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INTEGRATION OF ULTRASOUND AND FLIGHT INPUTS ON DESCENDING NEURONS IN THE CRICKET BRAIN

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Summary

In response to ultrasonic stimuli, tethered flying crickets perform evasive steering movements that are directed away from the sound source (negative phonotaxis). In this study we have investigated the responsiveness to ultrasound of neurons that descend from the cricket brain, and whether flight activity facilitates the responsiveness of these neurons.

1. Ultrasonic stimuli evoke descending activity in the cervical connectives both ipsilateral and contralateral to the sound source.

2. Both the amount of descending activity and the latency of this response in the cervical connectives are linearly correlated with ultrasonic stimulus intensity, regardless of the cricket's behavioral state.

3. Flight activity significantly increases the amount of descending activity evoked by ultrasound at all stimulus intensities, and significantly decreases the latency of the response in the cervical connectives compared with non-flying crickets. Flight activity, however, does not significantly affect the activity in an interneuron (Int-1) carrying ultrasound input to the brain. Thus, the increase in the amount of descending activity produced during flight activity is due to the integration of input from Int-1 and the flight motor system to ultrasound-sensitive neurons in the cricket brain.

4. Descending units recorded in the cervical connectives originate in the cricket brain. A reduction in the amount of descending activity is correlated with a decrease in the magnitude of the negative phonotactic response of the abdomen during flight activity, suggesting that these descending units play a role in eliciting negative phonotaxis.

Introduction

The behavior of animals often depends on the context in which a given stimulus is presented (Ritzmann *et al.* 1980; Hoy & Nolen, 1987). For example, the Australian field cricket, *Teleogryllus oceanicus*, exhibits negative phonotaxis in response to ultrasound only when flying (Nolen & Hoy, 1984). Negative phonotaxis consists of evasive steering movements directed away from the sound source (Moiseff *et al.* 1978; May *et al.* 1988).

Key words: cricket, ultrasound, negative phonotaxis, descending interneurons.

Ultrasound reception in the central nervous system of *T. oceanicus* is mediated primarily by a pair of interneurons, Int-1 (Casaday & Hoy, 1977; Moiseff & Hoy, 1983). The soma and auditory input region of Int-1 are located in the prothoracic ganglion, while the axon projects anteriorly into the brain, where it terminates (Moiseff & Hoy, 1983). Activation of Int-1 has been shown to be both necessary and sufficient for eliciting negative phonotactic behavior in flying crickets (Nolen & Hoy, 1984). Moreover, Int-1's connections in the brain appear to be required for eliciting negative phonotaxis in response to ultrasound (Moiseff *et al.* 1978; Pollack & Hoy, 1981). The motor output underlying negative phonotactic steering movements is produced by neurons located predominantly in the thoracic and abdominal segments (Moiseff *et al.* 1978; Pollack & Hoy, 1981; Hoy & Nolen, 1987). Thus, the neuronal pathway controlling negative phonotactic behavior originates in the prothoracic ganglion, extends to the brain, and descends to the thoracic and abdominal segments. Converging with this pathway is input from the flight motor system, since ultrasound only elicits negative phonotaxis in flying crickets. The nature of the descending input from neurons in the cricket brain and the site in the cricket central nervous system where activity from the flight motor system converges with ultrasound-induced, ascending activity is presently unknown.

In this paper we examine the nature of descending activity elicited by ultrasound and determine whether flight activity and ascending activity induced by ultrasound converge in the cricket brain.

Materials and methods

All experiments were performed on adult, female crickets, *Teleogryllus oceanicus,* 2–6 weeks after the adult molt. These animals were originally collected in Hawaii, and have been raised for approximately 3 years in our laboratory.

Initiation of sustained flight activity in dissected preparations

Two methods were employed to improve the reliability of eliciting sustained flight activity in response to wind puffs directed at the abdominal cerci in dissected preparations (see below for a description of the preparation). First, several days prior to use a large container of crickets was put on a reversed light cycle (10 h light: 14 h dark) and all experiments were performed during the subjective night (May *et al.* 1988). Second, 10^{-6} or 10^{-7} mol l^{-1} 3-isobutyl-1-methylxanthine (IBMX) was bath-applied to the exposed nervous system. IBMX dramatically increased the likelihood of eliciting flight in response to wind (G. S. Boyan, personal communication; P. D. Brodfuehrer & R. R. Hoy, personal observation). Within 3–5 min of bath application of IBMX to the exposed nervous system spontaneous flight activity often occurred. One disadvantage of IBMX was that it also reduced the sensitivity of Int-1 to ultrasonic stimuli. However, in many preparations the decrease in Int-1 sensitivity was almost completely reversed in approximately 10–20 min following replacement of IBMX with normal saline, ye.

the ability to elicit flight in response to wind remained elevated (P. D. Brodfuehrer & R. R. Hoy, personal observation). Thus all experiments reported here were performed at least 10 min following the removal of IBMX, and only in crickets which showed sustained flight activity in response to wind puffs. The results reported in this paper are only from crickets treated with IBMX, although similar results were also observed in non-treated crickets.

In this paper, the terms 'flight' and 'flying' are used to describe preparations in which rhythmic activity could be recorded in a flight muscle.

Physiological recording procedures

Extracellular recordings from the ipsilateral and/or contralateral cervical and circumesophageal connectives were made using suction electrodes, recording either from the cut end of the connective or *en passant* from the intact connective (Fig. 1A). In this paper, the terms ipsilateral and contralateral are used with respect to the location of the sound source and Int-1's ascending axon. To record extracellularly from the connectives in flying crickets, the animal was attached ventral side up by its head and pronotum onto a small wax platform, and the

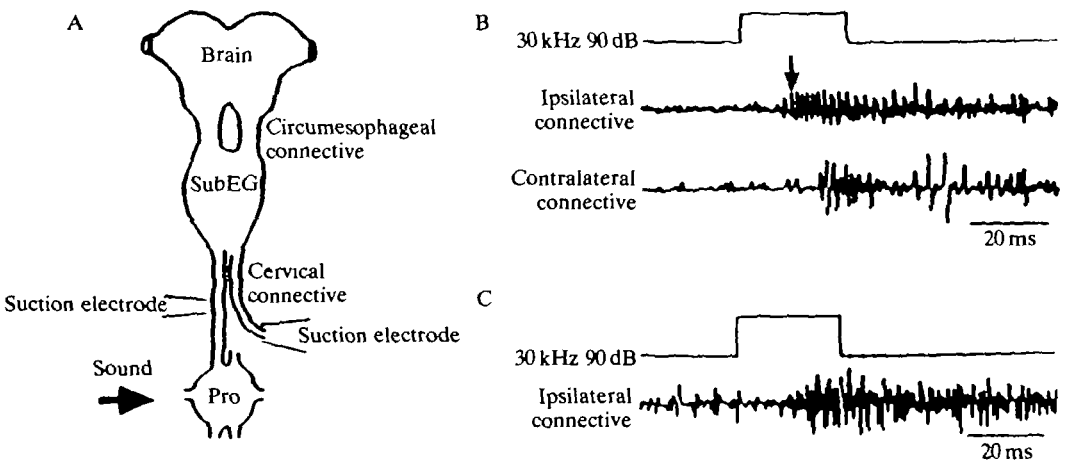

Fig. 1. Relationship between ascending and descending activity in the cervical connectives. (A) Diagram of the cricket anterior nervous system. SubEG, subesophageal ganglion; Pro, prothoracic ganglion. (B) Ultrasound elicits activity in both the ipsilateral and the contralateral cervical connective. Activity in the ipsilateral connective occurs prior to activity in the contralateral connective, but lasts for the same time in both connectives. The first Int-1 impulse is indicated by the downward arrow. Top trace, timing of the ultrasonic stimulus. Middle trace, *en passant* extracellular recording from the cervical connective ipsilateral to the stimulus. Bottom trace, extracellular recording from the cut end of the contralateral cervical connective. (C) Ultrasound elicits descending activity in the lateral half of the ipsilateral cervical connective. The ipsilateral connective was split approximately in half, leaving the medial half of the connective intact. Top trace, timing of the ultrasonic stimulus. Bottom trace, extracellular recording from the cut end of the lateral half of the ipsilateral connective. B and C are from different preparations.

prothoracic legs were waxed in approximately the natural flight posture. Both the mesothoracic and the metathoracic pairs of legs and wings were removed. With the cricket waxed in this position, the metathoracic wing stubs were free to move during flight. A large wax well extending from the pronotum around the head was built. The mouth parts and the jaws, and the soft cuticle covering the cervical connectives, were removed to expose the cervical connectives, subesophageal ganglion and circumesophageal connectives. In most preparations, the gut was also removed by cutting it just posterior to the subesophageal ganglion and pulling it out of the cricket through a slit in the abdomen. EMG recordings from a metathoracic wing depressor muscle (muscle 129a; Furukawa *et al.* 1983) were made using 50 μm diameter, copper wire insulated except at the tip. All electrical activity was recorded on a Vetter FM tape recorder and analyzed later. Permanent physiological records were made by photographing the oscilloscope screen.

To make quantitative comparisons between the amount of descending activity evoked in the cervical connectives by ultrasonic stimuli (50 ms in duration) during flight and non-flight (at rest), the descending electrical activity was first full-wave rectified and then integrated. The time constant of the integrator circuit was approximately 100 ms. The area under the integrated waveform was calculated using the peak area program of a Norland Prowler processing digital oscilloscope. In this program, the beginning and end of the integrated waveform were set manually. In five dissected preparations, the peak area was measured for four trials under flight and non-flight conditions at each stimulus intensity. The average peak area (±S.E.M.) for all trials during flight and non-flight was calculated and compared statistically using a paired *t*-test on all the data from each preparation. In addition, the latency from the start of the ultrasonic stimulus to the onset of the descending activity recorded in the cervical connectives was measured directly from the integrated waveform on the Norland oscilloscope. The average latency (±S.E.M.) was calculated and compared statistically (paired *t*-test) for flight and non-flight.

The average firing frequency of Int-1 in response to an ultrasonic stimulus in flying and non-flying preparations was used to determine if flight activity affected the response of Int-1 to ultrasonic stimuli. This parameter was chosen because Nolen & Hoy (1984) demonstrated that an average Int-1 firing frequency of greater than 220 Hz was necessary for Int-1 to elicit negative phonotactic responses to ultrasound. The average firing frequency of Int-1 was determined by counting, from stored traces on the oscilloscope screen, the number of Int-1 spikes elicited in the 50 ms following an ultrasonic sound pulse. In five dissected preparations, the average firing frequency of Int-1 in response to ultrasound was determined for five trials, at each stimulus intensity, during flight and non-flight. The average firing frequency of Int-1 (±S.E.M.) was calculated from all the trials and compared statistically (paired *t*-test).

Negative phonotactic behavioral assay

One reliable indication that ultrasound has elicited a negative phonotactic

response is an abdominal swing directed away from the sound source (Moiseff *et al.* 1978). We used this steering response as an indication that negative phonotaxis was occurring (Moiseff *et al.* 1978; Nolen & Hoy, 1984). Abdominal movement was monitored both visually and by a photocell. The photocell was mounted above the abdomen and a fiber-optic light guide was positioned below the abdomen of the dissected cricket, giving a qualitative indication of whether ultrasound elicited negative phonotaxis.

Auditory stimulus presentation

Acoustic stimuli used in this study were generated electronically. The carrier frequency of the sound pulses was generated with a B & K precision function generator, shaped by a custom-built trapezoid shaper (symmetrical sound pulse with rise and fall times of approximately 5 ms), amplified by a Nikko amplifier, and delivered through a pair of Motorola piezoelectric tweeters. The speakers were placed approximately level with the cricket and 90° to the left and right of its longitudinal body axis. Peak sound pressure levels (SPLs) are expressed in decibels (dB) relative to 20 μPa. All harmonics were at least 35 dB less than the fundamental carrier frequency, as measured by a Nicolet miniubiquitous spectrum analyzer. In these experiments, only 5 and 30 kHz sound pulses were employed.

Results

Relationship between ascending and descending activity

In non-flying crickets, a broad range of sound frequencies can activate descending units in the cervical connectives (Zhantiyev & Korsunovskaya, 1977; Brodfuehrer & Hoy, 1988). In Fig. 1B, the relationship between ascending Int-1 activity evoked by ultrasound (30 kHz) and the corresponding descending activity is demonstrated. The first Int-1 spike (indicated by a downward arrow) occurred approximately 12 ms following the onset of a 30 kHz (90 dB) sound pulse, while a barrage of descending activity in the contralateral connective began approximately 7 ms after the first Int-1 impulse. Moreover, ultrasound also caused an increase in activity in both the ipsilateral and contralateral cervical connectives which lasted over twice as long as the duration of the ultrasonic stimulus (Fig. 1B). In the contralateral connective, this activity represents descending units from the cricket brain and subesophageal ganglion, since we were recording extracellularly from the cervical connective posterior to the subesophageal ganglion. To determine if part of the increased activity evoked in the ipsilateral connective by ultrasound also came from descending units, we split the ipsilateral cervical connective with a sharpened tungsten wire, and recorded extracellularly from the cut end of the lateral half of the connective. The medial half of the cervical connective was left intact because Int-1's axon extends to the brain in the medial portion of the connective (Moiseff & Hoy, 1983). Ultrasound activated descending units in the ipsilateral lateral half of the cervical connective approximately 20 ms following the stimulus onset, and the activity greatly outlasted the duration of the stimulus

(Fig. 1C). Thus, descending units activated by ultrasound project posteriorly in the cervical connectives both ipsilateral and contralateral to the sound source. Since negative phonotactic responses can occur with a latency of approximately 25–35 ms (Nolen & Hoy, 1986; May *et al.* 1988), the short response latency of these descending units in the cervical connectives suggests that they could be involved in negative phonotactic behavior.

The relative strength of the ipsilateral descending activity compared with the contralateral descending activity could not be accurately measured in our experiments. However, in some preparations where only the ipsilateral cervical connective was connected to the thoracic and abdominal segments, ultrasonic stimuli evoked a 1–3 Hz increase in flight frequency (not shown) and also increased the amplitude of wing depressor muscle spikes (Fig. 2D). Thus,

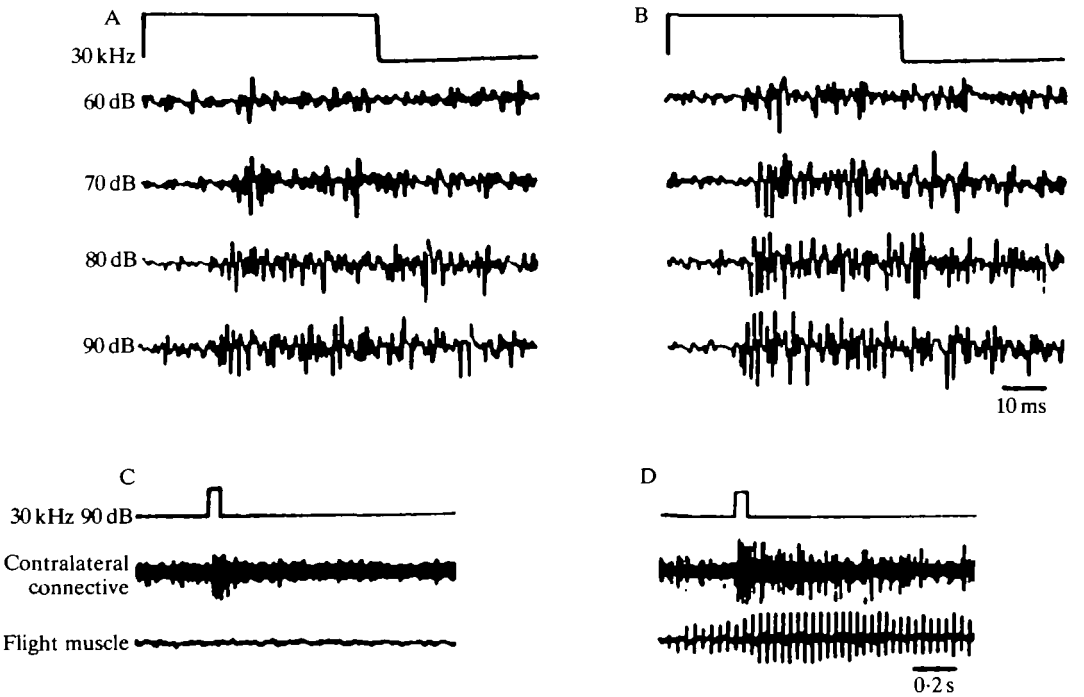

Fig. 2. Effect of flight activity on descending activity in the contralateral cervical connective. (A, C) Preparation not flying (at rest); (B, D) preparation flying. The amount and duration of the descending activity evoked by the ultrasonic stimulus increase with increasing stimulus intensity during both flight and non-flight. The response during flight was greater than that during non-flight. (D) Ultrasound also affects EMG recording from the metathoracic wing depressor muscle. (A, B) Top trace, timing of ultrasonic stimulus; lower traces, extracellular recordings from the contralateral cervical connectives at four different stimulus intensities. (C, D) Top trace, timing of ultrasonic stimulus; middle trace, extracellular recording from the cervical connective; bottom trace, EMG recording from metathoracic wing depressor muscle (muscle 129a).

ipsilateral descending activity is sufficient to produce changes in flight activity similar to those observed in intact, tethered flying crickets (May *et al.* 1988).

Facilitation of descending ultrasound units during flight

In crickets, negative phonotaxis only occurs if the flight system is active. To determine if flight affects the amount of the descending activity evoked by ultrasound, we recorded extracellularly from the cut end of the contralateral cervical connective posterior to the subesophageal ganglion during flight and non-flight. When the cricket was not flying, a 30 kHz sound pulse elicited descending neural activity, and as stimulus intensity increased, progressively more descending units in the contralateral connective were recruited for a longer period of activity (Fig. 2A). During flight, the response recorded in the cervical connectives was even greater, both in the number of spikes activated and in the duration of the response, than during non-flight periods at all stimulus intensities (Fig. 2B). These changes were especially evident when we split the cervical connective with a sharpened tungsten wire and recorded from approximately half of it. Both the medial and the lateral halves of the connective contain identifiable single units which are more strongly activated by ultrasound during flight than during non-flight (Fig. 3). For example, in Fig. 3C, an ultrasound pulse at 90 dB elicited three large spikes when the preparation was not flying. During flight, this same stimulus elicited at least 10 spikes in the connective and this unit was active twice as long as it was during non-flight (Fig. 3D).

Although flight activity increases the amount of descending activity elicited by ultrasound, no clearly identifiable, new single units appear to be activated by ultrasound during flight that were not also activated by ultrasound when the cricket was not flying (Fig. 3). However, the threshold of some descending units appears to be lower during flight than during non-flight. For example, a medium-sized spike (indicated by the dots) was activated by ultrasound at 60 and 70 dB when the cricket was flying, but only at 80 dB during non-flight (Fig. 3A,B). The background level of activity in the cervical connective recordings prior to the onset of the ultrasonic stimuli was approximately equal during flight and non-flight (Figs 2 and 3). Thus, flight does not increase the level of spontaneous activity in the connectives, which indicates that the increase in the descending activity that occurs during flight is associated with a change in the amount of ultrasound-induced descending activity, and not just with an overall increase in connective activity occurring during cricket flight.

To quantify the change in the amount of descending activity elicited by ultrasound during flight and non-flight periods, we calculated the area under the curve of our full-wave rectified and integrated records of descending activity in cervical connectives. Regardless of whether the cricket was flying, the amount of descending activity (normalized peak area) increased linearly with stimulus intensity (Fig. 4A). However, at all stimulus intensities the amount of descending activity was significantly greater (paired t-test, $P<0{\cdot}05$) when the cricket was flying than when it was not flying. The latency to the onset of descending activity

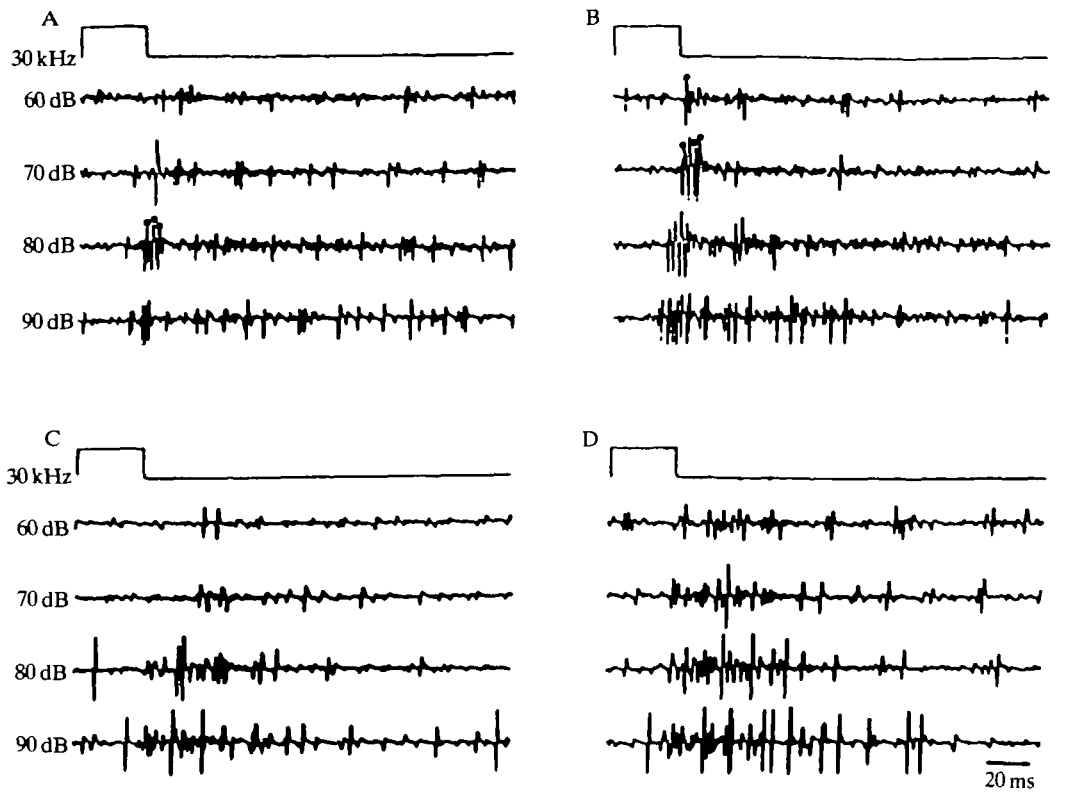

Fig. 3. Effect of ultrasound on descending units in the medial (A, B) and lateral (C, D) halves of the cervical connectives. (A, C) Non-flight; (B, D) flying. In both the medial and the lateral halves of the contralateral cervical connectives, flight increased both the amount and the duration of the descending activity evoked by the ultrasonic stimulus. In A and B dots identify the same unit. Notice that during flight this unit is activated at a lower threshold than during non-flight. (A–D) Top trace, timing of ultrasonic stimulus; lower traces, extracellular recording from split cervical connectives at four different stimulus intensities.

decreased linearly with increasing sound intensity during both flight and non-flight, and was significantly less (paired t-test, $P < 0{\cdot}05$) during flight than non-flight at all stimulus intensities (Fig. 4B). Thus flight appears to increase the amount of descending activity and decrease the latency of the descending response evoked by ultrasonic stimuli.

Changes in Int-1 activity during flight

The increase in the amount of descending activity evoked by ultrasound in flying crickets could be due either to a convergence of Int-1's input and the flight motor system's input to descending interneurons or simply to an increase in Int-1's response to ultrasound. To determine if the average firing frequency of Int-1 changes during flight, we recorded extracellularly from the cut end of the cervical connective anterior to the prothoracic ganglion. In five preparations, flight activity

did not significantly increase (paired *t*-test; $P > 0{\cdot}05$) the average firing frequency of Int-1 at all stimulus intensities (Fig. 5). In one preparation, Int-1 was recorded intracellularly in the brain, and again its response to ultrasound did not change during flight (not shown). Moreover, the duration of spiking activity in Int-1 (Fig. 5A,B) and the instantaneous firing frequency of Int-1 were also approximately the same during flight and non-flight episodes. Thus, it appears that the increase in descending activity is not due to a flight-induced increase in Int-1's response to ultrasound.

Localization of descending units

Int-1 has processes which extend into both the subesophageal ganglion and the brain (Moiseff & Hoy, 1983). Thus, descending neurons connected to Int-1 could be located in either the subesophageal ganglion or the brain. To determine whether ultrasound-induced descending activity originates from neurons located in the subesophageal ganglion or the brain, we performed the following experiment. First, we recorded from the cut end of the contralateral cervical connective posterior to the subesophageal ganglion in a preparation in which the brain was still attached to the subesophageal ganglion *via* the circumesophageal connectives (see Fig. 1A). In this preparation, ultrasound evoked a barrage of descending activity in the cervical connectives (Fig. 6A). Second, the contralateral circumeso-

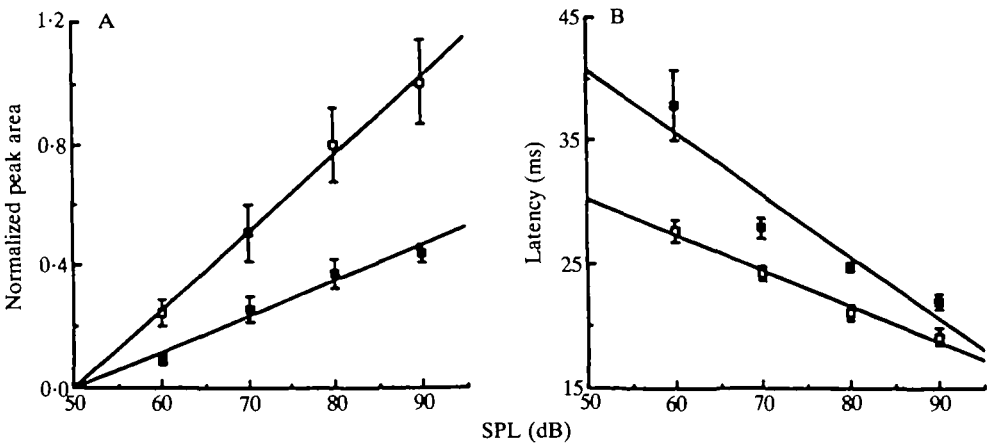

Fig. 4. (A) Change in the amount of descending activity elicited by ultrasound (30 kHz) during flight and non-flight as a function of stimulus intensity (sound pressure level, SPL). Peak area was measured from full-wave rectified and integrated extracellular recordings from the cervical connectives. Peak area increases linearly with SPL independently of flight activity (□ flight, line drawn is linear regression, $r = 0{\cdot}997$; ■ non-flight, $r = 0{\cdot}983$). Peak area is significantly greater for flight than for non-flight at all stimulus intensities (paired *t*-test $P < 0{\cdot}05$; $N = 20$). (B) Onset of descending activity recorded in the cervical connectives decreases linearly with SPL during both flight (□, linear regression drawn; $r = 0{\cdot}992$) and non-flight (■, linear regression drawn, $r = 0{\cdot}946$). Latency is significantly less during flight than during non-flight at all stimulus intensities (paired *t*-test $P < 0{\cdot}05$; $N = 20$).

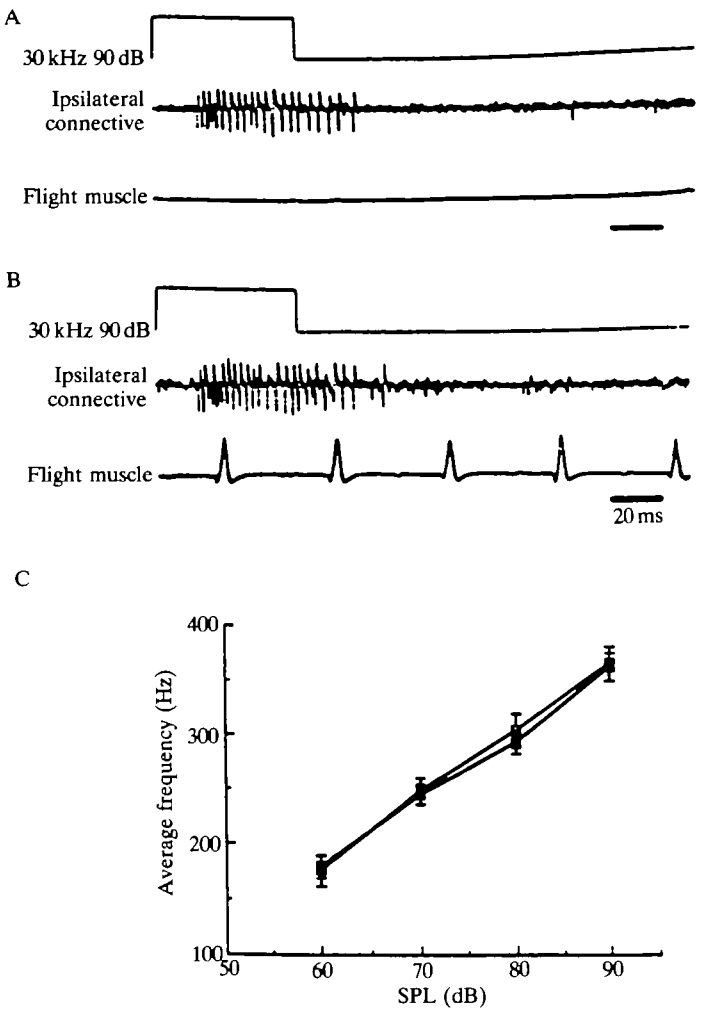

Fig. 5. Change in Int-1 activity during flight. Response of Int-1 to ultrasound during flight (A) and non-flight (B). (A, B) Top trace, timing of ultrasound stimulus. Middle trace, extracellular recording from the cut end of the cervical connective anterior to the prothoracic ganglion. Bottom trace, EMG recording from the metathoracic flight depressor muscle. (C) Average firing frequency in Int-1 is the same (paired t-test $P > 0{\cdot}05$; $N = 25$) during flight (□) and non-flight (■) at all SPLs.

phageal connective was severed, partially isolating the subesophageal ganglion. This procedure eliminated all the descending activity recorded in the cervical connective posterior to the subesophageal ganglion (Fig. 6B). Finally, we recorded from the contralateral circumesophageal connective posterior to the brain. Again, descending activity was observed in response to ultrasound (Fig. 6C). These results were observed in both flying and non-flying preparations. Thus Int-1 appears to activate descending neurons located primarily in the cricket brain.

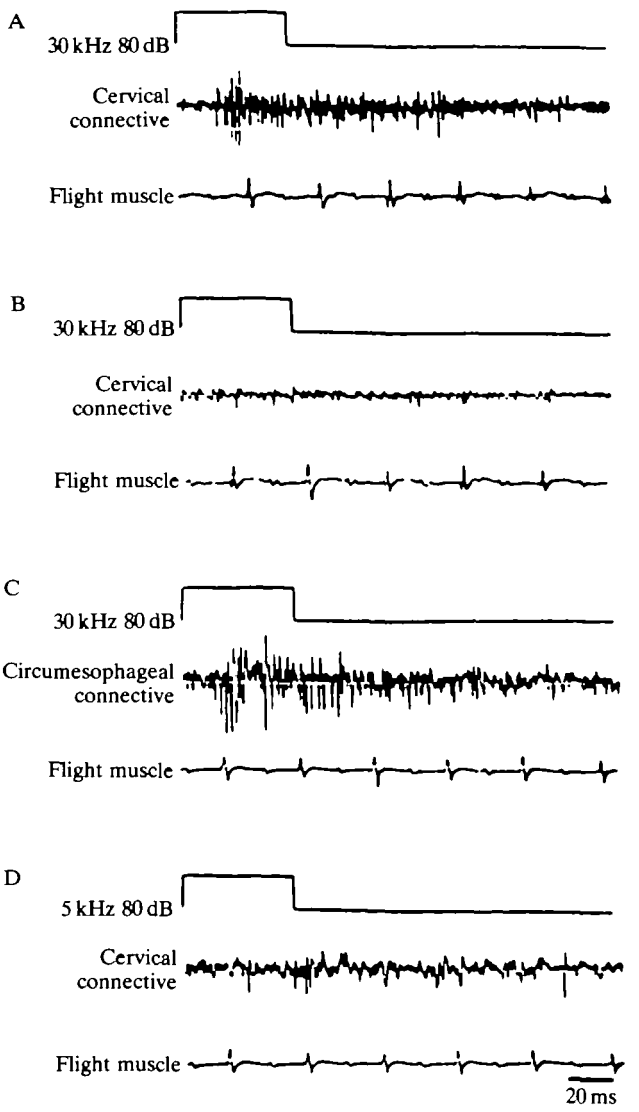

Fig. 6. Localization of ultrasound-sensitive descending units. (A–D) Top trace, timing of ultrasound stimulus pulse; bottom trace, EMG recording from the metathoracic wing depressor muscle. Wing elevator muscle activity is also evident. (A) Brain and subesophageal ganglion intact. Ultrasound activates descending units in the cervical connective (middle trace). (B) Contralateral circumesophageal connective cut with respect to the sound source. No ultrasound-sensitive units are active in the cervical connective (middle trace). (C) Brain intact. Ultrasound activates descending units in the circumesophageal connective (middle trace). Gain on the extracellular recording in C is different from that in A. (D) Brain and subesophageal ganglion intact. A 5 kHz sound pulse does not activate the same descending units in the circumesophageal connective as does a 30 kHz sound pulse (middle trace).

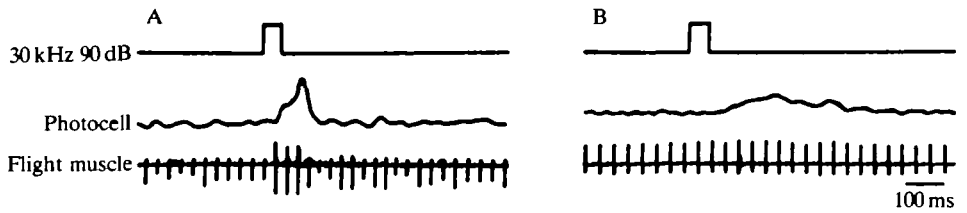

Fig. 7. Abdominal steering response. (A) Cervical connectives intact. A large abdominal swing occurs in response to ultrasound. (B) Lateral half of the contralateral cervical connective intact; medial half severed. An abdominal swing, although reduced in amplitude and with a slower time course, still occurs in response to ultrasound.

Since Int-1 also has a low threshold for 5 kHz sound pulses, we determined if the same descending units excited by ultrasonic stimuli are also excited by such pulses by recording extracellularly from the circumesophageal connective posterior to the brain. The 5 kHz sound pulses did not activate the same group of descending units in the contralateral cervical connective as did ultrasound (Fig. 6D). Moreover, in many preparations 5 kHz sound pulses did not reliably activate any descending units (Fig. 6D).

Correlation of descending activity with negative phonotactic steering

We have shown that (1) ultrasonic stimuli activate descending units which project posteriorly in both the medial and lateral halves of the cervical connectives, (2) the response of these units to ultrasound is enhanced during flight compared to at rest, and (3) their minimum response latency to high-intensity sound pulses is 20 ms. Thus, these three observations suggest that ultrasound-sensitive descending units in the cervical connectives could be involved in negative phonotactic steering movements. To determine this, we split the contralateral cervical connective approximately in half, leaving either the medial or the lateral half of the connective intact, and tested if this cricket demonstrated negative phonotaxis to ultrasonic stimuli, as assayed by an abdominal swing (Moiseff *et al.* 1978). In a flying preparation, where the contralateral connective was intact, ultrasound always evoked an abdominal swing away from the direction of the sound source (Fig. 7A). In three animals tested with only the lateral half of the contralateral connective intact, directionally correct abdominal swings were observed in all three animals, although the amplitude of the abdominal swing was reduced, while the onset and time course increased (Fig. 7B). In only one of three animals tested did ultrasound elicit correct abdominal swings when the medial half of the contralateral connective was left intact (not shown). Severing either one or both of the circumesophageal connectives always eliminated abdominal movements in response to ultrasonic stimuli (three preparations). Thus, descending units in each half of the cervical connectives appear to be associated with negative phonotactic behavior and are sufficient to elicit an abdominal swing in response to ultrasound.

Discussion

Integration of ultrasound and flight inputs

Cricket negative phonotaxis is a context-dependent behavior (Nolen & Hoy, 1984). That is, ultrasonic stimuli only elicit negative phonotaxis in flying crickets. Previous investigations have suggested that integration of ultrasonic input and flight input occurs in the cricket brain which 'allows' negative phonotactic responses to be elicited by ultrasound (Hoy & Nolen, 1987; Pollack & Hoy, 1981). Several lines of evidence from our data support this hypothesis. (1) The amount of descending activity in the cervical connectives elicited by ultrasonic stimuli is enhanced significantly during flight. (2) Decreasing the amount of descending activity reaching the thoracic and abdominal segments reduces the extent of the negative phonotactic response observed in the abdomen. (3) The average firing frequency of Int-1 elicited by ultrasound during flight and non-flight is the same. (4) Cutting the circumesophageal connectives eliminates all descending activity recorded in the cervical connective, but not in the circumesophageal connective. Thus, integration of ultrasonic and flight motor system inputs appears to occur in the cricket brain, such that it produces an increased level of ultrasound-induced descending activity that elicits negative phonotactic steering movements.

The mechanism by which ultrasound, a nonphase-locked sensory input, and the flight motor system interact to produce negative phonotactic steering movements is different from how other nonphase-locked sensory inputs have been shown to control flight steering movements in insects. For example, in the locust, integration of nonphased-locked sensory inputs from the ocelli, compound eyes, cephalic wind-sensitive hairs and antennae with input from the flight pattern generator occurs directly in thoracic ganglia on flight motor neurons and interneurons (Hensler, 1988; Rowell, 1988; Reichert & Rowell, 1985*a*,*b*, 1986; Arbas, 1986). The activity level of the multimodal descending interneurons (deviation-detector neurons) in the locust does not change during flight, which is clearly opposite to the response of descending interneurons involved in controlling ultrasound-induced negative phonotactic steering responses in crickets.

Negative phonotactic steering movements in the cricket, however, need not be controlled entirely by descending units whose activity level is facilitated by flight input to the brain. Two alternative possibilities could also function to control negative phonotaxis. First, descending units may also be located in the subesophageal ganglion, since our results only indicate that descending units recorded in the cervical connectives are not activated directly by Int-1 in the subesophageal ganglion. We cannot rule out the possibility that descending neurons in the subesophageal ganglion, which may be critical in controlling negative phonotaxis, are postsynaptic to ultrasound-sensitive neurons in the brain and, thus, are indirectly activated by Int-1. In fact, we have identified ultrasound-sensitive descending interneurons which have their cell bodies in the subesophageal ganglion (P. D. Brodfuehrer & R. R. Hoy, personal observation). Second, some descending ultrasound-sensitive interneurons could summate with flight motor system input directly on motor neurons and interneurons in thoracic and

abdominal segments to affect cricket steering movements, similar to the mechanisms controlling visually guided steering corrections during locust flight (see above). Descending ultrasound-sensitive interneurons whose activity is not facilitated by flight and which extend posteriorly to the thoracic ganglia have been identified in the cricket brain (Brodfuehrer & Hoy, 1988).

Organization of the pathway controlling negative phonotaxis

Besides demonstrating that input from the flight motor system facilitates the responsiveness of ultrasound-sensitive interneurons in the cricket brain, the results from this paper also suggest several interesting aspects about the nature and organization of the pathway controlling negative phonotaxis. (1) Ultrasound appears to activate many different descending units in the cervical connective. From our extracellular records it appears that at least five different descending units are activated by ultrasound. Thus, although ultrasound information is carried to the brain *via* one pathway, Int-1, many lines of descending information appear to control the negative phonotactic motor responses. This may be because negative phonotaxis is a complex behavior involving changes in several kinematic and aerodynamic flight parameters (Moiseff *et al.* 1978; Nolen & Hoy, 1984; May *et al.* 1988). (2) Ultrasound activates descending units which are ipsilateral and contralateral to the direction of the auditory stimulus. Two lines of evidence indicate that both ipsilateral and contralateral descending activity play a role in controlling negative phonotaxis. First, activation of ipsilateral activity alone is sufficient to increase the flight frequency and amplitude of EMG wing depressor muscle spikes. Second, reducing the amount of contralateral descending activity decreases the extent of an abdominal swing in response to ultrasound. The function of ipsilateral and contralateral descending units could be to produce the asymmetrical changes in fore- and hindwings that occur during negative phonotactic steering maneuvers (May *et al.* 1988). (3) Descending activity in the ipsilateral and contralateral connectives begins approximately 20 ms following a high-intensity sound pulse, and can last up to twice as long as the sound pulse. This is consistent with both the onset and duration of negative phonotactic steering movements observed in tethered, flying crickets (May *et al.* 1988). (4) Flight activity does not appear to recruit new ultrasound-sensitive descending units, but rather, increases only the number of spikes and the duration of the descending activity which was previously activated by ultrasound when the cricket was not flying. (5) The amount of descending activity elicited by ultrasound is linearly graded with increasing stimulus intensity. Thus, in the neuronal pathway controlling negative phonotaxis, a linear relationship has now been demonstrated between the intensity of an ultrasonic stimulus and the output of three different neuronal levels in this pathway: sensory (number of Int-1 spikes; Moiseff & Hoy, 1983), interneuronal (amount of descending activity) and motor (changes in several kinematic and aerodynamic flight parameters; Nolen & Hoy, 1984; May *et al.* 1988).

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