* competitive award

Contributions to Research (Refereed)

Refereed journal publications:

Yack, J. E., Otero, L. D., **Dawson, J. W.**, Surlykke, A. and Fullard, J. H. (2000) Sound production and hearing in the blue cracker butterfly, *Hamydryas feronia* (Lepidoptera, Nymphalidae) from Venezuela. Journal of Experimental Biology 203:3689-3702. (appears on cover of issue)

Fullard, J. H., and **Dawson, J. W.** (1999) Why do diurnal moths have ears? Naturwissenschaften 86:276-279.

Fullard, J. H., **Dawson, J. W.**, Otero, L. D. and Surlykke, A. (1997) Bat-deafness in day-flying moths (Lepidoptera, Notodontidae, Dioptinae). Journal of Comparative Physiology A 181:477-483.

Dawson. J. W.. Dawson-Scully. K.. Robert. D.. and Robertson. R. M.. (1997) Forewing asymmetries during auditory avoidance in flying locusts. Journal of Experimental Biology 200:2323-2335. (appears on cover of issue)

Fullard. J. H.. and Dawson, J. W. (1996) The echolocation calls of the spotted bat. *Euderma maculatum* are relatively inaudible to moths. Journal of Experimental Biology 200:129-137.

Robertson. R. M.. Kuhnert. C. T.. and Dawson, J. W. (1996) Thermal avoidance during flight in the locust. *Locusta migratoria*. Journal of Experimental Biology 199:1383-1393.

Dawson, J. W.. and Fullard. J. H. (1995) The neuroethology of sound production in tiger moths (Lepidoptera. Arctiidae) II. Location of the tymbal central pattern generator in *Cycnia tenera* Hübner. Journal of Comparative Physiology A 176:541-549.

Faure. P. A.. Fullard. J. H.. and Dawson, J. W. (1993) The gleaning attacks of the northern long-eared bat. *Myotis septentrionalis*. are relatively inaudible to moths. Journal of Experimental Biology 178:173-189.

Articles in review with refereed journals:

Dawson. J. W.. Kutsch. W.. and Robertson. R. M. (2001) Auditory evoked evasive manoeuvres in free-flying locusts and moths. Submitted to the Journal of Experimental Biology March 2001.

Dawson, J. W.. Leung. F.. and Robertson. R. M. (2001) Acoustic startle/escape reactions in tethered flying locusts: Motor patterns and wing kinematics underlying intentional steering. Submitted to the Journal of Experimental Biology March 2001.

Contributions to Research (Non-refereed)

Published Abstracts:

"Free-flight responses of locusts and moths to bat-like sounds"
Jeff W. Dawson. Wolfram Kutsch. and R. Meldrum Robertson
30th Annual Meeting of the Society for Neuroscience. November. 2000. New Orleans. La. (Abstract 368.13) *Soc. Neurosci. Abstr.* 26: 986.

"The great escape: Linking motor patterns with locust wing kinematics."
Jeff W. Dawson and R. Meldrum Robertson
Fifth International Congress of Neuroethology. University of California. San Diego. August. 1998.

"Bat-like sounds interrupt thermal avoidance in flying locusts"
Craig K. R. Willis. Jeff W. Dawson and R. Meldrum Robertson
36th Annual Meeting of the Canadian Society of Zoologists. University of Western Ontario. London. Ontario. May. 1997.

"Sound production in tiger moths: Rhythmic behaviour and simple ears."
James H. Fullard and Jeff W. Dawson
Third International Congress of Neuroethology. McGill University. Montreal.
Quebec. August. 1992.

"Gleaning insectivorous bats versus tympanate moths: who says cheaters never prosper?"
Paul A. Faure. James H. Fullard and Jeff W. Dawson
Third International Congress of Neuroethology. McGill University. Montreal.
Quebec. August. 1992.

Book Chapter:

Dawson, J. W.. and Robertson. R. M. (In Press. Oct 1996). Motor Patterning:
Electromyographic recording from wing muscles during flight in the locust. In:
Student Experiments in Physiology. Eds: Silverthorn. D. E.. Johnson. B.. and
Mills. A. C. Prentice Hall. New York.

Invited Talks and Seminars:

"Bat avoidance in flying locusts: From motor patterns to behaviour"
Departments of Biology and Psychology. University of Maryland. College Park
MD (July. 2000)

"Free-Flight responses of locusts and moths to bat-like sounds"
Neuroethology Journal Club. Cornell University. Ithaca. NY (April 27. 2000)

"How and why locusts steer"
Department of Zoology. University of Konstanz. Konstanz. Germany (May.
1999)

"How and why locusts steer"
Physiology Group. Department of Biology. Queen's University (April. 1999)

Talks Presented at Meetings and Symposia:

"Motor patterns for locust steering kinematics"
Jeff W. Dawson and R. Meldrum Robertson
25th Annual East Cost Nerve Net. Marine Biological Laboratory. Woods Hole.
Massachusetts. April. 1999

"Bat-deafness in Neotropical. day-flying dioptine moths of Venezuela."
James H. Fullard. Jeff W. Dawson. L. Daniel Otero and Annemarie Surlykke
Insect Sound and Vibration. 10th International Meeting. Marine Biological
Laboratory. Woods Hole. Massachusetts. September. 1996.

"Sound production and hearing in the butterfly. *Hamadryas feronia.*"
Annemarie Surlykke. L. Daniel Otero. Jeff W. Dawson and James H. Fullard
Insect Sound and Vibration. 10th Internation Meeting. Marine Biological
Laboratory. Woods Hole. Massachusetts. September. 1996.

"Evasive manoeuvres in flying locusts"
> Jeff W. Dawson. Ken Dawson-Scully. and R. Meldrum Robertson
> 22nd Annual East Coast Nerve Net. Marine Biological Laboratory. Woods Hole.
> Massachusetts. April. 1996.

"I beg your pardon? Auditory deafness in moths to the echolocation calls of the spotted
bat *Euderma maculatum*"
> James H. Fullard and Jeff W. Dawson
> 10th International Bat Research Conference. Boston. Massachusetts. August.
> 1995.

"Organization of the central pattern generator underlying sound production in tiger
moths"
> Jeff W. Dawson and James H. Fullard
> 21st Annual East Coast Nerve Net. Marine Biological Laboratory. Woods Hole.
> Massachusetts. April. 1995.

"Gleaners are cheaters: the auditory response of noctuid moths to the echolocation calls
of gleaning versus aerial bats."
> Paul A. Faure. James H. Fullard. and Jeff W. Dawson
> 21st North American Symposium on Bat Research. Austin. Texas. October.
> 1991.

Posters Presented at Meetings and Symposia:

"Heatshock protects action potentials from high temperatures in locust motor neurons."
> Bernhard Wu. Jeff W. Dawson. Virginia K. Walker. and R. Meldrum Robertson
> 25th Annual East Coast Nerve Net. Marine Biological Laboratory. Woods Hole.
> Massachusetts. April. 1999.

"M.E.L.: A locust controls the speed and direction of a mobile robotics platform."
> Jeff W. Dawson. Ronald B. Harding. and R. Meldrum Robertson
> 25th Annual East Coast Nerve Net. Marine Biological Laboratory. Woods Hole.
> Massachusetts. April. 1999.

"Evidence for hemioscillators in the organization of the CPG controlling sound
production in tiger moths (Lepidoptera. Arctiidae)."
> Jeff W. Dawson and James H. Fullard
> Insect Sound and Vibration. 10th International Meeting. Marine Biological
> Laboratory. Woods Hole. Massachusetts. September. 1996.

"Audition in the butterfly. *Hamadryas feronia*"
> Annemarie Surlykke. L. Daniel Otero. Jeff W. Dawson and James H. Fullard.
> 24th Göttinger Neurobiologentagung. Göettingen. 1996.

"Motor patterns underlying avoidance manoeuvres in flying locusts"
> J. W. Dawson. K. Dawson-Scully. and R. M. Robertson
> Neurons. Networks. and Motor Behaviour. Tuscon. Arizona. November. 1995.

"Wing movements during auditory avoidance in flying locusts"
> J. W. Dawson. R. M. Robertson. D. Robert. and K. Dawson-Scully
> Finding Food: Neuroethological Aspects of Foraging. Amherst. Massachusetts.
> October. 1995.

"Wing movements during auditory avoidance in flying locusts"
R. M. Robertson. D. Robert. K. Dawson-Scully. and J. W. Dawson
21st Annual East Coast Nerve Net. Marine Biological Laboratory. Woods Hole.
Massachusetts. April. 1995.

"DC electrical stimulation of the pterothoracic ganglion elicits tymbal CPG activity in the
dogbane tiger moth. *Cycnia tenera*"
Jeff W. Dawson
20th Annual East Coast Nerve Net. Marine Biological Laboratory. Woods Hole.
Massachusetts. April. 1994.

"Locating the neural circuit controlling sound production in the dogbane tiger moth
Cycnia tenera Hübner (Lepidoptera: Arctiidae)"
Jeff W. Dawson
19th Annual East Coast Nerve Net. Marine Biological Laboratory. Woods Hole.
Massachusetts. March. 1993.

"User-friendly signal processing software for PC-based analysis of echolocation calls and
other signals."
Jeff W. Dawson and James H. Fullard
21st North American Symposium on Bat Research. Austin. Texas. October.
1991.

Research and Field Work Abroad

May-Aug. 1999	University of Konstanz. Konstanz. Germany Worked with Prof. Wolfram Kutsch while studying responses of locusts and moths in free-flight to bat-like sounds.
Sept. 1998: July. 1999	Cambridge University. Cambridge. UK Worked with Prof. Charlie Ellington to develop a quasi-steady aerodynamic model of the effects of asymmetric wing depression in flying locusts.
Nov.-Dec. 1995	University of the Andes. Merida. Venezuela Worked with Prof. James Fullard (University of Toronto) on a National Geographic Society funded project to study day-flying dioptine moths.
June 1995	Field work in the Okanagan Valley. British Columbia. Canada Worked with Prof. James Fullard (University of Toronto) on a project studying the ability of moths to detect the echolocation calls of the western spotted bat. *Euderma maculatum*
July-Sept. 1991	Field work in North Eastern Ontario Worked with Prof. James Fullard and Paul Faure on a project studying the ability of moths to detect the echolocation calls of the northern long-eared bat. *Myotis septentrionalis*

Computer and Electronics Skills

Platforms:	80x86. Pentium. Macintosh (some). Atari 800 (an 8-bit blast from the past!) Much experience with hardware installation/upgrading/configuration including TCP/IP networking
Operating Systems:	MS-DOS. Windows 3.x/95/98/Me/NT. Unix (some)

Programming Languages: BASIC (Microsoft QuickBasic, VisualBasic, VisualBasic for Applications), C (some)

I have developed software for:
   Collecting data from human subjects presented with images in an interactive experimental design.
   Motor pattern and wing kinematic analysis
   Spectral analysis (fast Fourier transformations) of bat echolocation calls and other short duration acoustic signals
   Collecting frequency sensitivity profiles (Audiograms) from moth auditory nerves
   Phase analysis of neuronal spike data
   Spike counting and inter-spike-interval histogram analysis
   Nearest-neighbor distance calculations using video input
   Analysis and acquisition of fluorescence-spectrometer data

I have experience with GPIB bus and Parallel port interfacing.

I have extensive experience with HTML tagging and creating and managing WWW sites
   I have created WWW sites for:
      Office of the University Registrar, Queen's University
      Student Awards Office, Queen's University
      Department of Biology, Queen's University
      Many undergraduate courses, Department of Biology, Queen's University
      Many faculty homepages, Department of Biology, Queen's University

I established a facility for making 35mm presentation slides using a Polaroid HR-6000 Film Recorder for the Department of Biology, Queen's University (operated and maintained facility from April 1997 to May, 2000)

I was a computer resource person for the Department of Biology, Queen's University (from 1996 to 2000)

I have contributed significantly to the design and implementation of many electronic devices including:
   1999   A 16-channel amplifier for collecting electrophysiological data *
   1999   "M.E.L" A mobile robotics platform *
   1998   "DIO-64" A 64 channel switch monitor that connects to a PC Parallel port *
   1997   A sensitive (sub-mm accuracy) position transducer for monitoring limb movement and position in insects *

   * In cooperation with Waterloo Instruments and Ronald B. Harding.

Teaching and Other Contributions to Education

Thesis Student Supervision (Responsible for immediate day-to-day supervision)

1999	Fok-Han Leung	Tegula function during wing-opening in locusts
1998	Ben Bourke	Thermal avoidance in tethered flying locusts
	Mark Thompson	Effect of heat-shock on EMG recordings from flight muscles
1996	Craig C. K. Willis	Behavioural resolution of sensory conflict
1995	Ken Dawson-Scully	Motor patterns during auditory avoidance in flying locusts

Undergraduate Supervision

| 1999 | Briiann Dawson | Kinematic analyses of moths avoiding bat-like sounds |
| 1998 | David Chang | Abdomen movements during collision avoidance in locusts |

Guest-Lecturing

1999   "Cable Properties". 3 lectures
"Techniques in Neurobiology". 3 lectures
"Bat-Moth Interactions: A Case-Study in Neuroethology". 3 lectures
     A total of nine lectures presented to senior undergraduate neurobiology students (BIOL445. Queen's University)

1998   "Sensory Systems". 3 lectures
     Three lectures presented to senior undergraduate neurobiology students (BIOL445. Queen's University)

1996   "Neural basis of behaviour"
     Single lecture presented to undergraduate entomology students (BIOL327. Queen's University)

1994   "Neural basis of rhythmic behaviour"
     Single lecture presented to undergraduate neurobiology students (BIO304. University of Toronto)

Laboratory Exercise Development

| 1999 | "Arthropod Mechanoreception" An exercise for making single-unit recordings from sensory axons in a cricket leg |
| 1996 | "Insect Ears" An exercise for studying the morphology of cricket. grasshopper and moth ears. |

Teaching Assistantships

1999. 2000	Organization for Life – Biodiversity (BIOL200)	Queen's University
1997. 98. 99	Integrative Neurobiology and Neuroethology (BIOL445)	Queen's University
1997. 98	Introduction to Data Analysis and Statistics (BIOL343)	Queen's University
1996	Entomology (BIOL327)	Queen's University
1994. 99	Comparative Animal Physiology (BIOL338)	Queen's University
1993	Introductory Physiology (BIO204)	University of Toronto
1992	Evolution and Biodiversity (BIO151)	University of Toronto
1991	Diversity of Living Organisms (BIO201)	University of Toronto
1991. 92. 94	Neurobiology (BIO304)	University of Toronto

Other contributions

Apr. 1996	Judge at Frontenac. Lennox and Addington Districts Science Fair
Nov. 1994	Judge at Kingston Collegiate and Vocational Institute Science Fair
1988 to 1994	Organizer for the "Erindale College Science Expo". University of Toronto
1990 to 1992	Founded the "Erindale Biology Club". University of Toronto
1989 to 1991	Student Mentor. University of Toronto Mentorship Program:

	1989	Isabelle Okuda
	1990	Karen Milek
	1991	Abhijit Patel & Wayne Lau

Professional Development

1995-96 Teaching Assistant Associate with the Instructional Development Centre. Queen's University

Involved in the training and evaluation of teaching assistants (TAs) at Queen's University. Acted as a liaison with many departments to identify needs and concerns of TAs. Developed World Wide Web pages to facilitate advertising and distribution of materials to help TAs.

Sept. 1995-98 Organized and participated in TA workshops for the Department of Biology. Queen's University

Apr. 1994 Invited Speaker at Student Leadership Seminar. Erindale College. University of Toronto

Sept. 1994 Invited Speaker at Teaching Assistant Training Workshop. Erindale College. University of Toronto

Contributions to Administration

Sept. 1997 to May 1998 Graduate Studies Committee Representative. Biology Graduate Students Association. Biology Department. Queen's University*

Apr. 1995 to Aug. 1997 Staff Meeting Representative. Biology Graduate Students Association. Biology Department. Queen's University

Sept. 1993 to May 1994 Quality Services for Students Committee Representative. Association of Graduate Students at Erindale (AGSAE). Erindale College. University of Toronto

Sept. 1993 to May 1994 Student Centre Building Committee Representative. Association of Graduate Students at Erindale (AGSAE). Erindale College. University of Toronto

* May. 1998 "The 1998 Biology Graduate Student Cost Survey" Jeff W. Dawson and Darren Bos. Department of Biology. Queen's University.

I initiated this survey to quantify the living expenses and education costs of graduate students completing their degrees in the Biology Department at Queen's University. The purpose of the survey was to objectively quantify the financial needs of graduate students in the department. The survey results were quite surprising and received high acclaim from certain members of the department. the student awards office and the School of Graduate Studies and Research at Queen's