Rhythmic Modulation of the Responsiveness of Locust Sensory Local Interneurons by Walking Pattern Generating Networks

HARALD WOLF AND GILLES LAURENT

California Institute of Technology, Biology Division, Pasadena, California 91125

SUMMARY AND CONCLUSIONS

1. Spiking local interneurons in the thoracic ganglia of the locust are known to process mechanosensory information from leg receptors and to contribute to postural reflexes. It is not clear, however, whether these interneurons are also involved in the control of leg movement during walking or whether their role in mediating local reflexes is phasically modulated by the walking pattern generating networks. To address these questions, intracellular recordings from spiking local interneurons in a midline population were made both in a "fictive walking" preparation reduced to isolated thoracic ganglia and in semi-intact locusts walking on a treadmill.

2. In fictive walking (and therefore deafferented) preparations, rhythmic synaptic inputs were observed in 72% of the sampled local interneurons. These inputs resulted in a modulation of the interneurons' firing rate in phase with the centrally generated rhythm. A similar rhythmic drive was seen in spiking local interneurons recorded from semi-intact walking locusts. This drive persisted after amputation of the leg from which the interneurons received their sensory inputs. These results indicate that this population of interneurons receives inputs from central networks that participate in the generation of walking.

3. In fictive walking preparations where a hindleg was left attached to its segmental ganglion, individual local interneurons could be characterized physiologically by their receptive field properties. The central inputs were found to modulate the activity of members of all subclasses of interneurons (i.e., purely exteroceptive, purely proprioceptive, and with mixed receptive fields). The mode of modulation observed could oppose or assist the effect of sensory input expected to occur during normal walking.

4. The central drive could originate from the ipsilateral, the contralateral, or both central pattern generating networks of the corresponding ganglion. In this way the response of an interneuron to sensory inputs from its receptive field on one leg became phase dependent and linked to the step cycle of one or both legs of its segment.

5. These results indicate 1) that the mediation of local reflexes is phasically modulated by the action of the walking pattern generating circuits on the local sensory integrators, 2) that spiking local interneurons may contribute to the control of leg movement during walking, and 3) that the information about a sensory stimulus carried by the spikes of the local interneurons can be modified by the central drive to these interneurons.

INTRODUCTION

The multilayered sensory-motor neuronal networks that underlie postural reflexes in the locust have been investigated in considerable detail (see Burrows 1992 for review). These networks process signals from leg mechanoreceptors and are important for the maintenance and fine adjustments of posture. By their nature, however, these pathways must also be closely involved in the control of locomotion

(e.g., Bässler 1993). It is probable, therefore, that the neurons that subserve postural and avoidance reflexes in the quiescent animal also play an integral part in the control of leg movement during walking. This is true for the principal output elements of the segmental nervous system, the motoneurons, which supply the leg muscles, and for the premotor nonspiking local interneurons, which drive them by the graded and maintained release of neurotransmitter (Burrows and Siegler 1976, 1978; Pearson and Fourtner 1975; Schmitz et al. 1991). Other central components of the sensory-motor circuits in thoracic ganglia are the spiking local interneurons. Three discrete populations of such spiking local interneurons have been described, which share one main feature: all organize the afferent input from hundreds of leg mechanoreceptors into receptive fields that are (generally) composed of excitatory and inhibitory regions (Burrows and Siegler 1982, 1984; Nagayama and Burrows 1990; Siegler and Burrows 1983). The spiking local interneurons in a midline population, which have been best studied, appear to shape subregions of the receptive fields of motor neurons (Burrows and Siegler 1982) or, more commonly, of other spiking local interneurons (Nagayama and Burrows 1990), of premotor nonspiking local interneurons (Burrows 1987b), and of intersegmental interneurons (Laurent 1987). These neurons have therefore often been considered as the "primary integrators" of the sensory signals originating from the leg of the animal (Siegler and Burrows 1983).

Although some of these spiking local interneurons make monosynaptic connections with leg motor neurons (Burrows and Siegler 1982; Nagayama and Burrows 1990), it is not known whether they might participate both in the sensory-motor reflex loops and in the generation/control of the motor output during locomotion. The goal of the present investigation is therefore to examine the possibility that spiking local interneurons are influenced by the networks generating the leg motor patterns and therefore are involved either in the direct control of the locomotor output or in the phase-dependent control of reflex pathways. Because intracellular monitoring of the activity of spiking local interneurons during locomotion is difficult (because of the small size of their somata) we chose first to study their activity in a deafferented or reduced "fictive" walking preparation (Ryckebusch and Laurent 1993). This preparation allowed for stable intracellular recordings from the midline group of spiking local interneurons (Siegler and Burrows 1984) as well as easy manipulation of sensory inputs from the leg. The validity of these results was then tested in tethered locusts walking on a treadmill, where normal sensory feedback pathways could be left intact and later severed.

METHODS

Adult *Schistocerca americana* or *S. gregaria* of both sexes were obtained from crowded breeding colonies at the California Institute of Technology and the University of Konstanz. Animals were used for experiments ~4 days after their imaginal moult.

Fictive walking preparation

Locusts were immobilized ventral side up on a plasticene platform. An incision was made around the sternal cuticle of the pterothorax and the cuticle was removed by transecting sternal apodemes and flight and leg muscles near their attachment sites. Salivary glands, fatty tissue, and several thoracic muscles and their apodemes were removed to expose the meso- and metathoracic ganglia. The ganglia were then supported on a stainless steel platform coated with dental wax. They were immobilized with minuten pins and superfused with locust saline containing (in mM) 140 NaCl, 5 KCl, 5 CaCl₂, 4 NaHCO₃, 1 MgCl₂, and 6.3 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.1, at room temperature (~25°C). Fictive walking motor activity was elicited in the reduced locust preparations by application of $3\text{--}5 \cdot 10^{-5}$ M pilocarpine (Ryckebusch and Laurent 1993) in the saline used for superfusion. The abdominal and meso-metathoracic connectives and most nerve roots of the metathoracic ganglion were routinely crushed or transected. Metathoracic nerve 5B was initially left intact to retain the afferent supply from the hindleg and was severed later for complete deafferentation. The cut ends of metathoracic nerves 3B or 5A were inserted bilaterally into suction electrodes to monitor activity of the motoneurons supplying leg muscles 131 (levator trochanteris) and 135 (extensor tibiae, slow motoneuron) or 133a (depressor trochanteris), respectively (numbering of muscles according to Snodgrass 1929). As an additional monitor, the activity of a flexor tibiae motoneuron (136) was sometimes recorded intracellularly.

Semi-intact locusts walking on treadmill (adapted from Wolf 1992)

Locusts were glued to a holder by their thoracic pleurae and placed inside a pair of lightweight treadwheels in an inverted position. Each treadmill supported the legs on one side of the body, and the sternal cuticle was accessible through the slit between the two wheels. The wheels were suspended on a balance lever to allow for adjustment of the load carried by the animal's legs. The meso- or metathoracic ganglion were exposed by excising a small flap of cuticle and removing overlying tracheal sacs and fatty tissue. The ganglion was supported on a stainless steel platform that had been molded to snugly fit the ganglion. Gentle suction was applied through a central hole in the platform to immobilize the ganglion during walking movements. Walking behavior was monitored by electromyographic (EMG) recordings from muscle 92 (anterior rotator coxae) and leg movements were monitored in the anterior-posterior axis by an optoelectronic camera (modified after von Helversen and Elsner 1977).

Intracellular recordings

Soma recordings were made from a midline population of spiking local interneurons (Burrows and Siegler 1982). Interneurons in the meso- or metathoracic ganglion receive mechanosensory input from the middle or hindlegs, respectively, and were characterized physiologically by their receptive field properties (Burrows and Siegler 1984; Siegler and Burrows 1984). In the fictive walking preparation, recordings were made almost exclusively from metathoracic neurons; the few recordings performed from mesothoracic spiking local interneurons demonstrated qualitatively similar patterns of activity. In the semi-intact walking locusts, mesothoracic neurons were preferentially impaled. The chosen ganglion was treated with a small amount of protease crystals (Sigma P-5147) for ~1 min and then thoroughly rinsed with saline so as to allow easier penetration of the neural sheath. Glass micropipettes filled with 3 M potassium acetate and with resistances ranging from 50 to 100 M Ω were used for intracellular recordings.

Experimental procedure and stimulation

The motor pattern was monitored with extracellular recordings from nerves 3B or 5A (see above) and spiking local interneurons were characterized by examination of their receptive fields immediately after impalement. Leg mechanoreceptors (hair sensillae, campaniform sensillae, pulvilli, or tibial spines) were generally stimulated by hand. Better-controlled stimulation was achieved by using a small probe coupled to an electromechanical transducer (altered dynamic loudspeaker; limit frequency 200 Hz) and driven by a function generator. For stimulation of the hindleg femoral chordotonal organ (fCO) the receptor organ was exposed by removing a small flap of cuticle just proximal to the femorotibial joint (Burrows 1987a). The tendon of the extensor strand of the fCO was then clamped into miniature forceps attached to the transducer.

In locusts walking on a treadmill, spontaneous walking episodes were recorded after locomotion had been elicited by stroking the animal's abdomen. Impaled spiking local interneurons were again characterized by examination of their receptive fields while the animal was quiescent. Gentle mechanical stimuli were applied by a hand-held probe. Unequivocal characterization of a neuron's receptive field was possible only if the release of reflex movements could be avoided. After a few walking episodes had been recorded the leg from which the afferent input originated was amputated at midcoxa level and the remaining stump was immobilized with insect wax. Electrophysiological data, stimulus monitor, and leg position signals were stored on magnetic tape (8-channel DAT Sony/Biologic, 5-kHz sampling rate, or RACAL Store 4DS) for subsequent display on a chart recorder (Gould TA4000 or ES 1000).

RESULTS

Activity patterns of spiking local interneurons in fictive rhythmic preparations.

Fictive walking patterns were elicited in immobilized, dissected, and extensively deafferented locusts by application of $3\text{--}5 \cdot 10^{-5}$ M of the muscarinic agonist pilocarpine

A nerve 5B intact **B** nerve 5B severed

FIG. 1. Spiking local interneurons receive phasic synaptic inputs locked to the fictive locomotor rhythm before (*A*) and after (*B*) section of the main leg nerve. In this and the following figures the receptive fields of the interneurons are indicated in the *inset*. These receptive fields are based on data not shown. In this and following insets a plus sign denotes excitation and a minus sign inhibition. Arrows in *insets*: movement sensitivity. *A*: spiking local interneuron (*top trace*) was excited by pressure exerted on the anterior cuticle of the femur. This stimulus is most probably encoded by campaniform sensillae. Tonic activity was interrupted during the bursts of depressor activity. Fictive locomotor activity was monitored by recording from nerve 5A (*bottom trace*: Ds, spikes of slow coxotrochanteral depressor motor neuron; Df, spikes of fast coxotrochanteral depressor motor neuron; Cl, spikes of common inhibitory motor neuron 1). *Inset*: schematic drawing of a hindleg indicating the interneuron's receptive field (sensitive to pressure applied on the anterior face of the proximal femur). *B*: transection of the main (and only remaining) leg nerve (5B) did not affect the phasic inhibitory influence on this interneuron, which remained phase-locked to the ipsilateral centrally generated rhythm. Calibrations: 10 s, 15 mV.

(Ryckebusch and Laurent 1993). These motor patterns were irregular but resembled those observed during normal forward walking in many respects. For example, both the coordination between motor neurons supplying muscles acting on different joints of a leg and that between the motor neurons innervating different legs (preparations with intact thoracic connectives) corresponded to intra- and intersegmental patterns observed during normal walking (see Ryckebusch and Laurent 1993). The major difference between the fictive rhythms observed in isolated preparations and the walking patterns observed in intact locusts was a greatly increased cycle period (ranging from 5 to 20 s per fictive rhythm cycle), generally due to a long delay between the end of the fictive stance phase and the start of the following fictive swing phase.

Of 61 intracellular recordings from isolated metathoracic spiking local interneurons in the midline group, 44 (or 72%) exhibited rhythmic oscillations of membrane potential that were phase-locked to the fictive rhythm and persisted after deafferentation of the metathoracic ganglion. Often a strong correlation between the fluctuations in membrane potential of a spiking local interneuron and the discharge of an ipsilateral leg motoneuron (whose activity was recorded extracellularly from nerves 5A or 3B) was evident. In the remainder of the interneurons (28%), no oscillations of membrane potential or rhythmic activity were clearly discernible, although the preparations from which they were recorded showed clear rhythmic activity. No obvious features in their receptive fields allowed us to distinguish them from those of the local interneurons driven by the central rhythm generator.

The recording in Fig. 1*A* shows the activity of a spiking local interneuron that was excited by slight pressure exerted on the anterior cuticle of the proximal hind femur (*inset*). This interneuron exhibited pronounced and rhythmic hyperpolarizations that were associated with the peak discharge of the slow and fast trochanteral depressor motoneurons (corresponding with the stance phase of the fictive rhythm). Transection of nerve 5B completely deafferented the metathoracic ganglion but did not alter the inter-

neuron's activity pattern (Fig. 1*B*). This indicates that the rhythmic hyperpolarizations were not generated by phasic sensory feedback from the leg, as might have resulted from the activation of the trochanteral depressor muscles or other coactive muscles.

Members of all subclasses of spiking local interneurons (i.e., purely exteroceptive, purely proprioceptive, or with mixed receptive fields) were found to be modulated by the pilocarpine-activated central oscillator. The recordings in Figs. 2 and 3 provide examples of such rhythmic modulatory patterns. Figure 2*A* shows the activity of a spiking local interneuron with purely proprioceptive input: this interneuron was exclusively responsive to sensory inputs evoked by movements about the femorotibial joint (Fig. 2*A*, *inset*). In a rhythmic preparation, however, pronounced hyperpolarizations of the interneuron occurred in synchrony with peak discharges of the ipsilateral slow extensor tibiae motoneuron, corresponding with the fictive stance phase (Fig. 2*Ai*). This was particularly evident during episodes of irregular activity (Fig. 2*Ai*). As observed with the previous interneuron, this rhythmic modulation of tonic activity remained after deafferentation and was therefore not caused by movement-related sensory feedback (Fig. 2*Aii*).

The rhythmic activity of a spiking local interneuron with pure exteroceptive input is shown in Fig. 2*B*. Pronounced hyperpolarizations were here associated with activity of the contralateral levator trochanteris motor neurons, that is, with the fictive swing phase of the contralateral leg. Only rarely could rhythmic fluctuations in the activity of this interneuron be correlated with ipsilateral motor output. Numerous smaller fluctuations in membrane potential, however, could not be correlated with any particular phase of the fictive step cycle or the discharge of a motor neuron on either side of the ganglion.

The interneuron in Fig. 3*A* received sensory inputs from both proprioceptive and exteroceptive afferents, being excited by tactile contact of the dorsal tibia and inhibited by flexion of the femorotibial joint. In a rhythmic preparation this interneuron was depolarized during and just after the swing phase of the fictive rhythm (i.e., the phase of trochan-

FIG. 2. Spiking local interneurons with input from either proprioceptive or exteroceptive mechanoreceptors are driven by the networks underlying the fictive locomotor rhythm. *A*: interneuron inhibited by tibial extension and excited by tibial flexion, as recorded before (i) and after (ii) section of the only remaining leg nerve (5B). Fictive locomotor rhythm monitored extracellularly from ipsilateral nerve 3B. Note that hyperpolarizations coincide with bursts of ipsilateral slow extensor tibiae (SETi) activity. *B*: interneuron with tactile inputs from hair sensillae on the dorsal tibia recorded before (i) and after (ii) section of nerve 5B. Note that the modulation of this interneuron's firing rate was coupled to the locomotor rhythm on the contralateral side, monitored from nerve 3B. SETi, SETi motor neuron; lev. troch, levator trochanteris motor neurons. Calibrations: 10 s, 15 mV (*A*); 10 mV (*B*).

teral levator activity). Intracellular recording from a flexor tibiae motor neuron provided additional definition of the fictive swing phase in the smooth modulation of this neuron's membrane potential. Transection of nerve 5B resulted in minor changes of the interneuron's activity pattern, indicating 1) that the main drive for this interneuron was of central origin in the rhythmic preparation and 2) that sensory input resulting from the activation of muscles supplied by nerve 5B contributed only a little to the activity observed before complete deafferentation. The interneuron recorded in Fig. 3*B* received excitatory inputs from leg

campaniform sensillae, i.e., from receptors that may respond to signals of proprioceptive as well as exteroceptive origin. The activity of this interneuron, too, was modulated in phase with the fictive rhythm, being increased during the fictive swing phase and largely unaffected by deafferentation. Finally, we often observed a strong positive correlation between the excitatory drive to an interneuron and that to ipsilateral leg motor neurons. This can be seen in Fig. 4, where the amplitude and duration of the phasic depolarizations of an interneuron and those of a flexor motor neuron are generally matched.

FIG. 3. Spiking local interneurons with combined exteroceptive and proprioceptive responses are also driven by the central pattern generating networks. *A*: interneuron with excitatory tactile receptive field on the dorsal tibia. This neuron was excited during and shortly after the "fictive" swing phase (period of levator trochanteris activity) and inhibited during the fictive stance phase (period of SETi activity) of the ipsilateral rhythm. The depolarization evoked during the 2nd swing phase was terminated by an active flexion of the tibia that had not been immobilized (*). *B*: interneuron with tibial campaniform sensilla input, therefore responding to imposed as well as self-generated cuticular stress. In both cases (*A* and *B*), the rhythmic drive remained after complete deafferentation. Calibrations: 5 s, 15 mV (*A*); 10 s, 15 mV (*B*).

FIG. 4. The strength of the rhythmic drive to spiking local interneurons is positively correlated with that to certain leg motor neurons. This spiking local interneuron (*top trace*) was excited by tibial extension and inhibited by tibial flexion, as shown in the *right panel*. Both amplitude and duration of the depolarizing waves observed in the interneuron were correlated with those in a flexor motor neuron (*2nd trace from top*) and with the duration of the burst of levator trochanteris action potentials (*3rd trace from top*). Calibration: 5 s, 15 mV (interneuron); 25 mV (motor neuron).

Interactions between centrally generated and peripheral inputs

The central drive observed in fictive preparations could modulate the response of a spiking local interneuron to a sensory input. The interneuron in Fig. 5*A*, for example, was activated by rhythmic mechanical stimulation of campaniform sensillae in its receptive field (i.e., ventral bending of the otherwise immobilized tibia). Each stimulus normally evoked a burst of two to four spikes in the interneuron. If the stimulus coincided with the fictive swing phase, however, the interneuron was transiently hyperpolarized by the central oscillator drive and only one action potential was generated (Fig. 5*A*, stimuli occurring during levator bursts). In a walking locust one might expect this spiking interneuron to be activated by sensory inputs occurring during the stance phase (because it is excited by tibial extension or by a tibial bending stimulus). A central hyperpo-

larizing drive during the swing phase would therefore act in synergy with the excitatory sensory input expected to occur during the stance phase.

The activity of a different interneuron with a mixed receptive field is shown in Fig. 5*B*. This interneuron was responsive to touch of the ventral tibia and to movement about the femorotibial joint. The extensor strand of the fCO (which monitors tibial flexion) was selectively moved during generation of the pilocarpine-induced locomotor rhythm (Fig. 5, *Bii-Bv*). Although the interneuron clearly responded to this stimulus, no action potentials could be elicited during periods of quiescence, corresponding with the fictive stance phase (Fig. 5*Bii*). At times following (Fig. 5*Biii*), or precisely corresponding with, the fictive swing phase (Fig. 5*Biv*), however, the synaptic potentials generated by the fCO stimulus were superimposed on a depolarizing drive resulting from the central oscillator input. The

FIG. 5. Influence of the rhythmic central drive on the processing of mechanosensory input. Spiking local interneurons were recorded from during fictive locomotor rhythm (monitored from nerve 3B) while sensillae in their receptive fields (*inset*) were repetitively stimulated. *A*: campaniform sensillae were stimulated by bending the tibia. *B*: in a different interneuron, sinusoidal stretch was applied to the extensor strand of the femoral chordotonal organ (fCO) (*bottom trace*). Additional recording from a flexor tibiae motor neuron is given in the *2nd trace* and selected recording segments are shown. See text for details. Calibrations: 5 s, 15 mV (*A*); 10 s, 15 mV (interneuron), 25 mV (motor neuron) (*B*).

stimulus-evoked modulation of the interneuron's discharge during the fictive swing phase is most clearly seen on an expanded time scale (Fig. 5Bv). It is therefore only during this phase of the rhythmic motor output that the contribution of this interneuron to the sensory-motor pathways could become effective. [It is also noted that tibial flexion and extension respectively had a (weak) inhibitory and (strong) excitatory effect on this interneuron.] Here, therefore, central drive and sensory input expected to occur during normal walking would counteract each other during the swing phase of the step cycle. This interneuron would therefore presumably best signal an imposed extension occurring during the swing (flexion) phase. The information carried by spikes from this interneuron is thus increased by combining central and peripheral drives, precisely because these two types of input are not normally expected to occur simultaneously.

Bilateral interactions

Bilateral interactions between peripheral and central drives were often observed in the midline spiking local interneurons. This coupling could take two manifestations¹: 1) the interneuron's receptive field was on one hindleg, whereas the central drive to this interneuron was correlated exclusively with the activity of motor neurons controlling the contralateral hindleg (for example, see Fig. 2B); 2) the spiking local interneuron received central inputs correlated with the fictive locomotor patterns of both sides of the mesothoracic ganglion. These bilateral inputs could either oppose or assist each other. The interneuron in Fig. 6A, for example, was depolarized during the fictive swing phase of the contralateral hindleg. Spike activity in this interneuron was most closely related to bursts of activity in the contralateral trochanteral levator motor neurons (Fig. 6A). Central inputs from the ipsilateral side were not clearly evident when the interneuron was held at resting potential. Injection of a small depolarizing current, however, induced a tonic background discharge and revealed rhythmic inhibitory input correlated to the ipsilateral fictive swing phase (Fig. 6B), whereas the excitatory drive corresponding to the contralateral rhythm persisted.

In Fig. 6C the pattern of activity of a different interneuron, which received hyperpolarizing input from both left and right central drives, is shown. On average, hyperpolarizations associated with the contralateral rhythm were more pronounced as recorded from the soma. Sensory input exciting this interneuron would therefore be most efficient at the times when both hindlegs are simultaneously engaged in their stance phase.

Activity of spiking local interneurons in walking locusts

The above results demonstrate that mechanosensory spiking local interneurons receive synaptic drive of central origin that is phase-locked to the fictive rhythm induced by pilocarpine. To test whether similar rhythmic input to the

spiking local interneurons is present during normal walking behavior, intracellular recordings were made from tethered locusts walking on a treadmill. Recordings from the local interneurons were performed in the mesothoracic ganglion of intact locusts before and after removal of the middle leg that contained the interneuron's receptive field. In this way we could discriminate those aspects of the rhythmic activity that were produced by phasic sensory feedback from those originating from the walking pattern generator.

Figure 7 provides three examples of activity recorded from spiking local interneurons in walking locusts before (*left panels*) and after (*right panels*) amputation (see METHODS) of the leg containing the neuron's receptive field. The following observations were made. 1) In intact animals, rhythmic activity phase-locked to the movement of the leg was recorded from all the spiking local interneurons examined ($n = 9$). 2) Leg amputation was generally followed by a change in the rhythmic pattern of myographic activity recorded from the anterior rotator muscle of the stump. The cycle period was often decreased (Fig. 7, B and C) and strict coupling between the stump EMG and the movements of the remaining adjacent legs was often difficult to establish. This rhythmic activity was thus similar to that underlying "searching" behavior. 3) After leg amputation, however, rhythmic activity of the interneuron (sub- or suprathreshold) remained and was phase-locked to the EMG taken from the stump (Fig. 7, A-C). Just as the EMGs from the stump were less regular than those taken from the intact leg, the rhythmic activity recorded from the interneuron was correspondingly less regular than that in the intact animal. 4) Such rhythmic activity during walking persisted after leg amputation in all spiking local interneurons examined and therefore appeared to be independent of the phasic sensory feedback from the leg afferents forming their receptive fields.

DISCUSSION

Spiking local interneurons in the thoracic nervous system of the locust are known to receive mechanosensory input from the leg and to play a crucial role in the control of postural reflexes (Burrows 1992; Burrows and Siegler 1982; Siegler and Burrows 1986). Intracellular recordings were made from these interneurons in a fictive rhythmic preparation (see Ryckebusch and Laurent 1993) that allowed stable penetration. In ~70% of the recorded interneurons, rhythmic synaptic input was observed during fictive walking that resulted in a modulation of the interneurons' membrane potential and/or firing rate in phase with the centrally generated rhythm. This rhythmic drive could be observed in completely isolated (and thus deafferented) thoracic ganglia, thereby demonstrating that spiking local interneurons, in addition to their well-established sensory inputs, receive inputs from central networks that generate the fictive locomotor pattern. Moreover, similar rhythmic inputs were recorded from spiking local interneurons in semi-intact locusts walking on a treadmill, even after amputation of the leg from which the sensory inputs originated. This finding further supports the hypothesis that the rhythmic input observed in fictive walking preparations reflects functional rather than artifactual aspects of the walking controlling circuits.

¹ In all cases, the sensory receptive field of a spiking local interneuron was restricted to one leg only, as originally described by Burrows and Siegler (1984) and Siegler and Burrows (1984). Ipsilateral here refers to the side from which the sensory inputs originated and not the side where the soma was located.

FIG. 6. Bilateral influences of central pattern generating networks. *A* and *B*: recordings from a spiking local interneuron with a mixed receptive field (*inset*). This interneuron was inhibited during the ipsilateral fictive swing phase and excited during the contralateral fictive stance phase. *B*: interneuron was held depolarized by injection of small DC current to make inhibitory drive more apparent. *C*: interneuron with inhibitory input from hair sensillae on the tibia (*inset*). This interneuron was inhibited during fictive swing phases on both sides. Vertical dashed lines: correlations between onsets of events in intracellular and extracellular traces. Calibrations: 10 s, 15 mV.

Phase-dependent modulation of reflex pathways

Because spiking local interneurons receive rhythmic central inputs, all the neurons that they affect should in turn also see their activity modulated by the same central oscillator. Spiking local interneurons in the midline group are known to make inhibitory synapses with spiking local interneurons in the anteromedial population (Nagayama and Burrows 1990), nonspiking local interneurons (Burrows 1987b), motor neurons (Burrows and Siegler 1982), and intersegmental interneurons (Laurent 1987). In all cases the pathways through the inhibitory midline spiking local interneurons are the principal ones that shape the inhibitory receptive fields of those postsynaptic targets. Our present results therefore have three main consequences: First, they suggest that the effectiveness of a sensory signal in generating a response in any of these target populations will be inexorably linked to the excitability or state of the interposed spiking local interneurons. The size and shape of the receptive fields of those targets could therefore be dynamically modulated by the central pattern generating networks underlying locomotion. This would indeed be most dramatic if the edges between the excitatory and inhibitory regions of a receptive field, for example, were formed by an overlap between, or intertwining of, excitatory and inhibitory microregions. Second, our results imply that the effectiveness of a sensory stimulus in correcting an ongoing movement will be phase dependent. This principle is similar to that demonstrated in the locust flight system by Reichert and Rowell (1985) or in the spinal circuits underlying

swimming of *Xenopus* embryos (Sillar and Roberts 1988) or walking in cats (Moschovakis et al. 1991), and adds to modulatory mechanisms acting directly on presynaptic afferent terminals (see below). Third, these results imply that the spiking local interneurons could be directly implicated in the generation and control of the motor output and therefore rather be considered as integral or peripheral components of the walking pattern generating networks. We never observed that depolarizing or hyperpolarizing one of the spiking local interneurons during rhythmic activity could reset or even affect the ongoing rhythm.² This was not surprising, however, considering that any one interneuron probably contributes relatively little to the gross output of these highly distributed sensory-motor circuits. Given that the spiking local interneurons are a site of convergence for central and peripheral inputs, one could thus consider them either as mediating central modulation of sensory feedback or alternatively as mediating sensory modulation of the output of a central command. This distinction appears to us as only being one of semantics.

Increased specificity in signal processing

Our results show that the excitability of spiking local interneurons could be modulated by the oscillatory networks underlying walking. One interneuron, for example, re-

² A clear demonstration of reset would be difficult to obtain, considering the irregularity of the rhythms observed in fictive walking preparations (see Ryckebusch and Laurent 1993).

FIG. 7. Activity of 3 (A–C) spiking local interneurons in locusts walking on a treadwheel, before (*left panels*) and after leg amputation (*right panels*) at midcoxa level. For practical reasons the recordings were made from mesothoracic spiking local interneurons belonging to the same midline population (receptive fields indicated in *insets*). Position of the corresponding middle leg in the anterior-posterior axis (*top traces*, anterior is to the *top*). Electromyograms (EMGs) from the anterior rotator of the coxa muscle (*bottom traces*, EMG 92) served as monitors for walking behavior. Note that the rhythmic drive to the interneurons persisted after leg amputation and immobilization of the stump. Calibrations: 2 s, 10 mV (A); 2 s, 25 mV (B and C).

sponded to movements about the femorotibial joint but produced action potentials in response to an imposed extension only when the leg received the central command to engage in its swing phase. A different interneuron was affected by tactile inputs from one hindleg but was inhibited by the pattern generating networks whenever this or the contralateral hindleg was engaged in its swing phase. Both interneurons, therefore, could signal a sensory event best when it occurred during a specific phase of the step cycle. It is not known whether the phase dependency of the response of the interneurons to a sensory signal was due solely to the modulation of their excitability or to additional presynaptic regulatory mechanisms acting on the afferent terminals, as has been shown in this and other animals (Alford and Grillner 1991; Burrows and Laurent 1993; Burrows and Matheson 1993; Cattaert et al. 1992; Gossard et al. 1990; Rudomin et al. 1983). Whatever the mechanisms, it remains that the signals conferred by spikes from the spiking

local interneurons acquire an increased specificity as a result of the drive that they receive from the central walking pattern generating networks.

H. Wolf was supported by a Heisenberg Scholarship from the Deutsche Forschungsgemeinschaft (Germany). G. Laurent was supported by grants from the Office of Naval Research and the National Institutes of Health (USA). G. Laurent is a Searle, McKnight and Sloan Fellow.

Present address of H. Wolf: Fakultät für Biologie, Universität Konstanz, Postfach 5560, 7750 Konstanz, Germany.

Address reprint requests to G. Laurent.

Received May 1993; accepted in final form 27 August 1993.

REFERENCES

- ALFORD, S. AND GRILLNER, S. The involvement of GABA-B receptors and coupled G-proteins in spinal GABAergic presynaptic inhibition. *J. Neurosci.* 11: 3718–3726, 1991.
- BÄSSLER, U. The walking-(and searching-) pattern generator of stick in-

- sects, a modular system composed of reflex chains and endogenous oscillators. *Biol. Cybern.* 69: 305–317, 1993.
- BURROWS, M. Parallel processing of proprioceptive signals by spiking local interneurons and motor neurons in the locust. *J. Neurosci.* 7: 1064–1080, 1987a.
- BURROWS, M. Inhibitory interactions between spiking and nonspiking local interneurons in the locust. *J. Neurosci.* 7: 3282–3292, 1987b.
- BURROWS, M. Local circuits for the control of leg movements in an insect. *Trends Neurosci.* 15: 226–232, 1992.
- BURROWS, M. AND LAURENT, G. Synaptic potentials in the central terminals of locust proprioceptive afferents generated by other afferents from the same organ. *J. Neurosci.* 13: 808–819, 1993.
- BURROWS, M. AND MATHESON, T. A presynaptic gain control mechanism among sensory neurons of a locust leg. *J. Neurosci.* In press.
- BURROWS, M. AND SIEGLER, M. V. S. Transmission without spikes between locust interneurons and motoneurons. *Nature Lond.* 262: 222–224, 1976.
- BURROWS, M. AND SIEGLER, M. V. S. Graded synaptic transmission between local interneurons and motor neurons in the metathoracic ganglion of the locust. *J. Physiol. Lond.* 285: 231–255, 1978.
- BURROWS, M. AND SIEGLER, M. V. S. Spiking local interneurons mediate local reflexes. *Science Wash. DC* 217: 650–652, 1982.
- BURROWS, M. AND SIEGLER, M. V. S. The morphological diversity and receptive fields of spiking local interneurons in the locust metathoracic ganglion. *J. Comp. Neurol.* 224: 483–508, 1984.
- CATTAERT, D., EL MANIRA, A., AND CLARAC, F. Direct evidence for presynaptic inhibitory mechanisms in crayfish sensory afferents. *J. Neurophysiol.* 67: 610–624, 1992.
- GOSSARD, J.-P., CABELGUEN, J.-M., AND ROSSIGNOL, S. Phase-dependent modulation of primary afferent depolarization in single cutaneous primary afferents evoked by peripheral stimulation during fictive locomotion in the cat. *Brain Res.* 537: 14–23, 1990.
- LAURENT, G. The role of spiking local interneurons in shaping the receptive fields of intersegmental interneurons in the locust. *J. Neurosci.* 7: 2977–2989, 1987.
- MOSCHOVAKIS, A. K., SHOLOMENKO, G. N., AND BURKE, R. E. Differential control of short latency cutaneous excitation in cat FDL motoneurons during fictive locomotion. *Exp. Brain Res.* 83: 489–501, 1991.
- NAGAYAMA, T. AND BURROWS, M. Input and output connections of an anteromedial group of spiking local interneurons in the metathoracic ganglion of the locust. *J. Neurosci.* 10: 785–794, 1990.
- PEARSON, K. G. AND FOURTNER, C. R. Nonspiking interneurons in walking system of the cockroach. *J. Neurophysiol.* 38: 33–52, 1975.
- REICHERT, H. AND ROWELL, C. H. F. Integration of nonphaselocked exteroceptive information in the control of rhythmic flight in the locust. *J. Neurophysiol.* 53: 1201–1218, 1985.
- RUDOMIN, P., JIMENEZ, I., SOLODKIN, M., AND DUEÑAS, S. Sites of action of segmental and descending control of transmission on pathways mediating PAD of Ia and Ib afferent fibers in the cat spinal cord. *J. Neurophysiol.* 50: 743–769, 1983.
- RYCKEBUSCH, S. AND LAURENT, G. Rhythmic patterns evoked in locust leg motor neurons by the muscarinic agonist pilocarpine. *J. Neurophysiol.* 69: 1583–1595, 1993.
- SCHMITZ, J., BÜSCHGES, A., AND KITTMANN, R. Intracellular recordings from nonspiking interneurons in a semi-intact, tethered walking insect. *J. Neurobiol.* 22: 907–921, 1991.
- SIEGLER, M. V. S. AND BURROWS, M. Spiking local interneurons as primary integrators of mechanosensory information in the locust. *J. Neurophysiol.* 50: 1281–1295, 1983.
- SIEGLER, M. V. S. AND BURROWS, M. The morphological diversity and receptive fields of spiking local interneurons in the locust metathoracic ganglion. *J. Comp. Neurol.* 224: 483–508, 1984.
- SIEGLER, M. V. S. AND BURROWS, M. Receptive fields of motoneurons underlying local tactile reflexes in the locust. *J. Neurosci.* 6: 507–513, 1986.
- SILLAR, K. T. AND ROBERTS, A. A neuronal mechanism for sensory gating during locomotion in a vertebrate. *Nature Lond.* 331: 262–265, 1988.
- SNODGRASS, R. E. The thoracic mechanism of a grasshopper, and its antecedents. *Smithson. Misc. Collect.* 82: 1–111, 1929.
- VON HELVERSEN, O. AND ELSNER, N. The stridulatory movements of acridid grasshoppers recorded with an optoelectronic device. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* 122: 53–64, 1977.
- WOLF, H. Reflex modulation in locusts walking on a treadmill. Intracellular recordings from motoneurons. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* 170: 443–462, 1992.