The Role of Sensory Signals for Interjoint Coordination in Stick Insect Legs

(*Carausius morosus* and *Cuniculina impigra*)

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Contents

Zusammenfassung

Eine effektive Fortbewegung von Tieren mit mehrgelenkigen Extremitäten bedingt die Koordination der Bewegung aller Gelenke der einzelnen Extremität während der Schreitbewegung. An der Stabheuschrecke *Carausius morosus* konnten Heß und Büschges (1997, 1999) Mechanismen der Koordination von zwei benachbarten Gelenken nachweisen. Hierbei handelt es sich um die Kopplung zwischen dem Femur-Tibia-Gelenk (FT-Gelenk) und dem proximal vom FT-Gelenk liegenden Nachbargelenk, dem Coxa-Trochanter Gelenk (CT-Gelenk). Es wurde gezeigt, daß der Propriorezeptor des FT-Gelenks, das femorale Chordotonalorgan, das die Position und die Bewegung des FT-Gelenks mißt, die Aktivität der Motoneurone (MN) des CT-Gelenks beeinflußt. Dabei werden Positions- und Bewegungsinformationen „reflex-artig“ auf die MN des CT-Gelenks vermittelt. Bewegungssignale beeinflussen auch das zentrale rhythmusgenerierende Netzwerk (ZRG) zur Kontrolle des CT-Gelenks.

Von Gerharz (1999) wurde gezeigt, daß Koordination zweier Gelenke nicht immer von Gelenkstellungsrezeptoren vermittelt werden. So hat eine Bewegung des CT-Gelenks einen Einfluß auf die Aktivität der Motoneurone des FT-Gelenks (distal vom CT-Gelenk) und der Motoneurone des Thorax-Coxa Gelenk (TC-Gelenk, proximal von CT-Gelenk). Hierbei wurde jedoch die Bewegung des CT-Gelenks nicht durch die Gelenkstellungsrezeptoren signalisiert, sondern durch Belastung messende Sinnesorgane, die Campaniformen Sensillen (CS). Der Einfluß auf das FT-Gelenk wurde von den auf dem proximalen Femur liegenden femoralen CS (fCS, ein Feld), der Einfluß auf das TC-Gelenk von den auf dem Trochanter liegenden trochanteralen CS (trCS, drei Felder) vermittelt.

In der vorliegenden Arbeit wird zunächst untersucht, ob die Position des FT-Gelenks ebenfalls eine Einwirkung auf den ZRG des CT-Gelenks hat. Anschließend werden die Einflüsse der fCS und der trCS auf das FT-Gelenk bzw. das TC-Gelenk untersucht. Dabei stand die Frage im Vordergrund, ob es sich hierbei um „reflex-artige“ Einflüsse, oder um Einflüsse auf den ZRG des CT-Gelenks oder um beides handelt. Schließlich werden die Einflüsse der fCS-Signale auf das Netzwerk, das den FT-Gelenkreflex kontrolliert, im Detail untersucht.

Um den Einfluß der FT-Gelenkposition auf den ZRG des CT-Gelenks zu untersuchen, wurden isolierte und fast komplett denervierte -nur die Innervation des fCO wurde intakt gelassen- mesothorakale Ganglien untersucht, in denen durch den muskarinischen Agonisten Pilokarpin rhythmische Aktivität induziert wurde. Durch Elongation und Relaxation der Sehne des fCO wurden verschiedene FT-Gelenkpositionen simuliert und dabei die Aktivität der CT-Gelenk-MN, also Depressor trochanteris (DepTr) und Levator trochanteris (LevTr), extrazellulär und/oder intrazellulär abgeleitet. Es wurden zwei verschiedene Stimulustypen benutzt. Erstens wurden die Burstdauer, die mittlere Aktivitätsrate innerhalb eines Burstes und der Verhältnis von Aktivität zu Zykluslänge bei vier verschiedenen FT-Gelenkpositionen (40° -gebeugt-, 80°, 120° und 160° -gestreckt-) untersucht. Zweitens wurde der Winkel des FT-Gelenks sehr langsam (20-30s) von 40° (gebeugt) zu 120° (gestreckt) rampenförmig verändert. Dabei wurden die Abhängigkeit von Änderungen der Zyklusperioden der CT-Gelenk-MN von der FT-Gelenk-Position untersucht.

Die Burstdauer der LevTr-MN, ihre mittlere Aktivitätsrate innerhalb eines Burstes und der Anteil an Aktivität innerhalb eines Zyklus nehmen mit zunehmender FT-Gelenk-Beugung zu- und mit zunehmender FT-Gelenk Streckung ab. Die antagonistischen DepTr-MN verhalten

sich genau umgekehrt. Ein wichtiger Befund ist, daß die Zyklusdauer mit zunehmender Beugung des FT-Gelenks zunimmt oder sogar in manchen Fällen die rhythmische Aktivität der CT-Gelenk-MN bei extrem gebeugten (40°) oder extrem gestreckten (160°) Positionen abgeschaltet wird. In diesen Fällen sieht man, daß bei gebeugten Extrempositionen des FT-Gelenks die DepTr-MN tonisch aktiv und die LevTr-MN inaktiv sind. Bei gestreckten Extrempositionen sind die Verhältnisse umgekehrt. Dies deutet eindeutig auf einen Einfluß der Positionsinformation vom FT-Gelenk auf den ZRG des CT-Gelenks hin.

Reizung der fCS durch Levation des Femurs, also Bewegung im CT-Gelenk, oder, spezifischer, durch Applikation von Druck in der Nähe der fCS mit einem piezoelektrischen Element oder mit einer Pinzette, führt zum Abschalten der Aktivität in den Extensor-MN und zum Anschalten der Aktivität von Flexor MN. Auch Aktivität in Extensor-MN die durch Pilokarpin induziert wurde, wird durch die fCS-Stimulation abgeschaltet. Dabei wird die rhythmische Aktivität der Extensor-MN neu gestartet (reset). Bei rhythmischer Stimulationen der fCS wurde nie beobachtet, daß der Rhythmus der FT-Gelenk-MN an den Stimulus ankoppelt. Dies deutet darauf hin, daß der Einfluß der fCS auf den ZRG des FT-Gelenks sehr schwach ist.

Die Stimulation der trCS durch Levation des Femurs im CT-Gelenk oder durch Biegung des Femurs nach hinten, bewirkt ein Umschalten der Aktivität der TC-Gelenk-MN vom Protraktor Coxae (ProCx) auf den Retraktor Coxae (RetCx). Stimulation der trCS kann die zentral generierte, durch Pilokarpin induzierte rhythmische Aktivität der TC-Gelenk-MN neu starten oder, wenn die trCS Stimulation rhythmisch erfolgt, die rhythmische Aktivität der TC-Gelenk-MN an den Rhythmus der Stimuli ankoppeln. Dies zeigt, daß die trCS einen Einfluß auf den ZRG des TC-Gelenks haben.

Untersuchungen mit intrazellulären Ableitungen von prämotorischen nicht-spikenden Interneuronen (NSIN) des FT-Gelenk-Reflex-Systems und von Extensor-Tibiae-MN während fCS-Stimulation zeigen, daß die fCS-Signale die Aktivität der MN sehr direkt erregend oder hemmend beeinflussen. Daneben beeinflussen fCS-Signale die Antworten der MN auf Signale des fCO. Bei gleichzeitiger Reizung des fCO und der fCS wird die Stärke des Widerstandsreflex im FT-Gelenks geringer als bei alleiniger Reizung des fCO. In inaktiven und taktil aktivierten Tieren sieht man, daß die NSIN mit erregendem Ausgang auf die Extensor-Tibiae-MN im allgemein durch fCS-Reizung gehemmt werden, wobei der Einfluß auf die NSIN mit hemmenden Ausgang auf die Extensor-MN sehr variabel sein kann. Bei manchen NSIN führt die gleichzeitige Reiz von fCO und fCS zur Reduktion der Potentialänderungen während des Widerstandsreflexes (zum Beispiel bei E2 und E7). Andere NSIN, zum Beispiel der E3, ändern ihren Antwortcharakter auf fCO-Reizung, d.h. E3 reagiert mit einer Depolarisation auf die exklusive Elongationsreizung des fCO und mit Hyperpolarisation auf eine Elongationsreizung des fCO während einer fCS Reizung. Zusätzlich wird gezeigt, daß die Wahrscheinlichkeit des Auftretens einer „Aktiven Reaktion“ auf eine Elongationsreizung des fCO signifikant größer wird, wenn das fCO mit den fCS zusammen gereizt wird.

Zum Schluß wird der Einfluß eines Ausschaltens der fCS auf MN-Aktivität im „Einbein-Präparat“ gezeigt. Die Zerstörung der fCS führt dazu, daß die Aktivität des Flexor Tibia während der Stemmphase, in der das Bein maximal belastet ist, reduziert wird. Änderung der Aktivität im Extensor-Tibiae-MN während der Schwingphase, in der das Bein nicht belastet wird, wurde durch das Ausschalten der fCS nicht beeinflußt. Diese Experimente deuten darauf, daß die fCS eine wichtige unterstützende Rolle zur Aufrechterhaltung der Flexoraktivität während der Stemmphase spielen.

Abstract

Interjoint coordination in multi-jointed limbs is essential for the generation of functional locomotor patterns. It has been previously shown that movement and position of the femur-tibia joint (FT-joint) measured by the proprioceptor femoral chordotonal organ (fCO), can shape activity of the motoneurons (MN) of the adjacent coxa-trochanteral (CT-) joint in the stick insect, *Carausius morosus* (Hess and Büschges, 1997 and 1999). This interjoint influence is mediated in part via "reflex-like" pathways. In addition, movement signals from the FT-joint have access to the central rhythm generating networks (CRG) of the CT-joint. Previous investigations revealed that CT-joint movement also influences motor activity of the FT- and the thorax-coxa (TC-) joint MN activity (Gerharz, 1999). However, this influence is not a result of movement or position information from the CT-joint, signaled by proprioceptors, but of load information, caused by femur movement, from cuticular strain-measuring campaniform sensilla (CS). Thereby, the influence on the FT-joint is caused solely by signals from the femoral CS (fCS), a group of CS located on the proximal femur, and the influence on the TC-joint is caused solely by signals from the trochanteral CS (trCS), three groups of CS located on the trochanter.

This study investigated three aspects of interjoint control in the stick insect middle leg: 1) the influence of the FT-joint position on rhythmic activity generated in MN of the CT-joint, 2) the role that sensory signals from the cuticular strain sensitive fCS and trCS play in patterning motoneuronal activity of the FT- and the TC-joint MN in the stick insect middle leg, and 3) the pathways by which the influence of fCS are mediated onto the network that governs the FT-joint.

1) The influence of different FT-joint angles on pilocarpine-induced ($5x10^{-4}$ M) rhythmic activity of depressor and levator trochanteris (DepTr and LevTr) MNs innervating the CT-joint was tested in the isolated and otherwise deafferented mesothoracic ganglion. FT-joint angle was mimicked by moving the apodeme of the fCO (40, 80, 120 and 160°). Activity of the MNs was monitored extracellularly or intracellularly. Burst duration, mean spike rate within bursts, and duty cycle were found to depend on FT-position for each MN pool. For LevTr MNs, these parameters gradually increased from extended to flexed FT-angles, the reverse was true for DepTr MNs. Cycle period of rhythmicity depended on FT-position as well. This became particularly obvious from applying slow (20-30s) periodic ramps over the range of 40° to 160°. Cycle periods of CT-joint MN activity increased from extended to flexed FT-joint angles. In addition, at extreme positions (40° and 160°), sometimes it was observed that MN activity was shifted completely to one MN pool. Either LevTr (40°) or DepTr MNs (160°) fired tonically, and there was often no suprathreshold activity in the antagonist, suggesting that the CRG for the CT-joint became locked in one phase. These results indicate that FT-joint position can modulate rhythmic activity in CT-joint MNs by having access to CRG of the CT-joint.

2) Levation of the CT-joint or selective stimulation of the fCS by applying pressure on the femoral cuticle close to the fCS both produced barrages of IPSPs in tibial extensor motoneurons and activated tibial flexor motoneurons. During pharmacologically activated rhythmic activity of the otherwise isolated mesothoracic ganglion, deafferented except for the CT-joint, levation of the femur also had an inhibitory influence on tibial extensor motoneurons. However, the influence of femoral levation on the centrally generated rhythm was rather labile, and femoral levation only induced the rhythm to reset in some of the trials.

In addition, femoral movement could not entrain the rhythmicity in any of the preparations, suggesting that sensory signals from the CT-joint only weakly affect the CRG of the FT-joint.

A much more pronounced influence from movements of the CT-joint was found on the activity of TC-joint MNs. Levation of the femur at the CT-joint or stimulation of the trCS by caudal bending of the femur caused a switch of the TC-joint MN activity from pro- to retractor coxae motoneurons (ProCx to RetCx). In rhythmic preparations, stimulation of the trCS by levating the femur at the CT-joint or by bending the femur caused a switch from ProCx to RetCx. This influence of femoral levation or femur bending on the centrally generated rhythm could reset and entrain the rhythmic activity in the TC-joint MNs, suggesting that signals from the trCS can affect the CRG of the TC-joint and pattern TC-joint MN activity.

3) Further investigations on the influence of the fCS signals on the FT-joint reflex system revealed that the fCS information was not simply added to the FT-joint MNs. That is, in resting animals, fCS stimulation causes a decrease in the intrajoint resistance reflex response of the extensor tibiae MN to the fCO stimulation. Intracellular recordings from premotor nonspiking interneurons (NSINs) showed that the information from the fCS is also fed into these NSINs. In general, NSINs with an excitatory output to the extensor tibiae MN are hyperpolarized (except for the E7, which is depolarized) by fCS stimulation in resting and in active animals. In contrast, the response of inhibitory NSINs to the fCS stimulation was more variable. In resting animals, the amplitude of the response of the NSINs to the fCO decreased during fCS stimulation in some excitatory NSINs (for example the E2 and the E7), and reversed in sign for other excitatory NSINs (for example the E3). In active animals, simultaneous stimulation of the fCS and fCO causes an increase of the probability of the "active reactions" compared to fCO stimulation alone.

The role of sensory signals from the fCS during walking was analyzed in the single middle leg preparation with fCS intact and then after fCS removal. These experiments showed that fCS activity plays an important role in generating tibial motoneuron activity during the stance phase of walking. That is, the ablation of the fCS causes a decrease in flexor tibiae activity during the stance phase, when the leg is loaded, and no change occurs in extensor activity during swing phase, when the leg is unloaded.

In summary, the present investigation has unraveled the following sensory influences that can contribute to the coordination of motor activity between leg joints:

1. Movement and position of the FT-joint influences the CT-joint MN activity via "reflex-like" pathway and by having access to CRG of the CT-joint.
2. Load information from the CS can pattern motor activity of the FT- and TC-joints via "reflex-like" pathway and by having access to CRG of the FT- and TC-joints.
3. Stimulation of the fCS can increase the probability of "active reactions" in the FT-joint.

1. Introduction

Locomotion in legged animals results from the interaction between central rhythm generating networks in the nervous system and the information about the actual leg movement, position and generated forces provided by sense organs (Bässler, 1983; Bässler and Büschges, 1998; Cruse, 1990; Grillner, 1981; McPherson et al., 1997; Orlovsky et al., 1999: Pearson, 1995). A functional locomotor pattern requires the coordinated action of several joints. The control and coordination of motor outputs in each joint encompasses three different levels: intersegmental coordination, intrajoint control, and interjoint coordination (Clarac, 1991; Cruse et al., 1995; Duysens et al, 2000; Grillner, 1981; Orlovsky et al., 1999; Pearson, 1995; Stein and Smith, 1997).

Voluminous information is available about intersegmental coordination in vertebrates and invertebrates (vertebrates: Cruse and Warnecke, 1992; Rossignol et al., 1993; invertebrates: Cruse, 1990). However, the information about intersegmental coordination is mainly at the behavioral level, and there is less information about how information is transmitted from one leg to the other at the neural network level (vertebrates: Rossignol et al., 1993; invertebrates: Brunn and Dean, 1994).

There is detailed knowledge about information processing for intrajoint control in the posture and the movement control system in insects and crustaceans (crayfish: ElManira et al., 1991a; locust: Burrows, 1992; summary in Burrows, 1996; stick insect: Bässler, 1993a; Bässler and Büschges, 1998). The most current knowledge about intrajoint control in stick insects concerns the femur-tibia joint (FT-joint) pathway, which controls the position and movement of this joint (summarized in Büschges, 1995 and Büschges et al., 2000). The movement of the FT-joint is carried out by two antagonistic muscles; the extensor and the flexor tibiae muscles. The extensor tibiae muscle is innervated by two excitatory (the slow and the fast extensor tibiae, SETi and FETi) motoneurons and one inhibitory (the common inhibitor 1, CI1) motoneuron (Bässler and Storrer, 1980). The flexor tibiae muscle is innervated by at least 14 or 15 excitatory motoneurons and two inhibitory motoneurons, the common inhibitor 2 and 3 (CI2 and CI3) (Debrodt and Bässler, 1989, Storrer et al., 1986). The information processing in this system is distributed and can be divided in five levels. 1) Presynaptic inhibition of the femoral chordotonal organ (fCO) afferent axons, responding to the same modality (Büschges, 1995; Sauer et al. 1997); 2) Simultaneous excitation and inhibition of premotor interneurons,

elicited by the same stimulus modality (Sauer et al 1995); 3) Motoneurons are concurrently excited and inhibited by premotor nonspiking pathways (Büschges, 1990; Sauer et al., 1996; Stein and Sauer 1998) ; 4) The excitatory and inhibitory motoneurons interact with muscle fibers (Bässler, 1993b); 5) Antagonistic muscles contract.

In terms of interjoint coordination, there is little information available about the neuronal mechanisms responsible for interjoint information processing, specifically for the generation of coordinated activities during locomotion (Bässler 1993b; Büschges et al. 1995; ElManira et al. 1991b; Heß and Büschges 1997). In some walking systems, like locust, cockroach, and crayfish, it has been reported that the central pattern generator organizes the motor output for all leg joints (locust: Ryckenbusch and Laurent, 1993; cockroach: Pearson, 1972; Pearson and Iles, 1970; crayfish: Chrachri and Clarac, 1990). The situation is different in the case of walking systems that are less centrally organized. For example, in the stick insect walking system, the centrally generated rhythmic motor output for each leg joint in the insect is usually uncoupled and oscillates with different frequencies (Büschges et al., 1995; Bässler and Büschges 1998). However, there is only occasionally a tendency for synchronized motor outputs, with different patterns of mutual phasing that correspond to the step phase transitions in either forward or backward walking (Büschges et al., 1995). However, during walking there is a continuous strongly coordinated activity of all leg joints (stick insects: Bässler, 1993b; Cruse and Bartling, 1995; vertebrates: Grillner, 1981; Rossignol et al., 1993). Here, two questions arise: How are the activities of the adjacent leg joints coupled during locomotion? What role do sensory signals play in coordinating the activity?

In walking animals with multi-jointed limbs it is pivotal that sensory information continuously supplies the central nervous system with information about movement, position, and the total force on each leg (Bässler and Büschges, 1998; Grillner, 1981; Orlovsky et al., 1999; Wendler, 1964). This sensory information allows the animal to maintain the functional coordination of each leg joint and each leg, even if unpredictable obstacles in a highly irregular terrain occur that disturb stepping cycles. There are a large number of mechanosensory organs on an insect leg. They measure the position, velocity, and acceleration of particular joints or the cuticular strain on the cuticle. Sense organs on the coxa, trochanter, and femur will be briefly described here.

Coxa: There are two hair plates located on the anterior side of the coxa: the ventral and dorsal hair plates (cxHPv and cxHPd) (Wendler, 1964). The cxHPv contains 20-30 hairs and the cxHPd contains 15-20 hairs. On the posterior surface of the coxa, four hair rows are located, those on the interior row composed of 4-5 hairs, those of the outer row composed of 8-13 hairs (Bässler, 1965; Tartar, 1976). These sense organs measure the position and movement of the Thorax-coxa joint (TC-joint) (Cruse et al., 1984; Schmitz, 1986a).

Trochanter: There are two hair plates on the trochanter. The trochanteral hair plate (trHP), located on the dorsal surface of the trochanter, responds to the levation movement of the trochanterofemur in relation to the coxa (Schmitz 1986a,b,c; Tartar 1976). The rhombal hairplate (rHP) is located on the ventral surface of the trochanter (Tartar 1976) and its hairs are bent by the joint membrane when the trochanter is depressed. Previous studies report the existence of an internal levator stretch receptor organ (levSR, Schmitz and Schöwerling 1992), situated inside the coxa parallel to the levator trochanteris muscle. This sense organ detects changes in the length of the levator trochanteris muscle and resembles the organ in the locust (Bräunig and Hustert 1985). In addition to sense organs that measure displacement, changes in cuticular stress at the trochanter are signaled by three different fields of trochanteral campaniform sensilla (trCS; Delcomyn 1991; Hofmann and Bässler 1982; Tartar 1976). Cuticular stress at the proximal femur is detected by a field of femoral campaniform sensilla (fCS, Hofmann and Bässler 1982; Tartar 1976), located on the posterior side of the femur, close to the borderline of the trochanter. Campaniform sensilla (CS) are sensitive to cuticular strain, which can be caused by leg movement or muscle contraction in the leg (Duysens et al., 2000). Cuticular strain could also be induced by forces generated at locations other than the leg, i.e. on the thorax that induces torsion of the leg (Ridgel et al. 2000)

Femur: The main proprioceptor measuring the FT-joint displacement is the femoral chordotonal organ (fCO). Morphologically the fCO can be divided in two distinct scoloparia: the dorsal and the ventral scoloparia (Füller and Ernst, 1973; Hofmann et al., 1985; Hofmann and Koch, 1985). Büschges (1994) has shown that the sensory neurons located in the ventral scolopidia measure position, velocity, and acceleration. Additionally, Stein and Sauer (1999) have shown that the same velocity or acceleration sensitive afferences could be sensitive to vibration, and that they identified purely vibration-sensitive sensory neurons as well. The function of the dorsal scolopidia is unknown, but it has been reported that its stimulation elicit either no changes in the extensor tibiae motoneurons or only minor ones (Field and Pflüger,

1989; Kittmann and Schmitz, 1992). The multipolar sensory cells on the FT-joint are another type of sense organ. Two of them are located on the dorsal side, on the femoral cuticle, close to the FT-joint, i.e. the dorso-antero-lateral (RDAL) and the dorso-posterio-lateral (RDPL) receptor organs, both of which are sensitive to the extension of the FT-joint (Bässler, 1977). The third multipolar sensory cell, the ventro-posterio-lateral (RVPL) receptor organ, is located on the joint membrane below the flexor tibiae muscle apodeme. The function of RVPL is unknown (Bässler, 1983).

There is considerable information on the role that sensory signals play in organizing the overall motor output for walking. Movement signals from leg proprioceptors serve two functions in the locomotor cycle: 1) they modify the amplitude of motoneuron activity in the locomotor cycle ("reflex like pathway"); 2) they directly affect the central rhythm generating networks of the leg muscle control system (influence on the central rhythm generating network), thus determining the actual phase of the locomotor cycle (for review see Pearson 1993,1995).

In terms of interjoint coordination, previous investigations on stick insects (Heß and Büschges 1997, 1999) have shown that proprioceptive signals from the FT-joint, i.e. the fCO, effect the motor activity of the adjacent leg joint when the stick insect locomotor system is active and the joint control networks are in the movement control mode ("active" behavioral state: for a definition see Bässler 1983 and Bässler and Büschges 1998). Signals related to the flexion movement of the FT-joint induce specific transitions in the activity of the coxa-trochanteral joint (CT-joint) motoneurons by eliciting levator and terminating depressor motoneuron activity. Extension movements induce the opposite response. During locomotion this could facilitate the initiation of leg levation due to flexion signals from the FT-joint, or the initiation of leg depression during extension signals from the FT-joint. They also show that this information processing -from the fCO to the CT-joint motoneurons- is transmitted not only via the "reflex-like" pathways, but also via central rhythm generating networks (Heß and Büschges, 1999).

Graham and Bässler (1981) offered the first indications that positional information from the FT-joint, signaled by the fCO, influence CT-joint motoneuron activity. In their experiments, by attaching the receptor apodeme to the flexor tendon, they permanently reversed the sensory input provided by the fCO. The stick insects with this "crossed receptor apodeme" were no

longer able to perform functional locomotor movements. In a later study it was shown (Bässler, 1993c) that when locomotor movements were generated, the middle leg with a "crossed receptor apodeme" was normally kept in a so-called "saluting" posture (completely extended FT-joint and maximally elevated CT-joint). This was a result of the sign-reversed sensory input from the fCO on the CT-joint muscle control system of the middle leg. This means that the flexion of the FT-joint, signaled by the fCO, can induce the activation of the levator trochanteris motoneurons, and maintain this activation. It is known that the position of TC-joints is important information for walking (Bässler 1977, Cruse et al., 1984). The question arises regarding whether the positional information from the FT-joint activates the motoneurons of the adjacent CT-joint via "reflex-like" pathways, or alternatively by affecting the central rhythm generating networks of the CT-joint. In the first section of this thesis work (section 3.1.) I made experiments in collaboration with Dr. Dirk Bucher to see whether position information from the FT-joint influences the central rhythm generating network of the CT-joint.

From these studies the question emerges as to whether such specific influences represent a common mechanism for interjoint coordination in the multi-jointed limb. Petra Gerharz (1999) first showed that moving the femur up and down at the CT-joint has an effect on the FT-joint and the thorax-coxa joint (TC-joint) motoneurons. In active animals, elevating the femur at the CT-joint terminated ongoing extensor tibiae and protractor coxae motoneuron activities, this was followed by onset of the antagonists flexor tibiae and retractor coxae motoneuron activities. Using ablation experiments, she showed that only cuticular strain sensing campaniform sensilla located on the proximal femur (femoral campaniform sensilla – fCS) were causing this effect on the tibial motoneurons. The effects on the TC-joint motoneurons were only mediated by the campaniform sensilla located on the trochanter (trochanteral campaniform sensilla –trCS). She showed that the proprioceptors measuring the movement and position of the CT-joint have no effect on the FT- and TC-joint motoneuron activity. This suggests that the proprioceptive mechanism for interjoint coordination described by Heß and Büschges (1997) cannot be generalized for all joints.

In further sections (from section 3.2 to section 3.5.) of this present thesis work, I describe experiments regarding how the information from the fCS controls activity of the FT-joint (sections 3.2.1, 3.2.2., and 3.4) and TC-joint (sections 3.2.3., 3.2.4., and 3.3.) motoneurons. The main question is whether the observed interjoint influences are mediated purely by

"reflex-like" interactions, or whether sensory signals from the campaniform sensilla (CS) have access to the central rhythm generating networks of the FT- and TC-joint. Additionally, experiments were done on the network that controls the FT-joint reflex to see at which level the information from the fCS is fed in and how possible interaction between the fCO and fCS take place. In a final series of experiments the role of this interjoint influence in controlling the motor activity of the FT-joint in the walking animal was investigated.

2. Methods

The experiments were performed on adult female stick insects (*Cuniculina impigra* and *Carausius morosus*) from our breeding colonies at the University of Cologne and, in few experiments, from the colony at Bielefeld University. The main experiments were carried out on both species. Later, I will specify which experimental protocols were carried out on only one of the species. All of the experiments took place under daylight conditions and at room temperature (20°-22°). The experiments were carried out on animals under three different conditions: in resting animals ("inactive" state), in "active" animals (activated by touching the abdomen with a paint brush), and during the rhythmic activity of coxal MNs in an isolated ganglion preparation produced by a bath application of the muscarinic agonist pilocarpine ($5x10^{-4}$M) (Büschges et al., 1995). In Bässler (1988) and Bässler and Büschges (1998) inactive and active behavioral states have been defined and their properties have been described in detail.

2.1 Preparations

2.1.1 Influence of position information from the femoral chordotonal organ on the rhythmic activity of the coxa-trochanter joint motoneurons

The animals with only the left middle leg left attached were glued with dental cement (Protemp II, ESPE) with the dorsal side up, on a foam platform. The left middle leg was then fixed on the platform perpendicular to the body axis. The femur-tibia joint (FT-joint) was set to approximately 120° and embedded in dental cement. Around the femur, a wall of Vaseline was built and filled with the saline composed in accord with Weidler and Diecke (1969, WD-saline) so that the femur could reside in WD-saline. A cut along the midline of the meso- and metathoracic tergum was made; both sides were folded apart and fixed with insect pins. The inside of the insect was filled with WD-saline. The gut, fat, and connective tissue was removed to expose the ventral nerve cord. Care was taken to leave as much of the tracheation intact as possible. Leg nerves are named after Marquardt (1940) and Graham (1985). All lateral nerves where cut except for nervus cruris (ncr). The motor activity of the motoneurons that innervate the depressor and levator trochanteris muscles was monitored by extracellular recordings with hook electrodes (modified after Schmitz et al (1991) at the workshop of the Zoological Institute of University of Cologne) from the coxal nerves C1 and C2 (levator, and

depressor). After positioning the hook electrodes, the nerves C1 and C2 were cut distal from the electrodes.

To stimulate the femoral chordotonal organ (fCO), a small window was cut on the dorsal side of the femur of the left middle leg. All nerves, muscles, and tracheae were removed, and only the apodeme of the fCO was left intact. The apodeme of the fCO was fixed in the clamp of the stimulation device (Hofmann et al., 1985) (Fig. 2.1). The different FT-joint angles were mimicked either by setting the femoral chordotonal organ (fCO) for 40s step by step to positions corresponding to FT-angles of 40°, 80°, 120° and 160° (for the relation between fCO apodeme displacement and FT-angle, see Weiland et al, 1986) or by applying very slow ramps from 40° to 160° (ramp velocity: 3.64°/s for flexion and 6.32°/s for extension).

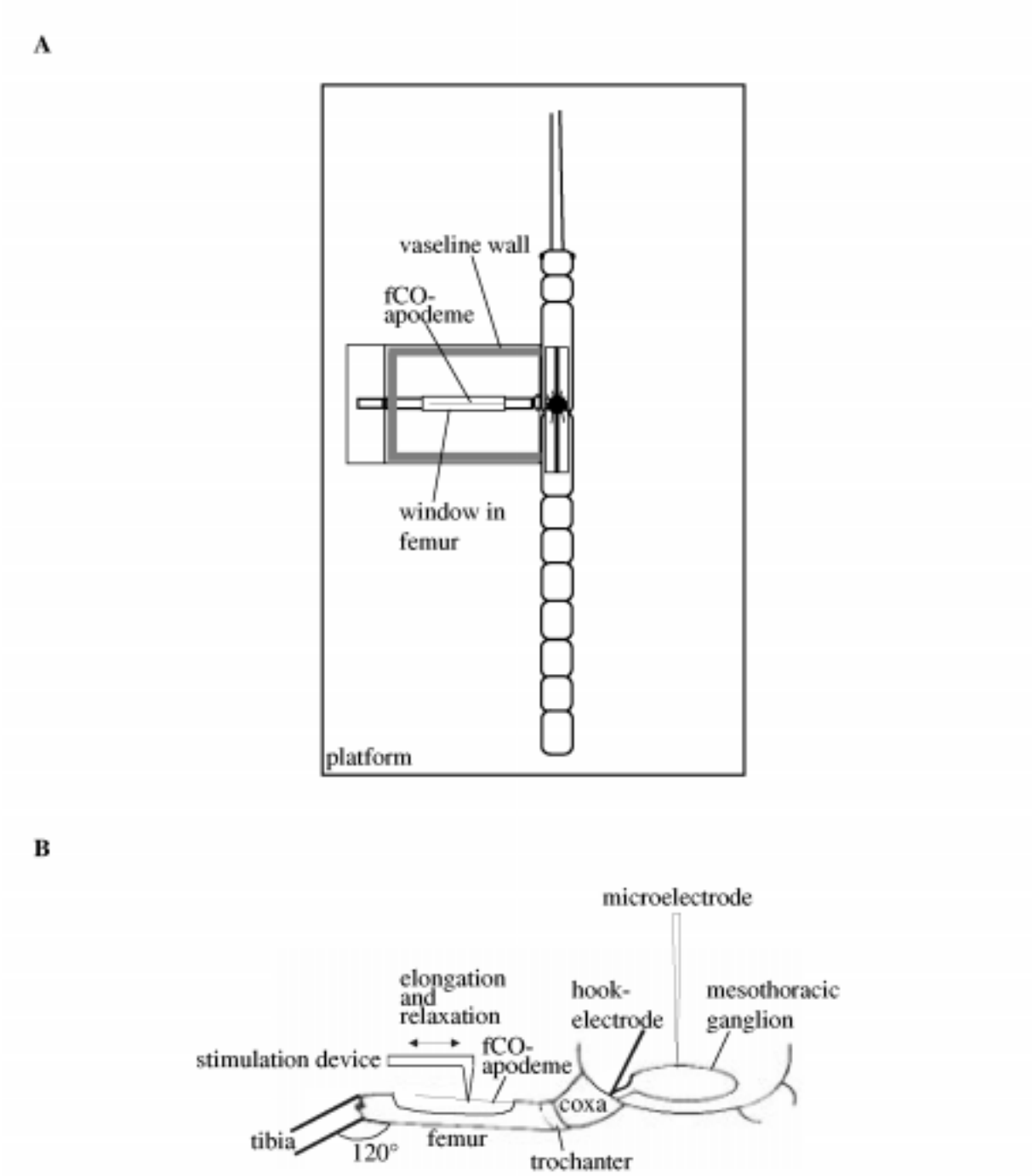


Figure 2.1. The preparation in which the fCO was stimulated by elongating or relaxing its apodeme. 100µm elongation or relaxation of the fCO apodeme corresponds to a femur-tibia joint movement (flexion or extension) of 20° (Weiland et al., 1986). **A:** dorsal view, **B:** schematic view from posterior of a cross section at the mesothoracic level.

To investigate the influence of the FT-joint position on the generation of rhythmic motor activity in the coxa-trochanteral joint (CT-joint), a preparation was used in which rhythmic activity of CT-joint MNs in the isolated and otherwise deafferented mesothoracic ganglion was induced by the muscarinic agonist pilocarpine (Büschges et al., 1995). Stock solution of 10^{-2}M pilocarpine was prepared in advance. Pilocarpine was applied by replacing the saline in the thorax of the experimental animal with a drug solution with a final concentration of

around $5x10^{-4}$M. Only those animals were used in which a stable long-lasting (20-40 minutes) rhythmic motor activity with a clear alternation between the depressor and the levator activity was established within a few minutes (4 out of 23 experiments).

I made these experiments -mentioned above and the results presented in section 3.1- in collaboration with Dr. Dirk Bucher.

2.1.2 Influence of coxa-trochanter joint movement on the thorax-coxa and femur-tibia joint motoneurons

The animals were fixed, dorsal side up, with dental cement (Protemp II, ESPE) along the edge of a foam platform. All legs except the left middle leg were removed. The coxa of this one remaining leg was embedded in dental cement so that the thorax-coxa joint (TC-joint) could not move. Care was taken not to disturb the elevation and depression movement of the CT-joint. A small window was cut in the meso- and metathoracic dorsal tergum, and the gut, fat, and connective tissue was removed to expose the ventral nerve cord (Fig. 2.2.A). To exclude indirect sensory influences, all lateral nerves of the mesothoracic ganglion were cut except for nerves innervating the sense organs of the CT-joint and the lateral nerve 3 (nl3) of the left middle leg. In the femur, the ncr was cut distal from the femoral campaniform sensilla (fCS). Only in experiments in which the activity of the flexor tibiae muscles were recorded by electromyograms (EMG) was the innervation of flexor tibiae muscle via ncr also left intact. The activity of the extensor tibiae motoneurons was recorded extracellularly with a hook electrode from nl3. In addition, the nl3 was crushed distal from the hook electrode. The measurements of the thorax-coxa joint (TC-joint) motoneuron activity were performed by extracellular recordings from the lateral nerve stumps 2 and 5 (nl2 and nl5, respectively). The activity of the flexor tibiae muscles was monitored by electromyographic recordings (EMG) with copper wires of 65µm diameter, insulated except for the tip. The femur was moved by a pen motor ±20° around the horizontal resting position of the CT-joint (Fig. 2.2.B).

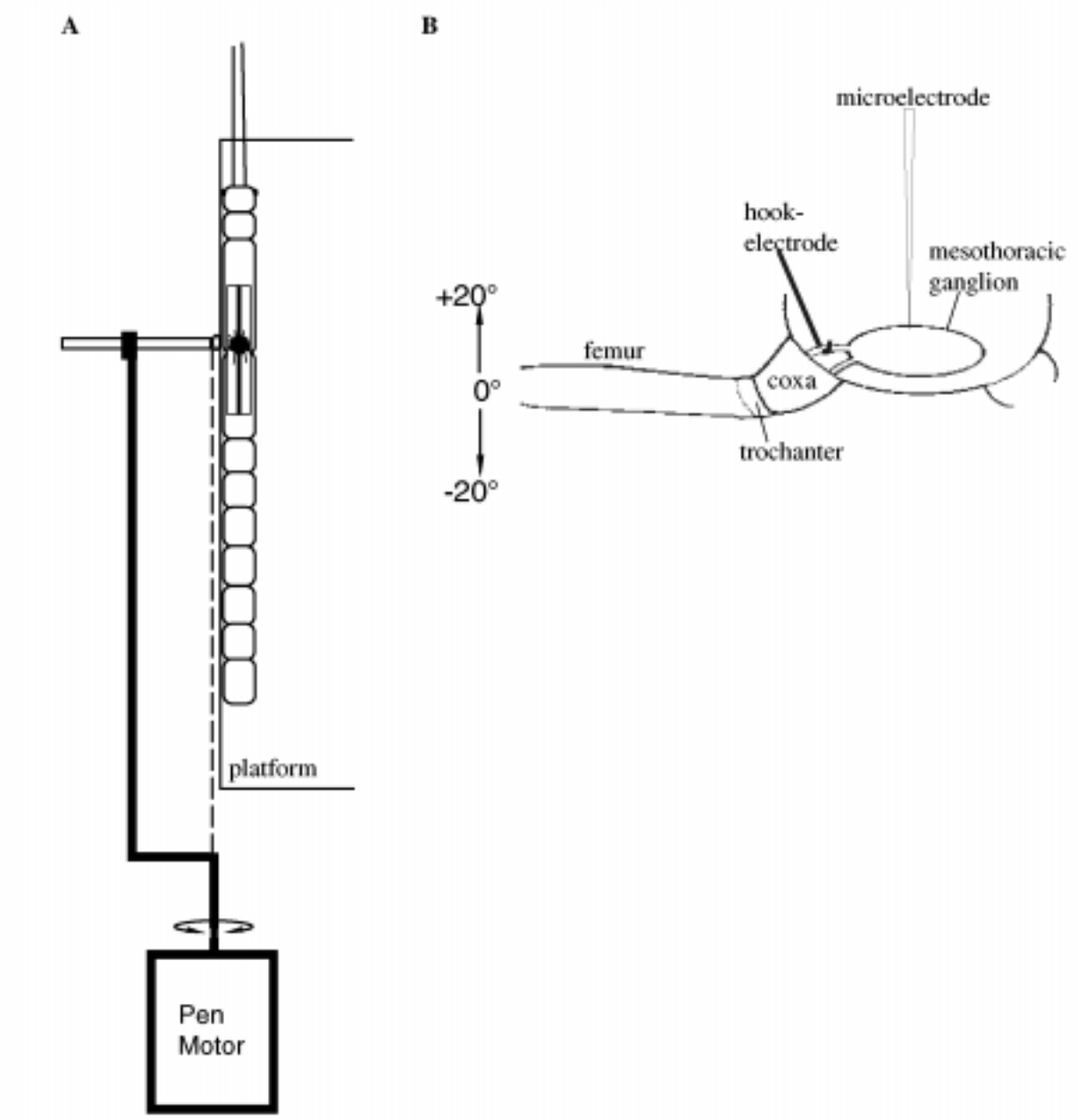


Figure 2.2. The preparation in which the sense organs signaling the movement of the trochanter were stimulated by moving the femur ±20° around the horizontal resting position of the coxa-trochanter joint with a pen-motor. **A:** dorsal view, **B:** schematic view from posterior of a cross section at the mesothoracic level.

2.1.3 Influence of strain information on the thorax-coxa and femur-tibia joint motoneurons

Two different procedures were used to stimulate the strain-measuring sense organs, campaniform sensilla (CS). First, the fCS were either stimulated being manually pushed with tweezers, or by means of a low-voltage piezo-electrical element (PI Physic), applying pressure to the proximal femoral cuticle close to the fCS. In this stimulation procedure, the sensory hairs at the femur were shaved with a splinter of a razor-blade, and the trochanteral

campaniform sensilla (trCS) were destroyed with a fine insect pin, which was pushed into the cuticle where they were located (Schmitz, 1993). The femur was totally fixed, fCO stimulation was applied along with extracellular recording of the femoral nerve 2 (F2), which contains the axons of the slow and fast extensor tibiae motoneurons (SETi and FETi), and the common inhibitor 1 (CI1) (Bässler and Storrer, 1980). The fCO stimulation was the same as in section 2.1.1 above. The only difference in this case was that the fCS was isolated from the saline-bath by a vaseline wall to prevent an electrical disturbance of the recording electrode from the piezoelectric element.

Second, the CS were stimulated by the caudal and rostral bending of the femur horizontally by means of a piezoelectric element or a pen motor with an amplitude of 200-340μm (Schmitz, 1993) (Fig. 2.3). During this procedure, protractor and the retractor motoneuron activity was recorded extracellularly from nl2 and nl5.

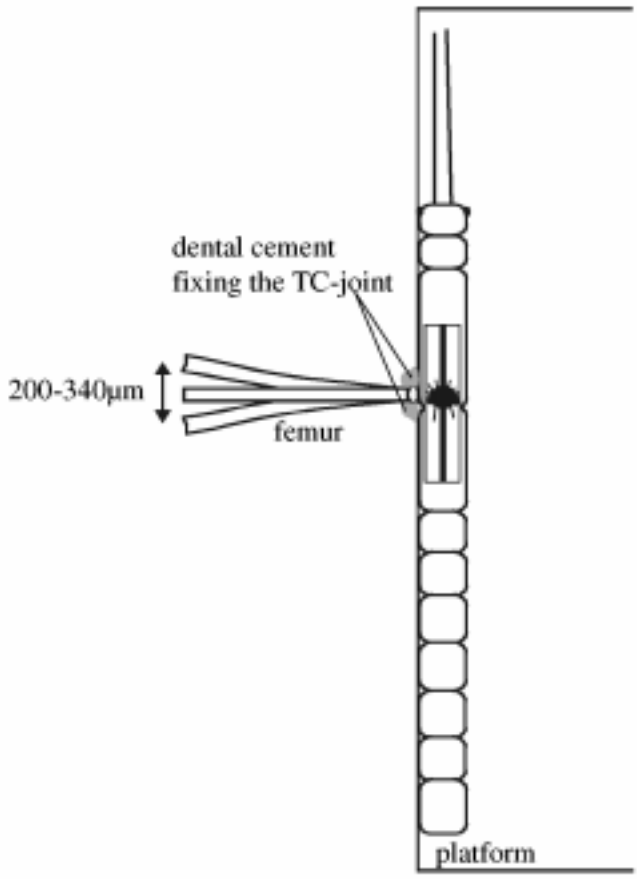


Figure 2.3. Dorsal view of the preparation in which the cuticular strain sensitive campaniform sensilla were stimulated by bending the femur 200-340μm horizontally by means of a piezoelectric element or a pen motor. During this procedure the thorax-coxa joint (TC-joint) was fixed with dental cement.

2.2 Intracellular recordings

To perform intracellular recordings from the neuropilar processes of motoneurons and interneurons, the mesothoracic ganglion was fixed on a wax-coated platform with fine cactus spines, in accord with the established procedures (Büschges, 1989). The ganglion sheath was treated with Pronase (Merck KGaA, 64271 Darmstadt) for 60 seconds to soften the ganglion-sheath and allow electrode penetration.

Intracellular recordings from all neurons were performed with thin-walled sharp microelectrodes in the bridge mode and in the discontinuous current clamp (DCC) mode by using intracellular amplifier (SEC-10L, NPI, Tamm, Germany). The electrodes had a resistance of 15-25MΩ when filled with 0.1M KCl/3M KAc or 0.05M KCl/2M KAc. The motoneurons were identified by a one to one correlation with the spikes in the extracellular recordings, and the interneurons were identified by their previously documented physiological properties and their morphology (Büschges, 1990; Sauer et al., 1996; Stein and Sauer, 1998).

To label the interneurons, dextran tetramethylrhodamine (3000MW, anionic, lysine fixable; Molecular Probes) was used. The tip of the electrode was first filled with 5% dextran in 0.1M KCl/3M KAc. The shaft of the electrode was then filled with 0.1M KCl/3M KAc solution. After finishing the experiment, each interneuron was filled with dye by passing a 5-7nA depolarizing current with 500 ms pulses at 1Hz for 10-30 minutes. After dye injection, the ganglion was desheathed and placed in 4% Paraformaldehyde in a 0.1M phosphate buffer (pH=7.4) for one hour. The preparations were dehydrated using an alcohol series by replacing the Paraformaldehyde solution for 20 minutes for each with 30%, 50%, 70%, and 90% ethanol, and two times 100% ethanol. Finally, the ganglion was transferred to a microscope cavity slide with a drop of methylsalycilate to clear the tissue. After adding the coverslip, the interneuron was viewed, and the images were collected by using a Zeiss 510 laser scanning confocal microscope equipped with a Axiovert 100M microscope. For imaging the interneurons a HeNeI Laser (543nm) and a LP 560 filter were used.

2.3 Behavioral analysis

For behavioral analysis, the stick insect single middle leg preparation was used (Fischer et al, 2001; Karg et al, 1991). In this preparation, all legs except the left middle leg of the animal

were removed. The left middle leg was fixed perpendicular to the thorax of the animal, extending over the rim of a foam platform. The TC-joint was immobilized, and the distal leg joints were free to move. The leg performed coordinated walking movements on a passive treadband (fig. 2.4) and searching movements in the absence of ground contact. Sequences of walking movements were elicited by touching the abdomen with a paintbrush. The activity of the tibial extensor motoneurons was recorded extracellularly from the nerve nl3 and the tibial flexor activity was recorded as EMGs from the muscle. The EMG signals were also recorded as rectified and low-pass filtered (time constant: 40ms) records. The animals were tested in three situations: 1) The control situation: the nerves innervating the muscles as well as the sense organs of the leg joints were left intact. In all of the behavioral experiments, the motor pattern was first recorded in a control situation (number of experiments, N=12). 2) After the removal of the fCS: the fCS of the leg was removed by destroying the field on the cuticle where the fCS is located with an insect pin (N=9). 3) Sham-operated animals: in this group, instead of destroying the fCS, we only made a small hole on the anterior side of the proximal femur (N=3). Therefore, any changes in the motor activity in the extensor and the flexor motoneuron pools due to the surgery at the femur could be monitored.

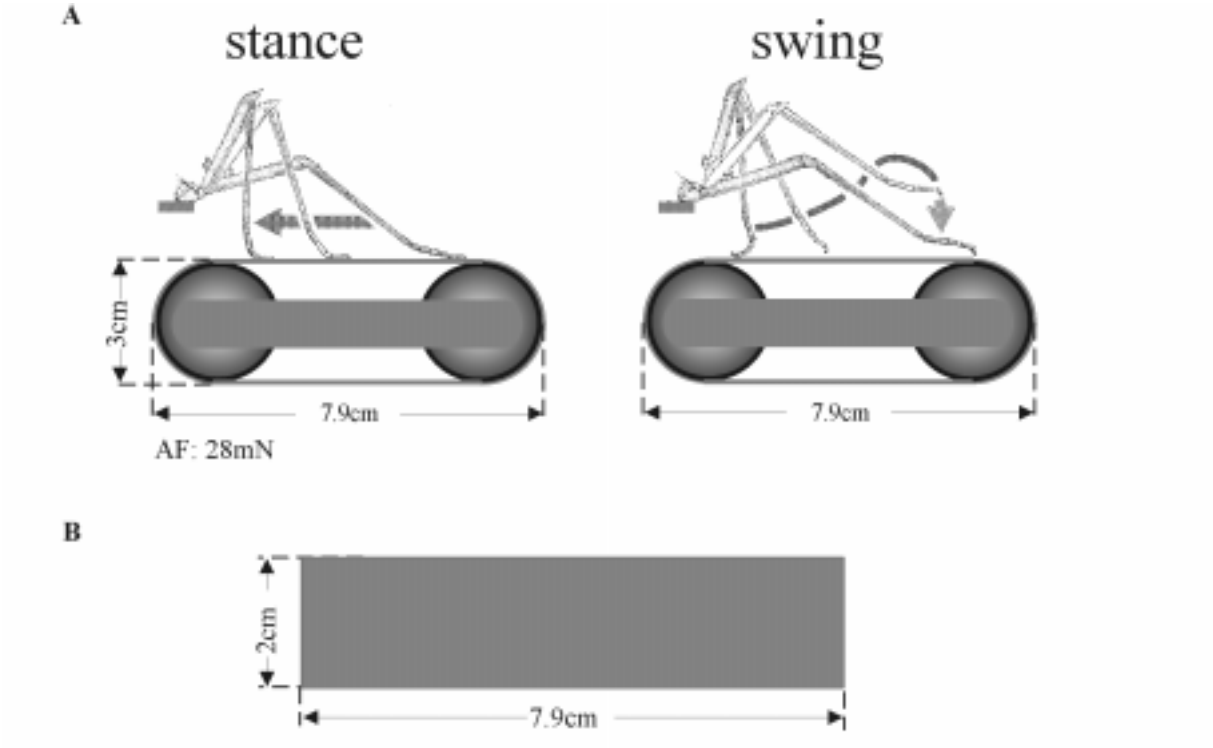


Figure 2.4. Schematic drawing of the treadband used in behavioral experiments. **A:** view from anterior (modified after Fischer et al., 2001). **B:** dorsal view. AF: adhesional friction.

2.4 Data analysis

Extracellular recordings, EMGs, and intracellular recordings were stored on a DAT-Recorder (SONY, PC 116) or on a FM-tape recorder (RACAL Store 7DS). Analog-to-digital conversion was performed (sampling rate: 12.5kHz for intra- and extracellular recordings) on a CED 1401plus interface (Cambridge Electronic). The recordings were analyzed with the Spike2 software (Versions 3.13-4.03). The statistical evaluation of the data and the plotting of the graphs was carried out with Statview (SAS Institute) or with Excel 97. In the text, “N” gives the number of experiments and “n” gives the sample size. The autocorrelations presented in figure 3.3B were made by calculating the mean spike rate (spikes/bin) of the levator trochanteris motoneurons within each bin (bin-width: 40ms): 6s before and after each levator trochanteris motoneuron spikes. In addition, the spike rates of one bin were normalized to the mean spikerate of all bins. Differences in the means of the samples were tested either by using the Student's t-test (Excel 97) or in behavioral analyses by a modified t-test (Dixon and Massey, 1969). Means were regarded as significantly different with $p<0.05$.

3. Results

3.1 Influence of position information from the femoral chordotonal organ on the activity of coxa-trochanter joint motoneurons

Heß and Büschges (1997 and 1999) showed that, in the stick insect (*Carausius morosus*), the position and movement of the femur-tibia joint (FT-joint), signaled by the femoral chordotonal organ (fCO), effect the activity of motoneurons, innervating the adjacent proximal coxa-trochanteral joint (CT-joint) muscles. They demonstrated that movement and position information from the fCO are mediated by a "reflex like" pathway. That is, they demonstrated that the FT-joint movement and position modulated the amplitude of the activity of the CT-joint motoneurons in animals at rest ("inactive" behavioral state) and in active animals ("active" behavioral state). Additionally, they showed that movement information from the fCO has access to the centrally generated rhythmic activity of the CT-joint motoneurons induced by pilocarpine. The timing cue of the pilocarpine-induced rhythmic activity in isolated ganglion (only the innervation of the fCO was left intact), could be influenced by changes of fCO receptor apodeme movements (corresponds to FT-joint movements). They could reset or entrain the pilocarpine induced rhythmic activity of the CT-joint motoneurons by fCO stimulation. However, their experiments did not clarify whether FT-joint position also influences the rhythmic activity of the CT-joint motoneurons. In this section the processing of FT-joint position information onto the CT-joint motoneurons was investigated. The influence of the FT-joint angle on the activity of CT-joint motoneurons was investigated to see whether position information from the fCO, in addition to the "reflex-like" pathway, is also mediated by affecting the centrally generated rhythmic activity of CT-joint motoneurons.

3.1.1 Effect of femur-tibia joint position on the coxa-trochanteral joint motoneuron activity in the resting animal

In resting stick insects, the CT-joint motoneuron activity is modulated by signals about FT-joint position (Heß and Büschges, 1997). Figure 3.1A shows an example of this dependency in the deafferented (except for the fCO) mesothoracic ganglion. With more flexed FT-joint positions, i.e. at 40°, the depressor motoneuron activity decreases and the antagonist levator motoneuron activity increases. The opposite was observed at more extended FT-joint

positions, i.e. at 160°. The phasic interjoint reflex response induced in trochanteral motoneurons by velocity signals from the fCO also exhibits a position dependency (Fig. 3.1A). The levator motoneuron activation was stronger for the flexion stimuli of a given amplitude when starting at rather flexed fCO positions, compared to extended positions. The above described influence was also substantiated by intracellular recordings from CT-joint motoneurons, fDepTr (N=8, Fig. 3.1B, *left*) and sDepTr (N=3, Fig. 3.1B, *right*), during which the position dependency of the motoneuron membrane potential was monitored.

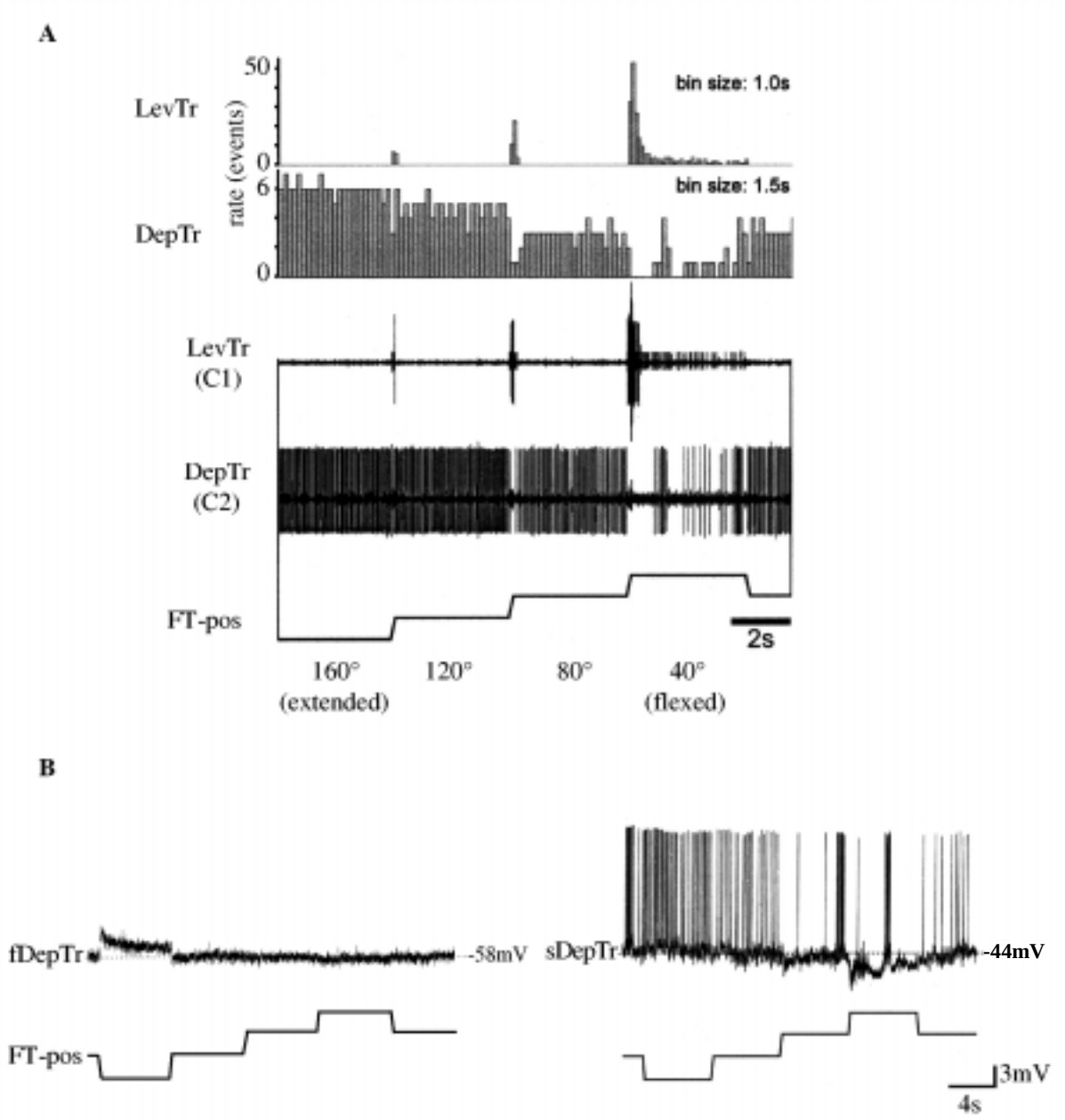


Figure 3.1. The tonic activity of the slow Depressor trochanteris motoneuron (sDepTr) gradually decreases as the leg is moved from extended to more flexed FT-angles in resting animals. Levator trochanteris motoneurons (LevTr) show the reverse dependence in their activity. **A:** Original recording from *Carausius morosus*. *Top histograms:* Histograms of spike rates of levator trochanteris (bin-width: 1.0s) and depressor trochanteris (bin-width:1.5s) motoneurons. *Top trace*: Extracellular recording from the coxal nerve C1 which contains the axons of the levator trochanteris motoneurons (LevTr). *Middle trace*: Extracellular recording from the coxal nerve C2 carrying the depressor trochanteris motoneurons. *Bottom trace*: FT-joint position. **B:** Intracellular recordings from fast (*left*) and slow (*right*) depressor trochanteris motoneurons (fDepTr and sDepTr) substantiate this result by showing subtle changes in the membrane potential with changing FT-joint angle.

3.1.2 Effect of femur-tibia joint position during pilocarpine induced rhythmic activity of the coxa-trochanter joint motoneurons

Applying pilocarpine to the isolated mesothoracic ganglion induces rhythmic alternating bursts of activity in antagonistic motoneuron pools of all leg joints, as described previously (Büschges, et al, 1995). The rhythmic activity of the antagonistic motoneuron pools is strictly alternating, with little or no overlap. However, pilocarpine-induced rhythmic activity in antagonistic motoneuron pools shows high variability, specifically with respect to cycle period and burst duration, these changes can even occur within one experiment. This variability made quantitative analysis impossible for all preparations, although the basic findings were similar for all of them. Therefore, the quantitative data evaluation was restricted to 4 out of 23 experiments. These 4 preparations showed a stable rhythmic activity for 20-40 minutes and were suitable for statistical evaluation of burst parameters. To examine the influence of FT-joint position on the rhythmic activity of the CT-joint motoneurons, two different stimulus protocols were tested: first, ramp and hold stimulus in which the fCO was set to a specific equivalent FT-joint position (40°, 80°, 120°, and 160°) and held for 40 seconds; second, very slow ramps from 40° to 160°, in which the velocity for flexion movement was 3.64°/s and the velocity for extension movement was 6.32°/s.

3.1.2.1 Experiments on the effect of femur-tibia joint position with ramp-and-hold stimuli

Figure 3.2A demonstrates a recording from an experiment in which the fCO was set at four different position (corresponding to 40°, 80°, 120°, and 160° FT-joint angle) and held for 40 seconds during pilocarpine-induced rhythmic activity in the CT-joint motoneurons. As shown above for the resting animal, the depressor activity decreased towards more flexed FT-joint angles, while levator motoneuron activity increased. This is obvious from the overall activity generated in the extracellular recordings. Additionally, the expanded sections of the recordings show this in detail.

The statistical evaluation of burst parameters is shown in figure 3.2B. The mean rate of motoneuron activity within a burst, the duration of the burst, and the duty cycle of the activity (burst duration/cycle period) clearly depend on the FT-joint position. These three parameters decreased with FT-joint movement from the flexed towards the extended position for the

levator motoneuron pool, while they increased for the depressor motoneuron pool. However, as seen in figure 3.2B only changes in these parameters from 40° to 80° and from 120° to 160° FT-joint angles differed significantly (except for the mean rate and burst duration of the levator motoneuron pool between 120° and 160°).

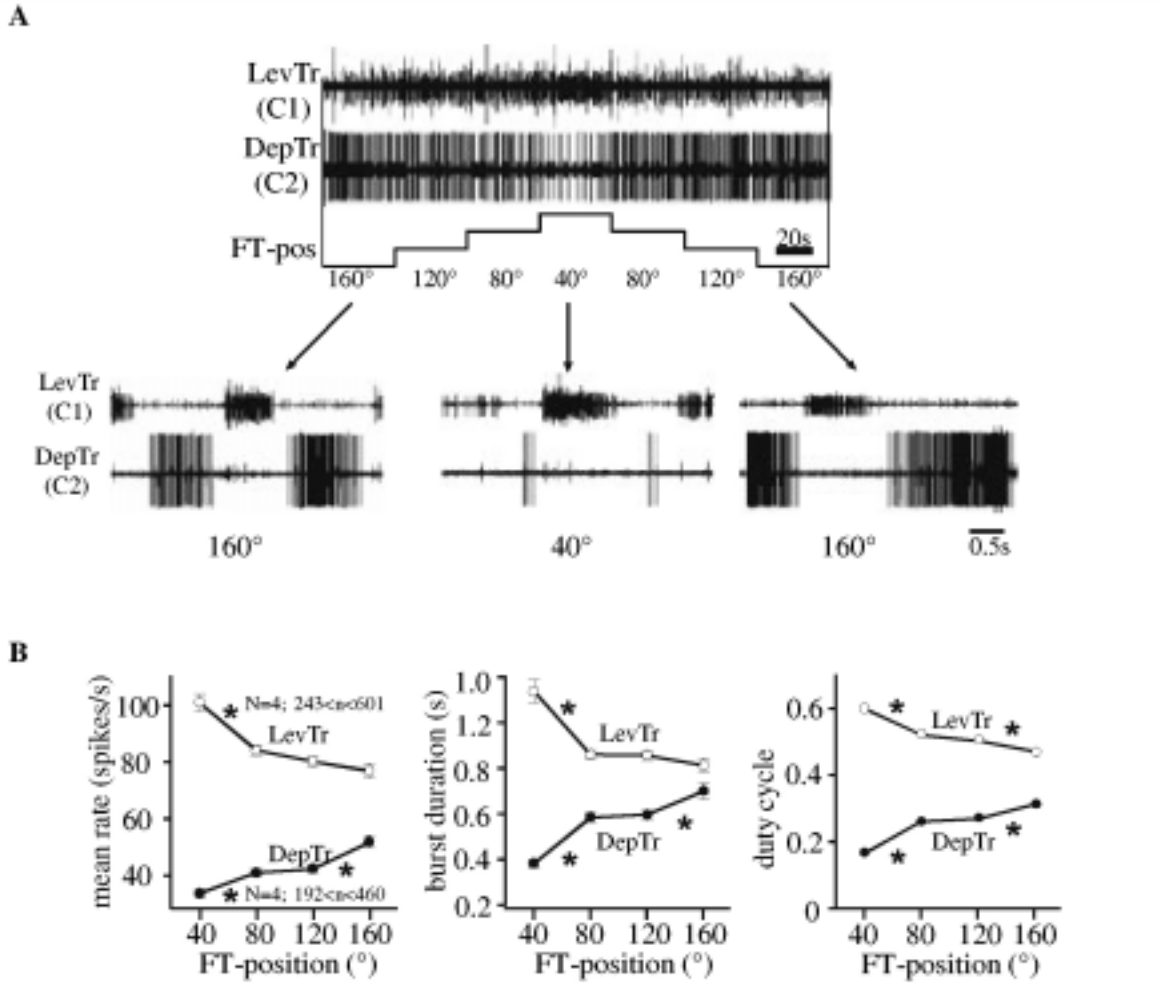
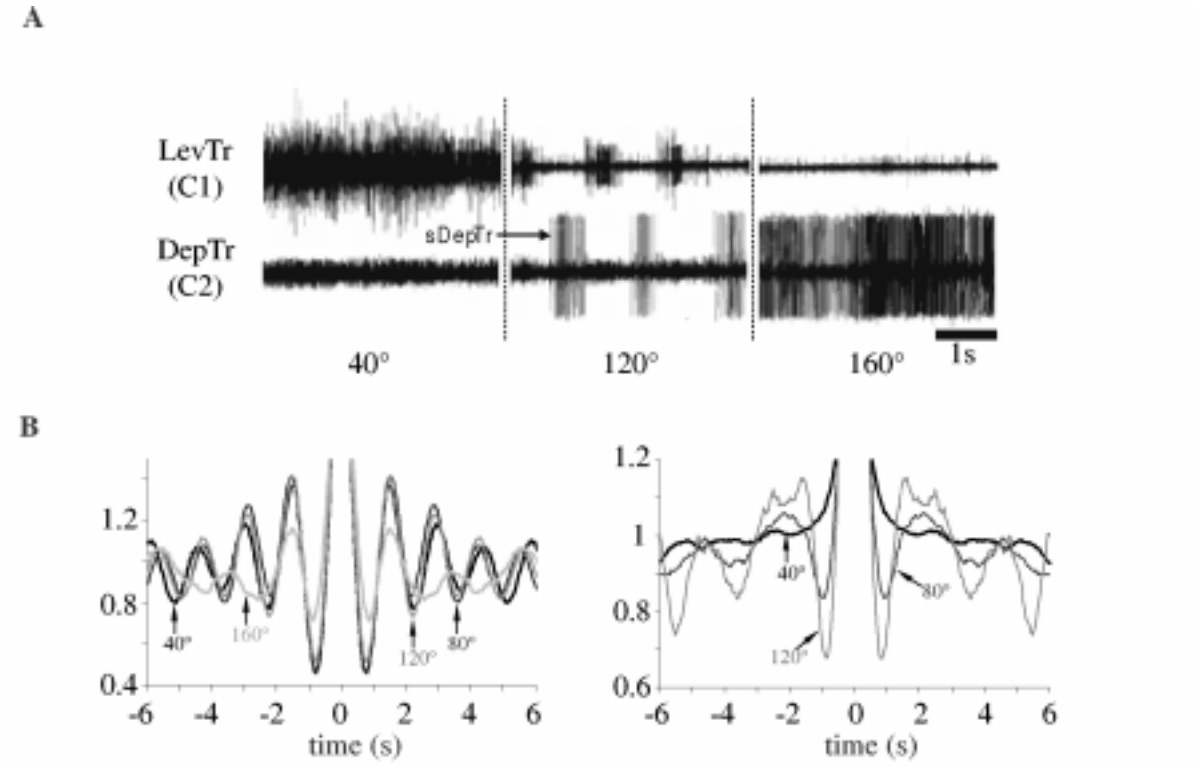


Figure 3.2. The pilocarpine-induced rhythmic activity of the CT-joint motoneuron in the isolated mesothoracic ganglion is modulated by position signals from the femoral chordotonal organ . **A:** An original recording from the coxal nerve C1 for levator trochanteris (*top trace*) and the C2 for depressor trochanteris (*middle trace*) motoneuron activity. *Bottom trace*: FT-joint position. **B:** Influence of the FT-joint position on the mean spike rate within bursts, the burst duration, and the duty cycle for each motoneuron pool. For LevTr motoneurons (*white circles*), these parameters gradually increase as the leg is moved from extended to flexed angles, whereas they decrease for DepTr motoneurons (*black circles*) (data +/- SEM; *=significant difference between adjacent data points, t-test).

Nevertheless, due to the variability of the pilocarpine-induced rhythmicity, the cycle periods were too variable to make it possible to find any significant dependence on the FT-joint angle. However, in some experiments the rhythmic activity ceased at the extreme joint angles (Fig. 3.3A), indicating that position-sensitive fCO afferents have access to the central rhythm-generating networks for the CT-joint. To clarify any influence of the FT-joint position on the pilocarpine-induced rhythmic activity of the CT-joint motoneurons, autocorrelation of spike

activity was made (Fig. 3.3B). Two different animals are presented in figure 3.3B. As seen in figure 3.3B (*left*), rhythmic activity was very regular between 40° and 120° and showed little change in cycle period (as indicated by the first peak, before and after the time zero). At 160° the rhythm is less stable, and the first harmonic peak broadens and becomes reduced in relative amplitude, indicating an irregular rhythmic activity. By contrast, the autocorrelation graph in figure 3.3.B (*right*) shows that there is no significant peak, before and after the time zero, when the FT-joint is extended at a 40° angle. This indicates that, in this experiment, the spiking activity was random at 40°, and it was not correlated with bursting activity seen at other angles.



Figur 3.3. Disruption of the rhythmicity at extreme FT-joint angles. **A:** In some experiments, activity in one antagonist ceased at extreme angles, whereas the other became tonically active. **B:** Autocorrelations of levator activity at different FT-joint angles. Data is normalized to the means. In animals with very regular activity (*left*), very little modulation in the cycle frequency is seen. However, at 160° (weakest levator activity), the rhythm becomes less regular. In animals with less regular activity (*right*), rhythmicity sometimes ceased at extreme FT-joint positions. In the example shown, the LevTr motoneurons are silent at 160° and tonically active at 40°. Autocorrelations show that the period of frequency modulation during this tonic activity do not correspond to the period of the rhythmicity seen at other angles.

3.1.2.2 Experiments on the effect of femur-tibia joint position with slow ramps

To determine a more direct assessment of cycle period changes, FT-joint angle changes were tested using very slow linear ramp stimuli (for elongating ramps (flexion): 3.64°/s and relaxation ramps (extension): 6.32°/s) (Fig. 3.4). During such ramps, the velocity information

remains constant, and the position information changes continuously until the end of one ramp. In addition, this movement velocity during one ramp is below the value (12,5°/s) reported by Büschges (1994) as being required to elicit responses in some of the movement-sensitive fCO afferents that he recorded. One can assume that the velocity afferents do not become active, or at least show little activity, during those slow ramps.

Figure 3.4A shows an example of pilocarpine-induced rhythmic activity in CT-joint motoneurons during the ramp-stimulation of the fCO. In two experiments (out of 6 experiments) the rhythmic activity usually occurred over the whole range of FT-joint angles (Fig. 3.4A). As seen in the insets in figure 3.4A, the cycle periods are shorter at more extended FT-joint angles (inset a) than in flexed FT-joint angles (inset b). However, in two experiments, rhythmicity ceased at extreme angles, and the motoneurons of one antagonist became completely silent, while the motoneurons of the other one were tonically active (Fig.3.4C). In the remaining two experiments the rhythmicity ceased at extreme positions in some stimulus cycles and in others they persisted continuously. Figure 3.4B shows the cycle period plotted versus the FT-joint positions from four experiments; it only shows stimulus cycles in which the rhythmic activity of the CT-joint motoneurons persisted during the whole stimulus cycle (N=4). Rhythmic activity significantly slows down when the joint is in a more flexed position, and it speeds up when it is at more extended angles. This demonstrates the influence of FT-joint position upon the central rhythm generating networks that control the activity of the adjacent CT-joint motoneurons.

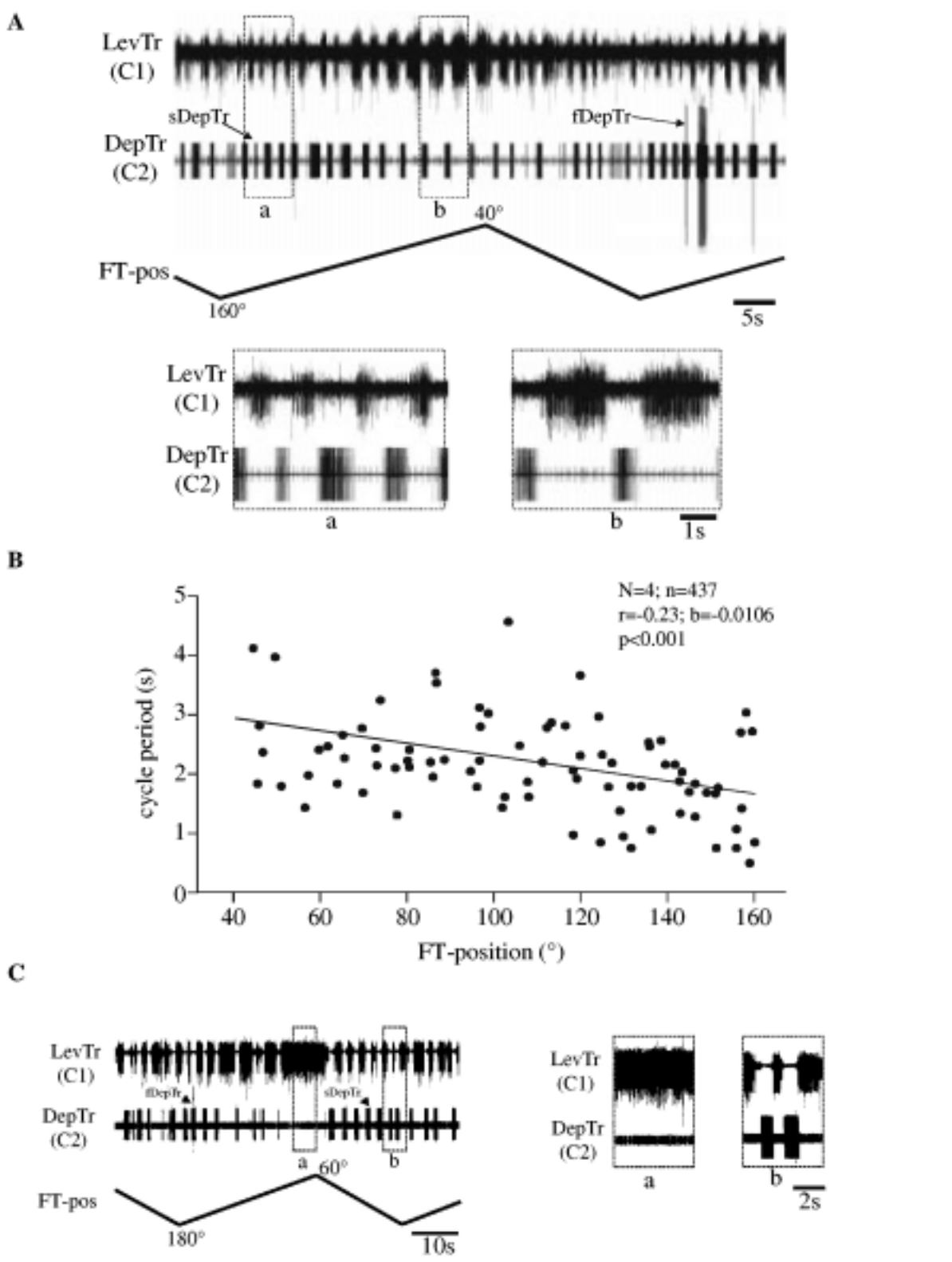


Figure 3.4. A: Application of stimulus ramps with very shallow slopes shows that the cycle period of rhythmicity depends on FT-position. This is shown in detail in insets a and b. **B:** The cycle period was significantly correlated with the FT-position. The data plotted derive from one experiment, whereas the regression line derives from the pooled data of four experiments. **C:** An example from an experiment in which the rhythmicity stopped at extreme positions (40° or 160°); rhythmicity stopped and tonic motoneuron activity occured in one phase (DepTr in extreme flexed positions or LevTr in extreme extended position).

In order to determine how position signals mediate the changes observed in motoneuronal rhythmicity intracellular recordings from trochanteral motoneurons were performed. Figure 3.5 shows an intracellular recording of the fast depressor trochanteris motoneuron (fDepTr). In a range of FT-joint angles, when the antagonistic Levator trochanteris (LevTr) motoneurons were tonically active, only minimal fluctuations in the membrane potential of fDepTr were detectable.

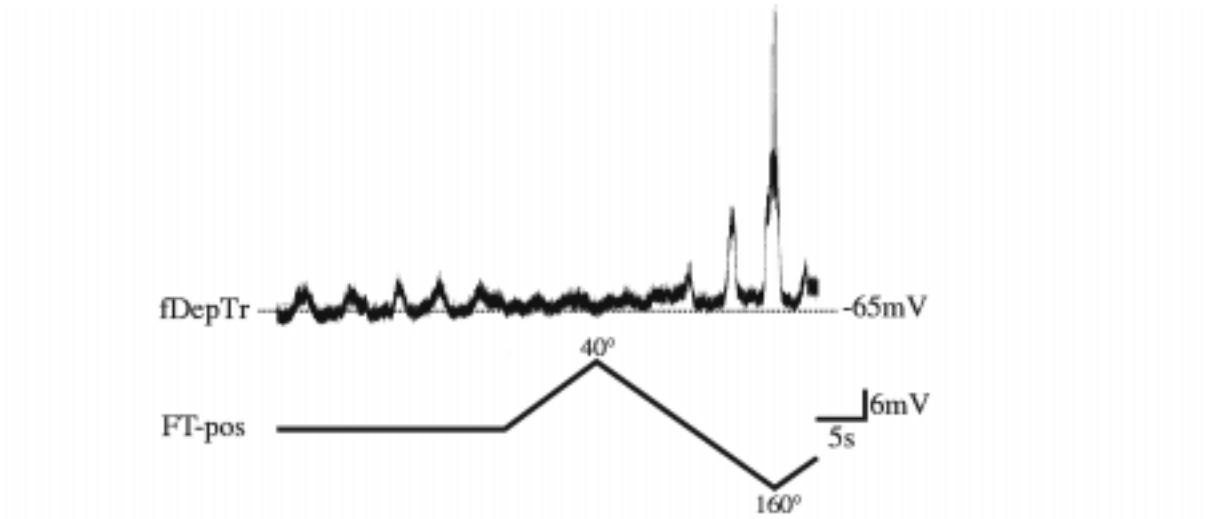


Figure 3.5. Intracellular recording of the fast depressor trochanteris motoneuron (fDepTr) during rhythmic activity in an animal where depressor activity was largely subthreshold and rhythmic activity ceased at flexed angles. Note that depolarizing phases in the fDepTr substantially decreased at flexed FT-joint angles.

3.2 Experiments on the influence of the coxa-trochanter joint movement on activity of the femur-tibia joint and thorax-coxa joint motoneurons

It was shown by Petra Gerharz (1999) that the movement of the trochantero-femur at the CT-joint has an effect on the motoneuron activity of the FT-joint and the TC-joint, in both resting and active stick insects (*Cuniculina impigra*). The effect was caused solely by the load measuring sense organs, campaniform sensilla (CS), which are located on the proximal part of the femur (femoral campaniform sensilla, fCS) and on the trochanter (trochanteral campaniform sensilla, trCS). The proprioceptors that measure the movement and position (rhombal and trochanteral hairplate, stretch receptors) have no effect on the FT-joint and the TC-joint motoneuron activity. Gerharz showed that levating the femur causes extensor tibiae motoneuron activity to end followed by an onset of flexor tibiae activity in the FT-joint and a termination of protractor coxae motoneurons, as well as an onset of retractor coxae motoneurons in the TC-joint. Additionally, her results indicated that the fCS (one group of CS) mediated the influence on the FT-joint motoneurons, and trCS (three groups of CS) mediated the influence onto the TC-joint motoneurons.

3.2.1 Influence of coxa-trochanter joint movement on the femur-tibia joint motoneuron activity in resting and in active animals

The influence of CT-joint movements were investigated with a fixed TC-joint and a completely denervated (except for the CT-joint sense organs) mesothoracic ganglion. This was done to exclude any indirect influence on tibial motoneuron and muscle activity. After activating the animals by touching the abdomen with a paintbrush, alternating activity in antagonistic muscles could be elicited. During this activity the femur was moved up and down with an amplitude of ±20° around the horizontal center position. In both species (*Carausius morosus* and *Cuniculina impigra*), upward movement of the femur applied during extensor activity caused an inhibition or a decrease in the ongoing extensor activity (Fig. 3.6A). Neither the levation of the femur during inactive phase of the extensor nor the depression of it during any phase of extensor activity resulted in any systematic change of activity. Figure 3.6B shows a peri-stimulus time (PST) histogram during upward movement in nine experiments (N=9) and 127 stimulus presentations (n=127) (69% of the total presented stimuli) in *C. morosus*. Additionally, figure 3.6C shows a PST-histogram during upward movement in seven experiments (N=7) and 154 stimulus presentations (n=154) (52% of the total number of stimuli) in *C. impigra*. The probability that extensor activity terminate by levation varied considerably from animal to animal, with extreme values ranging from 39% to 100% for individual preparations.

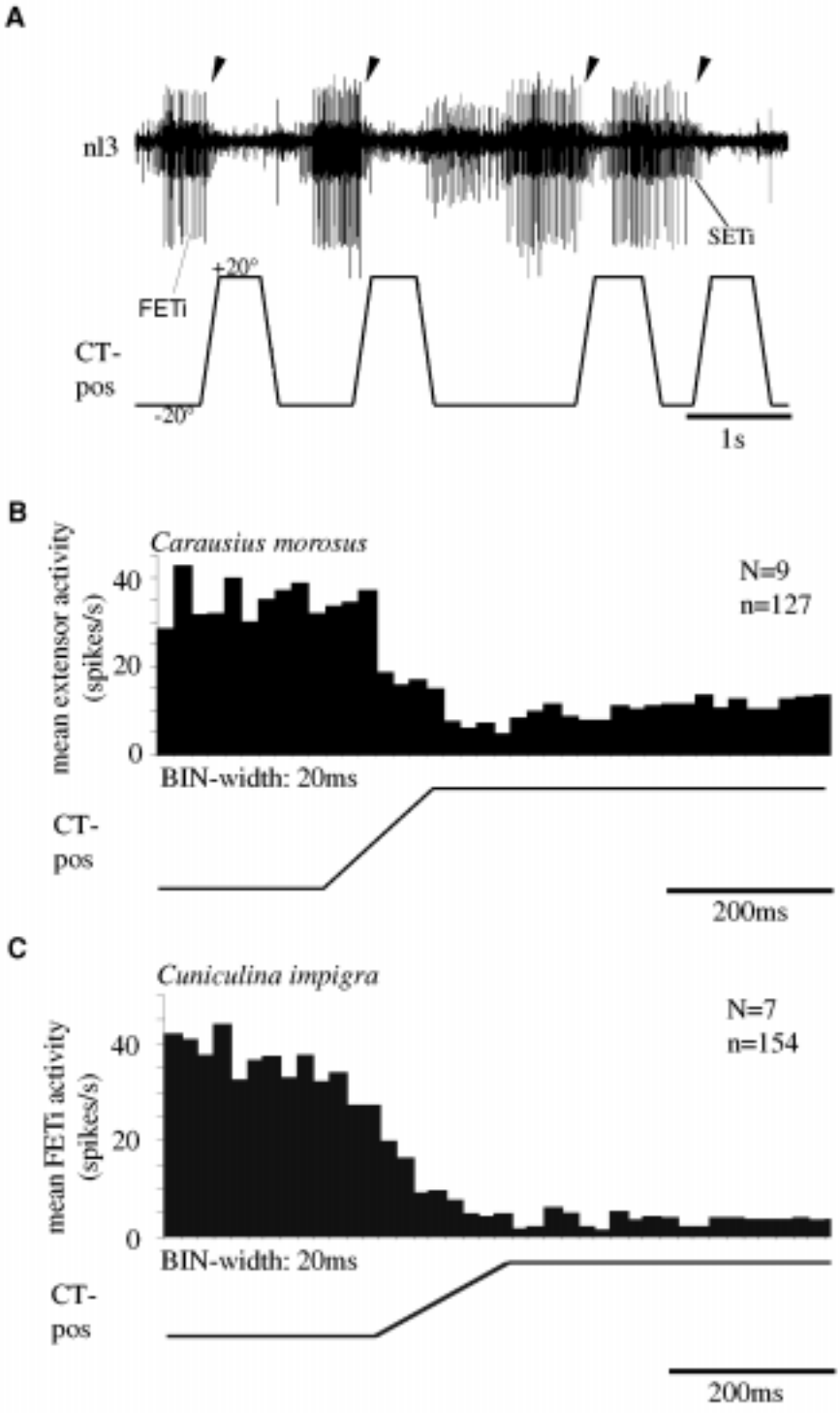


Figure 3.6. Influence of the levation and depression of the femur on the activity of extensor tibiae motoneurons in the active behavioral state of the stick insect. **A:** Original recording from *Cuniculina impigra*. *Top*: Extracellular recording from the lateral nerve nl3, carrying the extensor motoneurons. *Bottom*: Position of the femur. ▼ indicates termination of the extensor tibiae motoneuron activity. **B:** Peristimulus time (PST) histogramm of extensor motoneuron mean activity (FETi and SETi) in *Carausius morosus* for those 69% of the trials. **C:** PST histogram of FETi mean activity in *Cuniculina impigra* of those 52% of the trials.

Intracellular recordings from extensor tibiae motoneurons revealed that the termination of the extensor activity caused by femur levation is a result of inhibitory synaptic inputs to these

motoneurons (Fig. 3.7). Figure 3.7A shows that femur levation causes a termination of the ongoing extensor burst, followed by inhibitory postsynaptic potentials (IPSPs) in fast extensor tibiae motoneuron (FETi) (N=4) (Fig. 3.7B). The influence of femur movement on the extensor activity was different in resting and in active animals. Both the levation and the depression of the femur had only a weak influence on the activity of extensor motoneurons in resting animals. As shown in figure 3.7A (*right*), levation and depression movements in resting animals caused only one or two action potentials in slow extensor tibiae motoneuron (SETi), as visible from the extracellular recording of nl3. In general, during elevation of the femur FETi received small transient hyperpolarizing synaptic inputs of 1-2mV (N=9) (Fig. 3.7A and B, and Fig. 3.8). In addition, in four of these nine recordings, small depolarizing synaptic inputs were elicited by both levation and the depression of the femur. The inhibitory nature of these synaptic inputs was confirmed by current injection (Fig. 3.8). As seen in figure 3.8, the postsynaptic potentials in FETi caused by femur levation decreased in amplitude if -2.5nA hyperpolarizing current was injected. Injecting 2.5nA and 5nA depolarizing current increased the amplitude of the postsynaptic potentials, suggesting that these synaptic inputs, caused by femur levation, are inhibitory postsynaptic potentials (IPSP).

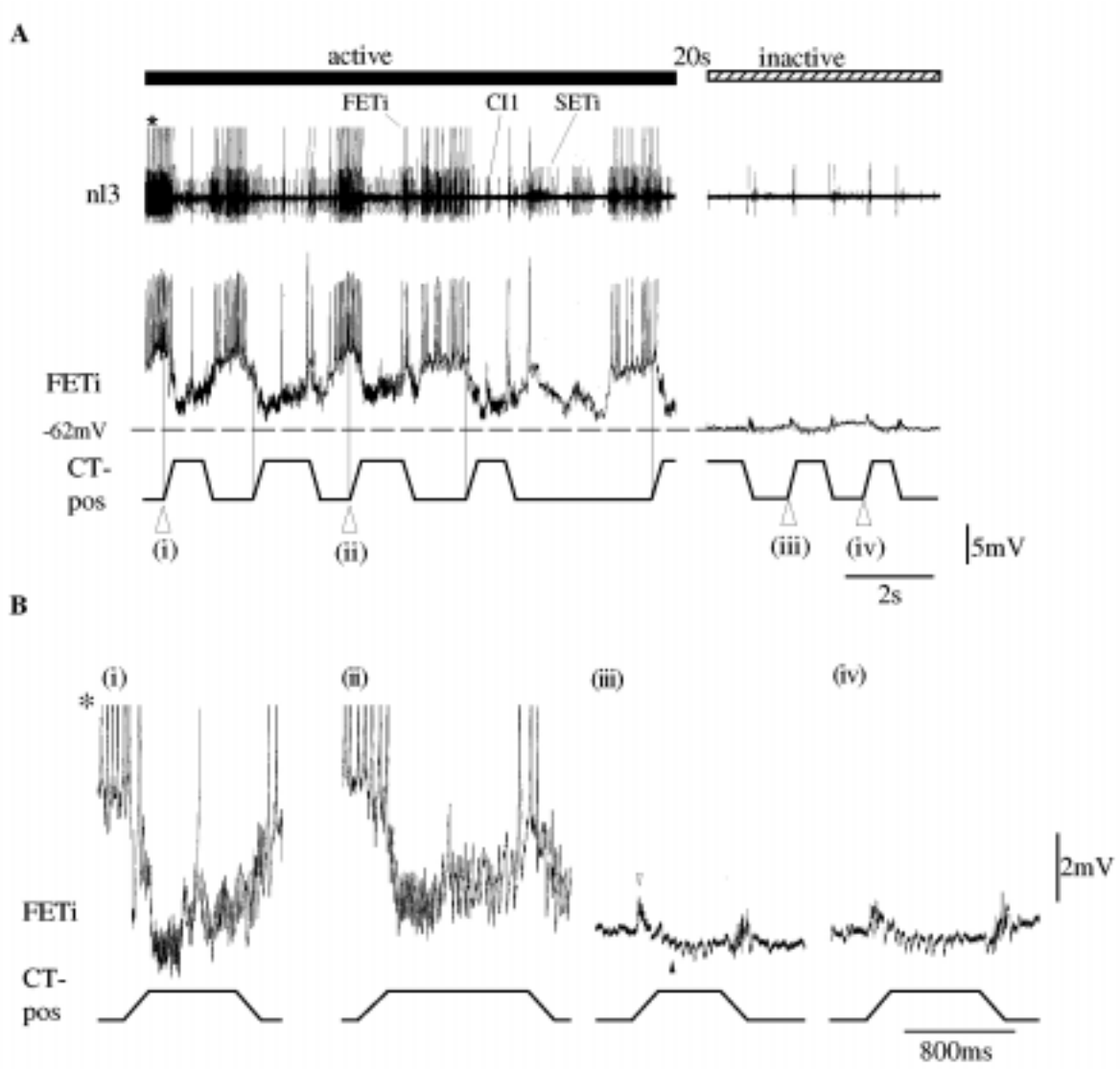


Figure 3.7. Intracellular recording from FETi in *C. morosus* during the levation and depression of the femur in the animal during the inactive and active behavioral state of the animal. The animals were activated by touching the abdomen with a paintbrush. **A:** Levation of the femur produces a hyperpolarization of the FETi in the active behavioral state. In inactive animals, the levation and depression of the femur elicit low amplitude synaptic inputs to the FETi, consisting of both IPSPs and EPSPs. **B:** Detailed presentation of four levation and depression stimuli from A. (i) and (ii) are from the active, (iii) and (iv) from the inactive behavioral state as indicated in A. In (iii) the depolarization is marked by an open arrowhead and the hyperpolarization by a filled arrowhead. The star indicates that the action potentials were clipped.

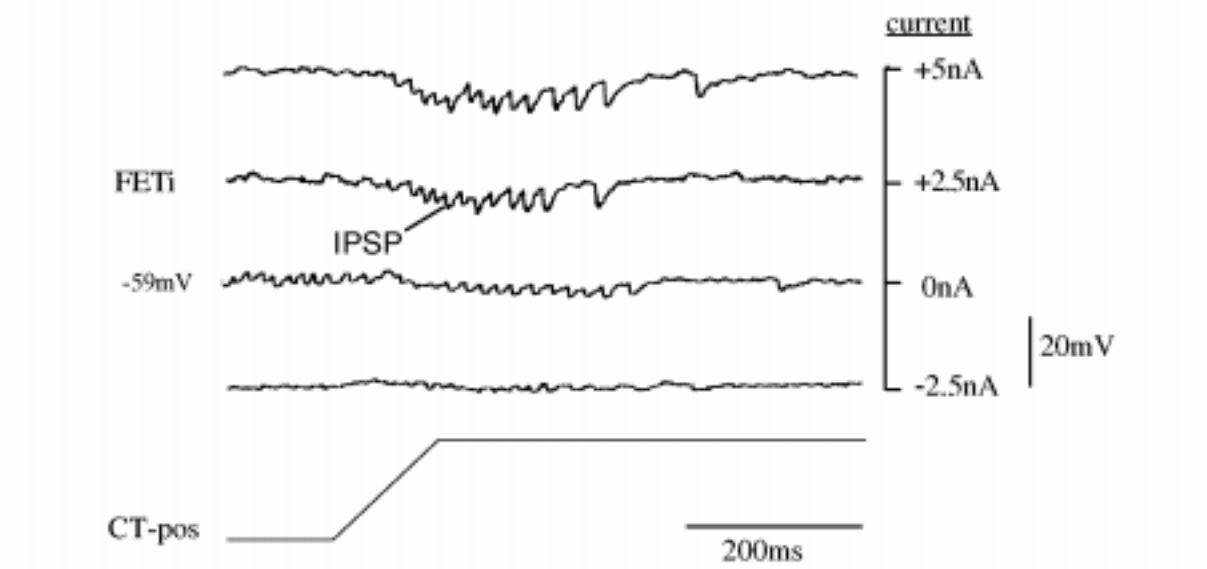


Figure 3.8. FETi receives hyperpolarizing synaptic input during the levation of the femur in the inactive animal. Note that the size of the individual IPSPs increases with the depolarizing current injection and decreases with the hyperpolarizing current injection. The recording derives from a set of experiments on *C. morosus*.

3.2.2 Influence of coxa-trochanter joint movement on the femur-tibia joint motoneuron activity during pilocarpine-induced rhythmic activity

With regard to the role of sensory signals in interjoint coordination, Heß and Büschges (1999) showed that the proprioceptive signals from the FT-joint effect centrally generated rhythmic activity in CT-joint motoneurons. In light of this finding, the question arose as to whether signals from the fCS have access to the central rhythm-generating networks (CRG) of the FT-joint. The relatively regular rhythmic activity of the CRG was elicited by topical application of the muscarinic agonist pilocarpine (Büschges et al., 1995), at a final bath concentration of $5x10^{-4}$M. During this rhythmic activity, the CT-joint was stimulated in the otherwise isolated mesothoracic ganglion (*C. morosus*), and the influence of the joint movements on the rhythmic activity of tibial extensor motoneurons was monitored (Fig. 3.9).

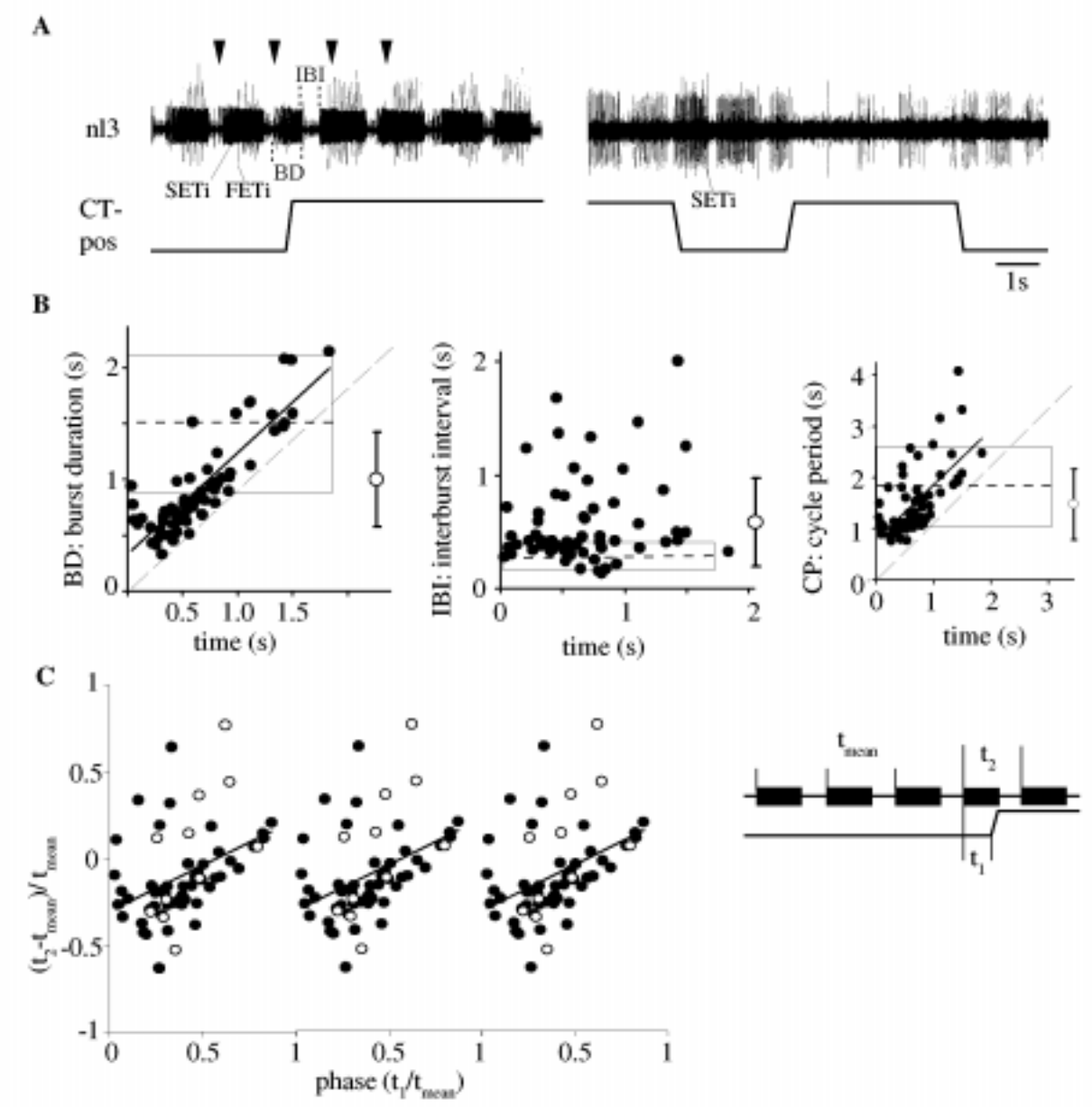


Figure 3.9. Influence of signals from the fCS during femur levation on centrally generated rhythmic activity in tibial extensor motoneurons (initiated by bath application of $5x10^{-4}$ M pilocarpine). **A:** In 57% (n=77) of the applied stimuli (N=5, n=135) we observed a shortening of tibial extensor activity upon femur levation. The arrowheads denote the time and expected time of the occurrence of burst onset in tibial extensor motoneurons (*left*). In 27 of these cases (20%) there was a long lasting inactivation of extensors after the stimulus was induced (*right*). **B:** Plot of the extensor burst duration (BD, *left*), interburst interval (IBI, *middle*), and cycle period (CP, *right*) as a function of stimulus time after the burst onset for those trials in which the femur levation was observed to have an influence (N=5, n=77). In addition, in each graph the mean ±S.D. for the parameters is given on the right-hand side (open circles). The dashed horizontal line in each graph indicates the mean value for the control cycles (n=87). The horizontal lines give their standard deviations. The thick solid lines give the regression lines for BD (corr. coeff.: 0.78, $p<0.001$) and CP (corr. coeff.: 0.59, $p<0.001$). **C:** Reset plot showing the relation between the stimulus phase and the influence of the stimulation on extensor activity (data derive from the same experiments as in B (N=5, n=77). The inset gives the paradigm of the evaluation of the data (t_{mean}: mean of control cycle period evaluated for each experiment for $7 < n < 38$ cycles, t_1: duration from onset of disturbed burst to start of stimulation, t_2: duration of disturbed cycle). Note that despite a considerable variability, a significant correlation exists between the phase of stimulation and the influence on rhythmicity (corr. coeff.: 0.39, $p<0.001$). Data points for one cycle have been repeated three times to better clarify the phasic influence. Open circles indicate data deriving from one experiment. All data presented derive from recordings in *C. morosus*.

In 57% (n=77) of the total number of trials (N=5, n=135), the levation of the femur was observed to shorten of the extensor activity (Fig 3.9A, *left*). In these 77 cases, the burst

duration (BD) of the tibial extensor motoneuron was significantly correlated with the latency between the onset of the extensor burst and the onset of the stimulus (Fig. 3.9B, *left*). In addition, not only was the BD effected by the stimulus; the first interburst interval (IBI) between extensor bursts, following the terminated extensor burst, was also altered by the stimulus, as it became more variable and increased, when compared with the control (Fig. 3.9B, *middle*). In 20% of the trials, a shortening of the extensor activity was followed by a long lasting interburst interval (Fig. 3.9A, *right*). In general, the duration of the IBI was not correlated with the latency between the onset of the extensor burst and the onset of the stimulus. Finally, the duration of the affected cycle period (CP=BD+IBI) was also significantly correlated with the latency between the onset of the extensor burst and the onset of the stimulus. The CP depended on the time of the stimulus, but with a high degree of variability (Fig. 3.9B, *right*). To illustrate the phase dependency of the effect of the CT-joint stimulation, figure 3.9C shows a phase response plot. Here it became clear that CT-joint stimulation resets rhythmic activity of the FT-joint motoneurons, but with a considerable amount of variability. As judged by the regression line, femoral levation delivered early in the cycle tended to shorten the cycle, while at higher phase values this influence reversed, showing a general tendency to slightly lengthen the cycle period. In the remaining cases, i.e. in 43% (n=58) of the trials (N=5, n=135), no change in motor activity could be detected. These results indicate that sensory influences from the fCS during leg levation do affect the patterning of extensor motoneuron activity, albeit in a rather variable and labile manner. Furthermore, depressing the femur was never observed to induce any detectable changes in tibial extensor activity (not shown).

3.2.3 Influence of coxa-trochanter joint movement on the thorax-coxa joint motoneuron activity in resting and in active animals

The influence of the CT-joint stimulation on the activity of the motoneurons innervating the TC-joint muscles was investigated by moving the femur at ±20° around its horizontal center position. The activity of the pro- and retractor motoneurons was monitored by extracellular recordings from lateral nerves, nl2 and nl5 respectively. The experiments in this section were performed only on *C. morosus*, and in resting and active conditions.

In resting animals, levating the femur caused an onset of retractor motoneuron activity in two out of six experiments. In one experiment spontaneous protractor activity decreased when the

femur was elevated. In the remaining three experiments, there was no spontaneous activity in any of the pro- or retractor motoneuron pool, and no effect of femur elevation could be detected.

In active animals, levation of the femur during protractor activity caused protractor activity to terminate and initiated retractor activity (Fig 3.10A). Figure 3.10B shows PST-histograms of pro- and retractor motoneuron activity during elevation movement in 6 experiments (n=47, 84% of total number of stimuli n=56) with *C. morosus*. Elevation of the femur during the retractor phase did not result in any systematic change. When the femur was depressed the response changed from experiment to experiment (i.e. in figure 3.10A depression of the femur increases the activity of retractor coxae motoneurons) and no systematic response could be detected.

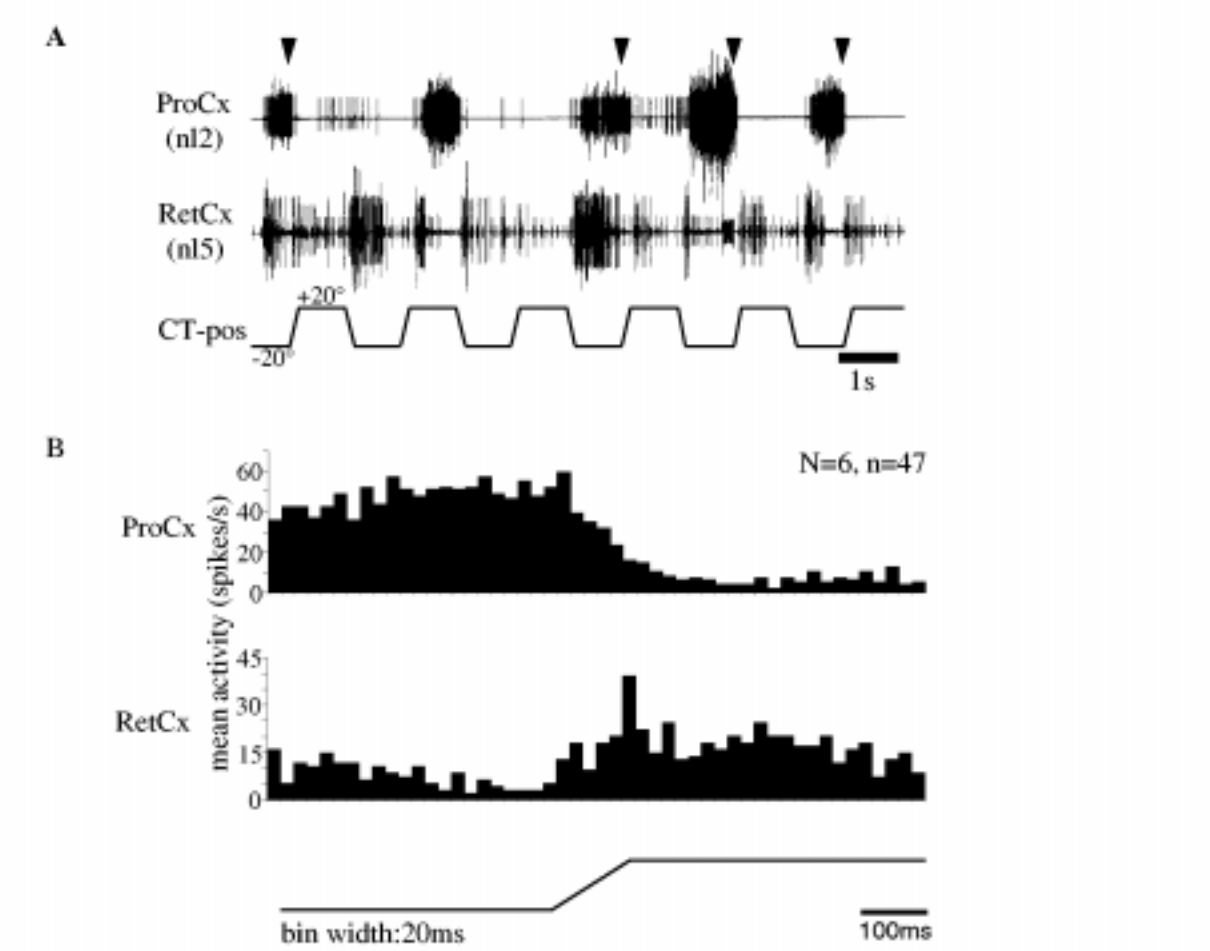


Figure 3.10. Influence of femoral levation on the activity of protractor coxae (ProCx) and retractor coxae motoneurons (RetCx) of the thoraco-coxal joint in active stick insects (*Carausius morosus*). **A:** An original recording from the lateral nerves nl2 (ProCx -*top*) and nl5 (RetCx -*middle*). *Bottom*: The position of the coxa-trochanter joint (CT-joint). ▼: Switch of activity from the ProCx to RetCx motoneurons. In the first femur levation only a termination of the ProCx motoneurons is observed. In the presented recording, levation of the femur also increases the RetCx activity (see text). **B:** Peristimulus time (PST) histogram of the ProCX (*top*), and RetCx (*bottom*) mean activity for those 84% of the trials in which a termination of the ProCx motoneurons was observed (N=6, n=47).

3.2.4 Influence of the coxa-trochanter joint movement on the thorax-coxa joint motoneuron activity during rhythmic activity

As in section 3.2.2, the influence of the CT-joint movement was tested in the pilocarpine-induced rhythmic activity of TC-joint motoneurons. In N=4 experiments, 63% (n=26) of the total number of levation movements (n=41) of the femur at the CT-joint during protractor activity caused a termination of the protractor burst, followed by an onset of the retractor motoneuron activity (Fig. 3.11A). A levation during ongoing retractor burst caused no detectable changes (only 4% of the total number of stimuli, n=84, caused a switch in the activity from re- to protractor; not shown). Depression of the femur had no systematic effects on the TC-joint motoneurons.

Closer inspection of the levation movements revealed that termination of protractor activity caused by levation of the femur did reset the rhythm of TC-joint motoneuron activity. This is shown in figure 3.11B. In the above mentioned 26 levation movements during ongoing protractor activity, protractor burst duration (BD) was significantly correlated (corr. coeff.: 0.94, $p<0.001$) with the latency between the onset of protractor burst and the onset of the stimulus (Fig. 3.11B *left*). In contrast to the BD, the first following interburst interval (IBI) and the period of the affected cycle (CP) were not correlated with latency between the protractor burst onset and the onset of the stimulus (Fig. 3.11B *middle* and *right*). The fact that CP is not correlated with the latency between the onset of the protractor burst and the onset of the stimulus could be due to the high variability of the rhythm, which can be seen in figure 3.11B, *right* (the dashed line denotes the mean cycle period, and the solid lines denote the standard deviations for undisturbed control cycle periods). To avoid a potential masking effect of this high variability in cycle period on a correlation, each disturbed cycle was normalized to control cycles and plotted versus the phase instead of the time of the stimulus onset (reset plot, Fig. 3.11C). In doing so, a significant correlation, which was masked by the variable cycle periods in figure 3.11B (*right*), was unraveled (corr. coeff.: 0.42, $p<0.05$; figure 3.11C): that is, that the CP is correlated with the phase of the stimulus onset suggesting a reset of the ongoing rhythmic activity of the TC-joint motoneurons. In other words, the femur levation has access to the CRG of the TC-joint.

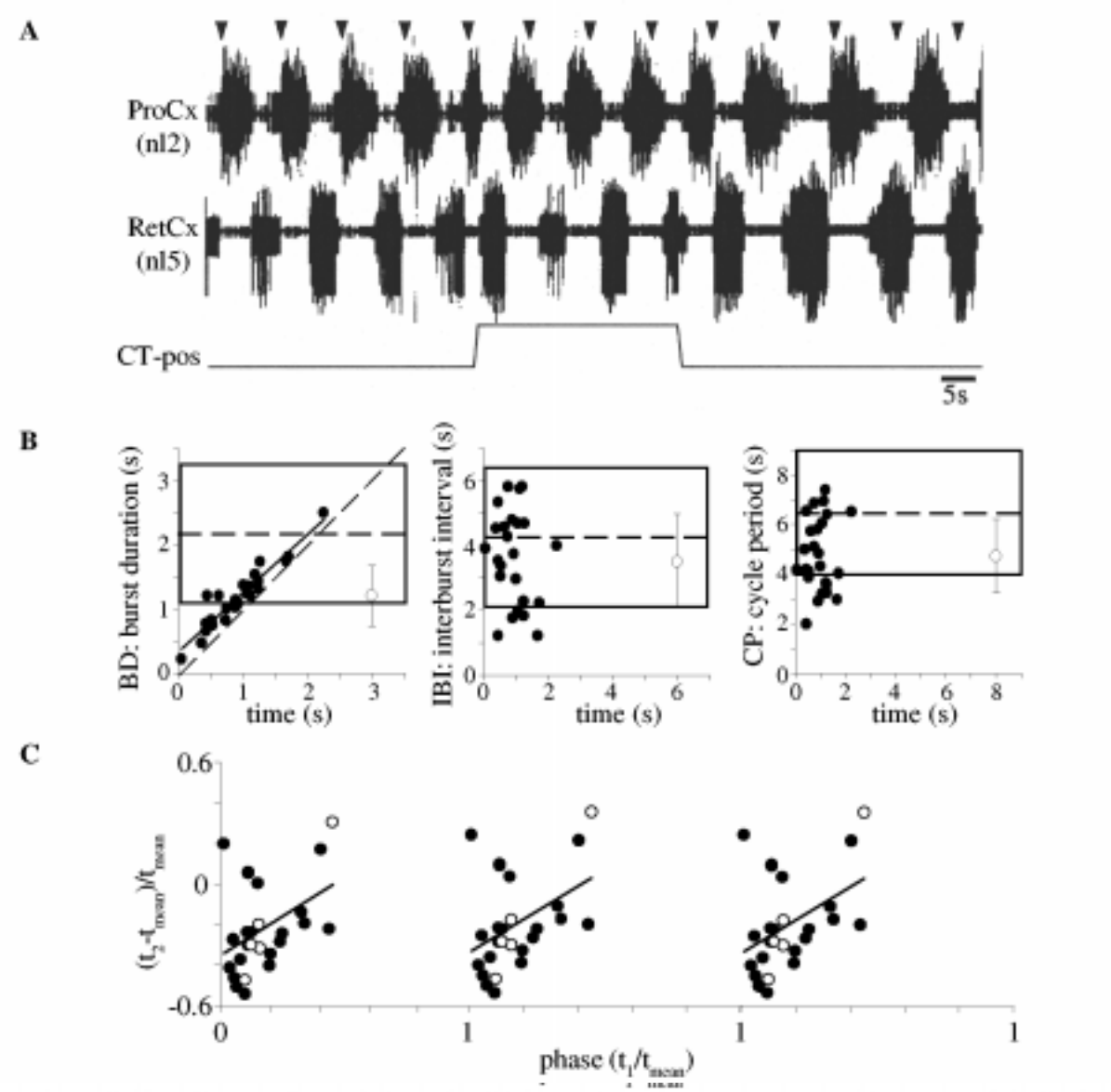


Figure 3.11. Influence of signals from the CS during femur levation on centrally generated rhythmic activity in thoraco-coxal joint motoneurons (initiated by bath application of $5x10^{-4}$ M pilocarpine). **A:** In 63% (n=26) of the applied stimuli (N=4, n=41) a shortening of protractor coxae activity upon femur levation was observed. The arrowheads denote the time and expected time of the burst onset in tibial extensor motoneurons. **B:** Plot of the protractor burst duration (BD, *left*), interburst interval (IBI, *middle*), and cycle period (CP, *right*) as a function of stimulus time after burst onset for those trials in which femur levation was observed to have an influence (N=4, n=26). In addition, in each graph the mean ±S.D. for the parameters is given on the right-hand side (open circles). The dashed horizontal line in each graph indicates the mean value for the control cycles (n=83). The horizontal lines give their standard viations. The thick solid lines give the regression lines for BD (corr. coeff.: 0.94, $p<0.001$). **C:** Reset plot showing the relation between the stimulus phase and the influence of the stimulation on protractor activity (data derive from the same experiments as in B (N=4, n=26). Note that a significant correlation exists between the phase of stimulation and the influence on rhythmicity (corr. coeff.: 0.42, $p<0.001$). The paradigm for evaluation is the same as in figure 11C (see inset in figure 11C).

3.3 Role of signals from the trochanteral campaniform sensilla in controlling the activity of thorax-coxa joint motoneurons

In sections 3.2.3 and 3.2.4 the influence of CT-joint movement on the TC-joint motoneuron activity in *C. morosus* was investigated. The results are in agreement with the experiments with *C. impigra* (Gerharz, 1999); i.e. in active animals a levation of the femur caused a termination of the protractor and an onset of the retractor motoneuron activity. Additionally, Petra Gerharz (1999) showed that this influence was mediated by load sensing campaniform sensilla (CS) and not by the movement- or position-sensing proprioceptors. In further experiments with *C. morosus*, load sensing CS were stimulated by caudal and rostral bending the femur horizontally by means of a piezoelectric element or a pen motor with an amplitude of 200-340µm (Schmitz, 1993) while pro- and retractor motoneuron activity was recorded extracellularly from the lateral nerves nl2 and nl5 in completely denervated mesothoracic ganglion (except for the CS). The experiments were carried out on stick insects in three different conditions: i.e. in inactive and in active behavioral states, and in pilocarpine-induced rhythmic conditions in isolated mesothoracic ganglion. The latter was performed to find out whether the trCS signals have access on the CRG of the TC-joint, or whether the influence is only mediated by “reflex-like” pathways.

3.3.1 Experiments on resting and active animals

As shown in figure 3.12A (*left*), bending the femur rearwards leads to the inactivation of spontaneously active protractor units and initiated retractor activity in resting animals. The opposite response was elicited by bending of the femur in the anterior direction (N=9). This phenomenon was previously shown by Schmitz (1993).

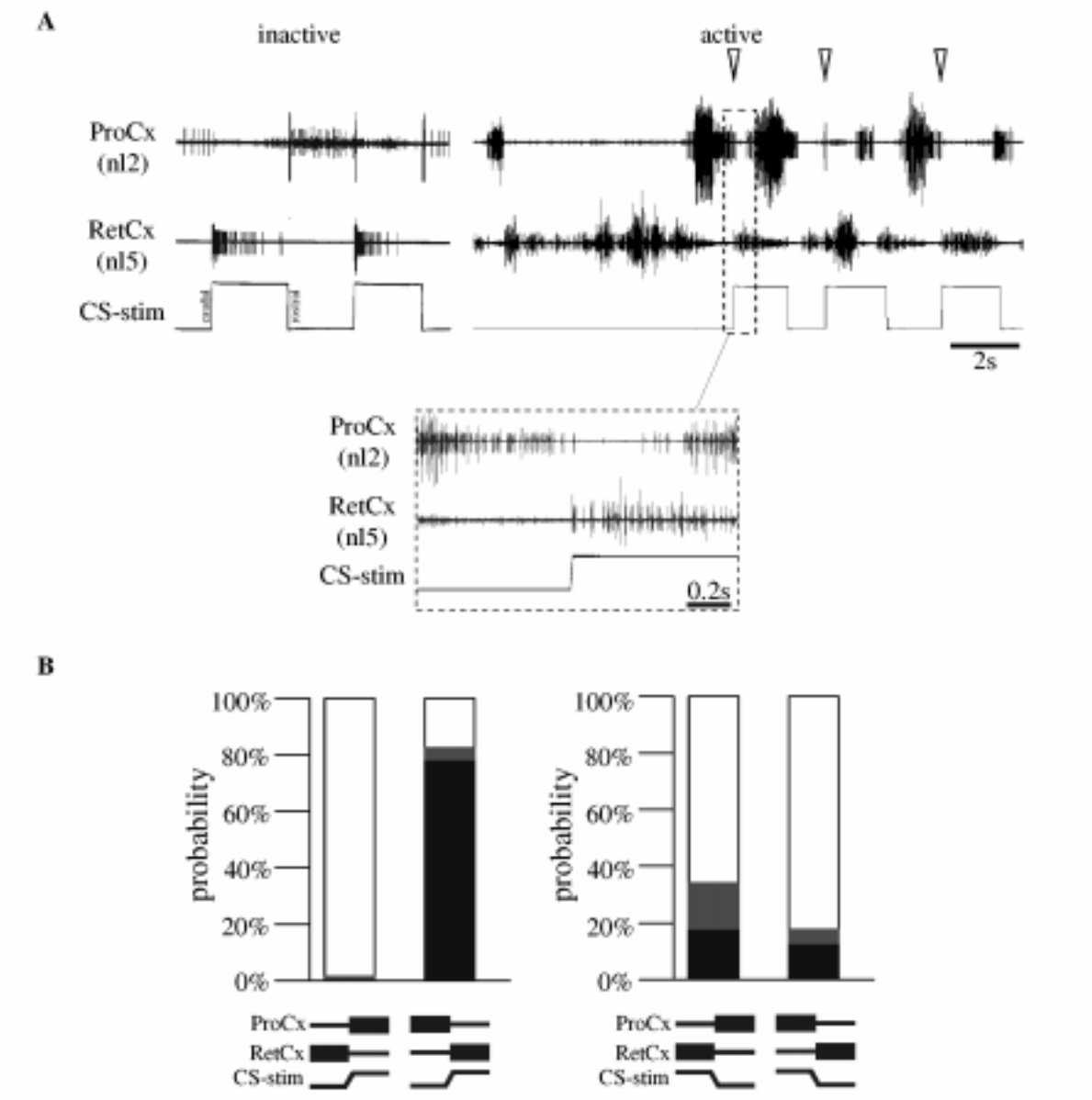


Figure 3.12. A: The stimulation of the CS by caudally bending of the middle leg femur switched the activity from protractor to retractor coxae motoneurons in inactive (*left*) and active (*right*) animals in nine experiments. Protractor (ProCx) and retractor coxae (RetCx) motoneuron activity was extracellularly recorded from lateral nerves, nl2 (*top*) and nl5 (*middle*). *Bottom trace*: CS stimulation by caudally and rostrally bending of the femur. **B:** Probability histograms show that the activity of ProCx is often switched to its antagonist if caudal bending of the femur is done during ongoing ProCx activity (*left*). Black area indicates switching of the activity from one antagonist to the other. Grey area indicates only the termination of the ongoing activity, with no activation of the antagonist. No systematic effect could be classified during rostral bending of the femur (*right*) (for details see text). The black area indicates a switch in the activity to the antagonist, whereas the grey area indicates a termination of activity without an activation of the antagonist. The sketches beneath the diagrams outline the two different cases, i.e. ProCx or RetCx motoneurons are currently active during caudal or rostral bending. Bars in outlines indicate activity.

In active animals the effect of femur bending was the same as in resting animals (Fig. 3.12A *right*). In nine experiments the protractor activity was terminated, and retractor activity started in 78% (n=124) of the total number of caudally directed bendings during ongoing protractor

activity (n=158). In 4% (n=6) of the cases only the termination of protractor, without retractor onset, was observed. In the remaining 18% (n=28) no changes in pro- or retractor activity could be detected. The caudal bending was not observed to cause systematic switching or a terminating effect during retractor motoneuron activity. In 300 rostral bendings (62 during ongoing protractor and 238 during ongoing retractor activity) no systematic effect could be classified. In 13% (n=8) of 62 trials a switch of activity from pro- to retractor was observed, and in 18% (n=43) of 238 trials a switch of activity from re- to protractor was observed. The protractor activity was terminated 3 times (in 5% of all trials), and the retractor was terminated 38 times (in 16% of all trials), without subsequent activation of the antagonist. This data is summarized in figure 3.12B.

In summary, in active animals, stimulation of the CS by the caudal bending of the trochantero-femur during ongoing protractor motoneuron activity causes a termination of the protractor motoneuron activity, which is followed by an initiation of the retractor motoneuron activity. The fact that the switching or terminating of the motoneuron activity in resting animals (where the system is inactive) and in active animals suggests that this influence is mediated via “reflex-like” pathways. To find out whether the CS signals also affect the CRG of the TC-joint, experiments during pilocarpine-induced rhythmic activity were performed in isolated mesothoracic ganglion (only the innervation of the CS was left intact) (see section 3.3.2).

3.3.2 Experiments on pilocarpine-induced rhythmic motor activity

To see whether the strain information caused by bending the femur has access to the central rhythm generating network of the TC-joint, the experiments in 3.3.1 were carried out under pilocarpine-induced rhythmic conditions. In total, n=440 caudal bendings (n=155 during protractor phase and n=285 during retractor phase) and n=450 rostral bendings (n=125 during protractor phase and n=325 during retractor phase) were tried in N=9 experiments.

In seven out of nine experiments, the effect of femur bending was the same in pilocarpine-induced rhythmic preparations as in resting and in active animals, shown above (Fig. 3.13A). In 68% (n=90) of the total number of caudal bendings during protractor activity (n=132), the stimulus caused a switch of activity from pro- to retractor motoneurons. In 14% (n=18) of the trials the protractor activity was terminated without an activation of retractor motoneurons. In

the rest of the caudal bendings (18%, n=24) no change in protractor activity could be observed. If the caudal bendings were applied during the retractor phase (n=207), a switch to the antagonist was only observed in 8% (n=16) of the stimuli, and a termination of the retractor was observed in only 20% (n=41). In 359 rostral bendings (97 during protractor phase and 262 during retractor phase) no systematic effect could be seen. When there was stimulation during the protractor or retractor phase, in 26% (n=25) of 97 trials a switch of activity from pro- to retractor was observed, and in 8% (n=22) of 262 trials a switch of activity from re- to protractor was observed. The protractor activity was terminated 11 (11%) times, and the retractor was terminated 80 (31%) times, without subsequent activation of the antagonist. The rostral bendings of the femur had no systematic effect on the TC-joint motoneuron activity (Fig. 3.13B). Those data are summarized in figure 3.13B.

In order to investigate whether the timing cues for the rhythmic activity of TC-joint motoneurons were affected by CS stimulation, data were inspected in order to evaluate whether the rhythmic motor activity could be reset by CS stimulation. To test this, experiments were selected for closer inspection in which the rhythm showed at least three undisturbed control cycles before the CS stimulation (caudal bending of the trochantero-femur) took place (N=4, n=46). The results of this data evaluation are shown in figure 3.14.

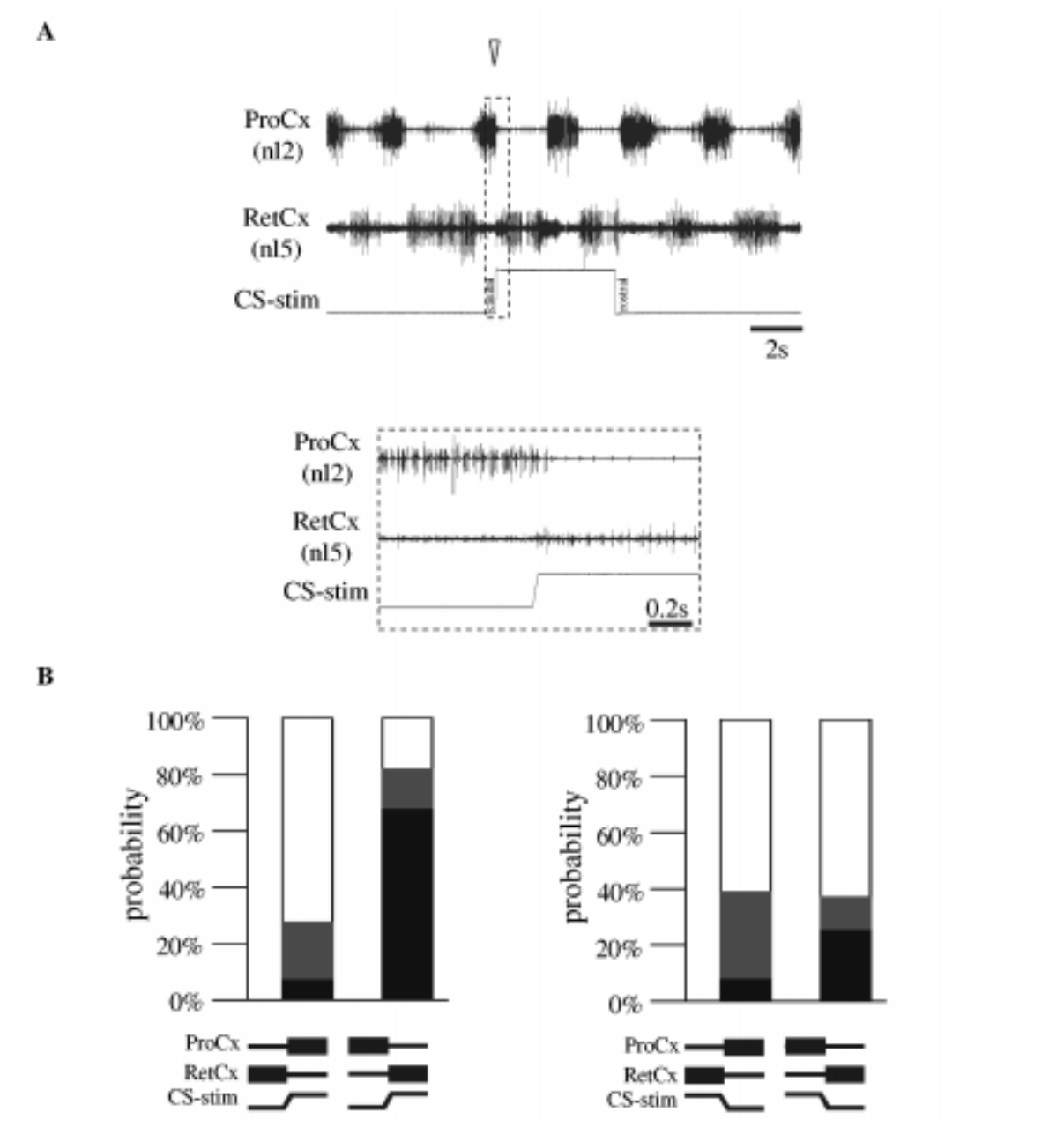


Figure 3.13. A: The stimulation of the CS by the caudal bending of the middle leg femur switched the activity from protractor to retractor coxae motoneurons during pilocarpine-induced rhythmic activity in seven of nine experiments (for the other two experiments see figure 3.17). Protractor (ProCx) and retractor coxae (RetCx) motoneuron activity was extracellularly recorded from lateral nerves nl2 (*top*) and nl5 (*middle*). *Bottom trace*: CS stimulation by the caudal and rostral bending of the femur. **B:** Probability histograms show that the activity of ProCx often switches to the antagonist if caudal bending of the femur occurs during ongoing ProCx activity (*left*). No systematic effect could be classified during rostral bending of the femur (*right*) (for details see text). The black area indicates that activity is switched to the antagonist, whereas the grey area indicates a termination of activity without activation of the antagonist. The sketches beneath the diagrams outline the two different cases, i.e. ProCx or RetCx motoneurons are currently active during caudal or rostral bending. Bars in outlines indicate activity.

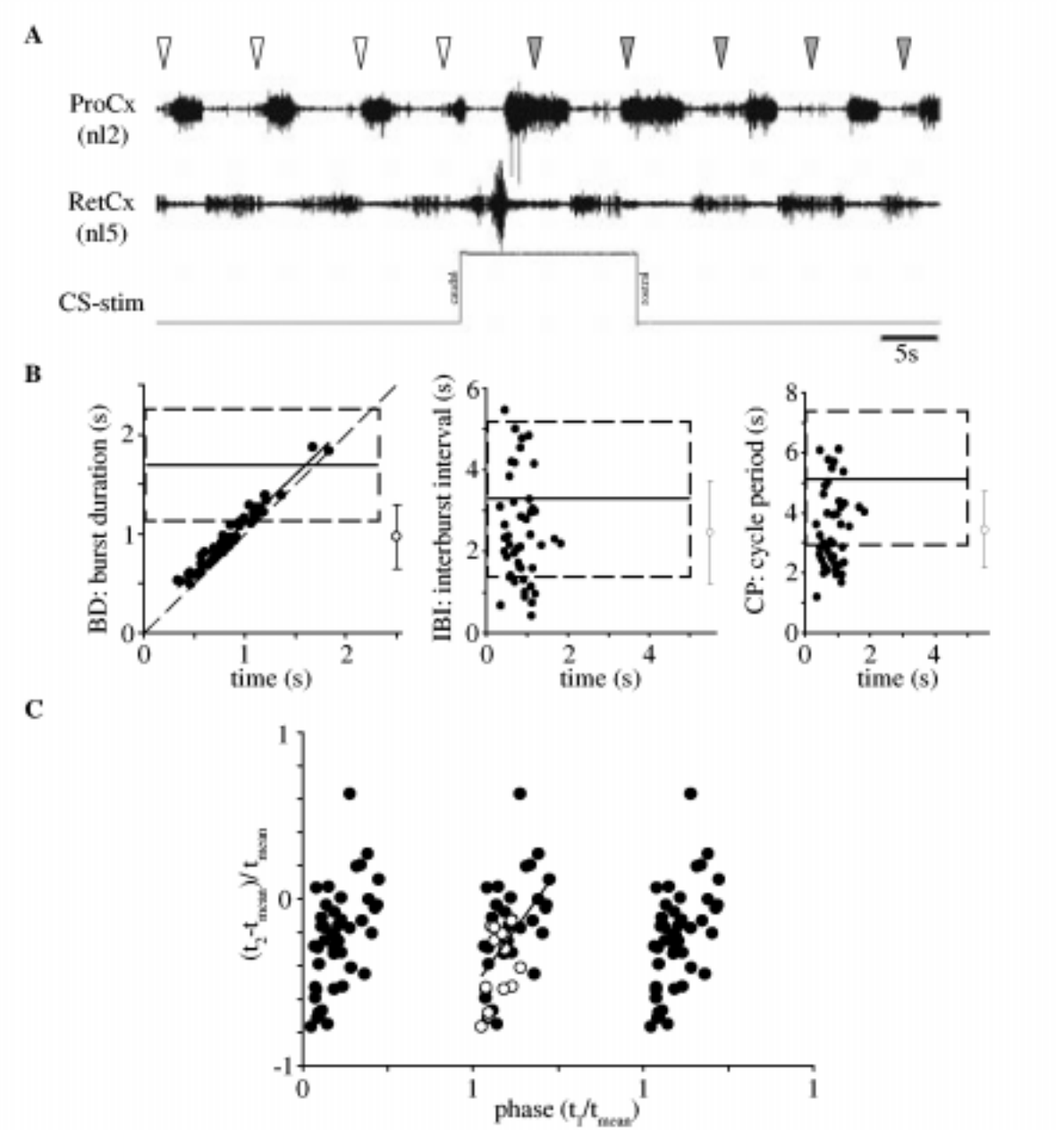


Figure 3.14. The influence of signals from the CS during femur bending on the centrally generated rhythmic activity in the thoraco-coxal joint motoneurons (initiated by bath application of $5x10^{-4}$ M pilocarpine). **A:** Original recording from the lateral nerves nl2 (ProCx, *top*) and nl5 (RetCx, *middle*) during pilocarpine-induced rhythmic activity. The white arrowheads denote the time, and the grey arrowheads the expected time of burst onset in protractor coxae motoneurons. **B:** Plot of the protractor burst duration (BD, *left*), interburst interval (IBI, *middle*), and cycle period (CP, *right*) as a function of stimulus time after burst onset for those trials in which femur levation was observed to exert influence (N=4, n=46). In addition, in the each graph the mean ±S.D. for the parameters is given on the right-hand side (open circles). The dashed horizontal line in each graph indicates the mean value for the control cycles (n=140). The horizontal lines give their standard deviations. The thick solid lines are the regression lines for BD (corr. coeff.: 0.98, $p<0.001$). **C:** Reset plot showing the relation between the stimulus phase and influence of the stimulation on protractor activity (data derive from the same experiments as in B (N=4, n=46). Note that a significant correlation exists between the phase of stimulation and the influence on rhythmicity (corr. coeff.: 0.54, $p<0.001$). The paradigm for the evaluation is the same as in figure 11C.

As seen in figure 3.14B (*left*), the protractor burst duration (BD) was significantly correlated with the latency between the onset of the protractor burst and the onset of the stimulus (corr. coeff.: 0.98, $p<0.001$). In addition, the following interburst interval (IBI) was not correlated with the latency between the onset of the protractor burst and the onset of the stimulus (Fig. 3.14B, *middle*). If the rhythm was reset, there would be an expectation that a significant correlation between the BD and the cycle period (CP) would occur, and that there would be no correlation between the IBI and the latency between the onset of the protractor burst and the onset of the stimulus. However, in these experiments the CP was not correlated with the latency between the onset of the protractor burst and the onset of the stimulus (Fig 3.14B, *right*). There could be two reasons for this: first, stimulation of the CS does not reset the rhythmic activity in TC-joint motoneurons. Second, the variability of the CP is too high, due to the high variability in IBI (see Fig. 3.14B, *middle*); it thus masks a potential correlation between CP and latency between the onset of the protractor burst and the onset of the stimulus. To overcome potential masking effects of variability of CP through IBI, the CP in which the simulations took place were normalized to the mean cycle period from three control cycles preceding this cycle that was influenced by the stimulus (Fig. 3.14C). After such normalization, indeed, a significant correlation between CP and the phase of stimulus was detected (corr. coeff.: 0.54, $p<0.001$), suggesting that CS stimulation resets the rhythmic activity in TC-joint motoneurons.

Random stimulation of trCS (N=3) over the whole cycle of rhythmic activity of the TC-joint motoneurons also shows the phase dependency of the reset (n=22, N=1, Fig. 3.15). From the reset plot presented in figure 3.15B it can be seen that the CP in which the stimulus occur decreased in duration, compared to the undisturbed control cycles, if the CS stimulation was applied approximately within the first half of the cycle (phase: from 0 to 0.5-0.6). CS stimulation within the second half (phase: from 0.5-0.6 to 1.0) increased the CP duration.

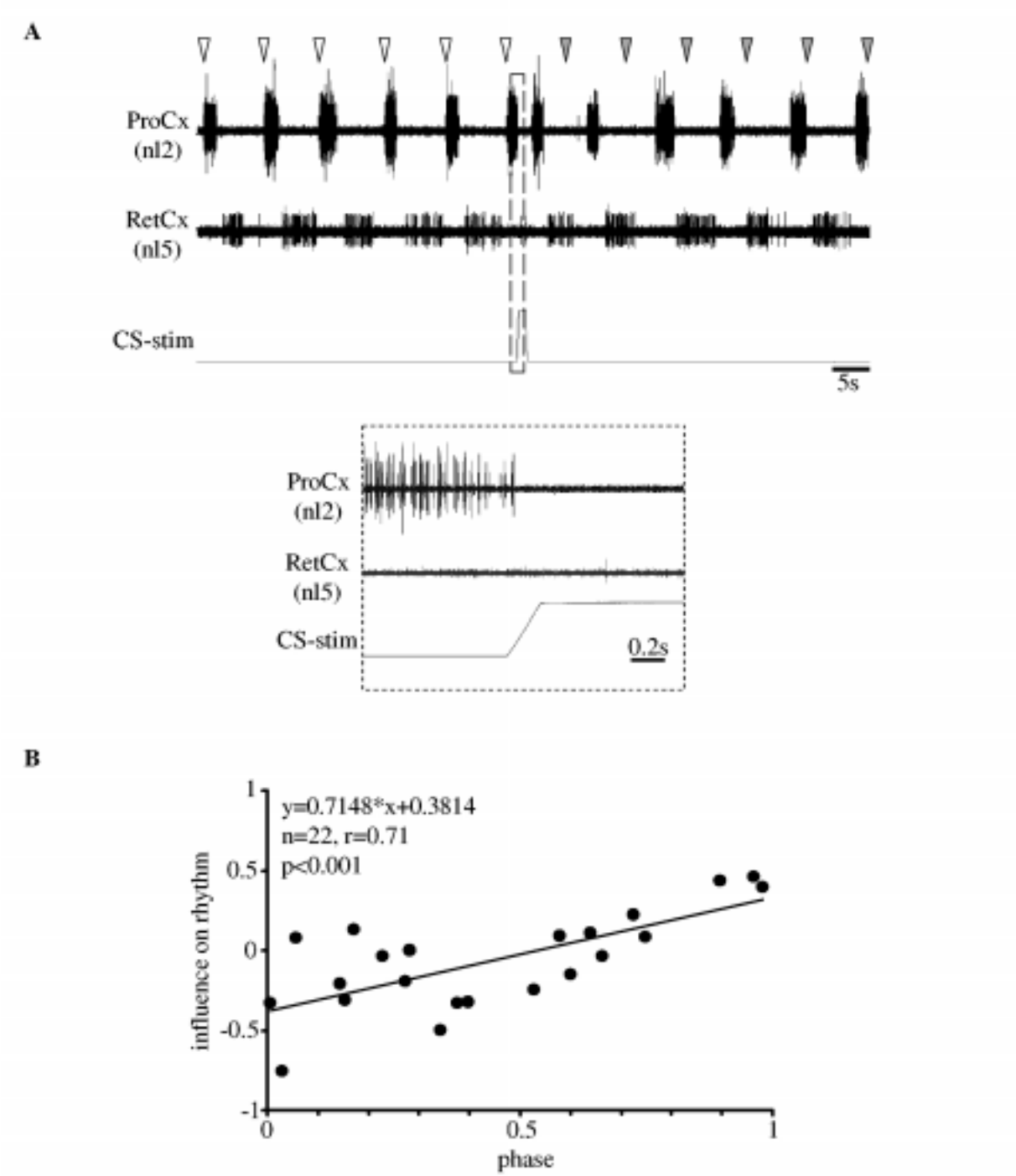


Figure 3.15. Caudal and rostral bending of the femur causes resetting of the rhythmic activity. **A:** Original recording from the lateral nerves nl2 (*top*) and nl5 (*middle*), monitoring the protractor coxae and retractor coxae motoneuron activity during pilocarpine-induced rhythmic activity. Black arrowsheads show the start of each cycle, wheras the grey arrowheads indicate the expected start time of a cycle if the stimulus were not applied. **B:** Phase response curve, including all trials (n=22) from one experiment, shows that the reset of the rhythmic activity is phase-dependent.

In the remaining two of nine experiments, the effect of the stimulus reversed during pilocarpine-induced rhythmic conditions; that is, caudal bending of the trochanterofemur switched the activity from the protractor coxae to the retractor coxae motoneurons (Fig. 3.16A). In these two experiments, there were 101 caudal (n=23 during protractor phase and

n=78 during retractor phase) and 91 rostral (n=28 during protractor phase and n=63 during retractor phase) bendings were applied. In most trials, the activity switched from retractor to protractor coxae motoneurons (32%, n=25); or the retractor coxae motoneurons activity was terminated (46%, n=36) if the CS stimulation (caudal bending of the trochantero-femur) was applied during retractor phase. Caudal bending during the protractor phase only had an effect in 17% (4) of the total number of trials (13%, n=3 switching the activity to retractor motoneuron activity, and 4%, n=1 termination of protractor activity) (Fig. 3.16B). As shown in figure 3.16B, the rostral bending of the femur had a weak but reversed effect: i.e. in 5% of the cases (n=3 out of 63 total number of stimuli) a switch to the antagonist retractor motoneuron activity, and in 5% (n=3 out of 63 total number of stimuli) termination of the protractor activity without subsequent retractor motoneuron activation could be observed if the CS stimulation was applied during retractor motoneuron activity. In 29%(n=8) of the cases (n=28) CS stimulation during protractor motoneuron activity caused a switch to retractor motoneuron activity and in 4% (n=1) it caused a termination of the retractor activity.

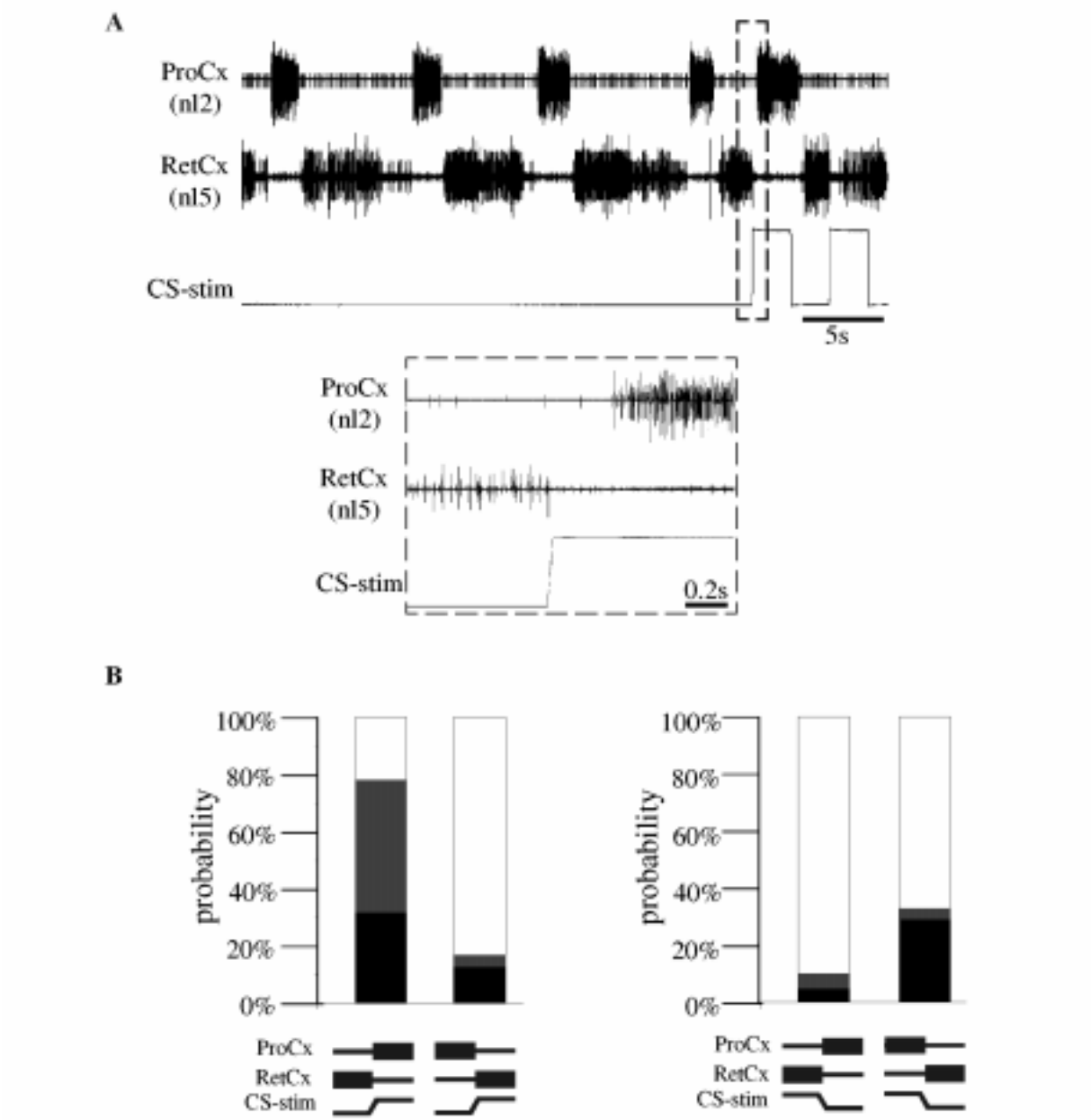


Figure 3.16. A: Stimulation of the CS by caudal bending of the middle leg femur switched the activity from the retractor to the protractor coxae motoneurons during pilocarpine-induced rhythmic activity in two of nine experiments (for the other seven experiments see figure 3.14). Protractor (ProCx) and retractor coxae (RetCx) motoneuron activity were extracellularly recorded from lateral nerves nl2 (*top trace*), nl5 (*middle trace*), and CS stimulation by the caudal and rostral bending of the femur (*bottom trace*). **B:** Probability histograms show that the activity of RetCx often switches to the antagonist if the caudal bending of the femur is applied during ongoing RetCx activity (*left*). Rostral bending often caused a switch from ProCx to RetCx if stimulation was applied during ProCx activity (*right*) (for details see text). The black area indicates the switch of activity to the antagonist, whereas the grey area indicates a termination of activity without activation of the antagonist. The sketches beneath the diagrams outline the two different cases: ProCx or RetCx motoneurons are currently active during caudal or rostral bending. Bars in outlines indicate activity.

Additionally, in these rhythmic preparations, it was found that the rhythmic activity of the TC-joint motoneurons induced by pilocarpine could be coupled to rhythmically applied CS stimulations (Fig. 3.17A). Figure 3.17B shows that the end and onset of the protractor bursts

could be phase coupled to rhythmic CS stimulation (white circles), whereas before and after the rhythmic stimulation no such phase relation existed (black circles). This shows that the CS stimulation clearly has an access to the CRG of the TC-joint.

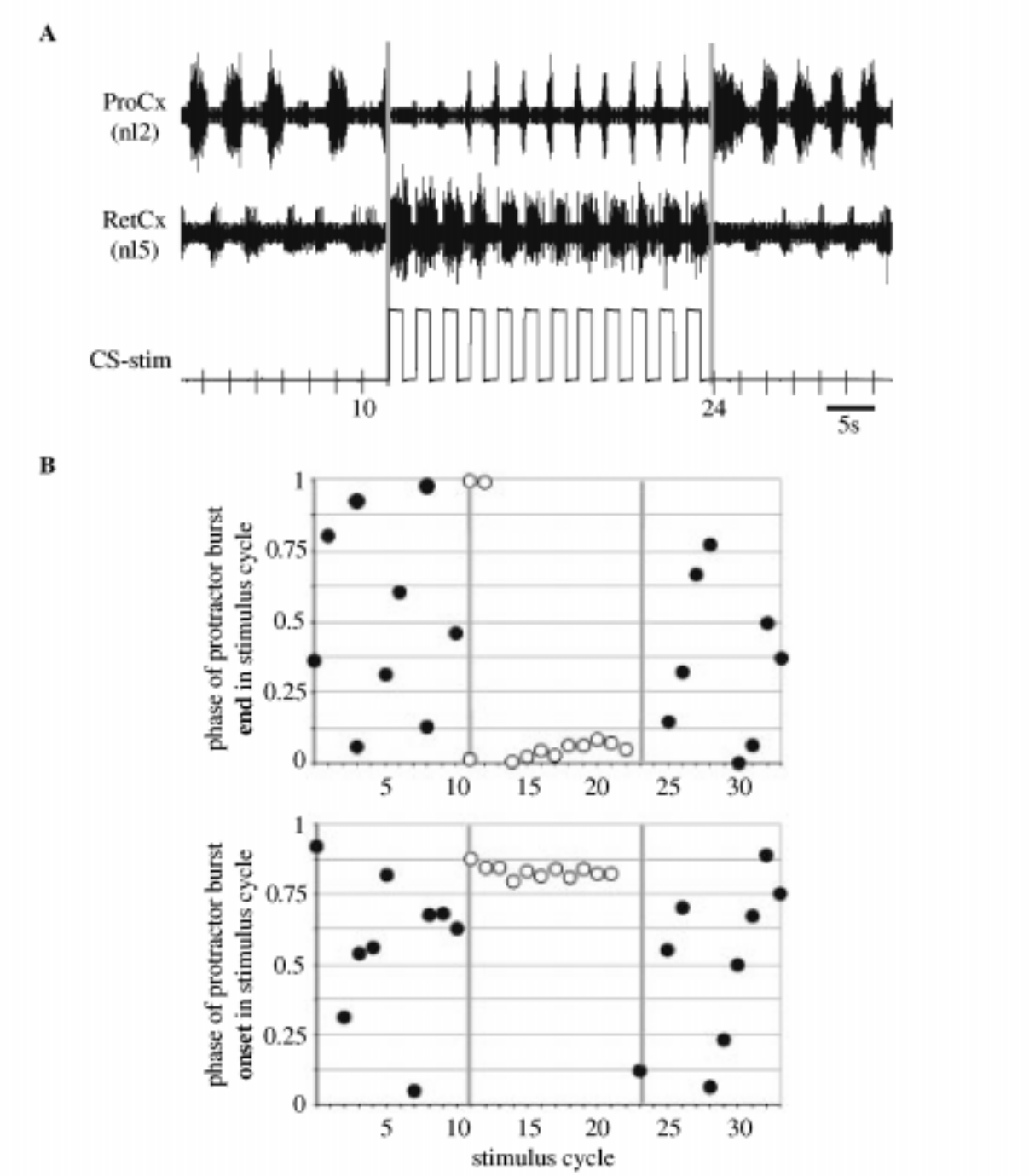


Figure 3.17. The rhythmic activity of the protractor and retractor motoneurons can be coupled to periodic stimulation with a frequency lower or higher than the inherent frequency of the centrally generated rhythmic activity. **A:** Original recording from nl2 (protractor coxae moroneurons, *top*) and nl5 (retractor coxae motoneurons, *middle*). *Bellow trace*: CS stimulation by femur bending. The small vertical lines indicate where those stimuli patterns would have begun, had stimulus been provided before and after the real stimuli. **B:** The onset and the end of bursts remain in a fixed phase relation to the stimulus, resembling entrainment. Vertical grey lines indicate the start and end of rhythmic stimulation as shown with vertical grey lines in A.

Successive ablation of the fCS and the trCS revealed that sensory signals from the trCS mediate this influence. Figure 3.18A shows that the effect of the CS-stimulation on TC-joint motoneuron activity was not affected by ablation of the fCS in three different experiments. In these three experiments additional ablation of all trCS (i.e. all CS where destroyed) abolished the influence of the femoral bending. In a further three experiments, only the trCS were destroyed, and the fCS was left intact. In these experiments, the influence of femoral bending decreased dramatically, but a little effect still remained in two experiments (exp. no. 4 and 6). The reason for this in these two experiments (exp. no. 4 and 6) could be that the destruction of the trCS in these experiments was not successful or that there is a subtle influence from the fCS on the TC-joint motoneurons. From these data it is clear that the strain information from the trCS are mainly responsible for patterning the centrally generated rhythmic activity of the TC-joint motoneurons. However, we can not exclude the possibility that the fCS yield a subtle influence (see Fig. 3.18B).

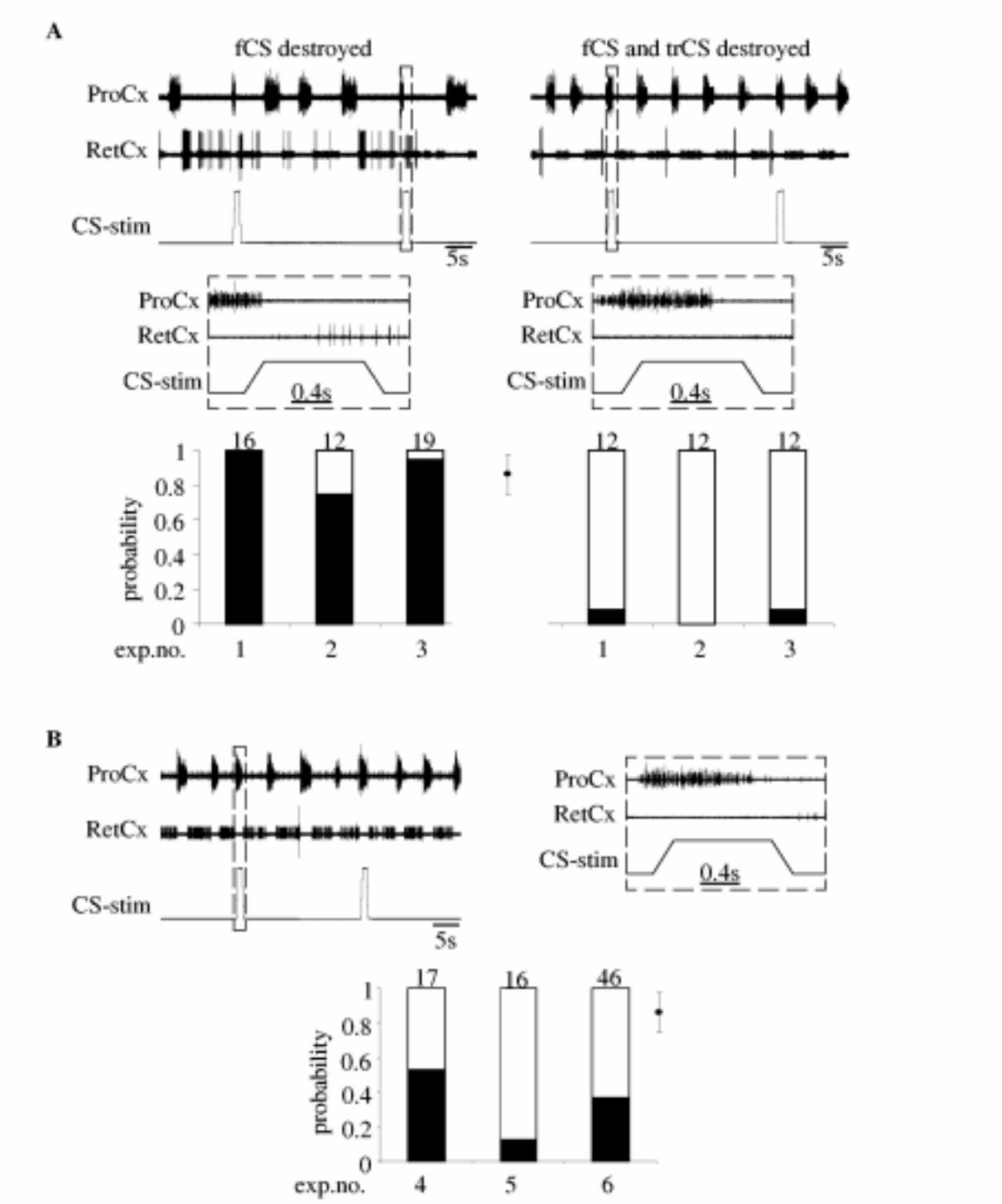


Figure 3.18. A: The influence of the bending of the femur persists after the removal of the fCS (*left*). The affect ended after the additional ablation of the trCS (*right*). **B:** Ablation of the trCS caused a decrease in the probability of the response. In A and B, original recordings from the nl2 (ProCx, *top trace*), nl5 (*middle trace*), and CS stimulation (*bottom trace*) are shown on the top. In A below, and in B beside this, traces of an enlagment of the indicated parts are shown. The probabilities of the CS effect (terminaton of the ProCx or switching from ProCx to RetCx -black area) are presented for each experiment. exp.no.: experiment numbers. The numbers on the top of the bars give the number of trials.

The data in figure 3.14, 3.15, and 3.17 clearly demonstrate that the cuticular strain information from the CS has access to the central rhythm generating network for the TC-joint.

3.4 Role of signals from the femoral campaniform sensilla in controlling activity of extensor tibiae motoneurons

In further experiments I focused on the detailed influence that signals from the fCS exerted on the extensor tibiae motoneurons. In order to investigate their influence on tibial motoneuron activity the fCS were selectively stimulated by applying pressure on the femoral cuticle where they are located. At first, the activity generated by the fCS in response to the application of pressure on the femoral cuticle was recorded (Fig. 3.19A; N=3; *C. morosus*). In order to monitor their activity, an extracellular recording was made from the nervus cruris in the coxa, while it was cut proximal to the electrode and distal to the fCS in the femur. Applying pressure to the femoral cuticle close to the fCS induced barrages of spikes in the sensory neurons of the fCS field (*top trace*). This response ended after the fCS was destroyed (*bottom trace*), verifying that only the fCS afferents were firing in response to cuticular pressure (Fig. 3.19A). These experiments revealed that applying pressure to the femoral cuticle can induce activity in fCS neurons and can serve as a tool to selectively investigate their influence on motoneurons.

Applying pressure to the fCS in the resting, inactive animal always decreased spontaneous activity in SETi, while no influence was detectable in the flexor EMG recording (Fig. 3.19B). In these experiments the femur was deafferented, except for the fCS, and they were also deefferented, except for the flexor muscle. In the active behavioral state, pressure applied to the fCS terminated activity in extensor motoneurons (Fig. 3.19B) and elicited activity in flexor motoneurons, as seen in the flexor EMG recording (arrows in Fig. 3.19B). Intracellular recordings from FETi also revealed the inhibitory synaptic drive caused by fCS stimulation in the inactive and active behavioral state (Fig. 3.19C). This verifies that the inhibitory effect of femur levation on the extensor tibiae motoneurons is caused by the fCS (Gerharz, 1999).

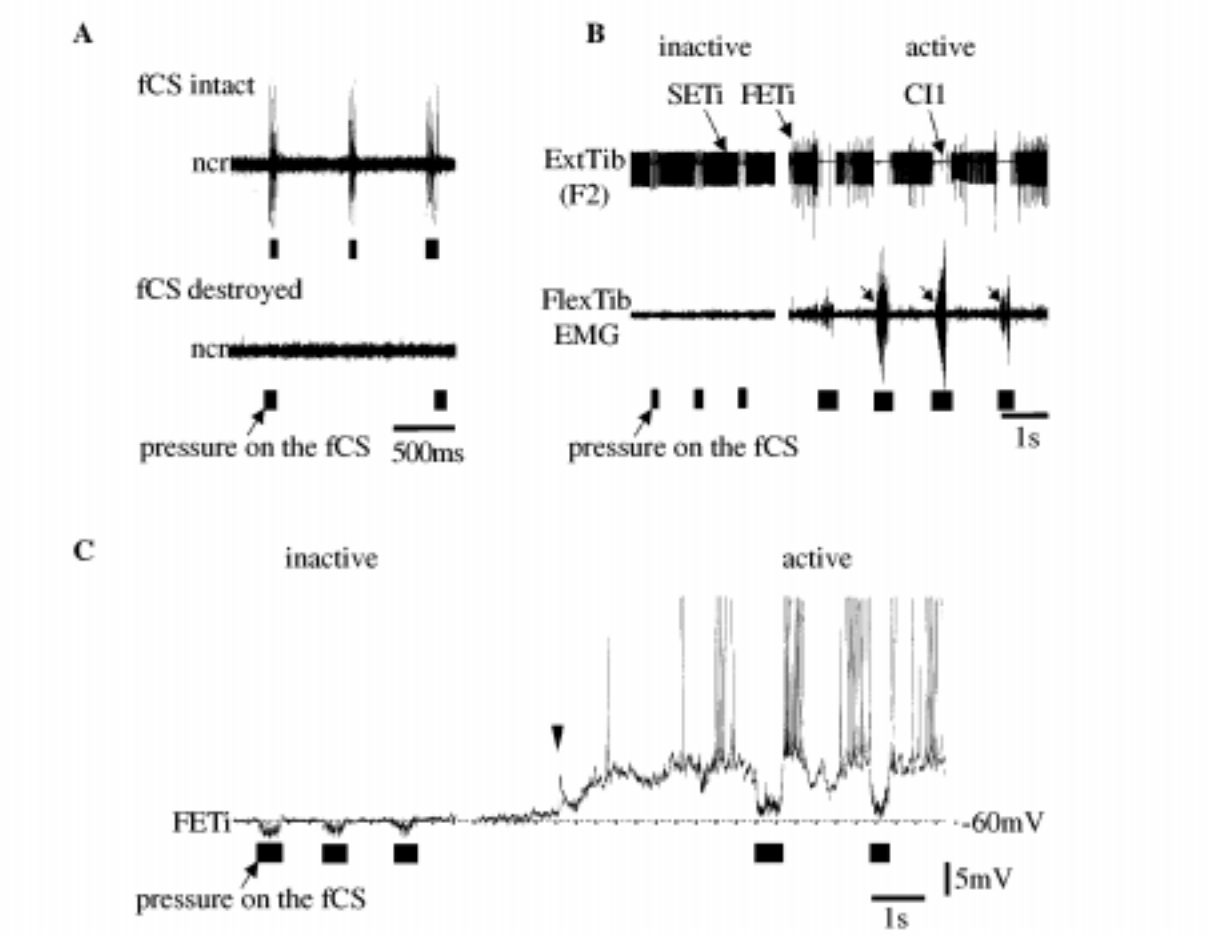


Figure 3.19. Influence of specific stimulatin of the fCS on tibial motoneuron activity in the inactive and active stick insect (*C. morosus*). In these experiments, the femur was completely deafferented (except for fCS) and deefferented (except for the flexor muscles). **A:** Recording from the fCS afferent activity in the nervus cruris (ncr) on the mechanical stimulation of the fCS. Note that application of pressure to the cuticle around the fCS induced sensory activity in ncr (*top*), which was abolished after the fCS was destroyed (*bottom*). **B:** Original recording from the femoral nerve, F2, for extensor motoneuron activity and flexor EMG in the inactive and active animal. Pressure application on the fCS terminates the activity in extensor tibiae motoneurons in resting and active animals. In active animals the termination of activity in extensor tibiae motoneurons is followed by the activation of the flexor tibiae motoneurons, shown by EMG recordings from the flexor muscles (arrows). **C:** Intracellular recording from FETi in the same experimental paradigm as shown in B. The arrowhead indicates the time when the animal was activated by tactile stimulation. Black bars indicate the time when pressure was applied to the fCS.

3.5 Convergence of displacement and load information on the reflex system of the femur-tibia joint

It was shown by Bässler (1988) that, in the forelegs of the *C. impigra*, strain information from the CS shifts the activity toward the flexor tibia motoneuron pool and decreases the probability that “reflex reversals” of the FT-joint reflex system will occur. In this work the CS afferents were activated by electrically stimulating the trochanteral nerve 1 (Tr1), which contains the axons of the trCS (except the posterior trCS) afferents. However, in the middle

leg no effect of the strain information from the trCS could be detected on the FT-joint motoneuron activity (Gerharz, 1999). The only group of CS which influence the FT-joint motoneurons is the one group located proximal on the femur, that is the fCS.

In the following sections the effect of the strain information from the fCS on the reflex pathway of the FT-joint is investigated. The fCS (load information) was stimulated mechanically by applying pressure to the femoral cuticle close to the fCS. This was done manually with tweezers or by means of a piezoelectric element, with the simultaneous stimulation of the fCO (displacement information of the FT-joint). The activity of the extensor tibiae motoneurons was monitored by extracellular recordings from the lateral nerve, nl3, or the femoral nerve, F2. Simultaneous intracellular recordings were made from FT-joint motoneurons or from premotor nonspiking interneurons known to transmit fCO signals onto FT-joint motoneurons (Büschges, 1990; Sauer et al., 1996; Stein and Sauer, 1998). The investigations were performed on *C. morosus* under two different conditions: (i) at rest (postural mode) and (ii) in the "activated" animals (locomotor mode).

3.5.1 Influence of stimulation of the femoral campaniform sensilla on the resistance reflex in the femur-tibia joint

In resting stick insects elongation of the apodeme of the femoral chordotonal organ (fCO), which mimics an flexion (see methods) of the FT-joint, initiates or increases extensor tibiae motoneuron activity; it also decreases or terminates flexor tibiae motoneuron activity. The reverse happens during relaxation of the apodeme of the fCO (mimicking extension of the FT-joint). This is called the resistance reflex, because the system tries to resist the imposed movement (for review see Bässler, 1993a). In this section I will demonstrate the influence of the fCS stimulation on the resistance reflex.

The influence of strain information from the fCS was investigated by stimulating the fCS, i.e. by applying pressure to the proximal femur in resting animals. The pressure on the proximal femur was applied either manually with tweezers or by a piezoelectric element. Interestingly, these two different applications had two different, opposite-directed effects on the extensor tibiae motoneurons in the resting animal. Applying pressure manually with tweezers always decreased the spontaneous activity in SETi (section 3.4), in resting and in active animals. By contrast, in only four of 55 experiments (7%) did application of pressure by means of a

piezoelectric element decreased the extensor activity in resting animals. In most experiments (N=39, 70%) in resting animals, an increase in the extensor activity was observed in extensor tibiae motoneurons (Fig. 3.20A-E). In three experiments, in which the extensor motoneuron activity increased with fCS stimulation, intracellular recordings from flexor tibiae motoneurons revealed that the antagonists also get excitatory input from the fCS stimulation, with the piezoelectric element (Fig. 3.20F). These results suggest that, in resting animals, fCS stimulation by means of a piezoelectric element is not a suitable stimulation because the reaction of animals varies highly from experiment to experiment, and co-contractions of the antagonistic muscles occur. This contrasts with the situation in the active animals, in which both kinds of stimulation induced inactivation of extensor tibiae motoneurons and activation of flexor motoneurons. Therefore, in the following, the stimulation of the fCS with piezoelectric elements will only be considered in active animals. In resting animals, only manually simulation with tweezers will be considered.

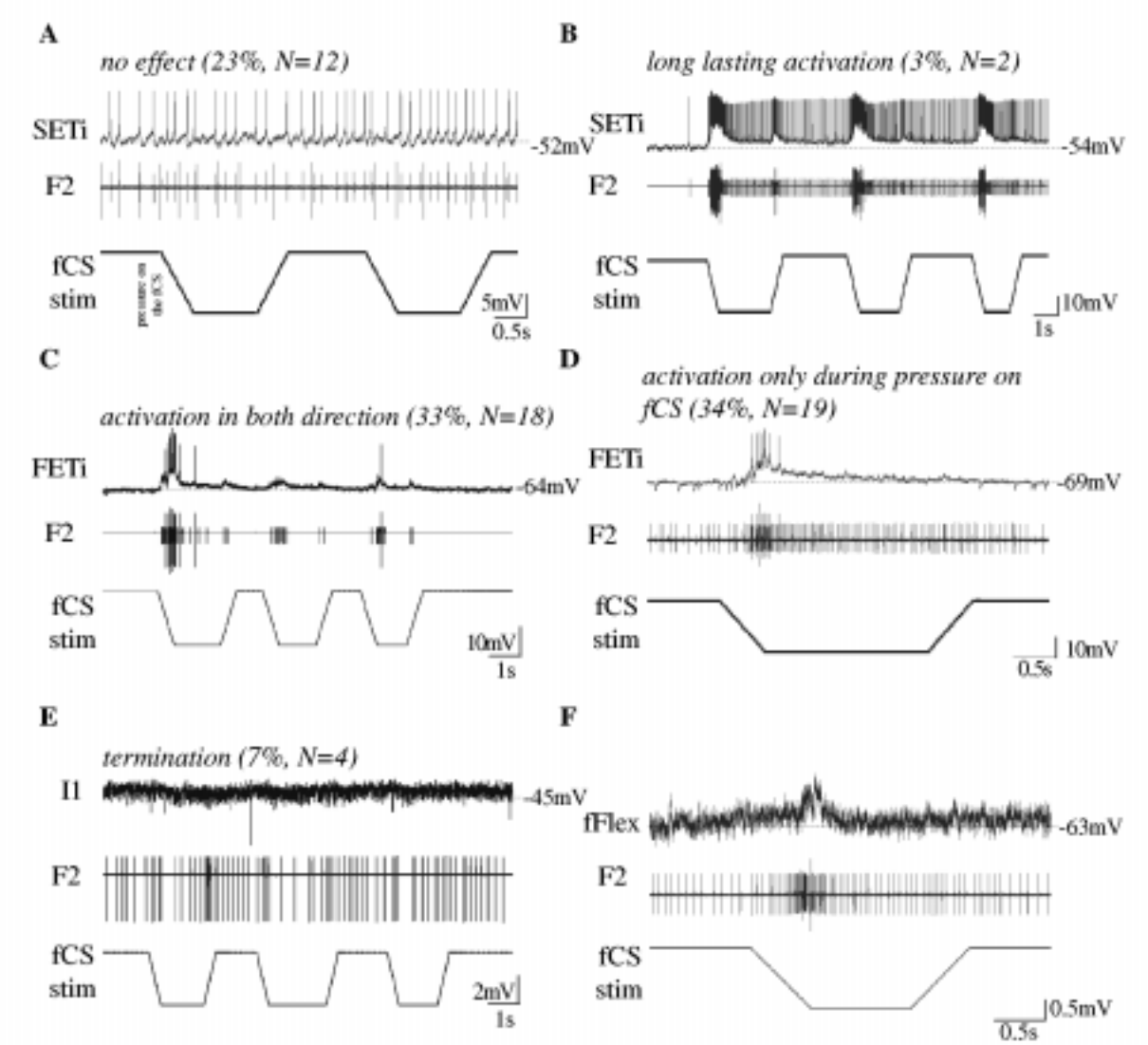


Figure 3.20. Stimulating the fCS by means of the piezoelectric element causes different effects on the tibial motonuerons in resting animals (N=55). **A-E:** All observed effects are classified into five different classes. **F:** In experiments in which the fCS stimulation caused an activation of extensor tibiae motoneurons, the flexor tibiae motoneurons were activated simultaneously, suggesting co-contraction (N=3). In all recordings, *top traces* are intracellular recordings from the slow extensor tibiae motoneuron (SETi), from fast extensor tibiae motoneuron (FETi), from the premotor nonspiking interneuron that has an inhibitory output on the extensor tibiae motoneurons (I1), or from the fast flexor tibiae motoneuron (fFlex). *Middle traces* are the femoral nerve F2. *Bottom traces* show the fCS stimulation (downward ramp means pressure was applied to the fCS).

In resting animals, combining fCS stimulation with tweezers and fCO stimulation showed that an inhibitory influence of fCS signals on the extensor tibiae motoneurons was also manifest as a change in the strength of the intrajoint reflex activation (resistance reflex) of the extensor motoneurons (N=4) (Fig. 3.21A,B). As seen in figures 3.21A and B, the strength of the resistance reflex in the FT-joint decreased if the fCO stimulation was applied simultaneously with fCS stimulation. This indicates that extensor motoneuron activity is determined by signals from both intra- and interjoint sensory signals.

These data suggest that the information from the fCS is not only directly transmitted to the FT-joint motoneurons, but also that the information processing from the fCO to the FT-joint motoneurons on the premotor level is influenced by fCS stimulation (see discussion section 4.3).

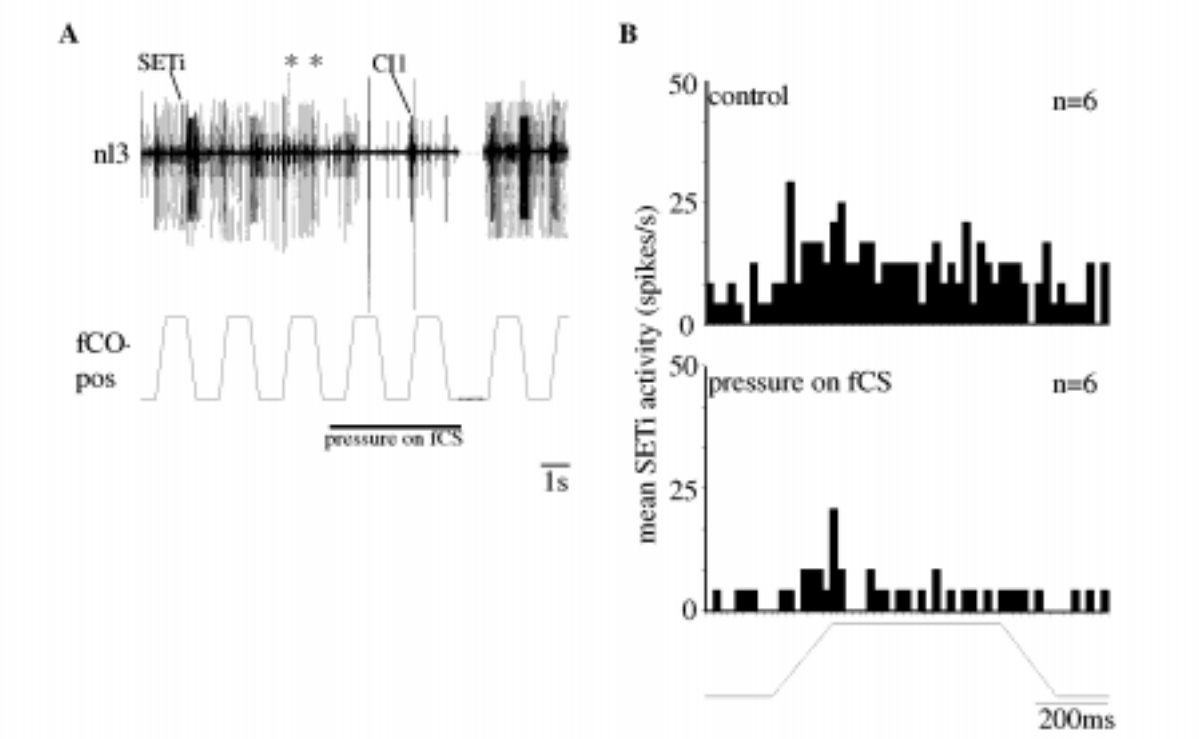


Figure 3.21. A: Decrease of reflex activation in extensor motoneurons upon stimulation of the femoral chordotonal organ (fCO) by applying pressure to the fCS in the inactive animal. Asterisks denote two artifacts during the positioning of the stimulation probe. **B:** PST histogram of the average SETi activity for the experiment shown in A (bin-width: 20ms).

3.5.2 Influence of stimulation of the femoral campaniform sensilla on the "reflex reversal" in the femur-tibia joint

The influence of fCS signals on the action of the FT-joint control network was also tested in active animals. In insects it has been demonstrated that the reflex action of the FT-joint reverses its sign: a resistance reflex (negative feedback) is generated towards fCO signals in resting animals, while an assistance reflex (positive feedback) can be generated in active animals (Bässler, 1976). That is, a resting animal resists any externally applied movement to the FT-joint: for example, an applied flexion movement at the FT-joint activates the extensor and inhibits the flexor tibiae motoneurons. If the animal is activated by tactile stimulus to the body, the same applied movement can release an assistance (reinforcement) by the nervous system; for example, an applied flexion movement at the FT-joint activates the flexor tibiae motoneurons and inhibits the extensor tibiae motoneurons. This reversed response of the

reflex system is followed by a position-dependent activation of extensor motoneurons; i.e. at a given position the activity switches from the flexor back to the extensor tibiae motoneurons and it resists the applied flexion movement at the FT-joint. This two parted motor response has been called “active reaction” (Bässler, 1988).

As shown in section 3.4, stimulation of the fCS caused a termination of the ongoing extensor tibiae motoneuron activity and induced activation of the antagonist flexor tibiae motoneurons. Here, the influence of the fCS on the generation of the “active reactions” by fCO stimulation (Fig. 3.22A) was investigated. This was done to see whether the cuticular strain information from the fCS influences the probability of the “active reactions” or is simply added to the fCO response. Three different aspects of the data were evaluated. First, the response of the extensor motoneurons during the “active reactions” was compared exclusively during fCS stimulation, than exclusively during fCO stimulation, and finally with simultaneous fCS and fCO stimulation (figures 3.22 and 3.23). Second, the timing of the extensor motoneuron activity was analyzed to compare the “active reaction” generated by fCO stimulation, with (i) the motor responses during simultaneous stimulation of the fCO and the fCS and (ii) with the motor activity during fCS stimulation only (figure 3.24A and B). Finally, the probability of occurrence of the “active reaction” was evaluated (figure 3.24C).

In figure 3.22A, three different combinations of fCO and fCS (from left to right; exclusive fCS stimulation, exclusive fCO stimulation and simultaneous fCS and fCO stimulation) are presented while extensor tibiae motoneuron activity is recorded extracellularly from the femoral nerve F2 during active animals. The PST-histograms from these experiments are presented in figure 3.22B.

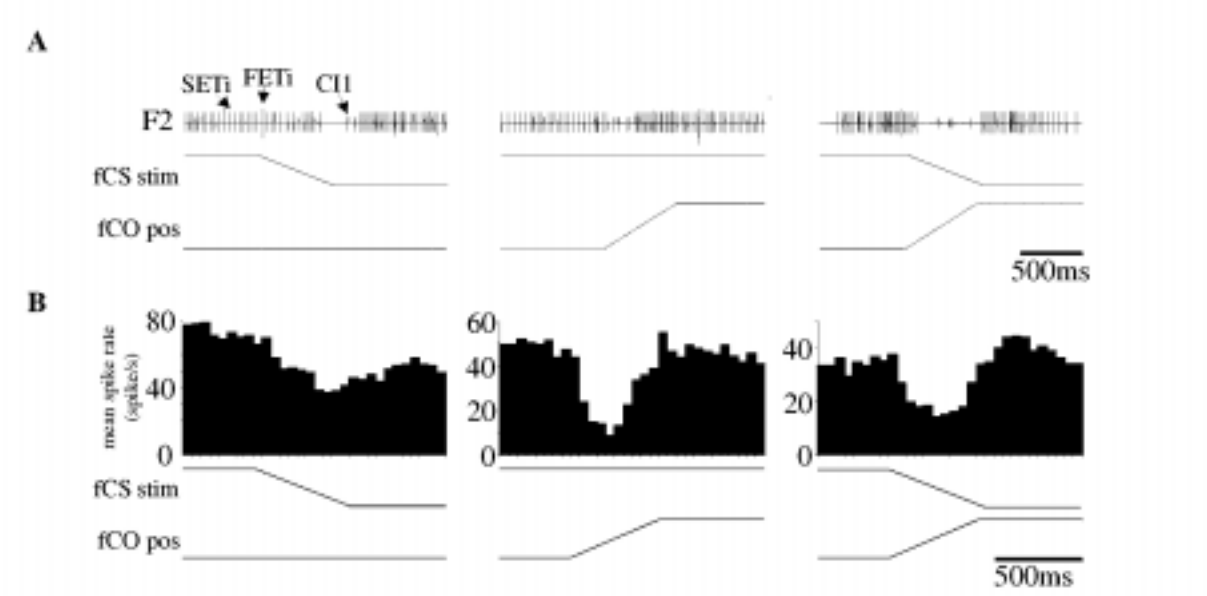


Figure 3.22. A: Original recordings from experiments where three different combinations of fCS (*middle trace*) and fCO (*bottom trace*) stimulation were applied while the activity of the extensor tibiae motoneurons were recorded from the femoral nerve F2 (*top trace*). *Left*: only fCS stimulation, *middle*: only fCO stimulation, and *right*: simultaneous fCS and fCO stimulation. Notice that in both the exclusive fCO stimulation and in fCS and fCO stimulations, ''active reactions'' could be elicited. **B:** Peristimulus time histograms are shown from situations depicted in A of the trials whith an effect from seven experiments are presented (*left*: n=52, *middle*: n=29, and *right*: n=58).

Intracellular recordings from the extensor motoneurons (SETi, N=2 and FETi, N=2) were made during fCO stimulations, fCS stimulations and simultaneous fCO and fCS (fCS+fCO) stimulations to see whether the inhibitions during the fCS+fCO stimulations resemble “active reactions” or whether the inhibition coming from fCS stimulation is simply added to the motor response. This is shown in figure 3.23. As seen in figure 3.23, inhibition of FETi motoneuron during fCS stimulation (i) increased gradually during the stimulus ramp. During fCO and fCS+fCO stimulation the inhibition effects the membrane potential toward -60mV similarly fast, and close to the end of the ramp, the FETi gets depolarized again.

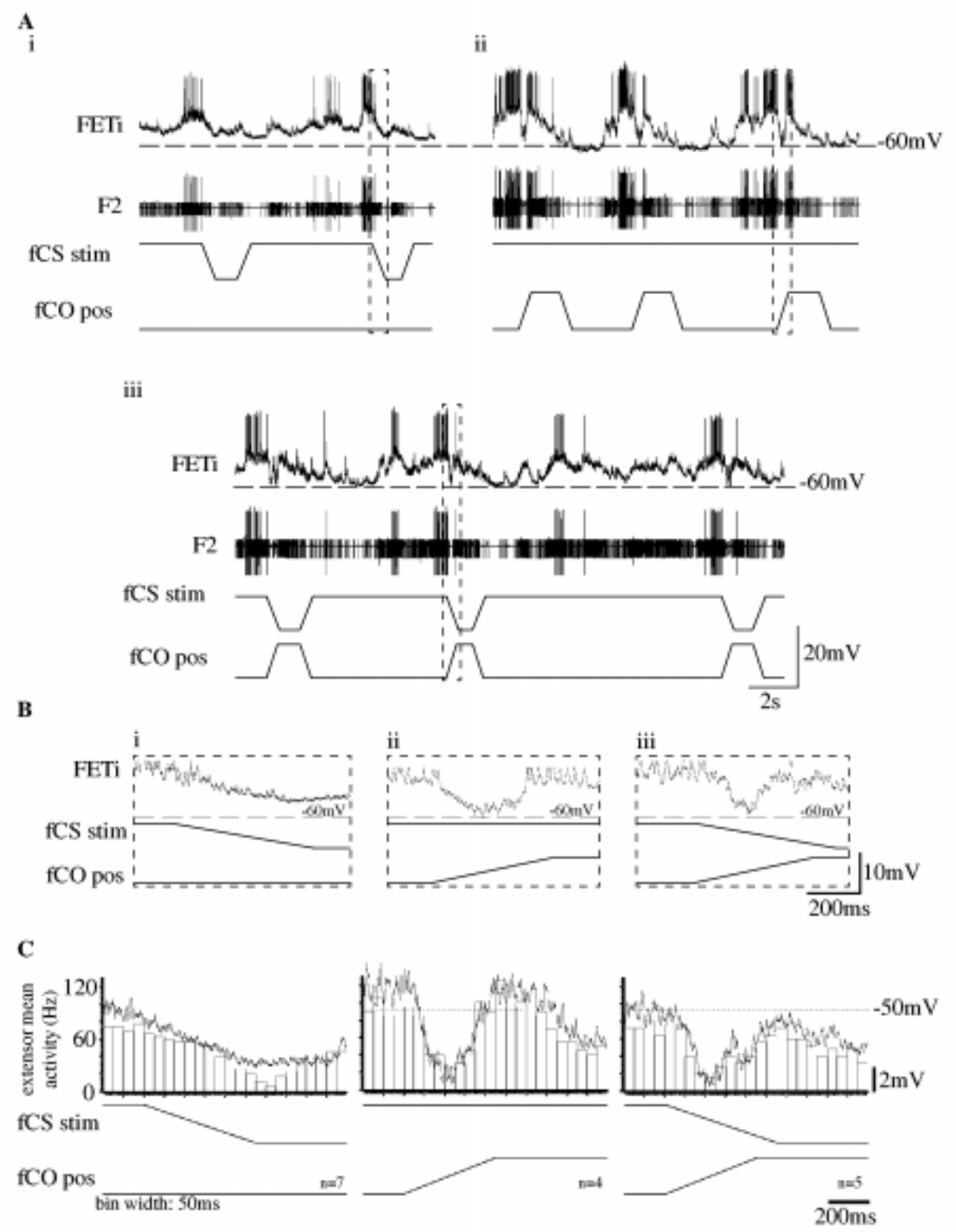


Figure 3.23. A: Intracellular recording from FETi during the stimulation exclusively to the fCS (i), stimulation exclusively to the fCO (ii), and during simultaneous stimulation of both (iii). **B:** Enlarged presentation of trials indicated with rectangles in A. **C:** PST histogram of extensor mean activity and averaged intracellular FETi trace (spikes are excluded by cutting them out from original traces) during fCS stimulation, fCO stimulation, and simultaneous fCO and fCS stimulation. Data derive from the same experiment presented in A.

In figure 3.24 the latencies between stimulus onset and the beginning and the end of the extensor tibiae activity during an “active reaction” are compared between exclusive fCO (n=29) stimulation and the inhibitions during simultaneous fCO and fCS stimulation (n=58) (*top*), and exclusive fCS stimulation (n=48 for offset and n=51 for onset of the extensor tibiae motoneurons) and the simultaneous fCO and fCS stimulation (*bottom*) (N=7). As seen in the *top* two graphics, the times of the termination of the extensor activity during the fCO ramp (0.12s±0.1s -mean±SD) do not change if the fCO stimuli are presented together with the fCS stimuli (0.16s±0.11) (*left*). By contrast, the delay in the onset of the extensor motoneuron activity (0.32s±0.12s) significantly increases ($p<0.001$) if the fCO and fCS are stimulated at the same time (0.45s±0.14s) (*right*). However, if we compare the coincident fCS and fCO stimulation with the exclusive fCS stimulation, both the off- and the onset of the extensor activities are significantly different. That is, the delay in the offset of the extensor activity significantly decreases (fCS: 0.38s±0.19s, fCS+fCO: 0.16s±0.11s, $p<0.001$), and the delay of onset of the extensor activity increases (fCS: 0.54s±0.19s, fCS+fCO: 0.45s±0.14s, $p<0.01$) if the fCS stimulation is coincident with fCO stimulation.

Figure 3.24C *left* show that the total probability the “active reactions” during fCO stimulation increased from 34% (n=92) to 68% (n=85, N=7) when both fCO and fCS were stimulated. The graph on the *right* in figure 3.24C presents the means and the standard deviations of the probability of “active reactions” calculated from each experiment (N=7; $6<n<25$ for only fCO and $7<n<20$ for combined fCO and fCS stimulation), which show that the increase in probability is statistically significant ($p<0.05$).

In summary, the data presented in figure 3.23 and 3.24 indicate that the observed extensor motor activity for simultaneous fCO and fCS stimulation highly resembles the “active reaction” and is similar to the “active reaction” observed when only the fCO is stimulated. The only detectable difference from the control “active reaction” was that part two of the “active reaction” (position dependent onset of the extensor motoneuron activity) significantly shifts to a more flexed position ($p<0.001$) if the fCS are stimulated at the same time as the fCO (Fig. 3.24B, top right graph).

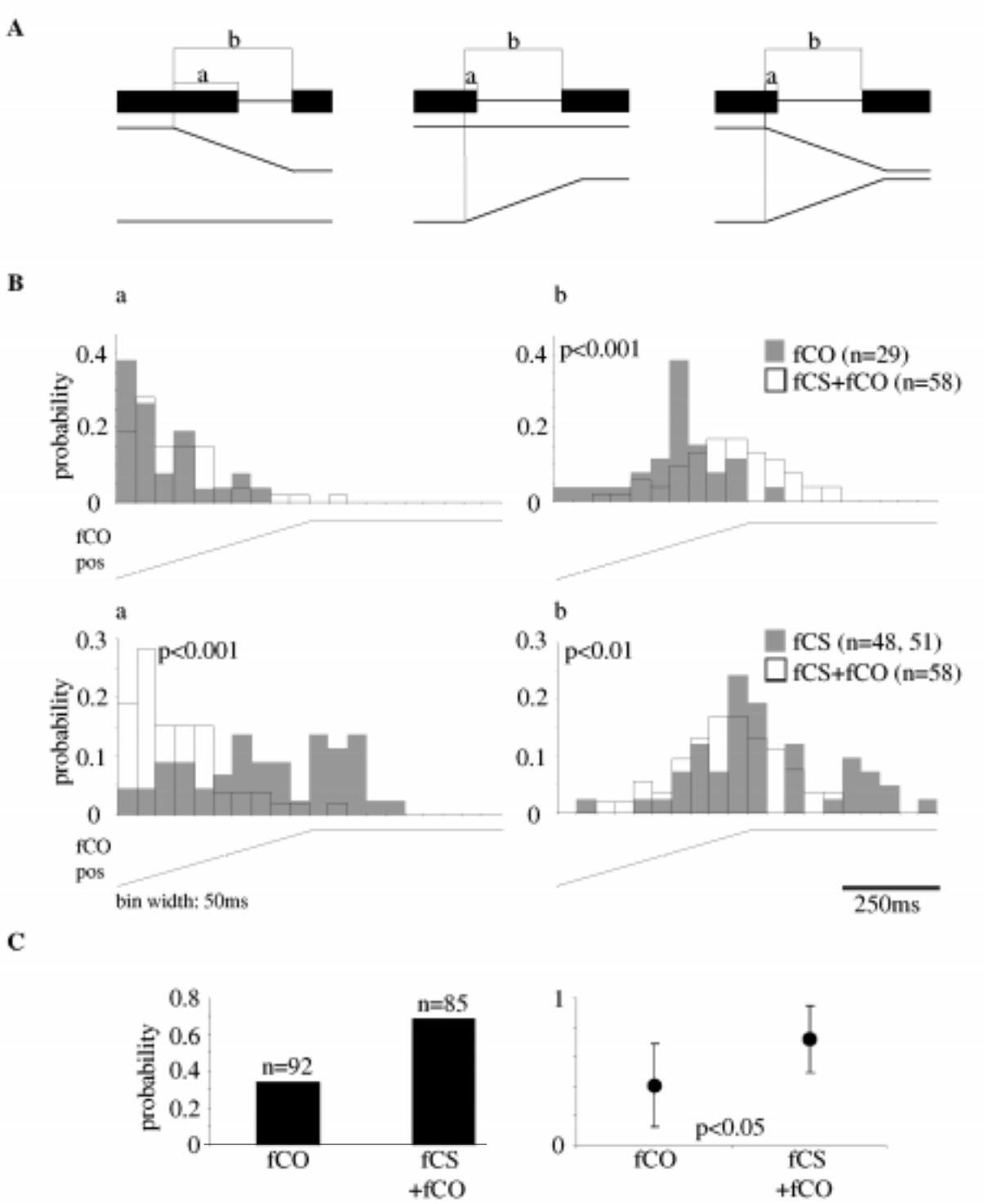


Figure 3.24. Comparison of the end (a) and the start (b) of extensor tibiae motoneuron activity. **A:** A drawing showing evaluation parameters. **B:** Histograms comparing the probabilities of delays of the off- and onset of the extensor tibiae motoneuron activity in fCO versus simultaneous fCS and fCO stimulation (fCS+fCO) (top two histograms), and fCS versus fCS+fCO (bottom two histograms). For details see text. **C:** 32% of the stimuli elongation signals from the fCO in tactile activated animals evoked a reflex reversal in FT-joint MNs. Elongantion signals, when combined with strain signals from fCS, increased the probability of the reflex reversals to 68%. This is shown as a bar diagram on the *left*. On the *right*, the mean and standard deviations of the probability of the "active reactions" were calculated from each experiment, showing that the increase is statistically significant ($p<0.05$) (only fCO: $6<n<25$, fCS and fCO: $7<n<20$).

3.5.3 Influence of fCS stimulation on the premotor nonspiking interneurons involved in reflex system of the FT-joint

3.5.3.1 Influence of fCS stimulation on the premotor nonspiking interneurons in resting animals

Intracellular recordings from the identified premotor nonspiking interneurons (NSIN) that are known to transmit sensory information from the fCO to tibial motoneurons (Büschges, 1990; Sauer et al., 1996; Stein and Sauer, 1998) revealed that fCS signals converge in the femur-tibia loop in resting animals in a systematic way. The NSINs providing excitatory synaptic drive onto extensor tibiae motoneurons are hyperpolarized during fCS stimulation (N=10). For excitatory NSINs, this result is exemplified by recordings from the NSINs E2, E3, and E7 in figure 3.25A-C. By contrast, the NSIN that provide inhibitory synaptic drive to extensor tibiae motoneurons do not get any systematic input from the fCS (N=6); this is exemplified by the recording from the NSIN I4 in figure 3.25D. The total number of recordings of the nonspiking interneurons in resting animals are summarized in Table 1.

Table 1 Summary of all recorded premotor nonspiking interneurons in resting animals.

Premotor nonspiking interneurons	Number of recordings	Observed reaction to the fCS stimulation	
		Hyperpolarization	Depolarization
E1	0	-	-
E2	4	4	0
E3	1	1	0
E4	3	3	0
E5/6	0	-	-
E7	1	1	0
E8	1	1	0
I1	4	0	0
I2	1	0	0
I4	1	0	0

When the fCO was stimulated while pressure on the fCS was applied, the reaction of these NSINs in response to fCO stimulation was altered, compared to fCO stimulation without fCS stimulation. This is shown in experiments with three excitatory NSINs: E2, E3, and E7 (Fig.

3.25A-C). The amplitude of the response to the fCO stimulation of E2 and E7 decreased and the sign remained unchanged. By contrast, during the elongation of the fCO-apodeme (flexion of the FT-joint), the response to fCO stimulation in E3 was reversed. Without fCS stimulation, E3 was depolarized by fCO elongation (flexion of the FT-joint), and it hyperpolarized during relaxation (extension of the FT-joint). By contrast, fCO elongation during continuous fCS stimulation hyperpolarized the E3, and the influence of relaxation remained the same as in fCO stimulation alone, i.e. hyperpolarization.

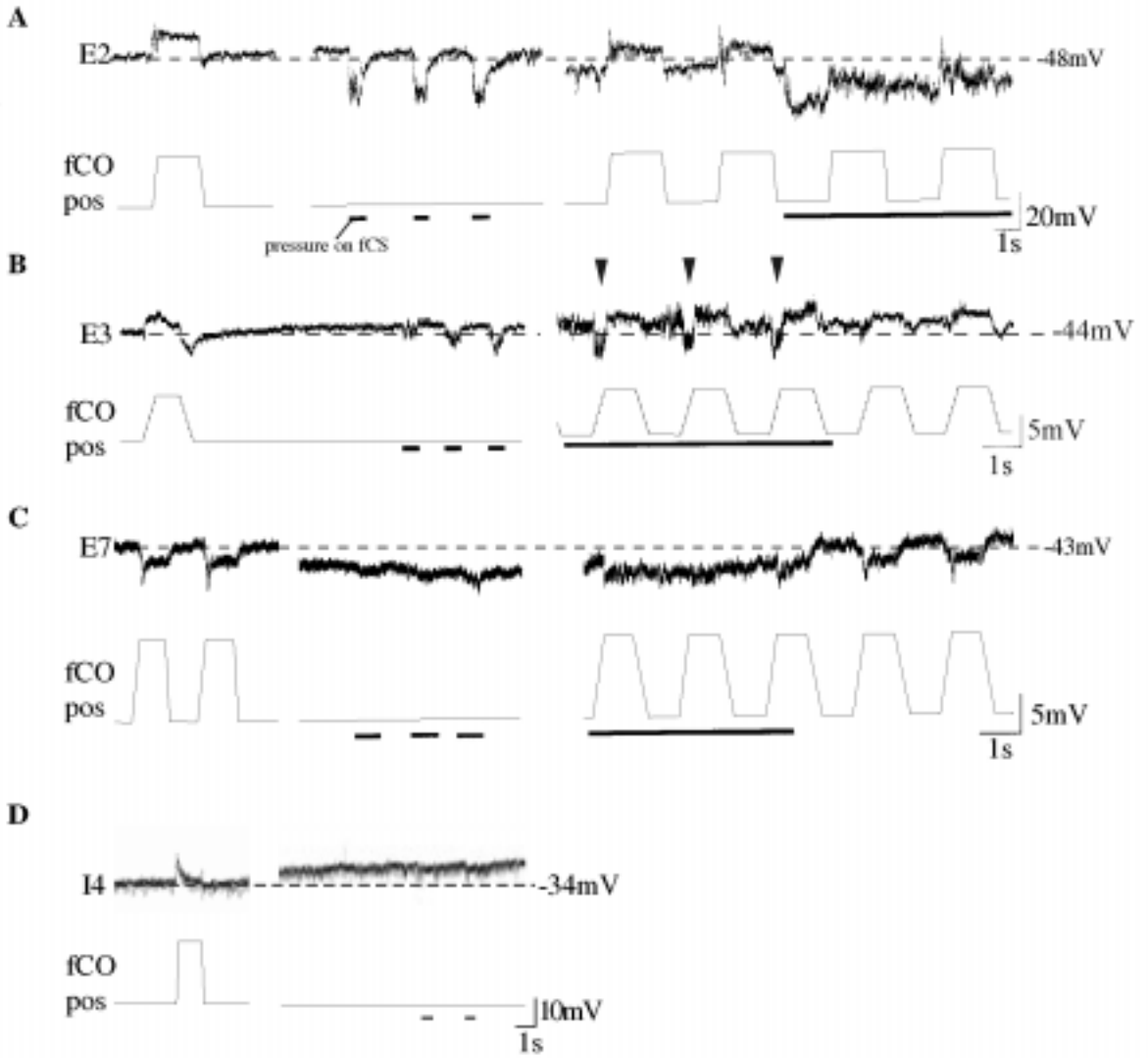


Figure 3.25. Premotor nonspiking interneurons providing excitatory drive onto extensor tibiae motoneurons get inhibitory inputs from the fCS stimulation (**A:** E2, **B:** E3, **C:** E7). If the fCO is stimulated during fCS stimulation, E2 and E7 only decrease the amplitude of the response of the fCO stimulation, whereas E3 reverses its sign for fCO elongation (see arrowheads). **D:** Inhibitory nonspiking interneurons (examplified with I4, *fourth recording*) show no systematic postsynaptic reaction to the fCS stimulation.

3.5.3.2 Influence of fCS stimulation on the premotor nonspiking interneurons in active animals

Additionally, to get more detailed information of the information processing of the fCS signals on the tibial motoneurons, intracellular recordings were performed on the premotor nonspiking interneurons of the FT-joint control network in active animals. Figure 3.26 shows the reaction of the nonspiking interneurons of the fCS stimulation in active animals. As seen in figure 3.26.A, all recorded nonspiking interneurons with excitatory influence on the extensor tibiae motoneurons (E2-E8, -E1 were never recorded in this project) were inhibited by the fCS stimulation. Only E7 was found to be depolarized during the fCS stimulation. The nonspiking interneurons with inhibitory influence on the extensor tibiae motoneurons (I1, I2, and I4) were either depolarized (I1) hyperpolarized (I4), or hyper- and depolarized (I2) (Fig. 3.26.B). The total number of recordings of the nonspiking interneurons in active animals is summarized in Table 2.

Table 2 Summary of all recorded premotor nonspiking interneurons in active animals. Only interneuron E1 was never recorded. (*: I2 was first hyperpolarized and then received additionally depolarizing input during each stimulation).

Premotor nonspiking interneurons	Number of recordings	Observed reaction to the fCS stimulation	
		Hyperpolarization	Depolarization
E1	0	-	-
E2	1	1	0
E3	1	1	0
E4	5	5	0
E5/6	2	2	0
E7	1	0	1
E8	3	2	1
I1	4	0	4
I2	1	1*	1*
I4	1	1	0

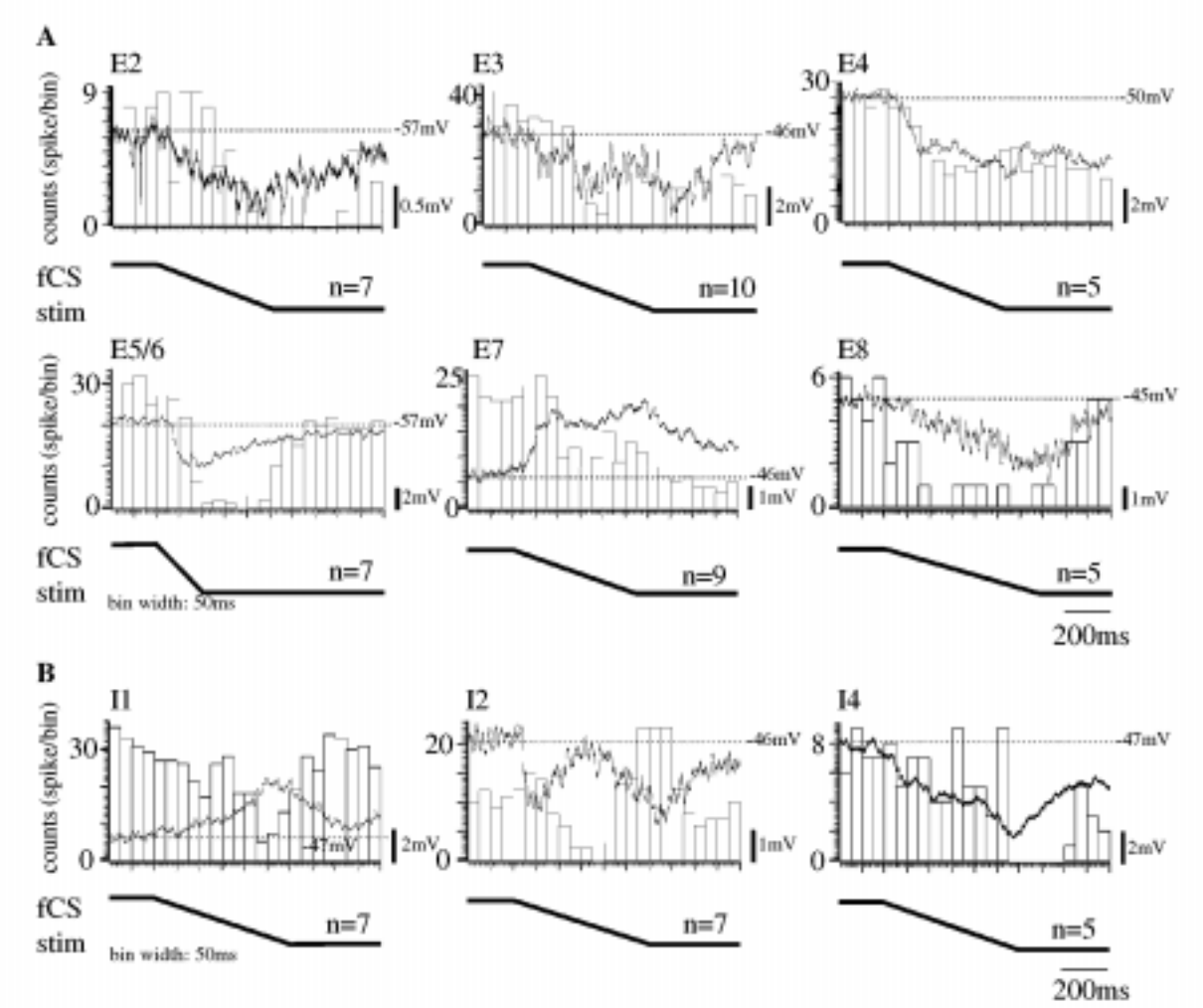


Figure 3.26. Intracellular recordings from already known premotor nonspiking interneurons (except E1) which transmit information from the fCO to the extensor tibiae motoneurons and have an excitatory (A) or inhibitory influence on the extensor tibiae motoneurons. Averaged intracellular traces are superimposed with peristimulus time histograms of extensor motoneuron activity during fCS stimulation in active animals. **A:** All excitatory nonspiking interneurons (except the E7) receive inhibition, and only the interneuron E7 gets excitation during fCS stimulation. **B:** Each inhibitory nonspiking interneuron has its own characteristic response. The I1 receives inhibition, I2 receives inhibition with deleyed excitation, and I4 receives only inhibition during fCS stimulation. n: number of trials included in the histograms and average traces.

3.5.3.3 New identified premotor nonspiking interneurons in the femur-tibia joint reflex loop

During this project, five new types of premotor nonspiking interneurons were recorded and labeled, which were physiologically and morphologically clearly different from the previously identified interneurons (Büschges, 1990; Sauer et al., 1996; Stein and Sauer, 1998). Two of these interneurons decreased the activity of extensor motoneurons (I3 and I8) when depolarized by current injection, and two of them increased the spiking activity of extensor motoneurons (E9 and E10) upon depolarization. These interneurons were named according to

the naming scheme by Büschges (1990). One other nonspiking interneuron identified here was named an IE1 (inhibitory and excitatory interneuron 1), because this neuron has antagonistic influence on the slow and fast extensor tibiae motoneurons: that is, when it was depolarized, this interneuron decreased the activity of the SETi and initiated activity in the FETi and the common inhibitor 1 (CI1)

3.5.3.3.1 Nonspiking interneuron I3

The gross morphology of the nonspiking interneuron I3 is shown in figure 3.27A. As mentioned above, interneuron I3 decreased the activity of the SETi when depolarized (Fig. 3.27B). When the fCO was stimulated, this neuron was depolarized during elongation (flexion of the FT-joint), and a slight position influence of membrane potential was observed (Fig. 3.27C, *top*). During active reactions, this interneuron stopped responding to the fCO stimulation (Fig. 3.27B, *bottom*). Finally, I3 was hyperpolarized by fCS stimulation (Fig. 3.27C). This interneuron was only recorded and labeled once.

3.5.3.3.2 Nonspiking interneuron I8

Figure 3.28A shows the gross morphology of the nonspiking interneuron I8. Depolarizing current injection into interneuron I8 decreased the activity of the SETi (Fig. 3.28B). Both elongation and relaxation of the fCO in resting animals caused hyperpolarization of the motoneuron (Fig. 3.28C). This response to fCO stimulation resembles the response of the excitatory nonspiking interneuron, E8 (Stein and Sauer, 1998). I8 was depolarized by fCS stimulation (Fig. 3.28C). This interneuron was recorded and labeled three times.

3.5.3.3.3 Nonspiking interneuron E9

The gross arborization of nonspiking interneuron I3 are shown in figure 3.29A. Injection of a depolarizing current into E9 caused an increase in the activity SETi (Fig. 3.29B). The elongation of the fCO (flexion of the FT-joint) caused depolarization; and relaxation of the fCO (extension of the FT-joint) caused hyperpolarization with a clear position component (Fig. 3.29C, *top*). During the “active reaction,” this interneuron was depolarized, but the position component was no longer detectable (Fig. 3.29B, *bottom*). Finally, I3 was

hyperpolarized by fCS stimulation (Fig, 3.29C). This interneuron was recorded and labeled three times.

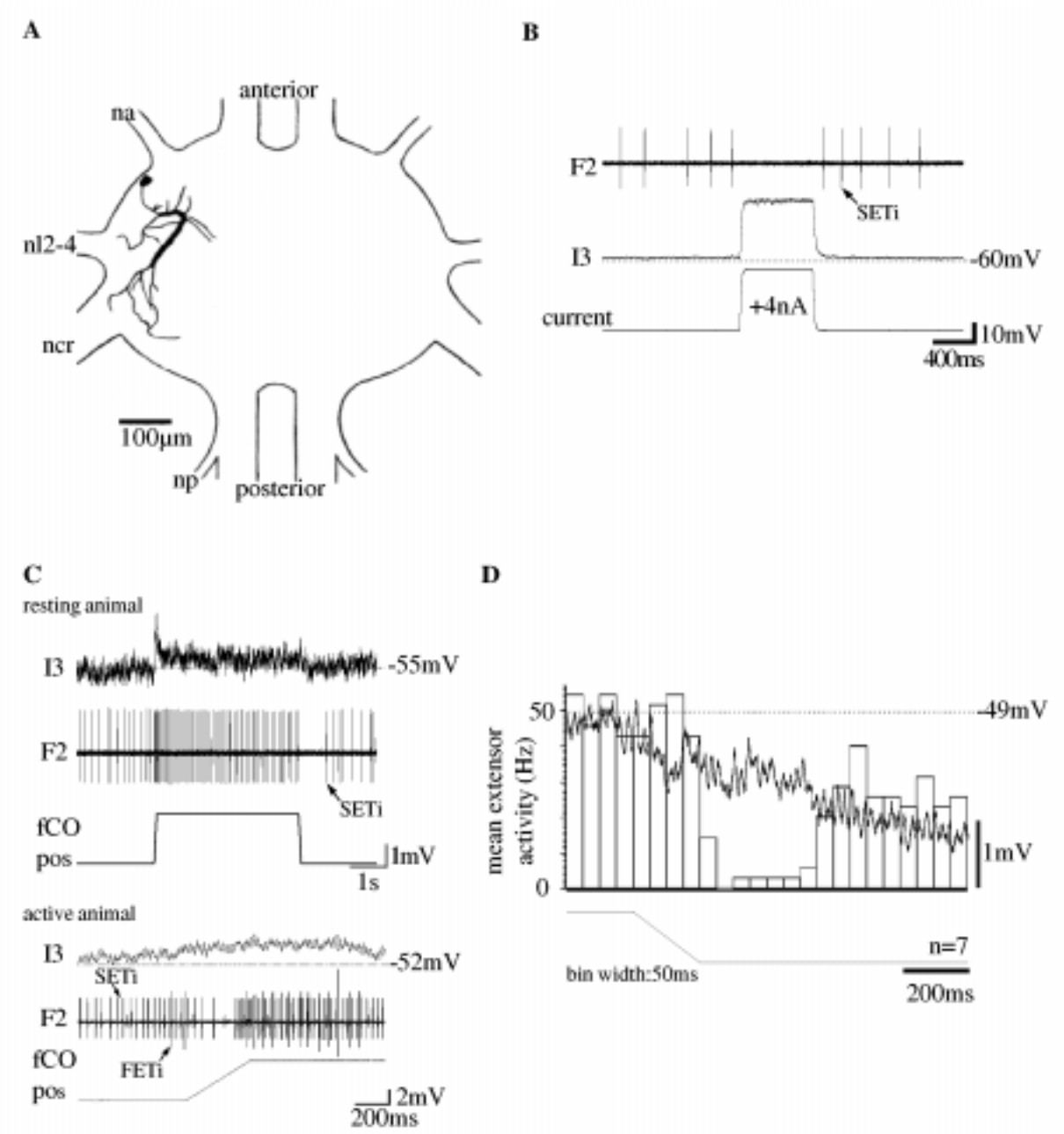


Figure 3.27. Morphological and physiological properties of the premotor nonspiking interneurons of type I3. **A:** Morphology of the interneuron I3 (na: nervus anterior, nl2-4.: nervus lateralis 2-4, ncr: nervus cruris, np: nervus posterior). **B:** Injection of depolarizing current (*bottom trace*) into the interneuron I3 (*middle trace*) terminates the ongoing spontaneous spike activity of the extensor motoneurons (recorded from the femoral nerve F2 -*top trace*). **C:** Response of the interneuron I3 (*top trace*) to ramp and hold stimuli at the fCO (*bottom trace*) in resting (*top*) and active (*bottom*) stick insects. Notice that in resting animals this interneuron receives depolarization during elongation (flexion of the FT-joint), with little position effect. The response of fCO stimulation ends in active animals during "active reaction." **D:** Response of the interneuron I3 (*intracellular recording trace*) to fCS (*bottom trace*) stimulation during active animal. The responses of the interneuron I3 were averaged during seven trials. The stimulus histogram shows the mean extensor activity during fCS stimulation (bin width: 50ms).

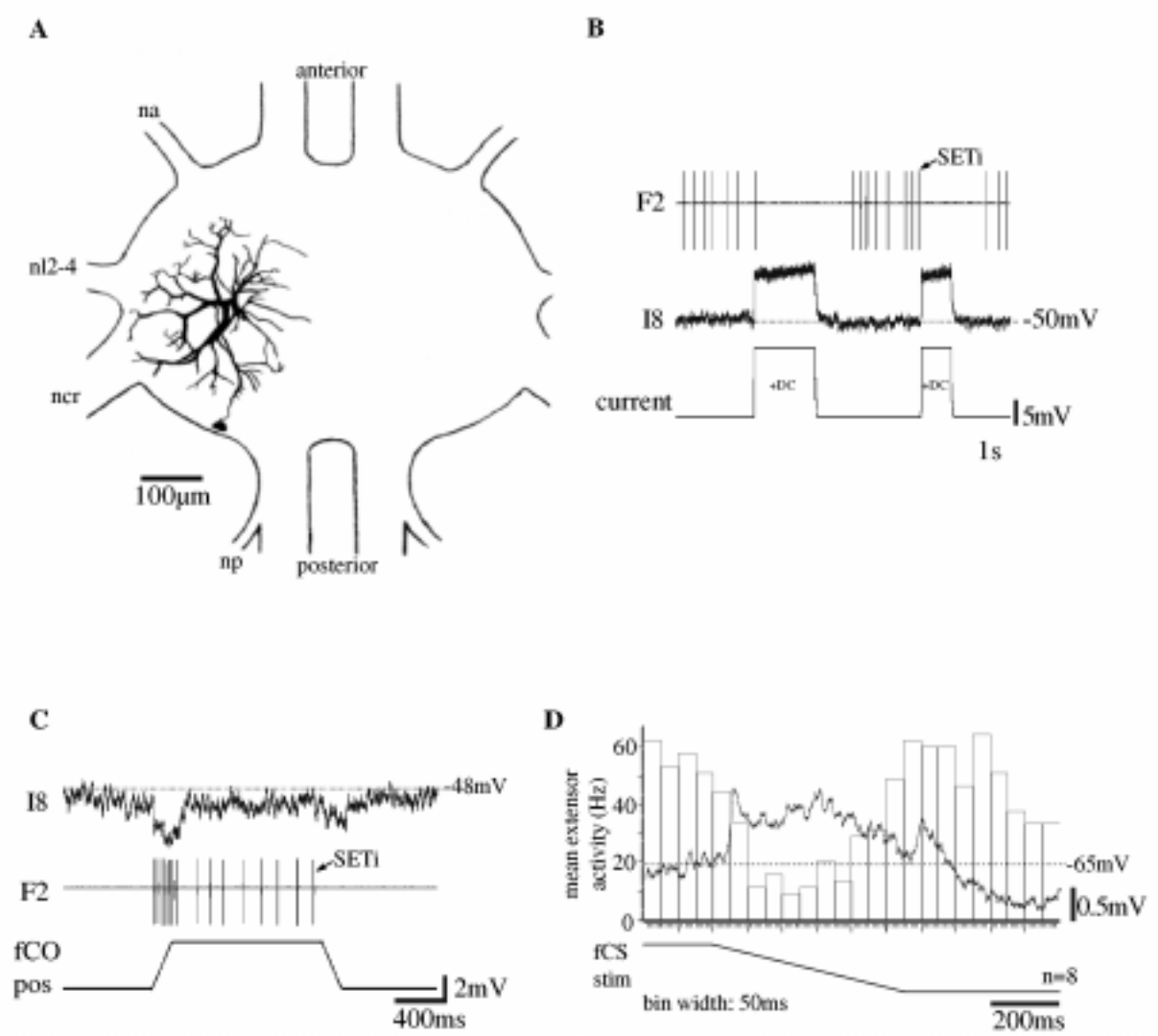


Figure 3.28. Morphological and physiological properties of the premotor nonspiking interneurons of type I8. **A:** Morphology of the interneuron I8 (na: nervus anterior, nl2-4.: nervus lateralis 2-4, ncr: nervus cruris, np: nervus posterior). **B:** Injection of depolarizing current (*bottom trace*) into the interneuron I8 (*middle trace*) terminates the ongoing spontaneous spike activity of the extensor motoneurons (recorded from the femoral nerve F2 -*top trace*). **C:** Response of the interneuron I8 (*top trace*) to ramp and hold stimuli at the fCO (*bottom trace*) in resting stick insect. Notice that this interneuron receives hyperpolarisation in both elongation (flexion of the FT-joint) and relaxation (extension of the FT-joint) stimuli. **D:** Response of the interneuron I8 (*intracellular recording trace*) to fCS (*bottom trace*) stimulation in active animal. The responses of the interneuron I8 were averaged during eight trials. The stimulus histogram shows the mean extensor activity during fCS stimulation (bin width: 50ms).

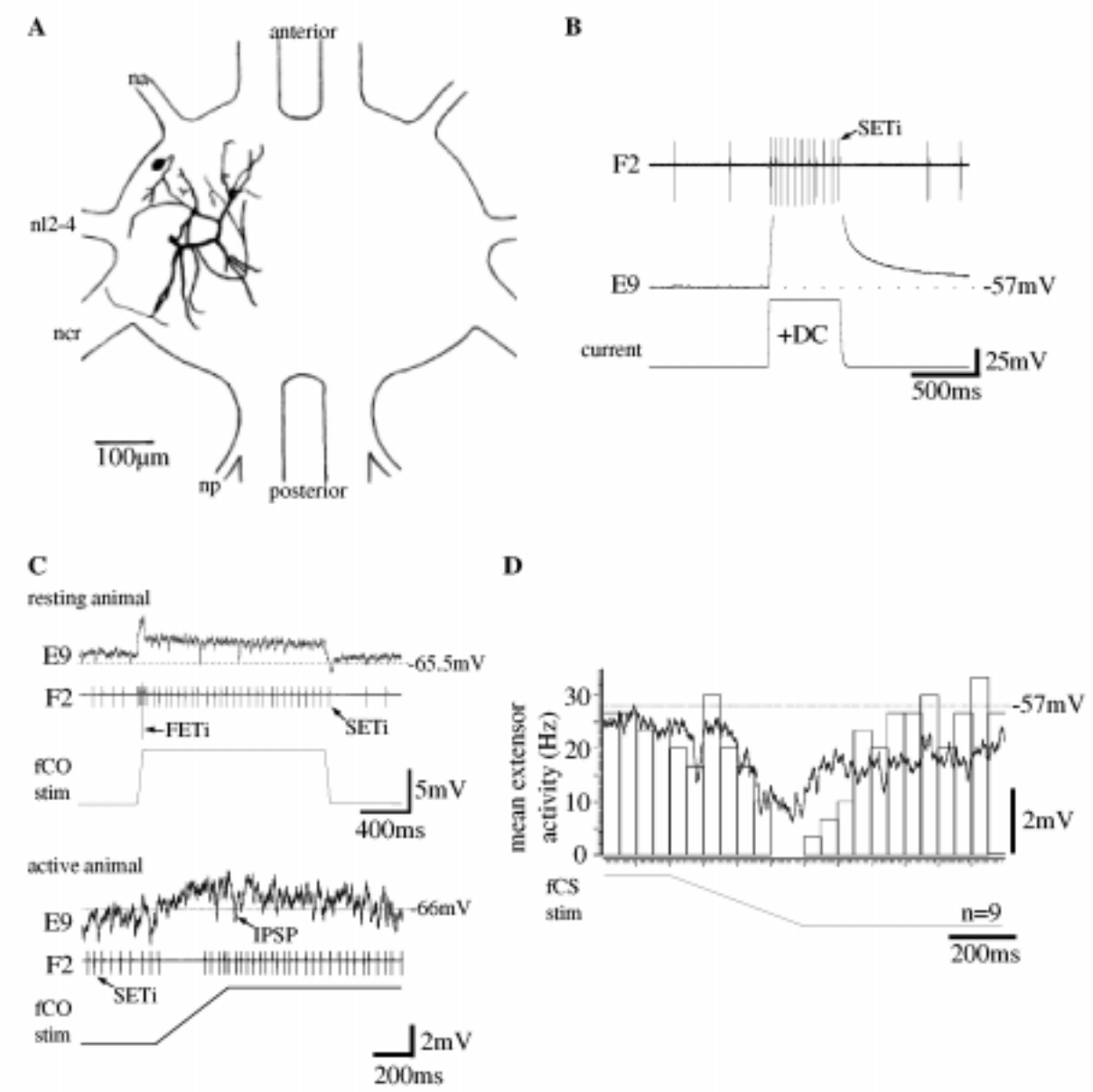


Figure 3.29. Morphological and physiological properties of the premotor nonspiking interneurons of type E9. **A:** Morphology of the interneuron E9 (na: nervus anterior, nl2-4.: nervus lateralis 2-4, ncr: nervus cruris, np: nervus posterior). **B:** Injection of depolarizing current (*bottom trace*) into the interneuron E9 (*middle trace*) increases the ongoing spiking frequency of the extensor motoneurons (recorded from the femoral nerve F2 -*top trace*). **C:** Response of the interneuron E9 (*top trace*) to ramp and hold stimuli at the fCO (*bottom trace*) in resting (*top*) and tacile activated (*bottom*) stick insect. Notice that in resting animals this interneuron receives phasic depolarization during elongation (flexion of the FT-joint), with position effect, and it hyperpolarizes during relaxation (extension of the FT-joint). In tactile activated animals, during 'active reaction' there is only a tonic depolarization. Notice that the IPSPs are abolished during the ramp. **D:** Response of the interneuron E9 (*intracellular recording trace*) to fCS (*bottom trace*) stimulation in active animal. The responses of the interneuron E9 were averaged during six trials. The stimulus histogram shows the mean extensor activity during fCS stimulation (bin width: 50ms).

3.5.3.3.4 Nonspiking interneuron E10

The physiology of a nonspiking interneuron similar to E10 was first observed by Prof. Dr. Ralph A. DiCaprio during his stay in our lab (personal communication). This neuron is here first successfully labeled. The morphology of the nonspiking interneuron E10 is shown in figure 3.30A. Upon injecting a depolarizing current, E10 increased the activity of SETi (Fig. 3.30B). This interneuron only displayed the position component of the fCO stimulation in its membrane potential. Figure 3.30C demonstrate the responses of E10 to fCO stimulation with three different ramp velocities (152°/s, 430°/s, and 1116°/s). There was no phasic component in the responses of E10, even with very fast stimuli; this suggests that this interneuron only mediates position information from the fCO to the extensor tibiae motoneurons. This interneuron was recorded and labeled only once.

3.5.3.3.5 Nonspiking interneuron IE1

The gross morphology of the nonspiking interneuron IE1 is shown in figure 3.31A. Interestingly, depolarization of IE1 led to a termination in the spiking activity of the SETi or at least a decrease in this activity; and it initiated or increased the activity of FETi and common inhibitor 1(CI1). An elongation stimulus at the fCO in resting animals led to depolarization in IE1 during movement; and IE1 showed a small position sensitive depolarization (Fig. 3.31C). This interneuron was recorded and labeled three times.

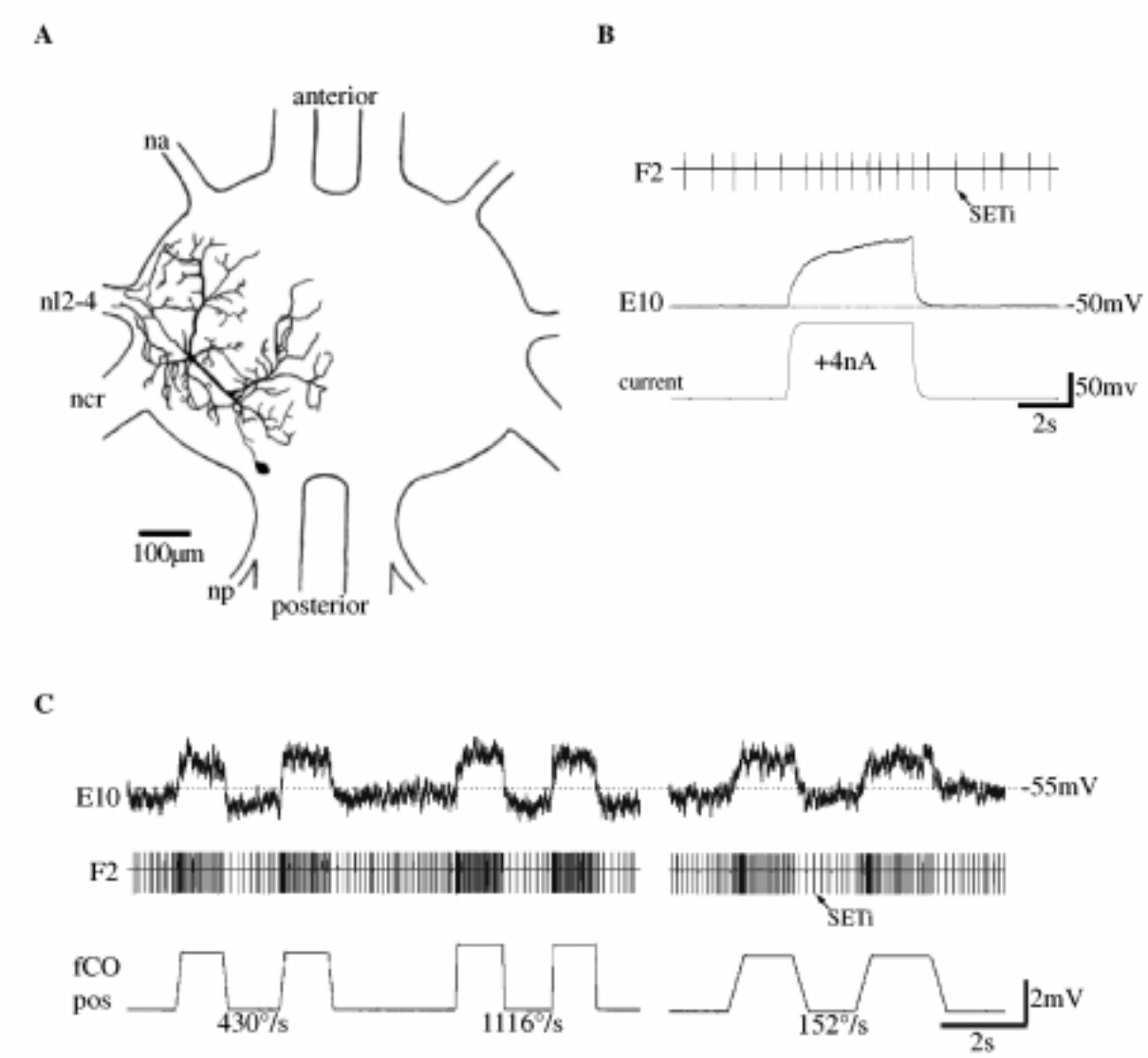


Figure 3.30. Morphological and physiological properties of the premotor nonspiking interneurons of type E10. **A:** Morphology of the interneuron E10 (na: nervus anterior, nl2-4.: nervus lateralis 2-4, ncr: nervus cruris, np: nervus posterior). **B:** Injection of depolarizing current (*bottom trace*) into the interneuron E10 (*middle trace*) increases the ongoing spiking frequency of the extensor motoneurons (recorded from the femoral nerve F2 -*top trace*). **C:** Response of the interneuron E10 (*top trace*) to ramp and hold stimuli at the fCO (*bottom trace*) in resting stick insects. Three different ramp velocities (430°/s, 1116°/s, and 152°/s) are shown. In none of these stimulus velocities could a phasic response be detected.

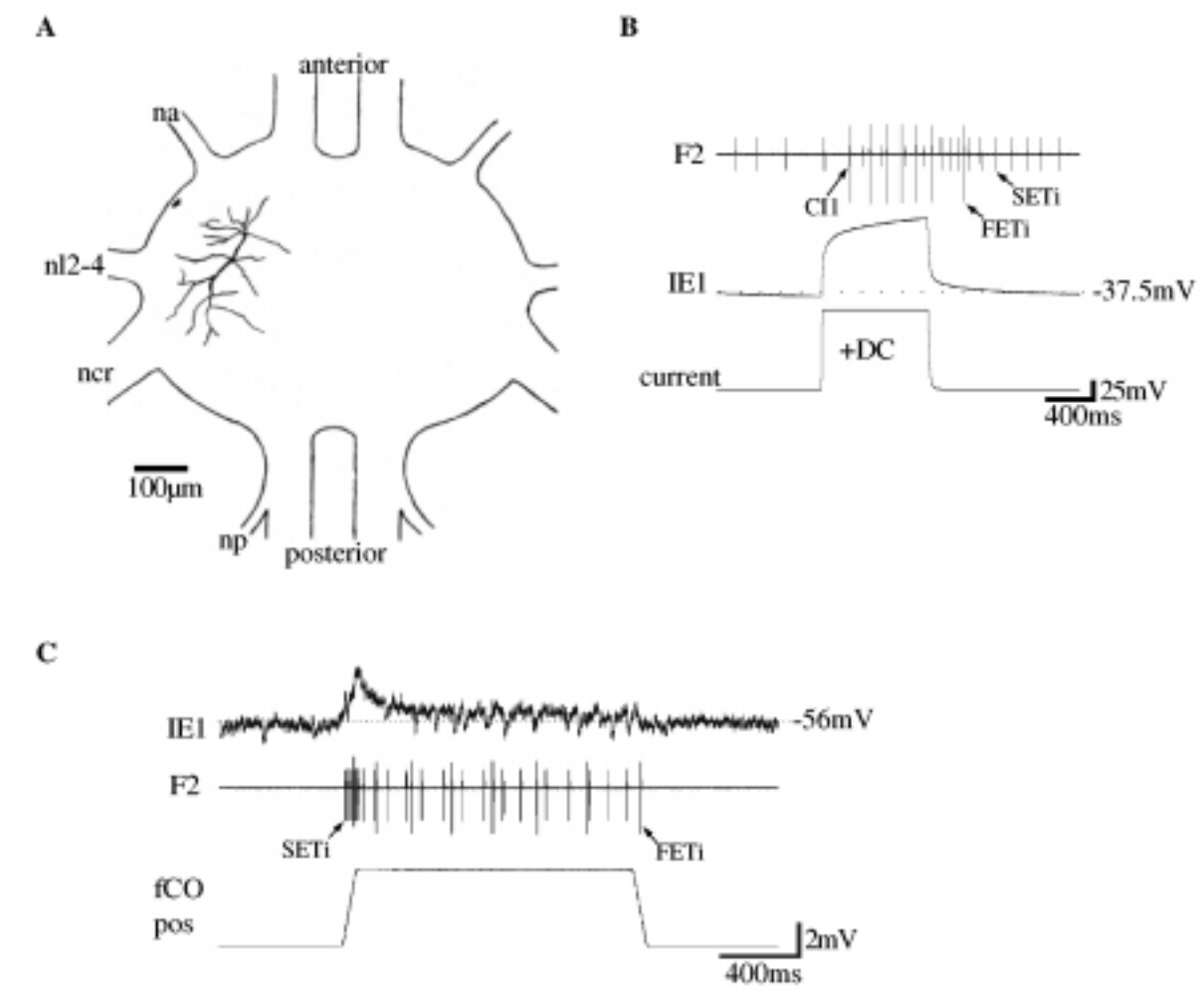


Figure 3.31. Morphological and physiological properties of the premotor nonspiking interneurons of type IE1. **A:** Morphology of the interneuron IE1 (na: nervus anterior, nl2-4.: nervus lateralis 2-4, ncr: nervus cruris, np: nervus posterior). **B:** Injection of depolarizing current (*bottom trace*) into the interneuron IE1(*middle trace*) decreases the ongoing spiking frequency of the slow extensor tibiae motoneuron (SETi) and initiates spike activity in the fast extensor tibiae motoneuron (FETi) and the common inhibitor 1 (CI1) (recorded from the femoral nerve F2 -*top trace*). **C:** Response of the interneuron IE1 (*top trace*) to ramp and hold stimuli at the fCO (*bottom trace*) in resting animals. The interneurone responses with a phasic depolarization to an elongation of the fCO (flexion of the FT-joint) and it is tonically depolarized during the elongated holding phase of the fCO (flexed FT-joint).

3.6 Role of femoral campaniform sensilla in walking pattern generation in the single middle leg preparation

In sections 3.4 and 3.5 it was shown that stimulation of the fCS inhibited extensor motoneurons and elicited activity in flexor motoneurons (e.g. Fig. 3.19B). In a final set of experiments, the role of the fCS in controlling tibial motoneuron and muscle activity in walking was examined. To investigate this question, the single middle leg preparation was chosen (Fischer et al., 2001; *C. impigra*). Two different conditions, fCS intact and fCS ablated walking sequences were compared, and any changes in the walking motor pattern were

analyzed. This preparation is appropriate for such investigations because segmental mechanisms of walking pattern generation can be investigated without the influence of intersegmental coordinating influences from the other legs.

Figure 3.32A shows recordings from the middle leg preparation when the animal is walking with the fCS intact (Fig. 3.32A, left) and after the removal of the fCS (Fig. 3.32A, right). The most obvious difference between these situations is the reduced flexor activity during stance after the fCS has been removed. This is seen both in the flexor EMG and in the rectified and integrated EMG activity. Such a decrease in the flexor activity during stance was found in all tested animals (N=9). Removing the fCS also caused a slight, but significant change in the mean flexor burst duration when the animal was walking (Fig. 3.32B). It increased from 1.31±0.58s (n=486) to 1.51±0.94s (n=475).

The decrease in flexor activity during stance was also obvious from plotting the average amplitude of the rectified and integrated EMG within a normalized burst, comparing animals before and after the removal of the fCS (Fig. 3.32.C). After removing the fCS, the amplitude of the flexor activity was significantly reduced in all bins, except for the first 6 bins at the beginning of stance (see Fig. 3.32.C,D; first 6 bins in C and first bin in D). The same was true for the average normalized amplitude of the flexor activity of all the investigated animals for the normalized flexor burst duration (N=9; Fig. 3.32.D), and it was true over time (N=4; Fig. 3.33A). To do this, bin values were normalized to the maximal bin value of the intact situation (“fCS intact”). However, no significant decrease in flexor activity could be observed in sham operated animals (N=3, Fig. 3.32E). By contrast, no changes were detectable in extensor activity when legs were in swing, as measured by the mean spike activity of the extensor motoneurons, FETi and SETi (Fig. 3.33A).

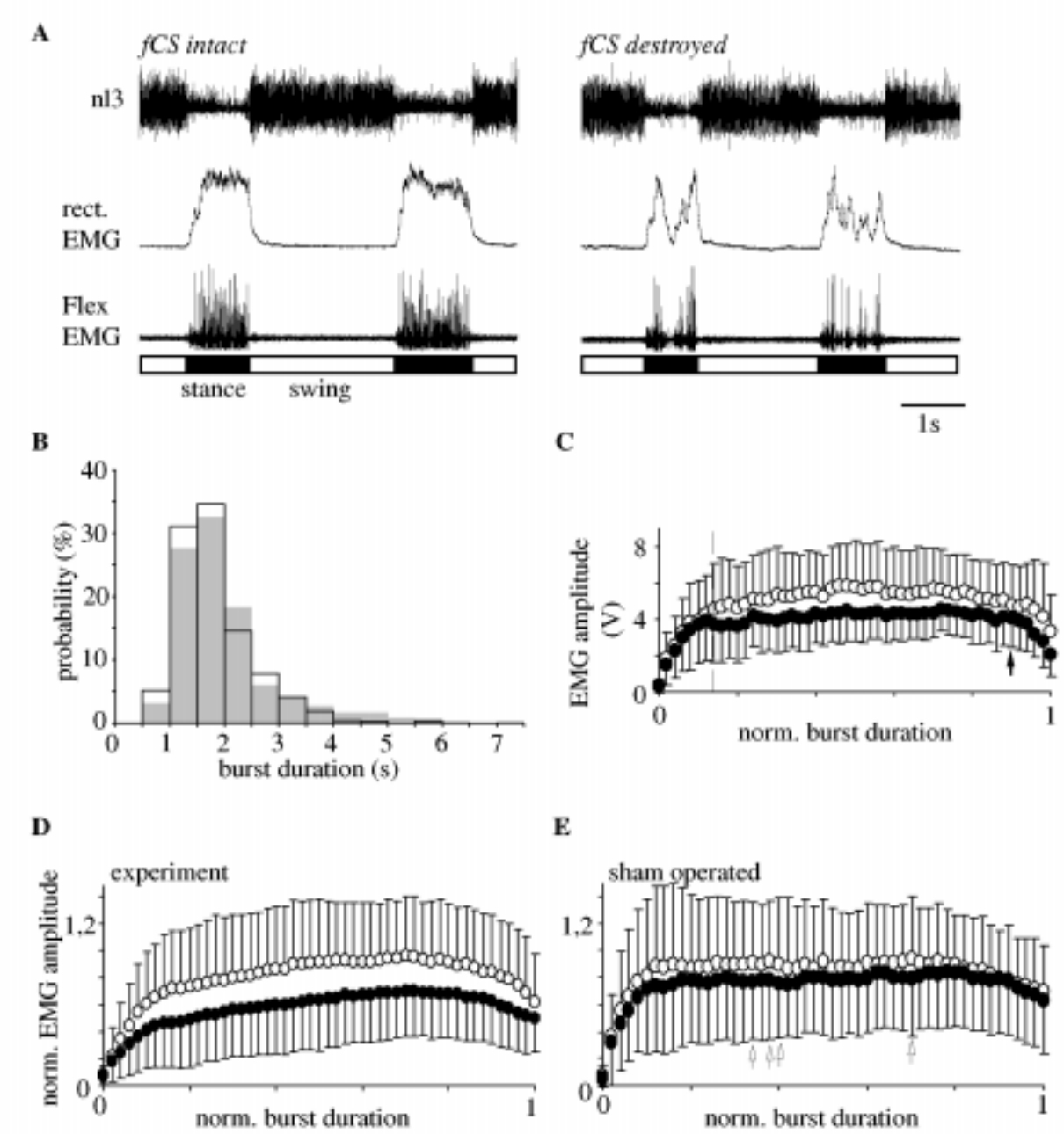


Figure 3.32. Influence of fCS ablation on the walking motor pattern in motoneurons and muscles of the FT-joint. **A:** nl3 nerve recording (*top trace*), rectified (time constant 40ms) flexor EMG (*middle trace*), flexor EMG (*bottom trace*) from the middle leg preparation during walking in the control animal with an intact sensory supply of the middle leg (*left*) and after ablation of the fCS (*right*). Black bars indicate the stance phase and white bars indicate the swing phase of the middle leg in a step cycle determined by the activity pattern of the motoneurons of the FT-joint (see Fischer et al. 2001). **B:** Probability histogram of flexor burst duration during stance (bin width: 0.5s). There is a slight, but significant ($p<0.05$) increase in burst duration after the ablation of the fCS (N=9; n=486 control- open bars; n=475 with fCS ablated - grey bars). **C:** Plot of the average rectified EMG amplitude in one animal under control conditions (open circles, n=65) and after ablation of the fCS (filled circles, n=57) during a normalized flexor burst. Flexor bursts were divided in 50 bins of the same duration. Notice that the amplitude of the flexor activity is significantly decreased after fCS were ablated in all bins, except for the first seven bins and one bin in late stance (filled arrow). This was observed in all experimental animals. **D:** Comparison of normalized rect. EMG amplitude in the nine experiments during a normalized flexor burst (n=486 in control, and n=475 after ablation of fCS). Bin values for each experiment were normalized to the maximum bin value in the intact situation ("fCS intact") of each experiment. Maximum bin value was set to 1. **E:** Comparison of normalized rect. EMG amplitude of three control animals before and after a sham operation, in which just a hole was made in the cuticle on the anterior side of the femur (n=96 in intact, open circles and n=95 after the sham operation, filled circles). Otherwise like C and D, no significant change was observed, except in the four bins marked by open arrows (see also text). Vertical lines in C, D, and E are the standard deviation.

Finally, I investigated whether sensory information from the fCS plays a role in generating the step phase transitions from stance to swing, and vice versa, when the animal walks on the treadband. The average latencies between the activities of the two antagonistic tibial motoneuron pools at both transitions, with intact fCS and after fCS removal (Fig. 3.33B) were compared. For the transition from swing to stance, the time was measured between the termination of the tibial extensor motoneuron activity in an extracellular recording from the extensor nerve nl3 and the start of the tibial flexor activity of the flexor EMG. No significant change in latency was found in any of the tested animals (N=4) (Fig. 3.33B, *left*). The same was true for the transition from stance to swing (Fig. 3.33B, *right*), although in one animal the overlap between the end of the flexor and the beginning of the extensor activity was significantly smaller after the removal of the fCS.

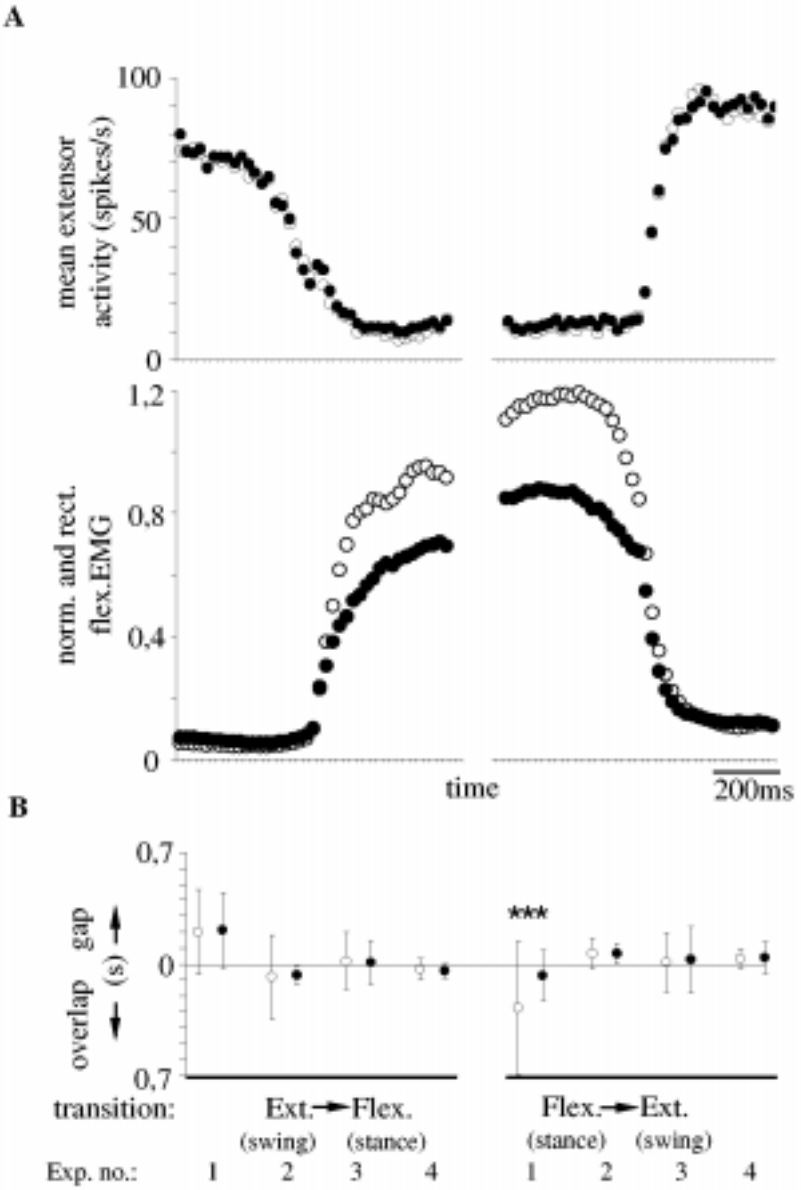


Figure 3.33. A: Comparison of the mean extensor activity (*top*) and the mean normalized and rectified flexor muscle activity (*bottom*) over the time of the transition from swing to stance, i.e. from extensor to flexor activity (*left*) and the time of the transition from stance to swing, i.e. from flexor activity to extensor activity (*right*) for intact legs (open circles, n=238) and after ablation of the fCS (filled circles, n=246). Normalization procedure was similar to the one in figure 3.27. Data were pooled from four experiments. Note that no change could be detected for the mean extensor activity in both situations. **B:** Comparison of the means±SD of latencies between extensor activity offset and flexor activity onset during the transition from swing to stance (Ext.→Flex., *left*) and between the flexor activity offset and the extensor activity onset during the transition from stance to swing (Flex.→Ext., *right*) in four experiments (28<n<60). I could find a significant decrease in latency for the transition from flexor to extensor activity in only one animal.

4. Discussion

4.1. Role of femur-tibia joint position in patterning rhythmic activity in coxa-trochanter joint motoneurons

Heß and Büschges (1999) have shown that movement of the femur-tibia joint (FT-joint) (as mimicked by elongation and relaxation of the femoral chordotonal organ -fCO- receptor apodeme) can induce transitions in the ongoing alternating activity of depressor and levator trochanteris motoneurons (DepTr and LevTr) when the animal is in the locomotor mode ("active" animal, summary in Bässler and Büschges, 1998), and it can also reset and entrain ongoing rhythmic activity in coxa-trochanteral joint (CT-joint) motoneurons elicited by application of pilocarpine.

In section 3.1 it has been shown that burst parameters during pilocarpine-induced rhythmicity in trochanteral motoneurons are also influenced by the FT-joint angle, measured by the fCO. For example, the mean spike rate within a burst, the burst duration, and the duty cycle of the spike activity are modulated, depending on the FT-joint angle, with reversed signs for LevTr and DepTr motoneuron pools (Fig. 3.2). In principle, there are two ways that such influences can be mediated: (i) via a tonic modulation of baseline membrane potentials in the motoneurons as seen in the resting animal (Fig. 3.1); and (ii) by affecting central rhythm-generating networks (CRG) that control motoneuron bursting during rhythmic locomotor activity.

Shifting the baseline membrane potential would bring the motoneurons closer or further away from the spiking threshold, and additional rhythmic input of constant amplitude would result in changes in the burst duration, in the spike frequency within the burst, and in the duty cycle. However, as shown in figure 3.5, there is no evidence of such shifts in baseline membrane potential, and there appears to be a much more significant influence from changing amplitudes of rhythmic input. The latter provides clear evidence of a phasic influence, e.g. by modulation of the premotor CRG of the CT-joint with changing FT-joint angle.

There are two other lines of evidence supporting this finding: (i) The disruption of the rhythmic activity of CT-joint motoneurons that sometimes occurs at extreme FT-joint angles (Fig. 3.3). (ii) The change of the cycle period of the rhythm that occur when the fCO position

is changed (Fig. 3.4). Both effects can only be explained if fCO signaling has access to the CRG activity of the CT-joint.

The previous findings (Hess and Büschges 1997 and 1999) and the presented data in section 3.1 suggest that the position and movement of the FT-joint, signaled by the main proprioceptor fCO, patterns the motoneuron activity of the CT-joint. The CT-joint motoneurons, LevTr increase and DepTr decrease their activity with the flexion movement and the flexed position of the FT-joint. The opposite happens with extension movement and extended position. This influence can be observed in resting animals and under pilocarpine-induced rhythmic conditions. In summary, position and movement signals from the fCO pattern the CT-joint motoneuron activity via "reflex like" pathways and by influencing the central rhythm generating network of the CT-joint (Fig. 4.1).

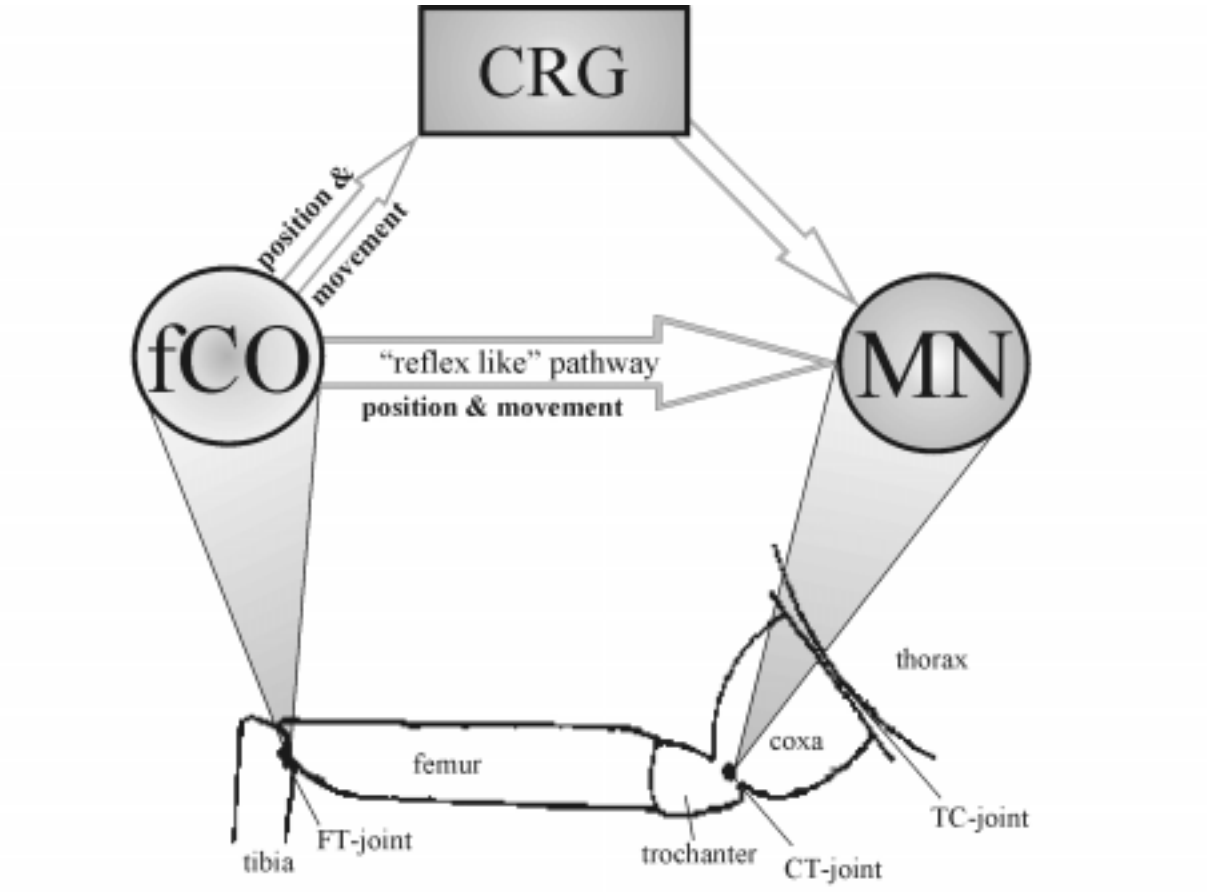


Figure 4.1. Both position and movement information of the femur-tibia joint (FT-joint) signaled by the femoral chordotonal organ (fCO) influence the coxa-trochanteral joint (CT-joint) motoneuron (MN) activity via a 'reflex-like' pathway and by influencing the central rhythm generating network (CRG) of the CT-joint.

There is some information about positional influences in controlling central pattern-generating networks of other leg joints during cat locomotion. Anderson and Grillner (1981) have shown that position signals from the hip influence the activity of the extensor and flexor motoneurons of the knees and ankles of cats. In the locomotion of spinal cats this influence can be seen as the dependence of the transition of activity from stance on swing to the hip position (Anderson et al., 1981; Pearson and Duysens, 1976): that is, more extended hip angles (end of stance phase of a walking cycle) enhance the initiation of a swing phase, the termination of extensor motoneuron activity, and the initiation of flexor motoneuron activity.

Such a mechanism has been shown in stick insects, i.e. the end of the stance phase depends on the position and the load information from the leg (Bässler, 1977; Cruse 1985). The data presented in section 3.1 could suggest a mechanism underlying this observation: that is, with an increase of the flexion of the FT-joint angle, the levator trochanteris motoneuron activity increases, and during rhythmic preparations, the burst duration and the burst proportion also increase. This could be an explanation for increasing probability of that a swing phase is initiated, in which the LevTr motoneurons are active (Fischer et al., 2001). A similar mechanism has been shown for the swing-stance transition (Cruse et al., 1998). If an obstacle disturbs a swing phase, the walking stick insect will have one of two different reactions. Either the animal will perform an avoidance movement and continue the ongoing swing, or it will terminate the swing, grasp the obstacle and start a stance. The probability of one rather other reaction depends on the distance of the leg to the anterior extreme position (the position at which the swing phase terminates and the new stance begins). The more the leg approaches the anterior extreme position, the more probable the transition from swing to stance phase is. The data presented in section 3.1 could suggest a mechanism that underlies this as well. With the increasing extension of the FT-joint angle, the depressor trochanteris motoneuron activity increases, and during rhythmic preparations the burst duration and the burst proportion also increases. This could be an explanation for increasing probability of an initiation of a stance phase, in which the DepTr motoneurons are activated (Fischer et al., 2001). The dependence of the cycle periods of the rhythmic activity of the CT-joint motoneurons on the FT-joint position could be due to the increased disposition for a transition from depressor to levator trochanteris motoneuron activities.

4.2. Role of sensory signals from the coxa-trochanter joint in controlling the motoneuron activity of the adjacent femur-tibia and thorax-coxa joints

It has been shown by Gerharz (1999) that the interjoint influence previously shown by Heß and Büschges (1997 and 1999) is neither a general rule for all other adjacent leg joints, nor a direction-specific rule. Levation of the femur was able to inactivate tibial extensor and coxal protractor motoneurons and to initiate activity in tibial flexor and coxal retractor motoneurons. The proprioceptors measuring the CT-joint position and movements, including the trochanteral hairplate (trHP, Tartar 1976; Bässler 1983), the rhombal hairplate (rHP, Tartar 1976; Bässler 1983), and the levator stretch receptor organ (levSR, Schöwerling 1993) did not effect the patterning of the motoneuron activity of the thorax-coxa joint (TC-joint, proximal direction) and the FT-joint (distal direction). Instead of proprioceptors, femoral movements signaled by cuticular strain sensing sense organs consisting of groups of campaniform sensilla (CS) were able to effect the FT- and TC-joint motoneuron activity.

There are four fields of CS at the CT-joint, that have largely been treated as “one” sensory system in the past (Hofmann and Bässler 1982; Schmitz 1993; Schmitz and Stein 2000): three on the trochanter and one on the very proximal femur. Gerharz (1999) and the present work (section 3.3.2) show specific influences of these four fields. Specifically, only the CS located at the proximal femur close to the CT-joint, i.e. the femoral CS (fCS), influenced the FT-joint motoneuron activity; and only the CS located at the trochanter, i.e. the trochanteral CS (trCS), influenced the TC-joint motoneuron activity.

However, given the design of the experiments by Gerharz (1999) and experiments presented here (section 3.2.1), we cannot exclude more subtle influences of the proprioceptive sense organs, trHP, rHP, and levSR, on the magnitude of activity in the motoneurons of the FT-joint (Bässler 1993c). In contrast to our findings, Bässler (1993c) showed that proprioceptive signals from the trochanteral hairplate at the CT-joint influenced interjoint coordination between the CT- and the FT- joint, an influence which was not detectable in our studies on the middle leg. There are three possible factors that may account for this difference. First, the differing results may be due to differences in mechanisms of interjoint coordination between the middle leg and the foreleg and/or a differing architecture in the trochanter and femur basis in these legs. Second, influences of the trochanteral hairplate on tibial motoneuron activity may only be effective during the production of leg movements and may not be effective in

reduced preparations. Third, the efficacy of proprioceptive signals from the CT-joint on the activity of the FT-joint may depend on the actual phase of motoneuronal activity in the TC- and CT-joint, an uncontrolled variable in the present investigation.

The experiments on pharmacologically activated rhythmic preparations show that fCS and trCS signals do affect centrally generated activity in motoneurons supplying the FT- and TC-joint (figures 3.9, 3.11, 3.14, 3.15, and 3.17). However, the influence of the fCS on the central rhythm generating network (CRG) of the FT-joint was rather labile and variable, compared to the influence of the fCO signals from the FT-joint on the motor activity that drives the CT-joint (Heß and Büschges, 1999) (Fig. 4.2). Figure 4.2 shows the outlines for the view that the fCS stimulation influences the FT-joint motoneurons via a “reflex-like” pathway and weakly influences the timing of the CRG of the FT-joint, and the trCS stimulation influences the TC-joint motoneurons via a “reflex-like” pathway and has some influence on the CRG of the TC-joint, which is stronger than the influence of the fCS in the distal direction.

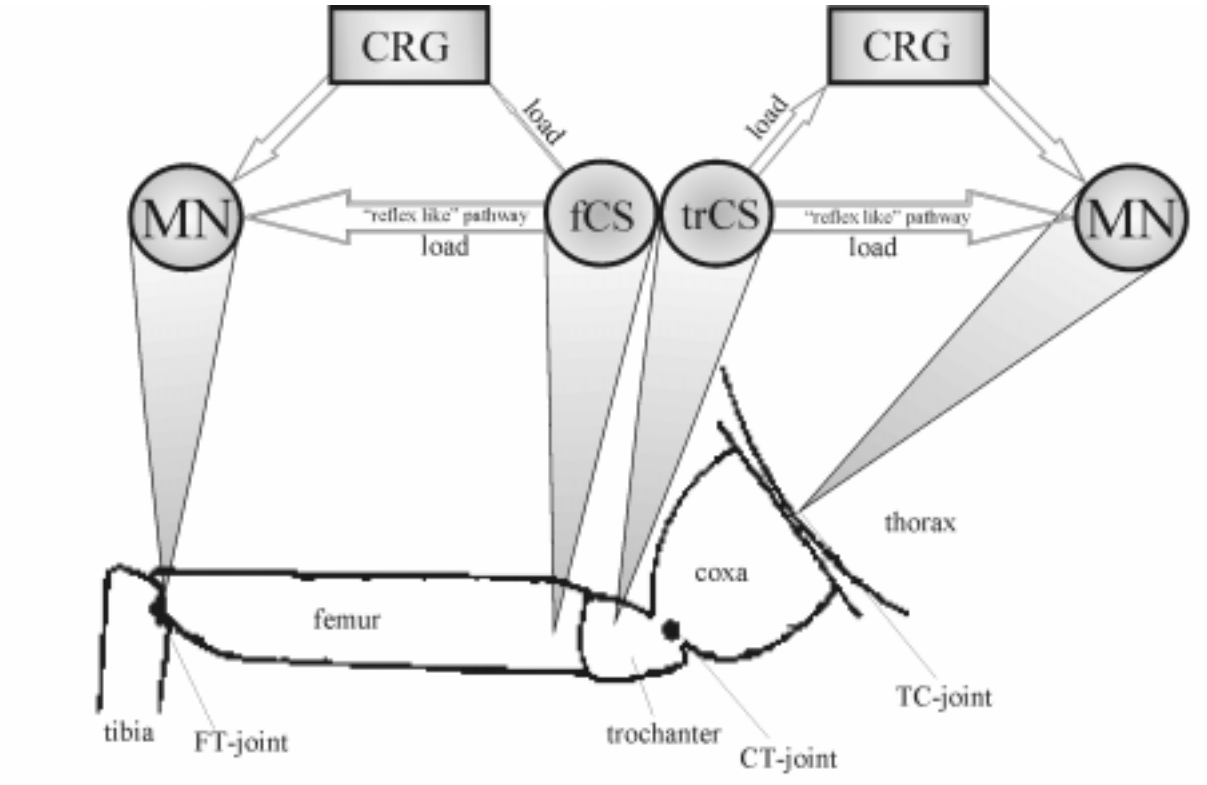


Figure 4.2. The load on the leg, sensed as a cuticular strain by femoral and throchanteral campaniform sensilla (fCS and trCS), influences the femur-tibia joint (FT-joint) and thorax-coxa joint (TC-joint) motoneuron (MN) achtivity via a “reflex-like” pathway and by affecting the central rhythm generating network (CRG). The influence of the fCS on the CRG of the FT-joint is labile and variable compared to the influence of the femoral chordotonal organ on coxa-trochanteral joint (CT-joint) and the influence of the trCS on the TC-joint.

The present results, combined with previous findings suggest the following scheme for the information flow between the three adjacent leg joints, i.e. the FT-joint, the CT-joint, and the TC-joint: proprioceptive information flow is specific in interjoint coordination from the FT-joint onto the CT-joint. No movement or position information (trHP, rHP and levSR) flows from the CT-joint to TC- or FT-joint. Instead, the cuticular strain-measuring campaniform sensilla were found to pattern the two adjacent leg joint activities, influencing via "reflex-like" pathways and having access to the central rhythm generators (Fig.4.3). Figure 4.3 shows that movement and position information, measured by the fCO, influences the CRG of the CT-joint. However no such influence on the movement or the position measuring sense organs (trHP, rHP, and levSR, see legend of figure 4.3) was observed. Only the load measuring sense organ -the fCS- influences the CRG of the FT-joint and the three trCS influence the CRG of the TC-joint. The influences from the TC-joint sense organs (cxHPd, cxHPv, and cxHR) are unknown.

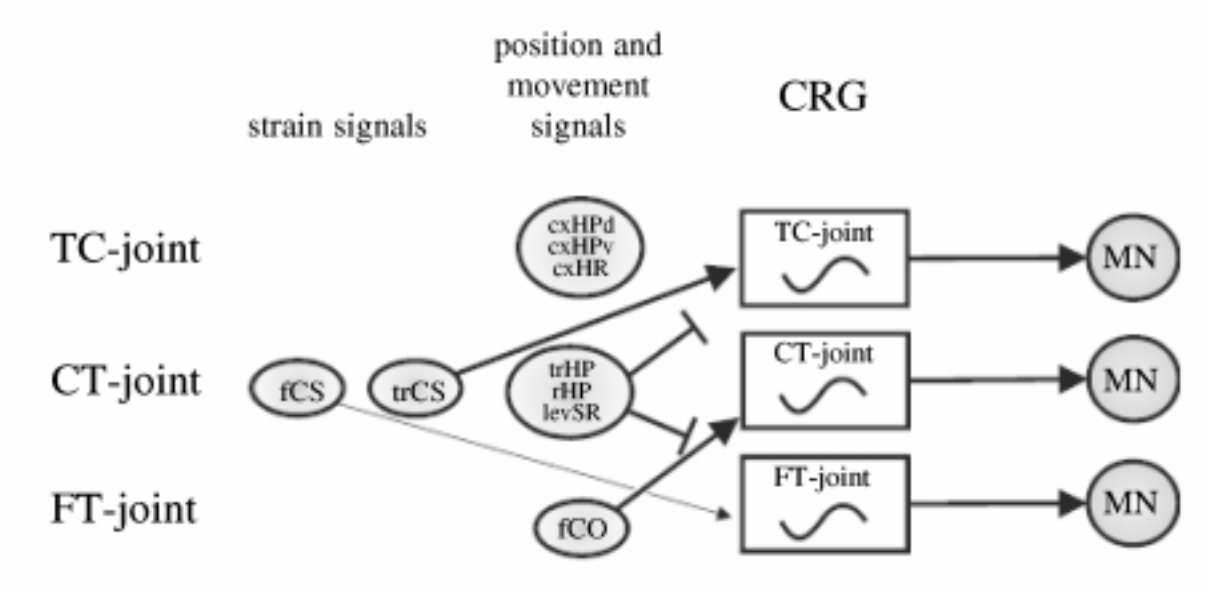


Figure 4.3. From already known interjoint coodination mechanisms, proprioceptive information flow from the femur-tibia joint (FT-joint) to the coxa-trochanteral joint (CT-joint). Influences from the thorax-coxa joint (TC-joint) to the distal FT- and CT-joint are unknown. Postion and movement of the FT-joint signaled by the femoral chordotonal organ (fCO) influences the central rhythm generator (CRG) of the CT-joint. Only the cuticular strain-sensitive femoral and trochanteral campaniform sensilla (fCS and trCS) were found to influence the CRG of the adjacent FT-joint and TC-joint. No proprioceptive information flows from the CT-joint to the FT- or TC-joint. (trHP: trochanteral hair plate, rHP: rhombal hair plate, levSR: levator stratch receptor, cxHPd and cxHPv: dorsal, and ventral choxal hair plate, cxHR: coxal hair rows, MN: motoneuron)

4.3. Convergence of displacement and load information on the reflex system of the femur-tibia joint

In invertebrate systems, it has been shown that intrajoint reflexes can change their gains or even their signs to adapt the reflex system to the required task, such as walking or standing (for review see Pearson, 1993). For example, it has been shown that the gain of the resistance decreases during rocking movements (Bässler, 1983) and is reversed, i.e., during walking the resistance reflex becomes an assistance reflex (Bässler, 1986). This plasticity of a reflex system can also occur in a phase dependent manner, that is the reflex can change its sign or gain, depending on the cycle of movement. This could occur for two reasons: First, the release of the phase dependent alteration of the reflex could be generated centrally (Knop et al., 2001). Second, the phase dependent alteration of the reflex could be made by interactions with other sense organs. Since the leg is maximally loaded during stance, where the leg is on the ground and supports the body and propels in the walking direction, and unloaded during swing, where the leg is lifted from the ground. For this reason, load-sensing sense organs such as the campaniform sensilla would be a good candidate for reflex altering input to the system.

Intracellular recordings from the extensor tibiae motoneurons during femur levation, and selective stimulation of the fCS, revealed that the fCS has an inhibitory influence on tibial extensor motoneurons and an excitatory effect on tibial flexor motoneurons (figures 3.7, 3.8, and 3.19). Intracellular recordings from premotor nonspiking interneurons that transmit information from fCO to the FT-joint motoneurons revealed that seven of eight recorded nonspiking interneurons with an excitatory output on the tibial extensor motoneurons (E2-9) were inhibited by fCS stimulation, while interneuron E7 received excitation. By contrast, the inhibitory nonspiking interneurons (I1-4 and I8) had a more variable response to fCS stimulation. The I1 and I8 were clearly depolarized by the fCS; I2 showed overlapping inhibition and delayed excitation; and the I4 was inhibited. The response of the I3 to the fCS looks like a slow drift to a more hyperpolarized membrane potential (figures 3.25, 3.26, and 3.27-3.31) (Fig. 4.4). Figures 3.7 and 3.8 show distinct inhibitory postsynaptic potential (IPSP) in fast extensor motoneuron, caused by fCS stimulation due to femur levation. Distinct IPSP suggest that the premotor neuron can only be a spiking interneuron (sensory cells with inhibitory output on postsynaptic cells are not known in the insects nervous system). This suggests that the fCS afferents are directly connected with specific spiking interneurons that

make direct inhibitory output connections with extensor tibiae motoneurons (stick insect: Schmitz and Stein, 2000; locust Newland and Emptage, 1996).

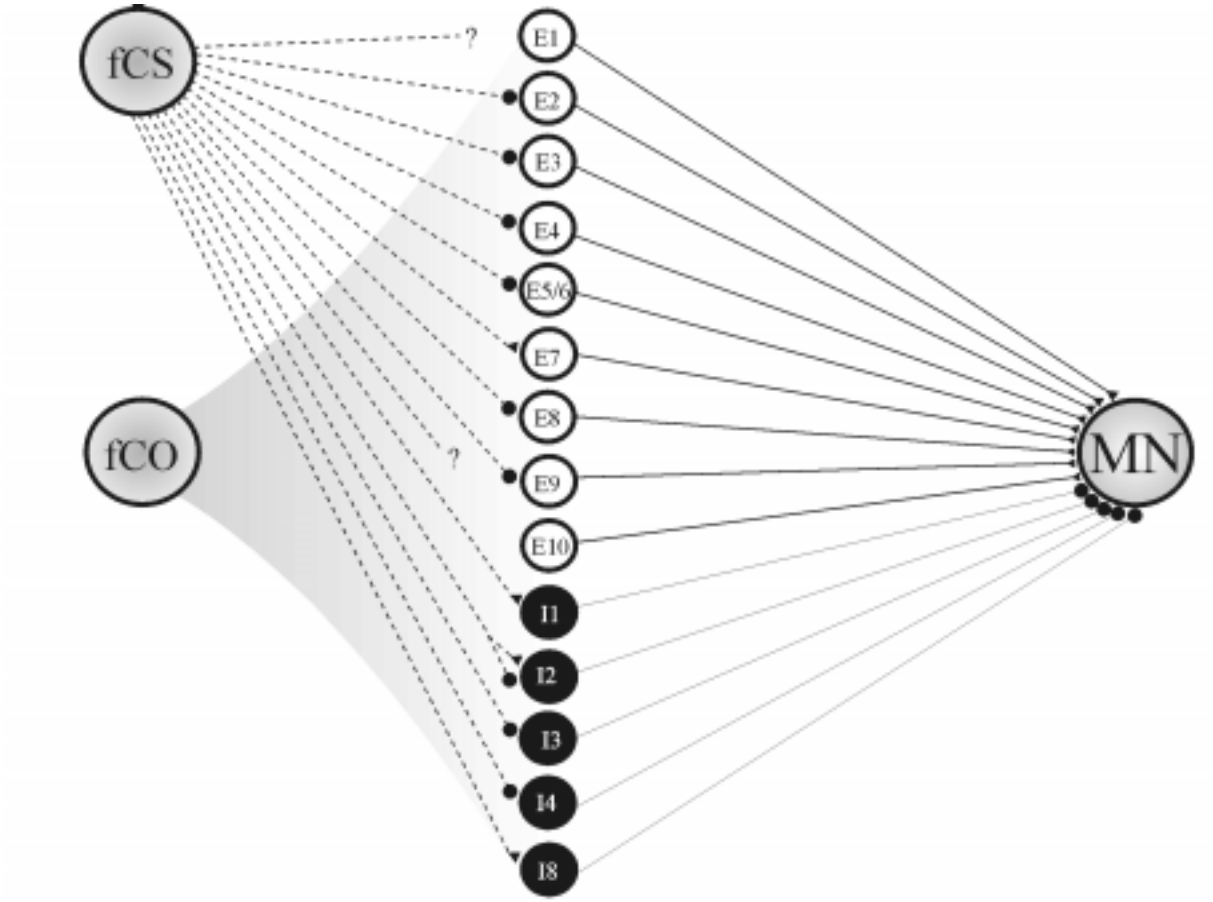


Figure 4.4. Premotor nonspiking interneurons that transmit information from the femoral chordotonal organ (fCO) onto the extensor tibiae motoneurons (MN) with excitatory (E1-10, white circles) or inhibitory (I1-4 and I8, black circles) output on the extensor tibiae motoneurons are affected by signals from the femoral campaniform sensilla (fCS). Excitatory nonspiking interneurons are generally inhibited (except for the E7, which is excited). The effect on the inhibitory nonspiking interneurons is more variable. (?: this influence is unknown, ▲: excitatory influence, ●: inhibitory influence)

Section 3.5 in this thesis work shows that the combined stimulation of the femoral campaniform sensilla (fCS) and the femoral chordotonal organ (fCO) decreases the gain of the resistance reflex in resting animals (figure 3.21 and 3.25) and increases the probability of the "active reactions" in active animals, compared to "active reactions" in stimulations exclusively of the fCO (figures 3.22, 3.23, and 3.24).

In resting animals, stimulation of the fCS during the elongation (flexion of the FT-joint) and the relaxation (extension of the FT-joint) of the fCO decreases the gain of the resistance reflex. Intracellular recordings from the premotor nonspiking interneurons (NSIN) that

transmit the information from the fCO to the tibial motoneurons revealed that the NSINs change their response if the fCS is simultaneously stimulated with the fCO. As shown in figure 3.25, the NSIN with an excitatory output to the extensor tibiae motoneurons E2 and E7 decreased the motoneurons response to the fCO signals when pressure was applied on the fCS. Decreases in the effect of fCO stimulation during fCS stimulation could be due to the fact that the influence of the fCO afferences are presynaptically inhibited by the fCS afferences, and their influences are weakened on the postsynaptic cells, the NSINs (see level 1 in figure 4.5). Another possible reason for the weakening of the postsynaptic effect of the fCO on the NSIN could be that the membrane resistance of the NSIN are decreased by the fCS afferences such that the synaptic currents cause changes smaller membrane potential. The second possibility was not tested here. Presynaptic inhibition of two different sense organs, measuring two different modalities, has been previously shown by Stein and Schmitz (1999). They showed that two sense organs, CS (measuring cuticular strain) and cxHPv (measuring position of the TC-joint), presynaptically inhibit the fCO afferences (sensitive to FT-joint movement, position). In addition, figure 3.25 shows that E3 changes the sign of its response to the fCO when the fCS is simultaneously stimulated. Previously it has been shown that E3 receives monosynaptic depolarizing and delayed (with specific spiking interneurons) hyperpolarizing input from the fCO afferents (Sauer et al., 1996). This suggests that the spiking interneurons that inhibit the E3 receive excitatory input from the fCS afferents (see level 2 in figure 4.5); this than strengthens the inhibitory pathway so that the response of this NSIN to fCO stimulation reverses. Figures 3.25 and 3.26 show that the NSINs receive excitatory or inhibitory input from the exclusive fCS stimulations, suggesting that fCS afferents have an immediate influence on NSINs (see level 3 in figure 4.5). Finally, figures 3.7, 3.8, and 3.19 show that the fast extensor tibiae motoneuron (FETi) receives distinct inhibitory postsynaptic potentials (IPSP) during fCS stimulation. This suggests that the fCS afferents are directly connected with extensor motoneurons with interposed spiking interneurons (see level 4 in figure 4.5).

In summary, figure 4.5 shows that the information from two different sense organs, i.e. the fCS (sensitive to cuticular strain) and the fCO (sensitive to movement and position of the FT-joint), do not only converges on the output elements of the network, the extensor tibiae motoneurons. The convergence of these sense organs is also realized in the premotor level. The levels of convergence 1 and 2 in figure 4.5 have to be considered as a hypothesis, because no evidence could be shown (Fig. 3.25). In addition, section 3.5.2 shows that the activation of

the fCS afferents also increases the probability of “reflex reversal” in the FT-joint reflex system (Figs. 3.22, 3.23, and 3.24). This suggests a second possibility for the phase dependent alteration of the intrajoint reflex, observed by Bässler (1986). Bässler (1986) showed that the reflex from the fCO changes from exciting the flexor motoneurons (reflex reversal) in the early part of the stance phase to inhibiting the same motoneurons (resistance reflex) at the end of the stance phase. Since the leg is maximally loaded during stance, where the leg is on the ground supporting the body and propelling it in the walking direction, and unloaded during swing, where the leg is lifted from the ground, load-sensing sense organs, campaniform sensilla, could be responsible for altering the reflex.

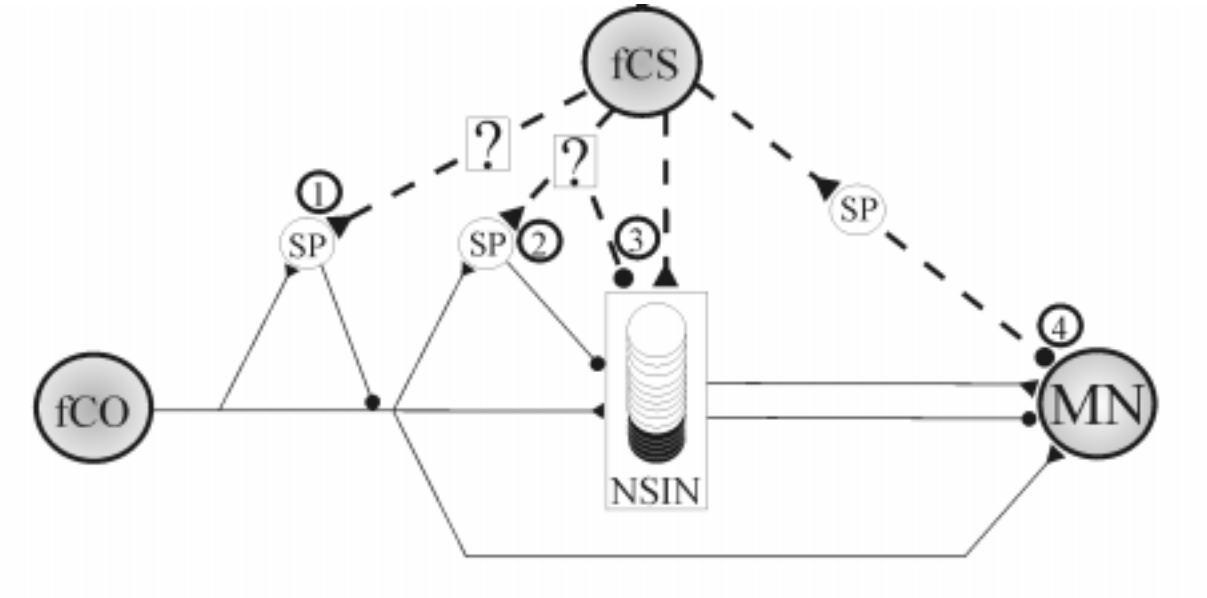


Figure 4.5. The influence of the femoral campaniform sensilla (fCS) on the network that governs the intrajoint reflex of the femur-tibia joint (FT-joint) occurs at four levels (bold black circles with numbers 1-4). No monosynaptic connections of these influences are shown in this thesis (dashed lines). For details, see text. For a review of the network of FT-joint intrajoint reflex, see Büschges et al. 2000. (fCO: femoral chordotonal organ, NSIN: premotor nonspiking interneurons, SP: spiking interneurons, MN: motoneurons, ?: details unknown, ▲: excitatory influence, ●: inhibitory influence)

4.4. Role of load information from the femoral campaniform sensilla in the generation of walking pattern in the single middle leg preparation

Ablation experiments in section 3.6 show that the sensory signals from the fCS affecting tibial motoneurons influence the magnitude of activity in the flexor muscle during stance in the single walking leg preparation. These results are consistent with previous findings on the role of the CS in controlling the stance-phase motor output of the leg, those results indicated that sensory information about the load on the leg from the CS reinforces stance-phase motor output (Pearson 1972) and specifies the role of one field of CS in this functional task. Interestingly, fCS ablation had no influence on the timing of the step-phase-transitions in the

walking cycle, the transition from stance to swing, or on the transition from swing to stance. There could be two reasons for this: 1) measurements of latencies with EMGs are not precise enough to detect changes; or 2) other sense organs play a role in transition of activity from extensor to flexor or flexor to extensor tibiae motoneurons.

4.5 General Discussion

This thesis work shows that during stick insect walking each joint of the middle leg, which is centrally uncoupled, is coupled through the peripheral signals from different sense organs measuring different modalities. The fCO measures the position and movement of the FT-joint, which contributes to patterning the CT-joint motoneuron activity. Interestingly, there is no such influence if we consider the influences of the sense organs that measure the position and movement of the CT-joint on the FT- and TC-joint motoneurons. The FT- and the TC-joint motoneuron activities are coupled by cuticular strain-sensitive sense organs, the CS. The fCS contributes to patterning the FT-joint motoneuron activity, whereas the trCS contributes to patterning the TC-joint motoneurons. All three of these influences are mediated via the "reflex-like" pathways; additionally they have access to the CRG of the effected leg joints; but the fCS has a relatively weak influence on the CRG of the FT-joint.

During the single middle leg walking preparation (Fischer et al., 2001), the flexor tibiae, the depressor trochanteris, and the retractor coxae motoneurons are active during stance phases, and the extensor tibiae, the levator trochanteris, and the protractor coxae motoneurons are active during swing phases. The interjoint mechanisms presented here can explain these pattern: 1) flexion of the FT-joint during stance phase initiates the levator trochanteris motoneuron activity, which causes the leg to be lifted (beginning of the swing phase). 2) At the end of the swing the leg touches down on the treadband, which increases the load on the leg and initiates the CS to fire. 3) The fCS signals cause the flexor tibiae motoneurons to become active and they maintain the activity until the next swing starts and the leg is unloaded again. 4) During stance, at approximately the same time as the fCS is active, the trCS causes the retractor coxae motoneurons (TC-joint is immobilized and denervated) to fire.

Point 1 mentioned above is different for free forward walking or forward walking on a treadwheel (Cruse and Bartling, 1995). Here, the FT-joint of the middle leg is more extended toward the end of the stance phase. The results presented in section 3.1 explain two different

behaviors: 1) the behavior during single middle leg preparation; and 2) the “saluting” movement shown by Graham and Bässler (1981).

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