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ACTIVITY OF GIANT INTERNEURONES AND OTHER WIND-SENSITIVE ELEMENTS OF THE TERMINAL GANGLION IN THE WALKING CRICKET

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Summary

Using intracellular recording techniques in stationary walking crickets (*Gryllus bimaculatus*), we have investigated the relationship between locomotion and the activity of interneurones ascending from the terminal ganglion. Nine different types of giant interneurones (GI) were characterized during walking and standing. One third of them reduced their activity, while the others enhanced their spike rate, during walking. These physiological properties were strictly correlated with morphological characteristics such as axon position in the longitudinal tracts of the terminal ganglion. In general, ventral GIs reduced and dorsal GIs increased their spike frequency during walking. In some of them, there was a weak but significant correlation between the spike rate and translational speed, but no correlation with rotational speed.

In all GIs except 10-3a, the changes in activity occurred at the start of walking. In GI 10-3a, an increase in membrane potential and spike rate was observed before the start of locomotion. Therefore, an intrinsic mechanism within the central nervous system operating on GI 10-3a is suggested.

Additionally, the activities of filiform hair receptors and of previously undescribed small ascending interneurones (SAI) have been studied during walking. About 80 % of the receptors slightly increased their spike rate during walking, while one SAI became more active during walking and another one was hardly affected.

The physiological properties of ascending interneurones are discussed with respect to their modulation and particular function during walking.

Introduction

Orthopterous insects are well equipped for detecting wind and low-frequency sound using filiform hairs (Nicklaus, 1965; Palka *et al.* 1977; Gnatzy and Tautz, 1980; Boyan and Ball, 1990) on a pair of caudal appendages, the cerci. On its deflection caused, for example, by air puffs, each hair transmits information *via* a sensory cell at its socket to a set of ascending interneurones within the terminal ganglion (TG) (Palka and Olberg,

Key words: *Gryllus bimaculatus*, cricket, filiform hairs, ascending interneurones, giant interneurones, terminal ganglion, walking.

1977; Westin *et al.* 1977; Heußlein, 1989). Some of these interneurones have been studied intensively and are well known as giant interneurones (GI) (Roeder, 1948; Edwards and Palka, 1974; Mendenhall and Murphey, 1974; Jacobs, 1984). The GIs contribute significantly to the initiation and modification of walking behaviour of cockroaches and crickets (Ritzmann, 1984; Kanou and Shimozawa, 1984). Conversely, the activity and responsiveness to wind of some ascending interneurones are influenced by the locomotion of the animal, as has been shown for *Periplaneta americana* (Delcomyn and Daley, 1979; Daley and Delcomyn, 1980). In crickets, the two largest GIs reduce their response to tone pulses when the animal walks (Murphey and Palka, 1974). The available data on the cercal sensory system of crickets are, however, incomplete.

To study more thoroughly the interdependence of walking behaviour and the activity of the cercus-to-GI wind-detector system, we carried out intracellular recordings of GIs, of hair receptors and of some small ascending interneurones in the TG of the cricket *Gryllus bimaculatus*. During the experiments, the animals were able to walk on the spot (stationary walking) in a normal manner, while the intended path, orientation and velocity of locomotion were measured and correlated to the neuronal activity. On the basis of these combined behavioural and physiological data we asked the following questions. (1) Does the spontaneous activity of wind-sensitive receptors and ascending interneurones change during locomotion and, if so, can we identify specific neuronal groups with either increased or lowered activity? (2) Are wind puffs processed differently by receptors and interneurones depending on the actual walking behaviour? (3) What mechanisms may be responsible for, or at least may contribute to, a functional modification of the GI system in the context of walking? Both reafferent sensory input because of the air turbulence evoked by leg movements and central mechanisms in the nervous system must be considered.

We found that most filiform hair receptors generate more action potentials during walking than during standing phases. From a total of 12 different ascending interneurones, 7 increased, 3 decreased and 2 retained their spontaneous and wind-evoked activity at the onset of locomotion. These physiological differences between the GIs match precisely the morphological classifications of Jacobs (1984) and Heußlein (1989). One GI showed enhanced wind-evoked EPSPs and spike numbers before the onset of walking. This indicates a centrally controlled modification of the receptor–GI system. The physiological and functional meaning of the behaviour-related changes in spike activity are discussed with respect to walking behaviour in crickets and cockroaches. Some of the results have been published previously in abstract form (Kohstall-Schnell and Gras, 1992).

Materials and methods

Adult male and female crickets, *Gryllus bimaculatus* de Geer, held at 28 °C and in a dark:light cycle of 12 h:12 h were taken from a crowded laboratory breeding colony. Only animals with intact legs, cerci, antennae and mouth parts were used for the experiments, which were performed at room temperature (21–24 °C).

Measurement of walking activity

The animal was fixed with wax at the head and prothorax to a holder passing ventrally around its neck. Care was taken to ensure that the legs were free to move and were in a natural position for normal tripod walking when the insect was positioned on the top of a hollow styrofoam sphere (mass 2.6 g, diameter 120 mm). This sphere rested on an air cushion produced by an a.c.-operated compressor. Its output carried a distinct 50 Hz modulation, which was reduced in initial experiments by passing the air stream through a water-filled vessel. While this arrangement was sufficient to prevent vibrations of the sphere, some filiform hair receptors detected the remaining air modulation. Therefore, we used a 30 l container for pressure balance, which reliably prevented any unintended 50 Hz stimulation of the wind receptors. After this modification, no differences in GI activity were obtained except for that of GIs 10-2a and 10-3a. These two GIs showed a decrease of 15–25 % in spontaneous activity after removal of the 50 Hz background stimulation. The reaction to wind-puff stimulation remained unaltered in all GIs and no behavioural differences were observed with or without the unintended stimulation. Therefore, data from all recordings were evaluated.

An optical system placed in front of the cricket at the equator of the sphere detected rotation of the ball caused by walking movements of the animal. Data were recorded by a microcomputer (Apple IIe or AT-compatible). All movements were regarded as a combination of translational (forward/backward) and rotational (left/right) elementary components, measured with a resolution of 1 mm and 1 °, respectively. The time resolution was 1 ms. A detailed description of the experimental apparatus is given by Gras and Hörner (1992).

Electrophysiology

To record intracellularly from neurones in the terminal ganglion of the crickets, the wings were removed and the abdomen was glued laterally and ventrally to a forked holder, which crossed the metathoracic segment dorsally and reached with its endings on either side to the tip of the abdomen. The cerci were not touched by the holder or restricted in their natural movements. The body cavity was opened dorsally along the midline from the TG to the first free abdominal ganglion (AG1) and the cuticle was fixed laterally to the holder. The gut and genital organs were removed. The TG was stabilized on a silver holder and by a small ring of silver placed carefully on the ganglion from above. The body cavity was filled with cricket saline (Honegger and Schürmann, 1975).

Thin-walled glass microelectrodes were filled with 4 % Lucifer Yellow (Sigma) in 0.1 mol l^{-1} LiCl at the tip and with 0.1 mol l^{-1} LiCl in the shaft and had resistances of 50–120 MΩ. The silver holder served as the reference electrode. After electrophysiological recording, the cells were injected with Lucifer Yellow for 10–60 min by 2–8 nA of hyperpolarizing d.c. current. After fixation in 4 % paraformaldehyde and conventional histological processing, labelled neurones were reconstructed from whole mounts.

The recordings were stored on magnetic tape (Racal Store 4DS) and documented off-line with a thermoelectric printer (Picker, Uniscript UD 210). Moreover, spikes were detected and normalized by a window discriminator and sampled by the microcomputer

at the same time as the behavioural data were obtained from the walking movements of the animal on the sphere.

Stimulation

Two nozzles with an inner diameter of 2 mm were positioned 10 mm apart from each other and about 10 mm behind the cricket, pointing at its cerci. Wind puffs of 50 ms duration and an air velocity of 0.5–1 m s^{-1} were delivered in a continuous series with a frequency of 10 Hz for 5–30 s from either the left or the right nozzle. The wind puffs reached the base of the cerci 20–23 ms after triggering, as measured by an anemometer. Depending on cercus length and position, filiform hairs near the tip would have been reached earlier. We estimated the minimal latency to be about 18 ms. Wind puffs from either direction displaced filiform hairs on both cerci, but to different degrees.

Instead of applying a predefined stimulus programme, we triggered stimuli by hand to adapt them to the actual type of behaviour of the animal. The intervals between the wind-puff series were at least 30 s long.

Data processing

All data were evaluated using an AT-compatible microcomputer. Behavioural data were analysed by automatically separating standing phases from walking phases. The data were arranged in subsequent time intervals lasting 50 or 75 ms. For each interval the translational (v_t) and rotational (v_r) velocities were computed, and all sequences comprising at least two successive intervals with $v_t \geqslant 10$ mm s^{-1} were defined as walking phases. Correspondingly, standing phases were defined as sequences with $v_t < 10$ mm s^{-1}.

For each behaviourally defined phase, the mean spike frequency of the simultaneously recorded neurone was correlated with v_t and v_r. Moreover, for each 50 ms interval, the instantaneous spike frequency was calculated and correlated with the actual walking velocity. Both methods revealed essentially similar results. Linear regression lines for the relationship between actual v_t and the spike rate in subsequent intervals of 50 ms were determined and tested for statistical significance (Spearman test; P=0.05, if not stated otherwise). The U-test (P=0.05) was applied to spike interval data, calculated separately for standing and walking phases.

To evaluate synaptic membrane potentials, intracellular recordings were sampled off-line with an A/D converter by a microcomputer and further processed by a special software package (Hedwig and Knepper, 1992*a*,*b*). A threshold value of 2 V s^{-1} of dV/dt was defined as the criterion for a spike filter. To evaluate specifically the subthreshold membrane potential, spikes were removed and the membrane potential was substituted by interpolated values (Hedwig and Knepper, 1992*b*). In this way, subsequent averaging of the graded membrane potential was not biased by action potentials.

Latencies of responses to wind-puff stimulation were measured from the time of triggering the wind puffs. Post-stimulus time diagrams (PSTD) were computed (Gras and Hackenberg, 1992) selectively for standing and walking phases. Within one type of neurone, the reactions to the stimulation were similar for all individual cells, as judged from the PSTDs. Minor inter-individual differences were due to slight deviations in the

positions of the cerci with respect to the nozzles. For each GI, the latency values of one typical recording are given as mean ± 1 S.E.M.

The terms ‘ipsilateral’ and ‘contralateral’ always refer to the position of the axon of an individual neurone.

In all figures, v_t is given in mm s^{-1} (backward negative) and spike rates in Hz or in numbers per time interval. Spike responses to wind puffs were counted over the 100 ms after stimulus onset and are given in spikes per stimulus.

Results

Walking behaviour

Only crickets walking with a normal tripod pattern of leg movements were used for the experiments. Some dissected animals showed reduced walking activity compared with controls, perhaps because the attachment to the holder prevented movements of the abdomen and hampered normal respiration. Nevertheless, normal leg coordination was always preserved and the general behavioural variables were similar to those of intact specimens (Gras and Hörner, 1992; Gras *et al.* 1994).

Filiform hair receptors

The sensory cells of filiform hairs project to the TG through the cercal nerve. Next to its root the very thin axons (diameter 1–5 μm) were recorded for 5–15 min. Because of the small axon calibre and the distance between the recording site and the cell body, we stained the fibres but not the somata. Therefore, classification of the sensory cells was determined by their reaction to wind puffs and/or by their arborizations within the TG (six successful stainings; Fig. 1A), which have already been described in detail (Dumpert and Gnatzy, 1977; Palka *et al.* 1977; Bacon and Murphey, 1984).

While most receptors generated action potentials irregularly with a frequency of less than 70 Hz (Fig. 1C, right-hand part), two showed very regular activity containing a distinct 50 Hz component in the standing animal (Fig. 1D, right-hand part). This was attributed to the compressor producing the air supply for the walking sphere. When walking began, the spike pattern became irregular in these cells as well. All receptors reacted to air puffs by coupling to the rhythm of the repetitive stimulation. In different preparations, this coupling was not uniform in its precision (Fig. 1C,D). Wind puffs from the two tested directions led to different discharge patterns in the receptors. Some of them showed a biphasic on–off reaction to each wind puff (Fig. 1C). The latencies of on- and off-responses to wind puffs were 20–30 ms and 30–45 ms, respectively, in different preparations. None of the sensory cells habituated during extended stimulation.

During walking, one receptor cell showed a decrease in spike frequency and one retained its activity. All other receptors increased their activity by 10–30 % (Fig. 1B). The additional spikes were probably the result of air turbulence caused by leg movements during walking.

Giant interneurones

The giant interneurones (GIs) of *Gryllus bimaculatus* and other crickets have been

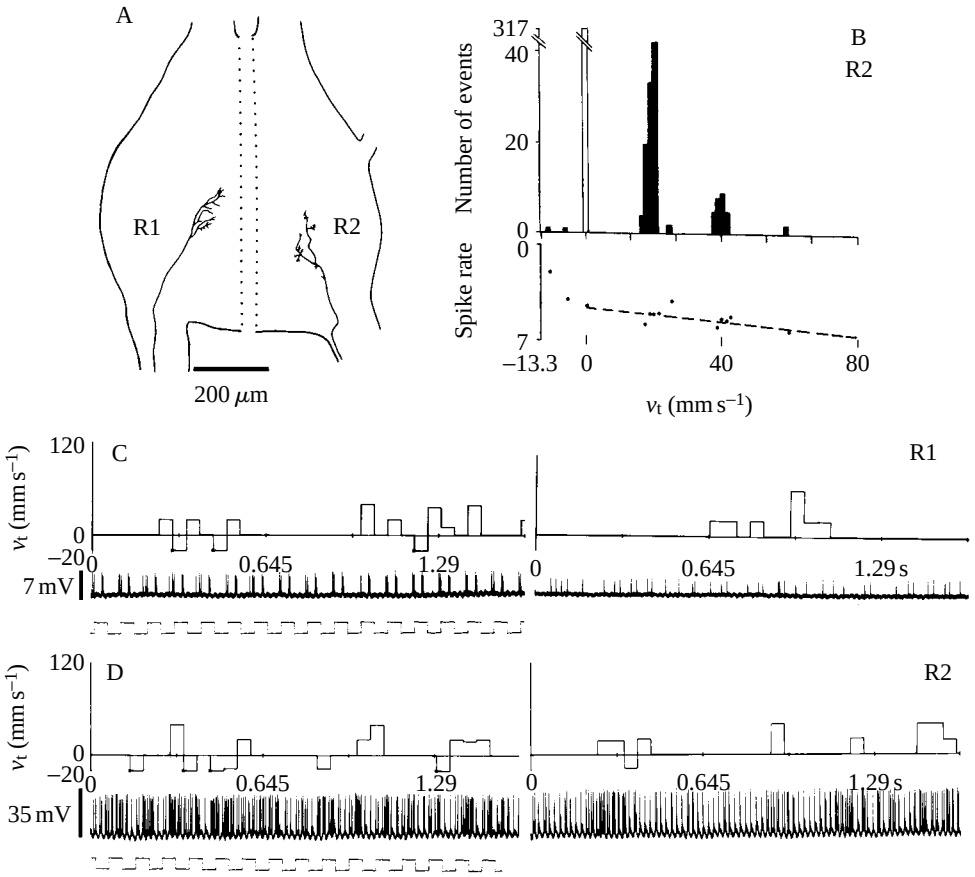


Fig. 1. Morphology and physiology of two sensory cells of filiform hairs. (A) Reconstructions of the two receptors R1 and R2 are from Lucifer Yellow stainings, as in all the other figures. The cells were recorded in two different specimens; the dotted lines indicate the midlines of the ganglia. (B) The histogram in the upper part of the figure illustrates the distribution of translational velocity (v_t) calculated for subsequent time intervals of 50 ms during spontaneous locomotion without wind stimulation. The discontinuous form of the histogram is caused by interference between the resolution of the optical detector and the temporal measuring interval (see Gras and Hörner, 1992). Interruption of the *y*-axis is valid only for the zero bin (white), which contains the intervals with v_t=0 mm s^{-1} (i.e. standing). The abscissa of the histogram also applies to the lower part of the figure, which illustrates the correlation between spontaneous v_t and the actual spike rate of R2. For each interval the spike rate (spikes per time interval) was calculated. The mean of the spike rates for all intervals with the same v_t (which belong to the same histogram bin) is indicated by a dot below each bin. The ordinate is inverted (increasing values downwards). Broken line, linear regression of original (not mean) data. There is a significant correlation between spike rate in R2 and increasing v_t (r=0.19, P<0.01). (C) Activity of R2 with (left) and without (right) contralateral wind puffs. No walking-dependent changes in activity are obvious. Traces from top to bottom show translational velocity (mm s^{-1}), intracellular spike activity and the stimulus marker. (D) Intracellular recording of R1. The cell responds to contralateral wind-puff stimulation by coupling to the stimulus. When no wind puffs are given, the activity of the receptor is higher when the animal walks than when it stands (right-hand part of the figure). The 50 Hz modulation of the membrane potential is a stimulus artefact caused by the walking apparatus, as explained in Materials and methods. The technical explanations given for Fig. 1 apply similarly to all other figures.

described morphologically and physiologically in detail (Mendenhall and Murphey, 1974; Jacobs and Murphey, 1987; Heußlein, 1989). In this study, we investigated the activity of the neurones 7-2a, 8-1a, 8-2a, 9-1a, 9-2a, 9-3a, 10-2a, 10-3a and 11-1a (nomenclature adopted from Mendenhall and Murphey, 1974) in the context of different walking activities. Each neurone has been recorded in at least two different specimens.

The GIs were classified into two distinct groups according to the alterations of their activity during walking. The activity of GIs 8-1a, 9-1a and 11-1a was reduced during locomotion, while it was enhanced in the others. The neurones belonging to the same group will be discussed together. For response properties (spikes per stimulus, latency) of the GIs to the wind-puff stimulation, see Table 1.

GIs enhancing their activity during walking

GI 7-2a (Fig. 2A) had a spontaneous activity of 70–80 Hz in the standing animal. Wind puffs did not evoke well-defined bursts of action potentials. Instead, ipsilateral wind puffs caused a slight increase in spike rate after about 35 ms followed by a period of about 10 ms with reduced spiking. Similarly, contralateral wind puffs evoked a slight increase and then a decrease in spike rate 38–45 ms after the stimulus. The mean spike frequency remained constant.

During walking, there was a slight but significant increase in spike rate of about 5 %, but the reactions to wind puffs did not change compared with standing.

During standing phases, GI 8-2a (Fig. 2B) had a spontaneous activity of 10–20 Hz. Wind puffs caused an increased spike rate and a coupling of the spike train to the rhythm of stimulation. The spontaneous activity rose by 70–80 % and the wind-evoked spike rate by 100 % during locomotion. Additional spikes occurred during the regular 50 ms intervals between wind puffs (Fig. 2C); notice that each wind puff arrived at the cerci after a delay of about 20 ms (see Materials and methods).

GI 9-2a (Fig. 3A) generated single, irregularly dispersed action potentials in the resting animal. Wind puffs evoked additional spikes phase-coupled to the stimulus train. When the cricket walked slowly, spontaneous spike activity was not affected, but it increased by 20–50 % at v_t>50 mm s^{-1} (Fig. 3C,E). This was weakly correlated to v_t (coefficient of regression, r=0.46) but not to v_r. Spike frequency during the wind-puff reaction rose by 50–70 % during walking, compared with standing. The additional spikes were strictly correlated to the stimuli, so the reaction to wind puffs during locomotion was only broadened compared with pauses (Fig. 3F).

GI 9-3a (Fig. 3B) was spontaneously active with spike rates of 2–10 Hz in the standing cricket. On air-puff stimulation, the neurone increased its spike rate and coupled its discharge pattern to the stimulus rhythm (Fig. 3D). In one preparation, not every wind puff evoked spikes, but stimulus-correlated EPSPs were always detectable. Walking behaviour caused a distinct increase in the spontaneous activity, which was not correlated to v_t or v_r. Wind puffs were represented in the spike pattern less exactly during locomotion than in the standing animal (Fig. 3D,G) because of additional spikes. They also caused an increase in the mean spike rate of 50–70 %.

GI 10-2a (Fig. 4A) was spontaneously active with a frequency of 45–75 Hz in different preparations. It produced more or less irregular spike bursts elicited by large EPSPs,

Table 1. *Spontaneous activity and reaction to wind-puff stimulation of ascending giant and non-giant interneurones in the standing animal and modification of these variables during walking*

	During standing					During walking			
		Reaction to wind-puff stimulation					Modification of reactions to wind puffs		
		Ipsilateral stimulation		Contralateral stimulation					
Cell	Spontaneous activity (Hz)	Spikes per stimulus	Latency (ms)	Spikes per stimulus	Latency (ms)	Change of spontaneous activity (%)	Number of spikes (%)	Response pattern	Latency (ms)
Dorsal giant interneurones									
7-2a	70–80	See text				+1–5*	–	Unchanged	Unchanged
8-2a	10–20	On 1–2 Off –	33.9±1.0 –	On 1–2 Off 0–2	29.4±1.5 43.6±1.6	+70–80*	+100*	Blurred	Unchanged
9-2a	2–10	On 1–3 Off –	30.2±2.9 –	On 1–2 Off 1–2	29.5±0.5 43.2±1.3	+20–50	+50–70	Unchanged	Unchanged
9-3a	2–10	On 2–3 Off 1–2	22.1±1.7 25.0±3.7	On 1–3 Off 1–3	21.3±2.3 26.2±17.3	+50–70*	+50–70	Blurred	Unchanged
10-2a	45–75	On 3–4 Off 3–4	35.3±2.2 48.3±3.5	On 3–5 Off 2–4	25.4±2.3 41.4±4.2	+2–7*	+7–13	Unchanged	Unchanged
10-3a	30–75	On 3–5 Off –	35.3±2.2 –	On 1–3 Off 2–4	38.5±4.4 42.0±4.4	+25–40[b,*]	+60[b,*]	Blurred	Unchanged
Ventral giant interneurones									
8-1a	2–10	On 3–7 Off –	25.6±1.8 –	On 2–3 Off 1–2	28.3±2.0 44.8±2.2	–30–60*	–50*	Unchanged	Unchanged
9-1a	2–6	On 1–3 Off 0–1	26.5±1.3 42.1±4.5	On 1–2 Off 1–2	26.4±5.5 48.9±1.3	–70*	–30–70*	Less specific	Unchanged
11-1a	20–30	On 9–12 Off –	33.8±3.2 –	On – Off –	– –	–10–30*	–30*	More specific	Unchanged
Small ascending interneurones									
AS1	2–5	On 1–2 Off –	25.5±3.5 –	On 2–3 Off –	34.5±3.5 –	+20–40*	+20–40*	Blurred	i 22.0±9.9 c 37.5±16.3
AS2	45–60	On† 2–3 1–2 Off 1–2	28.3±6.0 44.8±5.4 26.8±1.4	On 1–2 Off 1–3	27.0±1.3 36.2±3.6	+1–5*	–	Unchanged	Unchanged

i, ipsilateral; c, contralateral; b, change before start of walking; †two spike bursts in response to stimulus onset; *significant difference between standing and walking phases ($P<0.05$).

Values for latency are meann ± S.D.

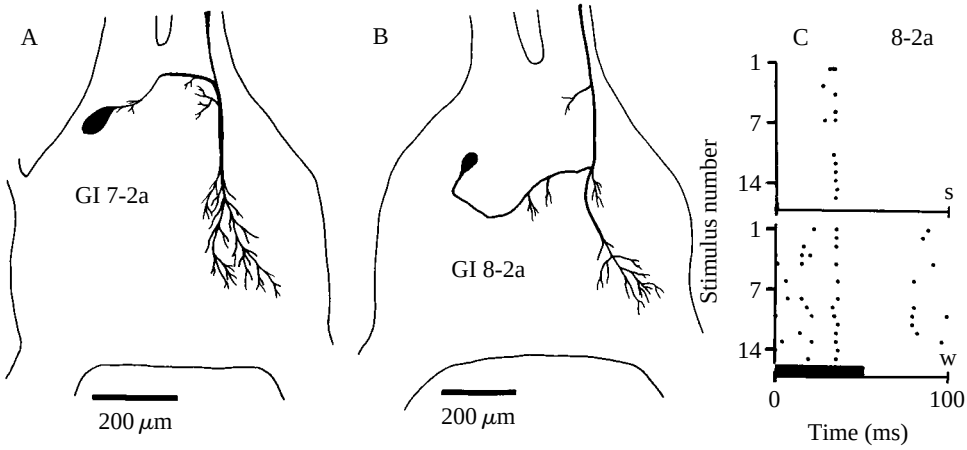


Fig. 2. Morphology and physiology of the GIs 7-2a and 8-2a. (A) Reconstruction of GI 7-2a. (B) Reconstruction of GI 8-2a. (C) The PSTD (each dot indicates one spike) of the responses of GI 8-2a to ipsilateral wind puffs applied to the standing (s) and to the walking (w) animal reveals the occurrence of additional spikes during walking; black bar, stimulus marker.

which were often followed by rebound periods of 50–100 ms without spikes. GI 10-2a reflected wind-puff stimulation in its discharge pattern. During walking phases, the mean spontaneous spike rate was slightly increased by about 2–7 % (Fig. 4C). Responses to wind puffs were very similar to those in standing phases: only a few additional spikes led to an increase in mean frequency of 7–13 % (Fig. 4B).

GI 10-3a (Fig. 5A) had a spontaneous spike frequency of 30–75 Hz in the resting animal. The activity in the neurone coupled to the rhythm of air-puff stimulation, but its mean spike rate remained constant. The beginning of each ipsilateral stimulus in a continuous series caused one or two spikes after a latency of 35.3±2.2 ms. Apart from the first puff, however, most stimuli were followed by action potentials after just 13.5±2.8 ms. These could not be caused by the actual stimulus, since it reached the filiform hairs about 18–20 ms after triggering. Instead, the spikes were probably caused by the end of the preceding wind puff, giving a true latency of 63.5 ms (Fig. 5F).

During walking, the neurone discharged more irregularly, with bursts of instantaneous spike frequencies of up to 200 Hz. This led to an increase in mean spike rate of up to 60 % (Fig. 5F), which was weakly correlated to v_t (r=0.2, Fig. 5B) but not to v_r. Wind-puff representation was blurred by additional spikes occurring preferentially 20–40 ms after the end of each stimulus.

The spike rate of GI 10-3a was raised before the onset of locomotion. In one preparation where recordings were made from the most anterior parts of the dendrites of the neurone (recording site indicated by an arrow in Fig. 5A), the membrane potential after wind-puff stimulation was altered 50–100 ms before, as well as during, locomotion (Fig. 5C,D,E). This alteration made the membrane potential less negative because of an extended depolarization in two phases of the stimulus cycle. In the first of these phases, the decrease in membrane potential lasted about 29 ms during standing but about 25 ms before and during walking. Moreover, the slope was steeper during standing

($-0.41\,V\,s^{-1}$) than before and during walking ($-0.39\,V\,s^{-1}$ and $-0.36\,V\,s^{-1}$, respectively). In the second phase, the duration of the decrease in membrane potential lengthened from 6.2 ms (standing) to 7.7 ms (walking) and the slope was reduced from

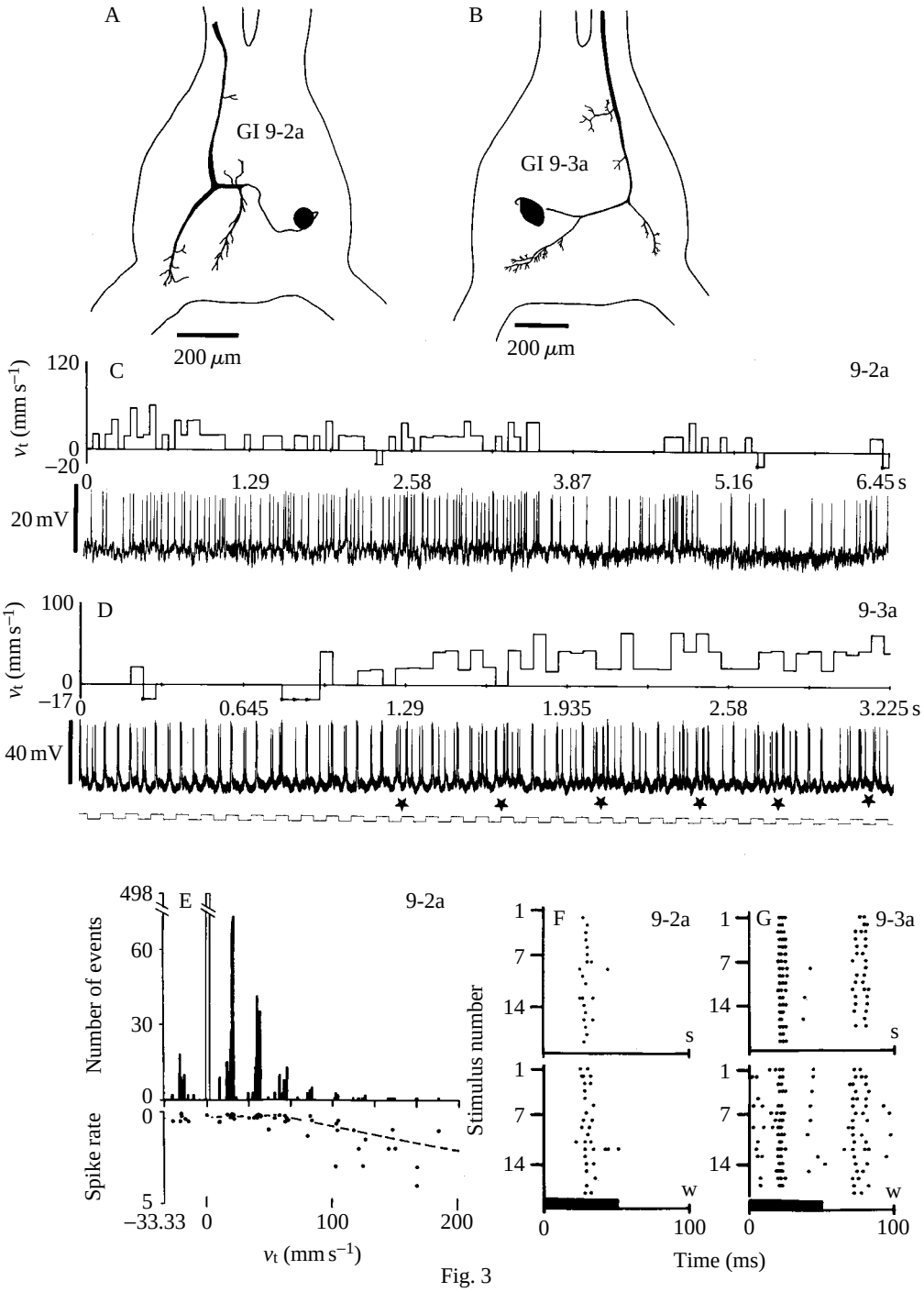


Fig. 3

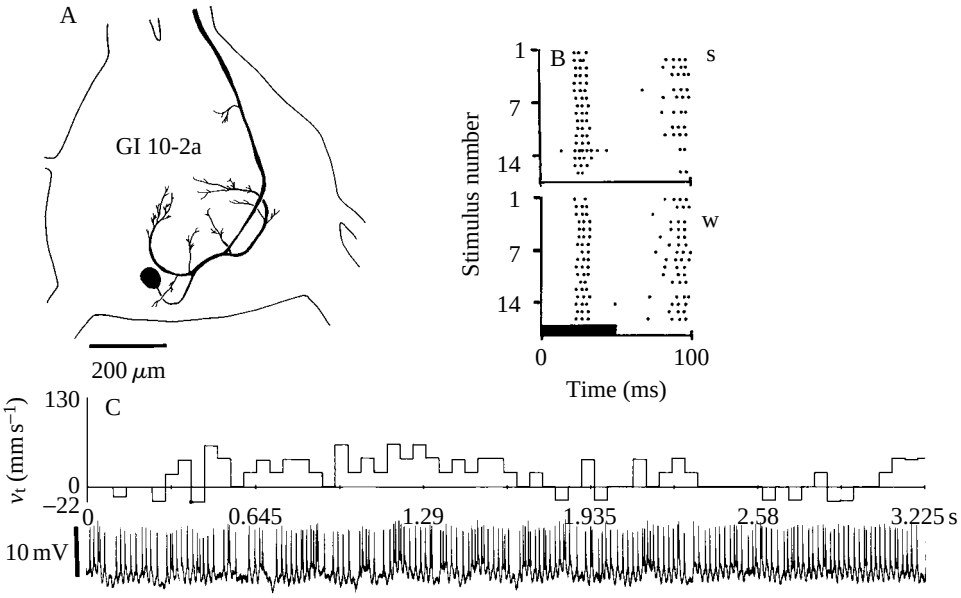


Fig. 4. Morphology and physiology of GI 10-2a. (A) Reconstruction of GI 10-2a. (B) When the animal walks during wind-puff stimulation, the neurone generates additional spikes, which are correlated to the stimulation and hardly alter the spike pattern compared with standing phases (contralateral stimulation; black bar, stimulus marker). (C) Without wind-puff stimulation, the spike frequency increases slightly during walking behaviour.

$-0.77\,\mathrm{V\,s^{-1}}$ (standing) to $-0.42\,\mathrm{V\,s^{-1}}$ (pre-walking) and $-0.31\,\mathrm{V\,s^{-1}}$ (walking). The entire decrease in membrane potential amplitude within this phase was reduced by 43 % immediately before walking and by 49 % during walking when compared with standing phases. As a common reference level, we used the most positive membrane potential at which spikes were observed. The membrane potential exceeded this reference level for 52 % of an average stimulus cycle during standing, for 86 % before walking and for 88 % during walking, thereby accounting for the enhanced spike rates.

GIs reducing their activity during walking

There are three GIs in this group, GI 11-1a and the two largest GIs within the TG, GI 8-1a (medial giant interneurone) and GI 9-1a (lateral giant interneurone).

Fig. 3. Morphology and physiology of GIs 9-2a and 9-3a. (A) Reconstruction of GI 9-2a. (B) Reconstruction of GI 9-3a. (C) Without wind-puff stimulation, the activity of GI 9-2a is higher during walking than during standing. (D) During walking, the responses to wind puffs in GI 9-3a are blurred. Some spikes (stars) occur in a temporal pattern similar to the step cycle of walking. (E) An increase of the spontaneous mean spike rate in GI 9-2a with increasing v_t is revealed by the histogram of v_t with corresponding mean spike rates (r<0.1 for v_t<50 mm s^{-1}, no significant correlation; r=0.46 for $v_t \geqslant 50$ mm s^{-1}, P<0.01). (F) Additional spikes during walking are also correlated with the stimulation, so the responses to wind puffs are broadened during walking compared with pauses (ipsilateral stimulation; black bar, stimulus marker). (G) PSTD of the responses of GI 9-3a to wind puffs applied to the standing (s) and walking (w) animal; additional spikes occur during walking (contralateral stimulation; black bar, stimulus marker).

GI 8-1a (Fig. 6A) had a spontaneous activity of 2–10 Hz in the standing cricket (six preparations); in one animal the spontaneous activity was 20–30 Hz. The spike rate increased to 30–45 Hz during wind-stimulation and the pattern synchronized with the wind-puff sequence in the standing animal. During walking, the spontaneous as well as the wind-evoked activities of GI 8-1a were diminished by roughly 50 % (Fig. 6C–G). The

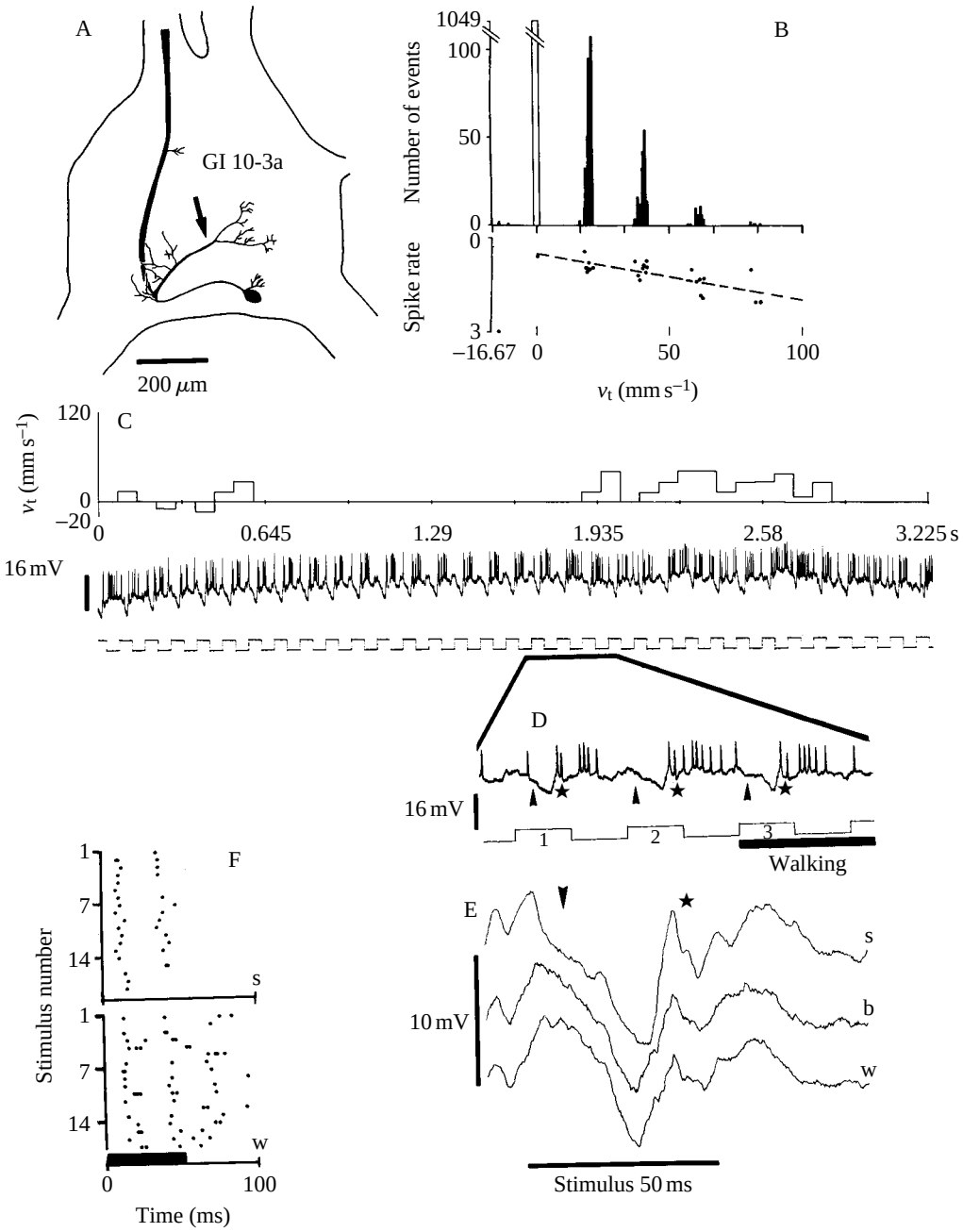


Fig. 5

EPSPs caused by the stimulation were smaller during walking than when standing (Fig. 6E). Therefore, spikes occurred preferentially during short pauses in locomotion (Fig. 6C–E).

GI 9-1a (Fig. 6B) had a spontaneous spike rate of 2–6 Hz in the standing animal. When wind-puff series were given, the neurone discharged in the stimulus rhythm (Fig. 6J). During walking, the spontaneous activity decreased by 70 % (Fig. 6H) and the response rate to air puffs was reduced by 30–70 % compared with that in standing phases (Fig. 6I,J). Immediately after a pause in locomotion, the full response of GI 9-1a to wind stimuli was frequently restored.

In the standing animal, GI 11-1a (Fig. 7A) had a spontaneous spike rate of 20–30 Hz, discharging in irregular bursts with momentary frequencies of up to 100 Hz (Fig. 7B). The spike rate was increased by stimulation with ipsilateral air puffs that caused a burst of 9–12 spikes after 33.8±3.2 ms. Contralateral wind puffs evoked only a slight increase in spike rate after about 41 ms. During walking, GI 11-1a reduced its mean spontaneous frequency by 10–30 % and membrane potentials became more negative. Thus, spike generation was prevented, or the spike frequency within the bursts (arrowhead in Fig. 7B) was reduced, during locomotion. Only 6–8 spikes per stimulus were generated (Fig. 7C,D). Compared with standing phases, this decrease resulted in a more precise representation of the stimulus in the spike train of the neurone (Fig. 7D).

Small ascending interneurones

The GIs are by no means the only fibres to carry information from cercal receptors to the anterior ganglia. Numerous interneurones that have small somata in the TG and send comparatively thin axons through the abdominal nerve cord respond to cercal stimulation (Dagan and Parnas, 1970; Heußlein, 1989). Although these neurones do not represent a homogeneous group, they are collectively referred to as ‘small ascending interneurones’ (SAIs). We present two examples of SAIs to demonstrate their individually different

Fig. 5. Morphology and physiology of GI 10-3a. (A) Reconstruction of GI 10-3a. The arrow marks a recording site discussed in the text. (B) The histogram with corresponding mean spike rates (spikes per time interval) reveals an increase of the mean spike rate with v_t during locomotion in GI 10-3a (no wind puffs given, r=0.21, P<0.01). (C) The intracellular recording of GI 10-3a shows a change in activity even before the cricket starts to walk. (D) Enlarged representation of the segment marked in C, showing the reaction to three wind puffs (1, 2, 3) applied to the animal during standing, immediately before walking and during walking. There are distinct alterations in membrane potential just before and during walking. At two positions of the stimulus cycle (indicated by arrowheads and stars), the depolarization lasts longer and the membrane potential remains higher than in the standing animal. This leads to the generation of more spikes. (E) Membrane potentials of GI 10-3a during wind-puff stimulation were evaluated by removing the spikes from the recorded signal and, thereafter, by averaging the responses in the standing animal (s; N=26), 50–100 ms before the start of walking (b; N=11) and during walking (w; N=21). Even before walking begins, the membrane potential is raised and becomes further enhanced during walking (horizontal bar, stimulus marker). (F) During walking, GI 10-3a generates additional spikes, which blur the representation of wind puffs as observed in the standing animal (preparation different from that shown in C–E; ipsilateral stimulation; black bar, stimulus marker).

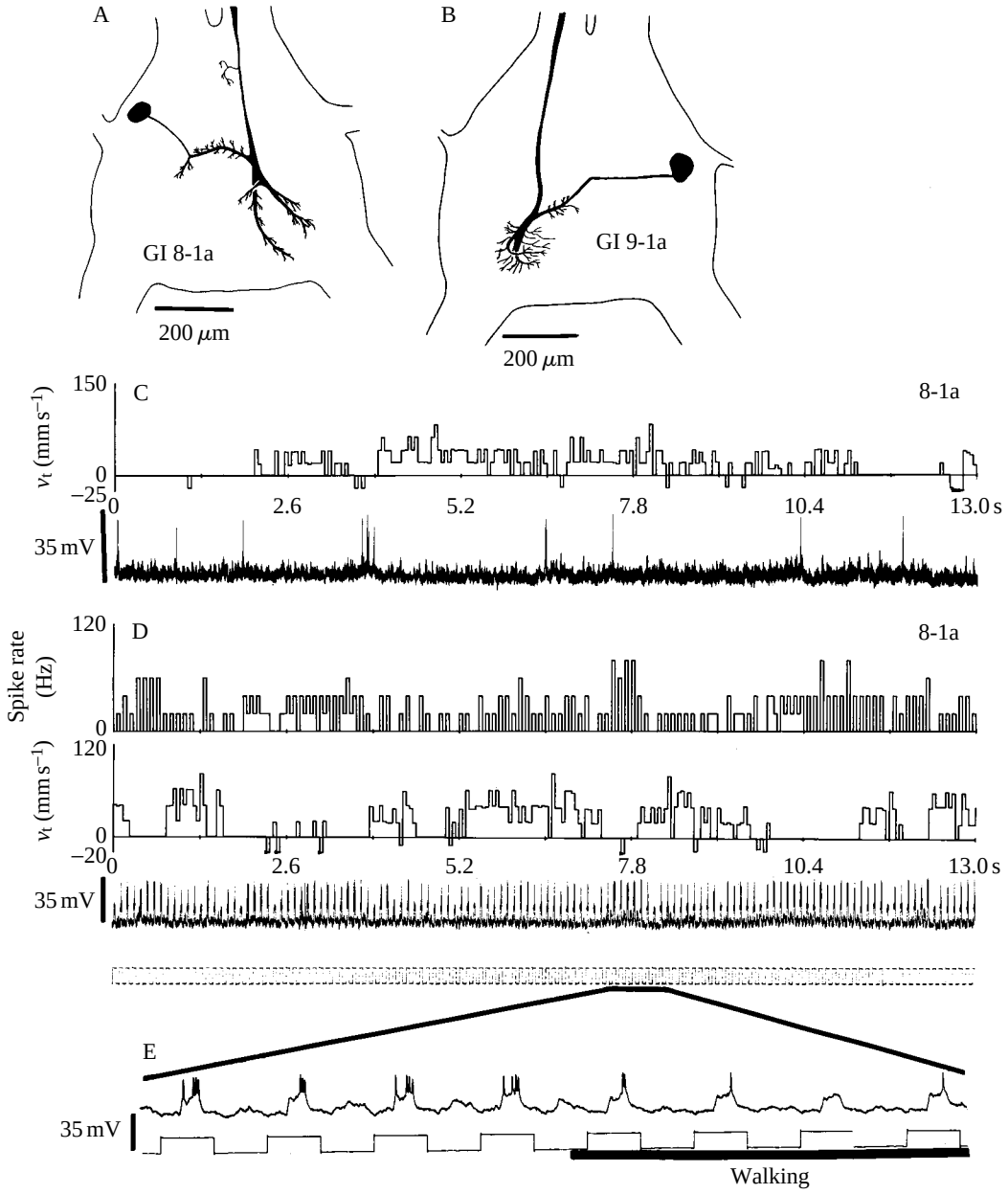
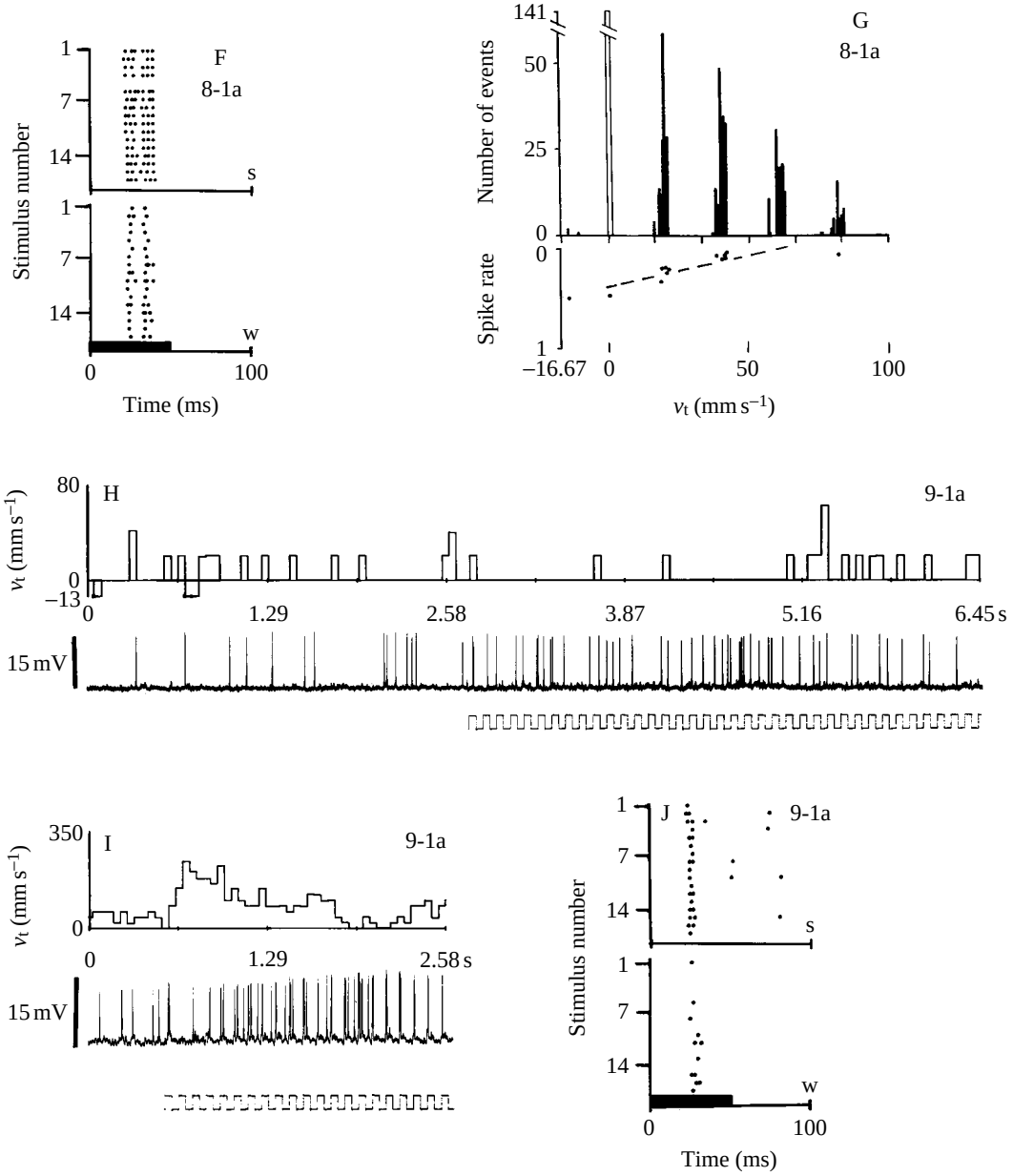


Fig. 6. Morphology and physiology of GI 8-1a and GI 9-1a. (A) Reconstruction of GI 8-1a (medial giant interneurone). (B) Reconstruction of GI 9-1a (lateral giant interneurone). (C) Without wind-puff stimulation, GI 8-1a generates spikes preferentially when the animal stands still. (D) The response of GI 8-1a to wind puffs mostly consists of 2–4 spikes per stimulus in the standing animal. During locomotion, this reaction is often reduced to only 1 spike per stimulus or even fails completely. Top trace, mean spike frequency of consecutive 50 ms intervals. (E) The sequence marked in D reveals the reduction of activity in GI 8-1a during walking. EPSPs are smaller during locomotion than during standing. (F) Spike response to wind puffs is reduced in GI 8-1a during walking (w) compared with standing (s) (preparation different from that shown in C–E; ipsilateral stimulation; black bar, stimulus



marker). (G) The histogram of v_t with corresponding mean spike rates reveals a significant reduction of spontaneous spike rate during locomotion in GI 8-1a ($r=-0.27$, $P<0.01$). (H) During spontaneous walking, GI 9-1a generates spikes in response to the first wind puff of a series but thereafter fails to react reliably to the stimulation. Further stimuli evoke spikes preferentially when the cricket walks very slowly or stops walking. (I) The onset of a wind-puff series evokes fast running in the animal (notice changed ordinate scale). While GI 9-1a reacts to the first wind puff by generating three spikes, it fails to respond thereafter. Responses are enhanced again when the animal walks slowly and stops walking. (J) The PSTD reveals the strong reduction of spike activity in GI 9-1a during walking compared with the activity during standing (ipsilateral stimulation; black bar, stimulus marker).

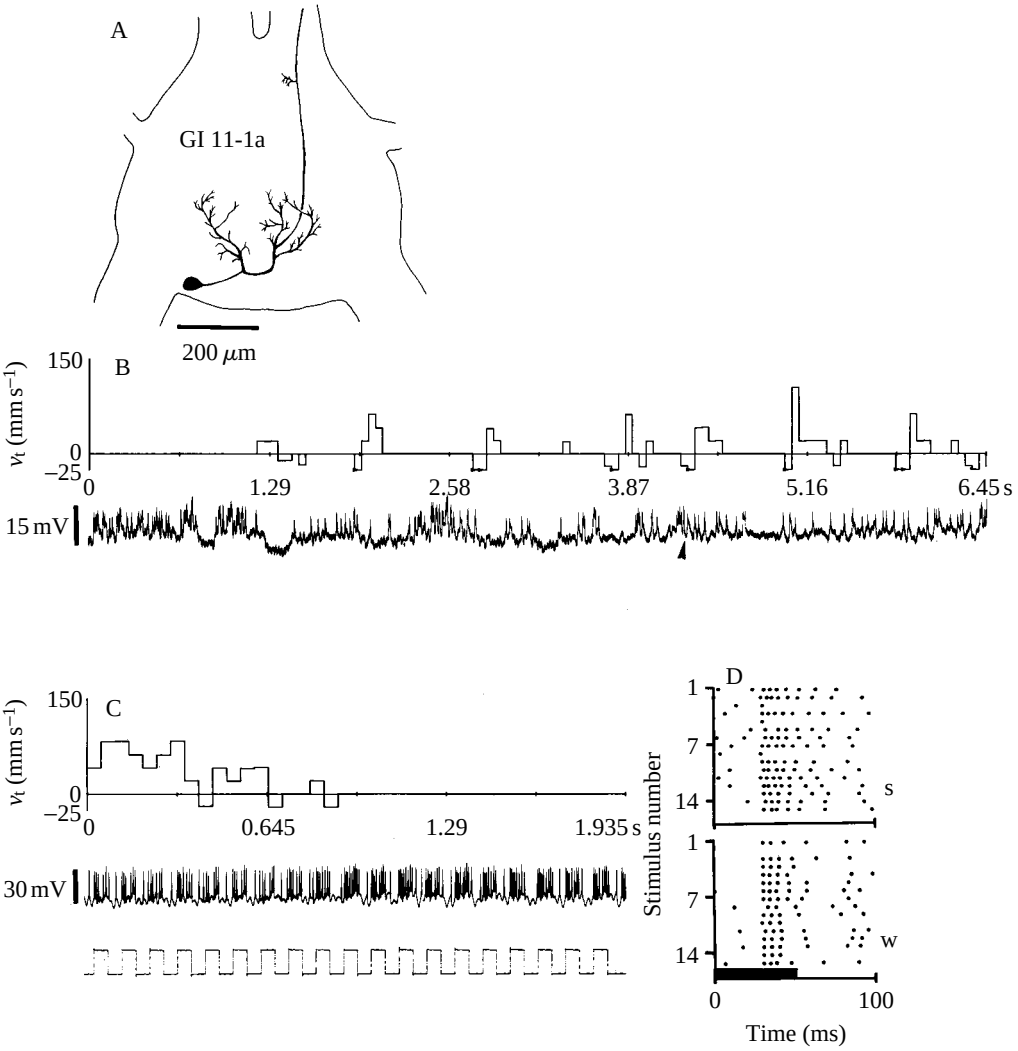


Fig. 7. Morphology and physiology of GI 11-1a. (A) Reconstruction of GI 11-1a. (B) Intracellular recording from the soma of GI 11-1a. In this preparation, the neurone discharges in irregular bursts, with the highest spike frequencies occurring in the standing animal. At the onset of walking, the neurone is hyperpolarised, so spike bursts often end or existing spike activity is reduced (arrowhead). (C) In another preparation, the recording was made from a dendrite. During locomotion, there are fewer spikes per stimulus than in the standing animal. (D) The PSTD confirms the reduction of spike activity in the walking (w) animal (s, standing phase; ipsilateral stimulation; black bar, stimulus marker).

relationship to walking behaviour. These two interneurones, which were named AS1 and AS2, have not, to our knowledge, been described previously.

Interneurone AS1 (Fig. 8A) had a small soma (diameter 15 μm) lying dorsally in the posterior half of the ganglion. Dendritic arborizations projected to the cercal glomeruli (Bacon and Murphey, 1984) in both hemispheres of the TG and an axon in a dorsal position

passed through the nerve cord at least as far as the third abdominal ganglion. In the standing animal, AS1 showed a spontaneous spike rate of 2–5 Hz. When stimulated by a wind-puff series, the spike rate increased and the neurone discharged in the pattern of the stimulation.

During walking, the spontaneous spike rate increased by 20–40 % (Fig. 8C,E) and the response to wind puffs was distinctly altered compared with that during standing. The mean spike frequency of the neurone was enhanced and additional spikes blurred the pattern of discharge (Fig. 8D,F). AS1 differed from all GIs, because response delay to wind puffs changed during locomotion. This latency was constant in the GIs, but it changed in AS1 in a contrary manner for ipsilateral and contralateral stimuli (Table 1): response latency to ipsilateral wind puffs decreased to 22.0 ms, but increased to 37.5 ms after contralateral stimulation. The standard deviation rose significantly as the response bursts broadened during locomotion.

The soma of AS2 (Fig. 8B) had a diameter of 17 μm and was positioned dorsally near the root of the cercal nerve. The neurone spread dendritic arborizations in the soma-contralateral posterior quarter of the ganglion, while the axon passed ventrally and gave off a small ramification within the seventh or eighth neuromere of the TG. In the standing animal, the spike frequency without stimulation was 45–60 Hz. When wind puffs were applied, the mean spike rate remained unaltered as the neurone coupled to the stimulation rhythm. Ipsilateral air puffs evoked a twin-peaked on-response (Fig. 8G). Walking had hardly any effect on the activity of the neurone: the spontaneous spike rate was enhanced by no more than 5 % (Fig. 8H) and reactions to wind puffs remained unchanged during walking.

Discussion

For the first time, correlations between walking behaviour and the activity of elements in the cercal wind-sensitive system have been investigated in crickets using a technique which allowed both quantitative measurements of locomotion and intracellular recordings of neurones in the TG. The diagram in Fig. 9 combines essential data obtained on filiform hair receptors and GIs in this study with previously demonstrated or suggested anatomical connections and physiological functions of the system. We will discuss these relationships in detail, focusing primarily on the GIs.

Response properties of GIs to wind puffs

Eight of the GIs (dGIs 8-2a, 9-2a, 9-3a, 10-2a, 10-3a; vGIs 8-1a, 9-1a and 11-1a) recorded in this study receive their main input from sensory cells of filiform hairs (Murphey *et al.* 1977; Palka and Olberg, 1977; Heußlein, 1989). All these GIs react distinctly to wind-puff stimulation with latencies of 22–35 ms. Part of this latency is a delay of 18–23 ms, which is due to the propagation of the air puff from the stimulus source to the cercal receptors. Thus, the physiological latency between displacement of the filiform hairs and the occurrence of stimulus-correlated spikes in the interneurones is 2–12 ms, which fits in well with data reported by other investigators (Palka and Olberg, 1977; Bacon and Murphey, 1984). These considerations also apply to the small ascending interneurones studied, AS1 and AS2.

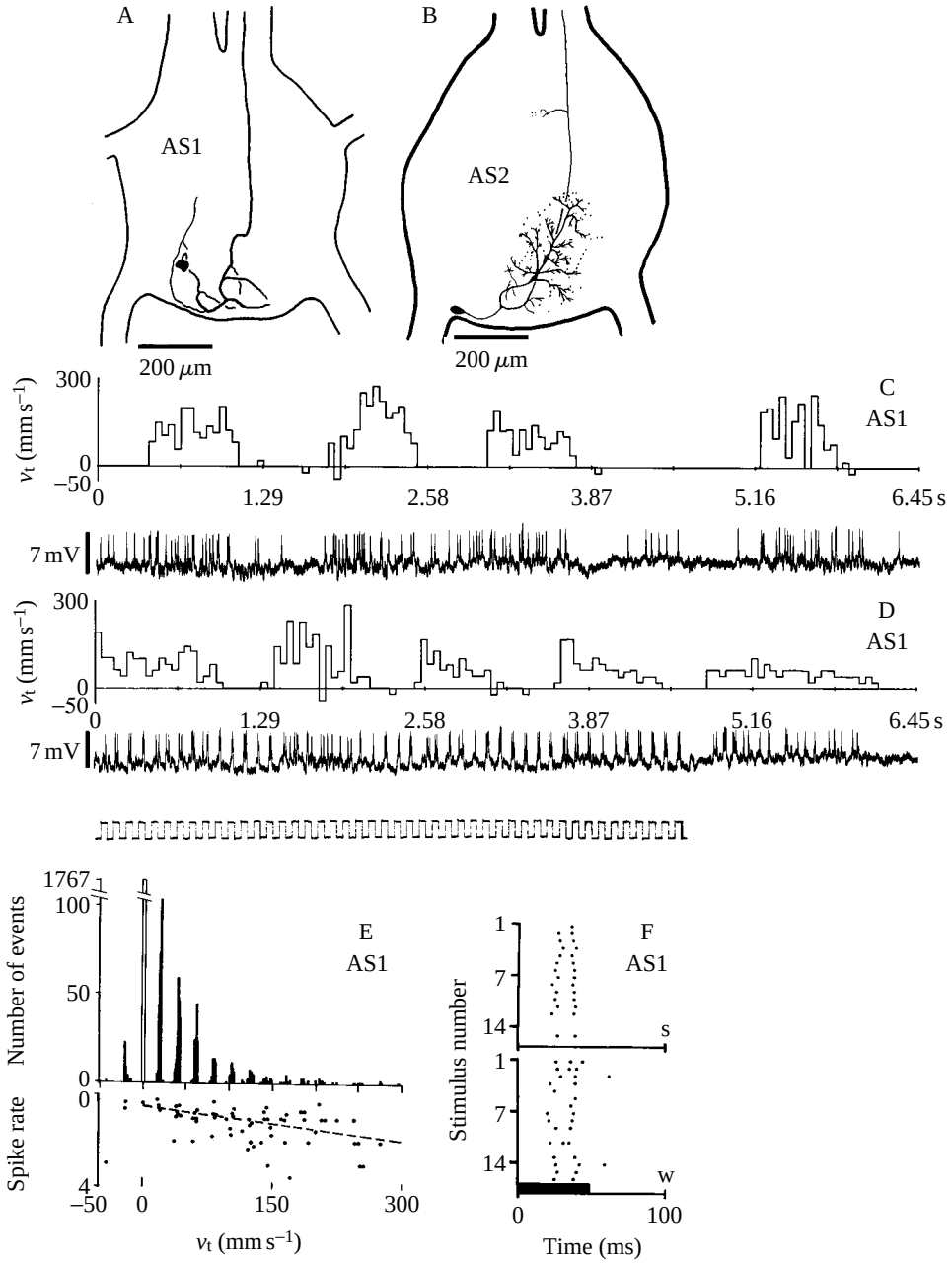


Fig. 8A–F

In contrast, GI 7-2a responds weakly to wind puffs and changes its activity during walking only slightly. It is the only dGI we recorded from that receives its main input not from filiform hair receptors but from clavate hairs (Sakaguchi and Murphey, 1983). These gravity receptors are positioned on the cerci and yield information about the spatial

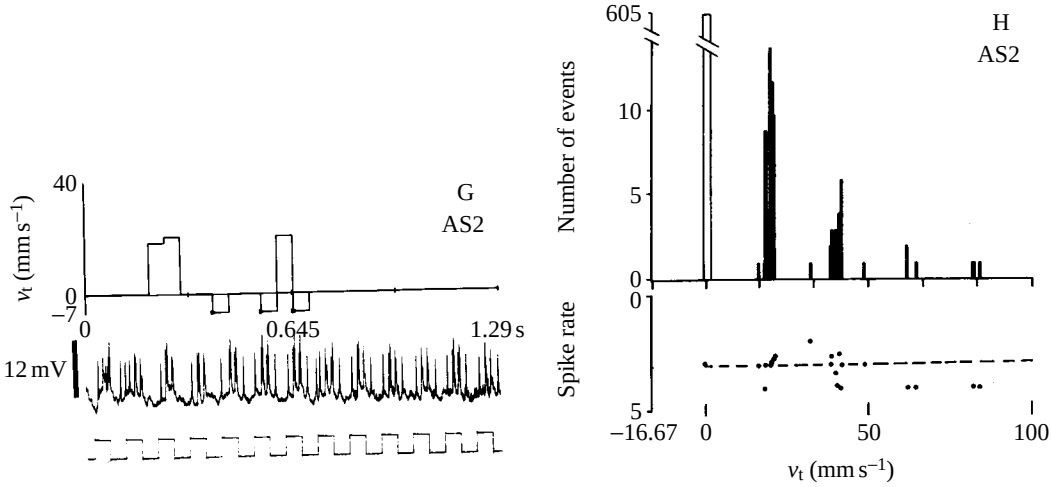


Fig. 8. Morphology and physiology of AS1 and AS2. (A) Reconstruction of AS1. (B) Reconstruction of AS2. (C) During spontaneous walking the spike rate in AS1 is increased. (D) In AS1, the representation of wind puffs is blurred during walking by additional spikes and the mean spike rate is increased. (E) The histogram of v_t with corresponding mean spike rates illustrates the increase in spontaneous spike rate in AS1 with faster v_t during walking (r=0.26, P<0.01). (F) The neurone responds to wind puffs applied to the standing animal more precisely than to those applied to the walking animal (ipsilateral stimulation; black bar, stimulus marker). (G) Responses of AS2 to wind puffs. Locomotion does not alter the spike sequence. (H) The histogram with corresponding spike rates reveals a slight increase in spike rate with enhanced v_t (r<0.1, not significant).

position of the animal (Horn and Föller, 1985). This fact may explain the weak reaction of the neurone to air puffs.

Correspondence of physiological and morphological characteristics of GIs

The GIs are classified into two groups according to the changes in their activity during walking. These physiological attributes correlate strictly with morphological characteristics described by Jacobs (1984) and Jacobs and Murphey (1987). All *ventral* giant interneurones, which arise from embryonic cluster 1 and send axons through the ventral tracts of the TG, reduce their activity during walking. In contrast, *dorsal* giant interneurones, which originate from cluster 2 or 3 and project in dorsal tracts, increase their spike rate during locomotion. Similarly, vGIs and dGIs of cockroaches (Roeder, 1948; Daley *et al.* 1981) show the opposite physiological characteristics with respect to their activity during locomotion: vGIs are suppressed during walking and flying, while dGIs are excited (Daley and Delcomyn, 1980).

Central and peripheral modulation of GI activity during walking

During walking, leg movements generate air turbulence that is likely to contribute to a reafferent input *via* the cercal receptors to the GIs. The filiform hair receptors of cockroaches and locusts are inhibited by different mechanisms to avoid reafferent

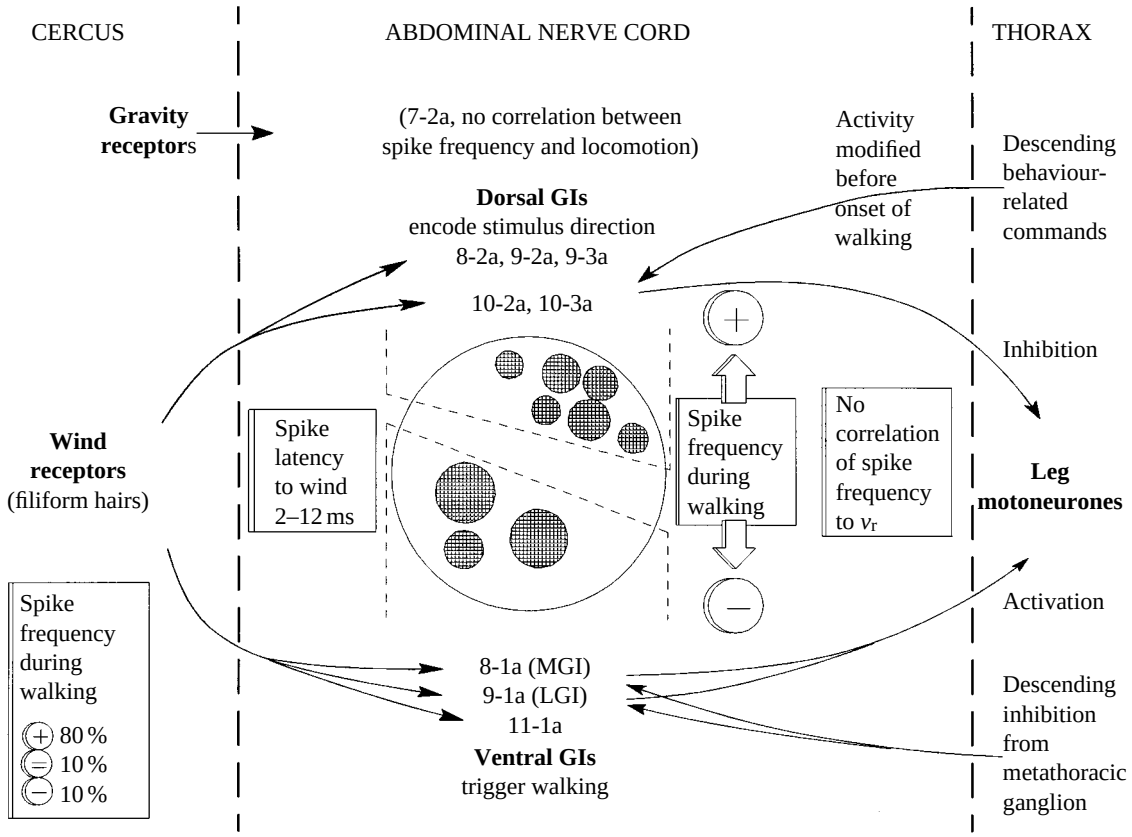


Fig. 9. Representation of the interdependence of cercal receptor cells and ascending interneurones of the terminal ganglion with walking behaviour and their presumed connections and functions within the nervous system of crickets. The scheme shows identified and presumed functional connections between abdominal and thoracic ganglia. It is based on the results of our investigation and on data adopted from previous studies referred to in the text. GI, giant interneurone; v_r, rotational velocity; LGI, lateral giant interneurone; MGI, medial giant interneurone. +, –, =, indicate increase, decrease and constancy of spike frequency in wind receptors and GJs.

excitation during flight and cercal movements (Libersat *et al.* 1987; Boyan, 1988). In crickets, however, there is no indication of any peripheral mechanism acting on sensory cells to reduce that reafferent excitation. The spontaneous spike rate of 80 % of the sensory cells is rather higher during walking than in the standing animal, and the receptors also generate accessory spikes during wind-puff stimulation of a walking cricket. This probable reafferent input to the interneurones could cause an increase in their activity, as was found in the dGIs. The step duration of slowly walking crickets in our experiments is about 200–350 ms (Gras and Setzkorn, 1993). This time interval coincides with the frequency of additional spikes (about 3 Hz) that have been observed in some dGIs (see Fig. 3D) during locomotion.

In addition to reafferent sensory input, central mechanisms are also likely to cause excitation during walking. In GI 10-3a, we found a distinct increase in membrane

potential preceding the onset of walking by 50–100 ms, which cannot be attributed to reafferent input from leg movements. The resulting increase in spike rate before the start of locomotion is maintained during walking. Similarly, in cockroaches the dGIs raise their activity during walking, but cutting the ventral nerve cord markedly attenuates this effect (Delcomyn and Daley, 1979). When reafferent input is subsequently prevented by covering the cerci with petroleum jelly, the dGIs show no more activity than in the standing animal (Daley and Delcomyn, 1980). This reveals a simultaneous influence of both peripheral and central mechanisms on the dGIs during locomotion.

A central mechanism within the central nervous system may also act on the vGIs, which reduce their activity during walking despite the enhanced activity of filiform hair receptors transmitting to them. At the onset of locomotion, the membrane potential of GI 11-1a becomes more negative and EPSPs in GI 8-1a are diminished. The generation of spikes, spontaneous or in response to wind puffs, is thus prevented or markedly decreased. On the basis of extracellular recordings from the ventral nerve cord, Murphey and Palka (1974) described a similar reduction of GI 8-1a and GI 9-1a activity during walking. Cutting the ventral nerve cord posterior to the metathoracic ganglion (MTG) resulted in uniform activity during walking and pauses, suggesting to these authors that there was an inhibitory pathway descending from the MTG and acting on GI 8-1a and GI 9-1a.

It should be noticed, however, that vGIs 8-1a and 9-1a are about 100 times less wind-sensitive than most of the dGIs in immobilized specimens (Kanou and Shimozawa, 1984; Kanou, 1991). In contrast to our results, a reduction of spike activity seems to be more appropriate in dGIs than in vGIs to prevent transmission of reafferent input induced by walking.

Functional aspects of GI activity during walking

With respect to locomotion, our study is restricted to spontaneous walking and slow walking during, or induced by, wind-puff stimulation. Sequences of fast escape running evoked by wind stimulation have been excluded from analysis. This escape behaviour of *Gryllus bimaculatus* consists of a succession of short constant running bouts and variable pauses (Gras and Hörner, 1992; Gras *et al.* 1994), but is prone to distinct habituation. Generally, GIs function as triggering elements for escape and defensive reactions in crickets (Gnatzy and Kämper, 1990), cockroaches (Camhi and Nolen, 1981), locusts (Boyan and Ball, 1989) and other orthopteran insects (Shen, 1983; Boyan and Ball, 1986). In cockroaches, the initial evasive turn in response to wind puffs is presumably triggered by the vGIs (Camhi and Nolen, 1981). These neurones transmit to a group of metathoracic interneurones that drive the motoneurones of leg muscles (Ritzmann and Pollack, 1986). For crickets, Kanou and Shimozawa (1984, 1985) attribute an indirect excitation of thoracic motoneurones to the two largest interneurones, vGIs 8-1a and 9-1a. They are presumed to initiate the behavioural escape reaction. Appropriately, these escape-triggering elements in both cockroaches and crickets reduce their spontaneous activity and also their responses to cercal stimulation during walking. For the dGIs, which enhance their activity during locomotion, no excitatory connection to leg motoneurones has been reported.

In *Gryllus bimaculatus*, many interneurones with somata in thoracic ganglia or in the brain exhibit a change of sensitivity to cercal wind stimuli similar to that of the GIs. Thoracic interneurones often fail to react to cercal stimulation during walking, while during short walking pauses their responsiveness to stimulation is restored and even enhanced compared with that in the inactive animal (Hörner *et al.* 1989; Gras and Hörner, 1992; Hörner, 1992). These neurones may either depend on the vGIs to receive cercal input or experience the same kind of central modulation as do the vGIs. In contrast, some descending brain neurones that influence locomotion show a functional similarity with dGIs, because they are more responsive to cercal stimulation during walking than during standing (Böhm and Schildberger, 1992).

One important variable of a wind stimulus is its direction, which is encoded mainly by the dGIs (Kämper, 1984). In our experiments, we found no preferred walking direction with respect to the stimulus; orientation seemed to be accidental. Additionally, the spike frequency of the GIs and the actual v_r of the animal are not correlated. At high v_r to either side, some dGIs (9-3a, 10-3a) enhanced their spike frequency, possibly because of air turbulence caused by leg movements. While vGIs are not likely to guide walking direction because of their inexact representation of stimulus direction (Kämper, 1984), the activity of the dGIs does not seem to influence the direction of slow walking either. However, in cockroaches they connect *via* thoracic interneurones to leg motoneurones of the ipsi- and contralateral leg (Ritzmann and Camhi, 1978) and thereby contribute to the guidance of evasive turning away from a possible predator during escape behaviour (Camhi and Nolen, 1981). Crickets also tend to orientate away from the stimulus during an escape reaction (Gras and Hörner, 1992; Gras *et al.* 1994). The output of the dGIs, which carries information on the stimulus direction, should be involved more directly in the control of walking direction during escape behaviour than during spontaneous walking.

Small ascending interneurones

Within the terminal ganglion (TG) of orthopteran insects, not only GIs but also numerous smaller ascending interneurones react to cercal stimulation (Dagan and Parnas, 1970; Boyan *et al.* 1989; Heußlein, 1989). Two previously undescribed SAIs of the cricket are described in this study. During walking, these are affected in different ways: AS1 distinctly enhances its spike rate while AS2 hardly does so at all. The only other neurone with nearly constant activity in our experiments was GI 7-2a, which is known to receive its main input from the sensory cells of clavate hairs (Sakaguchi and Murphey, 1983). In contrast, AS2 is most probably driven by filiform hair receptors, a conclusion drawn from the position of its dendritic arborizations and its strong reaction to wind puffs.

Unlike AS2, the spike rate of AS1 is enhanced by up to 40 % in walking specimens. Additionally, it is the only neurone studied that alters its response latencies to wind puffs in a behaviourally dependent manner. In AS1, the representation of cercal stimuli is not as well maintained during walking as it is in the GIs. Filiform hair receptors, which are strongly affected by leg movements, probably have a predominant influence on AS1.

Non-giant interneurones of cockroaches have been shown to drive leg motoneurones within the metathoracic ganglion (Dagan and Parnas, 1970). In crickets, SAIs have only

been investigated with respect to their responsiveness to cercal stimulation (Kämper, 1984; Heußlein, 1989) and nothing is known about their morphology and connectivity within the nerve cord. Summing up, AS1 and AS2 react to wind puffs in a similar way to the GIs (except for GI 7-2a), while alterations of their activity during walking distinguish them from the vGIs and dGIs and from one another. Although AS1 and AS2 are typical examples of small wind-sensitive interneurones that ascend from the TG to anterior ganglia, further SAIs with different functional modifications during locomotion may exist.

Our data reveal that the activity of interneurones ascending from the terminal ganglion contributes to the complex nervous processing within the thoracic ganglia and the brain and thus it influences the 'decision' of the animal to perform a behaviour pattern such as walking. In contrast, the activity of the cercal sensory system is determined not only by the input from the receptor cells but also by central mechanisms that control information transmission in a behaviourally dependent manner. Investigations aimed at a more complete understanding of the interrelationship between the behavioural status and the processing of wind stimuli in the ventral nerve cord are continuing. Preliminary observations reveal that local non-spiking interneurones in the TG, which respond to air puffs, are modulated in a walking-dependent manner (Kohstall-Schnell and Gras, 1992; D. Kohstall-Schnell, unpublished observations). This indicates that the cercal-receptor-to-interneurone system of *Gryllus bimaculatus* may be functionally even more complex than is suggested by the results of the present study.

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