AN EXPLORATION OF THE FUNCTION OF THE LATERAL NERVE AND THE FEMORAL CHORDOTONAL ORGAN IN THE AFRICAN DESERT LOCUST (SCHISTOCERCA GREGARIA)

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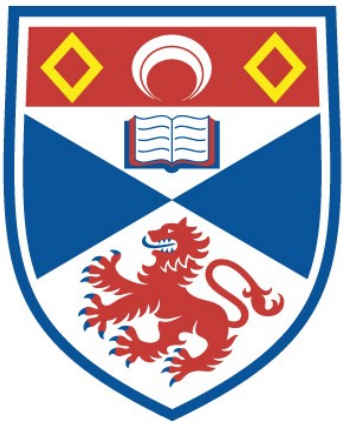
A Thesis Submitted for the Degree of MPhil
at the
University of St Andrews



1997

An Exploration of the Function of the Lateral Nerve and the Femoral Chordotonal Organ in the African Desert Locust (Schistocerca gregaria)

A thesis submitted by David Scott Tait, for the degree of Master of Philosophy, on the date of 31/3/96.

Abstract

An investigation of proprioception in the African Desert Locust (*Schistocerca gregaria*) is the basis of the following work. Experiments were performed on the adults and 5th instars to investigate the relation between the size of the animal and their jumping and kicking ability. This lead to a study of the effects of proprioception, specifically proprioception of the lateral nerve and the chordotonal organ on the jump and kick of adults.

In order to study these effects, the simplest method was used. The lateral nerves and chordotonal organs (of the metathoracic leg) were severed, both separately, and in combination. The consequences of this loss of information about the position of the tibia in relation to the femur resulting, was judged by the behavioural and electrophysiological effects on the jump and kick of the locust.

Results indicate that the loss of the information supplied via the lateral nerve is not sufficient to significantly alter the jumping or kicking performance of the animal, and neither is that loss resulting from the severance of the chordotonal organ. Loss of information from both proprioceptors appeared to prohibit kicking in the locust, but it did not prevent the locust from jumping. This surprising finding was taken as meaning that when the locust jumps, it can get positional information from other sources, such as tibial-tarsal chordotonal organs, which are in contact with the ground prior to the jump, and that this information is sufficient to enable the expression of the motor programme. However, these additional sources of information are not available when the locust kicks, and thus the motor programme is not expressed.

Declarations

i) I, David Scott Tait, hereby certify that this thesis, which is approximately 19,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

date signature of candidate

ii) I was admitted as a research student in October 1994 and as a candidate for the degree of M.Phil. in October 1994; the higher study for which this is a record was carried out in the University of St. Andrews between 1994 and 1996.

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iii) I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of M.Phil. in the University of St. Andrews and that the candidate is qualified to submit this thesis in application for that degree

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Contents

Introduction

Are the femoral chordotonal organ, and the lateral nerve of the locust required in order for it to jump and kick? This is, essentially, the question that is to be answered here. If the proprioceptive information from the femoral chordotonal organ is lost, will the locust still be able to jump and kick, or will there be some other effect, less drastic than total loss of jumping and kicking ability? If the lateral nerve is severed, there will be no information from the lump receptor, or the cuticle strain receptors, and will this affect the jump or the kick of the locust? Will any effect caused by the loss of proprioceptive input be the same in the 5th instars, and if not, what will be the effect on them?

An attempt will be made to explore the information that is required for the jump or kick to occur, with regard to the position of the tibia before, and during co-contraction.

The Locust Jump and the associated Motor Programme.

Sensory feedback in all animals is of utmost importance for locomotion. The position of limbs and the state of muscular activity must be known in order for the animal to produce a co-ordinated and effective behaviour in an unpredictable environment. The fundamental motor programme driving limb movement may result from a central pattern generator, but sensory feedback is usually essential in order to adjust and modulate the motor programme in response to external mechanical perturbation (Pearson, 1983)

Locusts have two methods of moving on the ground. They can either walk, or they can jump. The jump, as shall be discussed here, is used as a method of escape or to get airborne (in adult locusts). The hind legs can also perform a defensive kick, and it has been assumed that the neurological mechanisms for the jump and the kick are basically the same (Pflüger and Burrows, 1977). As it is very difficult to record neuronal and muscular activity in a jumping locust, most experiments are done on the kick. All the power for the locust's jump / kick comes from the metathoracic legs, and as such, these have greatly extended femurs and tibiae, compared to the prothoracic and mesothoracic legs. The metathoracic legs thus provide the lever with which the locust directs the force exerted during the jump / kick. One reason why sensory feedback is so important in the locust is that in order for the locust to jump, the tibiae must be fully flexed prior to the commencement of the main part of the motor programme. Why this is the case shall be described later as will the many pathways of the neurological network that control and monitor the jump / kick.

In order to fully understand the jump, it needs to be shown why the locust jump is so special. The main problem for the locust is that it cannot generate enough power with its muscles alone to perform the jump. The adult locust's peak power output during a jump can reach 5200 $W.kg^{-1}$ of living muscle (Katz and Gosline, 1993). However, the maximum power that any muscle can directly produce is between 500-

1000 $W.kg^{-1}$ of living muscle (and that for only a few seconds) (Alexander and Goldspink, 1977). So, somehow the locust is able to multiply the power exerted by its muscles by at least five times, assuming that the muscles are as efficient as they can be. Without this ability to amplify its power output the locust would probably be incapable of getting itself off the ground, certainly any jump that it could produce would be very short range, and the behaviour would be unlikely to be an effective escape response. Also the locust would have difficulty in launching itself for flight. It needs to achieve a certain velocity in order to become airborne (Pearson et al., 1986) and any reduction in the velocity might impair the initiation of flight.

The motor programme for the jump occurs in three separate phases. The first of these is the initial flexion, or cocking phase, the second is co-contraction and the third is triggering (Heitler and Burrows, 1977a). During cocking there is a rapid flexion of the tibiae in the two metathoracic legs. Once fully flexed, the tibiae are locked in this position by a division existing in the flexor tendon that allows it to slide down both sides of a cuticular lump on the distal femur. During cocking the extensor muscle is also activated, but the flexor tendon's special arrangement means that it has a mechanical advantage over the extensor (Heitler, 1974).

Stage two of the jump mechanism, co-contraction, lasts for up to 0.5 seconds and involves the storing of the energy required for the jump in elastic form. To do this there is repeated activity in the Fast Extensor Tibia motorneurone (FETi) and several flexor motorneurones (Godden, 1975; Heitler and Burrows, 1977a). Whilst this is occurring, the isometric contraction of the large extensor muscle in each tibia is causing a large amount of deformation in the cuticle in the distal part of the femur. This is where the elastic energy for the jump is stored, and this is why the tibia must be fully flexed for the jump to occur (Bennet-Clark, 1975). The extensor muscle itself cannot generate enough power for the jump, so elastic energy must be stored. The isometric contractions that occur during co-contractions can only occur when the tibiae are locked and the tibiae can only lock when they are fully flexed.

Stage three of the jump mechanism, triggering, involves the sudden inhibition of flexor motorneurone activity. The flexors thus relax, the tendon disengages from the cartilaginous lump and the energy stored in the cuticle is transferred to the rapid extension of the tibiae. The long length of the tibia forms a very effective lever, directing the power exerted into a strong downward force and resulting in a jump or a kick (Pearson, 1983).

The neural control system generating this three stage motor programme is very complex. Three pairs of large spiking interneurones have been specifically identified, although there are undoubtedly many other interneurones, both spiking (Gynther and Pearson, 1986), and non-spiking (Burrows, 1980), involved. The identified interneurones are the C-neurones and M-neurones (in the thoracic ganglia), which are responsible for generating stages 1 and 3 of the jump, and descending contralateral movement detector neurones (DCMDs), which are responsible for the link between the C- and M-neurones and the brain (Pearson, 1983). They allow the locust to produce either the cocking or the triggering stage in response to a movement in their field of view.

The C-neurones have their somatic and main neuropilar processes in the mesothoracic ganglion. The large axon of each of the two projects posteriorly and makes synaptic connections to the FETi in the metathoracic ganglion. It is also possible that there are connections between the C-neurones and the nine flexor motorneurones of the tibia (these shall be named and discussed later) (Pearson and Robertson, 1981). The connection to the FETi is very powerful, and a single spike in a C-neurone can synchronously activate the fast extensor and a number of flexor motorneurones. Thus the "C" of C-neurone stands for coactivator. The C-neurones themselves receive information from the DCMD neurones (visual) and also auditory and tactile. It is not clear how the C-neurones are activated however, as in the experiments the DCMD, tactile and auditory neurones do not seem to be enough to cause a spike. It is assumed however that in natural conditions these three must be able to excite the C-neurones.

The connection between the C-neurones and the motorneurones are also dependant on the extension of the tibia for their strength. Rapid tibial flexion only occurs when the tibiae are not extended beyond 20° to the femur. If the tibiae are extended beyond this angle, then the synchronous activation of the tibial extensor and flexor motorneurones will lead to an extension rather than the flexion (Pearson and Robertson, 1981). This is due to the advantage that the flexor muscles have over the extensor muscles being lost if the legs are extended too much. It is not yet clear how the modulation of the cocking action to deal with this is controlled.

The M-neurones have their somata and neuropilar processes in the metathoracic ganglion. The large lateral processes of these make inhibitory monosynaptic connections to the tibia flexor motorneurones (Pearson et al., 1979). They receive information from the DCMD, tactile and auditory neurones and also the hindleg proprioceptors. This multimodal input leads to the "M" of M-neurone. They have their axons projecting into the meso- and prothoracic ganglia, but the function of these has not yet been established. The M-neurones have a very high action potential threshold, indicating that a single sensory input would be insufficient to create a spike. High frequency bursts in the M-neurones precede the inhibition of the flexor motorneurones, resulting in the kick or jump (Pearson, 1983).

The final group of interneurones are the DCMD neurones. The axons of these descend from the brain to the thoracic ganglia via the connective contralateral to the eye, from which each DCMD receives its information. There are strong excitatory monosynaptic connections between the DCMDs and both the C-neurones and the M-neurones (Pearson and Robertson, 1981; Pearson et al., 1979). There are other connections to more interneurones and motorneurones, but these are weak compared to the ones between the C- and M-neurones. Movements of small objects and shadows, and especially "looming" movements of objects towards the eye, will all excite the DCMD neurones (O'Shea and Rowell, 1977; Schlotterer, 1977; Rind, 1996).

Having discussed the interneurones, the next important stage is the discussion of the motorneurones. There are two types of tibia extensor: the fast (FETi) and the slow (SETi). There are three anterior tibia flexor motorneurones: fast (AFFlTi), intermediate (AIFlTi) and slow (ASFlTi). Similarly, there are three posterior tibia flexor motorneurones: fast (PFFlTi), intermediate (PIFlTi) and slow (PSFlTi). There is a lateral fast flexor of the tibia (LFFlTi) and two flexor inhibitors of the tibia: anterior (AInFlTi) and posterior (PInFlTi) (Hoyle and Burrows, 1973; Heitler and Burrows, 1977a). During the cocking stage the flexor motorneurones cause flexion of the two tibiae. The FETi occasionally spikes, but due to the mechanical advantages of the flexor muscles over the extensor muscles (assuming less than 20° extension to begin with) (Heitler, 1974) there is no extension. In stage two, co-contraction, flexor and extensor motorneurones spike together for 300-600 ms (in the adult, less in the juvenile). The flexor neurones may spike before or after the FETi, but there is always an increase in their frequency when the FETi spikes (Heitler and Burrows, 1977a). During stage three, triggering, the co-contraction is terminated by inhibition of flexor excitor motorneurones. This allows the relaxation of flexor muscles and hence extension. The AInFlTi spike a few times during cocking but are inhibited during co-contraction. They begin to spike about 60 ms before the onset of the triggering stage, and this increases rapidly during this period. The PInFlTi is less inhibited during the co-contraction, but like the AInFlTi, is also excited just before the triggering stage begins. The decreased inhibition is probably caused by a lower threshold on the action potential of the PInFlTi. Both the PInFlTi and AInFlTi create a burst of inhibition of the flexor neurones before triggering, which result in increasing the speed of the flexor muscles relaxation. This occurs concurrently with the inhibition of the flexor excitor motorneurones (Heitler and Burrows, 1977a).

For effective jumping the locust must be able to synchronise the two hindleg tibiae. This is normally successful, with the kicks usually being only a few milliseconds apart, although with repeated jumping this difference can increase.

An unusual feature of the motorneurones involved with the jump / kick motor programme is that there is a strong excitatory connection from the FETi to the ipsilateral hindleg flexor motorneurones (Hoyle and Burrows, 1973). FETi is apparently unique amongst insect motorneurones in having this sort of central output, although central output is common in crustacean motorneurones (Heitler, 1978). Essentially, this means that signals are sent from the FETi to the flexor motorneurones during co-contraction that help to keep the tibiae flexed. These are not the only inputs that keep the tibiae flexed though, as the amplitude of the excitatory post-synaptic potentials decreases during repetitive activity of the FETi.

There are other control circuits too, involving auditory neurones and G-neurones (Pearson et al., 1979) mediating presynaptic inhibition of DCMD neurones from the brain (O'Shea and Rowell, 1977; Rowell and O'Shea, 1980). Co-ordination of the jump with the prothoracic and mesothoracic legs may be achieved with the axonal projections from the C- and M-neurones into the prothoracic and mesothoracic ganglia, although this is as yet uncertain.

It has been assumed that sensory feedback is required throughout the jump, so the hindlegs 'know' their status. Indeed it was thought that a major factor ensuring that the FETi continued to be stimulated throughout co-contraction was sensory feedback conveying a message saying that there was resistance to the extension of the tibia. This signal comes from the cuticular receptors in the distal femur and proximal tibia (Pflüger and Burrows, 1987), and possibly the subgenual organ in the proximal tibia (Heitler and Burrows, 1977b). This positive feedback would greatly simplify the system here, and take away the need for more central inputs, which at some point would need further inhibiting circuits. This is, however, not generally regarded to be the case now though. The flexor motorneurones do receive excitation through sensory feedback, and are also stimulated by the FETi (Pearson, 1983).

The locust also needs a way of desisting from jumping when it is not necessary. It must be able to be selective about what causes it to jump. The M-neurones that control triggering must not fire when the locust is not prepared to jump, i.e. has not

gone through cocking and co-contraction. As a result M-neurones have a very high spike threshold, and it has been suggested that during co-contraction inputs from sensory receptors, such as the femoral chordotonal organ, and the lump receptor (in the neck of the lump around which the flexor tendon divides), depolarise the M-neurones to such an extent that the visual, auditory and tactile stimulations as previously mentioned are sufficient to initiate spikes (Pearson et al., 1979; Steeves and Pearson, 1982). Therefore, co-contraction is actually necessary for a jump to occur.

This is essentially the mechanism that the locust uses to kick. We assume that this is also the mechanism to jump as it appears to involve basically the same procedure. There is however still a lot that is not understood about locust jumping. For example, comparisons between adults' and juveniles' jumping mechanisms, are very difficult to study, especially with the early instars as they are so small. They appear to jump slightly differently to the adults, in that they are far more willing to jump as a form of locomotion than the adults which use it as a way to escape or become airborne. The adults are actually able to initiate flight during the co-contraction part of the jump, caused by a direct stimulus to the flight systems (Pearson et al., 1986), that is similar to the signal they receive when there is wind passing over sensors on the head (this also initiates flight). The juveniles cannot fly so they have one less form of movement open to them. This indicates that there are definite neurological changes during the final moult to adulthood, as the 5th instars do not have this ability. There may also be mechanical differences. Altered cuticle or muscle properties are known to cause differences. After each moult, as the cuticle hardens there is a change in the jumping abilities, and the muscles also grow. Even during the adults' life the cuticle changes. The juveniles never tan the endocuticle, which the adults do. This means that the adult's cuticle is much stiffer and hence capable of storing more energy in a given deformation (Gabriel, 1985).

It is also possible that the differences are caused by size and weight changes. Adults are much heavier than juveniles. During the final moult they put on weight in

the form of wing muscles. The female puts on weight as she grows her eggs. Weight even changes within the instars; as muscles grow, the abdomen expands. There are changes in the centre of gravity during these changes. The relationships between the weight, size and musculature are constantly changing (Katz and Gosline, 1993), and so need to be taken into account. The most probable reason for the differences is a combination of these, so in studies of locust jumping it is important to look at, and categorise, as many of these differences as possible.

Having looked at the general method by which locusts jump, there shall now be a discussion of the areas that will be concentrated on in this thesis. These are the metathoracic femoral chordotonal organ, and the lateral nerve of the metathoracic femur.

The Metathoracic Femoral Chordotonal Organ and the Lateral Nerve

In each femoro-tibial leg joint in insects can be found several sensory organs. In the hind-leg of the locust there have been described three (Pflüger and Burrows, 1987), and these are, most importantly, the chordotonal organ (Usherwood et al., 1968), a strand receptor (Braunig, 1985), and five multipolar sensory cells (or multipolar joint receptors) (Coillot and Boistel, 1968). The chordotonal organ's functions are many, but all connected with leg movement and reflex activities (Field and Rind, 1981; Hustert, 1982). Position of the limb is of paramount importance both when voluntary movements and reflex movements are being mediated, so the locust must have sensory input to monitor this.

The chordotonal organ is known as a joint angle receptor, and mediates information important for posture and movement during locust jumping (Steeves and Pearson, 1982) and walking (Graham and Bässler, 1981). Other locust activities, such as flying (Wilson, 1961; cited by Zill, 1985a) and stridulation (Bässler, 1979) do not require input from a joint angle receptor.

The chordotonal organ is found attached to the dorsal surface of the distal femur. It is attached by a firm ligament, about 6-7mm proximal to the femoro-tibial joint (Zill, 1985a). This attachment is where the sensory neurones are located, and it is known as the scoloparium. There are normally two of these clusters, a distal scoloparium, and a proximal scoloparium, and these can be either partially separated, fully separated, or fused (Field and Pflüger, 1989). However, in the metathoracic leg of the locust it appeared that the proximal scoloparium was missing altogether (Fuller and Ernst, 1973; cited by Field and Pflüger, 1989; Burns, 1974; Field and Rind, 1976; Usherwood et al., 1968; Theophilidis, 1968; Burrows, 1987). The strand of the chordotonal organ is connected by a ligament to the tibia, just next to where the flexor tibiae muscle is inserted, so that when the leg is flexed the strand is distorted, and the sensory neurones fire. The dendrites from the sensory neurones are inserted into

scolopidia (complex mechanotransducer structures) within the strand (Matheson and Field, 1990). A second attachment occurs between the chordotonal organ and the tendon of the flexor tibiae muscle, about 5mm proximal to the femoro-tibial joint (Zill, 1985a). During tibial extension this ligament stretches the organ in a different direction. The organ is connected to the central nervous system by nerve 5b1, which the axons of the sensory neurones join (Campbell, 1961; cited by Zill, 1985a).

Within the chordotonal organ there are about one hundred neurones (Matheson, 1992b), and the cell bodies of these can be found in two distinct groups. One group is found ventrally and the other, dorsally. There is a third group of cells which has recently been discovered, distal to, and much smaller than, the other two (hence the only recent discovery), which is thought to be the remnants of the proximal scoloparium (Matheson and Field, 1990). Within each group, there are size differences, such that the larger cells are found proximally, and the smaller cells are found distally. The two groups thus formed can be so distinct that the axons often form two different bundles that join nerve 5b separately. There are four types of receptor cell in the chordotonal organ: tonic receptors, that fire in ranges of joint flexion or joint extension, and phasic receptors, that fire in response to either extension of the leg, or flexion (Zill, 1985a).

The tonic receptors fire depending on the position of the leg, and the phasic receptors fire depending on the movement of the leg. Rest position is about 80°, which is when tonic discharge is at a minimum. Repositioning of the leg at an angle greater or less than this leads to an increase in the firing of the tonic receptors. When one or the other of the main ligament or flexor ligament of the chordotonal organ is cut, there is a change in the firing pattern. If the main ligament is cut then the increase in firing rate that normally occurs when the leg is put in a more flexed position is abolished. If the flexor ligament is cut, then there is very little increase in response to the leg being more extended, and this is only at the extreme extension ranges (Zill, 1985a).

The tonic chordotonal organ receptors act as line labels, indicating leg position. Most such neurones also show range fractionation, in that they show maximum sensitivity over quite a narrow range of tibial positions (Matheson, 1992b).

Phasic activity is more complex than tonic, as there are more factors affecting movement than position. During rapid joint movement, the chordotonal organ registers discreet discharges, and these occur no matter what the starting position of the leg. Individual phasic units within the organ respond to either flexion or extension, but no matter what the angle of the joint, there is always the ability to respond to either extension or flexion. Those units that respond to flexion are found dorsally within the organ, and those that respond to extension are found ventrally, near to the flexor ligament. Some units respond only to movement (firing only once), but are very sensitive, whereas others are responsive to velocity of movement, and they fire according to the movement rate (Zill, 1985a). There is also range fractionation in the phasic neurones, with some responding with increased frequencies depending on the position of the leg during the movement. It should be noted that the response ranges of most neurones (both phasic and tonic) are wide, but that a few have narrow ranges, and these as, noted before, are generally sensitive to extreme leg angles, with just a few responding to middle angles (Matheson, 1992b).

Sensitivity of the metathoracic chordotonal organ to vibration is limited, but this is probably due the lack of the proximal scoloparium, which is devoted to the sensing of vibration in the mesothoracic chordotonal organ.

The chordotonal organ in the locust's mesothoracic leg is very different. It has both the distal and proximal scoloparia, unlike the metathoracic leg. The proximal scoloparium has only a few cells that respond tonically and phasically, and the tonic response is not the same as in the metathoracic leg. Maximum firing frequency occurs when the leg is at full extension, and minimum firing frequency occurs when the leg is at full flexion. This is quite different to the U-shaped curve generated by the tonic discharge from the metathoracic chordotonal organ. Most of the cells are devoted to the sensing of vibrations. The distal scoloparium is involved with reflexes and

position/movement, as is the case with whole of the metathoracic chordotonal organ (Field and Pflüger, 1989).

The reflex activity of the chordotonal organ occurs when it responds to joint movement, and muscles that oppose that movement are activated. It has two modes for doing this. The first is known as a resistance reflex mode. When this reflex is elicited, motorneurones to oppose any leg movement are excited. This is occurring constantly whenever the leg is supporting anything. The second is called a flexion reflex mode, and is when joint movement in any direction leads to the excitement of flexor motorneurones (Zill, 1985b).

Resistance reflex mode exhibits considerable variability, which is important, as it means that animal can alter its reflexes according to its current behaviour (Bässler, 1979). Thus, when the animal is walking, it does not exhibit resistance reflexes in response to it's own activities (Barnes et al., 1972), except where necessary. This plasticity is very important for the resistance reflexes if they are to act as load compensators. The animal can walk without the reflexes affecting the movement, but if a sudden, unexpected load is applied, then the animal can also compensate for that. It is suggested that the animal is continuously comparing afferent input with motor output, so that it doesn't respond to its own movement with a resistance reflex (Barnes et al., 1972).

The flexor reflex mode is more confusing, however. Input from the chordotonal organ, indicating either joint flexion or joint extension, leads to excitation of flexor motorneurones, but only when the tibia is free to move. It has also been shown that the reflex sign will change, when passive movement is forced after active movement (Vedel, 1982). In the case of the metathoracic chordotonal organ, these changes have only been seen in response to flexion movements and not extension (Zill, 1985b). Why this is the case is uncertain. It has gone unnoticed for some time as flexor activity has not always been recorded during these experiments. Suggestions that the changes represent the switching from a resistance response to movement assistance response have been refuted, mainly because assistance functions require

apparent joint flexions, not ligament relaxation and apparent joint extension. Furthermore, the activity that is required for effective load compensation (strong, tonic activity) (Melvill Jones and Watt, 1971)was not present. It is possible that the flexor reflex serves as a method for the animal to respond to a stimulus that affects leg position whilst the tarsus is in the air during walking (Zill, 1985b), and this has been seen before (Pflüger, 1980).

Another important aspect of the chordotonal organ is hysteresis. This occurs when the leg is moved from one position, and then returned to that position, and the tonic discharge is at a reduced level to that which it was at originally at that same leg position. This is, therefore, an apparent degradation of the sensory abilities of the joint receptor, but could be an adaptation to deal with muscle tension. It has been theorised that this hysteresis is primarily to reduce the effect known as 'catch' muscle tension, which is when there is residual tension in the muscle after activity. It has been seen in many vertebrates and invertebrates sensory systems (Grigg and Greenspan, 1977; Grigg et al., 1978; Krnjevic and van Gelder, 1961; Burns, 1974), indicating that other proprioceptive organs may be attuned to the muscles that they modulate. What is odd though is that hysteresis should create ambiguities in the encoding of signals for joint angles and position.

In recent experiments it has been shown that hysteresis is more complex than originally supposed. Of the approximately 100 neurones in the metathoracic chordotonal organ, 91 show a tonic response which codes to leg position. However, of these, 77 also have phasic properties. Those that are more flexion-velocity sensitive mostly have a tonic firing rate that is higher if the current angle of the leg is approached from a flexion, than it were approached from an extension. The opposite is true of those that are extension sensitive. However, it should not be taken from this, that hysteresis is merely an aftereffect of the phasic response to this movement. Four of the flexion-velocity sensitive neurones have a higher tonic firing rate when the current leg angle had been approached from an extension. Eight tonic neurones that respond maximally to the leg angle being in mid range (with response curves that

exhibit crossovers), showed highest tonic firing when the angle had been approached from a position further than the crossover angle. It is, thus, conceivable that some of the phasic-tonic neurones have phasic and tonic properties that are not linked to each other (Matheson, 1992b).

The chordotonal organ is very important in the proprioception of the locust, and as such has been studied extensively as an insect sensory organ. The locust requires the information from the chordotonal organ to provide it with the positional knowledge to produce the jump or the kick. When the chordotonal organ has been severed the locust loses the ability to kick (Heitler and Burrows, 1977b), although this ability does return, and this is what shall be looked at later.

The lateral nerve of the locust is a lot less well studied than the chordotonal organ. It is a branch of the nerve 5b2 (which is itself a branching of the nerve 5b) in the metathoracic leg. Its main functions are to innervate cuticular receptors in the proximal region of the femur, Brunner's organ, the 'lump' receptor, and other receptors in suspensory ligaments in the femoro-tibial joint.

Of these four systems, Brunner's organ is the least well understood. This organ is a protrusion found in the groove on the ventral surface of the femur, and consists of a small tubercle, with three sensory hairs and two campaniform sensilla at its base. No function has yet been found for Brunner's organ (Jannone, 1940; Joly, 1951; Uvarov, 1966: all cited by Heitler and Burrows, 1977b), although when the leg is fully flexed, and the tubercle is flattened against the cuticle, it does produce small amplitude excitatory post-synaptic potentials in the ipsilateral fast extensor tibiae motorneurone. These are never able to produce a spike in the FETi on their own, but only in combination with other excitatory inputs. There is no loss in kicking or jumping ability when Brunner's organ is excised, and so it is considered to have no importance (Heitler and Burrows, 1977b).

The cuticular receptors are ones that detect strain in the cuticle. This strain occurs when the tibia is extended to extreme levels, and results in large excitatory post-

synaptic potentials in the FETi and the SETi (Burrows and Horridge, 1974). The leg only achieves this level of extension after the kick or jump has occurred, and so these EPSPs do not directly affect the jump themselves. It is therefore possible, then, that these receptors are activated by another stimulus during the jump motor programme. Repeated high intensity stimulation of the lateral nerve will lead to a burst of EPSPs in the FETi as seen when the leg has been forcible extended. When the tibia is extended forcibly it produces a distortion in the cuticle of the femur that is similar to that experienced when the tibia is fully flexed, thus causing the EPSPs (Heitler and Burrows, 1977b). Thus the cuticular receptors are providing information on the position of the leg during the kick.

The other important function of the lateral nerve is to innervate the 'lump receptor' (Coillot and Boistel, 1968). The lump is a cuticular invagination in the ventral side of the distal femur. It is this lump that the flexor tendon fits over when the leg is fully flexed during the preparation for a kick. The lump receptor is a branch of the lateral nerve that innervates the lump, and is only activated when the leg is fully flexed (Heitler, 1974). In any position other than fully flexed, no spiking of the lateral nerve occurs when tension in the flexor tendon occurs (Heitler and Burrows, 1977b). This is a further way for the locust to receive information about the position of its leg.

When the lateral nerve is severed, it is difficult to obtain a jump or a kick from the locust (Heitler and Burrows, 1977b), but this shall be explored later.

Materials and Methods

Adult and juvenile locusts (African Desert Locust, *Schistocerca gregaria*) in all experiments were taken from a crowded population, maintained in the University's Gatty Marine Laboratory.

During the extracellular recordings 50μm insulated copper wire was used for the electrodes. Two Isleworth A103 pre-amplifiers were used to boost the signal from the locusts muscles (one for the extensor and one for the flexor) and a Tetronics 5 100 Series oscilloscope was used to display the results. These were recorded on a Racal Thermionic (D7) F.M.. recorder, and any results were printed out via an Astromed Thermal Array printer. During some experiments a Cadmium Sulphide Light Dependant Resistor (henceforth referred to as a photocell) was used to detect leg movement during an extension.

As each set of experiments uses different methods, the specific method to each chapter will occur at the beginning of that chapter.

Comparative Jumping Performance of Intact Locusts

Introduction

Various morphological parameters were measured for adult and 5th instar locusts to provide a general description of the sample population being used in the experiments to follow. The jumping performance of intact animals was then measured to provide a baseline against which future experiments involving sensory ablation could be judged.

The locusts' metathoracic legs were measured, as were their thoraxes. Of the body, the thorax alone was measured. The abdomen expands and contracts too much for one measurement to give an accurate picture of the size. The femur and the tibia of the metathoracic legs were measured.

Average jumping ability has already been established for the locust in many previous accounts. However, it is important to assess the ability of the animals that are going to be used in the experiments to follow, so that they can be compared to those that have been done already. It should be noted that at the time that the first experiments were being performed, the locust population in the Gatty laboratory was suffering from some unknown illness, and so expectations are that initially the jumping abilities shall be somewhat reduced compared to the averages seen before.

Methods

5th Instar Jumping.

Nine (the small sample is indicative of the illness of the locusts) 5th instar locusts were removed from their cages and encouraged to jump. They were placed on the floor (vinyl) of the laboratory in which they were kept (this would keep them at the same temperature as that which they were used to), and hand movements and

noises were used to elicit jumping activity. If this was not sufficient, then by gently touching the abdomen, it was possible to get them to jump.

The take-off and landing points were marked with chalk, and the distance between them was measured with a tape measure. Typically, the locust would perform several jumps, gradually getting smaller, before it was stopped and measuring would then take place. The floor was then cleared of chalk marks, and the animal would be encouraged to jump again. It was intended that approximately twenty jumps would be recorded from each animal, before the next was selected.

Adult Jumping.

Nine adult locusts were selected, and each one was encouraged to jump in the same way as the 5th instars had been previously. The difference here is that as the adults often use the jump to initiate flight, they needed to have their wings taped or removed. Taping the wings can lead to a preoccupation with trying to remove whatever is holding them together (usually insulating tape, as this does not damage the wings when it is removed after the experiments), so it was considered better in the long run to just remove the wings with a pair of scissors.

Therefore the clipped adults were placed on the floor of the locust room, and had their jump distances measured in the same way as before. With the adults, rather than go for the ten jumps each, as many jumps as possible were recorded (they are often reluctant to perform successive jumps).

Jumping on a different surface.

A wooden topped table was brought into the locust room, with the surface about one metre off the ground. The table surface was a lot rougher than that of the floor, and so it was hoped that it would provide a better indication of the jumping ability of the locusts than the floor. This should prevent, or reduce the skidding seen in the 5th instars when on the floor. Also, with it being higher off the ground, it was at a temperature closer to that which the locusts were used to. Ten 5th instar locusts

were selected, and placed on the table top, one at a time, and encouraged to jump as before. Each of the ten locusts made ten measured jumps, with the measurements being taken in the same way as before.

Results.

Size

The following table shows the averages of the lengths of the femur, tibia, and thorax of the adults and 5th instars (all results being in millimetres), and the ratios which they have with each other. The sample size is in brackets after the sample title, and the standard deviations follow the results in brackets.

	Averages		Ratios		
	Adults (n=10)	5th Instars (n=20)	Adults	:	5th Instar
Femur	22.3 (1.77)	16.7 (0.91)	1	:	0.75
Tibia	20.7 (1.64)	16.4 (0.74)	1	:	0.79
Thorax	15.7 (2.87)	11.4 (0.68)	1	:	0.73

This shows that there is not much difference in the ratios between the adults and the 5th instars. The indication, then, is that the whole body grows at the same rate after the final moult. The fact that the standard deviations of the adults' samples are so much higher than the 5th instars can be attributed to the sexual dimorphism that occurs in the adults. The female locust is larger than the male, and as there was no distinction being made when the adults were chosen, the variability of the sample would be expected to be bigger. The even higher standard deviation for the thorax for the adult indicates that there is greater sexual dimorphism in the thorax than there is in the metathoracic leg.

A t-test indicates that the two samples are, as expected, significantly different. For the femur, $t = 11.54$ with 28 d.f. at the 5% significance level. For the tibia, $t =$

9.98 with 28 d.f. , and for the thorax, t = 6.45 with 28 d.f., both at the 5% significance level.

A comparison between the femur and the tibia of the adult and the 5th instar would show that there is a slight difference between the ratios. Standard deviation for the adult is 0.03, and for the 5th instars it is 0.02. A t-test gives t = -4.90 with 28 d.f. at the 5% significance level, indicating that there is a significant difference between the two samples.

	Averages		Ratios		
	Femur	Tibia	Femur	:	Tibia
Adult	22.3mm	20.7mm	1	:	0.93
5th Instar	16.7mm	16.4mm	1	:	0.98

5th Instar Jumping Ability

From the experiments performed, the average 5th instar jump distance (all jump distances added, then divided by total number of jumps) is 17.1cm (S.D. = 8.01, n = 212), and the average maximum jump distance (maximum jump distance from each locust added, then divided by the number of locusts) is 28.2cm (S.D. = 13.27, n = 9). This compares unfavourably to results seen in previous experiments by Katz and Gosline (1993). They state that the average jump is between twenty and thirty centimetres. They use 25cm as the figure for establishing the specific peak power output of the muscle involved in the jump. Here, the figure that would be used is 28cm, as this is the average maximum jump. This figure does not compare favourably to previous work done in the same conditions, as part of an undergraduate project, and the illness that had been plaguing the locust population in the laboratory was given as reason. Also, the fact that many of the smaller jumps appeared to be affected by some degree of slipping on the vinyl floor, lead to the conclusion that a better surface could be used, and the experiment tried again.

Adult Jumping Ability.

From the experiments performed, the average jump of the adult locust is 47.11cm (S.D. = 21.26, n = 53), and the average maximum jump is 69.44cm (S.D. = 19.73, n = 9). The average jump is nearly three times as great as that of the 5th instar, and the average maximum jump is two and a half times that of the 5th instar. There did not appear to be any slipping in the adults jumping so it was deemed not necessary to repeat on a different surface. Also, a lot of the 5th instar jumps where slipping appeared to occur were towards the end of a succession of jumps, and so this could be attributed to fatigue, as well as the surface. This did not occur with the adults as they rarely took more than one jump before they stopped and the measurement was taken.

5th Instar Jumping on different surface.

The average jump for the 5th instar on the rough surface is 27.9cm (S.D. = 8.4, n = 100), and the average maximum jump is 40.5cm (S.D. = 6.45, n = 10). Thus, the average jump is 10cm greater than the previous experiment showed, or approximately one and a half (1.63 to be precise) times as much. The average maximum jump is 12 cm greater than before, or approximately one and a half (1.45) times as much. The following table shows this, and the standard deviations. For the average jump distances, t = 10.94 with 310 d.f., and for the maximum jump distances, t = -59.3 with 17 d.f.. At the 5% significance level there is a significant difference between the both the average and the maximum jump distances.

	Averages		Ratios		
	Floor (n=212)	Table (n=100)	Floor	:	Table
Jump	17.1 (8.01)	27.9 (8.40)	1	:	1.63
	Floor (n=9)	Table (n=10)	Floor	:	Table
Max. Jump	27.9 (13.27)	40.5 (6.45)	1	:	1.45

Most of the jumps that occurred in this experiment were long jumps, and very few short jumps were seen. The short ones are the ones that may appear to involve no co-contraction, and were seen in the experiment done on the floor. The size of the table does restrict the room that the animal has to jump, so less repeated jumpings were seen. It is feasible, then, that the short jumps seen were due to fatigue, however, there were some short jumps seen in this last experiment too, and ruling out lack of requirement for co-contraction would be wrong at this point.

Extracellular recordings from the extensor and flexor muscles during a jump and a kick

Introduction

Having established the normal jump ranges of the adult and 5th instar locust, the next goal was to be to insert electrodes into the extensor and flexor muscles in the femur of the animals metathoracic leg. This would enable extracellular recording of the impulses fired during the jump of the animal. Most of the previous work of this kind has been done on the kick, with the animal strapped down in plasticene. It was decided, however, that myograms of both kicking and jumping would be obtained, but that the free-moving experiments would be tried first. The first task was to perform a control experiment to establish that the animals jumping ability would not be impaired by the attachment of wires to the metathoracic leg. The wire to be used was very fine copper wire, which is very light to the human touch, but it had to be ruled out that it could itself affect the jump. This was particularly important for the juveniles, where the small size of the muscles would make them more susceptible to damage from the implanted wires.

Methods

Jumping with Electrodes.

The 5th instar locusts were placed in plasticene (mounted in a petri dish, with magnetic underside), and had their pro- and mesothoracic legs secured at the tibia with more plasticene, so they could not interfere with the preparation. The metathoracic legs were placed in plasticene grooves, with the ventral groove of the femur facing up. The tibiae of the metathoracic legs were secured with more

plasticene in a fully extended position, so that they too could not interfere with the preparation.

The wires that would normally be inserted into the muscles were then attached to the outside of the femur with bees wax (the same wax that would later ensure that the wires stayed in the muscles). The locust was then carefully released from the plasticene and taken through to the locust room, where it was placed on the table that had been used in the previous experiment. The locust was then encouraged to jump using the standard method of noise and movement near to it. Again, if this did not work, then it was gently rubbed on the dorsal surface of its abdomen.

Jumping with Electrodes in Muscle.

To record myograms in non-control experiments, the locusts involved were strapped down in the plasticene as before, but this time two small holes were made in the cuticle of the outer wall of the metathoracic femur, where the extensor muscle is inserted. Small needles were used to make the holes, which were about 1mm apart. The ends of the very fine copper wire were inserted into these holes, about 1-2mm into the leg. The wires were sealed in position by the same wax that had been used previously. This was melted with a hot wire and then placed over the holes with the wires in them. The locust was then carefully released from the plasticene, and placed on a rough surfaced wooden board in the wire cage where the recorded experiments take place. The temperature of this room was about 20°C, not the ideal condition for locust jumping, but necessary because the cage set-up could not be moved, due to the required electromagnetic isolation from the mains 50Hz wavelength. The fine copper wires were attached to a thicker wire that was in turn attached to a signal amplifier. The output from the amplifier goes into the oscilloscope, and also into a recorder, and from there it goes to a printer, with a readout facility. The thick wire going into the signal amplifiers was hung from the microscope in the cage, so that the only weight from the wire that the locust would have to encounter would be that from the very fine copper wire attached to its leg. This set-up gave the locust about 45cm jump

range in the direction that it was intended it should jump, and this was considered sufficient, given the average jump range of about 28cm, and the average maximum jump of about 40cm.

The tape recorder that was connected to the oscilloscope was switched on, and the animal was encouraged to jump. The locust, once it had jumped was then returned to the start position, so that it could jump again without getting tangled in the wires in the cage.

The extensor activity that occurred was printed out from the recordings made.

Recordings of the Control.

Recordings were also made of the animal jumping, but with the wires not in the leg. This was to see if any of the articles on the previous recordings had been caused by the wire moving through the air. Unfortunately, with the wires not in a conducting medium, there was little that could be distinguished, and so no conclusion can be drawn on this.

Adult Jumping with Electrodes.

The same procedure was adopted for adult locusts as for 5th instars, except that, as with all adult jumping experiments, the wings have to be taped or removed to stop them from taking off. Once the wings were removed and the wires inserted, the adults were placed on the board and encouraged to jump. There was no control performed on the adults, as it was assumed that as the wires did not drastically affect the 5th instars jumping ability (see results), then they would not affect the adults'. Recording of the muscle activity was operated as before.

5th Instar Kicks.

To further study the kick mechanism, the 5th instar locust was placed in plasticene again, and held there as when it was being prepared for the jump experiments. Holes were again made in the femur at the point where the extensor is

inserted, and they were also made on the ventral surface of the femur where the flexor muscle is inserted. This would allow the whole kick to be looked at, hopefully more clearly than when the jump was being studied.

The leg to be recorded from then had very fine copper wires inserted into both sets of holes. The new set of holes had wires that went to a separate signal amplifier box, which then lead on to the oscilloscope, the recorder and the printer. The leg was oriented so that the tibia moved in the vertical plane, allowing a photocell and light source to be set up either side of it. Attached to the distal end of the tibia was a flag made of insulating tape. This was necessary because the leg moves so quickly during the kick that the photocell did not register it well enough to see on the print-out. The flag passed through the beam of light going to the photocell, when the leg was at about 60°. The flag formed an area of about $1cm^2$. The photocell was attached to the printer directly, but as the printer had a display area on it, there was not a problem with monitoring its output.

The locust was then encouraged to kick by having its abdomen rubbed gently with a wooden rod or a paint brush. and recordings of any activity were made

Adult Kicks.

The same experiment was performed on the adult, for completeness sake, and also because it would further explain the kick of the animal. It would also provide a control for the experiments that were to be done later on the adult. The procedure done on the adult was exactly the same as the 5th instar

Control Kicks

To calibrate the ability of the photocell to detect tibial movement, experiments were performed with the adult locust secured in the plasticene as normal. The cuticle of the ventral surface of the thorax was cut away, allowing access to the nerves that control the leg movement. Once the fatty tissue and the tracheae were also cut away, the nerves that were required could be lifted clear of from the body cavity on hook

electrodes, and insulated with vaseline. The nerve used was nerve 5, and this allows stimulation of the extensor muscle. A wooden rod can be used to keep the leg fully flexed, meaning that when the extensor was stimulated, there would be distortion of the femoro-tibial joint (as there would be in real co-contraction), and that the removal of the rod would lead to a kick. A recording of the muscle activity would be made, and also the photocell would pick up the point when the leg passed it. A good indication of what the photocell spike would look like after a kick would be seen. This could then be compared to the results obtained from the real experiments.

The real kick experiments were first done on the adults, and involved the same procedure as before.

Results

5th Instar jumping with Electrodes.

This gives an average jump of 22.5cm (S.D. = 7.04), and an average maximum jump of 30.5cm (S.D. = 7.74). as these experiments were done on the rough table surface, they should really only be compared to the results from the last experiment, also done on the table.

	Averages		Ratios		
	Table (100)	Att. Wires (96)	Table	:	Att. Wires
Jump	27.9 (8.40)	22.5 (7.04)	1	:	0.81
	Table (10)	Att. Wires (10)	Table	:	Att. Wires
Max. Jump	40.5 (6.45)	30.5 (7.74)	1	:	0.75

This puts the average jump down by 6cm , or down by one fifth, to approximately four fifths (0.81) of the previous, and the average maximum jump down by 10cm, or three quarters of the previous. It would be expected for the attachment of wires to the leg to have some effect on the jump distance, and statistics show that there is a significant difference between the two samples (for the average

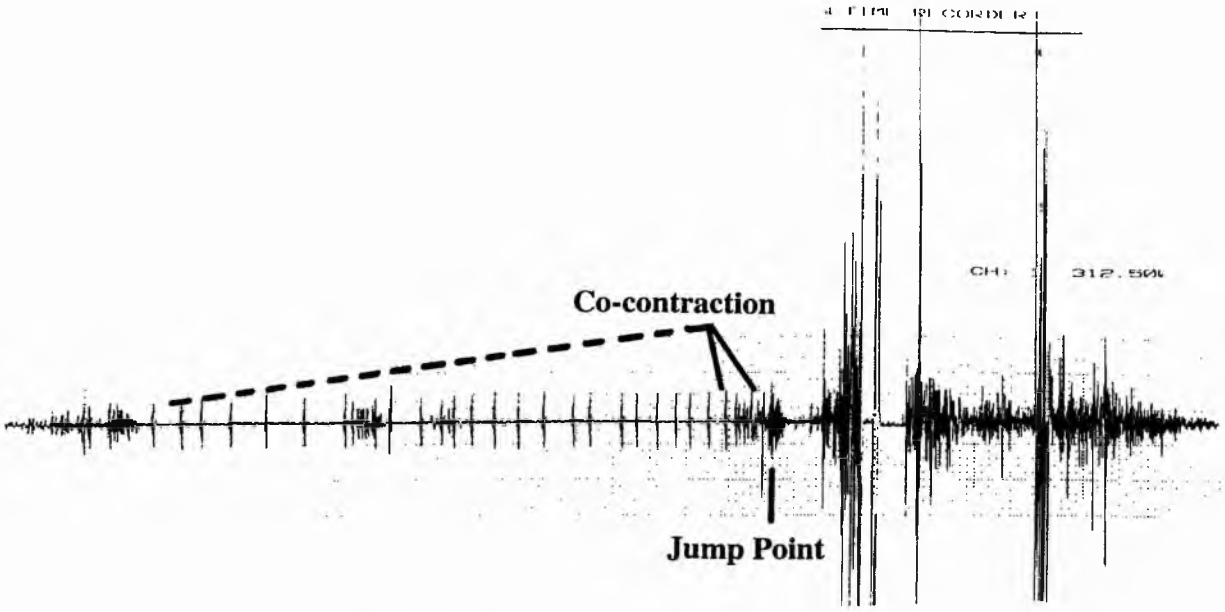

Fig. 1.1. Extensor activity during an adult jump.

Horizontal Scale: 20ms/mm. This jump shows exceedingly long co-contraction, followed by a jump, the take-off point of which is not very clear.

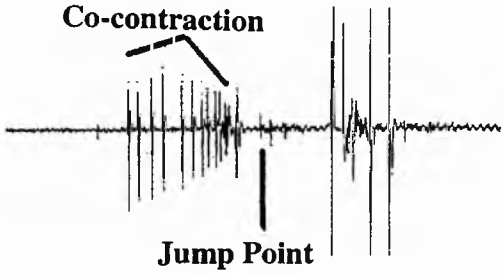

Fig. 1.2. Extensor activity during an adult jump.

Horizontal Scale: 20ms/mm. This jump shows very clear co-contraction, although the take-off point is again debatable.

jump, t = 4.93 with 194 d.f., and for the maximum jump, t = 9.41 with 18 d.f., both at the 5% significance level). This is still within the acceptable norms of the 5th instars jump, and so it is safe to continue the experiment, and put the electrodes into the locusts muscle.

Adult and 5th Instar Jumping with Electrodes in Muscles.

Once it was established that the very fine copper wire did not inhibit the jumping ability of the 5th instar too greatly, the distance that they jumped whilst their muscular activity was being recorded was not considered overly important. Obviously, it is important to some degree that they achieve a jump of some distance so that the muscular activity can be equated to a kick, but the exact distance achieved is not fundamental to this. Therefore the results from these experiments are in the form of myograms, printed from the recordings made during the experiments, showing when the extensor fires, and what pattern of activity it forms. At this stage there was no flexor activity being recorded. Having seen previous myogram printouts of the activity, it was thought that it would be easy to assess what was a jump and what was not. This was not always the case though, and it was at times difficult to see what was going on.

The traditional view of the jump and kick, is that there is a period of co-contraction, where both the extensor and the flexor fire together, whilst the leg is fully flexed. There is then a period when there is only extensor activity, with the flexor being inhibited, then the extension occurs. This will occur over about 300-600ms in total. Thus when the extensor is looked at on its own, a period of activity of this length is to be expected.

In one adult sample it is possible to see some good jumps. The first (shown in fig. 1.1) shows a very long period of co-contraction (about 1900ms, or nearly 2 seconds), followed by several large peaks. The second showed a clear co-contraction period of about 400ms, which fits in much better with the expected duration. The third jump showed co-contraction for about 600ms, followed by a gap of about

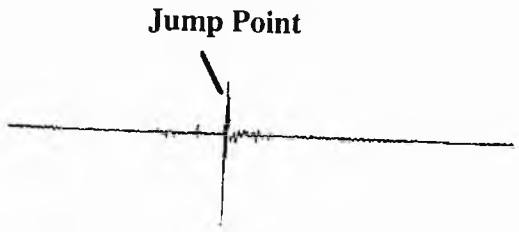

Fig. 2.1. Extensor activity during a 5th instar jump.

Horizontal Scale: 20ms/mm. The single middle spike appears to be the point where the jump occurs, although there are very small spikes either side. This does not look like co-contraction though.

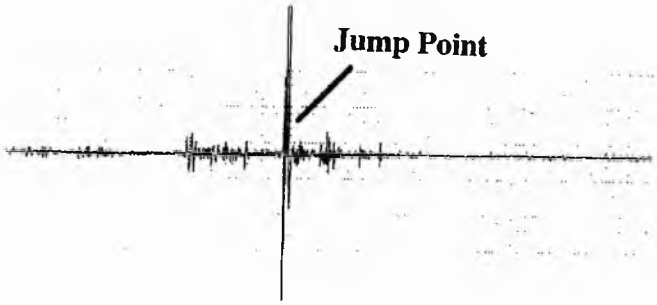

Fig. 2.2. Extensor activity during a 5th instar jump.

Horizontal Scale: 20ms/mm. The burst of spikes before the large spike(s) is thought to be co-contraction. The large spike(s) is where the take-off occurs.

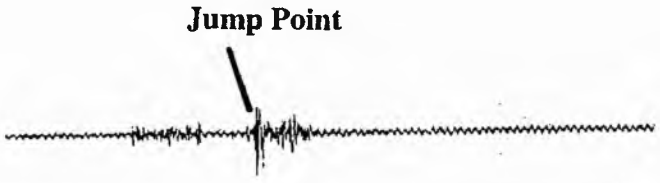

Fig. 2.3. Extensor activity during a 5th instar jump.

Horizontal Scale: 20ms/mm. Here, there is possible co-contraction, but the take-off point is less obvious.

150ms, then another burst of activity lasting about 70ms, which was presumably the jump take off point.

Another adult provided more good jumps (fig. 1.2). Here co-contraction lasts on average about 260ms, and then there is a break lasting about 300ms, before the next burst of spikes indicates the jump. The problem of the burst of spikes at the end signifying the take-off, can be explained by the movement of the very fine myogram wires moving through the air as the animal jumps. Recordings taken while the wires were not in the muscle showed nothing conclusive though.

In the 5th instar, one might expect the co-contraction period should follow the same pattern. However, what was seen here was not the same. It can be difficult to achieve this pattern in the 5th instar, but there should be some similarity. Here, however, it is often the case that the pattern seen before and during the 5th instar jump is totally unlike that which is expected. It is, therefore, nigh on impossible to give an average duration of the extensor activity that occurred before the jump. The best way to view the results is through description of some of the activity that is seen on the printouts.

In one sample there were four jumps. There was possibly co-contraction in the first and third, but even this was not as has been seen in the adults, nor as in previous 5th instar kicks. The jump shown in fig. 2.1 exhibits a single spike, followed about 10ms later by a second spike. This certainly does not appear to be co-contraction, yet it is the only activity on the tape where the jump occurred according to the recorded voice over.

Another sample showed several jumps, and some of these may have shown co-contraction, but still not in the form that was seen in the adult, or in 5th instars kicks. The second jump (fig. 2.2) in this recording (the first was very indistinct) consisted of about 170ms of small spikes, then 100ms later two large spikes, 10ms apart, followed by a 280ms long bursts of smaller spikes. It is possible that the first burst of spikes represented the co-contraction occurring, and that the middle two were the take-off point. The last burst could be leg movement whilst the animal was in the

air. The fourth jump (fig. 2.3) started with a burst of small spikes lasting 180ms. There was then a gap of about 140ms, and then a second burst of spikes lasting about 160ms occurred. The first two spikes in this second burst were similar in appearance to the two large spikes in the second jump, and so a comparison can be drawn between the two.

A lot of the jumps follow the pattern of the burst of spikes succeeded by a second burst of spikes, with two large spikes (or sometimes one). The pattern is not as expected, and so creates the need to look at the point where take off occurs, so that the activity causing the jump can actually be looked at. This requires the kick be monitored, and a photocell be used to look at the point when the leg extends.

With the kick it is much easier to look at the flexor as well as the extensor, and the pattern generated is assumed to be the same as that during the jump.

Adults and 5th Instar Kicks with Electrodes in Muscles.

Again the results here are primarily shown in the form of myograms recorded during the kicks themselves. With the flexor muscle also being recorded from, and the photocell giving accurate information about leg extension and flexion, this should provide ample information about the kick. Unfortunately, however, the same problem as before appears: the pattern is not as expected. One of the theories that was being worked on was that the 5th instars could jump and kick without using the co-contraction method. Here appeared to be some evidence to back the theory up (see results later). The procedure used was exactly the same as that used with the adults, but the pattern being seen was often different. If the procedure was not wrong then maybe the preconceptions about the 5th instar jumping / kicking mechanisms were.

To further explore the results here, it was decided to graph the relationship between the speed of the leg extension, and the activity in the extensor muscle. The extensor activity could be measured by the duration of the activity before an extension, or by the number of extensor spikes that preceded the extension. The initial

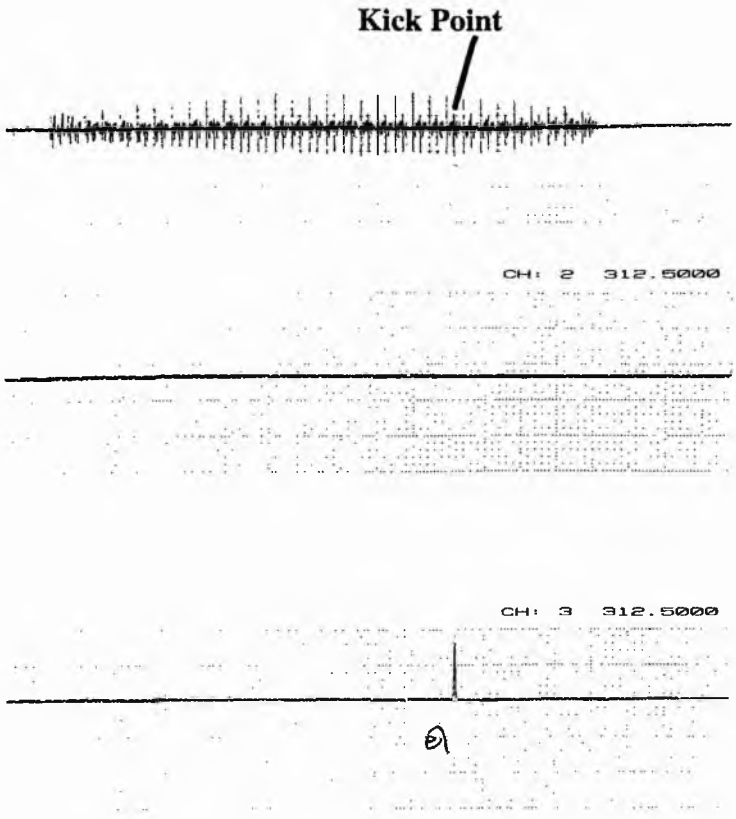

Fig. 3.1. Extensor activity (top trace) and photocell spiking (bottom trace) during an artificially created adult kick

Horizontal Scale: 20ms/mm. The long stimulation time leads to a small photocell spike, i.e. a fast extension.

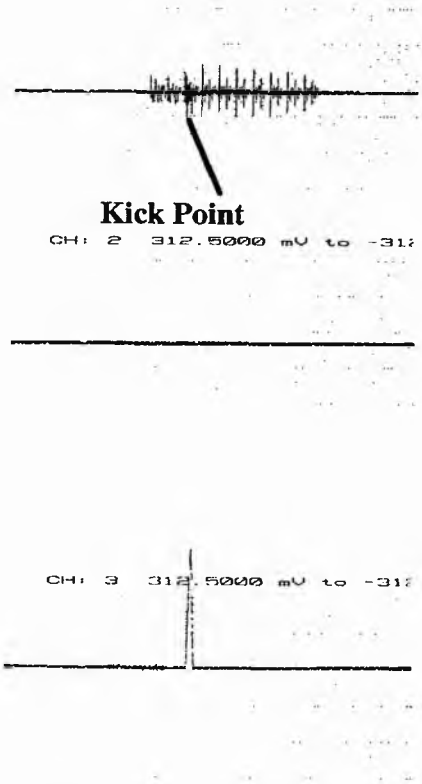

Fig. 3.2. Extensor activity (top trace) and photocell spiking (bottom trace) during an artificially created adult kick

Horizontal Scale: 20ms/mm.

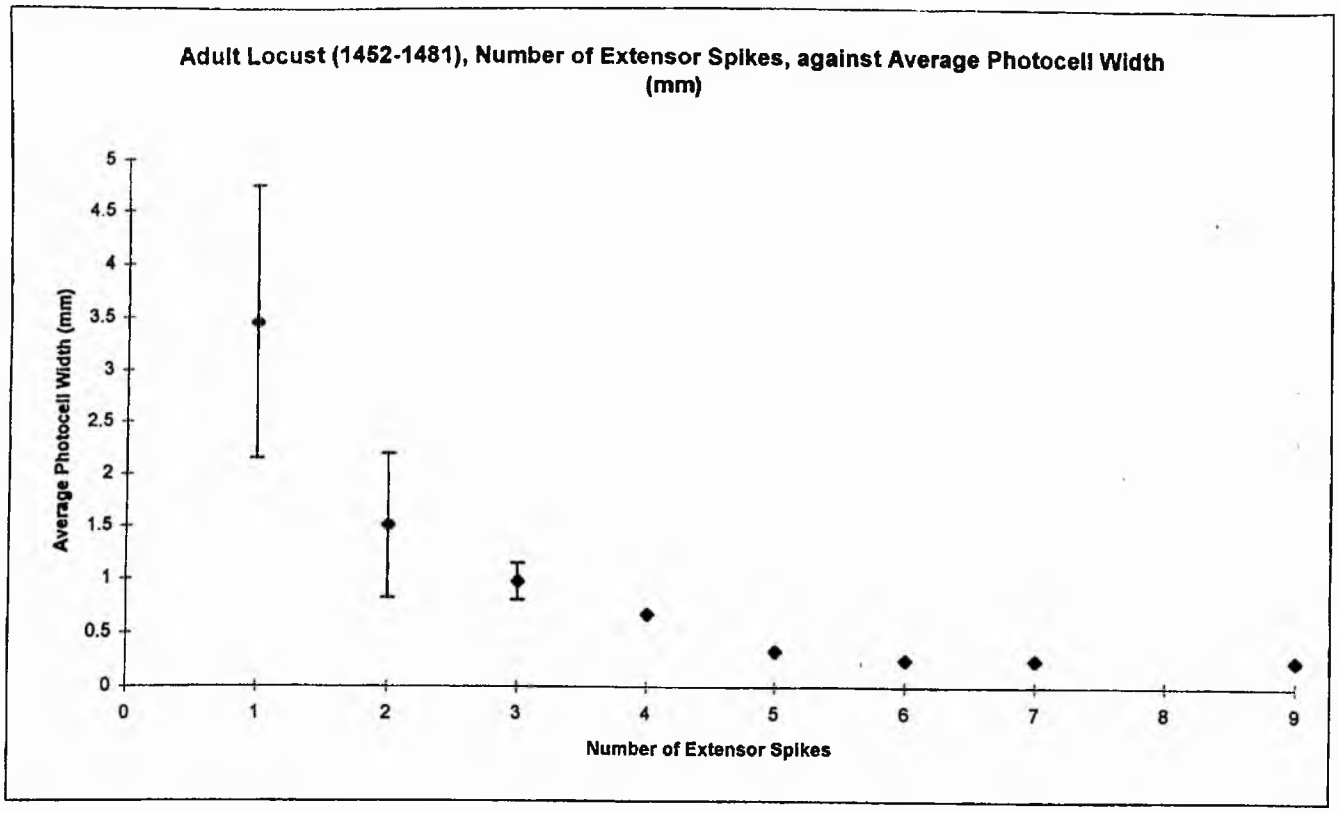
Adult Locust (1452-1481), Number of Extensor Spikes, against Average Photocell Width (mm)
Average Photocell Width (mm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
7
8
9
Number of Extensor Spikes

Fig. 4.1.

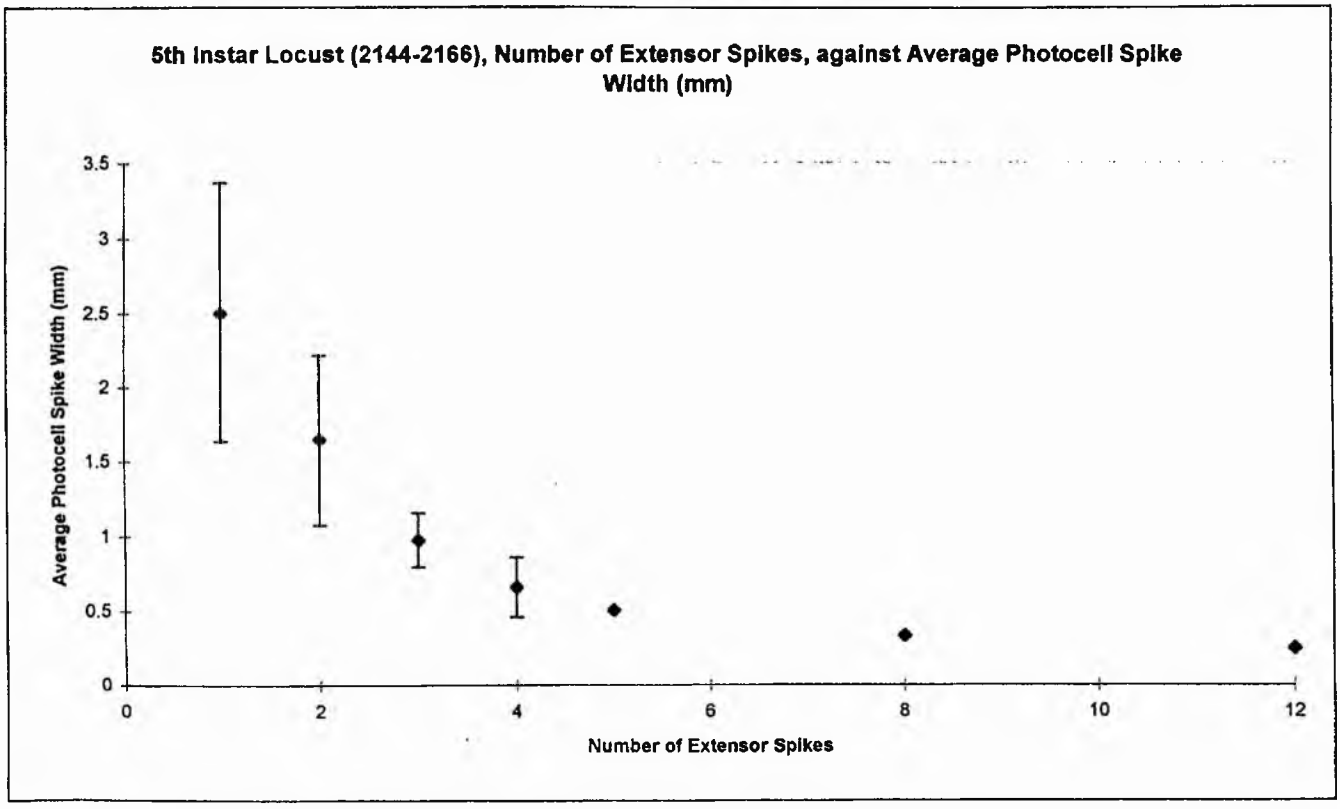
5th Instar Locust (2144-2166), Number of Extensor Spikes, against Average Photocell Spike Width (mm)
Average Photocell Spike Width (mm)
3.5
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
Number of Extensor Spikes

Fig. 4.2.

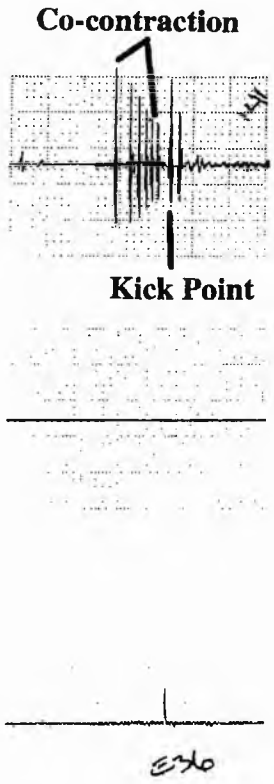

Fig. 4.1a. Extensor activity (top trace) and photocell spiking (bottom trace) during an adult kick

Horizontal Scale: 20ms/mm. Expected co-contraction followed by extension (from photocell spike).

attempts to graph the results used the number of spikes in the extensor, and the height of the spikes that the photocell registered when there was an extension.

Problems with the photocell arose when it was seen that there were some spikes that were unclear. These spikes appeared to be doubles, in that as one spike ended, another was forming after it. It was thought that this could be because the leg was not fully extending, and that this second spike was caused by the leg pulling back at the point where the photocell was positioned. However, after the double spike, there was always a very distinct spike caused by the flexion of the leg, as would be expected if it had fully extended beyond the photocell. For the double to have been caused by a mini-flexion at the photocell, there would have had to be another extension, to allow the full flexion that would then occur. It was therefore reasoned that looking at the width of the photocell would be a good idea, because if the double spike was not caused by a mini-flexion, then it might be a flaw in the photocell.

The approach used to combat this, was to try to establish what a kick looked like on the photocell by creating one through direct stimulation. The results from this are as follows. The recording showed no double spikes, and as the length of the stimulation time decreased, so did the speed of the kick (i.e. the size of the photocell spike increased) (Fig. 3.1, Fig. 3.2). There was a point where any further stimulation did not increase the speed of the kick though, and this appears to have occurred after 300ms, although this is not an absolute point. From previous results, it can be seen that the photocell responds about 10ms after the final extensor spike, assuming a kick is occurring. If a kick is not occurring, then the time period is greater, depending on the speed of the extension.

In a typical adult sample, the graph of number of extensor spikes against photocell spike width (fig. 4.1) shows a smooth curve. There is a steep decrease in the spike width at first (corresponding to the increase in extensor spikes), but as the number of extensor spikes increases, the rate of the decrease in width slows, and it levels out, showing no distinct break between a waggle extension and a kick (fig. 4.1a shows a typical kick, with expected co-contraction followed by extension). In this

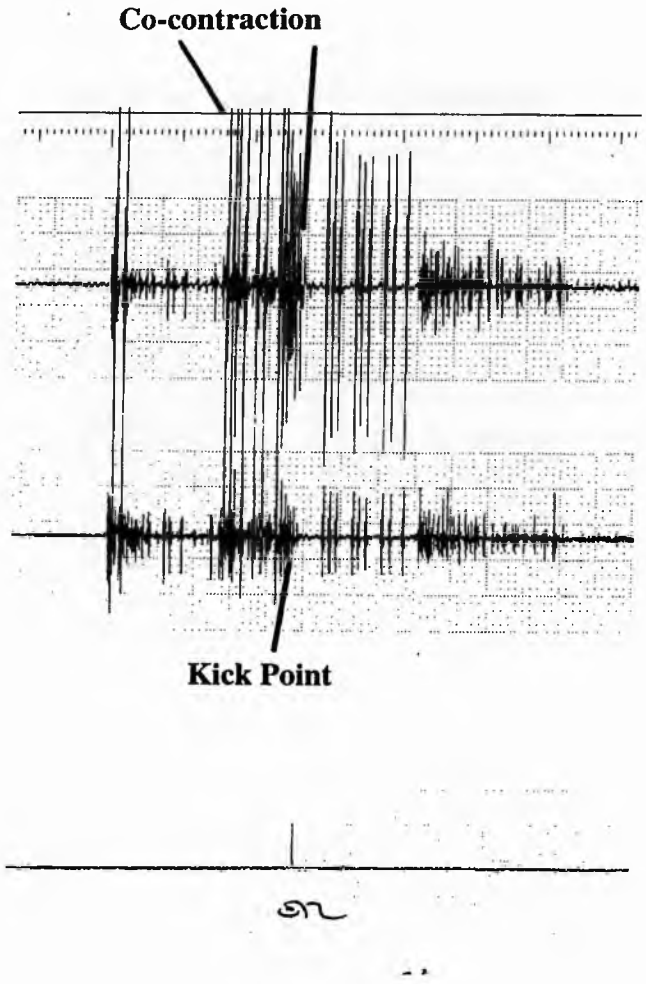

Fig. 4.2a. Extensor activity (top trace), flexor activity (middle trace), and photocell spiking (bottom trace) during a 5th instar kick

Horizontal Scale: 20ms/mm.

It is difficult to see the flexor activity underneath the extensor activity, as there is considerable crossover in this recording. The photocell spike indicates the extension.

animal, all the extensions with 5 or more extensor spikes were kicks, and most of these had a very low rate of spiking. They had a long duration of activity with proportionately fewer spikes than the waggle extensions. The most obvious result here is that the adult kick is the same as the jump. The fact that the expected co-contraction pattern can clearly be seen in both jump and kick indicates that any difference does not stem from the motor programme.

In the typical 5th instar sample, the graph of the number of extensor spikes against the photocell spike width shows a smooth curve (fig. 4.2, similar to the adults), with the photocell spike width decreasing rapidly as the number of extensor spikes increased, at first, and then smoothing out, as the number of extensor spikes increased to the point where a kick may occur. There are two kicks in this graph: the eight spike point and the 12 spike point. The 12 spike point is shown in fig 4.2a, and although the period of co-contraction is not as clear as in the adult (fig 4.1a), it is definitely there (as oppose to in the jump). The kicks have a lower spiking rate than the waggle extensions, as was seen in the adult sample described above.

In the 5th instar, the kicks that are seen are very different to the jumps. Most of the jumps show no co-contraction, and if there is any, then it is not the same as in the adult. The kicks of the 5th instar, however, are not the same as the jumps. There is co-contraction in the kicks, but this is never as easy to see as in the adult.

The speed of the metathoracic leg extension relies on both duration of the extensor muscle's activity, and the amount of spiking that occurs in this activity, before the kick. However, when co-contraction is occurring, there is a lower rate of spiking than that seen before a waggle extension. What was not seen, though, was a difference between the adult and the 5th instar results. Although the kicks from the 5th instars do not always look the same as the adults, the curve of extensor spike number against photocell spike size was the same. The initial expectation was that there would be a break in the adult curve, and not in the 5th instars'. The jumping evidence suggests that the 5th instars can jump without co-contracting, and it was thought that this might show up in the kicking experiments, and hence in the graph.

The 5th instars do jump differently to the adults, but this does not show up in this method of analysis. Their kick mechanism follows the same motor programme as the adult.

Extracellular recordings from the extensor and flexor muscles with either the lateral nerve, the chordotonal organ, or both, severed

Introduction

The next experiments that were to be undertaken were to investigate the role of proprioceptive information in the kick motor programme. These involved the severing of the lateral nerve, or the chordotonal organ (or both), and then looking for an effect on the kicking ability of the locust.

Methods

Loss of Lateral Nerve

The procedure was the same for both the adult and the 5th instar animals, in that the animal was secured in plasticene, with its ventral surface upward. The legs were secured in the plasticene, the pro- and mesothoracic legs so that they could not move, and the metathoracic legs, so that the inner wall was facing upward. A small window was cut into the cuticle on the inside surface of the metathoracic femur, near to the distal end. The lateral nerve was then revealed by carefully severing a couple of tracheoles that pass over the top of it from a trachea that runs parallel to it, and just underneath. The lateral nerve in older locusts often has a yellow tint to it, and can thus easily be spotted just above the trachea. It can then be hooked up if necessary, but in this case it was to be severed using a pair of very fine scissors.

Once the lateral nerve was severed, the window in the cuticle was closed. The cuticle often came off, but as the animal was not being tested for its survivability (in

this part of the experiment), this was not a problem. For the same reason, there was no need to seal the cuticle with bees' wax.

Loss of Chordotonal Organ

For the chordotonal organ to be severed, the metathoracic leg was secured with the outer side of the leg facing up. The leg was extended as fully as possible, without causing damage, so that the chordotonal organ was in the most accessible position. When the leg is fully extended, the ligament that connects the tibia to the strand of the organ, is at its most relaxed, and lies below the semi-lunar process. A window was cut into the cuticle that lies adjacent to the semi-lunar process, but the cuticle remained attached to the femur via a "hinge". There is a fine membrane just beneath the surface of the cuticle, and this must carefully be removed from the area where the ligament should be found. Beneath the membrane are trachea, and these were disturbed as little as possible. The initial aim of the chordotonal organ ablation procedure was to keep the animal in good health, and any damage to trachea at the joint may reduce the chance of survival, or result in damage to the leg, which might reduce the chance of jumping starting again. The trachea was thus moved aside to reveal the ligament, which was severed. The ligament was cut without stretching it. Once this had been done, the window of cuticle was replaced, and melted bees wax was used to seal it in place. During the whole procedure, the tissues were kept moist with locust saline. The seal is required to keep infection out. Everything must be done to keep the animal as healthy as possible, although the need for sterile saline did not arise. Both legs were done in exactly the same way.

The metathoracic leg was then repositioned so that the ventral surface was facing up, and the tibia had free movement. This would allow the tibia to be monitored with the photocell when the experiment was being prepared. It is not possible to film the femoro-tibial joint while the muscular activity is being monitored, because the electromagnetic field created by the camera is too great.

As before, two pairs of holes were made in the cuticle of the femur of the metathoracic leg. One pair in the outer side wall, where the extensor muscle is inserted, and one pair on the ventral surface, where the flexor muscle is inserted. A pair of fine myogram wires were inserted into each of the pairs of holes, and these were set up as before, going to amplifiers, then to an oscilloscope, then to an audio amplifier, then to the tape recorder, and finally to a printer, with a second oscilloscope. The flag was then attached to the end of the tibia, so that the photocell would more easily be able to detect its passing, and the animal was induced to kick by being rubbed with a brush.

Recordings of the extensor and flexor activity were made, and these were then printed out for analysis.

Results

Loss of Lateral Nerve

The following are the results that were obtained from the adults when their lateral nerves were severed, and their kicks were monitored. The kicks were monitored in the same way as before, so the results are in the form of myogram printouts. There are no results for the 5th instars, as when the severing of the lateral nerve was performed, the damage to the cuticle was such that the leg became useless, and the animal was no longer able to kick with it. The cuticle of the 5th instar is so soft, that when the small window was cut out from the leg, it buckled easily, and there was no way that the animal was going to be able to use it again. This happened with all the 5th instars that had their lateral nerves severed, and so trying to compare them to the adults' result was abandoned, and the only thing that would be comparable, would be the jumping and kicking before the lateral nerve was severed, and that which occurred after the severing, in the adult..

Thus these results describe the adult locust with a cut lateral nerve. In order to accurately compare kicking before and after, it was decided to first record the locust

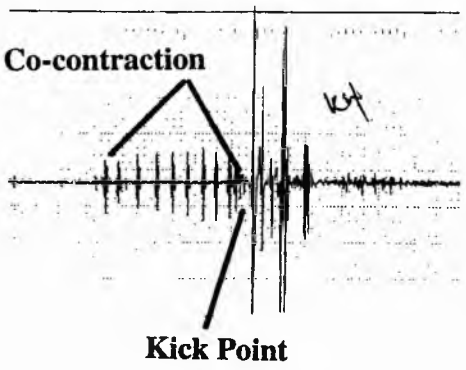

Fig. 5.1. Extensor activity during an adult kick.

Horizontal Scale: 20ms/mm.

Normal co-contraction followed by extension.

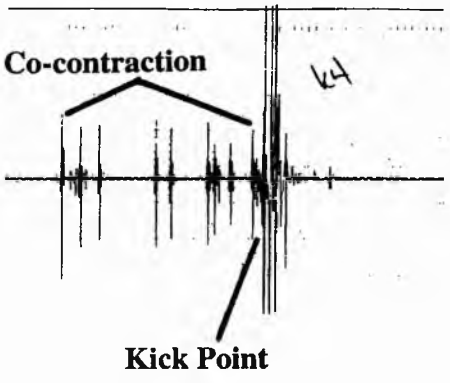

Fig. 5.2. Extensor activity during an adult kick after the lateral nerve has been severed.

Horizontal Scale: 20ms/mm.

Co-contraction is less regular after the severance.

with the lateral nerve intact, then cut the lateral nerve, and record the activity again. A typical sample provided the following results, an example of which can be seen in fig. 5.1.

The average co-contraction period before the lateral nerve was cut, is 333.3ms (S.D. = 61.5, n = 6), however, there were possible breaks in some of the co-contractions that could have reduced the average. After the lateral nerve has been cut (fig. 5.2), the average is 330ms (S.D. = 170.9, n = 4) (exceedingly close to the "before cut" result). These are remarkably similar results (a t-test showed them to be not significantly different at the 5% significance level, with t = 0.04 with 8 d.f.), and appear to show that there is little difference after the lateral nerve has been cut. The expected loss of positional knowledge would lead one to believe that there would be a difference, and that there would possibly be a more variable length to the co-contraction periods, or maybe just longer periods, that would result from the animal continuing to co-contract because it wasn't getting back the necessary information to cause the flexor muscles to be inhibited. The fact that the standard deviation is so high after the lateral nerve has been severed could suggest that there is more variability in the length of co-contraction once the information about the position of the tibia has been reduced. More information is needed before such judgements can accurately be made, though.

It was thought that the animal may be getting positional (with regard to the tibia) information from the other metathoracic leg, so a different method was approached. When the animal jumps, both metathoracic legs extend at the same time, and there is known to be communication between the two legs to accommodate this. If the other leg was cut off, then there would be no information passing about its position. Therefore, the other leg was cut off prior to the recording of kicks in this experiment.

A example of this gives an average co-contraction period of 185ms (S.D. = 72.4, n = 21), with the lateral nerve intact (fig. 5.3), and an average co-contraction duration of 341ms (S.D. = 300.6, n = 7), with the lateral nerve severed. However, the

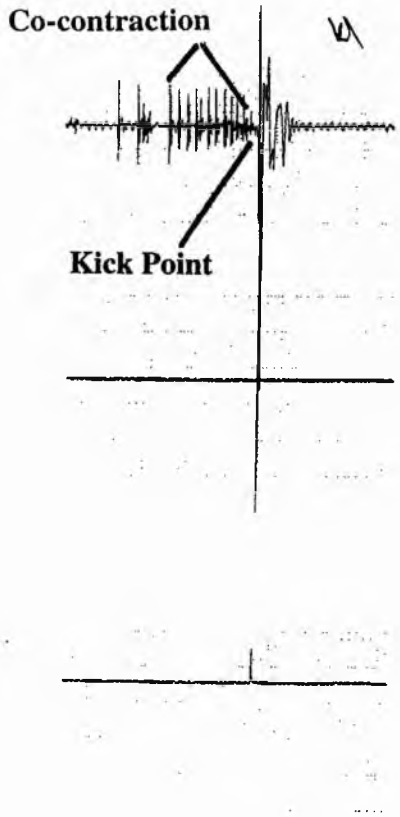

Fig. 5.3. Extensor activity (top trace) and photocell spiking (bottom trace) during an adult kick.

Horizontal Scale: 20ms/mm.

Normal co-contraction followed by extension.

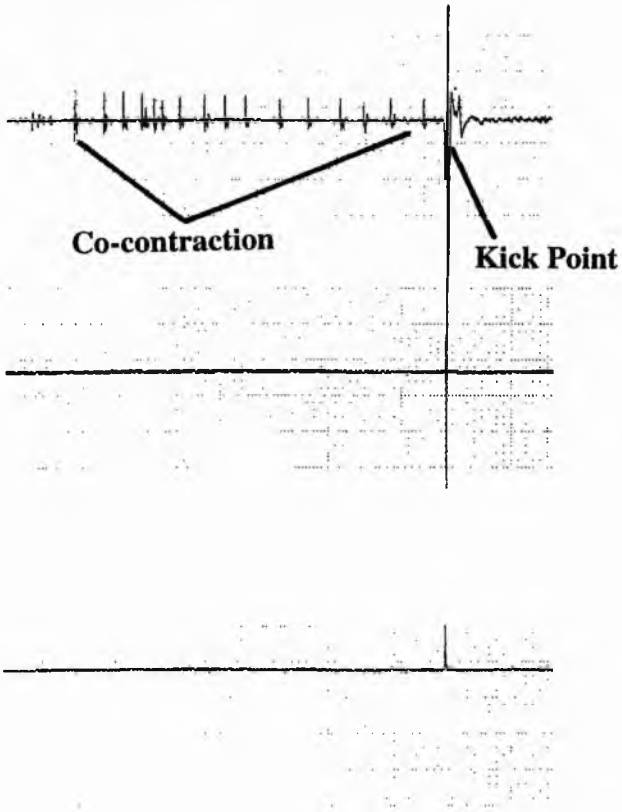

Fig. 5.4. Extensor activity (top trace) and photocell spiking (bottom trace) during an adult kick after the lateral nerve has been severed.

Horizontal Scale: 20ms/mm.

Co-contraction is much longer than seen before.

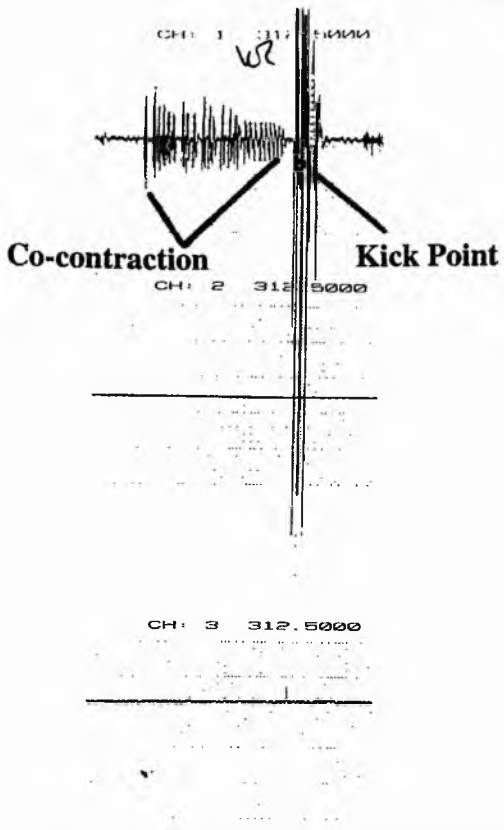

Fig. 5.5. Extensor activity (top trace) and photocell spiking (bottom trace) during an adult kick.

Horizontal Scale: 20ms/mm.

Normal co-contraction followed by extension.

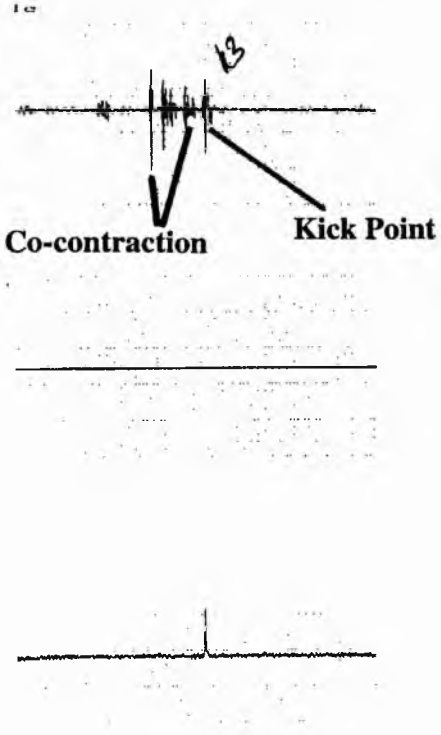

Fig. 5.6. Extensor activity (top trace) and photocell spiking (bottom trace) during an adult kick after the lateral nerve has been severed.

Horizontal Scale: 20ms/mm.

Co-contraction is much shorter than seen before lateral nerve severance.

Average Extensor Activity Duration before Kick		
Sample	Lateral Nerve Intact	Lateral Nerve Cut
1	333.3ms (n = 6, S.D. = 61.5)	330ms (n = 4, S.D. = 170.9)
2	190.8ms (n = 13, S.D. = 58.7)	212.1ms (n = 24, S.D. = 49.1)
3*	245ms (n = 14, S.D. = 76.2)	330ms (n = 9, S.D. = 64.8)
4*	185ms (n = 21, S.D. = 72.4)	341ms (n = 7, S.D. = 300.6)
5*	168.3ms (n = 12, S.D. = 54.4)	215ms (n = 6, S.D. = 211.5)
6*	257ms (n = 11, S.D. = 33.5)	143ms (n = 7, S.D. = 95.4)

Fig. 5.7. Table of average extensor durations before and after the severance of the lateral nerve in adult locusts. The "*" indicates that one leg has been removed from this animal.

last result from the lateral nerve severed experiment shows extremely long co-contraction (1000ms) (Fig. 5.4), and it is this result that causes the extremely high standard deviation. It is probably the case, though, that the high co-contraction time was caused by the loss of the information from the lateral nerve, about the position of the tibia during the co-contraction. A t-test gave a significant difference at the 5% level, with t = 2.26 for 26 d.f. (the critical value for t being about 2.06).

Another adult sampled, which also had one of its metathoracic leg removed, gave an average co-contraction duration of 257ms (S.D. = 95.4, n = 11), with the lateral nerve intact (fig. 5.5). After the lateral nerve had been cut the average co-contraction duration was 143ms (S.D. = 33.5, n = 7) (fig. 5.6). This is much lower than when the lateral nerve was intact, and is almost half (0.56) of that (t = 3.02 with 16 d.f. at 5% significance level, therefore a significant difference). This is very surprising, as the other locusts that have had one of their legs removed have all shown higher results for when the lateral nerve had been cut. This locust provided the longest average co-contraction period (before lateral nerve severance) of any so far, and then to produce such a short co-contraction period when the lateral nerve is cut, is very odd. It means that it is difficult to say what to expect when the lateral nerve is cut. The fact that the standard deviations are the reverse of the position normally seen (much higher after the cut of the lateral nerve) should, however, be viewed as an exception. Other results fell between those described here, and they can all be seen together in fig. 5.7. It looks like the co-contraction time becomes more variable after the loss of the lateral nerve. The locust does not require the lateral nerve in order to kick, but it clearly helps to modulate the kick, as can be seen in most of the results here.

Loss of Chordotonal Organ

The following are the results from the experiments performed on the adult locusts once their chordotonal organs had been severed.

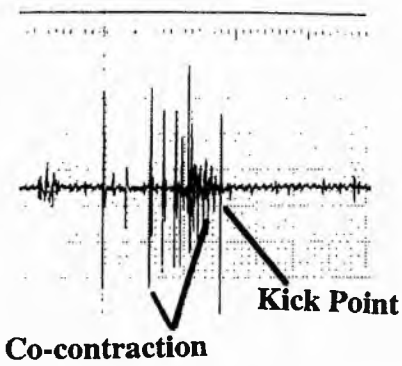

Fig. 6.1. Extensor activity 72 hours after the chordotonal organ of the adult has been severed.

Horizontal Scale: 20ms/mm.

The activity looks like co-contraction, although with no photocell this is hard to confirm.

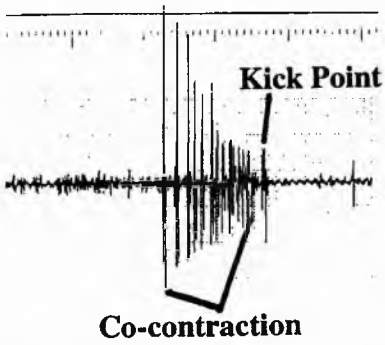

Fig. 6.2. Extensor activity straight after the chordotonal organ of the adult has been severed.

Horizontal Scale: 20ms/mm.

This is definitely a kick, even without the photocell confirmation.

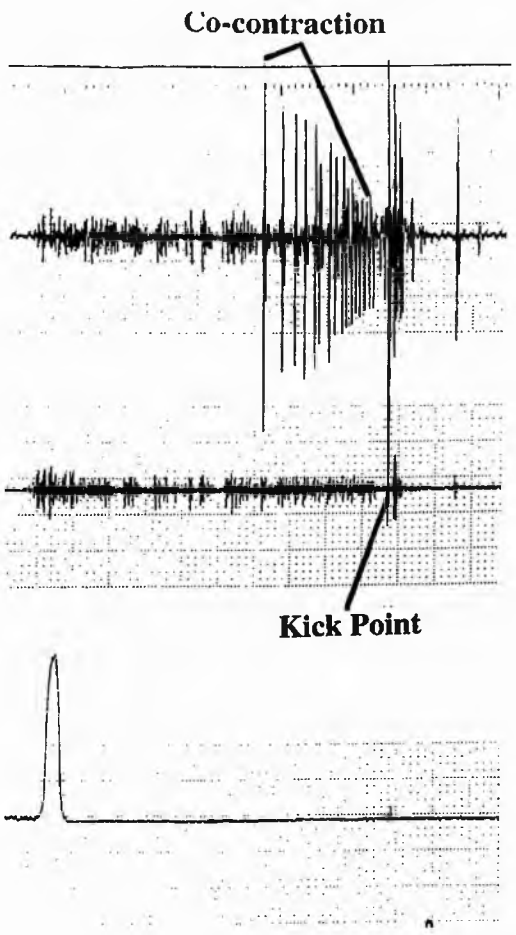

Fig. 6.3. Extensor activity (top trace), flexor activity (middle trace), and photocell spiking (bottom trace), straight after the chordotonal organ of the adult has been severed.

Horizontal Scale: 20ms/mm.

A typical kick.

The first few did not have their kicking ability tested immediately after the dissection of the chordotonal organ, as at this point the theory was that they would not be able to jump straight away, but would regain this ability at a later date. Therefore, the first time that the jumping ability was looked at was 72 hours after the organ was cut. The sample seen in fig 6.1 showed signs of the kicking ability having returned. There is extensor activity that looks like co-contraction. Once it was ascertained that the locust had regained its ability to kick, the animal was killed, and the legs dissected, primarily to make sure that the chordotonal organ had been severed from the tibia. In the above case, and all others where kicking ability returned, this was the case.

At this point there was no flexor or photocell reading, as the experiments were in the early stage. Shortly, however, they became essential.

The requirement for the photocell and flexor muscle recordings arose early, as a very surprising result was seen. One of the locusts had recordings taken from its extensor muscle straight after the dissection of the chordotonal organ had occurred. It produced some very odd patterns that were very similar to co-contraction, but there was also one definite kick (fig. 6.2). As stated earlier, it has been assumed that the locust could not kick immediately after the chordotonal organ had been cut, but this extension shows all the characteristics of co-contraction.

The clearest indication of co-contraction (and thus kicking ability) straight after the chordotonal organ was cut is shown in fig. 6.3. This kick shows an excellent representation of the expected pattern. The co-contraction is easily determinable, and the small photocell spike on the right of the myogram indicated the point where the tibial extension activates the photocell. There is little crosstalk between the two muscle traces (there is some from the flexor to the extensor, but none the other way). The co-contraction is about 350ms long, and the flexor activity cuts out about 60 ms before the leg crosses the photocell. There were three kicks during this recording, and all followed this pattern. This is excellent proof that the locust can kick straight after the chordotonal organ has been cut.

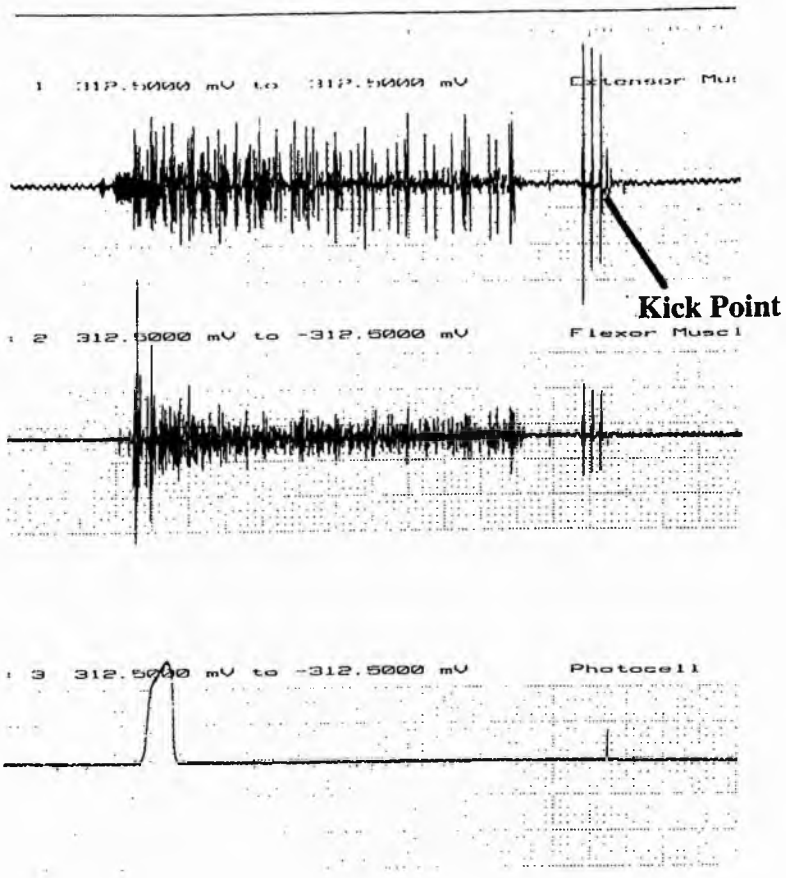

Fig. 6.4. Extensor activity (top trace), flexor activity (middle trace), and photocell spiking (bottom trace), straight after the chordotonal organ of the adult has been severed.

Horizontal Scale: 20ms/mm.

Long flexor bursts followed by short extensor bursts, but no co-contraction.

There were several possible theories that could have explained this strange result. Obviously, it might have been that the organ had not been severed, and that something else was cut. This was easily ruled out by reopening the window made in the cuticle, and looking for the intact tendon, which was not to be seen in either of the above cases. Another suggestion was that when the severance was performed, the tendon was stretched so much that the strand receptor of the chordotonal organ was still firing, and indicating that the leg was fully flexed (as it would do when the tendon was at full stretch), but this also was ruled out because even by waiting and testing the kick ability 30 minutes or an hour later the same findings occurred. The locust was capable of kicking straight after the chordotonal organ had been cut. There was no way that the locust was getting its information from the other leg, as this had also had the chordotonal organ cut.

This was the most important result seen. The original theory, put forward by Bässler, that the locust would lose the ability to jump, and then later regain it, has been seen to be wrong. Although not all locusts showed the same ability to kick straight after the ablation of the chordotonal organ, it shall be assumed that under otherwise perfect health conditions, sensory input from the chordotonal organ is not necessary for the animal to be able to kick.

Of further interest is a result seen in several of the locusts that were not able to kick straight after the ablation of the chordotonal organ. The extension described by fig. 6.4 was at first though to be a kick. The muscle activity that was taken during this, and many other such extensions, did not look like the kicks seen in the locusts that had retained the ability to kick. A long period of flexor activity was seen, possibly with extensor activity over the top. Unfortunately, the extensor trace was showing up flexor activity, and it was difficult to tell them apart. Certainly, between 50-350ms before the extension, the flexor activity stopped, and on the flexor trace there was no spiking. Then there were several extensor spikes in quick succession, resulting in the extension about 5ms later. It looked like the locust was trying to flex the leg continuously, so that it could get it into the fully flexed position, and start the

Fig. 6.5. Extensor activity (top trace), flexor activity (middle trace), and photocell spiking (bottom trace), straight after the chordotonal organ of the adult has been severed.

Horizontal Scale: 20ms/mm.

Plasticene near the femoral groove leads to extensor activity similar to that seen during co-contraction.

co-contraction, but that it couldn't tell that the leg was fully flexed (consistent with the loss of information from the chordotonal organ). This continuous flexing was mistaken, at first, for co-contraction, and the inability to distinguish the extensor trace meant that it was hard to see that there wasn't any co-contraction. It is possible that the prolonged extensor activity occurring before the kick caused some residual tension in the flexor muscle that was keeping the leg flexed. In some of the extensions, the flexor activity appeared to peter out, and there were clearly extensor spikes occurring whilst there was no leg movement. It was suggested that there may have been some plasticene in the joint, and that this might be helping to keep the leg flexed, and giving the extensor muscle something to work against, leading to a kind of artificial co-contraction. Thus the muscles were recorded from with the plasticene interfering with the femoral groove, to see what the activity would be like. This showed (fig 6.5) that, indeed, plasticene was able to provide a force sufficient to keep the leg fully flexed. Before the fast extensions (similar to those seen before, and thought to be kicks), there can be seen several extensor spikes. There is no accompanying flexor activity at all, so it is clearly not the flexor that is holding the leg fully flexed, but the plasticene instead. This means that an external force can combine with the extensor muscles and lead to joint distortion, and hence a larger than would be expected kick (without chordotonal organ modulation, and assuming some other damage (the reason for no co-contraction)), or also jump. The prime example of the external force here would be gravity. When the locust is preparing to jump, its tarsi are on the ground (and the legs are fully flexed), and so the extensor muscles can work against the weight of the locusts body rather than the flexor muscles. This could then lead to a jump without proper co-contraction, but that does involve joint distortion, and hence stored elastic energy.

Loss of Lateral Nerve and Chordotonal Organ

After the lateral nerves and the chordotonal organs had been cut, there was no kicking. An example of a fast extension can be seen in fig. 7.1, but this, like others,

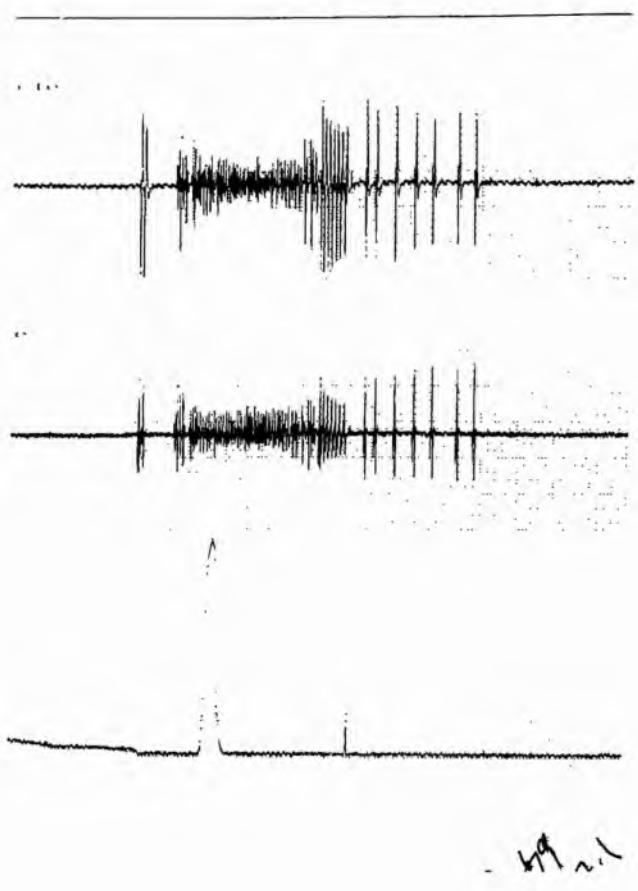

Fig. 7.1. Extensor activity (top trace), flexor activity (middle trace), and photocell spiking (bottom trace), after the chordotonal organ of the adult has been severed, and straight after the severance of the lateral nerve.

Horizontal Scale: 20ms/mm.

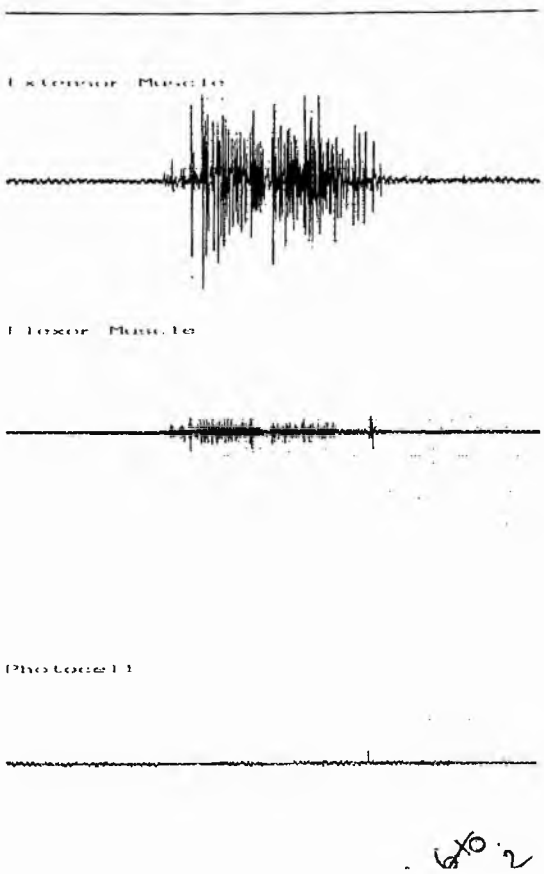

Fig. 7.2. Extensor activity (top trace), flexor activity (middle trace), and photocell spiking (bottom trace), after the chordotonal organ of the adult has been severed, and straight after the severance of the lateral nerve.

Horizontal Scale: 20ms/mm.

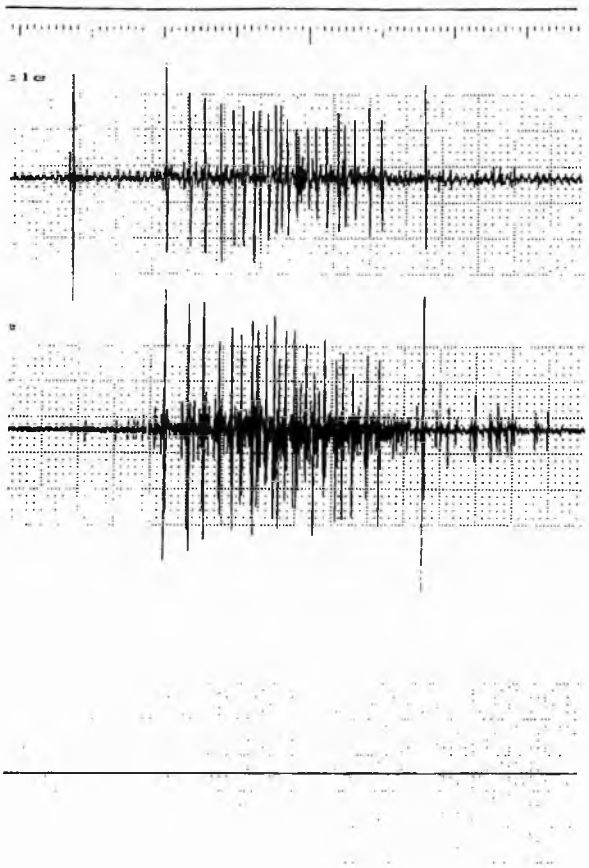

Fig. 7.3. Extensor activity (top trace), flexor activity (middle trace), and photocell spiking (bottom trace), after the chordotonal organ of the adult has been severed, and straight after the severance of the lateral nerve.

Horizontal Scale: 20ms/mm.

was not fast enough to be a kick, and appeared to be more like the extensions that occurred in the locusts that could not kick after the chordotonal organ only ablations. The same long flexion followed by a fast extension, with no visible co-contraction, can be seen. There was possibly one kick (seen in fig. 7.2), but it is not clear enough to be certain.

Fig 7.3 shows bursts of extensor activity that also had underlying flexor activity, and appeared, at first, to be co-contraction. There was no associated kick though. The extensor activity stopped before the flexor activity, indicating that the locust was not getting the information that it required for the kick to occur.

Thus it must be assumed that in order for the locust to kick, it requires certain sensory feedback, either from the lateral nerve, or the chordotonal organ. It can still kick when one source of information is lost, but when both are lost, it cannot kick.

Locust jumping with either the lateral nerve, chordotonal organ, or both, severed

Introduction

After seeing how the kick programme of the adult locust was affected by the loss of the lateral nerve and / or the chordotonal organ, it was necessary to observe the effects of these ablations on the jump programme. The adult kick motor programme is assumed to be the same as the jump motor programme, so the same effects would be expected to be seen. The adult should be able to jump having lost either the lateral nerve or the chordotonal organ, but not both.

Method

The same procedure was carried out as before on the locusts when the lateral nerve and / or chordotonal organ was cut, but this time rather than keep them strapped down, they were released on to the wooden table with the rough surface, to see how they would jump.

A control experiment was also performed. As normal, holes were cut in the cuticle where the chordotonal organs and the lateral nerves are. However, no sensory ablation was performed.

As before, the measurements of distance were made using chalk to mark the take-off and landing points. As with previous experiments done on the table, there was not room for the locust to jump more than about three times, so there was no continuous jumping as can occur on the floor.

Results

Control

Two jumps were observed from the control locust. One of 68cm and another of 62cm. These are both above the average of 47cm, and so it was assumed that in a healthy locust, the cutting of the holes in the cuticle would not affect the jumping adversely. As the assumption is that the adult jump motor programme is the same as the kick motor programme (and there has been no evidence to suggest otherwise), then it can be assumed that there is no significant effect on the kick of the animal either.

Loss of Lateral Nerve

Performance was variable. The adults were certainly less likely to jump when the lateral nerve had been severed, but they are reluctant to jump at the best of times. Some of the locusts would not jump at all, and some jumped quite far. None jumped as well as has been seen with the lateral nerve intact. An overall average jump distance of 29.2cm (S.D. = 12.57, n = 9) was obtained, which is close to half (0.62) of the average adult jump seen in the earlier experiments. There is no separate average maximum jump, as most of the animals only jumped once, and so it would be similar to the average jump. A t-test gave $t = 2.44$ with 60 d.f., indicating a significant difference at the 5% level.

It must be taken in to account that the dissection can sometimes lead to damage of the muscles, and often after the dissection, the locusts would lose a leg, or have one that was not working well. This could lead to the reduced jumping capacity. Some would not jump for up to a couple of minutes; instead, they would walk around holding there metathoracic legs in the air, not letting their tarsi touch the ground. Sometimes when they did this their legs would be fully flexed, and at other times there would be a state of partial extension. Then the legs would suddenly go back to the position for jumping, and the locust would jump. Often there would be a jump

when the animal got to the edge of the table, and then it would simply launch into the air, in attempt to fly. If the wings were held secure with tape, then they could be seen to vibrate as the locust attempted to fly.

Hence, damage to the legs can be attributed as some of the cause of the shorter distance of the jump, and also their reluctance to jump. However, the modulating effect of the lateral nerve on the kick must also be presumed to work on the jump, and so the loss of this can also lead to loss of jump capacity.

Loss of Chordotonal Organ

An average jump distance of 35.4cm (S.D. = 12.15, n = 9) was recorded. This is about three quarters of the average adult jump with the chordotonal organ intact. It is worth noting, though, that some of the jumps from locusts that had had their chordotonal organs severed were close to (and, in one case even greater than) the average adult jump. Indeed, a t-test showed there to be no significant difference at the 5% level, with $t = 1.59$ for 60 d.f..

The same pattern of behaviour appeared in some of these animals as occurred in those that had had their lateral nerves severed - walking with metathoracic legs seemingly disabled, then returning them to the flexed position, ready for a jump.

Loss of Lateral Nerve and Chordotonal Organ

The average jump distance was 40.4cm (S.D. = 9.26, n = 18), which is 0.86 times the average jump of the adult locust. The fact that the locust could jump at all, though, is the biggest surprise here. When the ablation of both the lateral nerve and the chordotonal organ occurred, there had been no kicking. Assuming the jump motor programme is the same as the kick, then it was expected that there would be no jumping after the loss of the these two sensory inputs. A t-test shows that there is again no significant difference at the 5% level, with $t = 1.29$ for 69 d.f..

Often, straight after the dissections, the locust would not jump, but instead hold its legs in a very awkward manner, as if it had no control over them (again, like

when either the chordotonal organ or lateral nerve on their own had been severed). About five minutes after the operations had been carried out, the locust would jump. The walking improved, and where previously it would hold its metathoracic femurs in an unnatural position, it would hold the legs so that the tarsi could touch the ground (with the femur at about 30° to the ground). This temporary loss of jumping ability is likely due to damage inflicted during the procedures. Although this was not seen on the control, there is more damage dealt when the ablation of the chordotonal organ and lateral nerve is taking place.

Muscle Mechanics

Of further interest, though, is whether all these jumps require co-contraction. It has been seen that the 5th instars can perform small jumps that are difficult to analyse, and could potentially have no co-contraction, so it needs to be demonstrated that the adults are not doing the same when the chordotonal organ and / or lateral nerve has been cut. As it is hard to record from both extensor and flexor muscles whilst the animal is jumping, equations, incorporating the weight of the locusts leg muscles (establishing this in the 5th instars proved difficult, and prevented the same equations being used on them) and their peak power output, can be used to determine whether a jump requires co-contraction. If the assumption is that the maximum output from muscle (specific peak power output) is 900 $W.kg^{-1}$ (from the locust this has been recorded as 450 $W.kg^{-1}$, (Bennet-Clark 1975) but the maximum from any muscle has been estimated at between 500 and 1000 $W.kg^{-1}$ (Alexander and Goldspink 1977), and as the highest distance that could be jumped without elastic energy storage is being sought, a figure in the higher range is being used) then the jump distance that could be achieved with this specific peak power output can be calculated.

Specific peak power output = P/ Muscle weight

P is the peak power output, and Muscle weight is the mass of the extensor muscles in the femur. This has been weighed, and the average is 0.00004 kg (Gatty

Marine Laboratory sample). However, as there are two legs, then this figure must be doubled.

$P = 900 * 2(0.00004)$

$P = 0.072$ Watts $= 0.072\ kg.m^2.s^{-3}$

The acceleration at take off can then be worked out with the following equation.

$a = P/Mv$

Where a is acceleration at take-off, P is the peak power output, M is the mass of the locust, and v is the velocity at take-off. There are two variables that are not known here - "a" and "v". Thus a second equation must be used to define "v" in terms of "a".

$v^2 = 2a.s$

Here, s is the length of the metathoracic leg, which is 0.043m (average adult, from previous data).

$a = v^2/2s$

This can be added to "a = P/Mv" to give

$v^2/2s = P/Mv$

M is 0.0021 kg (average adult in Gatty Marine Laboratory sample).

$v^3 = 2P.s/M$

$v^3 = 2*0.072*0.043/0.0021$

$v = 1.434\ m.s^{-1}$

The final equation that allows to find out the distance jumped is

$v^2 = d.g/\sin 2H$

d is the distance jumped, g is the gravity constant, $9.81 m.s^{-2}$, and H is the angle of take-off. As we are looking for the maximum jump distance from the specific power output, then the assumption is that H is 45^o, as this is the angle at which maximum distance can be achieved.

$d = v^2 \sin 2H/g$

$d = 2.056*\sin(2*45)/9.81$

$d = 0.21$ m

Thus, the furthest that a locust can jump, assuming that its muscles are working at their maximum level, and there is no co-contraction, is 21cm.

This does not mean that a jump distance of less than 21cm is not using co-contraction, as it is unlikely that the muscles will be working at their maximum output, however, it is possible to say that a locust jumping less than 21cm might not be using co-contraction. Any jump greater than 21cm must require co-contraction, or some form of elastic energy storage. It is possible that the locusts are using their own weight as a force to act against the extensor, creating elastic energy storage, as was seen in the chordotonal organ ablation kick results (the locusts used plasticene in the femoral groove to create a force against which the extensor could work, but the principle is the same for the jump), but these animals were ones that did not show the kicking ability after the loss of the organ, and the extensions that they did produce were not as fast as those that could still kick. The assumption is, then, that most of the locusts that jumped after the loss of the chordotonal organ (and lateral nerve) must have been using co-contraction to enable them to jump the distances that they managed. Hence this is further proof that the adult locust is capable of co-contracting after the chordotonal organ has been cut. This was expected after the kick results, however, it was not expected that they would be able to jump after the loss of both the lateral nerve and the chordotonal organ (especially after the kick results). Kicking and jumping was always expected after the loss of the lateral nerve, as the lump receptor and cuticular strain receptors are not as important to the jump / kick as the chordotonal organ.

Conclusion and discussion

In conclusion, the relevant results should first be summarised, so as to present them together, in a concise form, allowing them to be compared more easily.

The Motor Programme

Recordings taken from the adults extensor muscles during jumping showed extensor activity as expected during a jump or kick. This activity is characterised by a period of extensor activity, whilst at the same time there is flexor activity. This is the co-contraction, and lasts about 300-400ms, which is then followed by a short period (about 50ms),when there is no flexor activity. There is still extensor activity at this point, and this culminates in the extension of the leg, when the tension in the flexor muscle has reduced to the point where the force attempting to extend the leg is greater than the force keeping it flexed. The point usually is identifiable by a large spike on the extensor trace, as the extensor gives a final burst. All this can be seen on the adult read-outs, but is hard to identify on those of the 5th instar. When the extensor muscles of the 5th instars were recorded from during jumping there was no clear co-contraction. Rather, there were small bursts of activity, and / or single spikes indicating the jump. Never did these resemble the adults extensor activity, and this lead to the opinion that the 5th instars' jump motor programme could be different to the adults'. This forced the decision to return to looking at the kick rather than the jump, allowing comparison of the adults' jump / kick motor programme to the 5th instars' in full. The kick is easier to record in the adult (as it is more likely to kick than to jump (the only indication of any difference between the adults' jumping and kicking)), and the flexor muscle can also be recorded from. The flexor muscle provides valuable information on whether co-contraction is occurring or not .

The addition of the photocell to the normal set-up for recording the muscular activity, meant that the time at which the kick occurred could also be obtained. This is

very important in the 5th instars, as even recording their kicks can be difficult. They are less likely to kick than jump, which is the exact opposite of the adult - they are more likely to kick, than they are to jump. Why this is so, is unknown. It could have something to do with the fact that the adults jump to escape, or take-off, but the 5th instars use the jump as a means of locomotion as well. However, if, as has been seen here, the 5th instars can jump without co-contraction, then it is possible that they are not fatiguing themselves as much as they would be if they were co-contracting. Whereas a kick with co-contraction would fatigue them.

The photocell created a spike, which could be recorded, when the leg passed by it, cutting a beam of light from a lamp that was pointing at the receptor. What was seen on the new recordings could then be compared to the jumps, and whether or not the 5th instar co-contracted during every kick would then be able to be answered.

Thus, from the adults, the recordings were good, showing co-contraction as expected and further demonstrating that the motor programme for the kick and jump is the same. From the 5th instars, the recordings were variable. When the length of the co-contraction was compared to the speed of the extension (using the photocell spike as indication of speed), there was hope that some kind of distinction between the adults and the 5th instars would be seen. A graph showing an increase in extension speed (i.e.. decreasing photocell spike width) corresponding to an increase in the number of extensor spikes was seen, predictably. The surprise came in the curve of the graph. In both the adult and the 5th instar the curve was generally smooth. To begin with the increase in speed of the extension occurred at a high rate compared to the increase in number of extensor spikes. The rate then decreased until there was little increase in extension speed as the number of extensor spikes increased. This point was where the kicks began. It had been thought that there would be a gap on the graph of the adult where the speed of the extension would stop increasing as the number of extensor spikes, or duration of extensor activity did. Then, after this gap, the kicks would start. This would indicate that the adults' kick was different to the 5th instars, and that if there was no gap in the 5th instars graph, there would be seen a

"grey area" where an extension could be a kick or just a fast extension. This "grey area" in the kicking of the 5th instar could be looked at as some evidence of kicking without co-contraction. If this was the case, then the kick motor programme of the 5th instar could be equated with the jump motor programme. As the 5th instar has been seen to kick utilising co-contraction, and no clear indication of a kick without co-contraction has occurred, then it must be assumed that the 5th instar kick motor programme is the same as the adults jump / kick motor programme. Although not seen clearly, the 5th instars may be able to jump using co-contraction, and if this is the case then presumably the motor programme for this is the same as the adult also.

The Lateral Nerve and the Chordotonal Organ

Are the lateral nerve and the chordotonal organs' sensory inputs necessary for the locust to kick? Bässler (1979) stated that the ablation of the chordotonal organ resulted in the loss of jumping and kicking ability for a temporary period. The most important result of this thesis is to show that this is not the case. The locust can jump and kick immediately after the ablation of the chordotonal organ or lateral nerve, and can jump immediately after the ablation of both chordotonal organ and lateral nerve.

The first experiments performed were on the lateral nerves. The locusts involved were initially recorded from, then had their lateral nerves cut on one leg, and then were recorded from again. There appeared to be no real difference after the loss of the lateral nerve, and as they could have been getting positional information from the second leg, further experiments were performed with the second leg removed. Initial results suggested that there was an increase in the co-contraction time, after the lateral nerve was cut, which was interesting, but later, other locusts provided much shorter co-contraction times, more in line with those seen before the lateral nerve was severed.

When their jumping ability was tested they showed reduced jumping distances, but the damage caused by the dissection procedures can affect the jumping ability, although not overly so. From this, it appears that the loss of the positional

information from the lateral nerve does not significantly affect the locusts' ability to kick and jump. They get information from other sensory organs (such as the chordotonal organ), that are giving them sufficient information to allow the kick.

After the lateral nerve experiments came the chordotonal organ experiments. The procedure was essentially the same: sever the chordotonal organ, and look at the locust's ability to jump and kick afterwards. It was thought that after the chordotonal organ was severed, the locust would lose the ability to kick for a period of time, and the main aim of the experiment, originally, was to look at this duration, and then see how well the locusts could jump after the ability returned. The problem with keeping the locusts alive for long enough had to be addressed, and so to begin with the procedure involved careful resealing of all incisions with bees wax, and the possibility of the need for sterile locust saline. Fortunately, there did not appear to be too big a problem with mortality, and so the sterile saline was not needed. However, after a few experiments were performed, the question became one of whether the locust could jump and kick straight after the dissection, not how long it would take to regain the ability. This came as a surprise (as it contradicted the literature), and it was thought that the severance procedure could be overstretching the chordotonal strand, resulting in it firing for some time after the dissection was finished. This could then lead to the leg being perceived as fully flexed (the position where the strand is at its most stretched), and making a kick or jump possible.

When the procedure was repeated, with the utmost care not to stretch the strand as it was being severed was taken, the results were the same. Some of the locusts were able to kick straight after the chordotonal organ had been severed. Further experiments were done, and video recordings of the cuticle distortion during co-contraction were taken. Some of the locusts produced a strange fast extension after the procedure, and when there was plasticene in the ventral groove of the femur, or near the femoro-tibial joint, they were seen to produce distortion of the joint. This had been mistaken for co-contraction earlier, but when care was taken that there was no plasticene interfering, it was seen just to be fast extensions. It was as if the locust was

trying to co-contract, but whilst the flexor was firing there was no extensor activity. Then, when the flexor stopped firing, the extensor began to fire. There was still residual tension in the flexor muscle, so there was a period when the leg did not extend. This gave the appearance of co-contraction, and distortion of the cuticle, a number of times, but in all occasions there was no extensor activity whilst there was flexor activity.

Thus it was seen that locusts can kick after the chordotonal organ is ablated. It is probable that those that couldn't kick were damaged during the dissection procedure, and this also resulted in the strange activity. When the jumping of the locusts was looked at, they were seen to be able to jump, although not always as well as before the dissection. Again this reduction of jumping ability can be assumed to have been caused by the damage inflicted during the dissection procedure.

The second important find came when the chordotonal organs and the lateral nerves were severed in the same animals. This results in the loss of the two main positional information suppliers in the metathoracic leg, and the assumption here was that the locusts would no longer be able to kick or jump. This proved not to be the case. The locusts that jumped after both the lateral nerve and the chordotonal organ had been severed jumped well, although obviously not to their maximum capacity, but when these same locusts were secured in plasticene, and had electrodes implanted in the flexor and extensor muscles, they would not kick. Hence the loss of the chordotonal organ and lateral nerve did not affect the ability to jump, but did affect the ability to kick. This does not appear to indicate any differences in motor programme between the jump and the kick, rather that there is required more sensory input to make the locust kick.

It would have been good to have been able to look at the 5th instars in the same way, but their weaker cuticle made them incapable of jumping or kicking after the sensory ablations. This should not detract from the findings of the adults, though, and as the assumption is that the 5th instar jump motor programme is the same as the adults then the prediction would be that the same results would be seen in the 5th

instars kick, and probably not the jump (as this does not always require co-contraction).

A possible explanation for the different (from those seen before) results seen above is that the emphasis was on the whole animal. Important, at first, had been to keep the locust alive and in good health for as long as possible, and this lead to great care being taken when the dissection procedure was being performed. Obviously some of the locusts were damaged during the dissection, resulting in them not being able to jump straight away.

The chordotonal organ normally relays information about the position of the leg, and also the whether it is moving from being flexed or being extended. The loss of this information when the tendon attaching the strand to the tibia is severed, was thought to result in the loss of tibial positional information, resulting in the loss of the ability to kick. If the chordotonal organ is not getting information telling it where the leg is, and what it is doing, then it cannot pass this information on, which, it was thought, would mean that a kick could not occur. This, however, is not the case. The lateral nerve, which takes information from the lump receptor in the femoro-tibial joint, is also not needed for the kick to occur. The original tests performed on locusts with their lateral nerves severed was only intended to see what the effect of its loss would have on the kick. It was not expected that there would be a total loss in kicking or jumping ability, but some effect, other than one caused by the dissection procedure itself, was expected.

So, if the adult can jump without the lateral nerve and chordotonal organ, but not kick, then there must be a difference between the kick and the jump, at least as far as the stimuli required to initiate them. The motor programme is the same as has been seen on the myograms. The only difference immediately evident is the fact that the tarsi of the metathoracic leg are not in contact with the ground during the kick, which they are during the jump. If there is information passing from the tibial-tarsal chordotonal organ, then this could explain the ability to kick. The locust can be aware that the tarsi are on the ground, and the angle that the tarsi have to the tibia could give

valuable information about the angle of the tibia to the femur, allowing the jump to occur. When the locust is upside down in the plasticene, the tarsi are in the air, and the angle of the leg has no effect on their position, and so they will not be giving the locust the information that it needs to know the position of the leg.

The locust appears to have many ways of obtaining the information that it needs in order to be able to jump. The escape mechanism is far more important than the kick, and so it seems sensible that the jump has more back-up systems than the kick. This does assume that the kick has nothing to push against, which will often not be the case, and the chances of the locust losing the use of both the chordotonal organ and the lateral nerve at the same time are slim in the wild. The fact remains though that there are ways for the locust to be able to keep its ability to jump, even when several of its proprioceptive pathways are damaged. More work on the tibial-tarsal chordotonal organ (which appears to have been largely ignored in the metathoracic leg) will be able to say more clearly how many ways there are for the locust to get the information that it requires to jump.

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Acknowledgements

I would like to thank Dr. W. J. Heitler and Dr. T. Jellema for their help in the formulation and execution of the work involved in this thesis