PERIPHERAL CONTROL OF RESPONSIVENESS TO AUDITORY STIMULI IN GIANT FIBRES OF CRICKETS AND COCKROACHES

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SUMMARY

Auditory stimuli initiate ascending activity in large fibres of the ventral nerve cord of the cricket, *Acheta domesticus*, and the cockroach, *Periplaneta americana*. This auditory responsiveness is reduced during locomotion. An earlier study concluded that the depression of responsiveness was mediated by descending inhibition. However, the auditory responsiveness is reduced during locomotion even after section of the ventral nerve cord anterior to the abdominal recording electrodes. Further, auditory responsiveness of isolated abdomens attached to intact animals is inhibited during locomotion of their hosts. Laminar wind streams over the cerci depress responsiveness to sound, but only at velocities markedly higher than those encountered by freely walking animals. Although the exact mechanism is not known, the depressed auditory responsiveness can occur independently of any descending influences.

INTRODUCTION

Rather than being solely passive receivers of sensory input, central nervous systems may actually modulate that input. Centrifugal control of sensory systems is widespread, occurring in invertebrates (e.g. Kuffler & Ezyguirre, 1955; Cohen, 1963; Krasne & Bryan, 1973) as well as vertebrates (e.g. Loewenstein, 1956; Roberts, 1972; Flock & Russell, 1976) and taking place at different levels of neuronal processing from the primary receptor (Kuffler & Ezyguirre, 1955) to higher, central levels (Hernandez-Peon, 1961). Efferent control of sensory input is of particular importance when sensory stimulation occurs as the result of the animal's own behaviour. Self-stimulation could be averted by intercalating a subtraction system into the sensory pathway which would allow the central nervous system to separate exogenous stimuli from those self-generated during behaviour (von Holst & Mittelstaedt, 1950; Sperry, 1950). Other suggested functions of efferent control might be to modulate the sensitivity range of the receptors (Crowe & Matthews, 1964) or to protect afferent synapses from fatigue during phasic behaviours such as locomotion (Russell, 1971; Roberts & Russell, 1972).

In the present investigation, purported efferent control of a sensory pathway was studied in two orthopteran insects, *Acheta domesticus* and *Periplaneta americana*. At the posterior end of the abdomen of orthopteran insects are special sensory appendages

called cerci. Distributed over the surface of each cercus are numerous hair sensilla which are very sensitive to wind or sound over a wide range of frequencies (Nicklaus, 1965; Milburn & Bentley, 1971; Bentley, 1975; Counter, 1976; Schwab & Josephson, 1977). Movement of the sense hairs in response to wind or sound stimuli leads to excitation of the bipolar cells of the sensilla. The axons of these bipolar cells together form the medial cercal nerve which leads directly into the last abdominal ganglion. Here the axons synapse on and drive interneurones which ascend the ventral nerve cord (Pumphrey & Rawdon-Smith, 1936; Roeder, 1948; Farley & Milburn, 1969; Milburn & Bentley, 1971; Callec, 1974; Bentley, 1975).

Murphey & Palka (1974) recorded from large interneurones in the ventral nerve cord of intact *A. domesticus* and found that the responsiveness to sound was reduced during bouts of walking and grooming. In pinned-down preparations, intracellular recordings from one of these large interneurones revealed volleys of inhibitory post-synaptic potentials (IPSPs) which were correlated with activity in the main meta-thoracic leg nerve. The authors concluded that the interneurone's sensitivity was modulated during behaviour through descending inhibitory efference. We have re-examined the above phenomenon to further determine the inhibitory mechanism and have found that inhibition of sound responsiveness during locomotion does not require descending inhibitory pathways.

MATERIAL AND METHODS

Adult male *Acheta domesticus* and *Periplaneta americana* were selected for un-damaged cerci from laboratory cultures.

Freely walking preparations

Recordings were made from the ventral nerve cords of walking animals with fine copper wires, 60 μm in diameter and 50 cm in length, mounted in a polyethylene cuff. The cuff was 1·5 mm long and had an inside diameter of 0·4 mm. One side of the cuff was slit along its entire length, enabling it to be placed around the insect's ventral nerve cord. The wires were insulated except for a small portion near the ends. The uninsulated ends were inserted through the wall of the polyethylene tubing and fastened in place with a hot probe. Following CO_2 anaesthesia, the ventral nerve cord was exposed by cutting a window in the abdominal sternites. A portion of the nerve cord between the fifth and sixth abdominal ganglia was laid in the cuff which was then made fast around the nerve cord by sealing the cuff edges together with a hot probe. The sternites were pulled back into place and the abdomen sealed shut with wax. Animals in this condition without further surgery will be referred to as intact preparations. Animals subjected to these procedures appeared and behaved normal in all respects and suffered no obvious impairment to normal locomotion due to the presence of the recording leads.

After a recovery period of at least 3 h, each animal was placed in a circular activity arena, 30 cm in diameter, with a sand floor to minimize reflected sound. For the cricket, the acoustic stimuli were 600 Hz, 50 ms sound pulses. For the roach, the acoustic stimuli were 50 Hz and 50 ms in duration. Both types of stimuli were delivered from a loudspeaker mounted above the arena. The intensity of the sound, measured at the

floor of the arena, was 92 and 96 dB for the cricket and roach stimuli, respectively. The frequencies used were chosen because they fall within low threshold portions of the animals' response range (Edwards & Palka, 1974; Counter, 1976; Schwab & Josephson, 1977). Further, 600 Hz was the sound frequency used with *A. domesticus* by Murphey & Palka (1974), so results from that study and this one are comparable. For all stimuli, the sound amplitude was of trapezoidal shape with a rise time of 5 ms, thus avoiding on and off transients. The animals were observed and sound stimuli delivered either when they were motionless ('still presentations') or when they were walking ('walking presentations'). The minimum interval between successive stimuli was 5 s. At least 50 walking and 50 still presentations were made to each animal during each portion of the experiment.

Output from the recording leads was amplified and fed into an oscilloscope for display and into a threshold discriminator which passed suprathreshold spikes to a counter. The threshold of the discriminator was adjusted so as to count all spikes whose amplitude exceeded one-third the maximum spike height observed during the sound presentation. We assume that these larger spikes are generated by the larger interneurones in the ventral nerve cord. Spike counts were made in a 70 ms window opened at the onset of the sound stimulus. The percentage change in sound responsiveness during walking was calculated as:

$$\frac{N_s - N_w}{N_s} \times 100,$$

where N_s = average number of spikes per still presentation and N_w = average number of spikes per walking presentation.

Two methods were employed to isolate the large interneurones from sources of descending inhibition. In most cases, a tested, intact preparation was reanaesthetized, its abdomen reopened, and its ventral nerve cord severed anterior to the cuff. The abdomen was then resealed and the animal was allowed to recover prior to being retested as before. Animals subjected to this procedure will be referred to as 'cut nerve cord' animals. The second method for abdominal isolation was literally just that. A tested, intact preparation was reanaesthetized and the abdomen removed from the animal with the electrode still in place by cutting the abdomen–thorax junction. The open end of the abdomen was sealed with vaseline before the entire abdomen was fastened with wax to the back of another animal. After a recovery period, the 'epizoic abdomen' was tested as before as its host walked about the floor of the activity arena.

Applied wind and auditory responsiveness

The following method was used to determine the effect of wind on auditory responsiveness. Following CO_2 anaesthesia, the legs and forewings of the experimental animals were removed. The abdominal nerve cord was exposed by removing the tergites, gut, and gonads, and the preparation was then mounted on a pedestal 9 cm in height. The ventral nerve cord was cut between the fifth and sixth abdominal ganglia and the distal end sucked into a suction electrode. The output of the electrode was amplified and fed both into an oscilloscope for display and into a threshold discriminator. Threshold detector settings and spike counts were carried out as in the previous

section. The acoustic stimuli were trapezoidal 50 Hz, 80 dB, 50 ms sound pulses delivered 10 times at 0·5 Hz. The cricket was also tested with pulses of 600 Hz, 80 dB, and 50 ms duration. An air collimator was constructed by packing a length of plastic tubing, 20 mm o.d. with 1·0 mm o.d. capillary tubes. Wind velocities from the collimator were measured with a thermistor probe anemometer. The wind was directed along the midline of the animal in the anterior to posterior direction. Wind of eight different velocities were used for each preparation. Five replicates of the eight velocities were used for each preparation. The percentage change in sound responsiveness was calculated as:

$$\frac{N_a - N_p}{N_a} \times 100,$$

where N_a = average number of spikes per sound presentation in the absence of wind and N_p = average number of spikes per sound presentation in the presence of wind.

Applied wind and response of the cercal nerve

The effect of wind on the response of the cercal receptors to sound was tested in the following preparation. The right medial cercal nerve of a cricket was exposed by removing the abdominal tergites, gut, and gonads. The nerve was then cut as it entered the last abdominal ganglion and its distal end sucked into a suction electrode. The recorded potentials were displayed on an oscilloscope both directly and after passing the signal through a third-order, bandpass filter with a centre frequency of 50 Hz. The latter display emphasized components of the signal having the same frequency as the impinging sound waves. In addition, an indication of background activity was obtained with an a.c. voltmeter which measured the RMS voltage of the signal recorded from the cercal nerve. Wind was applied as before. The stimulus was a 50 Hz 50 ms, 80 dB sound pulse delivered at 0·5 Hz.

RESULTS

Free walking preparations

With each animal tested there was an obvious decrease in responsiveness to sound stimuli presented when the animal was walking (Table 1). This was even true in the cut nerve cord and epizoic abdomen preparations, indicating that descending inhibition from thoracic and/or head ganglia is not necessary for reduced auditory responsiveness during locomotion. Sham sound presentations, which consisted of opening the counting window in the absence of sound stimuli, were also presented to the animals. The spike counts during these sham stimuli increased noticeably during locomotion in intact animals and in animals with transected nerve cords, indicating that locomotion itself generates ascending activity in the ventral nerve cord. Fig. 1, which presents the data for a single cricket, illustrates the increase in background activity during locomotion (seen in the sham presentations) and the reduced auditory responsiveness to sound during walking.

In both species, cutting the nerve cord almost unanimously (10 of 11 instances) resulted in an overall reduction of the number of spikes counted per sound stimulus (Table 1) This suggests that anterior centres provide a tonic excitatory input to the

Table 1. *Responsiveness to sound and background activity in still and walking preparations (mean and range)*

	A. domesticus (600 Hz)					
	Intact			After nerve cord section		
	Still	Walking	% Change*	Still	Walking	% Change*
Spikes per sound pulse (N = 5 animals)	7·28 (2·77–11·20)	3·93 (1·89–5·78)	41 (24–72)	5·63 (3·20–8·81)	1·73 (0·76–3·09)	67 (49–85)
Spikes per sham presentation (N = 5 animals)	0·06 (0·00–0·15)	1·87 (0·50–4·30)	†	0·20 (0·00–0·50)	1·24 (0·15–1·40)	†
	P. americana (50 Hz)					
	Intact			After nerve cord section		
Spikes per sound pulse (N = 3 animals)	7·76 (6·10–9·08)	3·00 (1·34–4·36)	62 (45–78)	3·62 (2·43–5·69)	0·54 (0·45–0·71)	84 (81–88)
Spikes per sham presentation (N = 2 animals)	0·00 (0·000–0·00)	1·86 (0·34–3·38)	†	0·00 (0·00–0·00)	0·00 (0·00–0·00)	†
	Intact			Epizoic abdomen		
Spikes per sound pulse (N = 3 animals)	7·24 (5·40–10·81)	0·69 (0·22–1·33)	92 (88–96)	1·79 (1·48–2·15)	0·23 (0·07–0·49)	88 (77–95)
Spikes per sham presentation (N = 2 animals)	0·04 (0·00–0·08)	0·47 (0·00–0·93)	†	0·00 (0·00–0·00)	0·00 (0·00–0·00)	†

For each animal tested, the percentage difference between walking and still responsiveness, both for normal and sham presentations, was statistically significant ($P < 0.05$).

* Percentage change calculated as the average of the percentage for the individual animals.

† Undefined because no spikes at all were recorded during sham presentations from one or more animals.

cercal auditory system and removal of this input reduces responsiveness. This finding stands in contrast to Roeder, Tozian & Weiant (1960) who found that higher centres (specifically, the suboesophageal ganglion) maintained a tonic *inhibitory* input to motor systems in the last abdominal ganglion of a roach and a mantid.

Sound responsiveness and applied wind

It was hypothesized that the small wind currents generated during walking could play some role in auditory inhibition, for example, by displacing the sensilla against their sockets and preventing them from responding to sound stimuli. This idea was tested by presenting the sound stimulus concomitantly with a wind applied to the cerci. Indeed, as wind of increasing velocity is applied, the number of spikes counted per sound stimulus goes down markedly. Fifty per cent inhibition of responsiveness to 50 Hz, 80 dB sound pulses was achieved at wind velocities of 25 cm s^{-1} and at 40 cm s^{-1} for the cricket and roach, respectively (Fig. 2A, C). Fifty per cent inhibition of response to 600 Hz, 80 dB sound pulses presented to the cricket occurred at approximately 20 cm s^{-1} (Fig. 2B). It is important to note that these velocities are higher than those achieved by the animals in the free-walking experiments (estimated to be
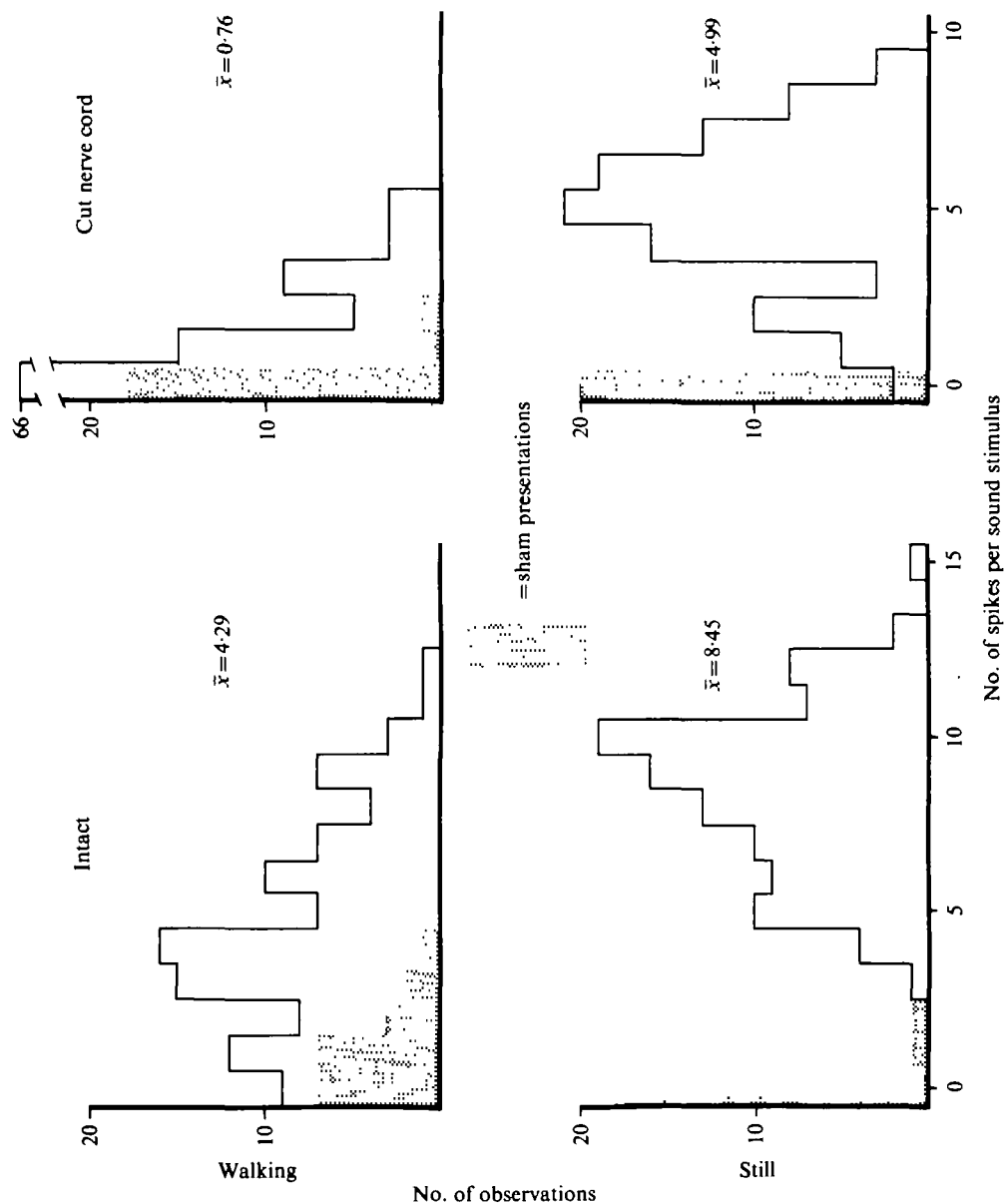
Fig. 1. Large interneurone spikes per sound stimulus in a cricket before and after cutting the ventral nerve cord. The upper histograms are for sound presentations to the insect while walking, the lower histograms from sound presentations to the animals while still. x's indicate the average number of spikes for a single sound pulse.
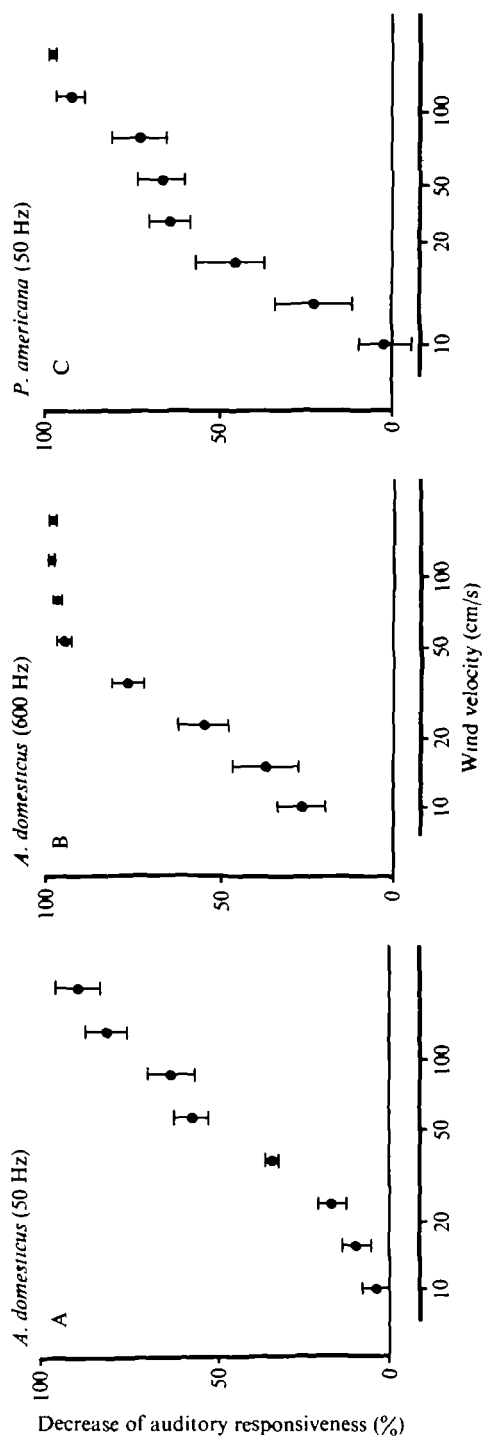
Fig. 2. Reduction in auditory responsiveness due to wind blown over the cerci.
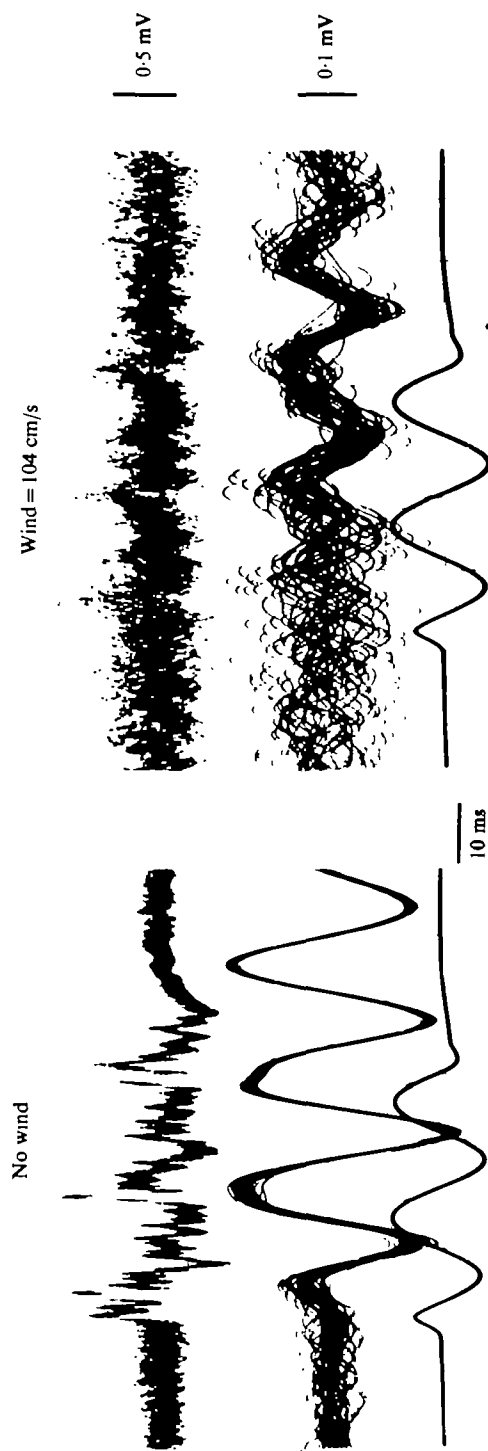
Fig. 3. Response of cercal nerve to sound stimuli in still air and with an air stream blown over the cerci. The sound stimuli were 50 Hz, 80 dB, 50 ms pulses. Each figure is made up of 50 superimposed traces. Upper trace: unfiltered signal; middle trace: filtered signal; lower trace: voltage applied to the speaker.
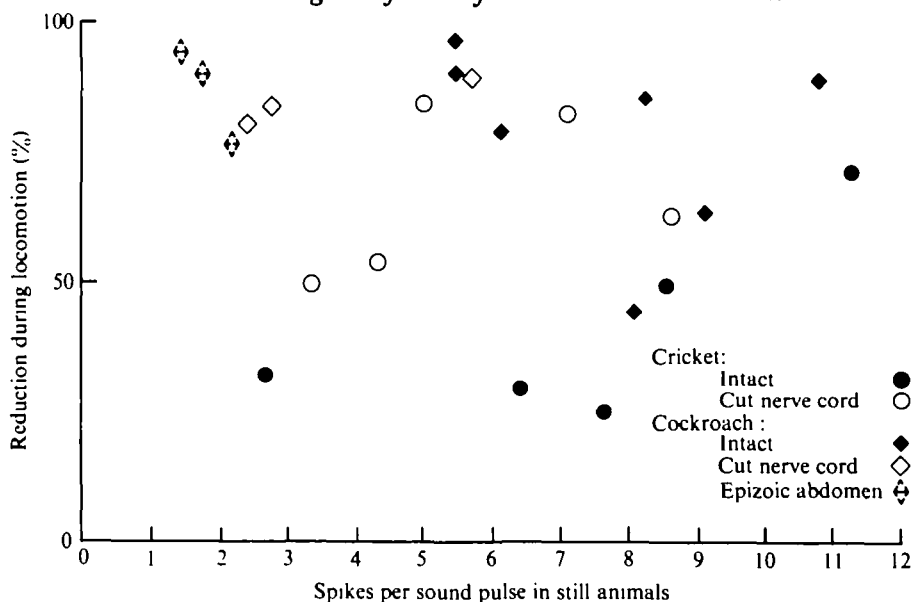
Fig. 4. The relation between the auditory responsiveness in still preparations and the percentage inhibition of responsiveness during locomotion. No correlation is evident.

2–5 cm s^{-1}). However, self-generated breezes about the cerci are unlikely to be continuous and laminar. It remains to be seen if turbulent micro-currents mediate any inhibition.

Applied wind and response of the cercal nerve

Decreased responsiveness of large interneurones in the ventral nerve cord is associated with a reduced input from cercal receptors. The left side of Fig. 3 illustrates the response of the cercal nerve to sound in the absence of wind. Note the waves of activity at the sound frequency. These waves are the 'synchronous responses' of Pumphrey & Rawdon-Smith (1936). As the wind velocity was increased, the amplitude of the response in the normal and filtered traces decreased *pari passu*. The background activity, measured with an a.c. voltmeter, also increased with increasing wind velocity (Fig. 3). The average increase of background activity over windless conditions was 41 % (± 6 % S.E.) at 22 cm s^{-1} and 59 % (± 7 % S.E.) at 104 cm s^{-1}. Thus, wind has two effects on the cercal nerve response: (1) It reduces the activity synchronized with sound waves during the pulse, and (2) it increases the background asynchronous activity between sound pulses. Either or both of these effects may be involved in the suppression of the interneuronal responses to sound during applied wind.

DISCUSSION

The auditory responsiveness of sound-driven interneurones in the abdominal nerve cord of a cricket and a cockroach was found to be markedly depressed during locomotion. This result agrees with that of Murphey & Palka (1974) who made similar observations on the cricket. Largely because inhibitory postsynaptic potentials associated with leg movements were recorded from sound-driven interneurones,

Murphey & Palka (1974) concluded that the inhibition of sound responsiveness during locomotion resulted from descending inhibition. However, we have found that inhibition of auditory responsiveness during locomotion also occurs following sectioning of the abdominal nerve cord which removed all descending neural information. The most dramatic demonstration that inhibition can occur independently of the anterior portion of the nervous system comes from the experiments with epizoic abdomens which also became less responsive when their host animals walked about. Thus, inhibition of sound responsiveness in animals with sectioned nerve cords depends on peripheral events (i.e. exogenous signals generated by locomotory movements) rather than central neural signals associated with activation of the motor programme.

The magnitude of the inhibition during movement in animals with sectioned nerve cords is surprising (see Table 1). Given that there are descending inhibitory pathways (Murphey & Palka, 1974) one might have expected less inhibition in cord-sectioned animals with descending influences removed than in intact animals. In fact, in nine of the eleven animals in Table 1, the percentage reduction in responsiveness was greater after cutting the ventral nerve cord than it was before section. There are several possible explanations for this. The velocity and regularity of movement in cord-sectioned animals might have been sufficiently altered to make the peripheral inhibitory components somehow greater; but we have no direct evidence suggesting this. Alternatively, there might be net descending excitation in the nerve cord during locomotion which partially compensates for peripheral inhibition in intact animals, rather than descending inhibition. One confounding factor is that the responsiveness to sound is reduced following cord section in both still and moving animals (Table 1), making it difficult to compare quantitatively intact animals and animals with sectioned nerve cords. It may be that the peripheral inhibitory component is more effective when the general excitatory level is reduced following cord section than it is in intact animals. If this were so, one would expect a relation between the overall level of excitation, measurable by the responsiveness to sound when the animal is still, and the percentage reduction in responsiveness when the animal moves. In fact, no such relation is seen (Fig. 4). The percentage reduction in responsiveness during locomotion is not obviously related to the responsiveness of the animal when still. We are left with the somewhat unsatisfactory conclusion that while there are inhibitory circuits activated when the legs move (Murphey & Palka, 1974), the overall inhibition during locomotion is greater without these circuits (and probably other circuits as well) than it is with them.

Wind applied to the cerci did indeed reduce sound responsiveness of the interneurones, but this effect was significant only at velocities higher than one might expect to be generated during walking. The wind used in the experiments was continuous and laminar. The effects of irregular, turbulent flow, which might occur across the cerci during normal locomotion, were not examined. Therefore, the possibility of effective inhibition due to self-generated air currents cannot yet be ruled out. In addition, the sensilla on the cerci are subject to acceleratory and vibratory forces during locomotion which could also be involved in the inhibition.

The actual mechanisms of the inhibition are unknown. Part may be due to altered responsiveness of cercal sensilla as occurs at high wind velocities (Fig. 3) but there may be inhibitory interactions within the last abdominal ganglion as well. All or pa

of the peripheral inhibition might be mediated by unidentified cercal receptors with inhibitory inputs to the interneurones (Callec, 1974). Inhibitory circuits (e.g. Krasne & Bryan, 1973; Kennedy *et al.* 1974; O'Shea & Fraser Rowell, 1975) might also be operating in the last abdominal ganglion to protect the interneurones from habituation during walking.

It is interesting that locomotion itself generates ascending spikes in the large interneurones of the ventral nerve cord (compare spike counts during sham presentations for still and walking animals in Table 1). It was once thought that these large interneurones, the giant fibres, triggered escape responses (e.g. Roeder, 1948) but this interpretation is now in doubt (see Dagan & Parnas, 1974; Harris, 1977). Although their behavioural role is uncertain, the large size of the giant interneurones indicates that they are part of an important afferent pathway. The observation that locomotion increases the amount of large fibre firing raises the question of how the central nervous system distinguishes between exogenous stimuli and those self-generated during movement.

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