Activity-Dependent Sensitivity of Proprioceptive Sensory Neurons in the Stick Insect Femoral Chordotonal Organ

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DiCaprio, Ralph A., Harald Wolf, and Ansgar Büssges. Activity-dependent sensitivity of proprioceptive sensory neurons in the stick insect femoral chordotonal organ. J Neurophysiol 88: 2387–2398, 2002; 10.1152/jn.00339.2002. Mechanosensory neurons exhibit a wide range of dynamic changes in response, including rapid and slow adaptation. In addition to mechanical factors, electrical processes may also contribute to sensory adaptation. We have investigated adaptation of afferent neurons in the stick insect femoral chordotonal organ (fCO). The fCO contains sensory neurons that respond to position, velocity, and acceleration of the tibia. We describe the influence of random mechanical stimulation of the fCO on the response of fCO afferent neurons. The activity of individual sensory neurons was recorded intracellularly from their axons in the main leg nerve. Most fCO afferents (93%) exhibited a marked decrease in response to trapezoidal stimuli following sustained white noise stimulation (bandwidth = 60 Hz, amplitudes from ±5 to ±30°). Concurrent decreases in the synaptic drive to leg motoneurons and interneurons were also observed. Electrical stimulation of spike activity in individual fCO afferents in the absence of mechanical stimulation also led to a dramatic decrease in response in 15 of 19 afferents tested. This indicated that electrical processes are involved in the regulation of the generator potential or encoding of action potentials and partially responsible for the decreased response of the afferents. Replacing Ca2+ with Ba2+ in the saline surrounding the fCO greatly reduced or blocked the decrease in response elicited by electrically induced activity or mechanical stimulation when compared with control responses. Our results indicate that activity of fCO sensory neurons strongly affects their sensitivity, most likely via Ca2+-dependent processes.

INTRODUCTION

Adaptation of primary afferent neurons to maintained levels of stimulation is a common phenomenon in most sensory systems. Previous studies on mechanosensory systems have found that adaptation may be due to a combination of mechanisms intrinsic or extrinsic to the sensory neuron, including mechanical factors such as the viscoelastic behavior of the receptor or supporting structures, and intrinsic membrane properties that may effect the transduction current directly or the spike encoder mechanism (French 1988, 1992; French and Torkkeli 1994). For example, Pacinian corpuscles are rapidly adapting mechanoreceptors where the removal of surrounding structures eliminates most of the adaptation of the receptor current (mechanical mechanism) although the firing rate of the modified afferent still adapts to a maintained stimulus (ionic mechanism) (Loewenstein and Mendelson 1965; Mendelson and Loewenstein 1964). In most instances, intrinsic membrane properties have been found to play a dominant role in mechanoreceptor adaptation. Ion channels in the sensory neuron can mediate adaptation either by a direct effect on the receptor potential or by acting at the level of action potential encoding in the neuron. In studies of the tactile spine of the cockroach, French (1984) has shown that viscoelastic mechanisms and receptor current adaptation during the transduction of the mechanical stimulus into the receptor potential are not important factors in the adaptation of this receptor. Adaptation of the tactile spine has instead been attributed to the slow inactivation of voltage-activated sodium channels (French 1987, 1989) as well as a contribution from calcium-dependent potassium channels (French and Torkkeli 1994; Torkkeli and French 1995). The rapidly and slowly adapting stretch receptors of the crayfish (Swerup and Rydqvist 1992) have similar mechanical properties (Nakajima and Onodera 1969a), and the differences in adaptation have been ascribed to intrinsic membrane properties affecting the receptor current and spike encoding mechanism (Swerup and Rydqvist 1992), one of which is a calcium-dependent potassium current, I(Ca) (Erxleben 1993; Ottersen and Swerup 1985a,b).

One of the main functions that adaptation is generally thought to serve is regulating the sensitivity of a sensory system in order to enhance the response to transient sensory inputs superimposed on a sustained background. In locomotor systems, transient sensory information plays a significant role in the generation of a proper motor output by controlling the magnitude and time course of motor activity and by controlling phase transitions in rhythmic locomotor programs (Bässler and Büschges 1998; Cattaert and LeRay 2001; Grillner 1981; Pearson 2000). Therefore sensory adaptation may have important functional consequences in sensorimotor systems that continuously receive and process sensory information during the generation of functional motor programs such as postural control while standing and movement control during locomotion.

To assess the possible consequences of sensory adaptation in a terrestrial locomotor system, we investigated the adaptation

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of sensory neurons in a proprioceptive sense organ of the stick insect middle leg, the femoral chordotonal organ (fCO). The sensory neurons of the fCO encode signals relating to movement and position of the tibia at the femur-tibia (FT) joint (Bässler 1967, 1993; for a comprehensive review of insect chordotonal organs see Field and Matheson 1998). Sensory signals from the fCO are utilized for controlling posture and movement of the tibia during standing and voluntary movements, such as walking (Bässler 1974, 1988; Field and Burrows 1982; Zill 1985; summary in Bässler and Büschges 1998). Chordotonal organ afferents measure position, velocity, acceleration, vibration, or a combination of these movement parameters (stick insect: Büschges 1994; Hoffmann and Koch 1985; Hoffmann et al. 1985; Sauer and Stein 1999; locust: Matheson 1990, 1992), with similar properties seen in orthopteran COs (Field and Matheson 1998) and in other arthropod systems (Mill 1976). In the stick insect, as in the locust (Field and Pfliüger 1989), approximately 80 afferents arising from the ventral scleromarin of the fCO provide inputs to the femur-tibia control system (Kittmann and Schmitz 1992). These afferents project into the segmental mesothoracic ganglion (Schmitz et al. 1991), where their information is processed via direct monosynaptic and distributed polysynaptic pathways onto interneurons and motoneurones (reviews: stick insect: Büschges et al. 2000; locust: Burrows 1996; Field and Matheson 1998). Sensory signals from the fCO are utilized for intra- and interjoint control in the stick insect leg muscle control system. For example, signals from the fCO mediate reflexes regulating position and movements of the FT joint during standing and voluntary movements (Büschges et al. 2000). These reflexes are highly flexible and are adapted to suit the actual behavioral state, thereby exhibiting significant changes in gain or even a reversal in sign (for review, see Bässler 1993; Bässler and Büschges 1998). On the basis of this extensive knowledge of the role of specific fCO signals and their subsequent processing, fCO afferents are well suited for investigating the significance of adaptation in a sense organ contributing to motor control.

We used random (white noise) mechanical stimulation of the fCO to provide a broadband excitation of the chordotonal organ. White noise stimulation also elicits a high spike density over a short period of time, thereby providing a good stimulus for investigation of receptor characteristics (Marmarelis and Marmarelis 1978). The potential influence of membrane properties that may contribute to fCO afferent adaptation was also investigated by electrical stimulation of single afferents with intracellular current injection. Putative Ca2+-dependent mechanisms were investigated by altering the Ca2+ concentration of the saline surrounding the chordotonal organ. The results presented here indicate that intrinsic membrane properties contribute to adaptation of stick insect femoral chordotonal organ afferents. The adaptation depended on the generation of action potentials in these neurons and is likely mediated by a calcium-dependent mechanism. The possible functional consequences of this adaptation with respect to the motor control system of the leg was tested “downstream” by recording from identified nonspiking interneurons and motor neurons within the FT control network.

METHODS

Experiments were performed on adult female stick insects (Carausius morosus) raised in the animal facilities of the University of Cologne under daylight conditions at a temperature of 20–22°C. The animals were mounted dorsal side up on a foam platform with the forelegs and hindlegs fixed aside the longitudinal axis of the body (Hess and Büschges 1997). The proximal leg segments of the left middle leg, i.e., the coxa, trochanter, and femur (the trochanter and femur are fused into a single segment in the stick insect) were fixed with dental cement (Protemp, ESPE) onto a foam rim pointing slightly upward at an angle of 30°. The tibia was extended over the distal margin of the rim and the platform and the FT joint then was fixed with dental cement at an angle of 120°. The femur was enclosed in a 3–4 ml bath that was built of dental cement (Protemp, ESPE) applied around the femur of the leg and filled with stick-insect saline (Bässler 1977; Weidler and Diecke 1969). The thorax of the animal was opened by a sagittal cut along the dorsal midline and pinned to the substrate to form a cavity that was filled with saline. The mesothoracic ganglion was freed from the surrounding connective tissue, placed on a wax coated platform, and fixed with cactus spines. Extensor tibiae motor neuron activity was recorded extracellularly with a monopolar hook electrode (Schmitz et al. 1988) from the extensor nerve (F2) (Bässler 1977) that contains the axons of fast and slow extensor motoneurones (FEtI and SETi) innervating the muscles of the FT leg joint (Bässler and Storrer 1980). The mesothoracic ganglion was prepared for intracellular recording from sensory afferents according to established procedures (Sauer et al. 1997) and recordings were made using thin-wall glass microelectrodes filled with a solution of 2 M KAc/0.5 M KCl (electrode resistance: 15–20 MΩ).

The ionic composition of the saline was (in mM) 179 NaCl, 17 KCl, 7.5 CaCl2, 25 MgCl2, and 2 Tris-(hydroxymethyl)-aminomethane, pH 7.4 (Weidler and Diecke 1969). In some experiments, CaCl2 was replaced by BaCl2 at the same concentration. To test the influence of elevated CaCl2 levels, in some experiments MgCl2 was decreased and replaced by CaCl2 to a final concentration of 25 mM CaCl2 and 7.5 mM MgCl2. The different ionic solutions were applied specifically to the fCO by altering the saline surrounding the fCO while maintaining the ganglion in normal saline. Saline changes were made by exchanging normal saline with modified solutions four times every 2 min as well as a final change after 15 min. Ample time was given after saline changes (25–35 min after the first exchange) before the next measurement of neuronal activity was performed.

Mechanical stimulation of the fCO

Mechanical stimulation of the fCO of the left middle leg was performed by exposing the receptor apodeme and fixing it to the clamp of an electromechanical stimulator. The apodeme was then cut distal to the clamp. Elongation (signaling flexion of the FT-joint) and relaxation (signaling extension of the FT-joint) movements were applied to the apodeme over a range of positions corresponding to femur-tibia angles between 60 and 120°. Ramp-and-hold stimuli with different stimulus velocities and holding times were tested in most cases from a starting position of 120° with an amplitude of 300 μm (corresponding to a tibia movement of 60°) (Weidler and Koch 1987). Stimulus velocities were in the range of 20–1,200°/s. This range encompasses movement velocities that are generated by the stick insect leg system during locomotion (Bartling 1993; Bässler 1983). Maximum movement velocities during white noise mechanical stimulation were approximately 2,200°/s, with an amplitude range of 60°, and 1,100°/s over the amplitude range of 30° that was usually applied to the fCO. Acceleration of the stimuli was not controlled independently.

White noise was generated by a 32-bit pseudo-random number generator clocked at 100 KHz. The digital output of this generator was
filtered to the desired bandwidth of 60 Hz using a variable 8-pole low-pass filter (Wavetek 852) and then amplified as required. The DC position of the fCO was offset by +30° (i.e., to a joint position of 90°) before application of the white noise signal to bias the range of movement to the middle of the joint angle range of the ramp-and-hold stimuli. To minimize the transient response of the afferents at the onset of random stimulation, the gain of the output amplifier was increased to the final desired amplitude by manually adjusting the output amplifier gain over a 2- to 5-s period. As the minimum gain of this amplifier was not zero, the initial application of the noise signal always resulted in some very small amplitude movement of the stimulator and consequent firing of the afferent. This is evident in all records (see for example Fig. 1) where the afferent starts firing before there is a detectable signal from the movement monitor. This is due to a combination of the extreme sensitivity of the afferent to movement and the resolution and dynamic range of the A/D converters used to sample the data (12-bit, ±5 V range). Trapezoidal stimuli were generated by a custom-built waveform generator with variable rise/fall time, amplitude, and duration (Hoffmann and Koch 1985).

Data storage and analysis

All data were stored on an eight-channel DAT-recorder (Biologic DTR-1800) as well as sampled on-line by a CED 1401 data-acquisition interface using the CED Spike2 software package. The sample rate for the intracellular and position monitor channels was 4kHz while the extracellular recordings were sampled at 12.5 kHz. Individual spikes from intracellular and extracellular recordings were converted to event times by applying a voltage threshold to the appropriate data channel and mean firing rates were calculated by Spike2 using a 0.4-s window at each spike time. Tests for the homogeneity of slopes were performed using a general linear model with JMP software (SAS Institute).

Results

The data presented here were taken from 43 intracellular recordings of fCO sensory neurons in 18 experimental animals. The individual afferents were of various types responding either to position (P), velocity (V), acceleration (A) of the fCO, or combinations of these parameters of the mechanical stimulus applied to the fCO. Sensory neurons were identified, characterized, and named according to established criteria (Buschges et al. 1994; Hoffmann et al. 1985). The typical firing pattern of a fCO afferent together with the response of extensor motor neurons FETi and SETi during random mechanical stimulation is shown in Fig. 1A. This sensory neuron was activated phasically during fCO movement and fired on both the positive and negative velocity phase of the ramp-and-hold stimulus (Fig. 1B). This afferent was therefore identified as a velocity-sensitive afferent, responding both to elongation (V+) and relaxation (V−) velocities and is thus termed a V± afferent (Buschges et al. 1994; Hoffmann et al. 1985). After the application of a control ramp-and-hold stimulus, random movement was applied to the fCO. This stimulation produced an initial strong activation of the afferent as well as of both extensor motoneurons. During maintained stimulation, however, the firing rate of all three neurons decayed. While the mean firing rate of the afferent was 21 Hz just after the onset of the stimulus, the firing rate declined to 8 Hz when measured during a 5-s interval 60 s after the start of the stimulation. The rate of SETi activity declined by approximately 50% after 10 s of stimulation, and FETi activity decreased to almost zero after only 5 s of stimulation. The difference in response of the tibial motoneurons over time is typical for reflex activation of fast and slow motoneurons in the insect-leg control system (Bässler 1993; Burrows 1996), where fast motor neurons are usually activated transiently. When the response of the system to a ramp-and-hold stimulus was tested after the random stimulation of the fCO, a very large decrease in the stimulus evoked response was observed. Figure 1B, i and ii, compares the response of the afferent and extensor motor neurons during the control ramp-and-hold stimulus and for the same stimulus after the period of random fCO stimulation. The response of the V± afferent to fCO elongation and relaxation was essentially eliminated after random fCO stimulation, with only one spike evoked during fCO elongation and none evoked on relaxation. In addition, the reflex activation of FETi and SETi evoked by the fCO stimulus also decreased, presumably due to a decrease (but not complete extinction) in activity of multiple fCO afferents. The response of this afferent and all others that were tested returned to control values within 30–60 s after the end of random stimulation. Similar results were obtained for 16 sensory neurons sensitive either to elongation or to relaxation velocity (V+ or V− afferents), 9 sensory neurons sensitive to both positive and negative velocity (V±) and 13 neurons that were sensitive either to fCO position (P) or a combination of velocity and position (VP, Table 1). No attempt was made to determine if there were subpopulations of afferents that differed with respect to their sensitivity to adaptation.

Correlation of fCO adaptation with activity of premotor elements of the FT joint control network

The decrease in overall reflex activation of extensor motor neurons during and after fCO stimulation indicates a decrease in gain of the postural reflex motor output. In a preliminary attempt to determine the basis of this change in gain within the FT-joint control network, intracellular recordings were made from tibial premotor interneurons and motor neurons, specifically the fast extensor motor neuron, FETi, and nonspiking interneurons in the fCO reflex pathway (Buschges 1990). Tibial motor neurons are known to receive synaptic input from fCO signals via direct monosynaptic pathways and via polysynaptic pathways, with both types of pathways participating in the generation of reflexes (Bässler and Buschges 1998; Buschges et al. 2000). Intracellular recordings were made from FETi (n = 3), and the response of FETi to ramp-and-hold fCO stimulation was tested before and after random fCO movement (30-s duration, 60° amplitude). While there was no change in FETi resting membrane potential, FETi always exhibited a large decrease in stimulus-related synaptic inputs immediately after random fCO stimulation (Fig. 2A; gray, before stimulation; black, after stimulation). The normal reflex depolarization during fCO elongation and the hyperpolarization during fCO relaxation decreased after fCO stimulation.

The extensor motor neurons also receive inputs from interneurons that integrate fCO afferent input. Previous investigations have shown that the nonspiking premotor interneuron E1 contributes to the reflex activation of tibial extensor motor neurons by providing phasic excitatory synaptic drive to tibial extensor motoneurons during joint flexion in addition to a
small tonic depolarization related to joint angle ( Büschges 1990 ). Intracellular recordings from interneuron E1 ( n = 2; Fig. 2, B and C ) revealed that the decrease in synaptic input to FETi shown in Fig. 2A could also be due to a decrease in input from these interneurons. When the response of interneuron E1 to identical ramp-and-hold movement of the fCO was tested after random fCO stimulation, the depolarization that is correlated with the rising phase of the ramp decreased by approximately 50% ( Fig. 2B ). Changes in the mean membrane potential of interneuron E1 were also observed during a sustained period of random fCO stimulation ( Fig. 2C ). At the onset of the stimulation, there is a 1.8-mV depolarizing shift in the mean membrane potential of the interneuron that declines toward resting levels with a time constant of approximately 12 s, reflecting the presumed decreasing summated drive from all fCO afferents presynaptic to the neuron.

In addition to this DC component, the root mean square ( RMS ) amplitude of the membrane potential fluctuations in this neuron also decreased during random fCO stimulation ( Fig. 2Cii ). The RMS amplitude of the membrane potential before the start of the movement was 1.7 mV; it increased to about 6 mV at the onset of stimulation and then declined to 2.2 mV after approximately 20 s and remained near this amplitude for the remainder of the stimulus application. Adaptation of fCO afferents causes substantial changes in the strength of the inputs to premotor interneurons and motoneurons “downstream” in this network and therefore produces marked changes in the gain of reflex responses.

To determine if the afferent adaptation and associated decrease in reflex gain during prolonged mechanical stimulation might be physiologically relevant, a series of asymmetric ramp-and-hold stimuli was applied to the fCO. These movements mimicked the rate and amplitude of FT-joint angle changes that occur during voluntary tibial movements such as searching or walking ( Bässler 1983 ; Cruse and Bartling 1995 ; Karg et al. 1991 ). Figure 3 shows the response of a V− afferent during fCO stimulation with an amplitude of 60° and angular velocities of 120°/s during fCO elongation and 30°/s during fCO relaxation, at a repetition rate of 0.25 Hz. The mean firing rate of the fCO afferent was 35 Hz for the first ramp-and-hold stimulus and decreased progressively for each of the next seven stimuli in the following approximately 30-s period. The mean firing rate then remained relatively constant at 9–10 Hz for the remaining trapezoidal stimuli.

**Effects of amplitude and duration of mechanical stimulation**

Mechanical properties can contribute to the history-dependent changes in receptor activity ( French 1992 ). Definitive assessment of the role of mechanical factors in mechanoreceptor transduction and adaptation usually requires direct measurement of parameters such as displacement, length and force.
While simultaneously recording the receptor current and/or afferent membrane potential, this type of experiment is extremely difficult in a chordotonal organ, where the bipolar sensory neurons are embedded within the elastic tissue that comprises the organ. In our preparation, we instead tested the possible role of mechanical elements on fCO afferent response by stimulating the fCO with random movements at different amplitudes.

Figure 4A shows recordings from a V+ afferent with random fCO stimulation at three different amplitudes [30°, 20°, and 10° peak-peak (p-p) amplitude] for a period of 55 s each. The mean fCO firing rate was calculated for a 2-s interval during the period of maximum activity at the beginning of the stimulation and for the same time period near the end of the stimulation. Initial firing rates were 55, 50, and 35 Hz for each amplitude respectively, while mean rates at the end of the stimulation had declined to 30, 30, and 23 Hz, resulting in decreases in mean firing rates of 45, 40, and 35%. For all three movement amplitudes, the fCO afferent response to a ramp stimulus was completely abolished when tested after the random movement.

We evaluated the degree of adaptation of the afferent with respect to the duration of the stimulation at large (30° p-p) and small amplitudes (10° p-p) of movement by calculating the percentage of spikes evoked by a ramp-and-hold stimulus of the fCO after stimulation compared with the control spike number before white-noise stimulation of the fCO (Fig. 4B). The control and test ramp duration (constant velocity stimulation) was constant (300 ms), so the change in response is also equal to the change in mean firing rate during this interval. Afferents were tested with different stimulus durations and the afferent response to the ramp was allowed to recover to initial control values for 60–90 s between trials. The decline in the response of the afferent was proportional to the duration of the stimulus in both cases, but the rate of decrease was less for stimulation with small-amplitude movements (Fig. 4B). Stimulation with large-amplitude movements produced complete abolition of the response to the test ramp after 8 s of random stimulation, while the response after small-amplitude movement decreased by only 50% when tested 8 s after random stimulation. While this result is consistent with a mechanical component of the observed decrease in response of fCO afferents, it is not definitive, as the mechanical properties of the receptor may not be amplitude dependent. In addition, this observation does not distinguish mechanical from electrical effects, as different numbers of action potentials were produced with different stimulus amplitudes.

The mean activity of the afferent during the random movement was greater with large amplitude (55 spikes/s) than with small amplitude movement (26 spikes/s; Fig. 4A). We tested for a correlation between the response decrease and the number of action potentials generated in the afferent. Afferent response was proportional to the number of spikes generated for both movement amplitudes (Fig. 4C) and the slopes of the regression lines were not different ($P > 0.34$). On the basis of this result, the adaptation of fCO afferents to mechanical stimulation appeared to be independent of the movement amplitude but may instead depend on the membrane properties of the sensory neurons.

**Mechanical vs. electrical adaptation mechanisms**

Investigations of other invertebrate and vertebrate mechano-receptors have shown that a significant portion of sensory adaptation may be due to factors that affect the transduction...
current directly or indirectly by altering the spike-encoding mechanism (Eatock 2000; French 1992; French and Torkkeli 1994). The observation that fCO afferent adaptation was correlated with the number of spikes evoked by mechanical stimulation independent of movement amplitude (Fig. 4) indicated that intrinsic membrane properties of the afferents may also be important in this system. To assess whether such properties contribute to the decrease in fCO afferent response, we tested the influence of action potentials evoked by electrical stimulation of the axon (in the absence of mechanical movement) on the response of fCO afferents. Most of the fCO afferents could not be activated with depolarizing current injection into their

FIG. 3. Rhythmic movement of the fCO mimicking tibial movements comparable to those occurring during walking in the stick insect. The amplitude range was 60° with angular velocities of 120°/s during fCO elongation and 30°/s during fCO relaxation at a repetition rate of 0.25 Hz. The mean firing rate of this afferent was 35 Hz for the first ramp-and-hold stimulus and decreased steadily during the next seven movement cycles over a approximately 30-s period. The rate then remained relatively constant at a mean rate of 9–10 Hz for the remaining stimulus cycles.

FIG. 4. Adaptation of activity of fCO afferents with random mechanical stimulation at different amplitudes of movement. A: V+ afferent was driven by random fCO stimulation at 3 different amplitudes (30, 20, and 10° peak-peak amplitude) for a period of 55 s each. The mean firing rate of the afferent declined during each stimulus trial to between 35 and 45% of the initial firing rate. Note that when tested with a trapezoidal movement at the end of the random stimulation, the fCO response was completely abolished for all 3 amplitudes of stimulation. B: test ramp response as a percent of initial control response (before random stimulation for each trial) to trapezoidal stimulation vs. stimulation time (s) for large (30°)- and small (10°)-amplitude stimulation; large amplitude, $r^2 = 0.76$; small amplitude, $r^2 = 0.85$. C: test ramp response as a percent of initial control response to trapezoidal stimulation for each trial vs. number of spikes evoked by the mechanical stimulation for large (30°)- and small (10°)-amplitude stimulation (large $r^2 = 0.69$; small, $r^2 = 0.85$). All data points are single measurements in both plots.
axons, but in the majority of recordings, afferent neurons would generate action potentials on rebound after the injection of hyperpolarizing current pulses. These spikes travel orthodromically to the ganglion and antidromically to depolarize the soma and dendrites of fCO neurons.

Figure 5A shows a typical experiment with a recording from a V± afferent that showed a decrease in response after random mechanical stimulation (not shown). In this afferent, bursts of action potentials were evoked on rebound after injection of hyperpolarizing current pulses of −6 nA amplitude, 300 ms duration applied every 900 ms. The peak afferent firing rate produced by this stimulation was approximately 65 Hz with a mean rate of 52 Hz during each burst of action potentials. When current pulses were applied for a period of 30 s, the response of the afferent to a test ramp applied at the end of electrical stimulation was significantly reduced compared with control values. The mean firing rate for the control ramp-and-hold stimulus was approximately 14 Hz for elongation and relaxation. After electrical stimulation, these rates were reduced during the test ramp to 3.5 and 0 Hz, respectively. This decrease in responsiveness after electrical activity was found in the majority of fCO afferents tested (Table 1). As with mechanical stimulation, the response of this and all other afferents tested returned to control values within 40–60 s after the end of electrical stimulation (not shown). The decrease in response produced by mechanical and electrical activation of a single afferent was compared by plotting the decrease in response to trapezoidal stimulation for both modes of stimulation with respect to the number of spikes evoked by the adapting stimulus (Fig. 5B). Between trials, 60 s was allowed for recovery, and there was no significant difference in the afferent response after this interval. Both electrical and mechanical stimulation caused a decrease in responsiveness proportional to the number of evoked spikes, and the adaptation rates were not different ($P > 0.21$).

These results indicate there is an activity related component affecting the responsiveness of fCO afferents. Activity-related changes in the firing pattern of neurons, termed spike-frequency adaptation, are observed in many neurons due to the activation of a slow afterhyperpolarization during the generation of a train of action potentials. This long-lasting afterhyperpolarization is usually associated with the presence of a SK-type calcium-dependent potassium channel (Hille 2001). Calcium-dependent potassium currents have also been implicated in the adaptation of some mechanoreceptors (Erxleben 1993; French and Torkkeli 1994). To investigate the ionic basis of the adaptation and to assess the role of calcium ions in the activity related reduction in response, we varied the Ca$^{2+}$ concentration in the bath solution surrounding the fCO. In all experiments, the saline that bathed the leg and exposed chrorotonal organ was altered while the saline surrounding the ganglion was maintained at the normal composition.

We exposed the fCO to saline containing 0 mM Ca$^{2+}$ plus 7.5 mM Ba$^{2+}$ to block potential Ca$^{2+}$-mediated effects ($n = 4$). Figure 6A shows such an experiment for a VP afferent, stimulated first in normal saline (7.5 mM Ca$^{2+}$). After random fCO stimulation, the tonic component of the ramp response was completely eliminated and the peak phasic response was reduced by 48% from 97 to 50 Hz. In addition to this reduction in activity, the start of afferent discharge on fCO elongation was also delayed by 0.14 s (Fig. 6Ci), while the afferent discharge started immediately on the start of fCO elongation during the control ramp. This increase in delay to firing in velocity-sensitive afferents was observed in all experiments where mechanical or electrical stimulation decreased, but did not completely abolish, the subsequent test response. When the saline surrounding the fCO was changed to saline containing 0 mM Ca$^{2+}$ and 7.5 mM Ba$^{2+}$, both the phasic and tonic components of the control (initial ramp) response were larger than the control ramp in normal saline. The number of action

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FIG. 5. Adaptation of fCO afferents after electrical stimulation. A: spikes were produced in a V± afferent on rebound after injection of −6 nA, 300 ms duration current pulses applied every 900 ms. The response of the afferent to a test ramp was reduced after this period of stimulation with a reduction in the mean firing rate on the positive velocity ramp from 14 to 3.5 Hz and the complete elimination of the V− response. B: comparison of the reduction in response with mechanical and electrical stimulation. This same afferent was also driven by mechanical stimulation of the fCO and the reduction in response to a control ramp is plotted with respect to the number of spikes evoked by each stimulus; electrical, $r^2 = 0.78$; mechanical, $r^2 = 0.89$. All data points are single measurements.
potentials elicited during the 30 s movement was also 25% greater than the number produced in the same interval in normal saline; this should increase the amount of adaptation. After random fCO stimulation, there was still a decrease in response compared with the control but not to the extent observed in normal saline. The tonic component now persisted after random fCO stimulation, although with a 30% reduction in firing rate from 65 to 45 Hz. The phasic component of the response still decreased by a small amount (26%) from a mean firing rate of 120–88 Hz, but the absolute magnitude of the firing rate was larger than in normal saline, and there was no delay to the onset of afferent discharge during fCO elongation (Fig. 6Cii).

DISCUSSION

The present investigation has shown that as in most other mechanosensory neurons, afferent neurons of the stick insect fCO exhibit adaptation. This finding extends previous investigations describing adaptation in some position-sensitive fCO sensory neurons (Sauer et al. 1995, their Fig. 8). This work, however, provides no analysis of the underlying mechanism(s). We have shown here a clear correlation between the decrease in response of fCO afferents and the number of action potentials generated during fCO stimulation (Figs. 4 and 5). Although the broadband mechanical stimulation used in most of the experimental protocols was likely to be more intense than the animal would encounter during normal movements, a reduction in afferent sensitivity was also observed when the fCO was moved for shorter times or in a repetitive manner that mimics normal walking movement amplitudes and velocity (Fig. 3), indicating the relevance of the observed phenomenon under more physiological conditions. This activity-related decrease in fCO afferent response was dependent on the presence of Ca\(^{2+}\) ions in the saline. It was decreased or enhanced dependent on the concentration of Ca\(^{2+}\) ions, indicating the contribution of a Ca\(^{2+}\)-dependent mechanism to fCO adaptation. Finally, recordings from interneurons and motoneurons in
stimulation was not uniform as observed in experiments with normal Ca\(^{2+}\) re in response to a test ramp after the period of stimulation. In addition, the decrease in response of the fCO sensory neurons was accompanied by a marked decrease in gain of the FT-control network.

We also observed that the activity related to the decrease in response of the fCO sensory neurons was accompanied by a marked decrease in gain of the FT-control network. We will first discuss our findings in relation to the current knowledge on adaptation in mechanosensory sensory neurons and the underlying mechanisms. Second, we will address the functional consequences of the observed phenomenon with respect to sensorimotor processing in the leg muscle control system.

Prior work on mechanoreceptor adaptation

Mechanical parameters of sensory structures as well as intrinsic membrane properties of receptor neurons can contribute to mechanoreceptor adaptation (French 1992; French and Torkkeli 1994). In the abdominal muscle receptor organs (MRO) of the crayfish (Swerup and Rydqvist 1992), it is possible to separate mechanical factors, leading to generator potential adaptation, from ionic mechanisms that mediate spike-frequency adaptation. There are two MROs in each abdominal segment of the crayfish (Alexandrowicz 1951, 1967) each consisting of a receptor muscle, excitatory and inhibitory motoneurons, and a single sensory neuron. One MRO is slowly adapting (SA) and can maintain constant firing for very long times, whereas the rapidly adapting (RA) MRO fires only transiently when stimulated. The length-tension relation is similar for both RA and SA MROs and approximately 70% of the adaptation of the generator potential of these receptors was attributed to their (similar) viscoelastic properties (Nakajima and Onodera 1969b). However, in a later study (Rydqvist et al. 1994), the length-tension relationship for the RA and SA MRO were found to be different, with the tension at a maintained length declined faster in the rapidly adapting receptor.

When the firing properties of the MROs were evaluated with intracellular current injection, the RA MRO never produced sustained repetitive discharges while the SA receptor produced long-lasting trains of action potentials (Nakajima and Onodera 1969a). The remaining factor contributing to the adaptation of MRO firing rate, and the differences in their time course of adaptation, was therefore attributed to unidentified intrinsic membrane properties affecting action potential encoding. Stimulation of the crayfish RA MRO mechanically, or directly by intracellular current injection, showed that the duration and time course of action potential firing was essentially independent of the mode of stimulation (Rydqvist and Purali 1993). Similarly, the differences in adaptation of spider slit-sense organ afferents can be partially explained by differences in the dynamics of mechanotransduction from mechanical stimulus to receptor potential. However, bypassing the mechanotransduction stage with depolarizing current injection revealed similar differences in adaptation, indicating that adaptation characteristics are dominated by active membrane properties (Juusola and French 1998).

In the locust fCO, prolonged depolarizing current injection into the soma of phasic (velocity sensitive) afferents evoked a transient discharge in the neurons, while the firing rate of tonic afferents declined only slightly with constant current injection, although the tonic background activity of these afferents was suppressed after the current injection (Zill 1985). In addition, the decrease in activity of both phasic and tonic afferents was similar when evoked mechanically with constant fCO displacement or directly with constant current injection into their somata (Zill 1985). In the coxobasal chordotonal organ (CBCO) of the crayfish, antidromic activation of CBCO afferents decreased the sensitivity of the stimulated neuron and resulted in a transient reduction or elimination of tonic firing of sensory neurons, although the underlying ionic mechanisms are unknown (Bevengut et al. 1997).

fCO afferent adaptation and mechanical properties

At present we cannot assess the contribution of mechanical factors that may contribute to the adaptation of stick insect fCO sensory neurons. Recordings from the afferents were made from their axons as they enter the segmental ganglion and therefore electrotonically distant from the site of mechanosen-
sory transduction and action-potential encoding. However, some indirect evidence from our experiments indicates me-
chanochemical factors may play a role in fCO adaptation. Most afferents completely adapted to prolonged mechanical stimulation, that is, they no longer responded to physiological move-
ments of the receptor after 20–40 s of broadband mechanical stimulation (Fig. 1 and Table 1). The exceptions were afferents that were completely or partially sensitive to acceleration, as only 40% of these afferents adapted with random movement and a similar percentage adapted to electrical stimulation. A class of acceleration-sensitive afferents may therefore exist that are resistant to adaptation. All velocity-sensitive afferents adapted to mechanical movement, but a small fraction (2 of 9 tested) did not adapt to electrical stimulation, perhaps indicat-
ing that mechanical factors alone caused adaptation in these neurons. Another possibility is that ionic mechanisms respon-
sible for adaptation were not activated in these cells due to a failure of the antidromic spikes to invade the soma.

Adaptation of single afferents was found to be related to the amplitude of ICO stimulation with larger movement ampli-
tudes producing a given degree of adaptation at a faster rate as compared with lower (constant bandwidth) stimulus amplitu-
des (Fig. 4). This effect could be due to a diminished effect of the low-amplitude movement on receptor mechanics, al-
though another factor is that the range of velocity of ICO movement is also lower with lower-amplitude (but constant bandwidth) noise stimulation. However, lower-amplitude stimu-
lation also elicited a smaller number of afferent spikes for any stimulation time. When we assessed the adaptation of afferents with respect to the number of spikes generated during stimu-
lation, the rate of adaptation was found to be proportional to the number of spike evoked and independent of movement ampli-
tude (Fig. 5C).

**Stick insect fCO afferent adaptation and membrane properties**

In the stick insect, bursts of spikes could be elicited in the axons of fCO sensory neurons on rebound from injection of hyperpolarizing current pulses. When an afferent that adapted to mechanical stimulation was instead activated antidromi-

cally, afferent response to a subsequent test stimulus was decreased markedly (Fig. 5A). The response decrease com-
pared with control as a function of numbers of spikes generated with electrical and mechanical stimulation was similar for both stimulation regimes (Fig. 5B). This indicates that ICO afferent activity is a factor in the decrease in afferent response and that the intrinsic membrane properties of stick insect fCO neurons are involved in adaptation. In our experiments, most of the recorded afferents exhibited this activity-related decrease in response (Table 1). Whether this indicates that there are dif-
ferent classes of fCO afferents with respect to sensitivity to adaptation is presently unknown, and our limited data set and experimental protocols did not allow any further clarification of this question. However, it is clear from our results that the activity of fCO afferents activates a mechanism leading to adaptation.

Previous work on several mechanoreceptors has shown that various intrinsic membrane properties can contribute to sen-
sory adaptation and may be the primary determinants of this phenomenon (French and Torkkeli 1994). Calcium-dependent potassium currents and A-type potassium currents do not con-
tribute to the adaptation of spider lyriform slit-sense organs (Seckizawa et al. 1999) where sodium channel inactivation appears to be the main factor in adaptation (Torkkeli and French 2002; Torkkeli et al. 2001). Early work on the crayfish MRO suggested that a Ca$^{2+}$-dependent potassium current con-
tributed to adaptation (Ottoson and Swerup 1985a,b), but later studies attributed the adaptation to a slowly inactivating so-
dium current that mediates action potential encoding (Edman et al. 1987). No evidence for a $K_Ca$ current was found when the MRO was exposed to apamin, a selective blocker of SK-type $K_Ca$ channel, or charybotoxin, a blocker of the BK-type $K_Ca$ channel (Purali and Rydqvist 1992). However, a later patch-
clamp study of the crayfish MRO found that Ca$^{2+}$ entry through stretch-activated channels caused the activation of a $K_Ca$ channel (Erxleben 1993). Studies on the frequency re-
spone properties of mammalian muscle spindles (Kruse and Poppelle 1990) have demonstrated that the response properties of the spindle were not altered when the mechanical properties of mammalian muscle spindles were modified by disruption of the myofibrillar structure of intrafusal muscle fibers. However, the application of several Ca$^{2+}$ channel blockers (ZnCl$_2$, apamin and TEA) altered the response dynamics. They con-
cluded from these results that there was likely to be a $K_Ca$ channel present in muscle spindle afferents. In the femoral tactile spine of the cockroach, it is estimated that approxi-
ately 50% of the outward current that contributes to adapta-
tion in these cells is due to a $K_Ca$ current (Torkkeli and French 1995).

The ionic basis for the decrease in stick insect fCO afferent response to spikes produced by electrical or mechanical stimu-
lation was assessed using saline where Ba$^{2+}$ was substituted for Ca$^{2+}$ as well as with saline containing high extracellular Ca$^{2+}$. When Ca$^{2+}$ was replaced with Ba$^{2+}$ in the saline, the decrease in response caused by mechanical or electrical stimu-
lation was substantially reduced (Fig. 6) or in some cases, completely blocked. When saline containing high concentra-
tions of Ca$^{2+}$ was applied to the fCO, adaptation of the afferents was enhanced (Fig. 7), and the firing pattern of the afferent during mechanical stimulation was altered compared with normal saline. In normal saline, fCO afferents always maintained a relatively constant firing rate during mechanical stimulation (Figs. 1 and 4) while in increased extracellular Ca$^{2+}$, afferents fired in high-frequency bursts (approximately 50–70 Hz) of 100- to 200 ms duration. This firing pattern is similar to the behavior of bursting pacemaker neurons, behav-
ior that is partially determined by a $K_Ca$ current (Hille 2001). Burst firing of mammalian muscle spindle primary afferent neurons has also been observed when extracellular Ca$^{2+}$ is increased (Fischer and Schäfer 2000). These data support the hypothesis that the activity-dependent decrease in response of fCO afferents is due to Ca$^{2+}$ influx during the generation of action potentials and the activation of a $K_Ca$ current. Experi-
ments using specific blockers for Ca$^{2+}$ and $K_Ca$ channels need to be performed to verify the potential contribution of these mechanisms to adaptation of fCO sensory neurons.

**fCO afferent adaptation and the FT-control network**

Sensory input from leg proprioceptors plays a significant role in controlling motor output in vertebrates and invertebrates.
(reviews: Bässler and Büsches 1998; Cattaert and Le Ray 2001; Cruse 1990; Pearson 1993). In insects, detailed knowledge has been gathered about the structure of the underlying neuronal networks and the role of sensory signals from leg proprioceptors to the CNS in locomotor control (Bässler and Büsches 1998; Burrows 1996). Sensory signals participate in regulating the magnitude and time course of motor activity as well as in controlling phase transitions in locomotor programs (Bässler 1993; Büsches and El Manira 1998; Pearson 1993, 2000). Sensorimotor processing is highly flexible and depends on the state of the locomotor system as well as on the history of stimulation during repetitive movements. The state dependency of the sensorimotor processing assures proper functioning of the locomotor system in behavioral situations with different requirements, such as standing and walking (Bässler and Büsches 1998). For example, the gain of motor reflexes is modified substantially and may change in sign between different behavioral states (Büschges et al. 2000; Cattaert and Le Ray 2001; Pearson 2000).

With repetitive sensory stimulation, reflex motor responses often decrease in gain in a systematic fashion (Thompson and Spencer 1966), establishing a filter in sensorimotor systems that reduces the effect of stereotyped sensory inputs. Reduction of the open-loop reflex gain in the stick insect FT-joint control system was previously thought to be primarily mediated by the neuronal tuning underlying sensorimotor processing (Bässler 1983; Kittmann 1991, 1997). However, the activity-related adaptation of fCO afferents and the correlated decrease in activity of premotor interneurons and motoneurons in the FT-control network (Fig. 2), indicates that sensory adaptation is an important factor determining the overall gain of the leg control system and adds a new level of dynamics to sensorimotor processing.

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