CONTRALATERAL COORDINATION AND RETARGETING OF LIMB MOVEMENTS DURING SCRATCHING IN THE LOCUST

T. MATHESON*

Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

*e-mail: tm114@hermes.cam.ac.uk (web page: http://www.zoo.cam.ac.uk/zoostaff/matheson/index.htm)

Summary

Locusts, *Schistocerca gregaria*, in common with many limbed vertebrates, can make directed scratching movements in response to tactile stimulation. For instance, stimulation of different sites on a wing elicits different movements that are accurately targeted so that the hindleg tarsus passes across the stimulus site. I have analysed these limb movements to define the ability of a locust to target stimulus sites correctly under a range of experimental conditions. In particular, I describe aspects of the behaviour that reveal possible neuronal pathways underlying the responses. These neuronal pathways will be the subject of further physiological analyses.

Limb targeting during scratching is continuously graded in form; different patterns of movement are not separated by sharp transitions. The computation of limb trajectory takes into account the starting posture of the hindleg, so that different trajectories can be used to reach a common stimulus site from different starting postures. Moreover, the trajectories of the two hindlegs moving simultaneously from different starting postures in response to a single stimulus can be different, so that their tarsi converge onto the common stimulus site. Different trajectories can be used to reach a common stimulus site from the same start posture. Targeting information from a forewing is passed not only down the nerve cord to the ipsilateral hindleg but also across the nerve cord, so that the contralateral hindleg can also make directed movements. This contralateral transmission does not rely on peripheral sensory feedback. When the stimulus site moves during a rhythmical scratch, the targeting of subsequent cycles reflects this change. Both ipsilateral and contralateral hindlegs can retarget their movements. The trajectory of a single cycle of scratching directed towards a particular stimulus site can be modified after it has begun, so that the tarsus is redirected towards a new stimulus site.

Key words: scratching, targeting, reaching, directed limb movement, grasshopper, *Schistocerca gregaria*.

Introduction

Generating an efficient motor pattern to move a multi-jointed limb in three dimensions from an arbitrary starting point to a target location signalled by sensory neurones is a formidable computational task, yet it is accomplished by both vertebrates (Sherrington, 1906, 1910; Stein, 1983) and invertebrates (Cruse, 1979; Vandervorst and Ghysen, 1980; Meyer, 1993; Berkowitz and Laurent, 1996*a*,*b*). Tactile stimulation of different regions of the body or limbs of frogs, turtles, cats or locusts elicits appropriately directed scratching movements of a limb that enable it to hit the target site. Other tasks also require precise targeting; for example, primates are highly skilled at reaching towards and grasping an object in their visual field (Georgopoulos, 1996).

To generate a limb movement that is directed towards a target location, the nervous system must translate a representation of that location, encoded in the patterns of activity in sensory neurones, into an appropriate temporal pattern of activity in many motor neurones innervating muscles at several joints of the limb. The nervous system must solve two fundamental problems to achieve this. First, the sensory representation must take into account one or more frames of reference. For example, in visually guided reaching, the position of a target encoded by receptors on the retina (i.e. represented in a retinal frame of reference) can only be translated to a body-centred frame of reference if the position of the eye is known relative to the head and the position of the head is known relative to the body. Second, because multi-jointed limbs may have many degrees of freedom, there will be many possible motor outputs that can carry the limb to the target. How is limb movement direction towards the target encoded in the central nervous system in such a way that the most appropriate pattern of motor activity is selected?

In vertebrates, these coordination problems have been assessed behaviourally by challenging an animal with different reaching tasks and observing the trajectories and errors for each situation. One model that can predict some movement trajectories arises from computations of the weighted and summed costs of movement at each joint (for a review, see Rosenbaum *et al.* 1996). At the neuronal level, the mechanisms underlying targeting have been addressed largely under one or

other of two models: either by considering population vector coding of target location in neurones of the motor cortex (for a review, see Georgopoulos, 1996) or by considering the motor representation in the spinal cord (the equilibrium point hypothesis: for a review, see Bizzi *et al.* 1991). Recent evidence based primarily on loading experiments suggests that the equilibrium point hypothesis may not fully explain observed movement trajectories (e.g. Schenau *et al.* 1995; Gomi and Kawato, 1996; Bellomo and Inbar, 1997). This suggests that vertebrates require explicit internal representations of target position, limb posture and perhaps other dynamic variables such as muscle elasticity and joint stiffness. Difficulties encountered in attempting to assess the neuronal bases of targeting in vertebrates are the vast numbers of neurones in each layer of processing and the many layers between sensory input and motor output.

An invertebrate preparation that holds great promise in helping us to understand the neuronal mechanisms that underlie limb targeting is the scratching locust. Intact or restrained locusts respond to tactile or chemical stimulation of their wings or body with repetitive scratching movements of their hindlegs that are accurately directed towards the site of stimulation (Meyer, 1993; Berkowitz and Laurent, 1996*a,b*; Matheson, 1997). For example, tactile stimulation of proximal locations on the wings elicits proximally directed scratches and stimulation of distal locations elicits distally directed scratches (Meyer, 1993). Some aspects of the coordination underlying the rhythmical motor patterns may be generated centrally, but appropriate targeting relies on sensory feedback (Berkowitz and Laurent, 1996*a*). The behaviour can be elicited in a restrained locust and, more specifically, can be generated by the metathoracic ganglion in isolation from the remainder of the central nervous system (Matheson, 1997), thus providing the opportunity to make intracellular recordings from identifiable neurones in either reduced or largely intact preparations. Many of the sensory to motor pathways are short, involving only one or a few serial synapses.

In the locust, we have a detailed understanding of both the specific features and general principles underlying the processing of information in local and intersegmental networks, particularly during reflex movements of the hindlegs (for a review, see Burrows, 1996). For example, the mapping of leg tactile sensory neurones onto postsynaptic interneurones (Burrows and Newland, 1993) can explain the form of reflex avoidance movements made by the leg in response to stimulation of these receptors (Siegler and Burrows, 1986). Similarly, the responses and synaptic connections of proprioceptive neurones signalling hindleg position can explain the role of these neurones in leg reflexes (Field and Burrows, 1982; Burrows, 1987; Matheson, 1990, 1992). Particular descending interneurones influence directly the local processing of reflex information (Laurent and Burrows, 1989). This knowledge of sensory and motor processing at the level of individual, often identified, neurones, will permit a detailed analysis of the neuronal mechanisms underlying limb targeting.

I have shown that, although intact locusts correctly target their scratching movements towards different stimulus sites along the length of the wings, they fail to readjust this targeting when the wings are artificially held outstretched orthogonal to the body axis. As a result, each scratch is targeted towards the empty point in space where the stimulus site would have been if the wing had not been displaced (Matheson, 1997). This unexpected finding provides an important experimental method that I exploit here: scratching elicited by stimulation of an outstretched wing does not generate reafference in leg or wing tactile sensory neurones because the two appendages do not touch. Proprioceptive feedback from leg receptors is nevertheless unaffected, and the animal can make natural movements. Furthermore, because leg movements are not hindered by contact with the stimulus site, I can analyse precisely how a locust retargets its movements as the stimulus site is moved.

My goal in this paper is to define properties of locust scratching movements that help to elucidate underlying neuronal mechanisms, which will be the subject of further study. I therefore describe targeted scratching movements made by intact, deafferented or de-efferented locusts in response to stimulation of an outstretched wing.

Materials and methods

Locusts (*Schistocerca gregaria* Forskål) from our crowded colony were tethered above a light polystyrene ball (diameter 8 cm) so that they could stand or walk in any direction while being video-taped by one or two JVC TK-C1380E video cameras. Locusts were tethered to a holder by a flexible wire loop passed around the neck anterior to the mesothoracic legs, allowing them unhindered movements of their legs and wings. The eyes were covered with white typists' correction fluid to prevent any possible influence of visual input on leg targeting and to reduce struggling. Covering the eyes in this way did not alter leg targeting during scratching.

Movements of locusts were video-taped from a lateral view and, in some experiments, from a dorsal view. Video images were mixed with a time signal (For-A VTG-33), recorded in sVHS format and played back for frame-by-frame analysis on a Panasonic NV-HS900 recorder and Sony PVM 1450MD monitor. When more than one camera was used, their images were combined using a For-A MV-40PS multiviewer and Kramer VM-3D composite to Y/C decoder. The positions of the base of the tarsus of one or both hindlegs were plotted for individual frames (25 frames s^{-1}) on transparent acetate sheets. The following results are based on video-taped observations of 2927 scratches in 45 locusts. Animals that did not scratch in response to either tactile or electrical stimulation were discarded.

In most experiments, scratching was elicited by gently touching particular points on a wing with a fine paintbrush. The stimulus site was at most 10 mm in diameter. To investigate targeting of moving stimuli, different sites were sequentially touched at rapid intervals or the tip of the paintbrush was

dragged along the surface of the wing. These different protocols are noted where appropriate in the text. In other cases, two pairs of extracellular electrodes made from 0.1 mm minutien pins were inserted into wing veins to stimulate particular branches of wing sensory nerves. Both types of stimuli will activate many wing sensory receptors, probably including trichoid sensilla, campaniform sensilla, the mechanoreceptive neurones of basiconic sensilla and the chemoreceptive neurones of basiconic sensilla. Small movements of a wing caused by tactile stimulation may also stimulate proprioceptors such as the chordotonal organ and stretch receptor at the base of the wing. Electrical stimuli consisted of trains of 10–50 pulses at 100 Hz, 0.1 ms pulse duration. The voltage was different for each animal and was set at a level just sufficient to elicit scratching without also eliciting struggling or other behaviours. The timing of electrical stimulation was monitored by a pair of red light-emitting diodes (LEDs) visible in the video recording.

When the wings are folded, each forewing completely covers the ipsilateral hindwing, so touching the lateral (upper) surface of a forewing is also likely to stimulate tactile receptors of the hindwing through movements of the forewing. Similarly, movements of a folded wing caused by the stimulus could be transmitted to mechanoreceptors on the abdomen beneath the wing. In some experiments, therefore, these abdominal sensory pathways were ablated by cutting the connectives that link the abdominal ganglia with the metathoracic ganglion and the abdominal nerves entering the metathoracic ganglion (which contains the fused metathoracic and first three abdominal neuromeres). In some experiments, the metathoracic ganglion was further isolated by also cutting the connectives that link it with the mesothoracic ganglion. To perform the ablations, locusts were first restrained ventral surface uppermost and a flap cut in the thoracic cuticle overlying the metathoracic ganglion. The appropriate nerves and connectives were cut (taking care not to damage the longitudinal tracheae), the thorax was resealed by waxing the flap of cuticle back into position, and the locust was tethered above the walking ball. In animals with an isolated metathoracic ganglion, electrical or tactile stimulation was restricted to a hindwing. Throughout this paper, the term 'ipsilateral' refers to the side of wing stimulation. All figures show the hindleg in a standardised position.

Results

Tactile stimulation of the tip of a wing of a locust elicits directed scratching movements of a hindleg that cross the point of stimulation. Touching the base of the wing elicits a different pattern of scratching movements that are directed towards this more proximal site (Meyer, 1993; Matheson, 1997). Tactile stimulation of a wing that is held out laterally in a posture similar to that used during flight also elicits targeted scratching, but the animal does not compensate for the altered posture of the wing. Instead, it scratches at the empty point in space where the stimulus site would have been if the wing had been folded

in its resting posture (Matheson, 1997). This location will be referred to as the 'apparent stimulus site'.

Limb trajectory to target site

Brief tactile stimuli applied to different parts of an outstretched wing at intervals of a few seconds elicited discrete ipsilateral hindleg movements in which the tarsus passed across each apparent stimulus site (Fig. 1A). The trajectories followed by the tarsus to reach each site differed in a graded pattern, with no sudden transitions between different forms of movement. This suggests that limb targeting along a wing is accomplished by graded changes in a single motor pattern, rather than by the use of different motor patterns to reach different sites.

To determine whether the tarsal trajectory takes into account the initial starting position of the hindleg, tactile stimuli were applied to the distal end of an outstretched wing to elicit leg movements from different tarsal starting points (Fig. 1B–D). Stimuli at the same point on the wing elicited ipsilateral movements with clearly different trajectories for different tarsal starting points (Fig. 1B,C). When the starting point was relatively anterior, the tarsus moved directly towards the target (Fig. 1B). When the starting point was posterior, tarsal trajectories followed one of two patterns. Either the tarsus moved directly towards the target (Fig. 1C) or, alternatively, it first moved anteriorly before moving posteriorly along a trajectory similar to that taken from an anterior starting point (Fig. 1D). Movements beginning at the same starting point could therefore follow different trajectories to reach the target (compare Fig. 1C,D).

Contralateral targeting

During tactile stimulation of one outstretched wing, not only the ipsilateral, but also the contralateral, hindleg sometimes made movements towards the proximal–distal position of the apparent stimulus site. Contralateral scratches made up 732 of 2739 (27 %) of all scratches performed by animals with both hindlegs intact. This is perhaps unexpected, because each hindleg can only touch sites on its own side of the body (with the occasional exception of the dorsal edges of the folded wings). For example, an intact locust made a series of directed movements of the hindlegs in response to prolonged (30 s) tactile stimuli of one outstretched forewing (Fig. 2). Stimulation of a proximal site elicited anteriorly directed movements of the ipsilateral and contralateral hindlegs (Fig. 2A,B). While one leg moved, the other remained in contact with the substratum. The outward and return trajectories of the movements were very similar in lateral view (Fig. 2A), although the dorsal view (Fig. 2B) revealed some difference in lateral placement. The ipsilateral leg made a single cycle of movement, whereas the contralateral leg made a more prolonged rhythmical scratching movement in which the tarsus crossed the midline only at the peaks of its outward cycles. With the exception of these occasional midline crossings at the peaks of scratch cycles, contralateral tarsi never scratched on the side of stimulation, indicating that,
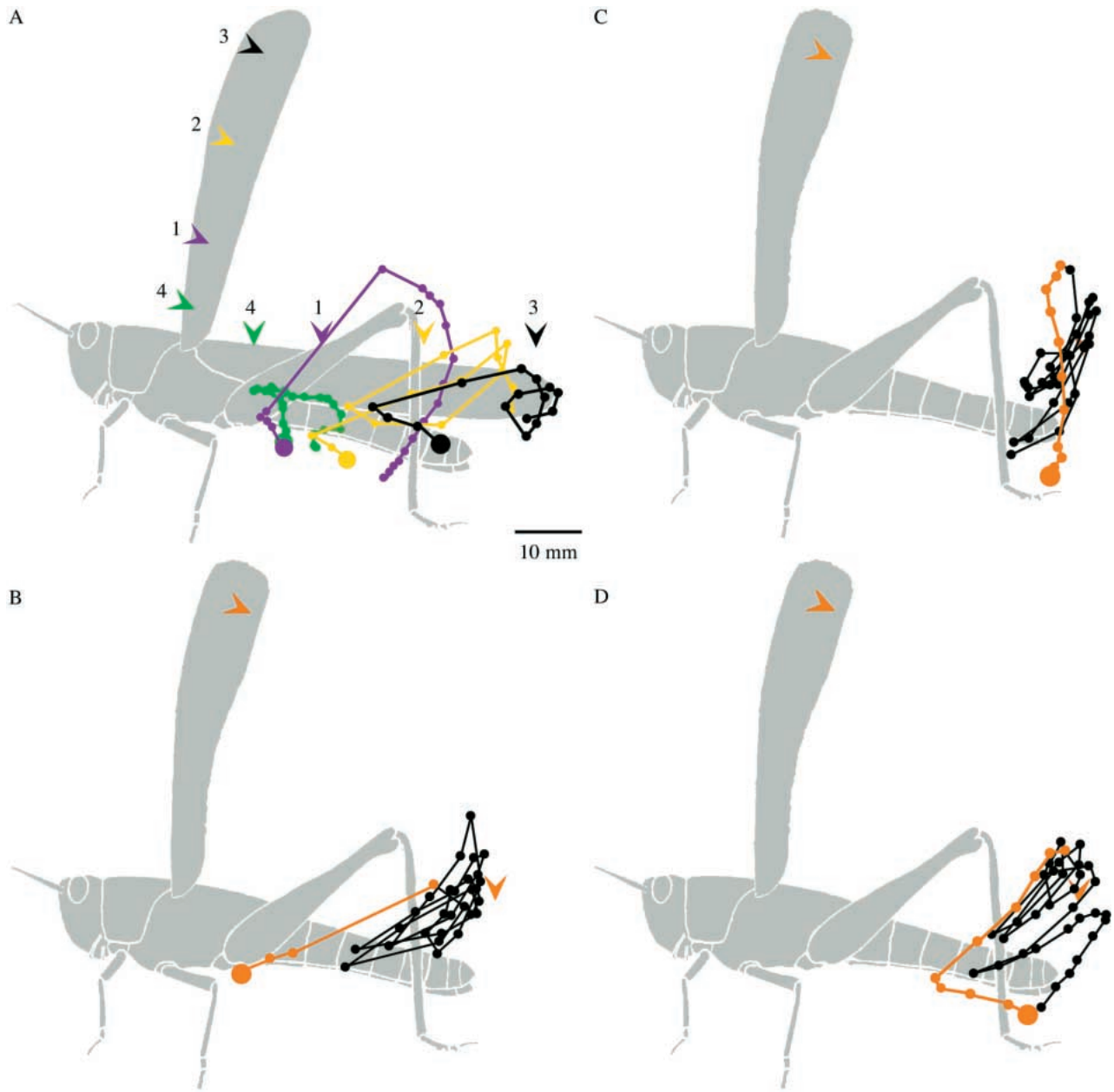
Fig. 1. (A) Tactile stimulation of four different sites along the outstretched forewing (arrowheads labelled 1–4 along the outstretched wing) of an intact locust elicited a series of targeted hindleg movements. The remaining wings were folded in the rest position. Numbers indicate the order of stimulation. The apparent positions of the stimulus sites (i.e. if the wing had been in its normal rest posture) are indicated by corresponding numbered arrowheads aligned along the body. Filled circles indicate the position of the base of the ipsilateral tarsus in successive video frames (25 frames s^{-1}). The first frame of each movement is indicated by a larger filled circle. Movements 1 and 4 were single-cycle scratches or pushes. Movements 2 and 3 were cyclic scratches. (B–D) In a different locust, tactile stimulation of an outstretched wing elicited sequential movements of the ipsilateral hindleg that converged from different starting points onto a common target site. The initial outward part of the movement is coloured orange for clarity. Movements from a single starting point could follow different trajectories to reach the same target (compare C,D).

although the contralateral limb is targeted appropriately along the proximal–distal axis, it is not targeted across the body. Stimulation of a distal site on the outstretched wing elicited single-cycle movements of both legs that were directed more posteriorly (Fig. 2A,B).

Three mechanisms may explain the ability to target the contralateral hindleg towards the proximal–distal location of a stimulus site. First, a targeted movement of the ipsilateral hindleg may stimulate mechanosensory receptors on the contralateral wing or abdomen which, in turn, generate similarly targeted movements of the contralateral leg. Second, targeted movements of the ipsilateral leg may be signalled by
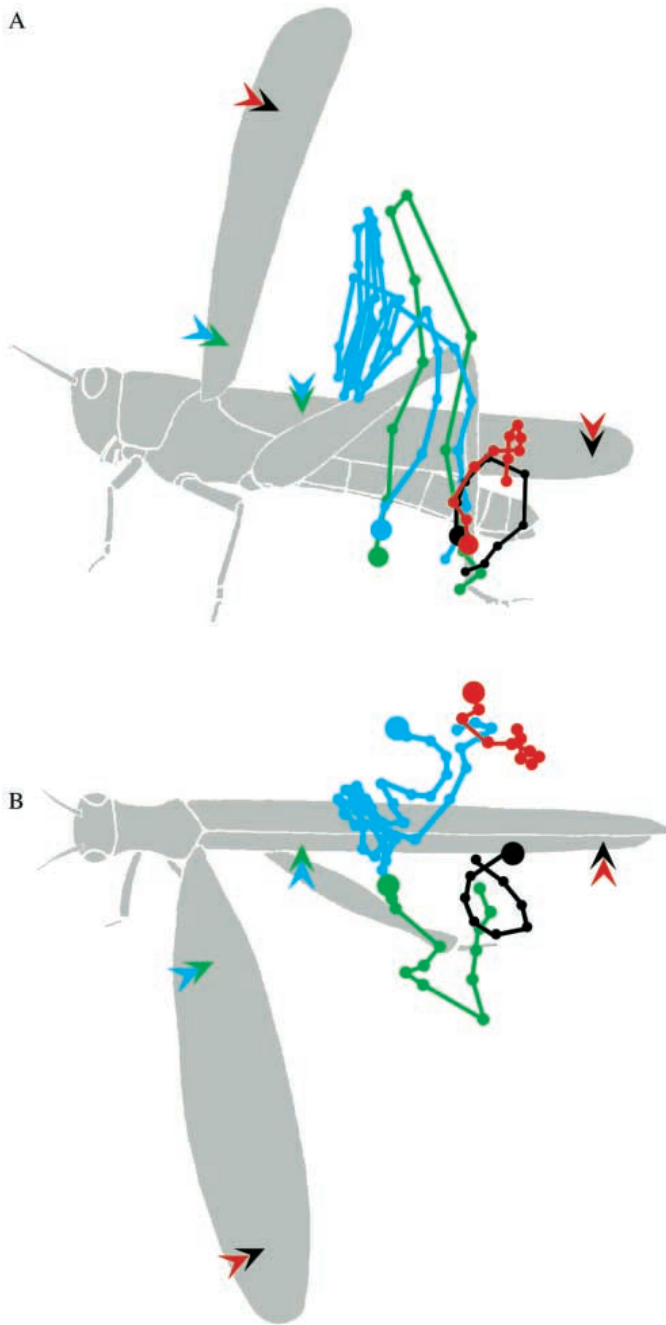
Fig. 2. Tactile stimulation of two different sites on the outstretched forewing (arrowheads) of an intact locust elicited a series of appropriately targeted movements of both the ipsilateral and contralateral hindlegs. The remaining wings were folded at rest. Positions of the tarsus are plotted in both lateral (A) and dorsal (B) views. Colour-coding of arrowheads and trajectories indicates the movements made by each leg in response to either proximal or distal stimulation: green and black, ipsilateral; blue and red, contralateral.
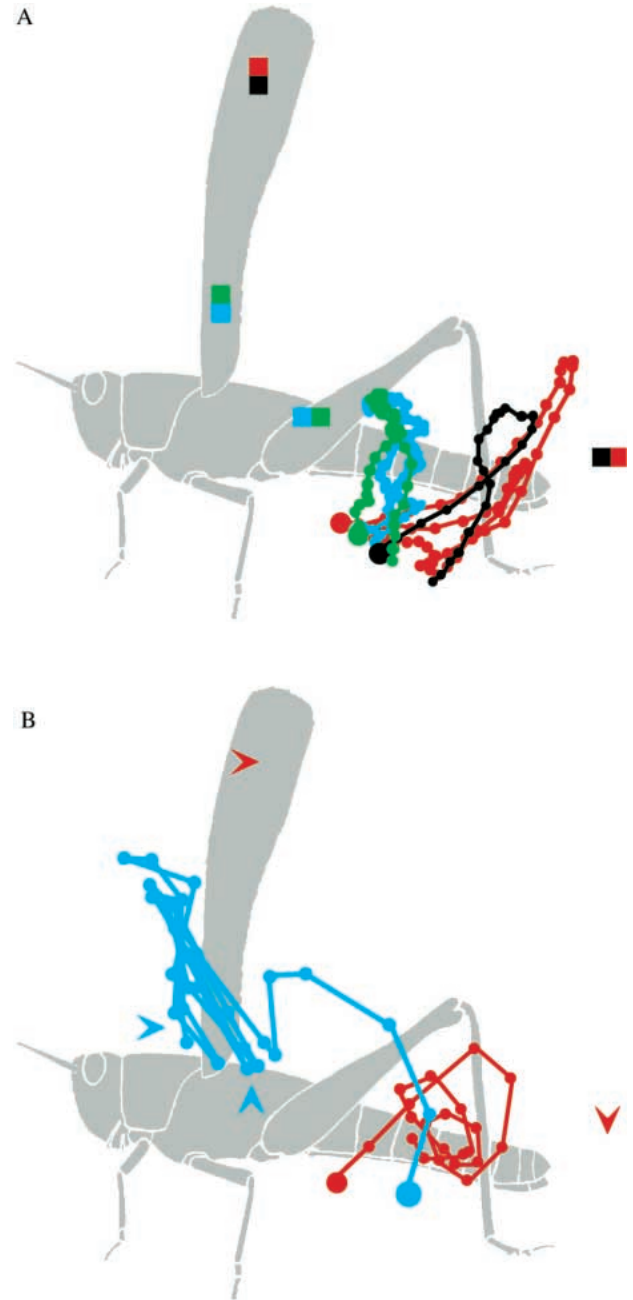
Fig. 3. (A) Electrical stimulation of two different sites on the outstretched hindwing (filled squares) of a partially deafferented locust elicited a series of appropriately targeted movements of both the ipsilateral and contralateral hindlegs. The sensory and motor innervation to the hindlegs and the ipsilateral hindwing were intact, but sensory inputs from the abdomen and other wings were prevented. Colour-coding of symbols and trajectories indicates the movements made by each leg in response to either proximal or distal stimulation: green and black, ipsilateral; blue and red, contralateral. (B) Tactile stimulation of an outstretched hindwing in a different locust whose ipsilateral leg was deafferented and de-efferented also elicited appropriately targeted contralateral hindleg movements (red and blue trajectories) in the absence of ipsilateral leg movement or sensory feedback. The sensory and motor innervation of the ipsilateral hindwing and contralateral hindleg were intact, but sensory inputs from the abdomen and other wings were prevented.

its own proprioceptors that convey targeting information to the contralateral motor networks driving the contralateral leg. Third, there may be central neuronal pathways by which ipsilateral targeting information is passed directly to

contralateral motor networks. To examine the first of these possibilities, contralateral targeting was examined in locusts whose sensory pathways from the abdomen and contralateral wings were removed. Ipsilateral wing receptors were stimulated electrically (rather than by touch) so that the stimulus did not produce vibrations that could potentially be detected by any other sense organs. In this situation, electrical stimulation of distal and proximal sites on a hindwing also elicited appropriately directed movements of both the ipsilateral and contralateral hindlegs (Fig. 3A). Further evidence that contralateral sensory feedback is not involved comes from the observation that contralateral scratching could precede ipsilateral scratching. To examine the second possibility, contralateral targeting was examined in similarly deafferented locusts which, in addition, were prevented from moving or sensing their ipsilateral hindleg by cutting the leg nerves that contain its motor and sensory axons. Tactile stimulation of an outstretched hindwing elicited correctly targeted movements of the contralateral hindleg, in the absence of ipsilateral hindleg movement (Fig. 3B). It therefore appears that the central nervous system of a locust can use the location of a stimulus site on one wing to target both ipsilateral and contralateral hindlegs towards the proximal–distal location of the apparent stimulus site, although the contralateral leg does not cross the midline.

Some tactile and electrical stimuli elicited simultaneous movements of both hindlegs that were directed towards the same target site. When the hindlegs moved simultaneously from different starting positions, their trajectories differed so that each tarsus moved directly towards the apparent stimulus site (Fig. 4A). The ability to generate different limb trajectories for the two hindlegs is emphasised by the movements illustrated in Fig. 4B. An intact animal with all its wings held out laterally responded to stimulation of one forewing by scratching with the ipsilateral hindleg while walking forward using the remaining five legs. During the 1 s period illustrated, the scratching limb made 5.5 cycles of movement, whereas the stepping limbs made 3 (pro-, meso-) or 3.5 (metathoracic) cycles.

When both hindlegs scratched together, the phase relationship between them could drift (Fig. 5A,B, inset). In this example, the peak of the first outward cycle made by the contralateral leg (point d in Fig. 5B) coincided with the peak of an outward cycle in the ipsilateral leg (d in Fig. 5A), but the peak of the second contralateral cycle (point 2) fell half-way through the next ipsilateral cycle, the peak of the third contralateral cycle (3) coincided with the trough of an ipsilateral cycle, and the fourth cycles (4) again coincided (Fig. 5 inset).

Retargeting during a rhythmic scratch

The data presented in Fig. 1A indicate that hindleg targeting can be modified within a few seconds to permit sequential scratching of different stimulus sites. To investigate this retargeting further, tactile stimuli were moved along an outstretched wing at different speeds in the range 2–15 cm s^{-1}.
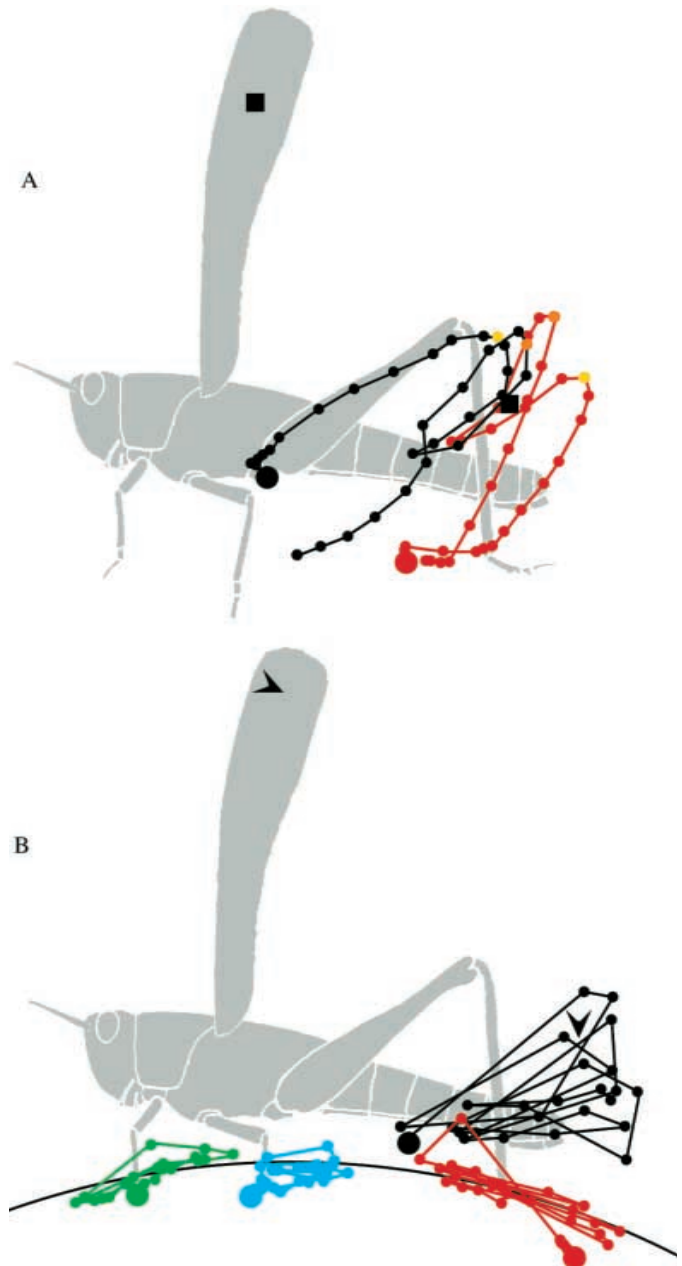

Fig. 4. Electrical stimulation of a distal site on an outstretched hindwing (A) elicited simultaneous movements of the two hindlegs that began at different starting points but converged towards the target by following different trajectories (red, contralateral; black, ipsilateral). Sensory inputs from the ipsilateral hindwing and both hindlegs were intact, but those from the other wings and abdomen were prevented, and the anterior connectives were cut. Yellow and orange dots indicate corresponding frames at the peaks of the two cycles. (B) Tactile stimulation of an outstretched wing elicited scratching by the ipsilateral hindleg (black trajectory) but walking by the other five legs (movements of contralateral fore-, middle and hindlegs are shown). The animal was intact, with all four wings held out laterally.

A stimulus moving at 2 cm s^{-1} takes approximately 2.5 s to travel the length of the wing, during which time a scratching limb can
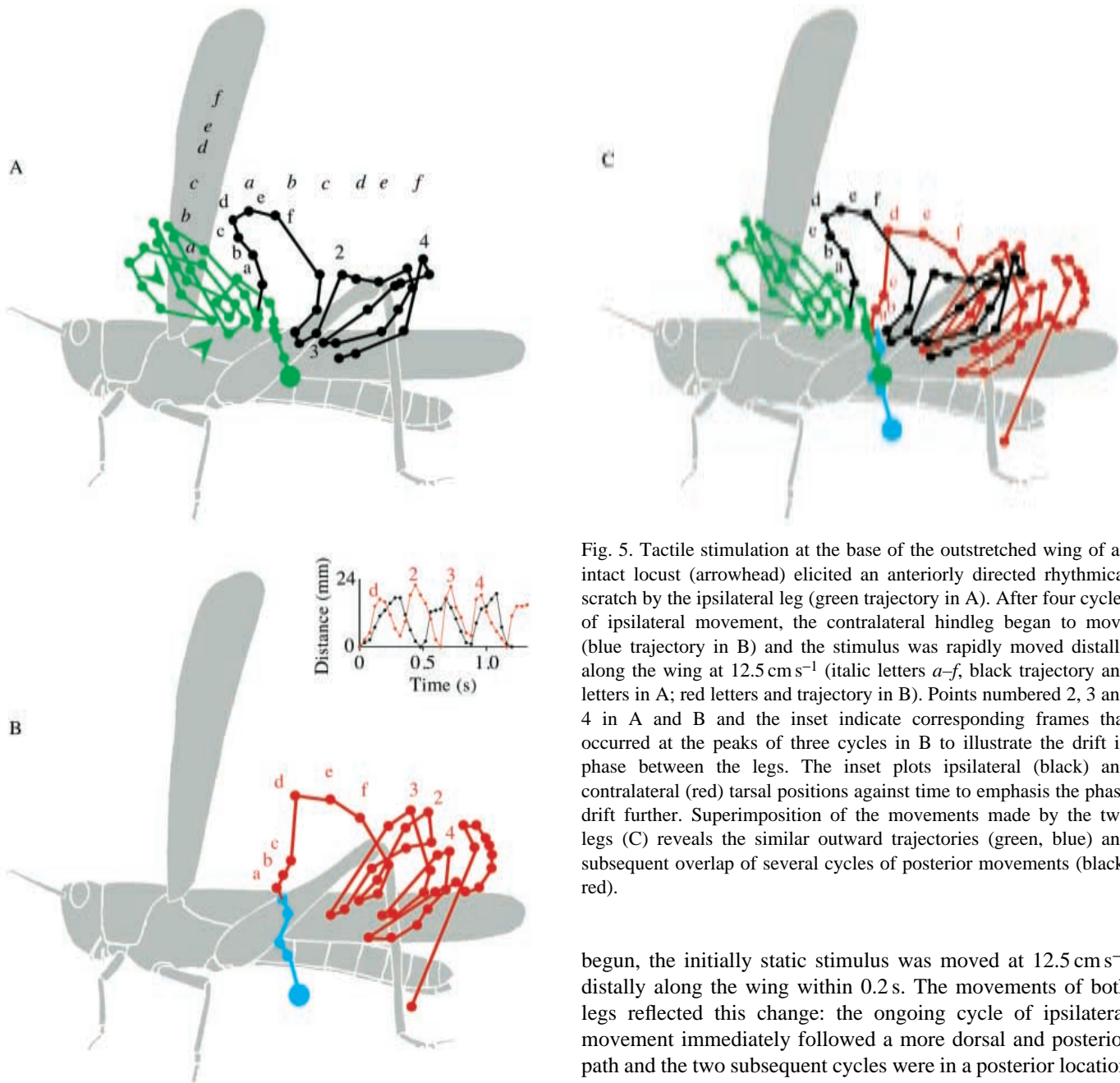
Fig. 5. Tactile stimulation at the base of the outstretched wing of an intact locust (arrowhead) elicited an anteriorly directed rhythmical scratch by the ipsilateral leg (green trajectory in A). After four cycles of ipsilateral movement, the contralateral hindleg began to move (blue trajectory in B) and the stimulus was rapidly moved distally along the wing at $12.5\,\mathrm{cm\,s^{-1}}$ (italic letters *a–f*, black trajectory and letters in A; red letters and trajectory in B). Points numbered 2, 3 and 4 in A and B and the inset indicate corresponding frames that occurred at the peaks of three cycles in B to illustrate the drift in phase between the legs. The inset plots ipsilateral (black) and contralateral (red) tarsal positions against time to emphasis the phase drift further. Superimposition of the movements made by the two legs (C) reveals the similar outward trajectories (green, blue) and subsequent overlap of several cycles of posterior movements (black, red).

make many cycles of movement. In contrast, a stimulus moving at $15\,\mathrm{cm\,s^{-1}}$ takes only 0.3 s to move the same distance, within which time a scratching limb can make at most one cycle.

The effects of rapid movements of the stimulus (e.g. $12.5\,\mathrm{cm\,s^{-1}}$) are illustrated in a locust that consistently scratched an appropriate anterior location with both hindlegs in response to tactile stimulation near the base of an outstretched wing. A static (non-moving) tactile stimulus of the base of the wing elicited an anterior scratch of the ipsilateral hindleg (green trajectory in Fig. 5A). After four cycles of ipsilateral movement, the contralateral hindleg also began to move along the same anteriorly directed initial trajectory (blue trajectory in Fig. 5B). Once this contralateral movement had

begun, the initially static stimulus was moved at $12.5\,\mathrm{cm\,s^{-1}}$ distally along the wing within 0.2 s. The movements of both legs reflected this change: the ongoing cycle of ipsilateral movement immediately followed a more dorsal and posterior path and the two subsequent cycles were in a posterior location (black trajectory in Fig. 5A). The outward path of the contralateral leg immediately turned posteriorly so that all the subsequent cycles were targeted towards a posterior location (red trajectory in Fig. 5B). Superimposing the trajectories of the two movements reveals the similar initial trajectories and the convergence towards a posterior location during and after movement of the stimulus (Fig. 5C).

When locusts scratched in response to slowly moving stimuli (e.g. $2–5\,\mathrm{cm\,s^{-1}}$), they were able to retarget each successive cycle of a rhythmical scratch (Fig. 6A,B). This retargeting occurred in response to stimuli moving either from proximal to distal along the wing (Fig. 6A) or from distal to proximal (Fig. 6B).

Brief electrical stimulation of wing sensory axons at proximal or distal locations elicits appropriately directed
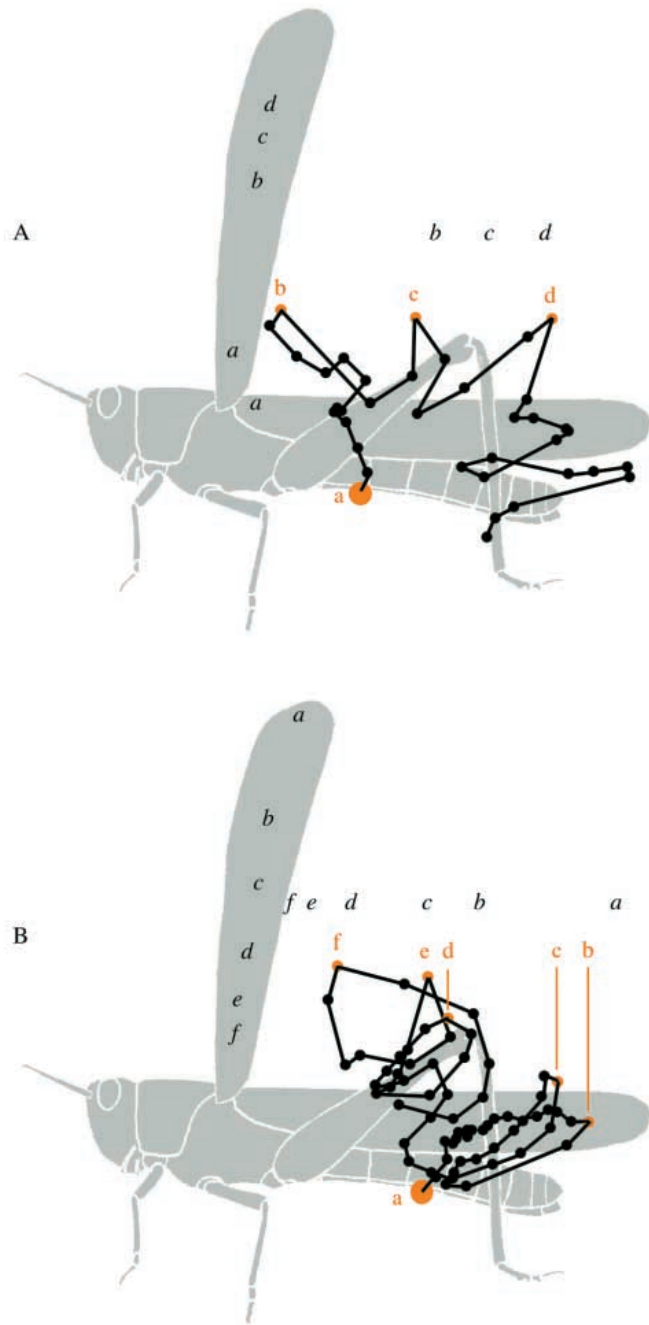
Fig. 6. (A) A tactile stimulus that slowly moved distally along the outstretched wing of an intact animal elicited a rhythmic ipsilateral scratch in which successive cycles were targeted progressively more posteriorly. Black italic letters *a–d* indicate the position of the stimulus along the wing at times when the tarsus reached the apex of each cycle (corresponding orange letters a–d). The stimulus moved at approximately $5\,\mathrm{cm\,s^{-1}}$ and lasted 800 ms. (B) A stimulus moving slowly from distal to proximal at $2\,\mathrm{cm\,s^{-1}}$ also elicited a progressively retargeted rhythmical scratch.
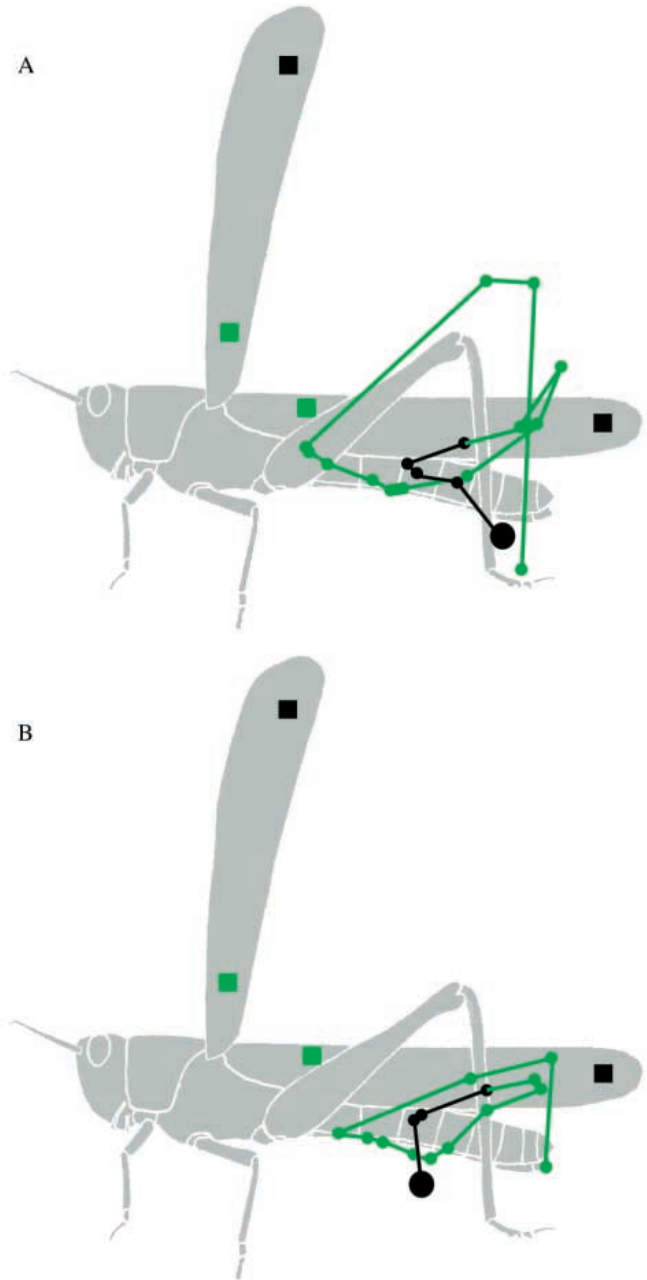

Fig. 7. Electrical stimulation (filled squares) of a distal site followed by stimulation of a proximal site after an interval of 120 ms (A) or 160 ms (B) elicited a posterior scratch cycle and then a more anteriorly directed cycle. The timing of stimuli is indicated by a change in colour of the trajectory corresponding to the colours of the square symbols.

hindleg movements (Fig. 3A). If such electrical stimuli at two locations follow each other with short latency (e.g. 120–160 ms, corresponding to stimulus movement velocities of $2.5–3.3\,\mathrm{cm\,s^{-1}}$ along the wing), locusts initially target a scratch towards the first site and then retarget their scratching for a subsequent cycle. For example, electrical stimulation of distal and then proximal sites on the outstretched wing of an intact locust repeatedly elicited a posteriorly directed cycle followed by a more anteriorly directed cycle (Fig. 7A,B). This indicates that retargeting can occur as a result of discrete sequential activation of wing sensory receptors in two locations
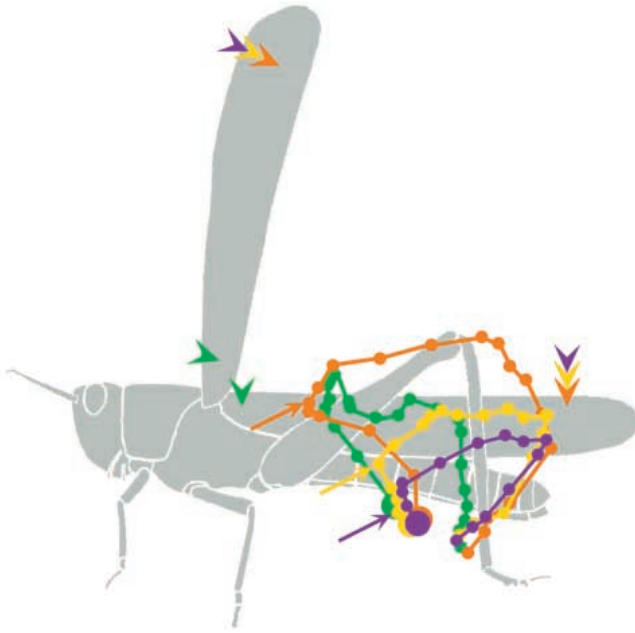
Fig. 8. Tactile stimulation at the base of the outstretched wing of an intact locust (green arrowhead) consistently elicited an anteriorly directed movement of the ipsilateral leg (green trajectory). Tactile stimulation at the base of the wing (green arrowhead) followed at different intervals by tactile stimulation of a distal site (orange, yellow, purple arrowheads) caused immediate changes in leg trajectory. The time at which the distal stimulus occurred is indicated for each trajectory by correspondingly coloured arrows.

and does not require other cues that may be present in a moving tactile stimulus.

Retargeting within a scratch cycle

Two strategies may be envisaged for retargeting: first, a locust may complete the ongoing cycle without retargeting, but modify the subsequent trajectory towards the new target site. Alternatively, the trajectory of the ongoing cycle may be modified so that the leg moves directly towards the new target position. To distinguish between these possibilities, brief static tactile stimuli were sequentially applied to proximal and distal locations of the outstretched wing of an intact locust. For example, in one animal, a brief proximal stimulation by itself consistently elicited single cycles of anteriorly directed hindleg movements with similar trajectories (e.g. green trajectory in Fig. 8). During a series of these movements, additional distal stimuli were applied to the wing after different intervals. When the distal stimulus occurred as the leg reached the apex of its anteriorly directed movement, the return trajectory was modified so that the tarsus moved posteriorly, directly towards the new apparent stimulus site (orange arrowhead and trajectory in Fig. 8). If the distal stimulus followed the initial proximal stimulus more rapidly, the anteriorly directed outward trajectory also turned posteriorly sooner (yellow and purple arrowheads and trajectories in Fig. 8). In all cases, the initial trajectory followed by the hindleg was very similar up until the time when

the distal stimulus was applied. Following distal stimulation, these outward trajectories were modified so that, in all cases, the tarsus moved directly towards the appropriate apparent stimulus site at the posterior of the animal within a single cycle.

Discussion

This paper shows that, in the locust, limb targeting along a wing is continuously graded, that different trajectories can be used to reach a common stimulus site from different starting points and that the trajectories of the two hindlegs moving simultaneously from different starting points can each converge directly onto a common stimulus site. Targeting information is passed both intersegmentally and contralaterally, so that touching one forewing can elicit targeted movements of one or both hindlegs. Locusts can retarget their movements during a rhythmical scratch, and this retargeting can occur within a single cycle of movement. These features of targeted limb movements provide important clues about the underlying neuronal pathways and information processing within the central nervous system.

Targeting is continuously graded

At least two distinct strategies may be envisaged for generating a movement directed towards a target site. The first, and simpler, would be to respond to stimulation by moving the scratching leg to a particular starting position common for all stimulus sites, and choosing from among a range of stereotyped motor patterns to move from this point to the target. A second, and more complex, strategy would be to compute a unique trajectory from any given starting position to the target. Locusts can use this second strategy, which presumably permits a more rapid and accurate response, but which may be more costly in terms of neuronal processing. Nevertheless, there is some evidence that they may also use the simpler strategy. For example, movements such as those illustrated in Fig. 1D suggest the presence of an initial movement component (a femoro-tibial flexion) that may take the leg to a stereotyped starting position, as suggested by Meyer (1993) in the locust, Giszter *et al.* (1989) and Fukson *et al.* (1980) in the frog, and Valk-Fai and Crowe (1979) in the turtle *Pseudemys scripta elegans*.

To generate a movement that takes a leg from any arbitrary starting position directly to a target, there must be neurones within the local networks that integrate proprioceptive information signalling the position of the leg with exteroceptive information from the wing signalling the stimulus site. Some spiking local interneurones may receive inputs from leg exteroceptors and leg proprioceptors (Burrows, 1985; Burrows and Newland, 1993), but their receptive fields are generally more restricted than those of interneurones that receive inputs of only one or other modality. This suggests that these particular interneurones are not well suited to integrating the position of the entire leg, signalled by receptors at several joints, with a tactile stimulus that could occur anywhere on the appendage. The possible convergence of exteroceptive and proprioceptive information at this neuronal level nevertheless

hints at the existence of appropriate interneurones with inputs from wing exteroceptors and leg proprioceptors.

In monkeys, many neurones in the dorsal premotor area respond preferentially to particular target locations (Shen and Alexander, 1997), whereas neurones in the motor cortex fire preferentially during movements of the arm in a particular direction, irrespective of the endpoint (for a review, see Georgopoulos, 1996). The preferred direction differs for each motor cortical cell, so that the population of neurones can encode movements in all three dimensions. The directional tuning of motor cortical cells results from an extremely complex interaction between excitatory and inhibitory inputs from many sources, probably including other motor cortical cells (through various pathways), neurones in the thalamus and in other ipsilateral and contralateral cortical sites (Georgopoulos, 1996). The cortical representation of movement direction is presumably passed to motor neurones in the spinal cord through one or more interneuronal layers. Intrinsic spinal circuitry appears to be responsible for the generation of simple movement components (Giszter *et al.* 1993). In the locust, it should be possible to use intracellular recordings to characterise the responses of interneurones that respond during targeted scratching and to identify key presynaptic and postsynaptic neurones.

Targeting information is conveyed intersegmentally

Sensory neurones from the forewings enter the mesothoracic ganglion, whereas those from the hindwing enter the metathoracic ganglion. Nevertheless, touching either a forewing or a hindwing elicits targeted scratching by a hindleg (Matheson, 1997), indicating that neuronal pathways exist to carry precisely encoded information about the target site from the mesothoracic ganglion to the metathoracic motor networks that generate hindleg movements. Tactile stimulation of sites on the thorax or abdomen can also elicit appropriately targeted scratching (Berkowitz and Laurent, 1996*a*), again suggesting intersegmental flow of targeting information. In vertebrates, tactile stimulation of sensory neurones that enter the spinal cord in one segment can lead to targeted scratching by a limb whose motor neurones are located in a distant segment (see Stein, 1983). The discrete nature of insect segmental ganglia and the relatively small number of intersegmental neurones that pass between the ganglia in the connectives provide an attractive opportunity to investigate the form in which this intersegmental information is encoded in identifiable neurones. In walking stick insects *Carausius morosus*, proprioceptive information from a middle leg is used to set the target position for the end of the swing movement of the ipsilateral hindleg (Cruse, 1979). Perhaps as few as 10 intersegmental interneurones that encode middle leg joint angles may contribute to this hindleg targeting (Brunn and Dean, 1994). In the locust, some descending intersegmental interneurones receive inputs from middle leg proprioceptors and make outputs onto hindleg local non-spiking interneurones (Laurent, 1987; Laurent and Burrows, 1989). Although possible inputs from wing hairs have not been investigated, these or similar interneurones would be ideally placed to carry targeting information from a forewing to a hindleg.

Targeting information is conveyed contralaterally

Touching the folded wings of a resting locust can elicit scratching by both hindlegs, but the neuronal pathways for this contralateral flow of information are unknown. Stimulating an outstretched wing when sensory pathways from the other wings and abdomen are ablated can also generate contralateral scratching, ruling out the possibility that the stimulus is indirectly passed to contralateral sensory neurones through mechanical pathways. Moreover, contralateral scratching can occur in the absence of ipsilateral leg movement in an animal in which the ipsilateral leg is deafferented and de-efferented. This rules out the possibility that contralateral leg movements are generated to match the pattern of sensory feedback from a moving ipsilateral leg. Finally, a single stimulus can elicit simultaneous movements of the two hindlegs from different starting positions that follow different trajectories to reach the same target site. All this evidence, taken together, indicates that information about the stimulus site is passed across the midline in a central neuronal pathway and that appropriate motor patterns are computed independently for the two legs taking into account their initial posture.

Further evidence for the existence of central pathways that can mediate contralateral leg coordination comes from an isolated metathoracic ganglion treated with the muscarinic cholinergic agonist pilocarpine (Ryckebusch and Laurent, 1993). This isolated ganglion produces a motor pattern in which contralateral leg motor neurones fire rhythmic bursts of action potentials, suggestive of a fictive walking motor pattern. The rhythms on the two sides may occur at different frequencies but, nevertheless, there is coupling between contralateral trochanteral depressor and levator motor neurones.

Berkowitz and Laurent (1996*a*) show that touching one ear (located on the abdomen) of a de-efferented locust preparation can elicit coordinated motor activity in the cut stumps of hindleg motor nerves on both sides of the body. The motor activity can have different patterns of coordination, so that in some cases trochanteral levator activity on one side is accompanied by trochanteral depressor activity on the other side, whereas in other examples there appears to be simultaneous activity of trochanteral levators on both sides. The variability of coupling in this fictive motor pattern is reflected by my finding that the contralateral coordination of hindleg movements during scratching is extremely complex. First, coupling can be present or absent, so that one leg or both legs can move in response to a unilateral stimulus. Second, the cycle period of repetitive scratching movements can differ on the two sides, so that there is no constant phase relationship between them. Third, the legs can follow different trajectories to reach a common target site. Fourth, one leg can make rhythmic scratching movements while the other legs make stepping movements with a different period. Cockroaches *Periplaneta americana* can also make single-cycle grooming movements of one hindleg while the other five legs walk but, in contrast to my observation, tibial extension of the scratching leg in cockroaches occurs at a constant phase with respect to the walking legs (Reingold and Camhi, 1977). This type of

grooming in the cockroach has therefore been described as 'slightly redirected walking movements', and both behaviours are hypothesised to be driven by the same central neuronal oscillator (Reingold and Camhi, 1977). This does not seem to be the case in the locust. The great adaptability and expression of different motor activity in different situations will make it extremely difficult to interpret fictive scratching motor patterns in a preparation that is unable to move.

What neuronal pathways could carry targeting information across the ganglionic midline? The central projections of tactile sensory neurones from the wings of locusts have not been described in detail but, by analogy with the equivalent receptors on the legs, most are unlikely to cross the midline (Pflüger *et al.* 1981; Newland, 1991). Prominent groups of local spiking interneurones that process inputs from leg tactile hairs and proprioceptors, and which contribute to the generation of reflex leg movements, are also mostly restricted to the ipsilateral hemiganglion (Burrows and Siegler, 1984; Siegler and Burrows, 1984; Nagayama, 1989). Nonspiking local interneurones that are thought to act together to coordinate hindleg movements have projections that, in most cases, are also restricted to one half of the ganglion (Siegler and Burrows, 1979; Watkins *et al.* 1985), although some have contralateral projections. These nonspiking interneurones and intersegmental neurones with branches on both sides of the metathoracic ganglion (Laurent and Burrows, 1988; Newland, 1990) are the most likely candidates to carry targeting information contralaterally.

In cat and turtle, contralateral motor neurones and interneurones are activated during an ipsilateral fictive scratch (Arshavsky *et al.* 1978; Deliagina *et al.* 1981; Berkowitz and Stein, 1994; Stein *et al.* 1995). This contralateral circuitry is important not only for the generation of contralateral motor output but also for the correct expression of ipsilateral motor output. In particular, ablation of the contralateral spinal hemicord usually prevents ipsilateral hip extensor activity. In the frog at least, there must be such a contralateral transfer because one hindlimb is able to scratch the other (Giszter *et al.* 1989). In an intact frog, both hindlimbs move to achieve a successful scratch, and the trajectories can vary greatly between trials of the same stimulus site. In a spinal frog, however, the movements are more stereotyped, and the animal can no longer successfully reach the contralateral target leg if it is artificially held in different positions (Giszter *et al.* 1989). In the locust, it remains to be determined whether the central representation of a target site on a wing is distributed bilaterally or whether there are separate representations on the two sides.

Limb trajectory can be modified during a targeted movement

Scratching in the spinal turtle is thought to be predominantly preprogrammed, rather than relying on sensory feedback to achieve precise targeting (Valk-Fai and Crowe, 1979; Robertson *et al.* 1985). Peripheral information is used before the movement starts, so that different stimulus locations give rise to different trajectories, but the motor pattern is not subsequently altered to compensate for external frictional forces or the effects of gravity. Impeding an ongoing movement so

that the limb cannot reach the stimulus site has no apparent effect on the pattern of muscle activity (Valk-Fai and Crowe, 1979). In frogs also, aiming is thought to take place at the start of a scratch when the scratching limb is flexed (Giszter *et al.* 1989), with the subsequent extension made largely under open-loop conditions. Spinal frogs cannot retarget a new stimulus site once a scratching movement has begun: they either miss the target or prematurely terminate the scratch (Giszter *et al.* 1989). Intact frogs reportedly do compensate for stimulus movement, but supporting data are not provided (Giszter *et al.* 1989).

A monkey that is trained to reach towards one target can modify the trajectory of its arm once the movement has started so that it can reach a new target at a different location, indicating that there is closed-loop visuomotor control (Georgopoulos *et al.* 1981). Locusts can also modify the trajectory of a targeted limb movement to reach a moving tactile stimulus. This retargeting can occur within a few tens of milliseconds in both ipsilateral and contralateral hindlegs. Moreover, retargeting can occur during the outward trajectory of a single scratch cycle, which implies that locusts can compute a limb trajectory from any point in space to any other point during an ongoing movement.

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