

MULTISEGMENTAL ANALYSES OF ACOUSTIC STARTLE IN THE FLYING CRICKET (*TELEOGRYLLUS OCEANICUS*): KINEMATICS AND ELECTROMYOGRAPHY

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Summary

Tethered, flying Australian field crickets (*Teleogryllus oceanicus*) stimulated with ultrasound respond with a rapid, short-latency turn from the sound source. We analyzed the kinematics of two behavioral components of this acoustic startle response and recorded electromyograms from the muscles involved in producing them. The two behavior patterns studied were the swing of the metathoracic leg, which has been shown to elicit a short-latency turn, and a lateral swing of the antennae, for which a direct role in steering has not been demonstrated.

The kinematic data showed that when a pulse of ultrasound was presented to one side of the animal (1) the contralateral metathoracic leg abducted and elevated, while the ipsilateral leg remained in place, (2) both antennae swung laterally, but the contralateral antenna moved farther than the ipsilateral antenna, (3) increases in stimulus intensity elicited larger movements of the leg and contralateral antenna, while the ipsilateral antenna showed little sensitivity to stimulus intensity, and (4) for the leg, the latency to the onset of the swing decreased and the duration of the movement increased with increasing stimulus intensity.

Electromyograms were recorded from the leg abductor M126 and two antennal muscles: the medial scapo-pedicellar muscle M6 and the lateral scapo-pedicellar muscle M7. M7 moves the antenna laterally, M6 moves it medially. Upon stimulation with ultrasound (1) both M126 and M7 showed increasing spike activity with increasing intensity of the ultrasound stimulus, (2) M126 showed a decrease in latency to the first spike and an increase in the duration of spike activity with increasing stimulus intensity, (3) latencies for M6 and M7 were not correlated with stimulus intensity, but M7 had significantly shorter latencies than M6 and the contralateral M7 had significantly shorter latencies than the ipsilateral M7, and (4) the ipsilateral M126 spiked in response to ultrasound in 6 of the 10 animals tested. In these cases, however, latency to the first spike was substantially longer, and the spike frequency was lower than for the muscle's response to contralateral stimuli. We attempt to correlate these electromyogram data with the

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kinematic data and relate them to the relevance of the two behavior patterns to the execution of an escape response.

Introduction

When stimulated with a pulse of ultrasound as brief as 1 ms, tethered flying Australian field crickets (*Teleogryllus oceanicus*) execute a short-latency turn away from the sound source (Nolen and Hoy, 1986). This negative phonotactic behavior is thought to serve as a mechanism for evading hunting bats. In addition to the changes in wing movements associated with steering in flight (May *et al.* 1988; Wang and Robertson, 1988), it also includes lateralized movements of a number of other body parts. Movements of the head and mouthparts and swinging motions of the antennae, abdomen, ovipositor (Moiseff *et al.* 1978; Hoy, 1989) and metathoracic leg (May and Hoy, 1990a) have been described. These characteristic responses have also been referred to as components of an acoustic startle response (Hoy, 1989). Acoustic startle has been defined as a set of relatively short-latency muscular responses to an unexpected, intense stimulus (Bullock, 1984; Davis, 1984). The muscular responses may be of small amplitude among a set of seemingly unrelated muscle and thus not necessarily produce an escape response. Some of the flying cricket's responses to ultrasound, like the movements of mouthparts and ovipositor, may well belong in this category. Here, we examine two components of the cricket's acoustic startle response, the swings of the metathoracic leg and antennae.

Recently, the movement of the metathoracic leg was shown to affect directly the yaw component of the cricket's response to ultrasound. An ultrasound stimulus presented to one side of the flying cricket causes the animal to swing its metathoracic leg on the opposite side of its body into the hindwing. The leg impedes the hindwing's downstroke and induces rotation about the yaw axis, thereby assisting in a turn away from the sound source (May and Hoy, 1990a). Similar motions of the metathoracic leg during steering have been described for a variety of insects: locusts (Gettrup and Wilson, 1964; Camhi, 1970; Baker, 1979; Cooter, 1970; Taylor, 1981; Arbas, 1986), flies (Hollick, 1940; Götz *et al.* 1979; Nachtigall and Roth, 1983) and bugs (Govind and Burton, 1970; Govind, 1972). However, aerodynamic consequences of this behavioral response are known only for the Australian field cricket.

The antennae of the flying cricket are held parallel to each other, straight out from the head. When presented with ultrasound from one side of the body, the animal swings both antennae apart laterally, with the antenna contralateral to the sound moving farther than the ipsilateral antenna (Moiseff *et al.* 1978). Antennal twitches induced by ultrasound have also been observed in flying locusts (D. Robert, personal communication), but have not been studied in detail. There is little information on the role of the antennae in flight for crickets, but work on locusts has shown several flight parameters that are affected by the antennae. In the locust, they influence the flight speed, wingbeat frequency and wing-stroke

angle (Gewecke, 1975). Antennal deflections caused by wind on the head have also been shown to induce a swing of the metathoracic leg (Arbas, 1986).

Here we examine the kinematics of the metathoracic leg swing, which has been shown to be directly involved in steering. We also analyze the antennal swing, which may affect steering indirectly, as in the locust, or may be among a set of generalized low-amplitude responses that are included in the cricket's acoustic startle response. We compare the kinematic results with electromyograms recorded from some of the muscles involved in generating both movements and relate these data to the relative importance of the two responses in executing escape behavior.

Materials and methods

Preparation and stimulus production

In all experiments we used adult *Teleogryllus oceanicus*, 1–4 weeks after their adult molt, obtained from our laboratory colony. The colony is maintained at a high density and on a reversed light cycle (12 h:12 h L:D). We have found that the animals are most likely to fly within the first 3 h of the onset of darkness.

Crickets were tethered upright in a wind stream. Using a small amount of dental wax, they were attached to a tether placed at their center of gravity on the dorsal surface of the first abdominal segment near the metathorax. For studying antennal movements, an additional strip of 0.8 mm diameter wire was waxed to the animal at the intersection of the head and pronotum and again at the intersection of the metathorax and abdomen. This prevented the animal from rotating its head and stabilized the preparation, enabling us more easily to observe the antennal movements. The same types of antennal movements have been observed previously in animals not immobilized in this way (Moiseff *et al.* 1978).

Ultrasonic stimuli were synthesized, using a 20 kHz sine wave (B+K model 3011 or 3011A) shaped (custom-built trapezoid shaper) and adjusted for duration (WPI pulse module model 831 or Grass SD9 stimulator). The signal was amplified (Nikko NA-790 or Harman/Kardon PM640 Vxi) and delivered to one of two piezoelectric loudspeakers placed in the cricket's horizontal plane at 90° to the animal's longitudinal axis and 67 cm from the animal's ear. The intensity of the sound stimulus was varied using a step-attenuator (Hewlett-Packard 350D). A sound level meter (Brüel & Kjaer 2209) fitted with a 1/4 inch microphone (Brüel & Kjaer 4135) was used to measure sound pressure levels at the position of the cricket's ear. These are given in decibels relative to 20 μ Pa (dB SPL). Spectral properties of the stimulus were determined by a real-time spectrum analyzer (Nicolet 444A), which showed that all harmonics were 30–40 dB less intense than the carrier frequency. For all experiments, the ultrasound stimulus was a pure tone pulse with a carrier frequency of 20 kHz, a pulse duration of 100 ms and a pulse rise–fall time of 5 ms. The stimuli were delivered over a series of intensities, in ascending and descending order, from the loudspeakers on either side of the animal.

Kinematic measurements

To measure movement of the metathoracic leg, we removed the hindwings to expose the leg during flight. Flight movements of tethered crickets ($N=10$) were recorded on video tape from the posterior aspect (NEC NC-8 color camera), and movements of both legs were analyzed with a video recorder (Panasonic NV-8950) and color monitor (NEC). The tether served as a linear reference for measurement of abduction and elevation of the leg during its swing. We defined abduction and elevation as the maximum lateral and vertical displacements, respectively, of the distal end of the femur. For each cricket we measured the length of the leg, from the proximal end of the coxa to the distal end of the femur. Given this length and the linear displacement of the distal end of the femur, we calculated the angular values ($\pm 0.5^\circ$) for abduction relative to the coxal joint. Antennal movements were analyzed from animals videotaped from above ($N=10$). The antennal angle was determined relative to the longitudinal axis of the animal, using the wire that held the head as a reference.

We measured the temporal characteristics of the leg swing for 10 animals. The animals were tethered as described above, but with a cadmium sulfide photocell placed beneath them and a fiber-optic light positioned above. Movement of the leg thus produced changes in a d.c. signal, which was stored on analog tape (Vetter model 420) for off-line analysis on a digital storage oscilloscope (Tektronix 2230). Both the latency and duration of the leg's movement could be measured from the photocell signal. Latency was defined as the time from the onset of the stimulus to the beginning of the leg swing. Duration was defined as the time from the onset of the photocell signal until its return to baseline. It was not possible to obtain temporal data for the antennal movements, because their much smaller diameters put them below the limits of resolution of the photocell.

Electromyography

Cricket were anesthetized by cooling, and placed on a Peltier plate or on ice to keep them cold during electrode placement. Earlier work on the metathoracic leg swing in tethered flying locusts (Dugard, 1967) showed that two leg muscles were involved in this movement: the anterior rotator, or muscle 121 (Albrecht, 1953), and the coxal abductor, or muscle 126 (Albrecht, 1953; Furukawa *et al.* 1983). We chose to examine muscle 126 (M126), which was one of the most external of the metathoracic muscles, and thus the more easily accessible of the two. M126 is innervated by a pair of metathoracic motoneurons (C. I. Miles, unpublished observations). A monopolar stainless-steel electrode, 50 μm in diameter and insulated except at the tip, was implanted in the centre of M126 in 10 animals. The electrode was inserted through a hole punched in the cuticle of the metathorax and held in place with a small drop of low-melting-point wax at its insertion point. A ground electrode was inserted into the abdomen. The other ends of the electrode and ground wires were brought to the ventral side of the animal, where they were attached at one point with a drop of wax and led posteriorly away from the animal

to an a.c.-coupled amplifier (A-M Systems) and oscilloscope. The muscle spikes were recorded (Vetter model 420F) for later analysis. There were no differences between muscle responses recorded from one muscle to stimuli presented from the ipsilateral and contralateral sides of the body and the responses obtained from bilateral muscle recordings to stimuli presented from only one side (R. Wyttensbach, personal communication). At the end of the recording session, we verified the position of the electrode in M126 by passing current through the recording electrode to make a small burn mark in the muscle.

For the antennal muscles, a similar technique was used. 50 μm stainless-steel electrodes were implanted into M6, the medial scapo-pedicellar muscle ($N=5$), or M7, the lateral scapo-pedicellar muscle ($N=5$). The electrode was inserted through a small hole punched in the scape and held in place with cyanoacrylate glue (Krazy Glue; B. Jadow and Sons Inc.). The ground wire was inserted into the dorsal metathorax and held in place with a drop of wax. Recording methods were as described for the leg muscle. Honegger *et al.* (1990) have shown that, in *Gryllus bimaculatus* and *Gryllus campestris*, M6 is innervated by three motoneurons located within the brain and M7 by four. We expect that these muscles in *T. oceanicus* are also multiply innervated.

For both the leg and antennal muscles, the latency to the first muscle spike was determined relative to stimulus onset. The number of spikes discharged in response to a given stimulus intensity was counted over the first 100 ms following stimulus onset, except for the ipsilateral leg muscle, which usually responded with a latency greater than 100 ms. The spike output was counted over the entire period that the ipsilateral leg responded. It was not possible to distinguish between potentially different motor units in a muscle. Muscle responses were, therefore, considered in terms of the total spike activity of the muscle. The duration of electromyographic discharge was also measured for the leg muscle. All data are presented as the mean $\pm 95\%$ confidence intervals (CI) on the mean.

Results

Kinematic measurements

When stimulated with ultrasound, the tethered crickets quickly moved the metathoracic leg contralateral to the stimulus up and away from the abdomen (Fig. 1). We did not detect any movement of the leg ipsilateral to the sound stimulus. Both the abduction and elevation angles of the contralateral leg increased with increasing sound intensity (Fig. 2). The temporal aspects of the contralateral leg's movement also changed with stimulus intensity. The latency to the onset of the leg swing decreased with increasing stimulus intensity (Fig. 3A), with a minimum latency measured in one individual of 29 ms. The duration of the leg swing outlasted the stimulus at all intensities and increased with increasing stimulus intensity (Fig. 3B). Thus, a cricket moved its metathoracic leg more quickly and farther and held it out for a longer period with increasing intensity of ultrasound.

Lateralization of the antennal movements was not as obvious as that of the metathoracic leg. Both antennae swung laterally when ultrasound was presented to one side of the animal, but there were differences in the degree of movement of the two antennae (Fig. 4). The antenna contralateral to the sound consistently moved farther than the ipsilateral antenna (Figs 4, 5). In addition, the distance

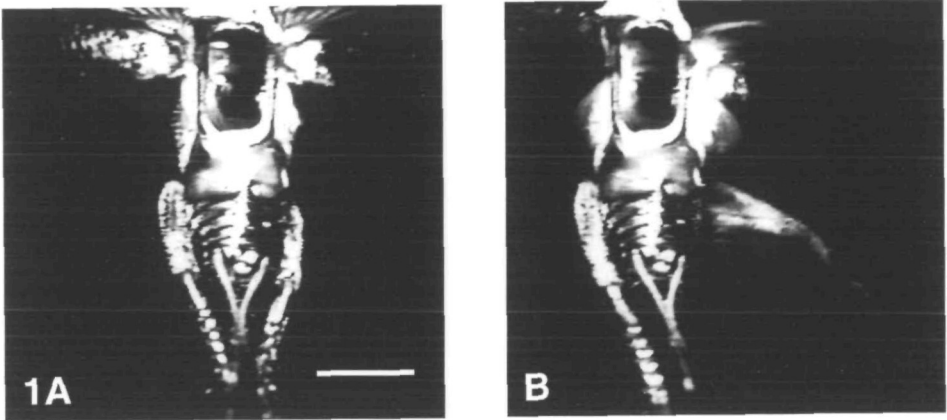


Fig. 1. The metathoracic leg's swing. As viewed from behind the flying cricket, these photographs were taken from the video screen of our recording equipment. (A) Straight flight in a tethered flying cricket, hindwings removed. (B) The right metathoracic leg swings out following a 92 dB ultrasonic stimulus from the left. Scale bar, 0.5 cm.

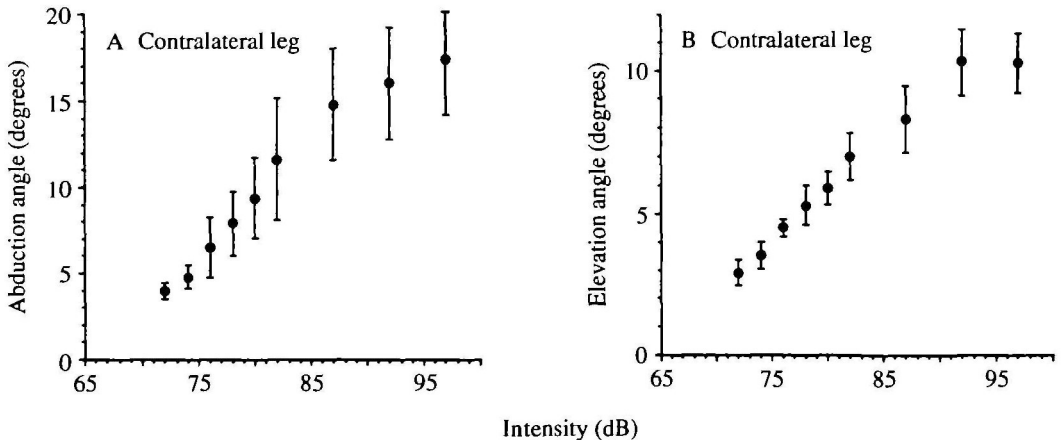


Fig. 2. Effect of stimulus intensity on the magnitude of the contralateral metathoracic leg's swing. All values shown are the mean \pm 95% confidence intervals (CI) on the mean, $N=10$ animals, two tests per animal. (A) Abduction angle of the leg as a function of intensity. These are positively correlated (Kendall's coefficient of rank correlation=1; $P<0.01$). (B) Elevation angle as a function of intensity. Elevation and intensity are positively correlated (Kendall's coefficient of rank correlation=0.94; $P<0.01$).

moved by the contralateral antenna increased with stimulus intensity, while the ipsilateral antenna showed no significant changes in the distance it moved as stimulus intensity increased (Fig. 5).

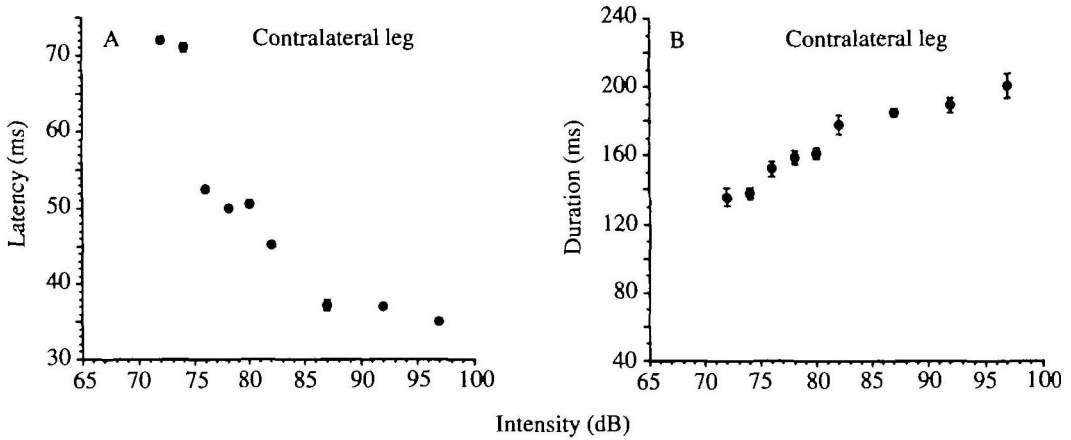


Fig. 3. Effect of stimulus intensity on the temporal properties of the leg swing. Mean \pm 95% CI on the mean, $N=10$ animals, two tests per animal. (A) Latency to the onset of detectable movement as a function of intensity. These are negatively correlated (Kendall's coefficient of rank correlation = -0.94 ; $P < 0.01$). (B) Duration of the leg swing as a function of intensity. Duration is positively correlated with stimulus intensity (Kendall's coefficient of rank correlation = 1.0 ; $P < 0.01$).

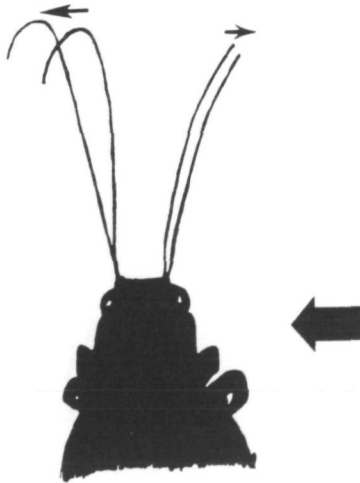


Fig. 4. Antennal movements elicited by a 100-ms pulse of ultrasound. Superimposed drawings from the video screen before and after an 85 dB ultrasound stimulus was presented from the side indicated by the large arrow. Antennal movements are indicated by the small arrows.

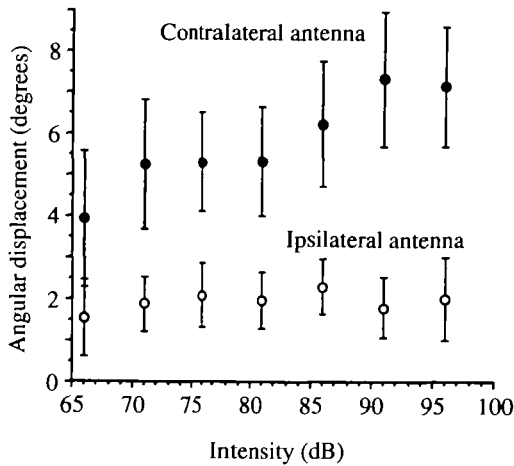


Fig. 5. Angular displacement of the antennae as a function of ultrasound intensity. Mean \pm 95% CI on the mean, $N=10$ animals. Open circles, ipsilateral antenna; filled circles, contralateral antenna. Antennal movement in response to contralateral stimuli was positively correlated with stimulus intensity (Kendall's coefficient of rank correlation=0.9; $P<0.01$). The response of the antenna ipsilateral to the sound was not significantly correlated with stimulus intensity (Kendall's coefficient of rank correlation=0.33; $P>0.1$).

Electromyographic analysis

Metathoracic leg

The leg abductor, M126, was normally silent during tethered flight. When the cricket was stimulated with directional ultrasound, the contralateral M126 fired a short-latency burst of spikes (Fig. 6A). Following this burst, the muscle frequently displayed a lower firing frequency that outlasted the stimulus and appeared to be phase-locked with the flight motor pattern. This can be seen in Fig. 6, where a number of the low-amplitude spikes from units rhythmically active in the flight rhythm are marked with asterisks. We believe that these units are the metathoracic flight muscles, since the recording electrode is in close proximity to these large muscles. The number of spikes fired by the contralateral M126 was graded with stimulus intensity (Fig. 7A).

Ipsilateral to the sound, 6 of the 10 animals tested showed activity in muscle 126, but with substantially longer latencies than for contralateral stimuli (Figs 6B, 8A). We could not detect any movement of the ipsilateral leg when this muscle activity occurred. When the muscle showed a response to ipsilateral stimuli, the first muscle spike did not occur until the last few milliseconds of the stimulus, or even after the stimulus had ended. Although the response could continue for up to about 350 ms, its spike frequency was lower than that for a contralateral stimulus and appeared to be phase-locked to the flight rhythm (Fig. 6B). For ipsilateral stimuli, the spike output of the muscles that responded was also graded with stimulus intensity (Fig. 7B).

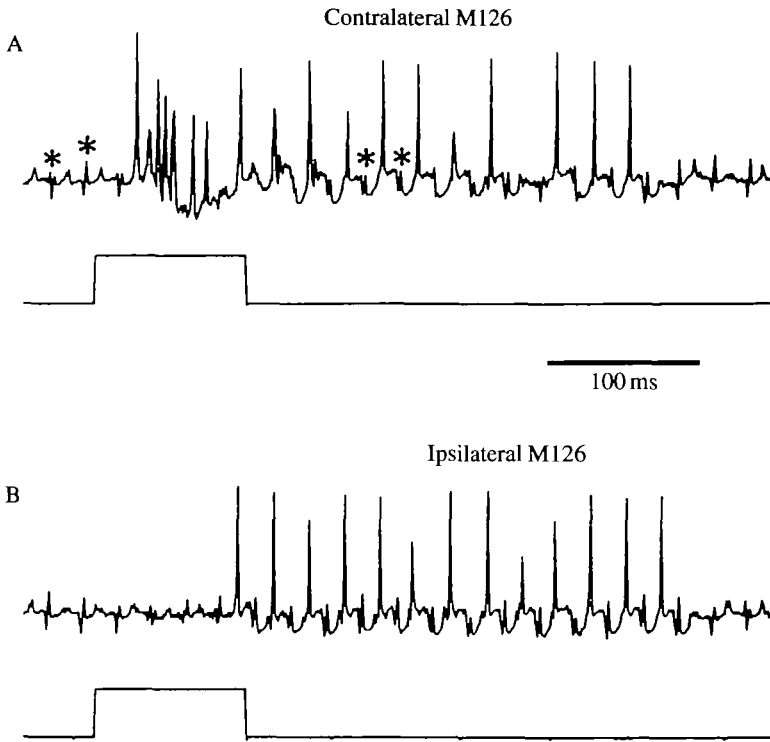


Fig. 6. Response of muscle 126 to ultrasound. The lower trace in each pair shows the timing of the sound stimulus. (A) Stimulus from the contralateral side. Asterisks mark low-amplitude spikes picked up by the recording electrode, which we believe are from metathoracic flight muscles, as discussed in the text. (B) Stimulus from the ipsilateral side. This type of response to ipsilateral stimulation was seen in 6 of 10 preparations. The remainder showed no response to sound stimuli presented to the ipsilateral side. The activity of the muscle is phase-locked to the flight rhythm, as indicated by the small-amplitude spikes, which we believe are from the flight muscles.

Some of the temporal aspects of the responses of M126 to ultrasound were also graded with stimulus intensity. For contralateral stimuli, the latency to the first muscle spike declined significantly with increasing stimulus intensity (Fig. 8A). The minimum latency recorded in an individual for activity in M126 (contralateral to the stimulus) was 20 ms. The duration of muscle activity always outlasted the contralateral stimulus and was positively correlated with stimulus intensity (Fig. 8B). As discussed above, the latencies for responses to ipsilateral ultrasound were substantially greater than for contralateral stimuli (Fig. 8A). In addition, although the responses to ipsilateral stimuli appeared to decline with increases in stimulus intensity, the trend was not significant. Similarly, the duration of the ipsilateral response did not change significantly with increasing stimulus intensity (data not shown).

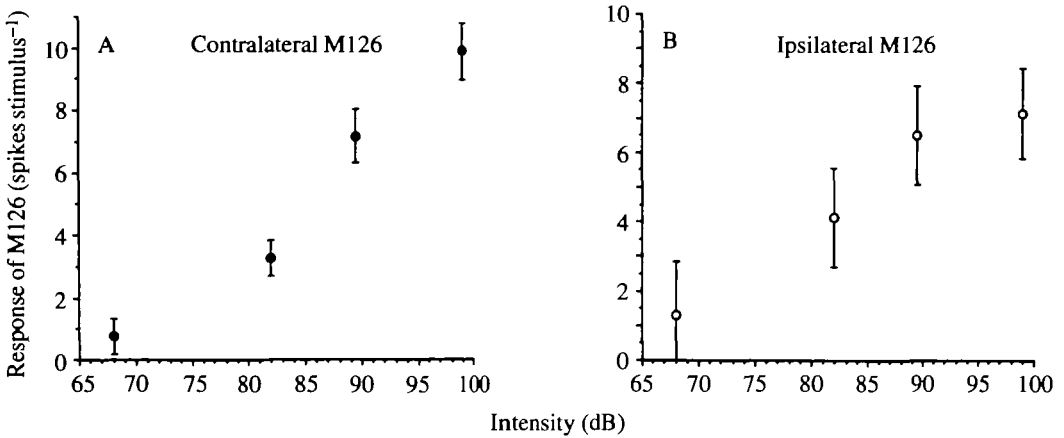


Fig. 7. (A) Response of muscle 126 to contralateral sound stimuli; the number of spikes fired in the first 100 ms following the onset of the stimulus as a function of intensity. Mean \pm 95% CI on the mean, $N=10$ animals. The number of spikes fired by the muscle is positively correlated with stimulus intensity (correlation coefficient $r=0.98$; $P<0.05$). (B) The number of spikes fired in response to ipsilateral ultrasound. Shown here are the means \pm 95% CI on the mean for the full duration of the response, which usually did not begin until 100 ms after the onset of the stimulus and lasted up to 350 ms. $N=6$ animals. The muscle response is positively correlated with stimulus intensity (correlation coefficient $r=0.98$; $P<0.05$).

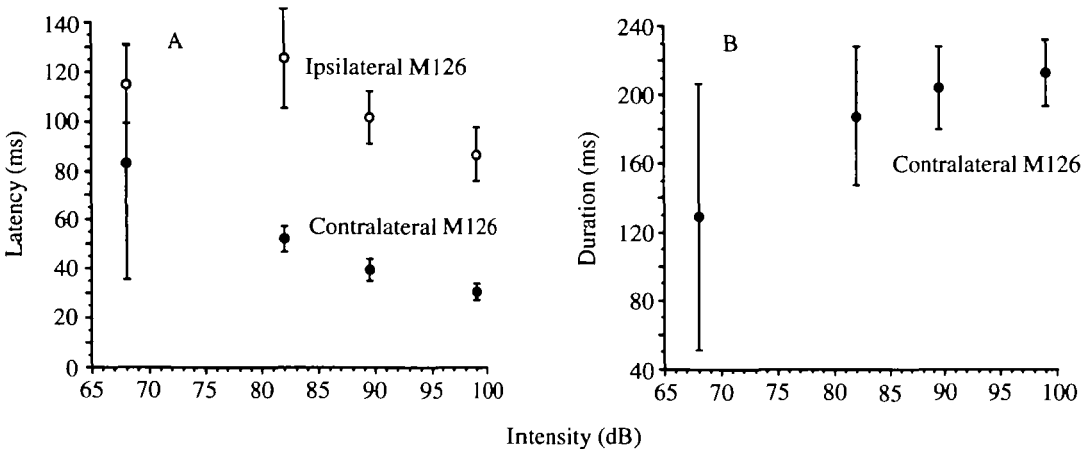


Fig. 8. (A) Latency to the first spike in muscle 126 as a function of sound intensity. Mean \pm 95% CI. For contralateral stimuli (filled circles), latency is negatively correlated with stimulus intensity ($N=10$ animals; correlation coefficient $r=0.98$; $P<0.05$). For ipsilateral stimuli (open circles), latency is not significantly correlated with intensity ($N=6$ animals; correlation coefficient $r=0.76$; $P>0.1$). (B) Duration of muscle 126 response to contralateral stimuli as a function of sound intensity. Mean \pm 95% CI on the mean, $N=10$ animals. These are positively correlated (correlation coefficient $r=0.96$; $P<0.05$).

Antennal muscles

The muscles M7 and M6 move the antenna in opposite directions in the horizontal plane (Honegger *et al.* 1990). M7 moves the antenna laterally, while M6 moves it medially. M7 was normally silent during tethered flight but, upon presentation of an ultrasound stimulus, it fired a burst of spikes. For both ipsilateral and contralateral ultrasound, the mean number of spikes fired by M7 increased significantly with increasing stimulus intensity (Fig. 9). M7 consistently fired more spikes per stimulus for contralateral stimuli than for ipsilateral stimuli at all stimulus intensities. However, because the 95 % confidence intervals on the mean values at each intensity overlap, we do not consider the differences significant (Fig. 9). During normal tethered flight, M6 showed occasional spontaneous spikes. When stimulated with ultrasound, M6 fired a burst of spikes. However, unlike M7, M6 spike activity was not correlated with stimulus intensity (Fig. 10).

We measured latency to the first spike in M6 and M7. Unlike the leg muscle M126, response latencies for the antennal muscles were not dependent on stimulus intensity. For contralateral stimuli, a plot of latency as a function of intensity had a correlation coefficient r of 0.8 ($P>0.05$) for M7 and 0.86 ($P>0.05$) for M6. For ipsilateral stimuli, a latency–intensity plot showed r to be 0.51 ($P>0.05$) for M7 and 0.29 ($P>0.05$) for M6. We therefore compared mean latencies for the two antennal muscles for all animals calculated over all intensities. These are shown in Table 1. Comparisons of these values revealed that M7 responded to contralateral stimuli with a significantly shorter latency than it did to ipsilateral stimuli. The

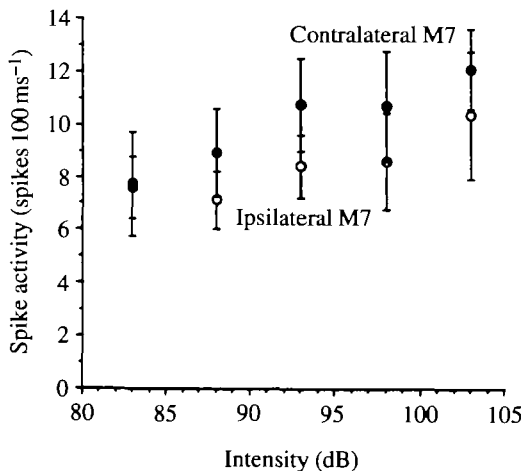


Fig. 9. Spike activity of M7 as a function of stimulus intensity. Mean \pm 95 % CI on the mean, $N=5$ animals. For both contralateral stimuli (filled circles) and ipsilateral stimuli (open circles) these are positively correlated (correlation coefficient $r=0.97$; $P<0.01$ for contralateral; correlation coefficient $r=0.89$; $P<0.05$ for ipsilateral). The mean numbers of spikes per stimulus are not considered significantly different because of the substantial overlap of the 95 % CI on the mean.

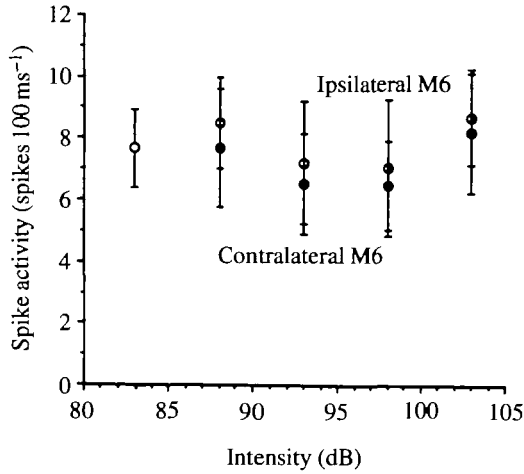


Fig. 10. Spike activity of M6 as a function of stimulus intensity. Mean \pm 95 % CI on the mean, $N=5$ animals. We did not consider these plots to show a significant relationship between intensity and spike number. Responses to contralateral stimuli (filled circles) have a correlation coefficient $r=0.23$; $P>0.1$; responses to ipsilateral stimuli (open circles) have a correlation coefficient $r=0.14$; $P>0.1$.

Table 1. Latencies of antennal muscle spike activity

	Latency (ms)	
	M6	M7
Ipsilateral stimulus (N)	53.1 ± 3.4 (73)	40.3 ± 2.7 (66)
Contralateral stimulus (N)	45.5 ± 3.3 (71)	34.5 ± 2.3 (59)

Values shown are mean \pm 95 % confidence intervals on the mean.

Five animals were used for M6 recordings; five animals were used for M7 recordings. The N value given is the total number of latencies measured at all intensities for the five animals in each set.

Mean values are considered significantly different where the 95 % confidence intervals do not overlap.

latency to activity in M6 was significantly longer than for M7 for both ipsilateral and contralateral stimuli. However, as was the case for M7, M6 responded to contralateral stimuli with significantly shorter latencies than it did to ipsilateral stimuli.

Discussion

Our kinematic studies reveal that for both the antenna and the metathoracic leg,

the response to ultrasound was strongest on the side of the body contralateral to the sound stimulus. In addition, the degree of movement of the leg or antenna was graded with the intensity of the sound. Similar results were obtained in earlier work for pitch, roll, yaw and forewing tilt (May *et al.* 1988; May and Hoy, 1990*b*). These observations are especially interesting since one of the characteristics of the mammalian acoustic startle response is its graded nature (Davis, 1984). Thus, the graded kinematic responses of the cricket's reaction to ultrasound can be added to a growing list of similarities between the acoustic startle of mammals and that of the cricket (for further discussion see Hoy, 1989; May and Hoy, 1991).

Other insects, most notably green lacewings (Miller, 1975) and a number of moths (Roeder, 1967), are well known for their responses to ultrasound. In moths, two distinct types of behavior can be elicited, depending on the intensity of the ultrasound stimulus. Low intensities cause the moth to steer away from the source of ultrasound, while high intensities result in an erratic flight path consisting of nondirectional nose-dives or a cessation of flight, producing a drop in height. Crickets also show these types of separate responses to low- and high-intensity ultrasound stimuli. Our study was concerned only with the behavior induced by the relatively low-intensity stimuli, a turn away from the source of ultrasound. While we have found that in the cricket this behavior is graded with the intensity of the ultrasound, this has not been systematically examined in moths. Like moths, however, crickets will also display erratic movements at very high intensities of ultrasound. Further comparisons of the responses of moths, lacewings and crickets and of the value of these strategies to the insects have been discussed in earlier work (Nolen and Hoy, 1986).

Our electromyogram data from the contralateral metathoracic leg muscle M126 and antennal muscle M7 correlated well with what would be expected from the kinematic data, showing increased spike activity with increasing stimulus intensity. Other aspects of the electromyographic data were generally in agreement with the kinematics, with a few exceptions. These will be discussed for the leg and antenna separately below.

Response of the metathoracic leg

The kinematics of the leg swing showed that this component of the cricket's acoustic startle response was strongly lateralized, with only the contralateral leg swinging out and away from the abdomen. In addition, the magnitudes of both the abduction and elevation angles of the leg, as well as the total duration of the leg swing, increased with increasing sound intensity. These results fit well with earlier work showing that the yaw rotation produced by the leg swing is graded with stimulus intensity (May and Hoy, 1990*a*). We also found that the latency to the onset of the leg's movement decreased with increasing sound intensity.

The responses of the contralateral M126 correlate well with these kinematic data. As already mentioned, the number of spikes we recorded in the muscle of the contralateral leg in response to the stimulus, like the magnitude of the leg's

movement, increased with the intensity of the stimulus. Likewise, the duration of the response in the muscle, which at each intensity showed similar mean values to the duration of the leg's movement, showed similar increases with increasing stimulus intensity. Finally, the latency to the first spike in the muscle and the latency to the onset of the leg's movement also showed similar decreases with increasing stimulus intensity.

One aspect of the response of M126 that was not expected from the kinematic data was its bilateral nature in 6 of the 10 animals tested, which showed responses to ipsilateral as well as contralateral stimuli. The number of spikes fired by a muscle with an ipsilateral response was graded with stimulus intensity, but the latency was substantially greater than the latency for contralateral stimuli and showed no significant changes with increases in stimulus intensity. We did not detect any movement of the ipsilateral leg accompanying this response. We believe it may be a secondary response, brought about by the actions of other muscles involved in negative phonotaxis. For example, many of the flight muscles in the cricket are bifunctional, serving also to move the legs. The hindwing elevators 118, 119 and 120 also have actions on the coxa (Furukawa *et al.* 1983). It has been shown that hindwing elevation increases on the side of the animal ipsilateral to the ultrasound stimulus, and this increase is graded with stimulus intensity (May *et al.* 1988). Perhaps in some individuals, ultrasound-induced increases in the contractions of the elevator muscles also pull the leg towards the abdomen, and muscle 126 serves to reposition it. Moiseff *et al.* (1978) clearly showed the ipsilateral leg being pulled into the turn during negative phonotaxis, but we could not confirm this in our kinematic study. This discrepancy may occur because Moiseff *et al.* (1978) tethered their crickets on the pronotum and flew them upside down, while we tethered them near the intersection of the thorax and abdomen and flew them upright. Although our tether system better models true flight, it can reduce the amplitudes of some movements, such as abdominal ruddering (May and Hoy, 1990*b*). The same may be true for movements of the ipsilateral leg.

Our electromyograms recorded from M126 indicate that its motoneuron may receive input from the flight motor, as has been shown for other non-flight muscles (Camhi and Hinkle, 1972), and/or from sensory inputs gated by the flight oscillator (Rowell, 1988). Following its initial burst of spikes, the response of the contralateral M126 usually slowed to fire in a pattern that appeared to be phase-locked to the flight rhythm, similar to all of the responses of the ipsilateral muscles 126 (Fig. 6). However, the initial burst of activity in the contralateral muscle 126 appeared to be independent of the flight rhythm. This indicates that contralateral stimuli initially provide sufficient input to the motoneuron to induce firing without the aid of the flight oscillator. Because this component of the cricket's startle response contributes directly to the animal's escape, it must be fast and it is, therefore, important that it should not be constrained to the timing of the flight rhythm.

In previous work, we found that the primary effect of the ultrasound-induced leg swing was to decrease the latency of yaw rotation (May and Hoy, 1990*a*). This

effect would require two temporal relationships. First, the leg's swing must precede the onset of yaw. This requirement is clearly met; for example, a 92 dB stimulus elicited a leg swing with a 37 ms latency, while the yaw response for this stimulus began at 108 ms (May and Hoy, 1990a), 70 ms later. This latency difference is not unusually long, given that with a typical wingbeat frequency of 32 Hz (May *et al.* 1988) 70 ms allows time for fewer than three wingbeats. Furthermore, some delay is likely because of the need to overcome inertial forces before the animal actually turns. The second requirement for the leg swing is that its duration be equal to, or longer than, the wingbeat period to ensure an interaction between leg and hindwing. For a 92dB stimulus, the duration of the swing is 190 ms, sufficient to impede the hindwing for at least five strokes.

We can gain some information about the extent of the neural circuitry underlying the leg swing from the temporal aspects of the activity of muscle 126. The circuitry of negative phonotaxis includes the receptor afferents of the tympanal organ, which provide input to a single second-order interneuron, Int-1. Int-1 ascends to the brain, from which the signal then descends to drive the behavioral response (Moiseff and Hoy, 1983). Brodfuehrer and Hoy (1989) showed that the first spike in Int-1 occurred about 12 ms after stimulus onset, and the first descending activity could be recorded extracellularly in the cervical connectives below the subesophageal ganglion at about 20 ms following stimulus onset. We found that the shortest latencies for M126, in the metathorax, were also about 20 ms, indicating that the connections between the descending neurons and motoneuron 126 must be fairly direct.

Responses of the antennae

Unlike the metathoracic leg swing, we do not know whether the ultrasound-induced antennal movements have a direct effect on steering in flight. In flying locusts, Arbas (1986) showed that movement of the antennae could induce movements of the metathoracic legs, although the delays he measured to the onset of leg movement were substantially longer than ours (averaging 145 ms). The antennae in locusts have also been shown to affect flight speed, wingbeat frequency and wing-stroke angle (Gewecke, 1975). However, it is not known whether the antennae serve similar roles in flying crickets. We do know that the antennal movements probably do not induce the leg swing of the cricket's startle response, since preventing antennal movements by waxing their proximal segments did not appear to affect the leg swing (C. I. Miles, personal observation). In addition, at higher stimulus intensities, the latencies we measured for the antennal muscles were not significantly different from the latencies for the leg muscles. If antennal movements induced leg movement, these would probably be detected by the antennal sensory neurons, and descending neural pathways would carry this information to motoneurons 126, which are located in the metathoracic ganglion. One would, therefore, expect a significant delay between the movement of the antenna and activity in the leg muscles, as reported by Arbas (1986). We did not

observe such delays. This does not, however, rule out a role in negative phonotaxis for antennae; they may well be involved in the directional steering that follows a turn initiated by the metathoracic leg swing.

The antennal movements were bilateral, although responses to contralateral stimuli were stronger than those to ipsilateral stimuli (Fig. 4). Antennal movements for contralateral stimuli were graded with stimulus intensity; those for ipsilateral stimuli were not. As might be predicted from this kinematic data, the contralateral antennal muscle M7, which moves the antenna laterally (Honegger *et al.* 1990), showed increasing spike activity with increases in stimulus intensity. Also consistent with the kinematic data, the muscle contralateral to the stimulus consistently fired more spikes per stimulus than the ipsilateral antennal muscle at all stimulus intensities, although the mean values were not significantly different. The M7 contralateral to the stimulus also fired with a significantly shorter latency than the ipsilateral muscle, although latency was not graded with stimulus intensity.

Unlike M7, ultrasound-induced activity in M6 did not change significantly with increasing stimulus intensity. This, coupled with the significantly longer latency of M6, would lead one to predict that, for the contralateral antenna, intensity-induced increases in M7 activity should produce a larger antennal swing, consistent with the kinematic data.

Much of the kinematic data from the ipsilateral antenna can also be explained by our electromyographic results. When stimulated with ultrasound from the ipsilateral side, the antenna moved laterally, but this response was not graded with stimulus intensity. Because the latencies for M7 activity were significantly shorter than those for M6, one would predict that the antenna would show some lateral movement. Further, while spike activity in the ipsilateral M6 did not increase with stimulus intensity, that in the ipsilateral M7 did. These data would predict that the antenna should move farther laterally with increases in stimulus intensity; this was not observed. However, it should be noted that the mean number of spikes fired per stimulus increased by only 2.7 when stimulus intensity increased from 83 to 103 dB. While we do not know the relationship between the number of spikes fired by the muscle and the distance that the antenna would move, it seems possible that so small a change in muscle activity might not produce a change in antennal angle large enough for us to observe. It is also possible that lateralized activities of the other antennal muscles, the scapal levators and depressors (Honegger *et al.* 1990), contribute to the reduced movement of the ipsilateral antenna.

While we know that the leg directly affects the cricket's flight path and elicits a short-latency turn away from the source of the ultrasound, it is not clear what role the antennal swing may play. If it has any effect on the cricket's flight path, it would probably be less direct than that of the metathoracic leg. This may be reflected in the less clearly graded responses the antennae show with changing stimulus intensities. In contrast, the movement of the contralateral metathoracic leg occurs with a very short latency and in a manner that is precisely graded with stimulus intensity. In this way, the cricket can respond to the aversive stimulus in a

manner appropriate to the degree of danger it may indicate, such as the proximity of a hunting bat.

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