Role of Presynaptic Inputs to Proprioceptive Afferents in Tuning Sensorimotor Pathways of an Insect Joint Control Network

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ABSTRACT: The femur-tibia (FT) joint of insects is governed by a neuronal network that controls activity in tibial motoneurons by processing sensory information about tibial position and movement provided by afferents of the femoral chordotonal organ (fCO). We show that central arborizations of fCO afferents receive presynaptic depolarizing synaptic inputs. With an average resting potential of $-71.9 \pm 3.72$ mV ($n = 10$), the reversal potential of these potentials is on average $-62.8 \pm 2.3$ mV ($n = 5$). These synaptic potentials occur either spontaneously or are related to movements at the fCO. They are thus induced by signals from other fCO afferents. Therefore, the synaptic inputs to fCO afferents are specific and depend on the sensitivity of the individual afferent affected. These potentials reduce the amplitude of concurrent afferent action potentials. Bath application of picrotoxin, a noncompetitive blocker of chloride ion channels, blocks these potentials, which indicates that they are mediated by chloride ions. From these results, it is concluded that these are inhibitory synaptic potentials generated in the central terminals of fCO afferents. Pharmacologic removal of these potentials affects the tuning of the complete FT control system. Following removal, the dependence of the FT control loop on the tibia position increases relative to the dependency on the velocity of tibia movements. This is due to changes in the relative weighting of the position and velocity signals in the parallel interneuronal pathways from the fCO onto tibial motoneurons. Consequently, the FT joint is no longer able to perform twig mimesis (i.e., catalepsy), which is known to rely on a low position compared to the high-velocity dependency of the FT control system.

INTRODUCTION

Individual leg joints of vertebrates and invertebrates are governed by neuronal networks controlling the position and movements of the leg joints on the basis of sensory information provided by proprioceptors (Bässler, 1993; Pearson, 1995; Prochazka, 1989). The different movement parameters detected by the transducers in charge (e.g., sensory organs measuring joint position or movement velocity) are processed differently within the joint control systems depending on the behavioral context (e.g., Bässler, 1986; Dean and Cruse, 1986; Zill, 1985). Neuronal networks that control leg joints in standing animals act in the posture control mode—i.e., they counteract every imposed joint movement. These resistance reflexes in the stick insect are based mainly on the processing of velocity signals from the joint transducer, and information on other movement parameters such as joint position is of minor importance. The dominance of velocity information
during resistance reflexes is crucial for the performance of catalepsy. Catalepsy is a behavioral component of twig mimesis that hides the insect from putative predators (Bässler, 1993). However, in actively moving stick insects, as in other vertebrates and invertebrates (Pearson, 1995), the joint control networks act in the movement control mode: on the one hand they assist ongoing joint movements, and on the other hand they terminate them at a certain position [e.g., the active reaction (Bässler, 1988)]. Apparently, position information from the leg joints becomes an important movement parameter in active animals (Bässler, 1986; Dean and Cruse, 1986; Weiland and Koch, 1987). Therefore, a mechanism must exist that strongly changes the relative weighting of position and/or velocity information when the animal alters its behavioral state.

Although the topology of neuronal networks governing leg joints in orthopteran insects is known in considerable detail (e.g., Burrows, 1987; Burrows et al., 1988; Büschges, 1990; Büschges and Wolf, 1995; Sauer et al., 1995; Büschges, 1995), it is still unknown which neuronal mechanisms contribute to the differences in the weighting of movement parameters that are processed within the joint control network (Büschges and Wolf, 1995).

One possible mechanism for the tuning of sensorimotor pathways would be presynaptic inhibition of sensory afferents, which is a common phenomenon in vertebrate and invertebrate central nervous systems. Several different functions of presynaptic inhibition of afferents have been identified: (a) protection of the sensory synapses from habituation (Krasne and Bryan, 1973; Levine and Murphey, 1980; Kennedy et al., 1974); (b) sharpening of the receptive fields of cutaneous afferents (Janig Schmidt and Zimmermann, 1968; Schmidt, 1971) or the response properties of sensory neurons (Blagburn and Satelle, 1987); (c) gating of sensory information in rhythm-generating networks and modification of the effectiveness of sensory signals in a phase-dependent manner (Gossard et al., 1977; El Manira et al., 1991; Rossignol et al., 1988; Sillar and Skorupski, 1986; Wolf and Burrows, 1995); and (d) presynaptic afferent inhibition acting as a gain control mechanism in sensorimotor pathways of joint control networks (Burrows and Matheson, 1994).

Little information, however, is currently available as to whether presynaptic inhibition plays a role in the tuning of sensorimotor pathways with respect to their dependence on and sensitivity to the different modalities of a movement stimulus (Sauer and Büschges, 1994).

In the present investigation, we describe depolarizing synaptic inputs occurring in the different types of sensory afferents of the femoral chordotonal organ (fCO) [the sense organ that measures the position and movement of the femur–tibia (FT) joint] of the stick insect middle leg. Specific reference is given to the kinds of synaptic input that different types of sensory neurons receive. On the basis of our results the properties of these potentials appear to be inhibitory. By removing these potentials we present evidence that they contribute to the actual tuning of information pathways in the FT joint control network with respect to the representation of the movement parameters transmitted. In summary, our results provide evidence that specific properties of inhibitory presynaptic inputs to fCO afferents contribute to the adaptation of the stick insect’s joint control networks to the performance of catalepsy.

**MATERIALS AND METHODS**

The experiments were carried out on adult female stick insects of the species *Cuniculina impigra* Redtenbacher (syn. *Baculum impigrum* Brunner) and *Carausius morosus* (Br.) from our colonies at the University of Kaiserslautern, under daylight conditions and at room temperature (20–26°C).

**Recordings from Afferents of the fCO**

The animals were fixed dorsal side up on a platform. The middle legs and mesothoracic and metathoracic segments were placed in an enclosure (20 × 60 mm) filled with saline (Bässler, 1977). The femur of the right or left middle leg was perpendicular to the body, with the tibia perpendicular to the femur. After removing a small dorsal part (30%) of the cuticle of the femur, the tendon of the extensor tibiae muscle was cut distally; the apodeme of the fCO was fixed in a clamp and cut distally to the clamp. The following muscles were then removed from the femur: retractor unguis, extensor tibiae, and flexor tibiae. Flexion movements of the tibia were mimicked by pulling the apodeme of the femoral chordotonal organ (starting position corresponding to 120° joint position) with an amplitude of 400 μm (corresponding to joint movements between 120° and 80° (Weiland and Koch, 1987). The stimuli were applied by a ramp-and-hold generator (for details of stimulation procedure, see Büschges, 1989). The applied stimulus velocities were in the range 0.067–9.4 mm/s (6.7–1240.7/s). Then the mesothoracic and metathoracic segments were opened by removing the tergum. After the gut, fat, and connective tissue were
removed, the mesothoracic ganglion and roots of the lateral nerves were fixed on a wax-covered ganglion holder. The neurilem was then treated with pronase (Sigma Chemical Co., St. Louis, MO) for 40–80 s. Intracellular recordings were performed from axons of fCO afferents in the ganglion close to the entrance of the nervus cruris into the mesothoracic ganglion using thin-walled glass microelectrodes filled, in the case of physiologic characterizations of fCO afferents (bridge mode and current clamp recordings), with a tip solution of 2 M KAc/0.05 M KCl (electrode resistance 12–20 MΩ) (see also Driesang and Büschges, 1993), and in the case of additional morphologic characterizations of fCO afferents, with 2 M KAc/0.05 M KCl/7% CoCl. Discontinuous current clamp recordings were performed with an NPI SEC1 L/H amplifier (NPI Electronic, Tamm, Germany) at switching rates of 10 kHz (25% duty cycle).

Measurement of Tibial Movements

The animal was fixed so that the longitudinal axis of its body was horizontal. The coxa and femur of the right middle leg were restrained in such a way so that its tibia was able to move freely in a vertical plane. The position of the tibia was measured with an optical detector described by Weiland et al. (1986). The voltage output of the detector was proportional to the position of the tibia. In this experiment, the FT control loop remained in the closed-loop configuration with the fCO apodeme intact.

Recordings from Motoneurons and Interneurons

The activity of the F2 nerve (innervating the extensor tibiae muscle) was recorded extracellularly using a hook electrode (Schmitz et al., 1991) to monitor the behavioral state of the experimental animal. The nerve F2 contains the axons of FETi and SETi (fast and slow extensor tibiae motoneurons) and of the common inhibitor 1 (Cl1). Only reactions that showed a clear resistance reflex of the extensor motoneurons in these recordings (Bässler, 1983) were used for further analysis.

To receive stable recordings, the mesothoracic ganglion was placed on a wax-coated platform, fixed, and treated as described above. Motoneurons and nonspiking local interneurons underlying the neural network controlling the FT joint (Büschges, 1990b; Sauer et al., 1996) were recorded in the dorsolateral neuropilar region of the mesothoracic ganglion. Individual nonspiking interneurons (NSI) were morphologically and physiologically identified according to Büschges (1990). Most of the recordings were performed using an NPI SEC1 L/H amplifier (NPI Electronic) together with thin-walled glass microelectrodes (wpi). For discontinuous current clamp recordings the electrodes were filled with 2 M KAc/0.05 M KCl. In the case of physiologic and morphologic characterization the electrodes were filled with 4% Lucifer Yellow (tip solution) and 1 M LiCl (shaft solution; electrode resistance 40–70 MΩ). The neurons were stained after physiologic characterization by applying −1 to −10-nA current pulses and were treated according to established procedures. Whole mounts of the stained neurons were drawn using a camera lucida.

Data Acquisition and Evaluation

Intracellular and extracellular signals, current, and stimulus traces were recorded on an eight-channel DAT recorder (Biologic DTR-1800; sample rate 12 kHz) and analyzed off-line on a 33-MHz 386 or 40-MHz 486 personal computer. The AD conversion was performed using a DT2821/F 16SE-channel AD/DA data acquisition board (250 kHz maximum sampling rate) and the program Turbolab V4.0 (Stemmer). The single-channel sampling rate was set to 0.5–5 kHz for analysis of the intracellular recordings of the NSI, and to 7.5 kHz for analysis of the extracellularly measured spike activity. The output files were converted with the program TL SPIKE (Hemcker et al., ver. 2.0, University of Kaiserslautern, 1991) to perform further analysis with the program Spike2 (Cambridge Electronics Co., Science Products, ver. 3.14). This program was used to average responses of NSI and produce peristimulus time (PSTH) histograms (bin width of 10 ms) of the extracellular F2 recording.

Differences in means of individual samples were tested using a modified t test after Dixon and Massey (1969). Means were regarded as significantly different at p < 0.05.

Solutions

The noncompetitive GABA antagonist picrotoxin (PTX) was diluted in dimethylsulfoxide (DMSO) in a relation of 1:10. Stock solutions of 10−4 M PTX in saline were prepared from this solution in advance and diluted to their final concentrations prior to application. Application of the drugs was performed by removing part of the saline from the thorax of the experimental animal and adding the drug solutions with concentrations in the range of 5 · 10−6 and 5 · 10−5 M.

RESULTS

Synaptic Potentials in Terminals of Afferents from the fCO

Intracellular recordings from axons of sensory cells located in the ventral scoloparium of the middle leg fCO were performed close to the location where the main leg nerve [nervus cruris (n,cr)] enters the mesothoracic ganglion. The membrane potential of
the recorded afferents ranged from −67 to −78 mV and was on average −71.9 ± 3.7 mV (mean ± S.D.) as evaluated for 10 of our recordings. The recordings showed not only orthodromically traveling spikes from the sensory cell to the central nervous system (CNS), but also depolarizing synaptic potentials that occurred either relative to fCO stimuli (Fig. 1) or spontaneously [Figs. 1 and 2(B)]. The amplitude of the unitary depolarizing potentials ranged from 0.5 to 3.5 mV and their duration was about 30–50 ms. These synaptic potentials appeared to be generated in the mesothoracic ganglion because (a) they persisted after removal of the fCO from the femur; and (b) in general, their amplitude was smaller with the recording electrode located more distal on the nts.

Properties of Synaptic Potentials in fCO Afferents. The amplitudes of these presynaptic potentials were dependent on the membrane potential of the afferent. Injecting constant hyperpolarizing current into the afferents increased their amplitudes, while injecting depolarizing currents decreased them (Fig. 2). This was true for spontaneously occurring postsynaptic potentials in the afferents (PSPs) [Fig. 2(B)], as well as for those occurring with fCO stimulation. Recordings under discontinuous current-clamp conditions revealed a cessation of the PSPs at membrane potentials ranging from −60 to −65 mV in different preparations, on average at 62.8 ± 2.3 mV (n = 5) (Fig. 2).

Figure 1  Synaptic potentials in afferents of the femoral chordotonal organ (fCO). Recording from a sensory axon sensitive to relaxation velocity (V−) at resting potential of −70 mV during ramp-and-hold stimuli at the fCO in an inactive stick insect. The upper parts of the action potentials are cut. Note the synaptic noise and depolarizing potentials in the afferent during elongation of the fCO. The behavioral state of the animal, as monitored by recording the activity of the extensor motoneurons extracellularly, is inactive owing to the strong resistance reflex.

Figure 2  Electrophysiologic and pharmacologic properties of depolarizing synaptic potentials in fCO afferents. (A) Discontinuous current-clamp recording from a V−-sensitive afferent at two different resting potentials. The stimulus-evoked depolarization disappeared at −62 mV. (B) Spontaneously occurring synaptic potentials in fCO afferents disappeared with a membrane potential of about 61 mV and reversed with more positive potentials. (C) Topical application of 1 × 10−5 M picrotoxin onto the mesothoracic ganglion led to a cessation of the synaptic potentials in a velocity (relaxation)-sensitive fCO afferent. Asterisks indicate clipped spikes in all figures.

Bath application of PTX, a noncompetitive blocker of GABAergic chloride channels, at concentrations of 1 × 10−5 M strongly reduced the amplitude of spontaneously occurring PSPs as well as the amplitude of PSPs occurring relative to fCO stimuli [Fig. 2(C)]. Within 5–10 min after application of PTX the PSPs completely disappeared (six of nine experiments) or were reduced in amplitude (three of nine experiments). In only one of the trials we found a complete recovery of the PSPs following removal of PTX from the bath solution. In the other experiments, recovery of the PSP amplitude was incomplete.

The occurrence of the synaptic potentials was correlated with a strong decrease in membrane resistance. In the recording shown in Figure 3(B), the input resistance decreased from about 26 to 21 MΩ during the occurrence of stimulus-
Figure 3  Influence of spontaneous as well as stimulus-induced synaptic potentials on the membrane resistance and action potential amplitude. (A) Stimulus-related PSPs lead to a strong depolarizing of a V± unit (for definition of + and –, see Table 1 legend) during elongation and relaxation. Afferent was held hyperpolarized (−5 nA). (B) Recording of the same afferent as in (A) in discontinuous current clamp mode. The afferent was held hyperpolarized (−5 nA) and current pulses with an amplitude of −2.5 nA were applied. The resulting change in membrane potential was smaller during the fCO stimulus ramp, indicating a decrease in membrane resistance. (C) Spontaneous and (D) stimulus-related PSPs led to a strong decrease in action potential amplitude in the afferent. (E) Plot of the action potential amplitudes in an fCO afferent versus depolarization of the afferent membrane potential from rest.

related synaptic potentials. As a consequence, the amplitudes of the afferent spikes recorded from fCO afferents were strongly reduced in the presence of the PSPs. This was true for action potentials of fCO afferents that coincided with spontaneous as well as stimulus-induced PSPs [Fig. 3(C,D)]. The amplitude of the action potential was found to decrease by up to 57% as evaluated for four recordings [Fig. 3(E)].

These data suggest that the depolarizing potentials recorded in the fCO afferents were inhibitory, similar to those found in the fCO afferents of lo-
Table 1 Relation between Afferent Modality (Rows 1 and 2) and Modality of Presynaptic Inhibition (Row 3) in Sensory Neurons of the Stick Insect fCO

<table>
<thead>
<tr>
<th>Classification</th>
<th>Response</th>
<th>Modality of Presyn. Inputs</th>
<th>No. of Records</th>
</tr>
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<tr>
<td>Position and velocity</td>
<td>P+V+</td>
<td>V+, V±, P+V+(4), P+V±</td>
<td>16</td>
</tr>
<tr>
<td>sensitive</td>
<td>P+V−</td>
<td>V±</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>P−V−</td>
<td>V±</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>P+V±</td>
<td>V±</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>P−V±</td>
<td>V−</td>
<td>2</td>
</tr>
<tr>
<td>Position and acceleration</td>
<td>P+A±</td>
<td>V±, P+V±</td>
<td>3</td>
</tr>
<tr>
<td>sensitive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity sensitive</td>
<td>V+</td>
<td>V+, V±(6)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>V−</td>
<td>V−(3), V±, P−V±</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>V±</td>
<td>V±</td>
<td>13</td>
</tr>
<tr>
<td>Velocity and acceleration</td>
<td>V+A±</td>
<td>V±, P+V+A±</td>
<td>3</td>
</tr>
<tr>
<td>sensitive</td>
<td>V−A±</td>
<td>V±</td>
<td>2</td>
</tr>
<tr>
<td>Acceleration sensitive</td>
<td>A+</td>
<td>A+</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>A−</td>
<td>A+, A−</td>
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<tr>
<td></td>
<td>A±</td>
<td>V±(3), V−, V±A±</td>
<td>14</td>
</tr>
</tbody>
</table>

1 Numbers indicate the number of recordings for the encountered type. The most common type within a group is boldfaced. Definition of stimulus modalities: A = acceleration sensitive; P = position sensitive; V = velocity sensitive; + = sensitive for elongation stimulus or positive acceleration; − = sensitive for relaxation stimulus or retarding acceleration.

Custs (Burrows and Laurent, 1993; Burrows and Matheson, 1994).

Synaptic Potentials in fCO Afferents Derive from Signals of fCO Afferents. Stimulus-related synaptic potentials occurring in fCO afferents were related to the stimulus modality position, velocity, and acceleration. We attempted to correlate the modality to which the afferent was sensitive with the stimulus modality of its presynaptic input. We investigated 17 of the 22 types of sensory cells that are known in the fCO of the stick insect middle leg (Hofmann et al., 1985; Hofmann and Koch, 1985; Büschges, 1994). In 95 of 119 recordings we detected antidromically traveling PSPs.

In general, we found position-sensitive units to receive PSPs correlated to fCO stimulus velocity or to a combination of position and velocity signals (Table 1 and Fig. 4). Sensory neurons that responded to both position and velocity signals received PSPs correlated to position and velocity (Table 1). The membrane potential of such an afferent was more depolarized at more flexed FT angles (i.e., elongated fCO position) and was more depolarized during the elongation movement [Fig. 4(A)]. This led to dependence of the afferent membrane potential on the fCO position [Fig.4(A,B)]. Sensory neurons responding to position as well as acceleration signals received similar synaptic inputs (Table 1). fCO afferents that were mainly velocity sensitive received PSPs that were dependent on stimulus velocity, but often also during the stimulus direction that did not increase the spike frequency [Table 1 and Figs. 1(A), 2(A,B), and 4(C,D)]. In only one recording was position dependence of synaptic potentials detected in a purely velocity-sensitive afferent. Sensory neurons responding to both velocity and acceleration received PSPs correlating to all three movement parameters (Table 1).

Femoral chordotonal organ afferents that were purely acceleration sensitive received depolarizing presynaptic inputs correlated to velocity and acceleration signals (Table 1). We did not observe position-dependent PSPs in this type of sensory neuron.
Figure 4  Relation between afferent modality and modality of synaptic inputs. (A) Recording of a position and elongation velocity–sensitive (P+V+) sensory neuron at different fCO positions. B Plot of the membrane potential in two position-sensitive afferents versus fCO position, i.e., FT angle. (C) Recording from an elongation velocity and position–sensitive (V+P−) fCO afferent receiving synaptic potentials during both directions of fCO movement, with the synaptic inputs induced by relaxation of the fCO attenuating over about 2 s following relaxation. (D) Elongation velocity–induced synaptic potentials in relaxation velocity–sensitive sensory neurons (V−).

PTX Selectively Blocks Presynaptic Inputs to fCO Afferents. To find out whether these synaptic inputs to fCO afferents contribute to the tuning of individual interneuronal pathways within the FT control network, we tried specifically to remove them from this network. In doing so, however, we had to look for a method that did not influence other inhibitory connections within this network as well (Fig. 5A). Within the FT control network of the stick insect identified nonspiking premotor interneurons exist that transmit position and velocity information from fCO signals onto the tibial motoneurons (Büschges, 1990). These interneurons receive inhibitory synaptic input from the fCO afferents via intercalated interneurons (Sauer et al., 1996) [Fig. 5(A)]. The representation of position and velocity signals is specific and characteristic for individual types of premotor interneurons (Büschges, 1990; Sauer et al., 1996).

We found that topical application of PTX on the isolated mesothoracic ganglion within the FT control network exclusively removed presynaptic inputs to fCO afferents (Fig. 5). Spontaneous or fCO position-related inhibitory postsynaptic potentials in premotor NSIs [Fig. 5(B)] were not abolished following application of PTX. Furthermore, stimulus-related inhibitory synaptic inputs to NSIs during elongation or relaxation stimuli at the fCO [Fig. 5(C,D)] were not blocked. Position- as well as velocity-dependent responses were even enhanced, as
Figure 5 Inhibitory synaptic interactions within the neuronal network controlling the FT control loop were not blocked by picrotoxin. (A) Schematic drawing of known inhibitory pathways processing sensory fCO information (cf. Büschges, 1995; Sauer et al., 1996). 1: Presynaptic inhibition of sensory afferents. Blocking of presynaptic inhibition by picrotoxin in fCO afferents is shown in Figure 2(C). 2: Spiking interneurons mediating delayed inhibition of nonspiking interneurons. 3: Inhibitory influence on nonspiking interneurons of unknown source. 4: Inhibitory influence of I-type nonspiking interneurons onto motoneuron. (B) Intracellular recording from nonspiking interneuron of type I1 before and after application of picrotoxin. The amplitude of the inhibitory inputs during elongation of the fCO was increased. (C) Intracellular recording of interneuron type E3 before and after application of picrotoxin. The amplitude of the inhibitory inputs during relaxation of the fCO was increased. (D) Intracellular recording from nonspiking interneuron of type E3 showing spontaneous inhibitory postsynaptic potentials before and after PTX application (arrows). (E) Intracellular recording of interneuron type H1 after PTX application. Note the inhibition of SETi activity upon injection of depolarizing current into H1 (arrow).

Figure 6 Influence of picrotoxin application on the membrane resistance of nonspiking interneurons of type E3. (A) Membrane resistance of E3 was measured by injecting hyperpolarizing current pulses into the interneuron before and after application of picrotoxin. (B) Quantitative evaluation of the alteration in membrane resistance at different holding potentials of the interneuron (100% = membrane resistance at resting potential before application of picrotoxin).

Application of PTX did not increase the membrane resistance in nonspiking premotor interneurons [e.g., type E3 in Fig. 6(B); also types E7 and I1] that would have indicated the existence of PTX-inputs ... of the FT control network were not sensitive to PTX.

Application of PTX did not increase the membrane resistance in nonspiking premotor interneurons [e.g., type E3 in Fig. 6(B); also types E7 and I1] that would have indicated the existence of PTX-sensitive ion channels. For example, in the case of interneuron E3 [Fig. 6(B)], the membrane resistance was found to decrease following application of PTX, from 5.6 ± 0.15 MΩ (n = 20) before to 4.8 ± 0.14 MΩ (n = 20) after the application. This must be the result of an increased position dependence of the interneuronal membrane potential following the removal of presynaptic inhibition by...
PTX (see below). The resting potential of this type of interneuron was not significantly affected by the application of PTX \( (n = 5) \).

In summary, it appears that topical application of PTX only removes inhibitory synaptic inputs to fCO afferents in the FT control network, and could therefore be used to study their role in the FT control network.

**Effects of Removal of Synaptic Inputs to fCO Afferents on Action of FT Control Network**

In general, we found that the parameter dependence of the membrane potential of a given NSI strongly increased following application of PTX. This was true for position- and velocity-dependent inputs from the fCO. The results are demonstrated in detail for interneurons E3, I1, and E4.

Interneurons of type E3 [Fig. 7(A)] are depolarized during fCO elongation movements and hyperpolarized during fCO relaxation movements. Their resting membrane potentials depend on position of the FT joint. It is more depolarized with elongated fCO positions (increasing joint flexion). Interneurons of type E3 provide excitatory synaptic drive onto the extensor motoneurons (Büsches, 1990). After removal of the presynaptic inputs by PTX, the amplitude of the depolarization in interneuron E3 during fCO elongation movements was increased and the position-dependent portion of the response was enhanced [Fig. 7(A–C)] [for a definition of position- and velocity-dependent portion of the response, see Fig. 7(B)]. In addition, the amplitude of the hyperpolarization during fCO relaxation stimuli was enhanced, indicating an increased activation of the pathways transmitting inhibition onto the NSI.

Similar results were obtained from recordings of interneurons of type I1. This type of interneuron is...
hyperpolarized by elongation signals from the fCO and depolarized during fCO relaxation movements [Fig. 7(D)]. Increased elongated fCO positions (corresponding to more flexed positions of the FT joint) induce a constant hyperpolarization of I1 [Fig. 7(D)], while relaxed fCO positions (extended joint) depolarize the membrane potential (Büschges, 1990). Following application of PTX the amplitude of the hyperpolarization during elongation of the fCO increased about three times [Fig. 7(D,E)] compared to the control. There was a two-fold increase in position dependence induced by application of PTX [Fig. 7(D,E)].

The alteration of parameter representation after the removal of the presynaptic inputs is even more obvious for NSI of type E4, as shown in Figure 8. This type of interneuron shows only a very weak response, or often none to the fCO position stimuli (Fig. 8). Following application of PTX the velocity component in the interneuron’s response was enhanced; the membrane potential of this type of interneuron also clearly showed position dependence (Fig. 8).

In summary, it is evident that with removal of presynaptic inhibition by application of PTX the neuronal pathways (i.e., the NSIs) showed increased dependence on the movement parameters measured by sensory cells in the fCO. Table 2 summarizes the qualitative results of the changes observed for the different types of interneurons recorded.

### Table 2. Summary of Changes Occurring in Premotor Nonspiking Interneurons following Removal of Presynaptic Inhibition

<table>
<thead>
<tr>
<th>Neuron Type</th>
<th>Effect of PTX Application on Synaptic Input during Elongation</th>
<th>No. of Records</th>
</tr>
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<tbody>
<tr>
<td>E3</td>
<td>Increase Position</td>
<td>5</td>
</tr>
<tr>
<td>E4</td>
<td>Increase Position 50% of records</td>
<td>4</td>
</tr>
<tr>
<td>E5/E6</td>
<td>Increase Position 50% of records</td>
<td>2</td>
</tr>
<tr>
<td>E7</td>
<td>No effect</td>
<td>2</td>
</tr>
<tr>
<td>I1</td>
<td>Increase Position 50% of records</td>
<td>2</td>
</tr>
<tr>
<td>I4</td>
<td>No effect</td>
<td>1</td>
</tr>
</tbody>
</table>

1. Nomenclature in row 1 is after Büschges 1990; Sauer et al., 1996. The specific effect on velocity (row 2) and position (row 3) sensitive–dependent portion of the membrane potential is indicated.

Removal of Synaptic Inputs to fCO Afferents Increases Excitatory Inputs on NSI. Which alterations within the neuronal network led to the increased synaptic inputs from fCO signals onto the premotor interneurons? For interneurons that receive mainly inhibitory signals during fCO movements such as interneuron I1, it is evident that this increase is due to an enhancement of the inhibitory pathways from the fCO onto them. However, the situation is more complicated for those interneurons that receive a net depolarization during fCO movements (i.e., interneurons E1, E2, E3, E4, E5, or E6). On the basis of the known network topology (Sauer et al., 1995, 1996), three possibilities exist: the increase in the amplitude of depolarizing synaptic inputs during fCO elongation could be caused (a) by an increase of stimulus-related direct excitatory inputs, (b) by a decrease of the stimulus-related polysynaptic inhibitory inputs onto the interneurons, or (c) by a combination of both. To check for one of the possibilities, we measured the membrane resistance of interneurons at different holding currents (i.e., membrane potentials) in discontinuous current clamp modus.
Figure 9 Alterations in the efficacy of excitatory inputs to nonspiking interneurons induced by the removal of inhibition. (A) Comparison of the change in input resistance in E3 due to fCO elongation before and after application of picrotoxin at (i) resting potential and (ii) holding the neuron hyperpolarized by current injection. (B) Quantitative evaluation of the decrease in membrane resistance during the ramp and holding phases of an fCO elongation stimulus.

Under control conditions the membrane resistance of interneuron E3 (n = 4) was markedly decreased by synaptic inputs during fCO elongation [Fig. 9(A[i])]. Following application of PTX this decrease was enhanced [Fig. 9(A[ii])], which becomes most obvious when the interneuron was held slightly hyperpolarized [Fig. 9(A[ii])]. For the individual recording the stimulus related decrease was enhanced from about 15% up to 25% [Fig. 9(B)]. Also, position-dependent alterations in membrane resistance were increased following application of PTX. This effect was weaker but significant [Fig. 9(B)].

These results indicate that excitatory synaptic inputs from fCO afferents onto the interneurons which are known to be mediated by direct connections (Burrows et al., 1988; Sauer et al., 1995) contribute to the enhancement of depolarization by fCO signals following the removal of presynaptic inputs to fCO afferents by the action of PTX.

**Alterations in Action of FT Control Network following Removal of Synaptic Inputs to fCO Afferents.** To investigate the effect of removal of the observed synaptic inputs to fCO afferents on the action of the complete FT control network, we recorded intracellularly and extracellularly from extensor motoneurons before and after application of PTX. The resting potential of FETi at a given fCO position, for example, changed only slightly following application of PTX (Fig. 10). However, the amplitude of the stimulus-related synaptic inputs from the fCO onto FETi was strongly enhanced (n = 2) [Fig. 10(A,B)]. The position-sensitive component of the FETi response, as measured in depolarization amplitude of FETi during the holding...
Figure 10 Alteration in motoneuronal responses to fCO stimulation induced by removal of presynaptic inhibition. (A) Sequences of a parallel intracellular recording from FETi motoneuron and extracellular motor activity of the extensor nerve during continuous rampwise stimulation of the fCO before (saline) and after application of picrotoxin (PTX). The upper trace gives a peristimulus time histogram (PSTH) of the SETi activity. The actual number of the stimulus is indicated below the stimulus trace. (B) Comparison of the average time course in membrane potential in FETi in response to fCO stimuli. Note that the amplitude of the depolarization during the ramp phase (velocity- plus position-sensitive component) and the depolarization amplitude during the holding phase of the elongation stimulus (position sensitive component) were enlarged. (C) Comparison of the amplitude of the velocity and the position component in FETi response before and after application of picrotoxin (n = 6). (D) Average PSTH of the activity of the slow extensor tibia motoneuron before (black bars) and after (white bars) application of picrotoxin during rampwise stimulation of the fCO.
Figure 11  Quantitative description of the removal of presynaptic inhibition on the activity of the extensor motoneurons in the FT control. (A) Change in the position dependence of the reflex loop response to different fCO holding positions. The fCO apodeme was held at four positions between −400 and +800 μm (corresponding to tibia positions of 60°–180°). The activity of the extensor motoneurons (SETi and FETi) were extracellularly measured (middle trace) and the instantaneous frequency was plotted (top trace; frequencies over 100 Hz were cut). (b) Quantitative change in the position and velocity dependence of the control loop response following removal of presynaptic inhibition. (Left) Dependence of SETi activity on fCO position in three animals (marked with different symbols). The regression line was plotted for the average of the activity in the three animals. (Right) Effect of removal of presynaptic inhibition on the velocity dependence of SETi activity in three animals (marked by different symbols).

phase after fCO elongation, was strongly increased. In most cases, FETi produced action potentials during the holding phase: i.e., there was a strong influence on the muscle that was not present in the untreated animal. In addition, we observed a moderate increase in the velocity-sensitive component of the FETi response [Fig. 10(C)].

Similar results were obtained from recordings from SETi motoneurons [Fig. 10(D)] (n = 2), but with a more pronounced increase in SETi discharge rate at a given fCO position owing to an increased position dependence of SETi activity (see below).

Removal of Synaptic Inputs to fCO Afferents Affects Position Dependence of the Motor Output. How does the action of the complete FT control network quantitatively change after the removal of the synaptic inputs to fCO afferents investigated with respect to position and velocity dependence? To answer this question, we analyzed the dependence of motoneuronal activity on the position and velocity signals from the fCO over a wide range of parameter values. Figure 11(A) shows a recording of the extensor nerve F2 at different holding positions of the fCO before and after application of PTX. It is obvious that the position dependence of extensor motoneuronal activity increased after application of PTX. This finding is substantiated by plotting the average SETi discharge rate versus fCO position [Fig. 11(B)]. A comparison of the dependence of extensor motoneuron activity on fCO position showed a difference between the slopes of the best-fit regression lines describing the relationship before and after application of PTX (average slope before: 0.9 Hz/100 μm; range: 0.8–1.02 Hz/100 μm; average slope after: 3.3 Hz/100 μm; range: 2.5–4.5 Hz/100 μm).

We also compared the dependence of the SETi
In summary, it appears that following application of PTX, the overall response of extensor motoneurons to fCO signals was strongly enhanced mainly owing to significantly increasing position dependence. The generation of catalepsy in the FT joint strongly relies on a high sensitivity of the joint control system to the velocity of a joint movement combined with a small sensitivity to the absolute position of the tibia (Bässler, 1993).

Movement of the Tibia in the Closed Loop. Because of the fact that the removal of synaptic inputs to fCO afferents leads to increased position dependence, we would expect the loss of the system’s capability to perform catalepsy.

To test this hypothesis, we measured the time course of tibial movements (see Materials and Methods) in response to passive displacements before and after application of picrotoxin in intact FT joints (closed loop) (Fig. 12). The FT joint was passively bent by manually moving the tibia with the help of a minuten pin. The tibia was held for a short time in this flexed position (much shorter than in previous experiments) (Bässler, 1983, 1993). After the release of the tibia, its return movement toward the starting position was recorded over time. Before application of PTX the tibia showed a time course of movement characteristic for the behavior of catalepsy: Immediately after its release the tibia extended quickly to a new, still-flexed position (arrows). (Right) Movement of the tibia after releasing it from an extended position.

discharge rate on stimulus velocity before and after application of PTX. We found that extensor activity was increased over the whole range of velocities tested. However, the dependence of SETi activity on stimulus velocity, compared by the slope of the regression lines before and after application of PTX, decreased (average slope before: 37 Hz/decade; range: 21–52 Hz/decade; average slope after: 16 Hz/decade; range: 13–19 Hz/decade). This result is in accordance with the wiring diagram of the network (Fig. 5(A)) and the fact that the direct excitation as well as the delayed inhibition of the NSI are influenced by presynaptic inhibition of afferent terminals. For slow fCO stimuli the delayed inhibition occurs during the stimulus ramp, and therefore decreases the response amplitude; for fast stimuli it occurs after the end of the stimulus ramp, and therefore only accelerates the response decline. Thus, removal of presynaptic inhibition should increase the amplitude of the response for slower stimuli more than for faster stimuli.
DISCUSSION

The present investigation has shown the following. (a) The terminals of the afferents in the stick insect fCO receive presynaptic inputs from afferents of the same sensory organ. The nature of these potentials appears to be inhibitory. (b) There is a specific interaction between afferent modality and modality of presynaptic inhibition received: Sensory neurons responding to position and velocity do receive presynaptic inputs mainly from sensory neurons responding to these movement parameters. In contrast, sensory neurons responding mainly to acceleration receive presynaptic inhibition that is almost exclusively induced by stimulus acceleration. (c) Removal of the synaptic inputs to fCO afferents altered information processing in identified interneuronal pathways in such a way that information about tibial position and velocity to the extensor motoneurons is enhanced significantly. As a consequence of the increase in position sensitivity, following removal of presynaptic inhibition the FT control network no longer performs the behavior of catalepsy.

Presynaptic Inhibition of Proprioceptive Afferents

The central terminals of fCO afferents receive synaptic inputs from afferents of the same sensory organ that are excited in part by the same movements. These synaptic inputs are associated with an increase in axonal membrane conductance and reduce the spike amplitude in the terminals. The reversal potential of the depolarizing postsynaptic potentials was about −63 mV (i.e., about 10 mV above the resting potential). The reversal potential and the fact that the potentials are blocked by PTX application indicate that they are mediated by GABAergic synaptic inputs. The latter are known to activate chloride channels in afferent membranes (Gallagher et al., 1978; Kennedy et al., 1980). Thus, the potentials recorded from fCO afferents show properties that are characteristic of inhibitory synaptic potentials. Furthermore, our results are in accordance with previous findings on presynaptic inhibition of proprioceptive sensory neurons in other arthropod species, e.g., the crayfish (Cattaert et al., 1992) and the locust (Burrows and Laurent, 1993). At present, it still remains unclear which neuronal pathways mediate presynaptic inhibition of afferents. It was suggested that intercalated spiking interneurons receiving sensory information from fCO afferents may inhibit the terminals of the afferents (Burrows and Laurent, 1993).

The interactions between fCO afferents sensitive to different stimulus modalities were found to be specific. Afferents that are sensitive to velocity and position, as well as those that are additionally sensitive to acceleration, mainly received velocity- and position-dependent inhibitory inputs. Only in a minority of recordings were acceleration-sensitive synaptic inputs onto such afferents detected. In contrast, purely acceleration-sensitive afferents received inhibitory synaptic inputs almost exclusively from acceleration-sensitive afferents. Presynaptic inhibition between velocity- or position-sensitive cells and acceleration-sensitive sensory cells thus seems to be rather rare. It therefore appears likely that the sensory cells of the fCO can be grouped into two functional groups: One group mainly measures movements and position of the tibia and a second one is sensitive to acceleration of the tibia.

Specificity of Removal of Presynaptic Inhibition by Bath Application of PTX

In part, the aim of our study was to investigate the effect of a removal of presynaptic inhibition on information processing in the FT control network. Therefore, we had to look for a way to remove presynaptic inhibition without affecting the other known inhibitory interaction in the FT control network (Büsches, 1990; Sauer et al., 1995, 1996; summary in Büschges, 1995). We were able to block the depolarizing synaptic inputs to fCO afferents by bath application of PTX. However, as PTX is reported to be a noncompetitive blocker of GABAergic chloride channels in general (Takeuchi and Takeuchi, 1969; Iversen, 1984), part of the experiments dealt with the question as to whether other inhibitory interactions within the FT control network are affected by PTX application as well. The results showed that obviously exclusively synaptic inputs to fCO afferents are affected by PTX in this network. The other known inhibitory interactions within the FT control network were found to be insensitive to PTX. This raises three hypothesis: (a) the latter ones might be mediated by another transmitter than GABA; (b) they are mediated by an ion other than CL−, i.e., potassium; or (c) they may be mediated by GABAergic CL− ion channels that are not sensitive to PTX. Driesang and Büschges (1996) reported that in the stick insect stimulus-related inhibitory synaptic inputs to NSIs of type II are based on outward currents with a
reversal potential of about −93 mV. In addition, recent experiments show that stimulus-related inhibitory inputs to individual NSI can be blocked by TEA, a specific blocker of potassium channels (Gramoll and Sauer, 1996) and that motoneurons possess transmitter-activated potassium channels (Büschges and Haas, 1996). These results indicate that the inhibitory inputs to NSI and to motoneurons during resistance reflex generation may be mediated by potassium channel activation, rather than by chloride channel activation. This evidence appears interesting in light of the detailed knowledge on GABAergic, chloride-mediated inhibition in interneuronal pathways in other orthopteran species (Watson, 1986; Watson and Laurent, 1990; Watson and Burrows, 1987).

Role of Presynaptic Inhibition in Information Processing

Removal of presynaptic inhibition in the terminals of fCO afferents had two effects on the processing of sensory information in identified premotor NSIs. First, the overall response to signals from the fCO in individual interneurons was increased. The synaptic inputs from phasic and tonic afferents to NSI were increased. This increase contributed to an increase in the gain of the motor output of the reflex loop. This finding is consistent with the hypothesis that presynaptic inhibition might act as a gain control mechanism in invertebrate motor control systems (Burrows and Matheson, 1993; Cattaert et al., 1992).

Second, removal of presynaptic inhibition altered the relative weighting of the movement parameters in the joint control loop. To perform the behavior of catalepsy, the joint control loops of the stick insect show high sensitivity to the velocity of an fCO movement and only little sensitivity to the position of the fCO (Bässler, 1983, 1993). This allows a limb to return very slowly to the starting position after the joint is passively bent or stretched. After application of PTX the sensitivity of the motor output to the position of the joint was increased. As a consequence, legs still showed resistance reflexes but no longer cataleptic behavior. This is true even though part of the increase in motoneuronal activity following application of PTX might be due not only to the removal of presynaptic inhibition from fCO afferents, but also from removal of presynaptic inhibition from other tonically active internal or external sensory organs which affect motoneuronal activity. However, these unspecific influences would not lead to alterations correlated to position or velocity signals from the fCO.

Our results suggest that alterations in parameter weighting among fCO signals by presynaptic inhibition of afferents may be a basic prerequisite for the performance of cataleptic behavior. Unfortunately it is not possible to remove presynaptic inhibition selectively from individual classes of afferents (e.g., position-sensitive sensory neurons). However, support for this idea could derive from removal of presynaptic inhibition in FT control networks of other orthopteran species that do not exhibit catalepsy and in which parameter weighting in FT joint control is consequently different. For example, in the locust, which does not exhibit catalepsy, one could therefore expect that removal of presynaptic inhibition with PTX (Burrows and Laurent, 1993) would have a different effect on the parameter weighting in the joint control network compared to the stick insect. This is because despite basic similarities in network topology (Burrows et al., 1988; Büschges and Wolf, 1995; for review see Büschges, 1995), the properties of FT control loop action are very different. In the locust control loop action, velocity dependency is less and position sensitivity is more pronounced compared to the stick insect (Bässler and Ebner, 1978; Field and Burrows, 1982; Field and Coles, 1994). In fact, preliminary experiments indicate that removal of presynaptic inhibition in the mesothoracic ganglion of the locust by application of PTX does not alter the relative weighting of movement parameters velocity and position (W. Stein, unpublished results) and thus support the idea that relative parameter weighting in the FT control network derives at least in part from the specific action of presynaptic inhibition among fCO afferents.

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