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Current injection into interneurons of the terminal ganglion modifies turning behaviour of walking crickets

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Abstract Ascending interneurons of the terminal ganglion of orthopterous insects are known to carry information on wind stimuli perceived by cercal receptors to thoracic and cephalic ganglia. Neurons of these anterior ganglia control evasive walking behaviour. We demonstrate that current injection into individual wind-sensitive local non-spiking interneurons and ascending giant interneurons of the terminal ganglion can influence the orientation behaviour of walking crickets. To induce a change of turning during “wind puff stimulation” by current injection into the lateral giant interneuron, its spike activity has to be modified by at least 100%. In 5 of 12 different types of non-spiking interneurons a moderate shift of the membrane potential results in a change of the mean speed of rotation and/or the frequency of turns. All preparations tested with different amounts of current injection showed a proportional change of turning frequency. Normally, the turning behaviour is evasive with respect to the wind source. During current injection this dependence is preserved, but the general orientation is readjusted. Taking into account known connections between some of these interneurons and ascending neurons the tested wind-sensitive local non-spiking interneurons of the terminal ganglion are likely to impose an offset on the mean direction of orientation controlled by cephalic and thoracic neuronal networks.

Key words Orientation · Walking · Local non-spiking interneuron · Giant interneuron · *Gryllus bimaculatus*

Abbreviations *GI* giant interneuron · *LI* local interneuron · *LNI* local non-spiking interneuron · *LSI* local spiking interneuron · *SAI* small ascending

interneuron · *TG* terminal ganglion · v_{Rot} rotatorial velocity · v_{Trans} translatorial velocity

Introduction

Crickets – like cockroaches and many other orthopterous insects – are extremely sensitive to air movements and low-frequency sounds which are perceived by numerous filiform hairs on two abdominal appendages, the cerci. Deflection of a hair evokes a change of spike activity in a mechanoreceptive sensory cell at its base, whose axon projects to the terminal ganglion (TG) of the ventral nerve cord. There, some bilateral pairs of giant interneurons (GIs) receive input from numerous hair receptors and ascend to the thoracic ganglia and the brain (Edwards and Palka 1974; Mendenhall and Murphey 1974; Jacobs and Murphey 1987; Heußlein 1989). Together with small ascending interneurons (SAIs) (Baba et al. 1991; Kohstall-Schnell and Gras 1994) they carry information about strength and direction of a wind stimulus necessary to induce and direct an appropriate behavioural reaction. Unrestrained crickets will respond to wind puffs with a turn away from the stimulus source and with short running bouts (Gras et al. 1989). This evasive behaviour occurs also in specimens mounted on a walking sphere to measure the intended locomotion (Gras and Hörner 1992).

In cockroaches, the escape reaction after wind puff stimulation is faster and more extended but otherwise similar to the reaction of crickets (Gras et al. 1994). The functional importance of this behaviour has been shown by Camhi et al. (1978), who found that air displacement caused by an approaching toad is sufficient to trigger escape after an initial rotation away from the predator, a “contraversive” turn as termed by Ye and Comer (1996). According to a mechanism proposed by Liebenenthal et al. (1994) and Levi and Camhi (1995) the turning direction is determined by a comparison of total spike activity in the GIs of the left side versus that one in the GIs of the

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right side of the nerve cord. Depolarization of a single GI, which evokes a moderate increase of its spike rate and results in an only slight imbalance of the bilateral GI activities, is reported to induce the cockroach to shift its orientation to the side contralateral to the stimulated axon.

In the course of an extended study on the mutual interactions of locomotion and neuronal activity in the TG we recorded intracellularly from all GIs of the cricket *Gryllus bimaculatus* and measured simultaneously the intended walking behaviour (Kohstall-Schnell and Gras 1994; Kohstall 1996). However, in only one case were we able to influence the orientation of a cricket by current injection into a GI, as will be shown in this paper.

Local interneurons (LIs) in the TG represent another component of the wind-sensitive neuronal network. There are at least 27 types of LIs which receive direct or indirect sensory input from the cercal filiform hairs and convey excitatory output to GIs and SAIs (Bodnar et al. 1991; Bodnar 1993; Baba et al. 1995; Kohstall 1996). Some of these LIs – the local spiking interneurons (LSIs) – generate action potentials spontaneously and during wind stimulation, while in others – the local non-spiking interneurons (LNIs) – spiking does not occur even during and after experimental de- or hyperpolarization. In the standing cricket, LNIs and LSIs show a complex and individually different sensitivity to wind from various directions around the animal. During locomotion many of the LIs modify their background activity (spike rate, mean membrane potential) and their response to wind (Gras and Kohstall 1995; Kohstall 1996). We will demonstrate in this paper that some of the LIs also influence the orientation of a walking cricket. During repetitive wind stimulation, hyper- and depolarizing current injection gradually modulates the walking behaviour in a reproducible manner. Presumably this effect is caused by a divergent information transfer from the LIs to many ascending interneurons, SAIs as well as GIs. While only small but significant effects of LI activity are observed in individual postsynaptic GIs (Bodnar 1993; Kohstall 1996), the simultaneous modification of the spike rate of numerous ascending interneurons may induce the visible change of walking behaviour. Our results indicate that some LNIs tonically influence the general orientation of walking. Onto this base level of rotation evasive turning is added during unilateral wind stimulation.

Materials and methods

Preparation and electrophysiology

Adult crickets (*G. bimaculatus* de Geer) of both sexes were used in the experiments. After clipping of the wings the insect was glued with its ventral side onto a brass holder from the mesothorax to the end of the abdomen, while the cerci remained free and could be moved normally. The abdomen was opened dorsally and the intestine and reproductive organs were removed. The TG was slightly

lifted onto a silver platform which also served as reference electrode for intracellular recording. Microelectrodes tip-filled with 5% lucifer yellow in $0.1 \text{ mol} \cdot \text{l}^{-1}$ LiCl had a resistance of 50–80 M Ω , through which currents could be injected into the neurones to: (1) verify the absence of spikes in LNI (–6 to +6 nA), (2) exert effects on the overall turning behaviour (–6 to +5 nA, 4 to 15 s), and (3) eject lucifer yellow at the end of the experiment to stain the neurone (–2 to –20 nA, 5 to 30 min). The recordings were stored on tape (Racal 4DS FM recorder) and documented with a digital paper chart recorder (Picker Uniscript 2108). The nomenclature of the interneurons follows the rules defined by Mendenhall and Murphey (1974) and Kohstall (1996).

Behaviour

During the electrophysiological experiments the crickets were mounted on top of a hollow styrofoam sphere (mass 2.4 g, diameter 120 mm) carried by an air cushion. Here, the insect performed normal walking behaviour on the spot. The resulting rotation of the sphere was optoelectronically detected. A personal computer stored the behavioural data together with markers for action potentials and stimuli and displayed the intended walking during the experiment. The translatorial velocity (v_{Trans}) of forward/backward movements and the rotatorial velocity (v_{Rot}) of left/right turns were computed off-line for consecutive intervals of 50 ms or 100 ms and correlated with the simultaneous spike activity or the graded potentials of the recorded interneurone. Furthermore, standardized criteria were applied to discriminate continuous walking and standing phases from short erratic movements which occur during grooming. Similarly, segments of continuous turning behaviour were automatically detected. A detailed description of the apparatus and the analytical methods can be found in Gras and Hörner (1992) and Gras et al. (1994). To quantify the experimental influence on orientation, we determined the mean v_{Rot} without and during current injection. Moreover, we counted the episodes of continuous rotation to one or the other side and computed the relative frequency of turns to the right side as $n_{\text{right}}/(n_{\text{left}} + n_{\text{right}})$. These two parameters have different functional meanings: in an unrestrained cricket the overall direction of orientation depends on the mean value of v_{Rot} , while the frequency and size of turns define the actual path of walking. Statistical significance of differences between means of v_{Rot} was tested with a *t*-test. The distributions of instantaneous v_{Rot} in 50-ms intervals as measured during injection of different amounts of current were compared with the Wilcoxon test. To compare the relative frequencies of turns to the right side we drew up 2×2 contingency tables to which the chi-squared test or, if necessary, Fisher's exact test was applied (Sachs 1974).

Stimulation

Wind stimuli were given as 10-Hz series of 50-ms pulses, controlled by electromagnetic valves and selectively delivered through one of ten nozzles (2 mm in diameter), which were horizontally arranged around the cricket at a distance of 15–35 mm from the cerci. The air velocity ($200\text{--}300 \text{ mm s}^{-1}$) was regulated by a precise valve calibrated for each nozzle with an anemometer.

Results

Giant interneurons

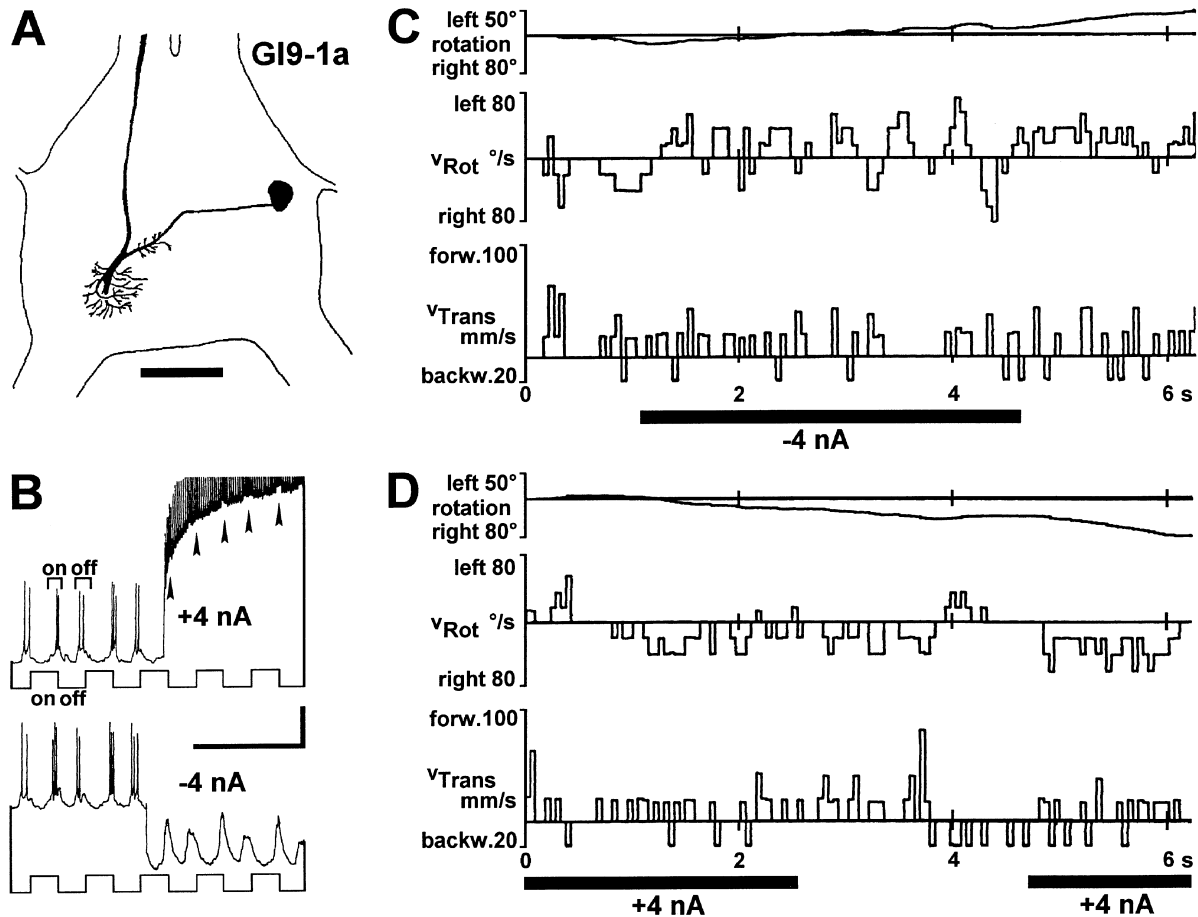
In the course of our experiments on GI activity during walking we obtained recordings from a total of 45 neurones of 9 different types (GI8-1a, GI8-1b, GI8-2a, GI9-1a, GI9-1b, GI9-3a, GI10-1a, GI10-3a, GI11-1a). All of them significantly changed their spontaneous and wind-evoked spike activity before and/or during walking

compared to standing (Kohstall-Schnell and Gras 1994; Kohstall 1996). GI8-1a (also known as “medial giant interneurone”), GI8-1b, GI9-1a (also known as “lateral giant interneurone”), GI9-1b, GI9-3a and GI10-3a were tested also for possible effects of current injection on walking behaviour. However, in no case could walking be induced in a standing cricket nor could spontaneous or wind-induced locomotion be terminated. It was generally difficult to transmit through the microelectrode a current sufficient to cause massive spiking or to suppress spontaneous or wind-evoked action potentials. Even during injection of lucifer yellow by large hyperpolarizing current we never observed a behavioural effect on v_{Trans} .

GI9-1a has been recorded in seven specimens, but only in one case did the applied current successfully modify the turning behaviour during extended series of wind puffs (Fig. 1). This neurone was impaled at the base of the axon next to the region of spike initiation. While during a 10-Hz series of wind puffs normally four to six action potentials were generated per stimulus (i.e. 40–60 spikes s^{-1}), hyperpolarization (–4 nA) suppressed any spiking and depolarization (+4 nA) increased the response to 280–300 spikes s^{-1} , now partly uncoupled from the stimulus pattern (Fig. 1B). In this experiment, all wind puffs were given directly from posterior. Simultaneous hyperpolarization of GI9-1a caused the cricket to turn preferentially to the left side (Fig. 1C): the frequency of left turns increased by 20%. An id-

ential enhancement of right turns occurred during depolarization (Fig. 1D). This neurone had its axon in the left connectives and its main dendritic input region in the neuropile at the base of the left cercus, as is known for

Fig. 1A–D Effect of current injection into the lateral giant interneurone GI9-1a. **A** This GI9-1a receives its dendritic input mainly from receptors on the left cercus and sends its axon through the left connective (scale bar 200 μm). **B** Normally, start and end of each wind puff (50 ms, 10-Hz series) applied directly from posterior to both cerci evoke an excitatory post-synaptic potential (EPSP) and two to four action potentials. At the cerci the wind arrives about 20 ms delayed relative to the control voltage of the magnetic valves (*lower traces*). The GI generates action potentials after a further latency of 20–25 ms. Depolarizing current injection largely obscures the response (*arrowheads*) to wind and results in continuous spiking with high frequency (spikes truncated). During hyperpolarization the EPSPs become larger but do not cause action potentials (scale bars 20 mV, 200 ms). **C, D** Examples for walking and turning behaviour during current injection (*bars*) and continuous wind stimulation from posterior as shown in **B**. The three corresponding diagrams show the total rotation to the left (*up*) or right (*down*) side of the animal, the instantaneous rotatorial velocity v_{Rot} , and the instantaneous translatorial velocity v_{Trans} forwards (*up*) and backwards (*down*) in consecutive intervals of 50 ms each. At that time of the experiment the specimen is behaviourally habituated to the wind-puff stimuli and shows only slow walking similar to spontaneous locomotion. It turns preferentially to the left side during hyperpolarization (**C**) and for some seconds after current injection has been terminated. Conversely, most turns are to the right side during and after depolarization (**D**)



this GI type (Bacon and Murphey 1984; Heublein 1989). While the mirror-symmetrical GI9-1a in the right connectives was not influenced by current injection, both cells received presumably similar amounts of excitation from filiform hair receptors on the two cerci. This means that the rotation has been increased always to the side with the GI9-1a axon carrying less spikes per stimulus. It should be noticed, however, that in this experiment the current injection modified the response of GI9-1a to a degree probably never attained under normal conditions. Moderate changes, e.g. depolarization of GI8-1a to an increase of its wind response by 10–15%, had no detectable effects on the orientation of walking in any of the tested GIs.

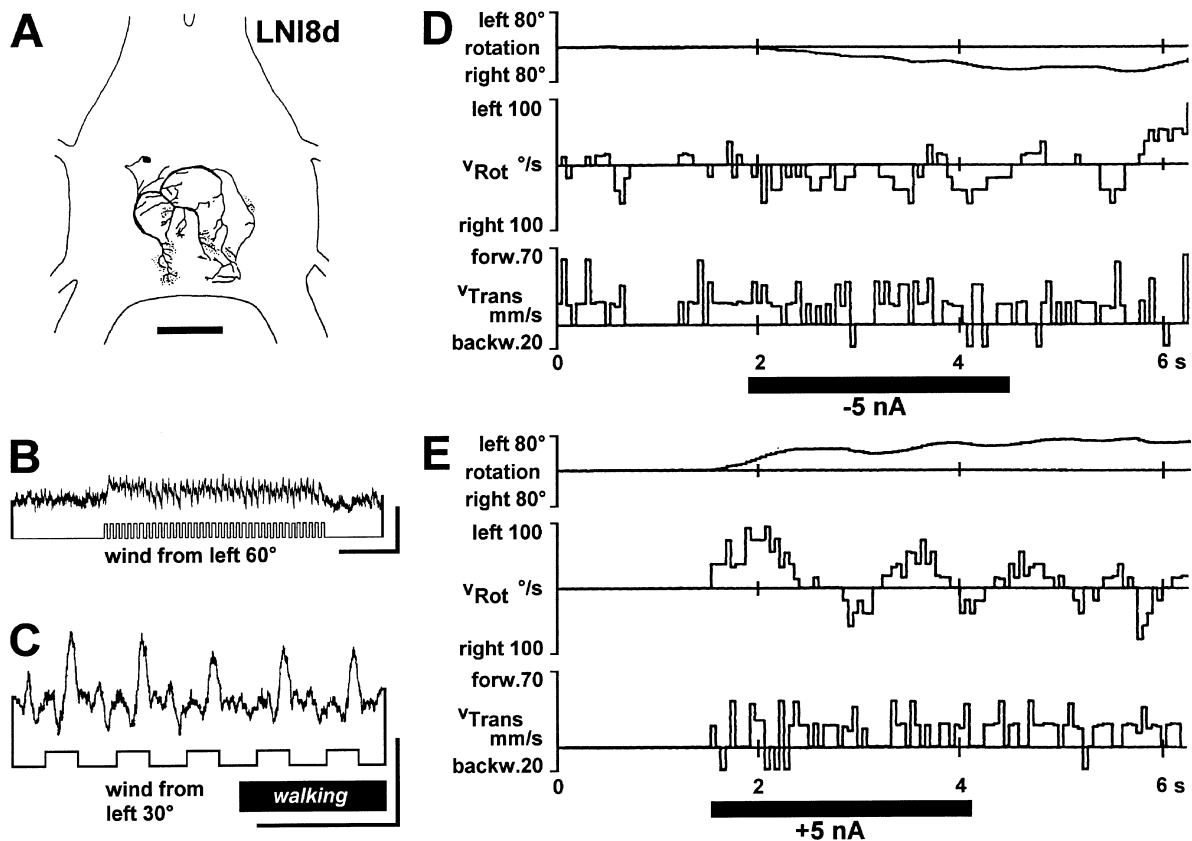
Local non-spiking interneurons

Twelve of 18 different types of LNI, which were impaled in the TG of walking crickets, were modified with regard to mean membrane potential, background activity and wind response during locomotion compared to standing (Kohstall 1996). We tested these LNIs for a possible effect of current injection on walking. As in the case of GIs we never observed any reproducible change of v_{Trans} or a stop in spontaneous walking during hyper- or depolarization. In one test, a standing cricket started extensive walking at the onset of current injection (Fig. 2E), whereas current occasionally induced only a short episode of walking or, most often, did not cause

any locomotion. In six experiments with LNIs of five different types, however, the orientation of walking was modified with v_{Rot} depending on the amount and polarization of the injected current.

Figure 2 shows results of an experiment on LNI8d. From a soma in the ventral left part of the TG prominent branches covered with numerous bleb-like varicosities reach to the cercal glomeruli of both TG hemispheres (Fig. 2A). During a wind-puff series, the mean membrane potential decreased tonically by 3–5 mV (Fig. 2B). This shift was not influenced by the actual walking behaviour. LNI8d generated a large depolarizing potential for each puff from any direction, but was most sensitive to stimuli from behind. This phasic wind response, however, was reduced about 100 ms

Fig. 2A–E The response of LNI8d to wind depends on the actual walking behaviour, but the membrane potential of this neurone also influences the orientation of walking. **A** This experiment has been done on an interneurone with soma position in the left half of the ganglion (scale bar 200 μ m). **B** The mean membrane potential depolarizes by 6 mV during a wind-puff series for 3.6 s (bars 20 mV, 1 s). **C** The response to each wind puff is largest for stimuli from behind. During and even before walking (*black rectangle*), however, the wind-evoked modulations of membrane potential are reduced (bars 20 mV, 200 ms). **D, E** Current injection induces a specific turning reaction during continuous stimulation with wind puffs from behind (0°). During hyperpolarization (**D**) the slowly walking cricket turns to the right side, i.e. soma-contralateral to the injected neurone. Depolarization during a standing phase (**E**) triggers walking and orientation to the soma-ipsilateral side



before and during walking (Fig. 2C). LNI8d not only received information about the behavioural status of locomotion, but its output also influenced the orientation of walking. During hyperpolarization of LNI8d (-5 nA, Fig. 2D) the insect started to turn preferentially to the right (i.e. soma-contralateral) side, while during depolarization ($+5$ nA, Fig. 2E) orientation was adjusted to the left (i.e. soma-ipsilateral) side. In both cases the insect did not rotate continuously to one side. Instead, as has been observed similarly during spontaneous orientation, it changed the relative frequencies and velocities of left and right turns. In another cricket we impaled the LNI8d of the right hemiganglion and measured a similar reaction to current injection: hyperpolarization caused turning to the left (again the soma-contralateral) side, while the animal oriented preferentially to the right side during depolarization. We obtained recordings of LNI8d in six other crickets; however, they showed only little walking activity during hyper- and depolarization of the neurone.

Effects of current injection on v_{Rot} and on the frequency of turning

Quantitative data from the two successful behavioural experiments on LNI8d are presented in Fig. 3. The mean v_{Rot} without current injection was not significantly different from 0° s^{-1} , i.e. both specimens walked forward during wind application, although the second one was stimulated from 60° left (Fig. 3B, C). During hyperpolarization the mean v_{Rot} increased significantly and resulted in an overall orientation to the soma-contralateral side. Conversely, depolarization evoked a quantitatively similar orientation to the soma-ipsilateral side. For the first animal, we obtained behavioural measurements for four different levels of hyperpolarization between -3 and -6 nA. Injection of -4 , -5 and -6 nA caused similar increases of v_{Rot} , while -3 nA was ineffective in this respect. The distribution of instantaneous v_{Rot} in intervals during turning behaviour often differed from a normal distribution and, therefore, mean and *t*-test might be inappropriate for a statistical analysis. We compared the distributions of v_{Rot} obtained during different current injections (Fig. 3A). Hyperpolarization of -4 nA to -6 nA yielded similar histograms which are skewed to right turns. The distributions for hyperpolarization of -3 nA and for controls were identical. Additionally, we counted the number of intended turns to the left and to the right side during current injection. Figure 3D, E shows the relative frequency of turns to the right side. Corresponding to the effect on mean v_{Rot} , hyperpolarization also enhanced the number of turns to the soma-contralateral side, while depolarization decreased it. In contrast to v_{Rot} , however, turning frequency was graded

with the amount of hyperpolarizing current injected (-4 to -6 nA), but -3 nA had no significant effect (Fig. 3D). For both crickets tested, we could completely suppress turning to the soma-ipsilateral side by hyperpolarization, while depolarization was not equally effective but reduced soma-contralateral turns to 20–30%. Similar results as those presented in Fig. 3C, E with wind stimulation from 60° left were also obtained with wind from 60° right and depolarization (data not shown), while hyperpolarization could not be tested. There was a small difference in turning frequency during stimulation from the left and from the right side: it remained constant without current (0 nA) and during depolarization ($+3$ nA) in spite of the described shift of general orientation during current injection. Therefore, the membrane potential of LNI8d possibly defines a kind of offset for the animal's mean orientation to wind.

During experiments on four other LNIs with impact on the orientation of walking we obtained data either for hyper- or for depolarizing current injection. These results, which are summarized in Fig. 4, are compared with those for LNI8d. Three of the LNIs – LNI9c, LNI9d, LNI9e – were more or less bilaterally symmetrical and possessed a characteristic neurite of large calibre transversing the TG. While these neurones rose from the left hemiganglion, the LNI9j in Fig. 4 had its soma and all its arborizations in the right half of the TG. In the following description and discussion of the behavioural effects during current injection we will refer to these basic morphological facts and relate turning direction to soma position.

Hyperpolarization of LNI9c gradually increased the relative frequency of turning movements to the right, i.e. soma-contralateral side during wind-puff stimulation from behind (0°). While this finding is similar to the results obtained for LNI8d (Fig. 3), the overall v_{Rot} was changed in a different way: during hyperpolarization of LNI9c with -3 nA the cricket's mean orientation was to the left side with a mean v_{Rot} of 9.9° s^{-1} . These intuitively contradictory behavioural data are explained by the fact that many turns to the right side with low v_{Rot} were interspersed with a few turns to the left side with high v_{Rot} (up to 55° s^{-1}). High-speed turning to the left did not occur without current injection in this specimen. Occasional jerky movements of this specimen contributed to the large standard deviation of v_{Rot} .

The cricket from which we recorded LNI9d increased the percentage of turns away from the wind source when stimulated from posterior 60° left or right, respectively (Fig. 4). However, directional discrimination was rather poor in all experiments because of strong habituation during extended stimulus series. When LNI9d was hyperpolarized, both the frequency of turns and v_{Rot} to the right, i.e. soma-contralateral side increased. As a result, the insect walked predominantly to the right even during

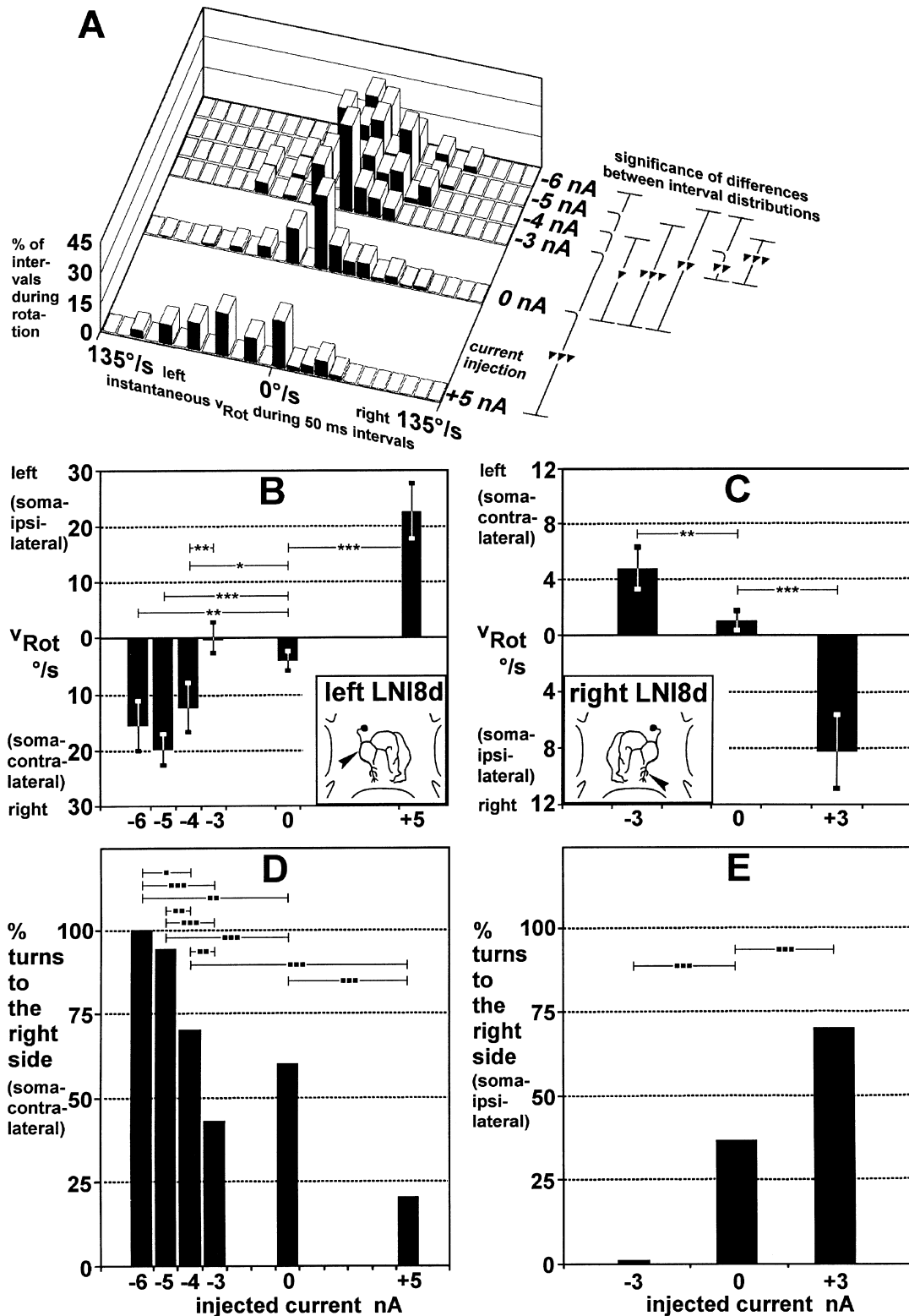


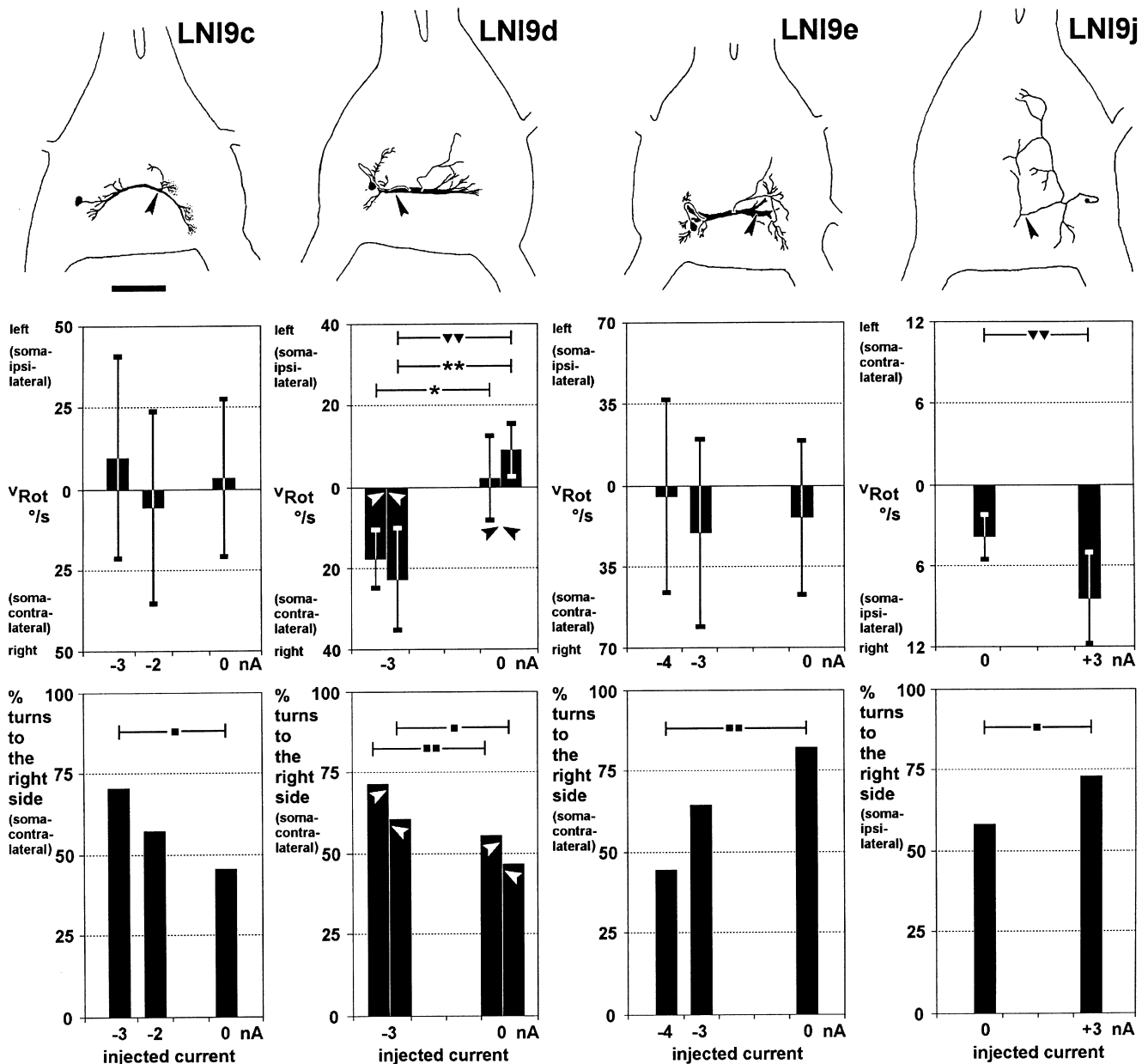
Fig. 3A–E Current injection into LNI8d influences turning behaviour during stimulation with a wind-puff series. **A,B,D** Data obtained from the neurone with soma in the left hemiganglion displayed also in Fig. 2. Stimuli were applied from 0° posterior. **C,E** Data measured for a complementary neurone originating in the right hemiganglion; compare schematic insets for neurone position and recording site (*arrowheads*). Stimuli were applied from 60° left. The mean velocity of rotation depends on the amount and polarity of injected current (**B, D**). The significance of difference of the means is indicated by *asterisks* (*t*-test: * 10%, ** 5%, *** 1%). The histograms of v_{Rot}

determined in 50-ms intervals during episodes of rotation often do not follow a normal distribution during current injection (**A**); *triangles* indicate the significance level of differences (Wilcoxon test: ▼ 10%, ▼▼ 5%, ▼▼▼ 1%). Current injection influences significantly also the frequency of turns to one or the other side (contingency test: ■ 10%, ■■ 5%, ■■■ 1%) (**D,E**). The qualitative mirror symmetry of the diagrams **B–C** and **D–E**, respectively, corresponds to the position of the two LNI8d studied in the terminal ganglion. Generally, hyperpolarization increases the velocity and frequency of turning to the soma-contra-lateral side, while depolarization has an opposite effect

wind stimulation from the right side. However, the behavioural difference in response to wind from left or right remained nearly unchanged with respect to the frequency of turns. As has been supposed for LNI8d also LNI9d seems to be able to cause an offset in the directional wind response of the cricket.

We recorded the LNI9e shown in Fig. 4 in an animal with a preferential general orientation to the right side during wind-puff stimulation from behind (0°). In control trials (0 nA) turns to the left side never exceeded 100 ms. They constituted only 20% of all rotation events and were performed with low v_{Rot} . When LNI9e was hyperpolarized, rotations to the left lasted for up to 350 ms and occurred more frequently. Especially during current injection normal locomotion of this specimen was interspersed with very short jerky turning bouts. While they hardly influenced the overall frequency of turns to one or the other side, they caused a large standard deviation of v_{Rot} . Therefore, the mean v_{Rot} during depolarization was statistically similar to the control value, while the distributions of v_{Rot} differed significantly. It should be noticed that, with respect to the neurones' position in the TG, the effect of LNI9e-

Fig. 4 Effect of current injection into four different LNIs. Each column shows a sketch of the neurone (scale bar 200 μ m, applies to all neurones; *black arrowhead* indicates the recording site), a diagram of the mean rotatorial velocity during and without current injection, respectively, and a further diagram of the relative frequency of turns to the right side of the animal. Data are from intervals with sequential wind puff stimulation. In the experiment on LNI9d, wind direction was from posterior 60° left and right (*white arrowheads*), in the other experiments wind was applied directly from behind (0°). *Asterisks, triangles and squares* indicate significance levels of statistical tests as described in Fig. 3. For further explanation see text



hyperpolarization on the turning tendency was inverse compared to that one observed for hyperpolarization of LNI8d, LNI9c, and LNI9d.

LNI9j is the only longitudinal and strictly unilateral LNI which we have found in the TG (Fig. 4). This morphological type is rather typical for LSIs of the TG, but physiologically the neurone has been verified to be unable to generate action potentials. While serial wind puffs were applied from behind (0°), depolarization of LNI9j by +3 nA increased the frequency and duration of turns to the right (soma-ipsilateral) side, while the remaining turning movements to the left side were not affected.

Discussion

Walking in orthopterous insects is controlled and modified by a multitude of interacting neuronal elements. Descending brain neurones become active before and during running bouts (Kien 1990; Hörner 1992; Staudacher 1994) and may induce or terminate walking (Böhm and Schildberger 1992). Thoracic spiking and non-spiking interneurons process proprioceptive as well as ascending and descending input to coordinate the activity of leg motoneurons (Burrows and Siegler 1978; Ritzmann and Pollack 1986; Laurent and Burrows 1989a,b; Burrows 1992; Büschges and Wolf 1995; Wolf and Büschges 1995). From cercal mechanoreceptors ascending neurones of the abdominal nerve cord, especially the GIs of the TG, carry information which may trigger escape running and influences the orientation of locomotion (Dagan and Parnas 1970; Ritzmann and Camhi 1978; Boyan and Ball 1989; Kohstall-Schnell and Gras 1994; Liebenthal et al. 1994).

Influence of GI activity on walking behaviour

In this study we have shown that in crickets an individual GI exerts a detectable influence on the actual walking behaviour only if its spike activity is completely blocked or increased to an unphysiological level. Because of the large calibre of the GIs it is difficult to inject sufficient amounts of current to achieve such a modification of GI spike rate. Although we have found an influence on turning only in one experiment on GI9-1a, it seems possible to obtain similar results with other GIs under favourable recording conditions. All GIs of the cricket project to the thoracic ganglia, and the axons of GI9-1a and some other GIs have been traced up to the brain (Kämper 1981; Hirota et al. 1993; D. Kohstall, unpublished observations). Nothing is known about the cerebral connections of the GIs, and information about thoracic GI output is incomplete in crickets (Kanou and Shimozawa 1985). In cockroaches, the GIs with ventral axon position (vGIs) make contact with thoracic interneurons (Casagrand and Ritzmann 1991) and metathoracic motoneurons (Ritzmann and

Camhi 1978). In this way action potentials in the GIs of one side of the nerve cord excite directly and indirectly motoneurons of the ipsilateral legs (Ritzmann and Pollack 1986). This results in a turn away from the side of the (more) active GIs. A similar mechanism may work in crickets, as GI9-1a activates leg motoneurons indirectly via thoracic interneurons (Kanou and Shimozawa 1985). These authors as well as Ritzmann and Pollack (1986) have suggested that the vGIs are engaged in triggering a wind-evoked escape, while the GIs with dorsal axon position (dGIs) primarily influence its orientation away from the wind source. Our results do not support this hypothesis, as an artificial change of activity in GI9-1a, which is a member of the vGI group, never induced or terminated walking but modified the mean orientation of walking to the side of the less active GI9-1a. A change of at least 100% in the activity of GI9-1a was required to change the cricket's orientation, while in cockroaches an increase from six to eight spikes per stimulus in the vGIs is sufficient to modify the initial orientation of the behavioural response (Levi and Camhi 1995).

In all experiments with current injection in dGIs (GI8-2a, GI9-3a, GI10-3a) we were unable to induce effects on coordinated walking, but often observed jerky leg movements. According to Hirota et al. (1993), however, walking should be reproducibly triggered by depolarization (+2 nA) of any of the dGIs in a cricket. In that study the insect was mounted a short distance above a rigid surface and leg movements were video taped. Therefore, it seems possible that unspecific motions because of convulsive muscle contractions have been misinterpreted as walking.

Influence of LNI membrane potential on turning

We have shown that it is much easier to modify walking behaviour by current injection into an individual LNI than into a GI. By changing the membrane potential of a single LNI we were able to influence reproducibly the orientation of walking during wind stimulation. This was observed for 5 of 12 different types of LNI which were recorded in spontaneously walking crickets. For 6 other types of LNI the available behavioural data are too scarce to assess an effect of these neurones on orientation. In contrast, none of 9 types of wind-sensitive LSI in the TG exerts a similar impact on walking behaviour (Kohstall 1996).

In experiments on LNI8d it was possible to apply hyper- as well as depolarizing current, which caused qualitatively and quantitatively opposite effects on the cricket's orientation. These results as well as the data from LNI9c and LNI9e suggest a linear relationship between the membrane potential of the specific LNI and the frequency of turns to one or the other side. Similarly, the mean v_{Rot} may depend linearly on the amount and polarity of the injected current (LNI8d). For LNI9c and LNI9e, however, the effect of membrane potential on

v_{Rot} is obviously more complex and partly unexpected: hyperpolarization of these LNIs evokes short bouts of high-speed rotation to the soma-ipsilateral side, a behaviour not observed under control conditions. Current injection into these neurones has somewhat contradictory effects on the mean v_{Rot} and the relative frequency of turning direction. This fact may indicate that these two parameters of rotation during walking are governed by different, at least partly independent control mechanisms.

Current injections into two mirror-symmetrical LNI8d neurones reveal an appropriate, inverse effect of current polarity on the direction of orientation. Similar in this respect to LNI9c, LNI9d and LNI9j, LNI8d causes an increased frequency of turning movements to the soma-contralateral side during hyperpolarization and an increased turning to the soma-ipsilateral side during depolarization. The contrary applies to LNI9e, although its morphology resembles that one of LNI9c and LNI9d. For a more thorough discussion of the relation of form and function of individual LNIs the distribution of input and output synapses on the arborizations of these neurones must be known. Those data, however, are not yet available.

Walking induced by a LNI

LNI8d not only exerts an influence on the orientation of walking but it is the only neurone which we have found to be possibly capable of triggering locomotion. Furthermore, while all LNI types presented in this study change their response to wind stimuli during walking, only LNI8d does so always about 100 ms *before* the onset of walking (Gras and Kohstall 1995; this study). Two other LNIs – LNI9i and LNI10b – also modify their activity before walking (Kohstall 1995, 1996). Otherwise, however, there is no obvious morphological or physiological similarity between these interneurones, and it is unknown whether they can influence walking behaviour as LNI8d does. Modification of activity preceding the behavioural change points to a descending control of these neurones.

Divergent information transfer between LNIs and ascending interneurones

The signals from the LNIs in the TG must be transmitted via ascending interneurones to the thoracic and/or cephalic ganglia to change the actual walking activity and orientation of the cricket. Most of the LIs are presumably connected to some of the GIs and SAIs of the TG. LNI9j, for example, produces excitatory post-synaptic potentials in the soma-ipsilateral GI8-1a, whose axon ascends through the contralateral connective (Kohstall 1996). Depolarization of LNI9j, therefore, will increase the activity of at least this GI, which may drive contralateral thoracic motor neurones

(Ritzmann and Pollack 1986) and induce a turn to the other, i.e. LNI9j-soma-ipsilateral side. This behavioural effect is documented in Fig. 4. LNI9e is similar in some way to the 9DLI-neurone (Bodnar 1993) which transmits both to the ipsi- and the contralateral GI10-3a in *G. bimaculatus*. This GI has previously been shown to change its wind response 50–100 ms before onset of walking (Kohstall-Schnell and Gras 1994), presumably in response to a similar descending control mechanism as has been discussed for LNI8d (Kohstall 1996).

Until now, no functional contacts between LNI8d, LNI9c or LNI9d and any ascending interneurones have been found. Intracellular double recordings have revealed excitatory transmission between seven different pairs of LIs and GIs, five pairs were not connected and only one LI inhibited (presumably indirectly) a SAI (Kohstall 1996). Inhibitory effects of LNIs of the TG on large ascending neurones have been reported for crustaceans (Reichert et al. 1983) but not so for orthopterous insects. Therefore, it may reasonably be assumed that excitatory contacts exist between about 50% of all LIs and GIs of the cricket. In double recordings from LI-GI-pairs (Kohstall 1996) current injection into a single LI never changed the activity of a postsynaptic GI to an extent similar to that enabling GI9-1a to influence the orientation of walking. This supports the hypothesis that information is divergently transferred from one LNI to many GIs. Therefore, a shift of a LNI's membrane potential may modify turning behaviour via an induced change of overall spike activity in GIs of one side of the nerve cord. The capability of LNI8d to trigger walking in a standing animal results probably also from simultaneous activation of multiple ascending units, as we never observed onset of walking in response to depolarization of an individual GI. Additionally, inhibitory interactions between LIs of the TG have been demonstrated (Kohstall 1996). Depolarization of LNI8d reduces the spike rate of a LSI, which presumably activates ascending interneurones. It is conceivable, therefore, that a single LNI may decrease the spike rate of GIs in one connective by a polysynaptic mechanism, while it activates GIs in the other one by direct excitation.

Functional significance of LNIs for the regulation of walking direction

In two experiments (on LNI8d and LNI9d) we applied wind puffs from different directions which caused differences in v_{Rot} and turning frequency as may be expected in the course of an evasive behavioural response. The rather small difference in orientation during wind stimulation from the left and from the right side, respectively, results from marked habituation of escape behaviour (Gras et al. 1994). It should be noticed, however, that this difference in turning frequency remains exactly constant when the orientation of the ani-

mal is modified by current injection into a LNI. This strongly suggests that some LNIs of the TG are capable to impose a general offset shift onto the orientation control taking place in the central nervous system. For this purpose the graded potentials of the LNIs seem to be ideally suited, as they can tonically influence the activity level of ascending interneurons, which transmit information relevant to orientation in response to wind stimuli. The fact that current injection into LSI never had a detectable effect on the walking behaviour in our experiments fits well into this view.

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