Task-specific modulation of a proprioceptive reflex in a walking insect

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Abstract

The generation of task-dependent and goal-directed walking behaviour requires feedback from leg sense organs for regulating and adapting the ongoing motor activity. Sensory feedback from movement and force sensors influences the magnitude and the timing of neural activity generated in the neural networks driving individual joints of a leg. In many animals, the effects of sensory feedback on the generated motor output change between posture maintenance and locomotion. These changes can occur as reflex reversals in which sensory information, that usually counteract perturbations in posture control, instead reinforce movements in walking. In stick insects, for example, flexion of the femur-tibia joint is measured by the femoral chordotonal organ, which mediates reinforcement of the stance phase motor output of the femur-tibia joint when the locomotor system is active. Flexion signals promote flexor and inhibit extensor motoneuron activity. However, the mechanisms underlying these changes are only partially understood.

Therefore, the purpose of the present thesis was to investigate whether the processing of movement and position signals of the FTi joint is task-specifically modified in the generation of adaptive leg movements, which is required when locomotion is adapted to changes in walking direction or in turning movements. To study the role of these task-dependent changes in walking behaviour on the processing of local sensory signals, the generation of reflex reversals mediated by the femoral chordotonal organ in the femur-tibia joint of the stick insect *Carausius morosus* was measured in a semi-intact walking preparation. In several experimental conditions either in front, in one or both middle or in hind legs, the femoral chordotonal organ was mechanically displaced and the motoneuronal responses in the *flexor* and *extensor tibia* were monitored, while the remaining legs performed either forward, backward or curve walking on a slippery surface.

I demonstrated that the occurrence of reflex reversals depends on the specific motor behaviour executed. While in forward walking flexion signals from the front leg fCO regularly elicit reflex reversal in the tibial motoneurons, this cannot be observed in backward walking. Similarly, during optomotor-induced curve walking, reflex reversal occurred reliably in the middle leg on the inside of the turn, however not in the contralateral leg on the outside of the turn. Thus, the experiments revealed that the nervous system modulates proprioceptive reflexes in individual legs during task-specific walking adaptation. Furthermore, I showed that nonspiking interneurons, known to be involved in the premotor network of the FTi joint, participate in reflex responses in both the inner and outer middle leg during curve walking. First results show that the reflex response in some interneuron types is altered between the inner and outer leg, while no differences were found in others.

Zusammenfassung

Zielgerichtete und verhaltensabhängige Fortbewegung setzt die Anpassung rhythmisch alternierender motoneuronaler Aktivität mittels sensorischer Rückkopplung durch Propriozeptoren und weitere Sinnesorgane voraus. Diese Signale von Bewegungs- und Belastungssensoren beeinflussen die Stärke und zeitliche Abstimmung der, von rhythmusgenerierenden Netzwerken erzeugten, neuronalen Aktivität. In vielen Tieren ändern sich die Effekte der sensorischen Rückkopplung in Abhängigkeit vom Verhaltenszustand. Diese Änderungen können als Reflexumkehrungen auftreten. Reflexe die zur Aufrechterhaltung der Positur dem sensorischen Eingang entgegenwirken, wirken im Falle der aktiven Bewegung, bei gleichem sensorischen Eingangssignal verstärkend auf den Bewegungsablauf. Dieser Mechanismus der Reflexumkehr tritt zum Beispiel im Femur-Tibia Gelenk der Stabheuschrecke auf. Während einer aktiven Beugung des Gelenks werden mittels eines propriozeptiven Sinnesorgans, dem femoralen Chordotonalorgan, Positions- und Bewegungssignale des Femur-Tibia Gelenks gemessen. Diese Beugungssignale führen dann, also bei aktiver Beugung, zur Verstärkung der Beugung und verhindern gleichzeitig die Streckung des Gelenks. Die Mechanismen die diesen Änderungen der Reflexantwort in der verhaltensabhängigen senso-motorischen Verarbeitung unterliegen, werden bisher nur teilweise verstanden.

In dieser Arbeit soll untersucht werden, ob und wie weit die verhaltensabhängige Verarbeitung sensorischer Bewegungs- und Positionssignale des femoralen Chordotonalorgans, im speziellen beim Vorwärts-, Rückwärts- und Kurvenlaufen, moduliert wird. Dazu wurde die Auftretenswahrscheinlichkeit der Reflexumkehr im Femur-Tibia Gelenk der Stabheuschrecke *Carausius morosus* in semi-intakten Präparationen untersucht. In verschiedenen experimentellen Ansätzen wurde das femorale Chordotonalorgan im Vorderbein, in einem oder beiden Mittelbeinen oder im Hinterbein mechanisch stimuliert und gleichzeitig die motoneuronale Aktivität des Femur-Tibia Gelenks gemessen, während die übrigen Beine auf einer rutschigen Oberfläche vorwärts, rückwärts oder eine Kurve liefen.

Es konnte gezeigt werden, dass es verhaltensabhängige Unterschiede in der Auftretenswahrscheinlichkeit der Reflexumkehr gibt. Während des Vorwärtslaufens im Vorderbein wurde die Reflexumkehr regelmäßig ausgelöst, im Hinterbein hingegen nicht. Ähnliches konnte während des optomotor-induzierten Kurvenlaufens gezeigt werden. Im Mittelbein, welches sich auf der Innenseite der Kurve befand, trat die Reflexumkehr signifikant häufiger als im kontralateralen Außenbein auf. Somit konnte im laufenden Tier eine Modifikation der Reflexverarbeitung während zielgerichteter, verhaltensabhängiger Lokomotion nachgewiesen werden. Außerdem konnten erste Charakterisierungen Nicht-Spikender-Interneurone, welche sowohl an der Reflexantwort im ruhenden Tier, als auch im aktiven Tier beteiligt sind, durchgeführt werden. Es wurden Hinweise darauf gefunden, dass in einigen Interneuronentypen die Reflexantwort, im Innenbein und Außenbein unterschiedlich ist und in anderen gleich ausgeführt wird.

1 Introduction

Task-dependent, active locomotion is of decisive importance for the survival of all animal species. In the course of evolution different locomotor strategies and also various locomotion systems have developed along with the diversity of species and their habitats. A wide range of terrestrial invertebrates and vertebrates have evolved highly adaptive walking gaits. Therefore, different numbers of limbs, ranging from two in humans up to 750 in myriapoda have to be adapted to different walking terrains, body postures and behavioural situations.

Rhythm generating networks, networks which mediate alternating leg muscle coordination, and networks for inter-limb coordination, which are modified by sensory and neuromodulatory influences, underlie the generation of walking. Since the beginning of the 20^{th} century, major advances have been made in the understanding of locomotion and the underlying processes that establish rhythmic motor patterns. Sir Charles Sherrington suggested that reflexes, in particular flexion and extension reflexes in spinalised quadrupeds, are integrated in the generation and control of movement, while proprioceptive sensory signals mediate phase transitions (Sherrington, 1910, 1913). At the same time, the first suggestion of an intrinsic pattern-generating mechanism, termed *half center*, arose from experiments, in which alternating muscle activities were still generated in the absence of sensory information (Brown, 1911, 1914). The idea of these functional networks was additionally underpinned by experiments, in which locomotor-like activity seen as alternating flexor and extensor activity in spinal cats was elicited by intravenous injection of L-dopa (L-3,4-dihydroxyphenylalanine) (Jankowska et al., 1967). The generation of rhythmic motor patterns was extensively studied in a variety of motor systems in vertebrates and invertebrates leading to the commonly accepted concept of central pattern generators (CPGs), neuronal networks which generate motor rhythms in the absence of descending inputs from higher centers and sensory feedback (for reviews see Bässler, 1986c; Delcomyn, 1980; Grillner, 1975, 1981, 1985; Grillner and Wallen, 1985;

Grillner and Zangger, 1979; Pearson et al., 1993; Selverston and Moulins, 1985).

Subsequently, motor patterns were found to execute different tasks with the same locomotor appendages, like walking, airstepping, scratching, and paw shake in the cat (Giuliani and Smith, 1985; Koshland and Smith, 1989; Pratt and Loeb, 1991). In chickens, the same interneuronal circuits were found to establish different behavioural tasks, like walking, scratching, and posture control (Berkinblit et al., 1978; Gelfand et al., 1988). Furthermore, in turtles (Berkowitz, 2002, 2005), similarities between scratching and swimming movements were found. Several studies in invertebrates reported a multitude of movements performed with the same consistent motor structures (stick insect: *searching, rocking, walking, grooming* (Bässler and Wegner, 1983); locust: *jumping and kicking* (Burrows, 1995; Gynther and Pearson, 1989; Hedwig and Burrows, 1996; Heitler and Burrows, 1977a,b); cricket: *flight and stridulation* (Hennig, 1990)). For example, Pflüger and Burrows (1978) demonstrated that the same motoneurons are involved in the movement generation of kicking, jumping and swimming in the locust. Interestingly, the three different motor outputs were generated similarly. The movements started with flexion of the FTi joint, followed by a co-contraction of flexor and extensor muscle and, finally, a rapid extension of the tibia. However, a major problem with the half-center organisation is that mixed-muscle synergies were found that are characterised by, at least, a partial co-activation of antagonistic muscles, which is not in conformity with the half-center model. Therefore, a more flexible modular concept was proposed, in which distinct behaviours were executed by units of a small number of interneurons or groups of functionally-related interneurons (Bässler and Büschges, 1998; Grillner, 1981; Stein and Smith, 1997). However, it is only partially understood which modules exist and what they are composed of.

Subsequently, researchers investigating such basic motor patterns found several mechanisms involved in the tuning motor patterns. First, afferent signals from the periphery are involved in the control of movement and posture, for example in chicks (Bekoff et al., 1989, 1987), in the cat (Grillner and Rossignol, 1978), in stick insects (Bässler, 1986a, 1988) and in turtles (Stein et al., 1986). An example of this is the study carried out by Bekoff and co-workers (1987) in which removing of sensory feedback from the legs results in a difference of the motor output of walking and hatching. In particular, the intralimb movements became more similar. Second, descending control signals from the brain, which is becoming an increasingly important research area, for initiation, maintenance and modulation of locomotion was studied extensively in vertebrates (*human*: Capaday et al., 1999; Gerloff et al., 1998; Petersen et al., 1998, 2001; Schubert et al., 1997; *monkey*: Eidelberg et al., 1981; Fetz and Cheney, 1980; Kobayashi and Isa, 2002; *cat*: Armstrong, 1986; Beloozerova and Sirota, 1993; Friel et al., 2007; Grillner, 1975; Lajoie and Drew, 2007; Shik and Orlovsky, 1976; *mouse*: Hagglund et al., 2010; *lamprey*: Shaw et al., 2010; Smetana et al., 2010) and invertebrates (*drosophila*: Strausfeld, 1999; Strauss, 2002; Strauss and Heisenberg, 1993; *cockroach*: Bender et al., 2010; Mu and Ritzmann, 2008a; Ridgel et al., 2007; Ritzmann et al., 2005; Schäfer and Ritzmann, 2001). For example, in cats, the medullary reticular formation is known to generate responses in limb extensors and flexors that are modulated during locomotion (Drew, 1991). In addition, recent studies in cats suggest that the posterior parietal cortex is involved in the fine-tuning of visually guided locomotion (Lajoie and Drew, 2007). Studies in insects further support the role of descending signals in the contol of locomotion. For example, in cockroaches, the central body complex (CBC) and the surrounding regions affect the control of turning (Ridgel et al., 2007). In this study, the researchers showed that lesions of the CBC or in regions immediately surrounding the CBC, results more likely in an abnormal turning behavior than lesions in other brain regions. Finally, neuromodulators play an important role in the shaping of rhythmic motor output (*pyloric rhythm*: Hooper and Marder, 1987; *feeding*: Kupfermann and Weiss, 1982; *swimming*: Sillar et al., 1998; *locomotion*: Brownstone et al., 1992; Wallen and Grillner, 1987; Zagoraiou et al., 2009). In neonatal rats and mice, recent studies using spinal cord preparations, pharmacological, and genetic approaches identified a variety of neurotransmitters and neuromodulators and, accordingly, several types of receptors involved in the control of locomotion. In a recent study, Zagoraiou and co-workers (2009) found evidence that cholinergic premotor interneurons are a defined class of intrinsic neuromodulatory neurons, which modulate the mouse locomotor activity. So far, however, these different sources of modulatory influences on the locomotor output that are known still fail to explain the generation of the tremendous flexibility in locomotor behaviour sufficiently.

The variability of the environment and the challenges animals have to overcome (such as foraging, avoidance of predators, and reproduction) requires flexibility and results in adaptive and goal-directed motor outputs that are modifiable with regard to walking speed, direction, and turning. Therefore, the output of pattern-generating networks, including the CPGs, has to be modified in the generation of different motor behaviours (reviewed in Büschges, 2005; Grillner, 1975; Marder and Bucher, 2001; Marder and Calabrese, 1996; McCrea and Rybak, 2008; Orlovsky et al., 1999; Pearson et al., 1993; Pearson, 1995a, 2004; Rossignol et al., 2006; Zehr and Duysens, 2004). In several studies on stepping in human infants, where descending supraspinal control is still in ongoing development, the flexibility of the locomotor system was studied. For example, in infants walking in various directions, mechanical disturbances elicited specific reflex responses and a modulation in their interlimb coordination (Lamb and Yang 2000; Pang and Yang, 2000, 2002; Pang and others 2003). Researchers have shown an increased interest in understanding the generation of task-dependent motor behaviour, such as forward and backward locomotion (*human*: Choi and Bastian, 2007; Pang and Yang, 2002; *cat*: Buford and Smith, 1990; *lamprey*: Islam et al., 2006; *salamander*: Ashley-Ross and Lauder, 1997; *crayfish*: Ayers and Davis, 1977), turning (*stick insect*: Dürr, 2005; Dürr and Ebeling, 2005; Gruhn et al., 2009; *cockroach*: Mu and Ritzmann, 2005; *drosophila*: Bender and Dickinson, 2006), gap-crossing (*stick insect*: Bläsing and Cruse, 2004a,b; *drosophila*: Pick and Strauss, 2005) and obstacle climbing (*cockroach*: Watson and Ritzmann, 2002; Watson et al., 2002). For example, in lampreys, it is known that during forward swimming, periodic waves of lateral body flexion propagate from head to tail. In a recent study, by Islam and co-workers (2006) it was demonstrated that during backward swimming this wave is reversed, thus forming a wave in tail-to-head direction. Similarly, during curve walking, changes in leg kinematics, step length, directions of the legs, stepping frequencies and the interleg coordination were described (Dürr, 2005; Dürr and Ebeling, 2005; Jander, 1982). These studies move the question on the underlying neural mechanisms into the focus of present neurophysiological research.

To date, several studies have provided first insights into the neuronal mechanisms underlying locomotor adaptation in vertebrates (Cheng et al., 1998; Gabriel et al., 2011; Gosgnach et al., 2006; Stein, 2005; Zagoraiou et al., 2009) and invertebrates (Akay et al., 2007; Bender et al., 2010; Briggman et al., 2005; Lockery and Kristan, 1990; Pick and Strauss, 2005; Ridgel et al., 2007; Ridgel and Ritzmann, 2005; Schäfer and Ritzmann, 2001). For example, in the control of locomotor speed, genetic and neurophysiological approaches show that V1 inhibitory spinal interneurons are involved in the frequency regulation of central pattern generated rhythm (Gosgnach et al., 2006). Also, in cockroaches, brain structures were identified that are involved in the control of locomotor speed (Bender et al., 2010). In this study, it was shown that neural activity in the central complex is correlated with the walking frequency of the cockroach and, further, that electrical stimulation in the same area could generate and alter walking (Bender et al., 2010). Another example of locomotor adaptation is a recent study of decision-making processes in the leech by Briggman and co-workers (2005). In this study it was reported that activity patterns of a small number of neurons are correlated with the leech's behavioural choice to swim or crawl. The authors successfully identified one single neuron that affected this choice by injection of a hyperpolarising or depolarising current (Briggman et al., 2005). However, there is still insufficient data to fully explain adaptive motor behaviour. One major issue in the research on locomotion concerns the role of sensory information. Changes in sensory feedback are an important component of locomotor adaptation (for review Pearson et al., 1993). Several studies have identified the role of sensory feedback, for example, in the transition from stance to swing phase in decerebrate and spinal cats. Sensory signals from Ib afferents of the Golgi tendon organ (GTO) in the ankle extensor muscle and afferent signals, measuring hip extension, mediate the transition from stance to swing at the end of the stance phase (Conway et al., 1987; Duysens and Pearson, 1980; Gossard et al., 1994; Hiebert et al., 1996; Pearson et al., 1992; Whelan et al., 1995; Whelan and Pearson, 1997). Further evidence, for phasic sensory signals being involved in the timing of phase transition is shown for insect walking (Büschges, 2005) and flight (Pearson and Ramirez, 1997).

In a variety of systems, the effects of sensory input during walking differ from those seen in postural control. Commonly, these changes occur as *reflex reversals* (Duysens et al., 2000). For example, during posture control, the GTOs of vertebrates generate reflexes (Prochazka, 1996). During walking, however, these receptors tend to amplify muscle tension in the stance phase (Pearson, 1993). Similar reflex reversals are known in other receptors of vertebrates (*human*: Duysens et al., 1990; *cat*: Forssberg et al., 1975; *rat*: Fouad and Pearson, 1997) as well as invertebrates (*crayfish*:DiCaprio and Clarac, 1981; Skorupski and Sillar, 1986; *locust*: Burrows and Pflüger, 1988; Theophilidis and Burns, 1990; Zill, 1985; *stick insect*: Bässler, 1976, 1986b, 1988). In some instances, the generation of reflex reversals depends on the phase of activity in rhythmic movements (e.g. Fouad and Pearson, 1997; Pearson and Collins, 1993; Skorupski and Sillar, 1986). For example, in crayfish, the thoracocoxal muscle receptor organ mediates reflexes that are known to activate promotor MNs when active that are, however, inhibitory when the remotor MNs are active (Skorupski and Sillar, 1986). More frequently, however, the generation of reflex reversals depends on the behavioural state of the animal (e.g. Bässler, 1988; Zill, 1985, for review see e.g. Büschges and El Manira, 1998; Clarac et al., 2000; Pearson, 1993). It is further known that locomotion patterns are extensively modified with regard to direction of progression and during visually guided stepping (Pang and Yang, 2002). So far, there is no satisfactory explanation how such changes affect the occurrence of reflex reversals, although their regulation must be part of the adaptation of walking patterns (Pang and Yang, 2002; Pearson et al., 1993).

The neural mechanisms underlying reflex reversal and the flexibility of adaptive locomotion have been extensively studied in the stick insect's walking system (for review see Bässler, 1983b; Bässler and Büschges, 1998; Büschges, 2005, 2012; Büschges and Gruhn, 2008). First studies of the walking pattern and, particularly, the generation of rhythmic leg movements in the stick insect date from the early 20^{th} century (von Buddenbrock, 1921). Nowadays, increasing knowledge with regard to the control of single leg movements and the underlying neuronal network architecture in stick insects is available. The insect leg consists of five main segments: the coxa, the trochantero-femur (which is fused in *Carausius morosus*), the tibia, and the segmented tarsus. Leg movements are mainly controlled by muscles of the thorax-coxa (ThC) joint, the coxa-trochanter (CTr) joint and the femur-tibia (FTi) joint. The muscles of the ThC joint move the leg forwards by the *protractor coxae* muscle and backwards by the *retractor coxae* muscle. The *levator* and *depressor trochanteris* muscles elevate and depress the leg in the CTr joint and the flexion and extension of the FTi joint is mediated by the *flexor* and *extensor tibiae* muscles (see Graham and Epstein, 1985. These antagonistic muscle pairs are alternately-active during the generation of a step, which can be divided into a stance and a swing phase. The transition between stance and swing phase is controlled by signals from a variety of leg sense organs, like hair fields, the campaniform sensilla and the femoral chordotonal organ (for review see Büschges, 2005, 2012).

Movements of the leg segments are measured by hair plates (fields) on the leg joints, particularly of the CTr joint. These hair fields consist of groups of hair sensilla that measure position and movement of the joint. The ventral coxal hair plate (vcxHP) comprises two groups of hair cells, group G1 and G2, which are located ventrally on the coxa and detect position and movement of the ThC joint (Büschges and Schmitz, 1991; Cruse, 1985b; Dean and Schmitz, 1992). The trochanteral hair plate (trHP) is situated on the dorsal side of the trochanter and measures the CTr joint position (Schmitz, 1986b). In addition, the rhombic hair plate (rHP) on the ventral trochanter was described by Tartar (1976) and Schmitz (1986a,b). Furthermore, the levator receptor organ, an internal sense organ, located inside the coxa parallel to the *levator trochanteris* muscle, detects movements of the trochanter (Schmitz and Schöwerling, 1992). It measures the length change of the *levator trochanteris* muscle and acts similar to strand receptors in the locust (Bräunig and Hustert, 1985a,b). Also, the campaniform sensilla (CS) are important leg sensors, which signal load information of the leg (Delcomyn, 1991; Hofmann and Bässler, 1982) and cuticular stress (Hofmann and Bässler, 1982; Pringle, 1938). To date, there are four CS groups known on the proximal leg joints. One is located on the proximal femur (fCS- femoral campaniform sensilla) and three are located on the trochanter (trCS- trochanteral campaniform sensilla). Akay and co-workers (2001) found that sensory signals of the fCS influence the activity of FTi joint motoneurons. Several studies revealed that signals of the fCS and trCS modifies the motor output of the CTr and ThC joint (Akay et al., 2004, 2007; Schmitz, 1993). Recently, Zill et al. (2011) identified two groups of campaniform sensilla, group 6A and 6B located distally from the FTi joint.

In addition to these groups of exteroceptors, a further sensory proprioceptor, the femoral chordotonal organ (fCO), is important for the control of leg movement and position. The fCO is located dorsally in the proximal part of the femur (Bässler, 1972; Bässler and Büschges, 1998; Field and Matheson, 1998; Kittmann and Schmitz, 1992). It extends through the femur with a thin receptor tendon and its distal end attaches to the tibia. It consists of two parts, of which the dorsal part contains more than 400 sensory cells and the ventral part more than 80 (Füller and Ernst, 1973). Füller and Ernst (1973) described the fine structure of the fCO and were able to show that every scolopidium comprises two sensory cells, one scolopale cell, a fiber cell, and at least one sheath cell. The sensory cells of the dorsal part of the fCO measure and control position, velocity, and acceleration of the FTi joint and combinations of these signals (Büschges, 1994a; Hofmann and Koch, 1985; Hofmann et al., 1985). In contrast, the fCO cells of the ventral part are not involved in the adjustment of the FTi joint (Field and Pflüger, 1989; Kittmann and Schmitz, 1992). The influence of sensory signals from the fCO has been studied under various experimental conditions and is known to produce resistance reflexes in resting animals that function in postural compensation (Bässler et al., 1974; for summary see Bässler, 1993). These reflexes have been shown to change when the animal generates active leg movements (Bässler, 1976, 1988). In such cases, afferents of the fCO that signal joint flexion also inhibit extensor firing and assist the generation of flexor activity. These changes represent a reflex reversal and occur as the first part of a sequence of muscle activities termed the *active reaction* (*AR*; Bässler, 1988). Together with inter-joint influences of the fCO on motoneurons of the tarsus, the active reaction appears to assist the generation of stance and the subsequent transition to leg swing (Bässler, 1988). It was also found that inter-joint reflexes were mediated by the fCO. It has been shown that sensory signals from the fCO in the inactive and active, but stationary, animal influence muscle activity of the adjacent CTr joint (Hess and Büschges, 1997, 1999).

Several studies have examined the underlying neural elements within the local premotor network that mediate both the resistance and assistance reflexes of the fCO in a distributed fashion (for review see Bässler and Büschges, 1998; Büschges and El Manira, 1998; Büschges and Gruhn, 2008). However, no information is currently available on how the reflex reversal is initiated and regulated *in vivo* during walking and, particularly, while performing task-dependent walking, such as curve or forward and backward walking when leg movement kinematics are specifically modified to the movement task to be executed. Several studies have demonstrated effects of descending input upon local reflexes in insects (Knop et al., 2001; Mu and Ritzmann, 2008a; Ridgel et al., 2007). However, these experiments were not performed in animals that were actually walking.

In the present thesis, I will present evidence for the task-specific modulation of a proprioceptive reflex in walking stick insects as the result of neuronal adaptation processes to specific walking tasks. The results section of my thesis is divided into two main parts. The first part addresses the modification of reflexes mediated by the fCO during forward and backward walking. The second part focuses on the adaptation during curve walking.

The first part of the results addresses the following questions:

1. Is there any difference in the segmental processing of fCO information during forward and backward walking, in particular between the front and hind leg?
2. How does sensory load and position information influence the adaptation processes in the FTi joint in forward and backward walking?
3. What are the kinematic differences in the front and hind legs of forward and backward walking stick insects?

4. Are the inter-joint reflexes from the fCO on the CTr joint affected by a change in walking direction (i.e., forward and backward walking)?

The second part of the results addressed two questions:

1. Is there any difference in the task-dependent processing of fCO information during curve walking?
2. How are these signals encoded in the premotor network of the FTi joint, in particular, in the responses of the membrane potential of nonspiking interneurons caused by fCO stimulation during curve walking?

2 Materials and Methods

2.1 Animals

Experiments were performed on adult female stick insects of the species *Carausius morosus*, Brunner (Phasmatodea) at room temperature ($20 - 22°C$) under reduced light conditions. Animals were obtained from a breeding colony maintained by the University of Cologne, Germany. The colony was kept under constant conditions at temperatures between 20°C and 25°C, high humidity ($55 - 70\%$) and under an artificial $12 : 12$ hours light/dark cycle. Animals were fed with blackberry leaves *(Rubus fructiosus)*. The experimental procedures reported in the study comply with the German national and state regulations for animal welfare and animal experiments.

2.2 Preparations and experimental design

Positioning of the experimental animal for walking and sensory stimulation

All experiments were performed on an air table (MICRO-g, TMC, Peabody, MA, USA) surrounded by a Faraday cage. Experimental animals were positioned above a plate (acrylic glass) at a height of about 8 - 12 mm to establish resting angles of the femur-tibia joints (FTi) in the middle and hind legs of roughly 90° (Fig. 2.1, Epstein and Graham, 1983; Graham and Wendler, 1981; Gruhn et al., 2006, 2011). To ensure free stepping movements of the tethered stick insect and to reduce the mechanical coupling between legs via ground contact, the surface of the plate was made slippery by covering it with a glycerine/water mix (95%/ 5%). Animals were fixated dorsal-side-up on a foam-covered metal rod with dental cement (ProTempII, 3M ESPE, Seefeld, Germany) applied to the meso- and metathorax (Fig. 2.1). The leg that was to be investigated was glued to an extension of the rod. Coxa and femur of this leg were immobilized and the tibia protruded over the edge of the rod extension with a fixed FTi joint angle of approximately 110°. Depending on the experimental setup, either the front, middle or hind leg was

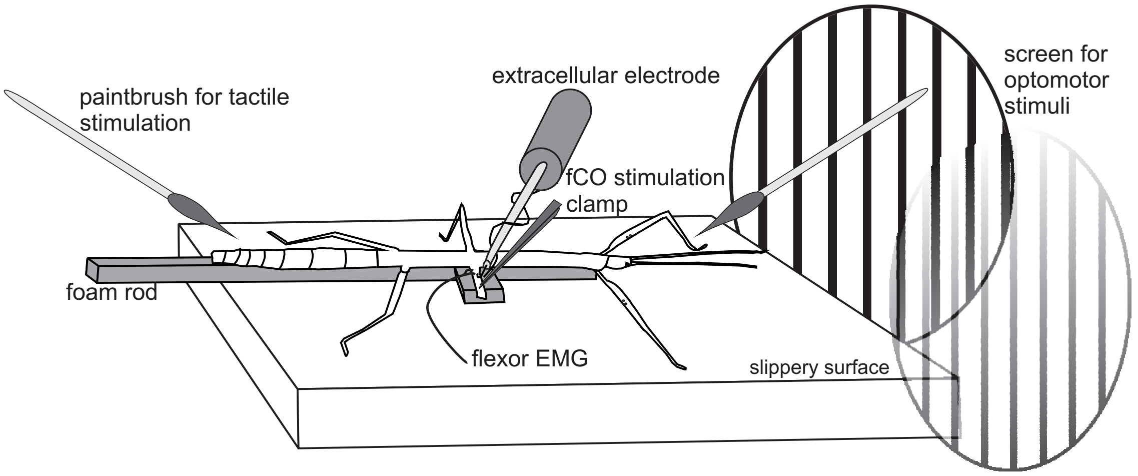

Figure 2.1: Preparation for studying reflex reversals in stick insect walking. The stick insect is mounted on a foam-covered metal rod above a slippery surface. Mechanical stimuli that mimic femorotibial (FTi) joint flexion are applied to the femoral chordotonal organ (fCO) of either the front, middle (shown here) or hind leg. Reflex responses to fCO stimuli are monitored in recordings of the femoral nerve 2 (F2), which innervates the tibial extensor (tib ext) and in an electromyogram (EMG) of the tibial flexor muscle. Forward or backward walking was induced by a mechanical stimulation on the antennae or the abdomen. Turning movements were induced by the display of optomotor stimuli. (Taken from Hellekes et al., 2012).

immobilised. When immobilised, the front leg was fixed at a position of 45° anterior with respect to the body axis, middle legs at 90° (Fig. 2.1) and the hind leg at 45° either anterior or posterior. At the same time, all other legs were free to move. In some experiments, the trochanteral and the femoral campaniform sensilla (trCS, fCS) were ablated by pushing an insect pin through the cuticle at the location of the CS (Schmitz, 1993). For better clarity, the different experimental conditions are displayed as simplified sketches in the results section, indicating the walking direction as well as the leg on which the fCO stimulation and the nerve recordings were performed.

Positioning of the experimental animal for walking and kinematic monitoring

In a further experimental study to understand the kinematics of the front and hind leg during forward and backward walking the animal was also fixated dorsal-side-up onto a foam-covered metal rod by means of dental cement and positioned above a slippery surface. Here, all legs were free to move (Fig. 2.2). The animal's body axis and the inspected leg were marked with dots of fluorescent dye. For this purpose, fluorescent pigments (Dr. Kremer Farbmühle, Aichstetten, Germany) were mixed with dental ce-

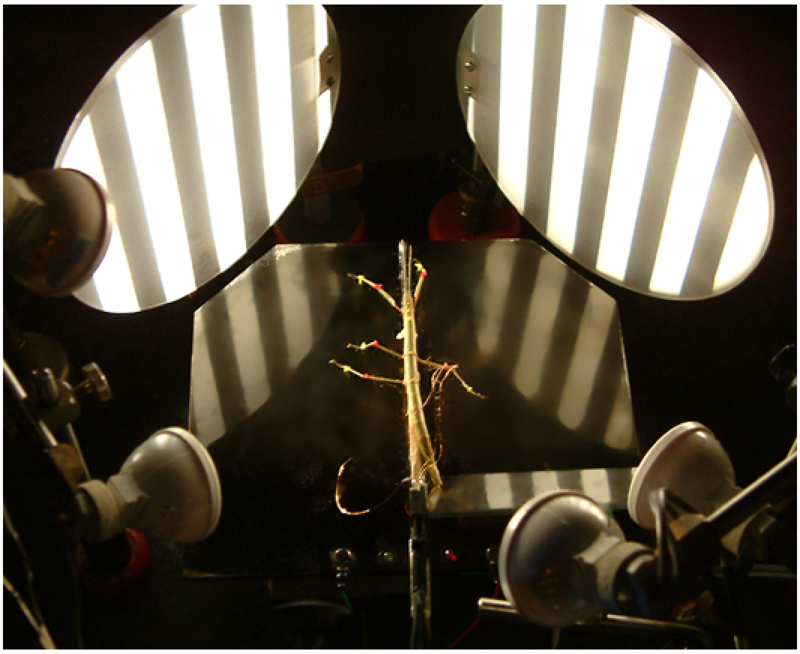

Figure 2.2: Preparation for studying stance phase kinematics of front and hind legs in stick insect forward and backward walking. The stick insect is mounted on a foam-covered metal rod above a slippery surface. Femur and tibia are marked with fluorescent pigments for leg tracking. The *flexor* and *extensor tibiae* muscle activity and the tarsal contact were measured. Forward or backward walking was induced by optomotor stimuli or mechanical stimulation on the antennae or the abdomen. (Taken, with permission, from Gruhn et al., 2011)

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ment and applied on the distal tibia, the distal femur, the head, and the pro-, meso- and metathorax. An externally triggered high-speed video camera (Marlin F-033C, Allied Vision Technologies, Stadtroda, Germany) recorded the walking animal at 100 fps (frames per second) from above. For an additional sideward view, mirrors were placed in a 45° position, either in front of the front leg or behind the hind leg, depending on the observed leg. The fluorescent markers were illuminated with arrays of blue LEDs (30 - 50 V DC, luminance 24 cd, Electronics Workshop, Zoological Institute, University of Cologne). The experiments were performed under low-light conditions. A yellow filter in front of the camera lens filtered out short wavelengths to ensure a higher contrast for the video recordings. Furthermore, to monitor leg ground contact, a tarsal contact signal was recorded. For this purpose, a current flow was measured between the tarsus and the slippery surface plate. A small voltage (2.4 mV) was applied to the slippery surface by a pulse generator (Model MS501, Electronics Workshop, Zoological Institute, University of Cologne). An isolated copper wire (47 μm diameter) was attached to the tibia and connected with a differential amplifier via an alligator clamp. The copper insulation was stripped and electrode cream (Marquette Hellige, Freiburg, Germany) was used on the contact points to allow for proper current conduction. When the tarsus touched the plate the electric circuit was closed and the current flow indicated stance phase. In contrast, when the leg did not have any ground contact the circuit was opened and no current flow was measured indicating swing phase.

Induction of stepping in different directions

Forward walking was induced by tactile stimulation of the animal's abdomen with a small paint brush (Fig. 2.1). Backward walking was elicited by tactile stimulation of the head or by pulling manually on the antennae (Graham, 1985). Once stepping was initiated, tactile stimulation was stopped. An animal was considered to be walking forward or backward when the unrestrained legs showed forward or backward stepping movement. In many of the backward walking experiments, walking activity was also monitored by myographic recordings of the *levator trochanteris* and the *retractor coxae* muscles of the middle leg to distinguish the walking direction by the different phase relationships in forward and backward walking (Graham, 1985). Curve walking was induced by optomotor stimulation. To this end, a vertical black-and-white stripe pattern was projected on two screens, placed laterally in front of the stick insect (Scharstein, 1989). To elicit curve walking in the stick insects, the stripes on both screens moved either to the right or to the left (Fig. 2.1, 2.2, Gruhn et al., 2011). Corresponding to the location of the leg, where the fCO stimulation was performed, and relative to the turning direction, the extremities are denoted as inner legs and outer legs throughout this thesis). In the kinematic studies, optomotor stimulation was also used to elicit forward walking, by progressive forward-directed stripe patterns.

Preparation for fCO stimulation

A small opening was cut dorsally into the femoral cuticle, allowing for mechanical stimulation of the fCO and for extracellular recordings from tibial muscles and motor nerves (Fig. 2.3, Büschges, 1989). The leg cavity was filled with saline (Weidler and Diecke, 1969), the apodeme of the fCO was cut and the distal ending attached to a moveable

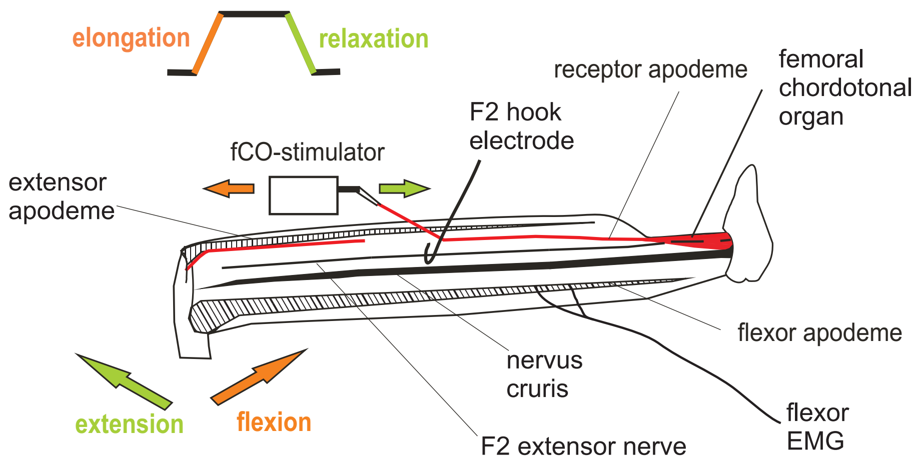

Figure 2.3: Leg anatomy and preparation for fCO stimulation, nerve and muscle recordings. The leg was opened dorsally and the receptor apodeme of the fCO was attached to a moveable clamp controlled by a linear motor. Mechanical displacements of the apodeme, like an elongation (red arrow) corresponds to upward ramp movement (red) and simulates a leg flexion (red arrow). Relaxation of the receptor apodeme (green arrow) corresponds to downward ramp stimuli (green) and mimics a leg extension (green arrow). Simultaneously, the extensor activity was measured by extracellular recording of the F2 extensor nerve with a hook electrode and flexor tibiae muscle activity by a flexor muscle electromyogram (EMG).

clamp controlled by a linear motor. Mechanical displacement of the apodeme parallel to the leg towards or away from the body were produced by applying voltages to the motor by a stimulus generator (Electronics Workshop, Zoological Institute, University of Cologne). The fCO was stimulated with ramp-and-hold stimuli which produced displacements of 300 - 400 μm (sometimes also higher displacements of up to 670 μm) from starting position. These displacements correspond to FTi joint angles (inner angle) from 110° - 60° or rather from 110° - 80° (Weiland et al., 1986).

Preparation for extracellular and intracellular recordings

For experiments investigating the influence of walking direction on the inter-joint processing of fCO signals in the ThC joint, the coxa nerve branch 1 (C1), which innervates the *levator trochanteris*, was recorded extracellularly in the mesothorax. In additional experiments to investigate the processing of fCO signals during curve walking, intracellular recordings of different neurons in the mesothoracic ganglion were performed. For both experiments, the animal was prepared as previously described. Additionally, the thorax was opened dorsally by a midline incision to gain access to the ganglion or to the C1 nerve (Fig. 2.4). The thorax cavity was filled with stick insect saline (Weidler and Diecke, 1969). The gut was placed intact besides the animal, and fat and connective tissue was removed. For the intracellular recordings, the ganglion was placed on a wax-coated holder (see also Büschges, 1989, 1990. In order to prevent movement of the ganglion, it was fixed with small cactus needles (*Nopalea dejecta*). The surrounding tissue was removed in the area of the intracellular recording. Afterwards, this region was treated for 60 s with a proteolytic enzyme (Pronase E, Merck, Darmstadt, Germany) to facilitate electrode penetration through the ganglion sheath. Finally, the enzyme was washed out by repeated rinsing with saline (Weidler and Diecke, 1969) and the thorax cavity was filled with saline.

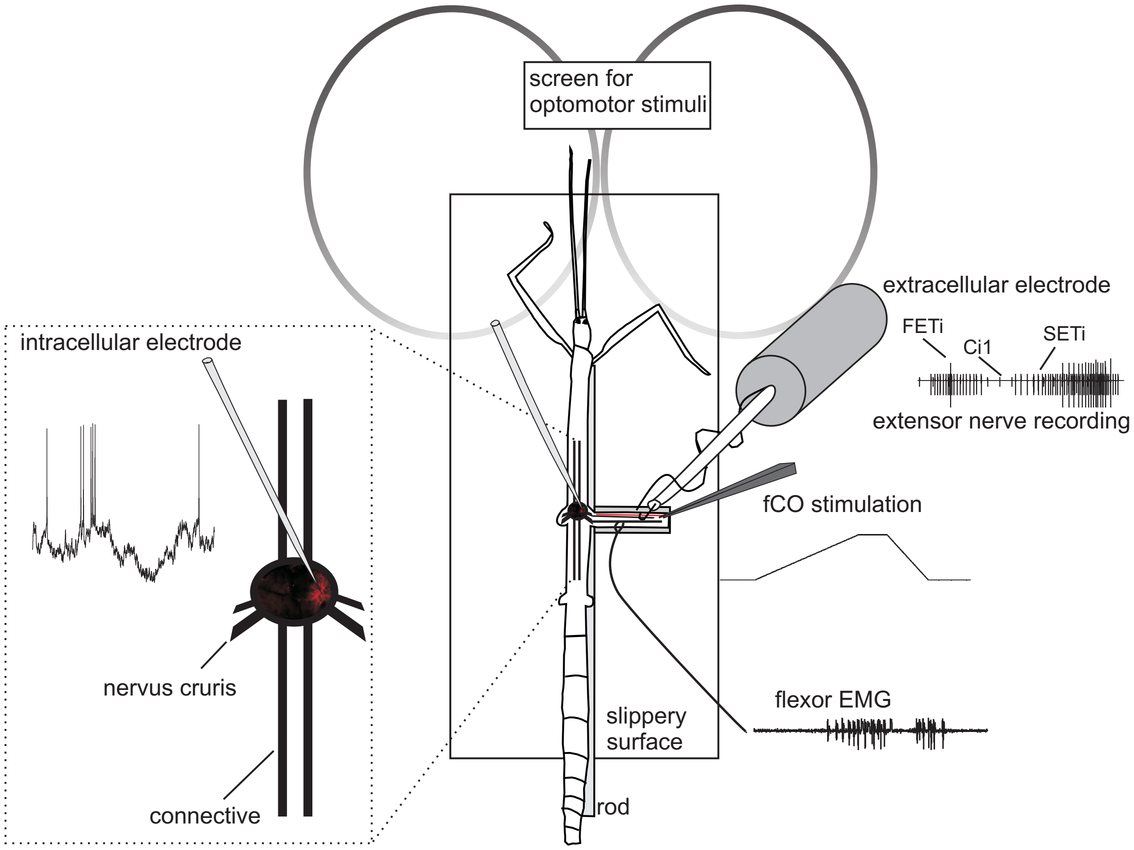

Figure 2.4: Preparation for studying fCO-mediated reflex responses in the FTi joint premotor network. Shown are the used recording devices and typical time courses of the acquired signals. Additionally to the preparation for extracellular analyses (shown in Fig. 2.1), the mesothoracic thorax was opened and intracellular recordings were obtained in the mesothoracic ganglion ipsilateral to the fCO stimulation. Extracellular recordings were performed by using myographic recordings (electromyogram, EMG) of the flexor muscle and extracellular extensor nerve recordings. In the extensor nerve recording, two excitatory motoneurons, the fast (FETi) and slow (SETi) *extensor tibiae* MNs and one inhibitory MN, the common inhibitor 1 (CI1) are identifiable.

2.3 Electrophysiology

Extracellular Recordings

Electromyograms (EMGs)

The activity of the *flexor tibiae* muscles in the fixated leg was monitored via an electromyogram (EMG; Fig. 2.1, 2.3, 2.4, e.g. Gruhn et al., 2011; Rosenbaum et al., 2010). The tips of two twisted copper wires (Elektrisola, Eckernhagen, Germany; 47 μm outer diameter), insulated except for the tip, were inserted into small holes of the cuticle (transfixed by insect minuten pins) in the ventral femur. In some experiments, flexor muscle activities were measured in the proximal and in the distal part of the ventral femur. The wires were fixated with small drops of dental cement. A silver wire was inserted into the abdomen to obtain a reference (indifferential) signal. Wires for EMG electrodes of the *retractor coxae* were placed inside the thorax anterior to the leg and for the *levator trochanteris* EMGs dorsally on the anterior side of the coxa.

Extracellular nerve recordings

The activity of the *extensor tibiae* motoneurons was recorded extracellularly from the femoral branch 2 (F2). The nerve was placed on a hook electrode (modified after Schmitz et al., 1988) and insulated with silicone oil (Baysilone-Paste mittelviskos, Bayer AG, Leverkusen, Germany). In further experiments, the motoneuronal activity of the *levator trochanteris* was recorded extracellularly via hook electrodes in the thorax from the coxa branch 1 (C1).

Intracellular recordings

Intracellular recordings were obtained from neurons involved in the femur tibia control loop. In the present thesis, only recordings from nonspiking interneurons (NSI), as well as flexor and extensor motoneurons (MNs) are shown. The recordings were collected with thin-walled borosilicate glass microelectrodes (GB100-TF8P, Science Products, Hofheim, Germany) from the neuropilar aborisations in the mesothoracic hemiganglion, ipsilaterally to the fCO stimulation (Fig. 2.4). The microelectrodes were pulled on a P-1000 filament puller (Flaming/ Brown Micropipette Puller, Sutter Instruments, Novato, USA) and filled with a solution of 3 M potassium acetate and 0.1 M potassium chloride (3 M KAc / 100 mM KCl) and had electrode resistances between 15-25 MΩ. Signals were amplified with an SEC-05 amplifier (NPI Electronics, Tamm, Germany) in bridge mode (switching frequency 12-25 kHz). The *extensor tibiae* MNs were identified by a one-to-one relationship of intracellularly recorded spikes compared with spikes in the extracellular F2-nerve recording. *Flexor tibiae* MNs were identified by a one-to-one relationship between the intracellular spikes and the flexor muscle potentials in the flexor EMG. Interneurons were identified as nonspiking interneurons if they were in accordance with the following six criteria (see also Burrows, 1981; Büschges, 1990; Hengstenberg, 1977; Siegler, 1985; Wilson, 1981: no generation of spike by 1) fCO stimulation, 2) unspecific tactile stimulation, 3) change of the behavioural state of the animal (Bässler, 1988), 4) after a long and large hyperpolarisation, 5) during depolarisation despite an increase of the amplitude of the EPSPs, and 6) also during graded effects on the activity of postsynaptic MNs. Furthermore, the identification of the different NSIs was accomplished by their characteristic responses to fCO stimulation and either their excitatory or inhibitory effect on extensor MNs activity (see also Akay, 2002; Büschges, 1990; Stein and Sauer, 1998). Recordings without any stable resting membrane potential were discarded.

2.4 Data recording and evaluation

Both the intra- and the extracellularly recorded signals were amplified 100-fold by a pre-amplifier (Electronics Workshop, Zoological Institute, University of Cologne). Subsequently, the extracellular recordings were amplified 10-fold and band-pass filtered (nerve recordings 300 Hz - 4.5 kHz / EMG recordings 30 Hz - 2 kHz) (4-Channel Amplifier / Signal Conditioner ModelMA102, Electronics Workshop, Zoological Institute, University of Cologne). The voltage output of the fCO stimulator and the electrophysiological signals were digitised using an A/D converter (MICRO 1401k II, CED, Cambridge, UK) and recorded with a sampling rate between 6.25 kHz and 12.5 kHz with SPIKE2 (data acquisition and analysis software; version 7.01; Cambridge Electronic Design, Cambridge, UK) on a personal computer (operating system: Microsoft Windows 7). Video files were analysed using motion-tracking software (WINanalyze, Vers.1.9, Mikromak Service, Berlin, Germany).

Analysis of extracellular recordings

To analyse extracellular nerve recordings, peristimulus time histograms (PSTHs) were generated. For this purpose, spikes were counted from 1 s before stimulus onset (fCO elongation) to 2 - 3 s after stimulus onset over a certain number of stimulations. To normalise the data, the counted spikes in each bin were divided by the total number of stimulus events. To evaluate the flexor muscle activities, EMG recordings were rectified and smoothed (time constant 1 ms) and averages of the waveforms were generated. The averages include the EMG signals also from 1 s before onset of fCO elongation up to 2 - 3 s after onset of the fCO elongation. With regard to differences between the location of the EMG, as well as differences of recording quality, the flexor activity was normalised between forward and backward walking in each animal and the relative change in the muscle activity is given by the here defined arbitrary units (a.u.).

Analysis of intracellular recordings

To examine the reflex responses induced by fCO stimulation on the level of the involved motoneurons and the premotor network, overdraws of the neuron membrane potential were created for all stimulations in the inner and outer leg during curve walking. Furthermore, the changes in membrane potential during reflex reversals were analysed by waveform averages. For averaging of the MN membrane potential, spikes were removed from the intracellular recordings and replaced by a straight line (maximal5 ms before and after the peak of the spike).

Statistical analysis

To determine the reliability of the frequency of reflex reversal, 95% confidence intervals for the different experimental situations were defined (Hayes, 1988). If the confidence intervals of the mean values do not overlap, the differences between the processing of fCO signals in the compared data are statistically significant. For further evaluation also between different experimental conditions, the differences in the frequency of reflex reversal were tested with (2×2) contingency tables, conducting either a one-sided or two-sided analysis depending on the test hypothesis. The Pearson's chi-squared test was used, if the sample size was > 60 with the expectancy value > 5, otherwise the Fisher's test was used (Agresti, 1992; Sachs, 1972). P-values of all combinations between the frequency of reflex reversal in different legs during forward as well as during backward walking were determined. Furthermore, the p-values for the differences in reflex reversal frequencies between forward and backward walking in the same leg and between the inner and outer leg during curve walking were calculated. The statistical significance is indicated as follows: (n.s.) not significant $p > 0.05$; $(^{*})p \leq 0.05$; $(^{**})p \leq 0.01$; $(^{***})p \leq 0.001$. Evaluation of the data and plotting of the graphs were performed with Matlab R2011b, Origin Pro 8.5G and Corel Draw X4. In the text and figures, N refers to the number of animals, and n refers to the sample size (steps or stimuli).

3 Results

3.1 Forward and backward walking

Converging evidence from a variety of animals suggests that reinforcement of movement is one important mechanism by which sensory feedback contributes to the generation of the motor output for walking (for reviews see Büschges, 2005; Clarac et al., 2000). For example, in stick insects, flexion of the femur-tibia (FTi) joint is measured by the femoral chordotonal organ (fCO). The fCO is known to reinforce stance phase motor output of the FTi joint when the locomotor system is active (Bässler, 1988). In active stick insects, reinforcement of flexor activity reflects the reflex reversal of a strong resistance reflex (RR). During this resistance reflex fCO elongation (indicating FTi joint flexion) excites extensor MNs and inhibits flexor MNs in resting stick insects (Bässler, 1983a). In active animals, the same elongation of the fCO reverses this reflex, seen as excitation in the *flexor tibiae* motoneurons (MNs) and as inhibition in the *extensor tibiae* MNs. This reflex reversal represents the first part of the so-called *active reaction (AR)* (Bässler, 1988). When the chordotonal organ signals a certain flexed-joint position, the *extensor tibiae* MNs are strongly excited and the *flexor tibiae* activity decreases (part II of the AR) (Bässler, 1976, 1983b, 1986a, 1988). The transition between part I and part II of the active reaction is independent of velocity, yet position-dependent and assumed to contribute to the stance-swing transition during walking. Furthermore, it is known that load signals from the femoral campaniform sensilla (fCS) inhibit *extensor tibiae* MNs, activate *flexor tibiae* MNs, and also increase the occurrence of the AR (Akay et al., 2001; Akay and Büschges, 2006). Akay and co-workers (2007) have recently shown that the influence of the CS signals on the thorax-coxa (ThC) joint is reversed in backward versus forward walking, and thereby assists the generation of stance phase muscles activity in both walking directions. With regard to the walking direction, it is also important to mention that the individual legs, in particular front and hind legs, show different leg kinematics during forward and backward walking. Cruse and Bartling (1995) have described that during forward walking, the FTi joint angle in the front leg decreases during stance phase and increases during swing phase. In the hind leg, a functional reversal was observed. This supports the assumption that stance and swing phase in the front and hind legs are mediated by different activities of leg muscles. One major objective of the present study was to investigate, if the processing of movement-related fCO signals in the individual legs differs during forward and backward walking. Furthermore, this study attempts to determine, if the processing of fCO signals is segment-specific. Therefore, the experiments were conducted separately in the front, middle and hind leg. Finally, a better understanding of the kinematics during forward and backward walking is anticipated, to correlate the sensory processing to the actual motor behaviour. Accordingly, the kinematics of front and hind leg stance phases was studied for both walking directions.

3.1.1 Influence of fCO signals on tibial MN activity in the front leg

To test whether the generation of reflex reversal depends on the walking direction, an experimental setup was used that allowed inducing animals to forward or backward walking by applying mechanical stimuli to the abdomen or antennae, respectively (Rosenbaum, Wosnitza et al., 2010). In general, forward walking was elicited more readily than backward walking. Sometimes, a puff of breath or a slight touch on the abdomen was sufficient to elicit long-lasting forward walking periods. In contrast, in almost all cases of backward walking, a continuous stimulation of the antennae was necessary to maintain sustained stepping. In stick insects walking with five legs either forward or backward on the slippery surface, the front leg fCO was stimulated, while the mesothoracic tibial motoneuron activity was monitored (Fig. 3.1). In general, backward walking was characterised by a higher ongoing activity in tibial extensor motoneurons compared to forward walking. At the same time, *flexor tibiae* activity was diminished (Fig. 3.1 A, B). During forward walking, elongation of the prothoracic fCO elicited a reflex reversal: extensor motoneuron activity was terminated by imposed FTi joint flexion, while flexor motoneuron activity and common inhibitor 1 (CI1) activity was initiated (Fig. 3.1 A, C). On the contrary, reflex reversals were less often observed during backward walking (Fig. 3.1 A,

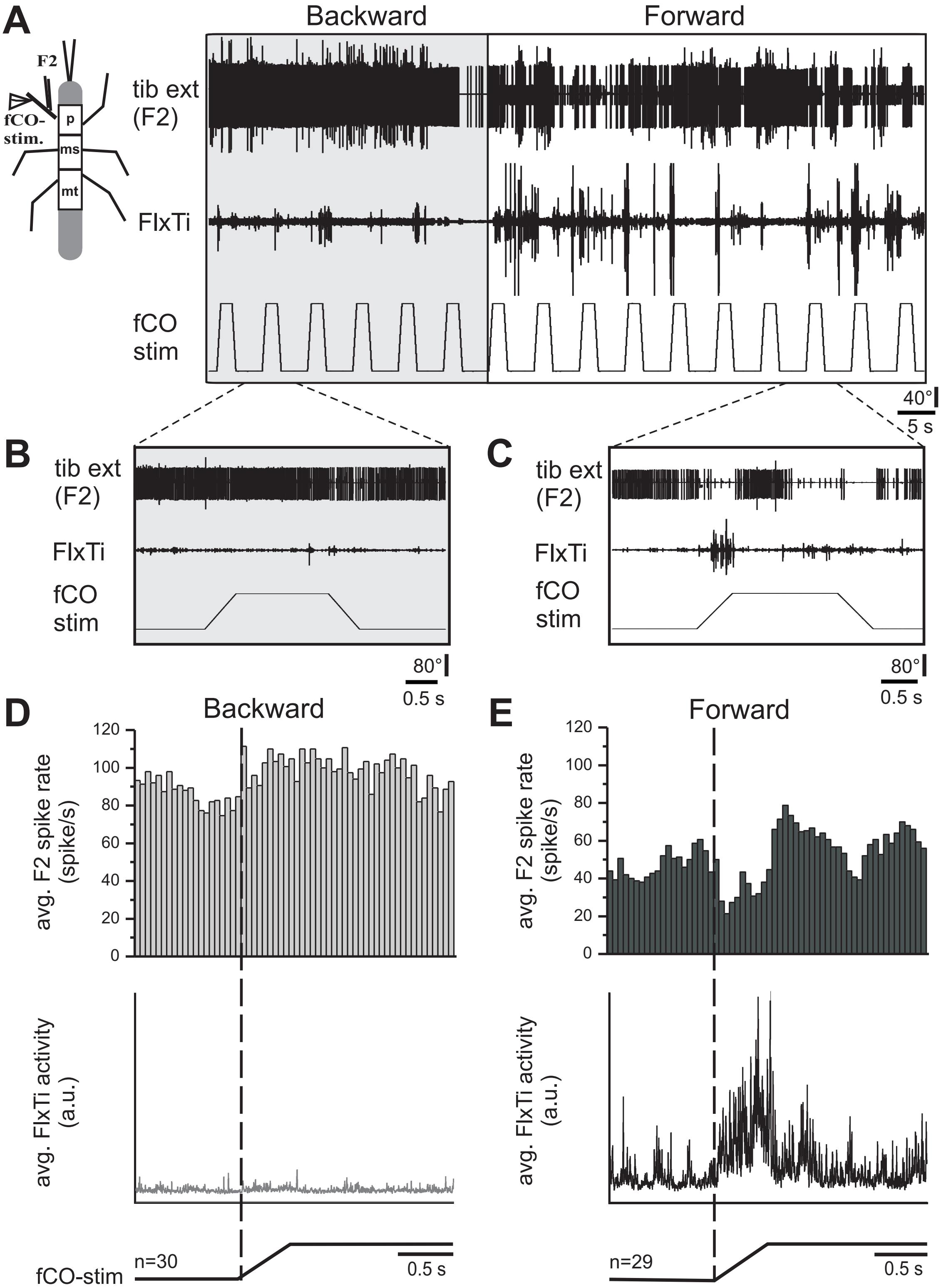

Figure 3.1: (A) Influence of fCO signals on the tibial MN activity in the front leg during forward and backward walking. The femoral chordotonal organ of the front leg was displaced (fCO stim) while monitoring the tibial extensor (tib ext (F2)) and flexor muscle activities (Flx Ti) in that leg. In the shown sequence, the animal was walking backward and then forward. Reflex reversals occurred during forward walking. B) and C) Expanded traces of responses during backward (B) and forward (C) walking. D) and E) Peri-stimulus time histograms of the firing frequency of the tibial extensor motoneurons (top) and rectified waveform averages of the flexor muscle activity (bottom) during fCO stimulation in the front leg during backward (D) and forward (E) walking (D: n=30; E: n=30, data from one animal).

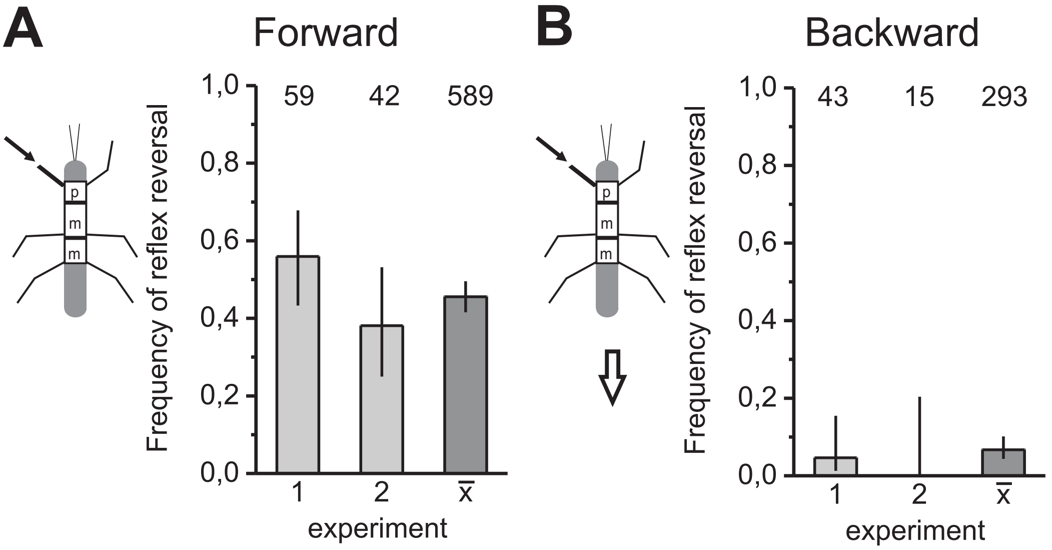

Figure 3.2: Reflex reversals in the front leg depend on walking direction. Bar histograms show the frequency of reflex reversals in the front leg's tibial muscles during displacement of the front leg fCO in animals that were walking forward (A) and backward (B). Each histogram shows two typical experiments (light bars) and the mean values of all experiments (dark bars; N=17). Reflex reversals occurred during forward, however, not during backward walking. Arrows indicate walking direction; lines designate 95% confidence intervals.

B). Stimuli applied to the fCO during backward walking did not show any reliable influence on the extensor activity (Fig. 3.1 A, B). This difference in forward and backward walking becomes evident in the PST-histograms of the averaged F2 extensor nerve activity (Fig. 3.1 D, backward; Fig. 3.1 E, forward). In backward walking stick insects the extensor firing frequency was, in general, enhanced and slightly increased caused by the fCO elongation (Fig. 3.1 D, top). Simultaneously, the flexor activity remained low and no change in response to fCO stimulation was found (Fig. 3.1 D, bottom).

Additionally, to examine the differences in the processing of fCO signals in the front leg of forward and backward walking stick insects, the frequency of reflex reversals was quantified for both walking directions. Reflex reversals were generated in front legs of forward walking animals in 45.5% of trials (N=17, n=589; Fig. 3.2 A), compared to 6.7% during backward walking ($N = 17$; $n = 293$; Fig. 3.2 B). In 16 of the 17 animals, a higher frequency of reflex reversals in forward walking than in backward walking was found (two typical experiments are displayed in Fig. 3.2 A, B). The frequency of occurrence of reflex reversals differed significantly between forward and backward walking in 10 of 17 animals (Fisher's exact test).

3.1.2 Influence of fCO signals on tibial MN activity in the middle leg

In order to investigate the segment specificity of the processing of fCO signals during forward and backward walking, in the following experiments the mesothoracic fCO was stimulated and the tibial motoneuronal activities were detected. In middle legs, the different influences of forward and backward walking on the processing of fCO signals were unincisive. Both, in the forward walking and in the backward walking stick insect, reflex reversals were elicited (Fig. 3.3 A, B, C). The enlarged view of one stimulus for each walking direction, highlights the reflex reversal during fCO elongation (Fig. 3.3 B, C). Although the inhibition in the F2 extensor nerve upon fCO elongation in this particular animal becomes more obvious during forward walking (Fig. 3.3 E, PSTH), the decrease in the averaged F2 spike activity is also identifiable during backward walking (Fig. 3.3 D, PSTH). Moreover, during forward and backward walking, the averaged *flexor tibiae* muscle activities increased during fCO elongation and, therefore, revealed the activity pattern of a reflex reversal (Fig. 3.3 D, E).

The frequency of reflex reversals in the bar histograms in exemplary animals reached a quite similar level in both walking directions (Fig. 3.4 A, B). The mean frequency of reflex reversals in forward walking (48.7%) is approximately 10% higher than in backward walking (34.4%). However, the overlap of the 95% confidence intervals indicates that the observed numerical difference is, in fact, not statically significant. In total, only one of seven animals showed a significant difference in the frequency of reflex reversal between forward and backward walking. Five of seven animals showed a higher frequency of reflex reversals during forward walking and two during backward walking. In summary, in the middle leg, only in rare cases an influence of walking direction on the probability of reflex reversals upon fCO stimulation was found.

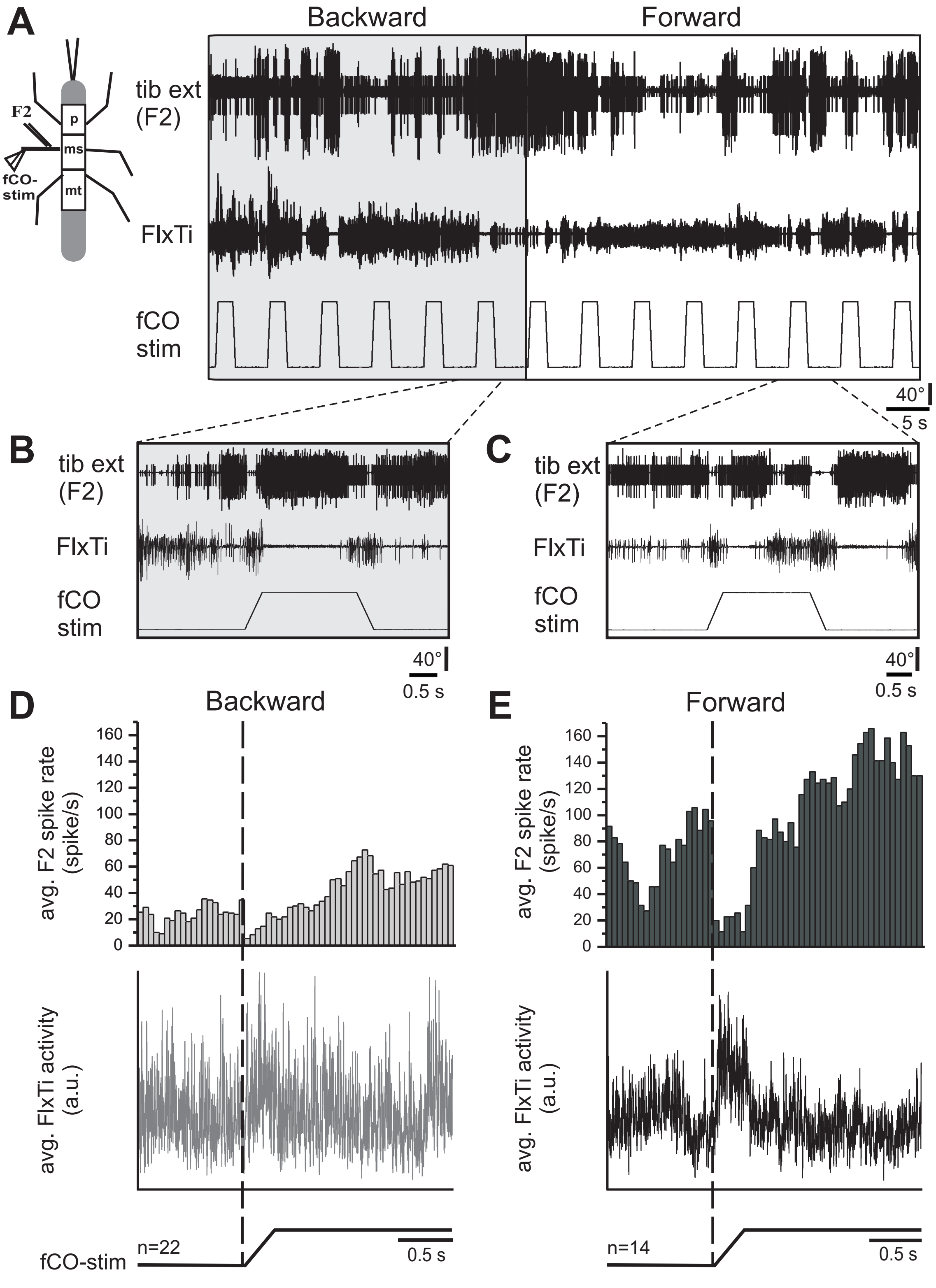

Figure 3.3: (A) Influence of fCO signals on the tibial MN activity in the middle leg during forward and backward walking. The femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor (tib ext (F2)) and flexor muscle activities (Flx Ti) in that leg. In this sequence, the animal was walking backward and then forward. Reflex reversals occurred during forward and backward walking. B) and C) Expanded traces of responses during backward (B) and forward (C) walking. D) and E) Peri-stimulus time histograms of the firing frequency of the tibial extensor motoneurons (top) and rectified waveform averages of the flexor muscle activity (bottom) during fCO stimulation in the middle leg during backward (D) and forward (E) walking (D: n=22; E: n=14, data from one animal).

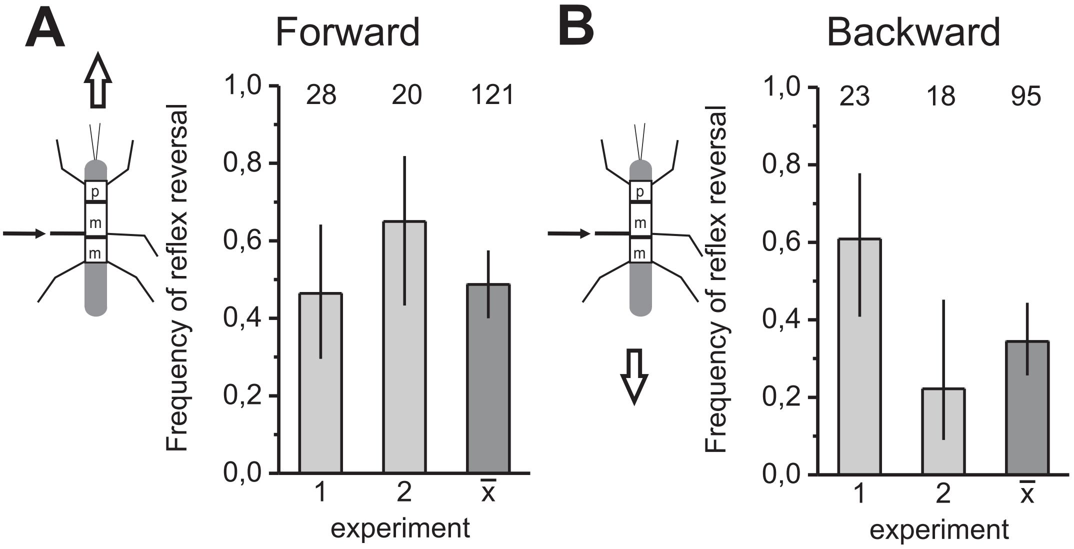

Figure 3.4: Reflex reversals in the middle leg do not depend on walking direction. Bar histograms show the frequency of reflex reversals in the middle leg tibial muscles during displacement of the middle leg fCO in animals that were walking forward (A) and backward (B). Each histogram shows two exemplary experiments (light bars) and the mean values of all experiments (dark bars; N=7). Reflex reversals occurred during forward and backward walking. Arrows indicate walking direction; lines designate 95% confidence intervals.

3.1.3 Influence of fCO signals on the tibial MN activity of the hind leg

In order to determine the role of the processing of fCO signals in hind legs while the remaining legs walked forward and backward, again, the activity of the F2 extensor nerve and the *flexor tibiae* muscle activity were recorded and simultaneously ramp-and-hold stimuli were applied to the fCO. During forward, as well as during backward walking, elongation of the fCO could generate reflex reversals (Fig. 3.5 A, B, C). However, during forward walking, only in some cases fCO elongation elicited a reflex reversal (Fig. 3.5 A). Mostly, however, the neuronal activity in the F2 extensor nerve remained high and no inhibition during elongation was found. This is also shown in the PSTH of the averaged F2 extensor nerve activity, in which only in the backward walking condition a slight decrease of the activity, caused by the fCO elongation, becomes apparent (Fig. 3.5 E). Nonetheless, averaged flexor muscle activity increased during fCO elongation in forward, as well as in backward walking (Fig. 3.5 D, E bottom).

Closer inspection of the frequency of reflex reversals during forward and backward walking revealed only a small difference in the metathoracic processing of fCO signals (Fig. 3.6 A, B). During forward walking, the frequency of reflex reversals did not ex-

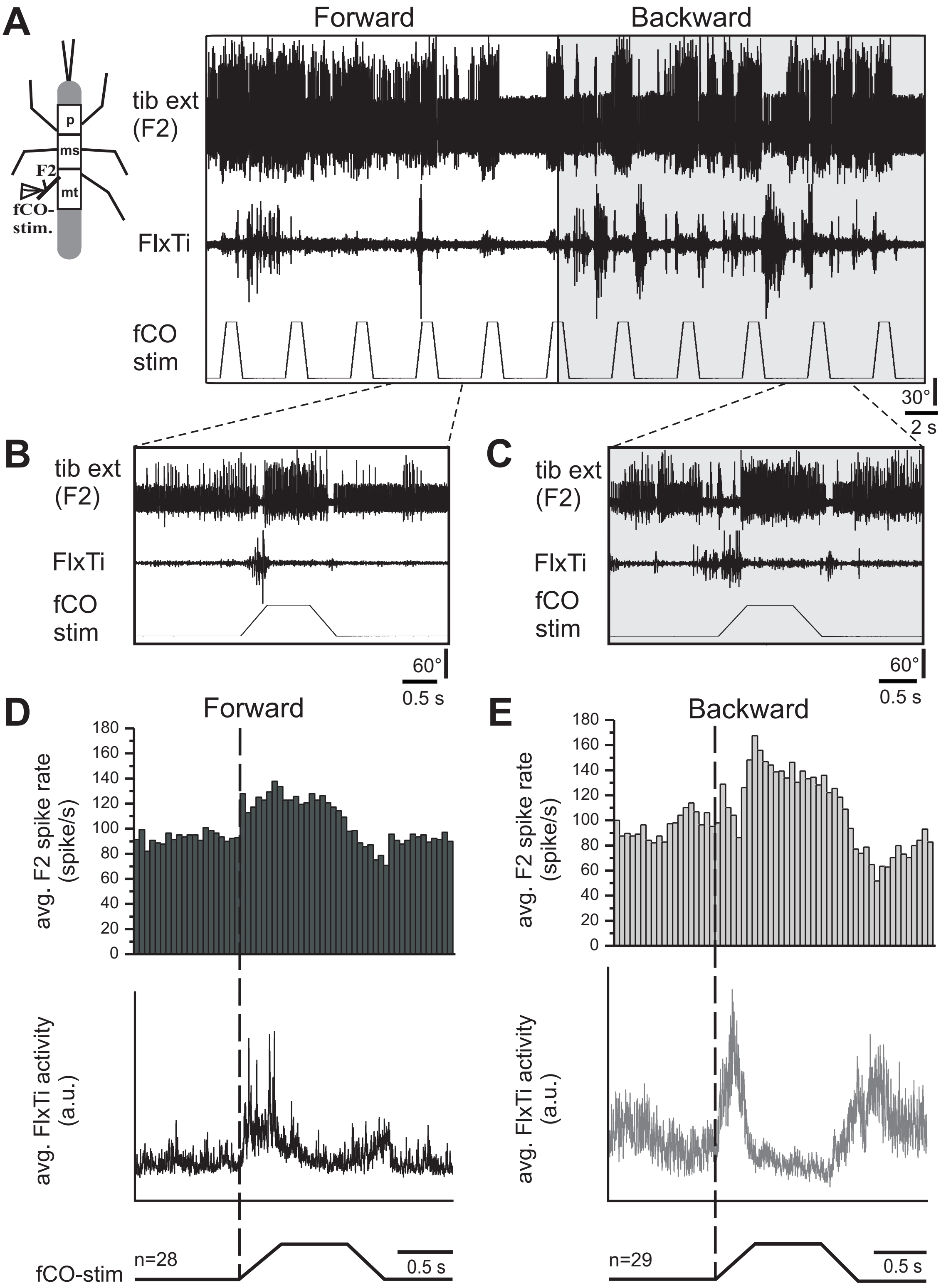

Figure 3.5: (A) Influence of fCO signals on the tibial MN activity in the hind leg during forward and backward walking. The femoral chordotonal organ was displaced (fCO stim) in the hind leg while monitoring the tibial extensor (tib ext (F2)) and flexor muscle activities (Flx Ti) in that leg. In this sequence, the animal was walking forward and then backward. Reflex reversals occurred during forward and backward walking. B) and C) Expanded traces of responses during forward (B) and backward (C) walking. D) and E) Peri-stimulus time histograms of the firing frequency of the tibial extensor motoneurons (top) and rectified waveform averages of the flexor muscle activity (bottom) during fCO stimulation in the hind leg during forward (D) and backward (E) walking (D: $n = 28$; E: $n = 29$, data from one animal).

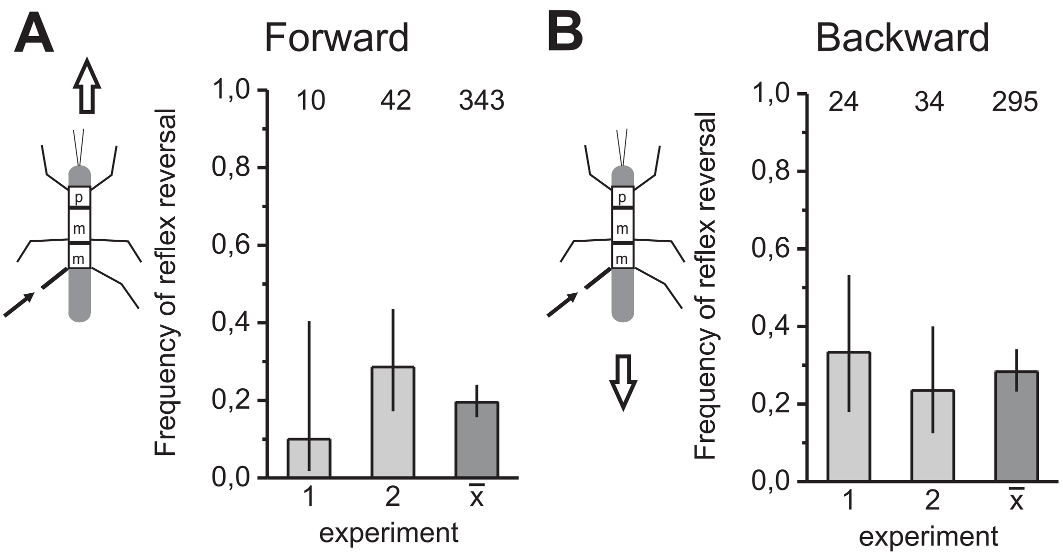

Figure 3.6: Reflex reversals in the hind leg depend on walking direction. Bar histograms show the frequency of reflex reversals in the hind leg's tibial muscles during displacement of the hind leg fCO in animals that were walking forward (A) and backward (B). Each histogram shows two exemplary experiments (light bars) and the mean values of all experiments (dark bars; $N = 11$). Reflex reversals occurred during backward, however, only rarely in forward walking. Arrows indicate walking direction; lines designate 95% confidence intervals.

ceed 20% (Fig. 3.6 A) and during backward walking, the frequency was 28% (Fig. 3.6 B). Moreover, only in three of eleven experimental animals the frequency of reflex reversals differed significantly between forward and backward walking. In conclusion, no noticeable difference in the frequency of reflex reversals between the two walking directions was observed. In four of eleven experiments, the frequency of reflex reversal was higher in forward walking than in backward walking, whereas in six of eleven experiments the frequency was higher in backward walking. Two of these exemplary experiments are shown in Fig. 3.6 (A) for forward walking and in Fig. 3.6 (B) for backward walking. Generally, in the hind leg, there was a slightly increased tendency for the generation of reflex reversals in backward walking, compared to the forward walking condition. However, altogether, the differences are only marginal and not significant.

3.1.4 Influence of ThC joint position on the processing of fCO signals in tibial MNs of the hind leg

During forward and backward walking, a difference in the processing of fCO signals in the front leg was demonstrated; in contrast, in the hind leg, only a slight influence of walking direction was found. Furthermore, for both walking directions, the frequency of reflex reversal in hind legs was generally lower than in front and middle legs. Therefore, it might be hypothesized that in the hind leg different or additional leg sensors, besides the fCO, are involved in the control of tibial MN activity. Hair fields, in particular the ventral coxal hair plate (vcxHP) located on the ThC joint of the leg (Bässler, 1983b; Dean and Schmitz, 1992; Graham and Wendler, 1981), are known to detect movements of this joint. These hair fields are formed by groups of hair sensilla (Dean and Schmitz, 1992). The ventral coxal hair plate is composed of two groups of hair sensilla, G1 and G2. It was shown that simulating a constant leg protraction, by fixating the ThC joint with a clamp, results in reinforcement of the stance phase, and even, in some cases, in a constant retraction, therefore, causing the leg to remain in the stance phase (Bässler, 1977).

To determine a potential influence of position signals from the ThC joint on the generation of the reflex reversal in the hind leg during forward and backward walking, the ThC joint position was changed. Thus, the hind leg was fixated at an angle of 45° anterior instead of 45° posterior to the body axis. Simultaneously, fCO stimulation and tibial nerve and muscle activity recordings were performed. During forward walking, fCO elongation elicited an increase in activity in the *extensor tibiae* nerve (Fig. 3.7 A, B, D), similar to the resistance reflex in the inactive animal. In backward walking, the same fCO stimulation more likely mediates reflex reversals (Fig. 3.7 A, C, E). The difference in the motoneuronal activities of the FTi joint in response to fCO stimulation becomes particularly obvious in the enlarged presentation of one fCO stimulus for the situation during forward (Fig. 3.7 B) and backward (Fig. 3.7 C) walking. Also, the *extensor tibiae* nerve activity in forward walking increases on average during fCO stimulation (Fig. 3.7 D). In backward walking, fCO elongation caused only a slight increase in the extensor activity in response to stimulus onset (Fig. 3.7 E). However, afterwards, during the hold phase of the stimulus, the extensor discharge rate further increased (Fig. 3.7 E). The complementary activity pattern to the extensor activity during fCO stimulation is given by the averaged *flexor tibiae* activity, thus, only in the backward walking situation a slight increase in the muscle activity in response to fCO stimulation was found (Fig. 3.7 E). This indicates that a change in the ThC joint position increases the frequency of reflex

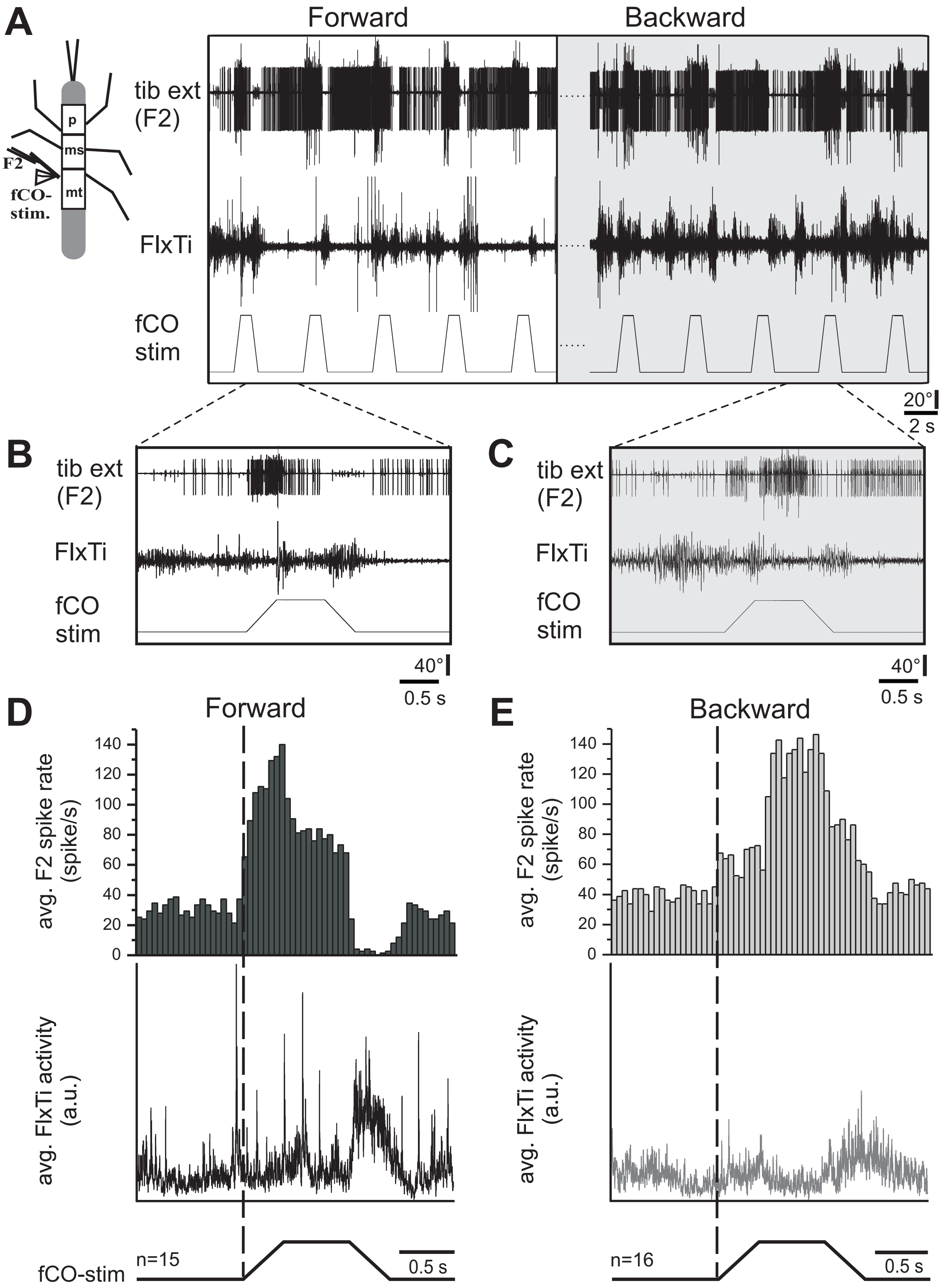

Figure 3.7: Influence of fCO signals on the tibial MN activity in the anterior-directed hind leg during forward and backward walking. The femoral chordotonal organ was displaced (fCO stim) in the anterior-directed hind leg, while monitoring the tibial extensor (tib ext (F2)) and flexor muscle activities (Flx Ti) in that leg. A) The animal walked forward (left panel) and backward (right panel). Reflex reversals occurred during backward walking. B) and C) Expanded traces of responses during forward (B) and backward (C) walking. D) and E) Peri-stimulus time histograms of the firing frequency of the tibial extensor motoneurons (top) and rectified waveform averages of the flexor muscle activity (bottom) during fCO stimulation in the anterior-directed hind leg during forward (D) and backward (E) walking (D: n=15; E: n=16, data from one animal).

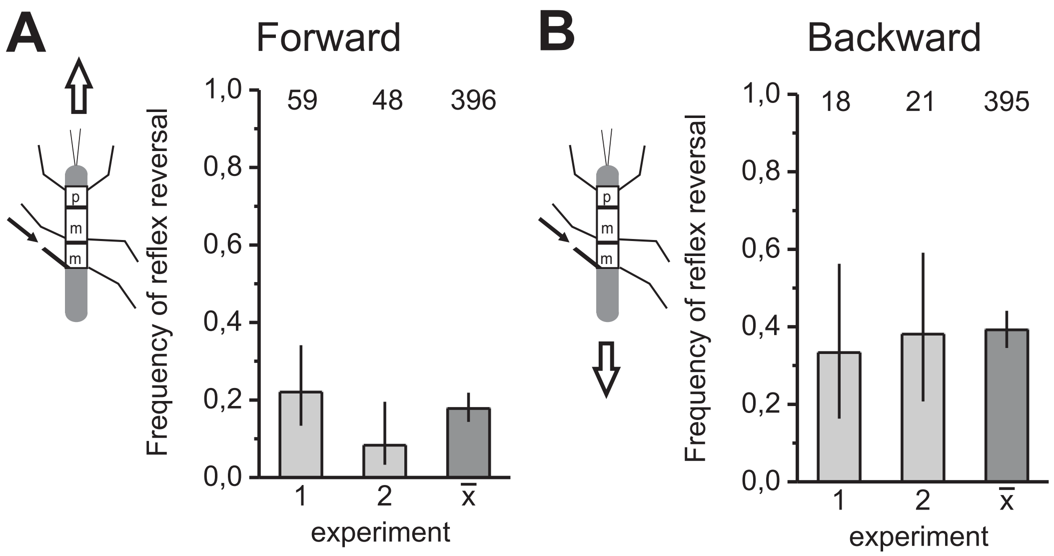

Figure 3.8: Reflex reversals in the hind legs fixated anteriorly depend on the walking direction. Bar histograms show the frequency of reflex reversals in hind legs fixated anteriorly during fCO elongation in animals that were walking forward (A) and backward (B). Each histogram shows two exemplary experiments (light bars) and the mean values of all experiments (dark bars; $N = 13$). Reflex reversals occurred during backward, yet rarely in forward walking. Arrows indicate walking direction; lines designate 95% confidence intervals.

reversals during backward walking. In more than one half of the experiments (eight of 13 experiments) the frequency of reflex reversals differed significantly between forward and backward walking (in three experiments $p < 0.01$; in five experiments $p < 0.001$). Moreover, the frequency of reflex reversals was enhanced during backward walking, compared to forward walking in eleven of 13 experiments. The frequency of occurrence of two of these exemplary experiments and averages of all investigated animals (N=13) are depicted in Fig. 3.8 for forward walking (A) and backward walking (B).

3.1.5 Influence of ThC joint position and CS ablation on the processing of fCO signals in tibial MNs of the hind leg

It was demonstrated that during backward walking the generation of reflex reversal was increased by changing the position of the hind leg in a 45° anterior directed leg position (as discussed in the previous section). To confirm that this effect is indeed mediated by position sensors, it was necessary to exclude that load information from the CS are responsible for the observed differences. It has been shown previously that sensory signals of the three fields of trochanteral campaniform sensilla (trCS), as well as of the femoral campaniform sensilla (fCS), are changed by cuticular deformation of the

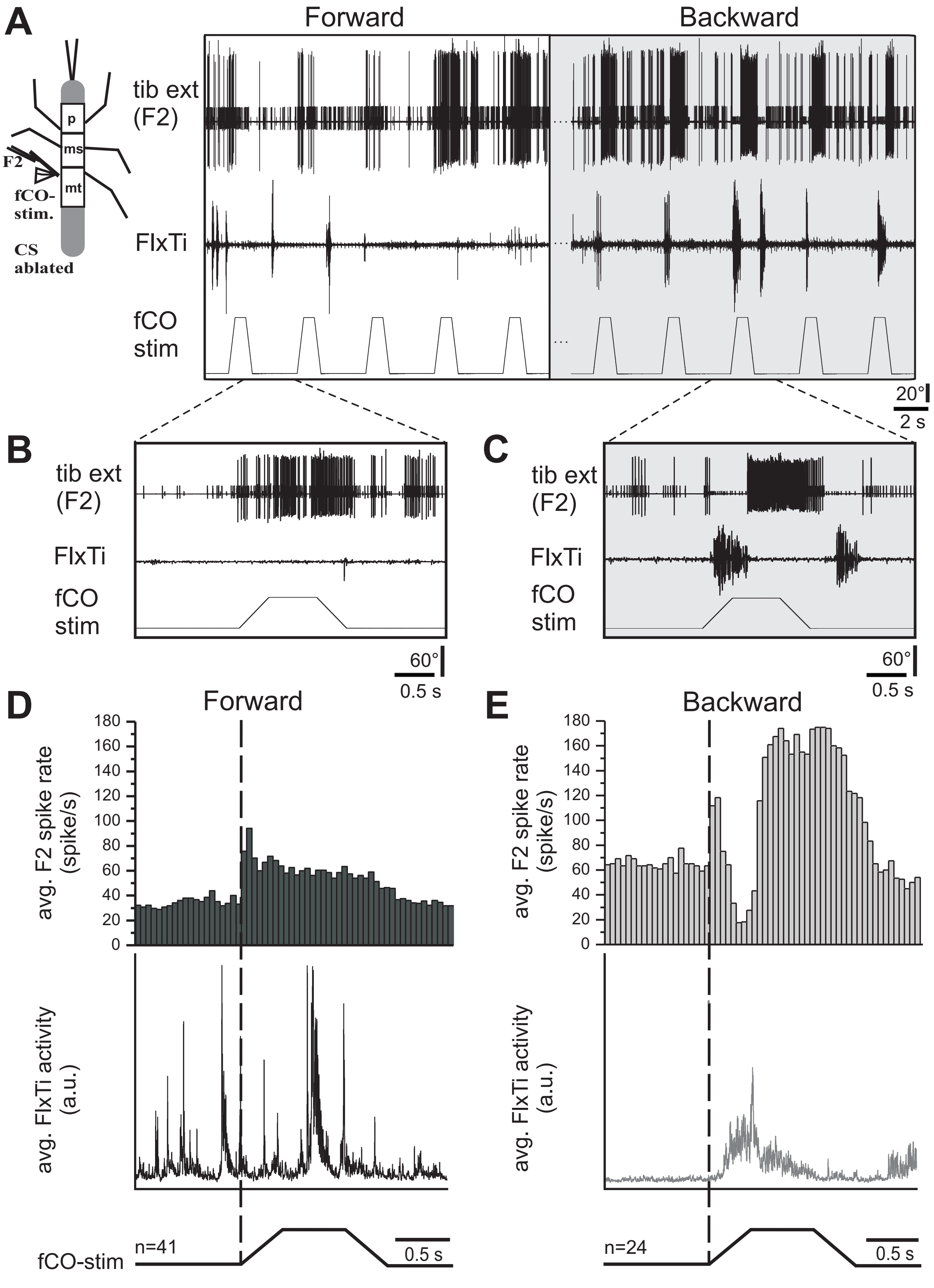

Figure 3.9: (A) Influence of fCO signals on the tibial MN activity in the anterior-directed hind leg with ablated CS during forward and backward walking. The femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor (tib ext (F2)) and flexor muscle activities (Flx Ti) in that leg. In the first sequence, the animal walked forward and in the second sequence, it walked backward. Reflex reversals occurred during backward walking. B) and C) Expanded traces of responses during forward (B) and backward (C) walking. D) and E) Peri-stimulus time histograms of the firing frequency of the tibial extensor motoneurons (top) and rectified waveform averages of the flexor muscle activity (bottom) during fCO stimulation in the anterior directed hind leg with ablated CS during forward (D) and backward (E) walking (D: n=41; E: n=24, data from one animal).

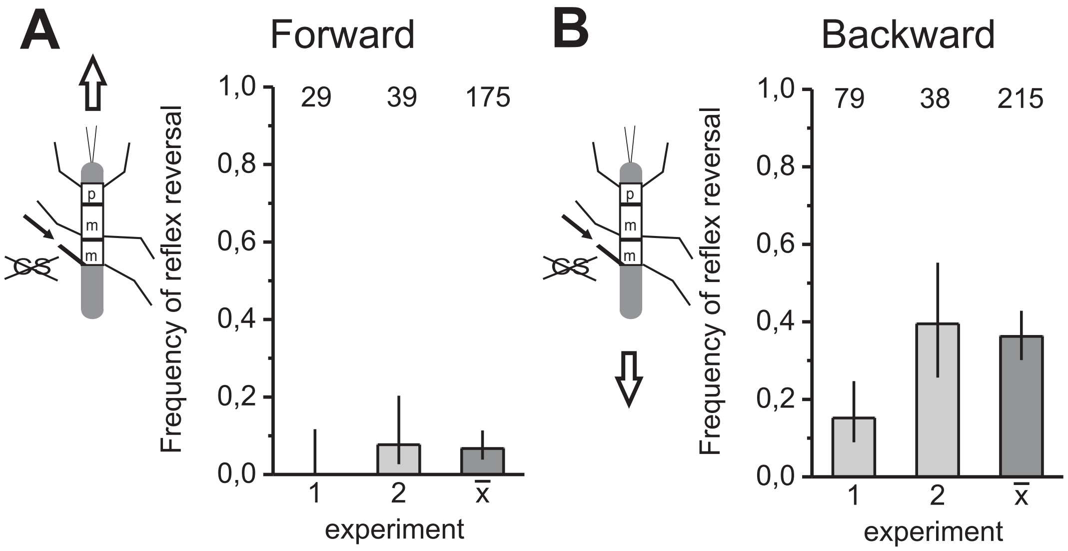

Figure 3.10: Reflex reversals in the hind legs fixated anteriorly depend on walking direction. Bar histograms show the frequency of reflex reversals in the hind legs fixated anteriorly and with ablated CS during displacement of fCO in animals that were walking forward (A) and backward (B). Each histogram shows two exemplary experiments (light bars) and the mean values of all experiments (dark bars; $N = 5$). Reflex reversals occurred during backward, yet rarely in forward walking. Arrows indicate walking direction; lines designate 95% confidence intervals.

trochanter (Delcomyn, 1991; Hofmann and Bässler, 1982; Tatar, 1976). Furthermore, load and position information influence the change between stance and swing phase (Cruse, 1985a). Akay and Büschges (2006) was able to show that a simultaneous increase in load information of the fCS and elongation of the fCO caused a higher frequency of AR. In the following experiments, all four CS of the trochanter were ablated, the hind leg was again fixated at 45° in the anterior-directed position, the fCO was stimulated by ramp-and-hold stimuli, and the extracellular activity of the tibial extensor nerve and the *flexor tibiae* muscle activity were recorded. As a result, elongation of the fCO mediates a reflex reversal in backward walking, yet rarely in forward walking (Fig. 3.9 A, B, C). During forward walking, the F2 extensor nerve activity increases upon fCO elongation and the averaged flexor muscle activity does not show any correlated activity as a consequence of the fCO stimulation (Fig. 3.9 D). The reflex reversal in backward walking becomes particularly obvious in the averaged PST-histograms of the *extensor tibiae* activity, displaying a decrease in activity due to fCO elongation and a simultaneous strong increase in the averaged flexor activity (Fig. 3.9 E). The emerging hypothesis that reflex reversals occur more likely in backward walking than in forward walking, can be supported by my empirical findings, as in three of five animals a significant difference between forward and backward walking was found. Furthermore, in all experiments ($N = 5$), the frequency of reflex reversals was lower in forward walking (Fig. 3.10 A) compared to backward walking (Fig. 3.10 B).

3.1.6 Summary 1: Influence of walking direction

To summarize the influences of position and movement signals of the fCO during forward and backward walking, the frequencies of all experimental conditions are displayed as box-and-whisker diagrams (Fig. 3.11). In the front legs, as well as in the hind legs, the frequencies of reflex reversals differed between forward and backward walking. Interestingly, the occurrence frequencies in the front legs are inversed to the occurrence frequencies found in the hind legs (Fig. 3.11): the frequency of reflex reversals in the front legs during forward walking is significantly ($p < 0.0001$) higher than in the backward walking animal (experimental condition 1, Fig. 3.11). In contrast, in all experimental conditions of the hind legs, reflex reversals in forward walking occurred less often than in backward walking. walking (experimental condition 3-5, Fig. 3.11). The frequency of reflex reversals in the posterior directed hind leg was decisively more frequent during backward walking than during forward walking (experimental condition 3, p = 0.0123) and was notably higher in the anterior-directed hind legs (experimental condition 4, p = 1.19E-13). The most distinct difference in the occurrence of reflex reversals during forward and backward walking in the hind legs (p = 4.05E-07) was found in the experimental condition with the 45° anterior-directed hind leg and simultaneously ablated CS (experimental condition 5, Fig. 3.11). In the middle legs, no significant changes between the frequency of reflex reversals in forward and backward walking animals were detected ($p = 0.0795$).

3.1.7 Summary 2: Influence of segment specificity

During forward walking, the highest frequency of reflex reversals was found in the front legs (Fig. 3.12). Even though there was a large variation of the frequency of reflex reversals during forward walking in the front legs, the median was higher than in the middle legs. Furthermore, the medians of both (front and middle legs) were higher than the

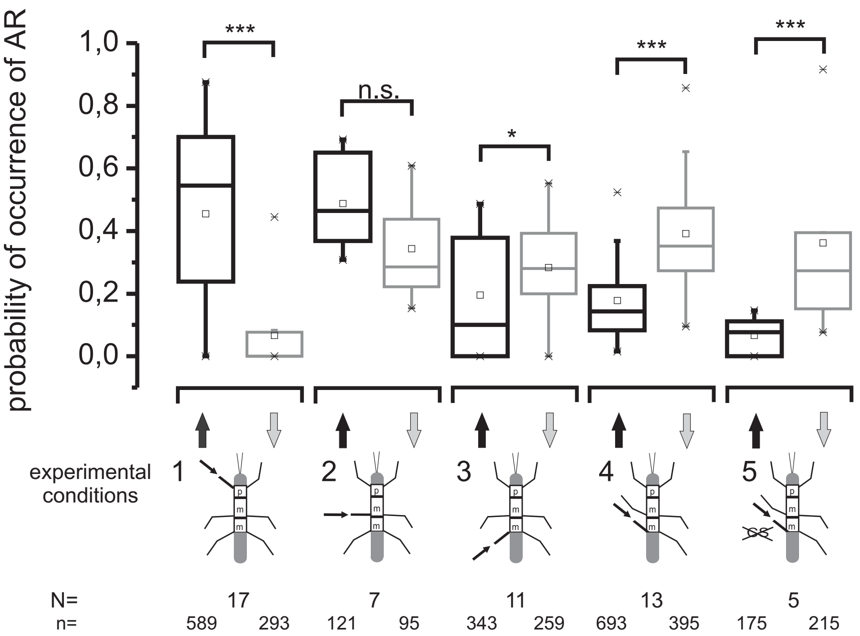

Figure 3.11: Frequency of occurrence of reflex reversals during forward and backward walking in front, middle and hind legs. Whisker box plots depict all tested animals of all different experimental conditions during forward and backward walking. The differences between forward and backward walking were tested with the chi-square test. The statistical significance is denoted as follows: (n.s.) not significant $p > 0.05$; *p ≤ 0.05; **p ≤ 0.01;***p ≤ 0.001.. Arrows above the stick insect schematics indicate walking directions and small black arrows highlight the investigated leg. N indicates number of experiments and n indicates sample size (i.e., number of stimuli).

medians of all experimental conditions in the hind legs during forward walking. In addition, the statistical tests supported the hypothesized difference in reflex reversal frequency between the front legs, the middle legs, and the hind legs. In the hind legs, during forward walking, the most outstanding difference of the occurrence of reflex reversals was found between the posterior-directed hind leg and the anterior-directed hind leg with CS ablation (p = 2.39E-04). In the forward walking stick insects, the frequency of reflex reversals was decisively larger in the anterior-directed hind legs with intact CS, than in the hind legs with ablated CS (p = 0.002). Contrary to the distribution of frequency of reflex reversals in the different experimental conditions in forward walking, in backward walking, the lowest frequency was detected in front legs. In backward walking stick insects, the frequency of reflex reversals varied considerably between the front legs and all other experimental conditions (Fig. 3.13, p < 0.001). Furthermore, a difference between the anterior-directed hind legs and the posterior-directed hind legs ($p = 0.031$), as well as to hind legs with ablated CS ($p = 0.021$) was found. Nevertheless,

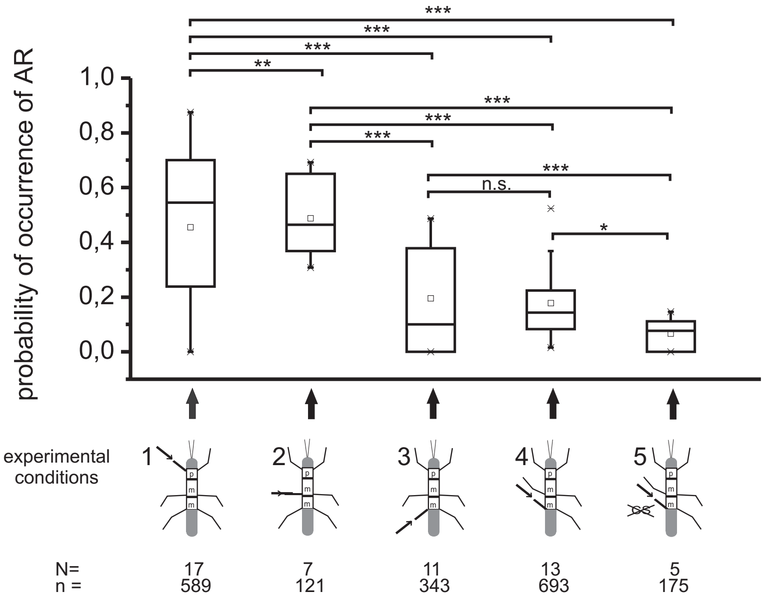

Figure 3.12: Frequency of occurrence of reflex reversals during forward walking in front, middle and hind legs. Whisker box plots depict data from all tested animals of all different experimental conditions during forward walking. The differences in the frequency of occurrence between the different experimental conditions during forward walking were tested with the chi-square test. The statistical significance is denoted as follows: (n.s.) not significant $p > 0.05$; *p ≤ 0.05; **p ≤ 0.01;***p ≤ 0.001.. Arrows above the stick insect schematics indicate walking directions and small black arrows highlight the investigated leg. N indicates number of experiments and n indicates sample size (stimuli).

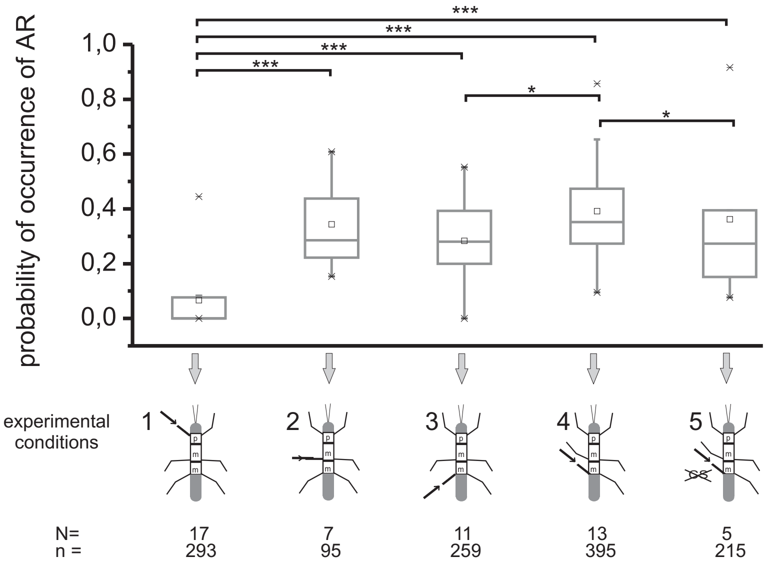

Figure 3.13: Frequency of occurrence of reflex reversal during backward walking in front, middle and hind legs. Whisker Box plots depicts all tested animals of all different experimental conditions during forward walking. The differences in the frequency of occurrence between the different experimental conditions during backward walking were tested with the chi-square test. The statistical significance is denoted as follows: (n.s.) not significant $p > 0.05$; *p ≤ 0.05; **p ≤ 0.01;***p ≤ 0.001.. Arrows above the stick insect schematics indicate walking directions and small black arrows highlight the investigated leg. N indicates number of experiments and n indicates sample size (stimuli).

in the middle legs and in all hind leg conditions, the means differ only by a maximum range of 10%.

3.1.8 Front and hind leg stance phase kinematics during forward and backward walking

In order to elucidate the differences of the frequency of reflex reversals in the different legs during forward and backward walking, the correlation of the described processing of flexion signals from the fCO with the corresponding stance phase kinematics was essential. Cruse and Bartling (1995) have described the changes in the FTi joint angle in the front leg, as well as in the hind leg, during forward walking. According to their description, during forward walking, the FTi joint angle (inner angle) of the front leg decreases during stance phase and increases in the swing phase. In the hind leg, this relationship is reversed. Consequently, during stance phase, the FTi joint angle increases. A recent study that addressed the muscle activities of the FTi joint reported that, at least in the middle leg, the *flexor tibiae* and *extensor tibiae* muscle activities are quite similar during forward and backward walking (Rosenbaum, Wosnitza et al., 2010).

To correlate the processing of position and movement information from the fCO during forward and backward walking with the leg movements, we performed kinematic investigations regarding the change of the FTi joint angle during stance phase in the front and hind legs in both walking directions (some experiments were performed under my supervision from T. Giang (student) and C. Glowania (Bsc. student)). For the experiments, animals were mounted above a slippery surface and walked with all legs either forward or backward. Movements of the front or hind leg FTi joints were recorded by the use of a high speed video camera. For data evaluation, the forward and backward steps were divided into three classes, regarding an increase or a decrease of the FTi joint angle. The first class contains steps during which the FTi joint angle decreased, which was classified as flexion. The second class contains steps during which the stance phase was mediated by flexion at the beginning of stance, followed by an extension of the FTi joint. The third class contains steps during which the stance phase was achieved by FTi

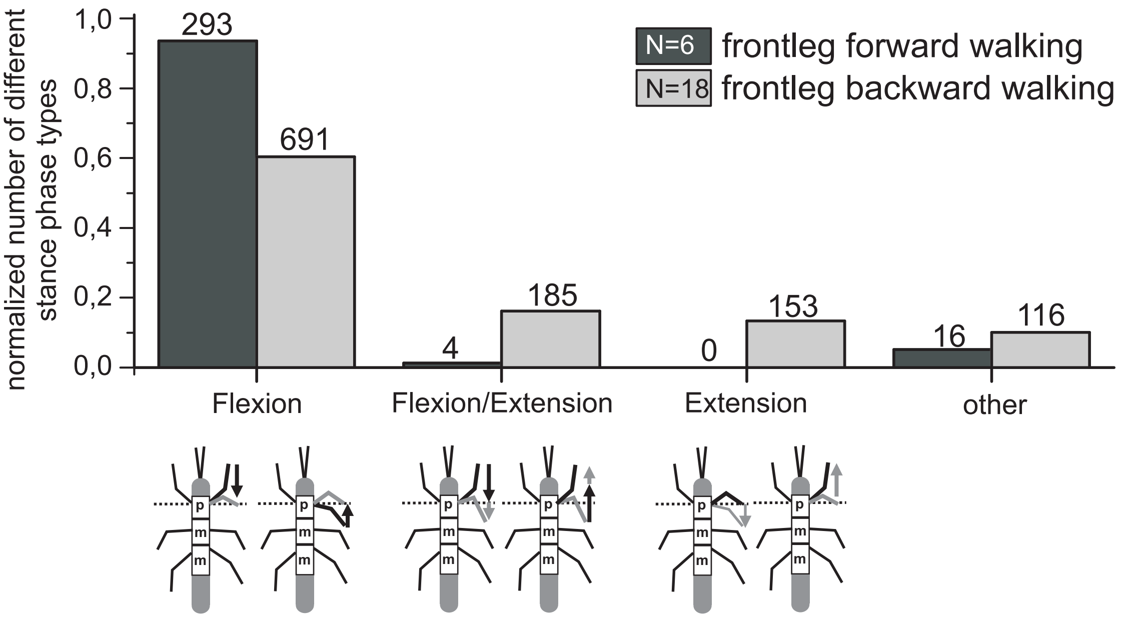

Figure 3.14: Stance phase directions in the forward and backward walking front leg. The FTi joint angles are divided into three classes of stance phase movements: flexion, flexion and extension, and extension. The normalized numbers of stance phase types during forward walking are shown in black ($N = 6$), while phase types during backward walking are shown in grey ($N = 18$). Arrows in the schemes indicate stance direction by flexion (black) and extension (grey); numbers above bars indicate quantity of steps.

joint extension, which means enlargement of the FTi joint angle. In the forward walking front leg, stance phase was mediated by flexion of the FTi joint in more than 90% of the steps (Fig. 3.14, black bars). In backward walking stick insects, the stance phase of the front legs was dissimilarly accomplished. In the majority of steps the FTi joint was flexed during stance (≈ 60%). However, stance phase was also realised by either both FTi joint flexion and extension or exclusively FTi joint extension (Fig. 3.14, grey bars).

In backward walking hind legs, the stance kinematics was comparable to the kinematics of the forward walking front leg. In 90% of all observed backward walking steps, stance phases were performed by FTi joint flexion (Fig. 3.15, grey bars). However, in forward walking hind legs, the stance phases were more diversified. This results in almost 40% of stance phases in which the FTi joint was flexed and further 40% of the steps in which the FTi joint was extended during stance phase (Fig. 3.15, black bars). Furthermore, in forward walking hind legs, stance phase was performed by FTi joint flexion, followed by an extension. However, in some steps no classification was possible (Fig. 3.15, black bars).

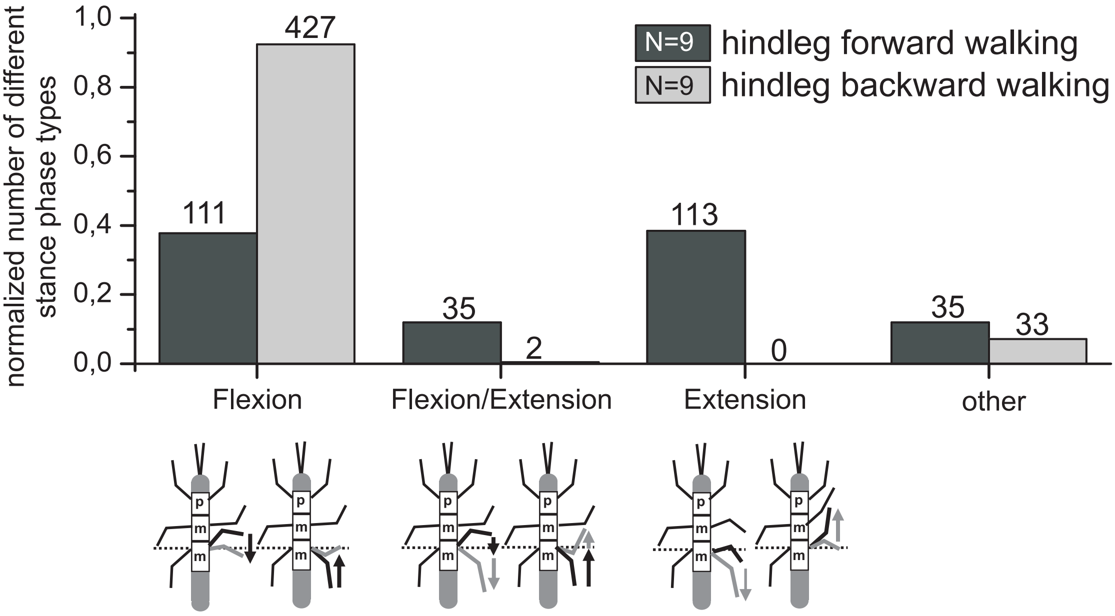

Figure 3.15: Stance phase directions in the forward and backward walking hind leg. The FTi joint angles are divided into three classes of stance phase movements: flexion, flexion and extension, and extension. The normalized numbers of stance phase types during forward walking are shown in black ($N = 9$), while phase types during backward walking are shown in grey ($N = 9$). Arrows in the schemes indicate stance direction by flexion (black) and extension (grey); numbers above bars indicate quantity of steps.

3.1.9 Influence of walking direction on interjoint reflex response to fCO stimulation

Previous studies reported that position and movement signals from the FTi joint processed by the fCO have influences on the coxa-trochanter (CTr) joint (Hess and Büschges, 1997, 1999). The authors identified reflex pathways controlled by the fCO, which modulate the *depressor trochanteris* and *levator trochanteris* activity in an active and inactive stick insect. In the inactive animal, elongation stimuli activate *levator trochanteris* MNs and relaxation stimuli activate *levator trochanteris*, as well as *depressor trochanteris* MNs (Hess and Büschges, 1997). The reflex response was also tested in an active, but not walking animal, in which the reflex responses increase (Hess and Büschges, 1997). It is still unknown how the fCO information is processed in the CTr joint of a walking stick insect, in particular, during different behavioural tasks, like forward and backward walking.

To investigate the influence of position and movement signals of the FTi joint on the

CTr joint during walking, the animal was again mounted above a slippery plate and the middle leg was fixated for mechanical stimulation of the fCO. Simultaneously, extracellular nerve recordings of the F2 extensor nerve and muscle recordings of the *flexor tibiae* muscle were performed. To monitor the activity of the CTr joint, the mesothoracic *levator trochanteris* nerve C1 was recorded extracellularly inside the thorax. In the walking stick insect, during forward as well as during backward walking, the motoneuronal activity in the *levator trochanteris* nerve increased upon fCO elongation (Fig. 3.16 A). This becomes particularly obvious in the enlarged presentation of one stimulus during forward (Fig. 3.16 B) and during backward (Fig. 3.16 C) walking.

Furthermore, the averaged neuronal spike activity of the C1 nerve in six animals (Fig. 3.17 A-F) during forward (black bars) and backward (white bars) walking was increased during elongation of the fCO. The increase in C1 frequency in forward and backward walking was similar; for example in Fig. 3.17 (B) a strong enhanced frequency was found for both walking directions, yet in Fig. 3.17 (D) the frequency was only rarely increased. In general, the spike activity of the C1 nerve was increased due to fCO elongation and decreased upon fCO release (Fig. 3.17 A-F). For a closer look at the activity distribution of the C1 during the stimulation period, the burst activity of C1 was quantified. Therefore, the stimulus duration and the time between two stimuli were divided into eleven time bins and each *levator trochanteris* burst onset was assigned to one bin class. The fCO stimulation, during both walking directions, elicited bursting activity mainly during elongation (Fig. 3.18 A, B; shown for two exemplary animals). In addition, for both walking directions the levator burst on- and offset during fCO elongation was analysed. The *levator trochanteris* activity started, or remained active, in approximately 40% of the applied fCO stimuli and only in approximatly 10% the levator burst activity ended upon fCO elongation (Fig. 3.19, N=3).

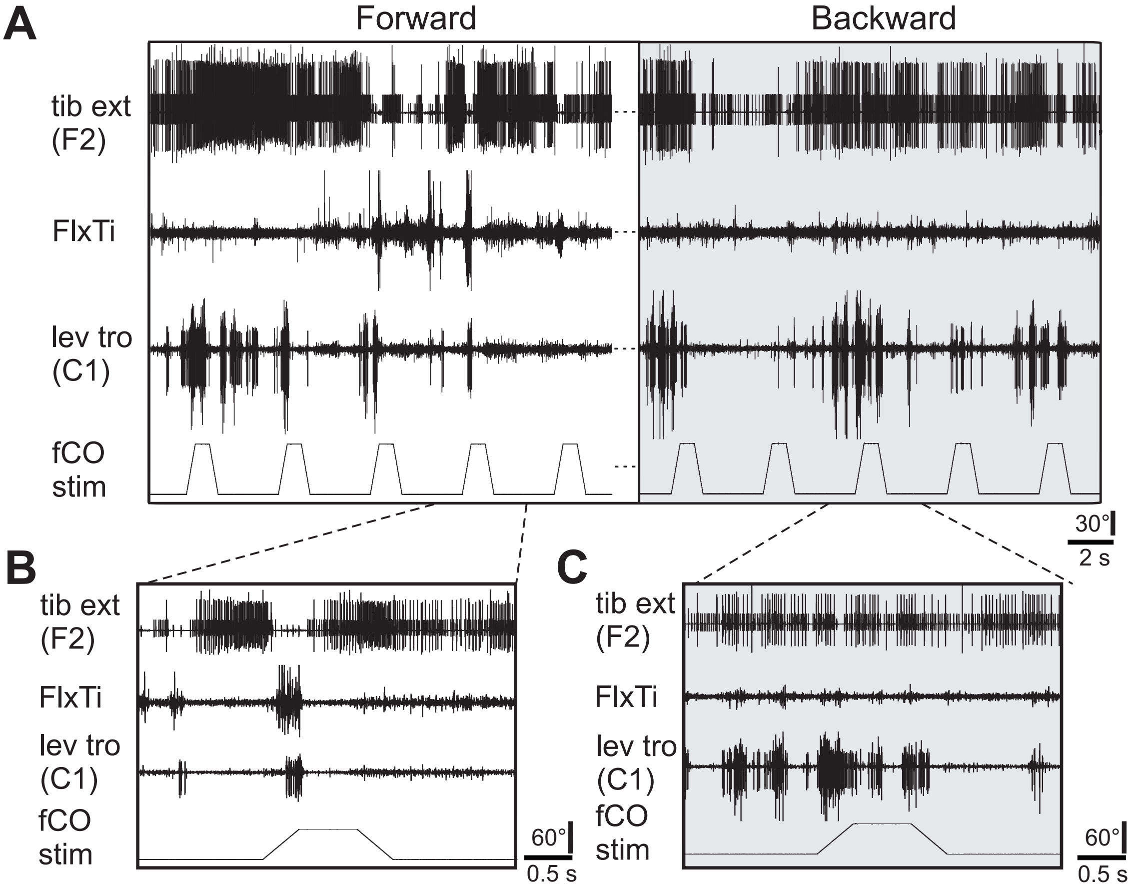

Figure 3.16: Influence of walking direction on the interjoint reflex between the FTi joint and the CTr joint in the middle leg of forward and backward walking stick insects. The mesothoracic femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor (tib ext (F2)) and flexor activities (FlxTi) in that leg and the neuronal activity of the *levator trochanteris* nerve (lev tro (C1)). A) In the first sequence, the animal was walking forward and in the second sequence backward. In forward and backward walking the *levator trochanteris* activity increases during fCO elongation. That becomes obvious in the expanded traces of one stimuli during forward (B) and backward (C) walking.

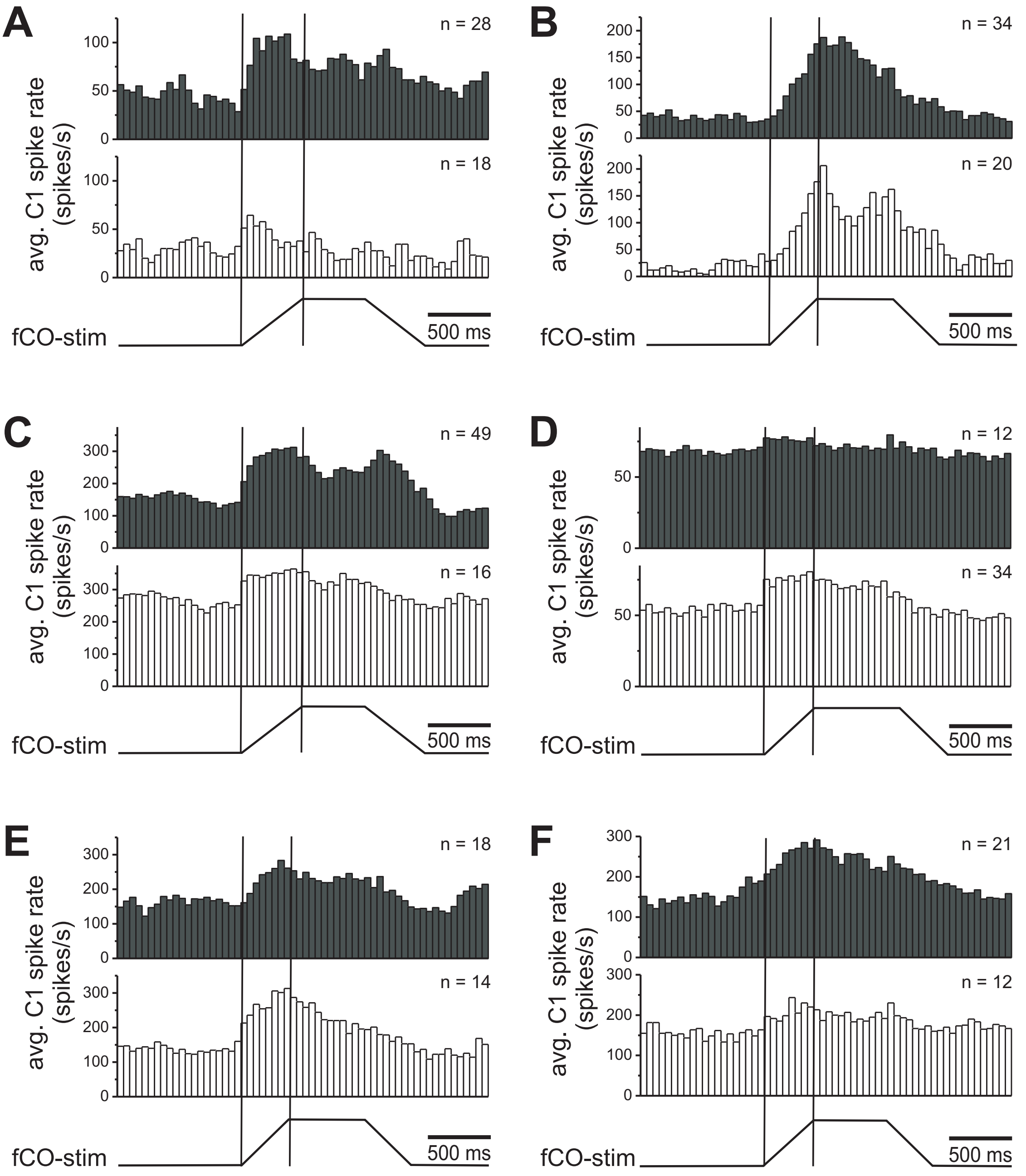

Figure 3.17: Mesothoracic *levator trochanteris* nerve activity in forward (black bars) and backward walking (white bars) during fCO stimulation in six animals. The schematic stimulus below the bar histograms gives the correct stimulus time course. However, note that the stimulus amplitude is normalized. Therefore, the varying stimulus velocity in the different experiments are the following $A = 98°/s$, $B = 303°/s$, $C = 148°/s$, $D = 173°/s$, $E, F = 98°/s$.

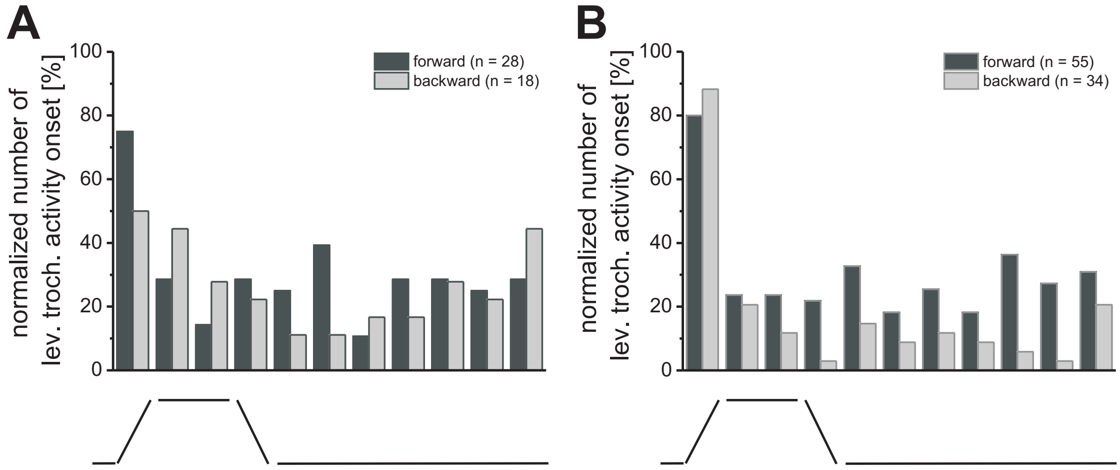

Figure 3.18: *Levator trochanteris* burst onset in two animals (A, B) during fCO stimulation in forward (black bars) and backward (grey bars) walking animals. Over the stimulus period eleven bin classes according to the elongation, holding, relaxation and time between two stimuli were used to evaluate the *levator trochanteris* burst onsets.

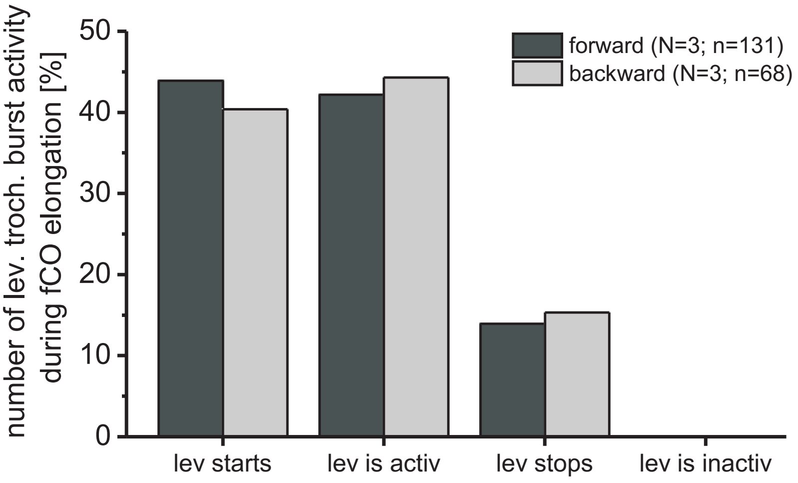

Figure 3.19: *Levator trochanteris* burst activity during fCO elongation in forward (black bars) and backward (grey bars) walking animals ($N = 3$).

3.2 Curve Walking

In stick insects walking a curve, the animal must adjust its motor output in order to generate a certain leg movement pattern that is capable of producing turning. The leg kinematics, step length, directions of the stance phase of each leg, stepping frequencies and the interleg coordination need to be modified to walk a curved path (Dürr, 2005; Dürr and Ebeling, 2005; Jander, 1982). The kinematics and muscle activities of tethered insects were investigated during optomotor-induced curve walking on a slippery surface (Gruhn et al., 2006, 2011, 2009). Under these experimental conditions, each leg participating in curve walking can be regarded as walking on either the inside or outside of the curve. The stance phase of a middle leg on the inner side of the curve is directed towards the body of the animal. In contrast, a middle leg walking on the outside of the curve pushes backward along the longitudinal axis of the animal (Gruhn et al., 2006). In order to understand the neural basis of these adaptations responsible for the leg coordination during curve walking, the present and recent studies (Hellekes et al., 2012; Hoffmann, 2010) investigated whether the occurrence of reflex reversal differed in the middle leg walking on the inner or outer side of a curve. In a second set of experiments, I investigated intracellularly the physiological changes in the mesothoracic neuronal network that controlls the FTi joint. Therefore, the physiology of identified premotor nonspiking interneurons was analysed in middle legs that were walking on either the inside or the outside of the curve. Their physiology was first compared between all fCO stimulations in the leg walking on the inside or on the outside of the curve, and secondly between only fCO stimulations which generate reflex reversals in the leg either walking on the inside or the outside of the curve.

3.2.1 Influence of fCO signals on the tibial MN activity during curve walking

In order to analyse the influence of walking direction on the processing of fCO signals, the animal was tethered above a slippery surface. One middle leg was fixated to stimulate the mesothoracic fCO and to monitor the motoneuronal activities of the *flexor* and *extensor tibiae*, while the animal performed optomotor-induced curve walking with the remaining legs (Hellekes et al., 2012; Hoffmann, 2010). According to the location of the fixed leg, in which the fCO stimulation was performed, and relative to the turning direction, the leg is denoted as inner or outer leg throughout this thesis (see Material and Methods 2, Fig.3.23 B, C). The frequency of reflex reversals in the inner middle legs (58%) was significantly higher ($p < 0.001$, $N = 8$) than in the outer leg (7.2%) (Fig.3.20, experimental condition 1, filled boxes recent data of Hoffmann (2010)). To determine the differences in the frequency of reflex reversal between the inner and outer middle leg, experiments were performed in which the outer and inner middle leg were investigated simultaneously (Fig. 3.20, experimental condition 2). In general, the frequency of reflex reversals was significantly higher, when the left or right middle leg was an inner leg compared to an outer leg (left leg $p < 0.001$; right leg $p = 0.0017$). To determine the different roles of the individual legs during curve walking, the task-dependent influences on the processing of fCO signals were evaluated in the front legs, where fCO stimulation was applied, while recording the tibial motoneuronal activities during curve walking. In 62% of all stimulations in the inner legs reflex reversals were generated and in 43% in the outer legs (Fig. 3.20, experimental condition 3). In addition to fCO stimulation

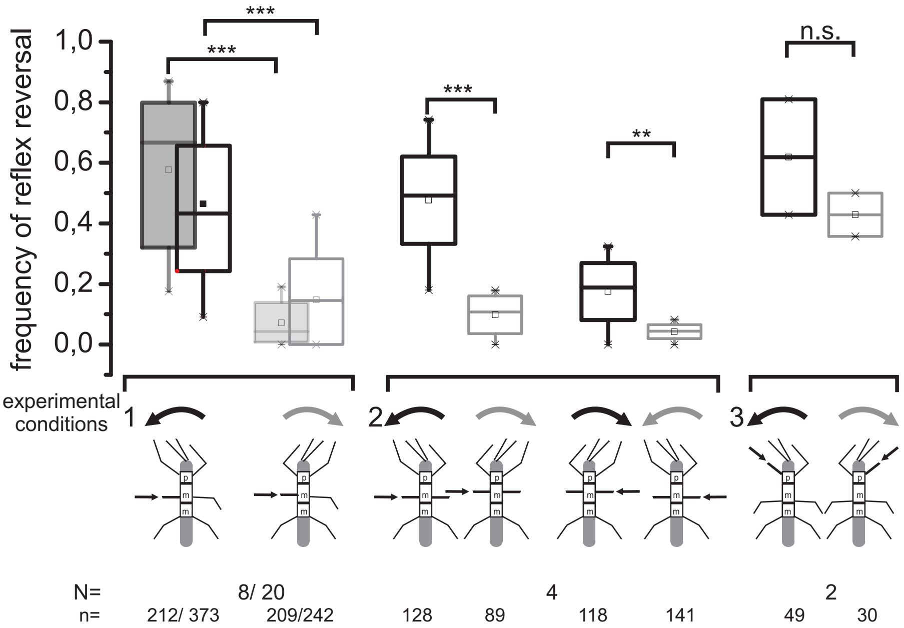

Figure 3.20: Frequency of reflex reversals in turning front and middle legs. Whisker box plots depict data from all tested animals of all different experimental conditions during walking to the left or right side. The differences between the leg on the inner side of the curve and on the outer side of the curve were tested with the chi-square test. The statistical significance is denoted as follows: (n.s.) not significant $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$;$^{***}p \leq 0.001$. Arrows above the stick insect schematics indicate walking directions and small black arrows highlight the investigated leg. N indicates number of experiments and n indicates sample size (stimuli).

and extracellular recordings, intracellular recordings were performed to investigate the physiological changes in the mesothoracic neuronal premotor network. Even in the experiments with enhanced surgery, the reflex reversal was more likely generated in the inner leg 46% compared to the outer leg 15% ($p < 0.001$; $N = 20$, Fig. 3.20 experimental condition 1, unfilled box plots). In summary, in all three experimental conditions (including the condition with additional intracellular recordings) the frequency of reflex reversal was higher in the inner leg than in the outer leg (Fig. 3.20).

3.2.2 Activity of *flexor tibiae* MNs during fCO stimulation in curve walking

The innervation of the *flexor tibiae* muscle and its function in the FTi joint control loop is highly complex. In a recent study, Goldammer and co-workers (2012) have identified up to 25 motoneurons innervating the flexor muscle of *Carausius morosus*. These are about six more flexor MNs than previously reported (Debrodt and Bässler, 1989; Storrer et al., 1986). In studies investigating the response properties of flexor MNs due to fCO elongation in resting stick insects, it was found that the changes in membrane potential in different flexor MNs can differ from each other in response to the same fCO stimulus (Debrodt and Bässler, 1989, 1990). Debrodt and Bässler (1990) found that in *Extatosoma tiaratum*, fast and intermediate flexor MNs are depolarised more strongly by fast fCO stimulation, while slow MNs are depolarised more strongly by lower stimulus velocities. Interestingly, it was further demonstrated that flexor MNs are depolarised most strongly during fCO relaxation, whereas fCO elongation was found to either hyperpolarise or depolarise flexor MNs (Debrodt and Bässler, 1990). Pfeiffer (1991) also reported different responses of flexor MNs due to fCO stimulation in the resting stick insect, but similar responses of slow and fast flexor MNs in the active animal. This was also suggested from studies in inactive (Field and Burrows, 1982; Siegler, 1981) and active locusts (Zill, 1985), in which the flexor MNs membrane potential was depolarised in response to fCO elongation as well as relaxation.

In order to investigate the specific function of flexor MNs in the FTi joint control, the physiological properties of flexor MNs in response to fCO stimulation were analysed in resting and curve walking stick insects. Interestingly, fCO stimulation in resting animals elicited in 12 of 22 intracellularly recorded flexor MNs a depolarisation due to fCO elongation and relaxation. Fig.3.21 displays exemplary data of one of these flexor MNs. The flexor MN was identified by depolarising current injection and spike correlation between the intracellular recording and the flexor EMG (Fig.3.21 A). The membrane potential of the flexor motoneuron was depolarised by current injection and the spike threshold was identified at approximately −41 mV by increasing the depolarised current injection (1, 2 nA and 1, 5 nA). Important to note is the response in the membrane potential of this flexor MN to fCO stimulation in the resting animal. During imposed fCO elongation and relaxation the membrane potential was depolarised and the depolarisation increased with higher stimulus velocities (Fig.3.21 B-D). As reported by Debrodt and Bässler (1990) in resting stick insects elongation of the fCO elicited in different flexor MNs a depolarisation of the membrane potential as well as a hyperpolarisation. In the conducted experiments here, the membrane potential of 8 flexor MNs (out of a total of 22) was hyperpolarised due to fCO apodeme elongation. In two recorded flexor neurons no response to fCO stimulation was found. Relaxation of the fCO apodeme in resting

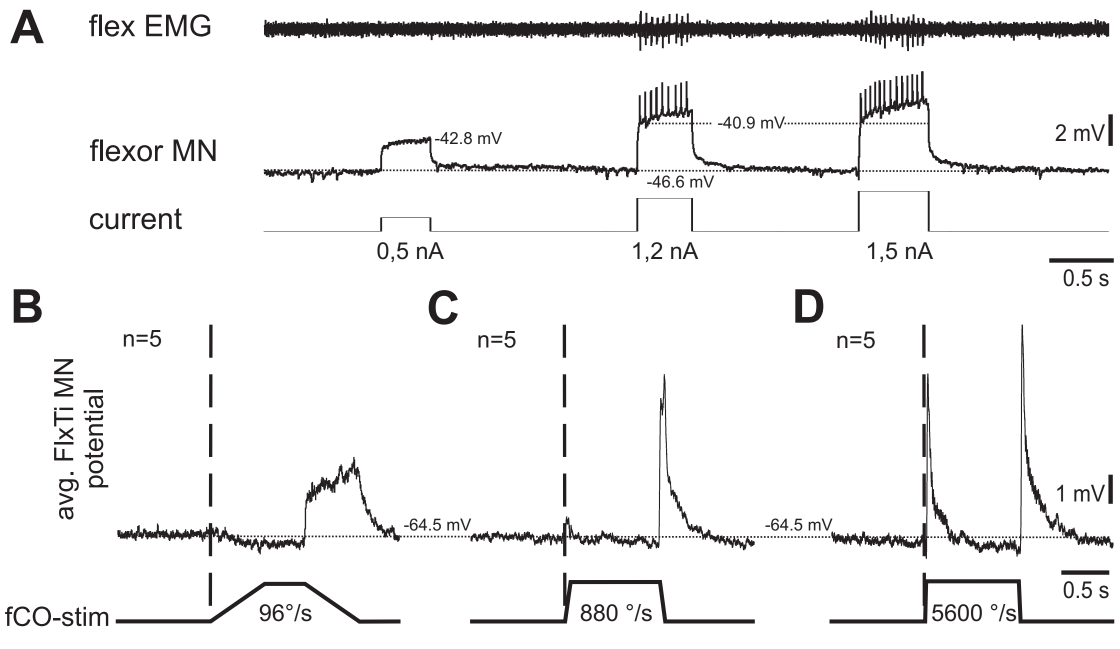

Figure 3.21: Physiological properties of a flexor MN. (A) Identification of the flexor MN by depolarising current injection and detection in the flexor muscle activity (flex EMG). B)-D) Influence of fCO stimulus velocity (B = 96 °/s, C = 880 °/s, D = 5600 °/s) on the averaged flexor MN membrane potential in resting animals. Note the increase in depolarisation caused by fast stimulus velocity during fCO elongation (D).

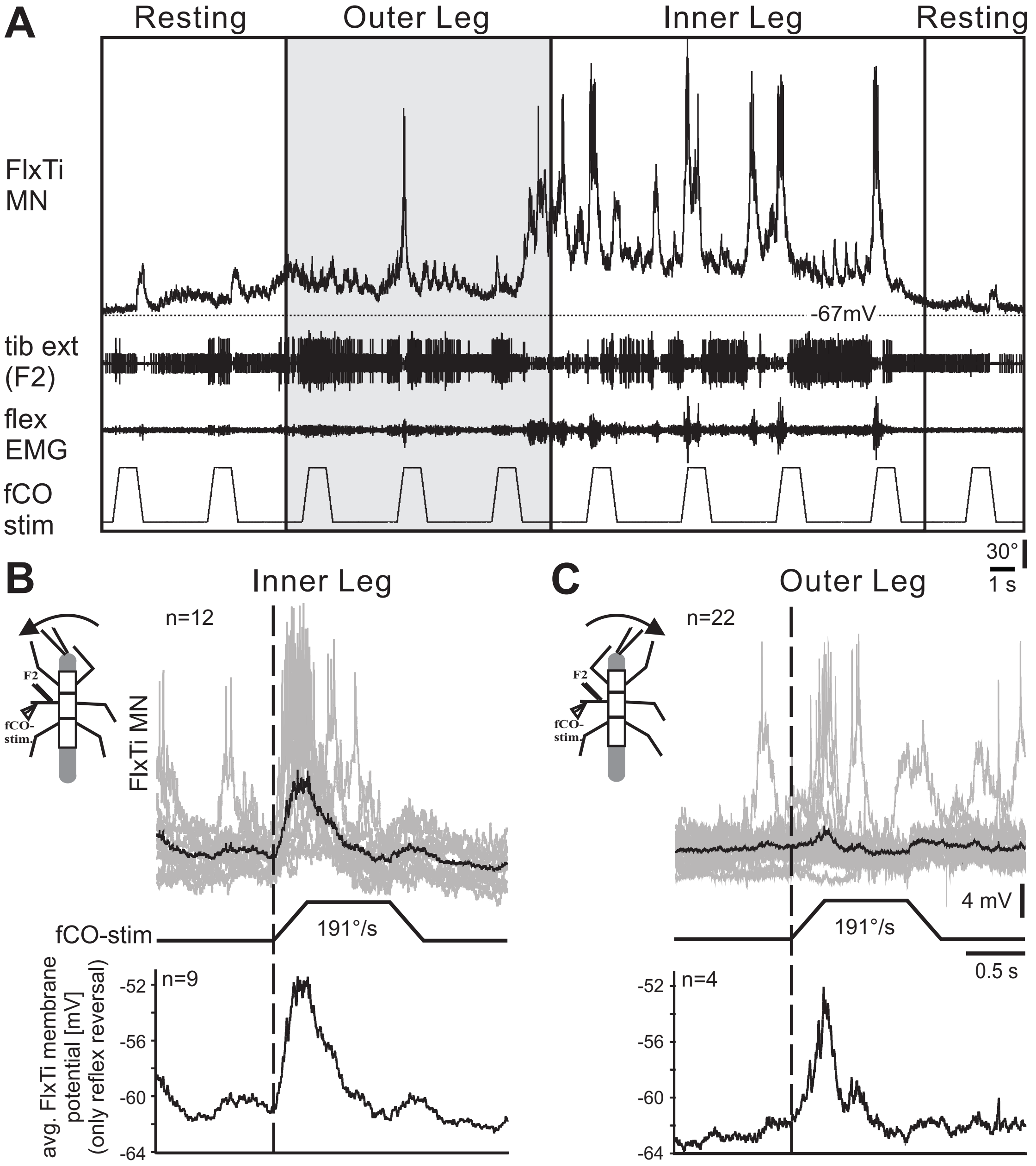

Figure 3.22: (A) Influence of fCO signals during turning in a mesothoracic flexor MN (FlxTi MN) in one exemplary animal. The femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor nerve activity (tib ext (F2)) and flexor muscle activities (flex EMG) in that leg. In this sequence, the animal was walking to the right side (outer leg, grey shaded area) and then walked to the left side (inner leg). The animal was standing before and after the walking sequence, where the fCO stimulation elicited the typical resistance reflex. (B) In the overlays of flexor MN membrane potential (spikes were removed) during all fCO stimulations in the inner leg ($n = 12$) as well as in the averaged flexor MN potential only during stimulations where reflex reversals occurred (B, $n = 9$), a strong depolarisation in the motoneuronal flexor activity due to fCO elongation is found. C) In the outer leg fCO stimulation did not elicit a clear response in the flexor MN membrane potential (C, $n = 22$). In the flexor MN averages of four stimulation with reflex reversals a depolarisation in the flexor membrane potential was found (C).

animals leads in all recorded flexor MNs ($N = 22$) to membrane potential depolarisation. However, during walking the membrane potential of the same flexor MN shown in Fig. 3.21 was slightly depolarised in the outer leg and tonically depolarised in the inner leg (Fig. 3.22 A). During both curve directions, a phasic modulation in the membrane potential becomes obvious, in which in the inner leg larger phasic depolarisations as well as large depolarisation due to fCO elongation occurred. The depolarisation of the flexor MN potential is shown in overdraws of 12 fCO elongations in the inner leg. This is even more obvious in the averaged potential (thick black trace, Fig. 3.22 B, top) and in the averaged potential of fCO stimulations, where only reflex reversals occurred (Fig. 3.22 B, bottom). However, in the membrane potential overlays of all stimuli in the outer leg no clear response to fCO stimulation was found (overlays, Fig. 3.22 C, top). Although reflex reversals occurred in four cases due to fCO stimulation in the outer leg, which is seen as a depolarisation of the flexor MN (Fig. 3.22 C, bottom), the effect during all stimulations is rare. In seven intracellular recordings performed in flexor motoneurons during curve walking, the frequency of reflex reversals altered between the inner leg (53%, $n = 104$) and the outer leg (14%, $n = 73$).

3.2.3 Activity of *extensor tibiae* MNs during fCO stimulation in curve walking

The *extensor tibiae* muscle is innervated by the femoral branch 2 (F2), which contains two excitatory MNs, the *slow extensor tibiae* (SETi) and the *fast extensor tibiae* (FETi) and one inhibitory neuron the common inhibitor 1 ($CI1$) (Bässler, 1989).

In resting stick insects, SETi spontaneously fires at a resting FTi joint position of about 90°. Elongation of the fCO depolarises both FETi MNs and SETi MNs and relaxation of the fCO hyperpolarises the membrane potentials (Bässler, 1983a). Depending on their resting membrane potential, fCO elongation elicits higher spike frequencies in SETi than in FETi. This response, described as resistance reflex, is known to reverse in the active animal (*active reaction*) (Bässler, 1973, 1976, 1986a). In active animals, fCO elongation causes an excitation of flexor MNs and an inhibition of extensor MNs during the first part of the AR, which also corresponds to a reflex reversal. In the second part of the

AR a position-dependent activation of the extensor MNs and inactivation of flexor MNs occurs.

During curve walking, the membrane potential of the *extensor tibiae* was more depolarised and the spiking frequency was higher in the outer leg compared to the inner leg (Fig.3.23 A). A tonic depolarisation in the outer leg was also observed by Hoffmann (2010). In the inner leg, fCO elongation elicited a reflex reversal, seen as a hyperpolarisation in the FETi MN and an inhibition in the extensor nerve (Fig.3.23 A, left). The response in the FETi MN membrane potential up to fCO stimulation is depicted in Fig. 3.23 (B), where an early latency depolarisation (see also Driesang and Büschges, 1996) is followed by a hyperpolarisation and a subsequent depolarisation. The influence of the fCO stimulation on the FETi MN membrane potential varied in the outer leg. However, the average of 10 stimuli indicates a depolarisation elicited by fCO elongation. Therefore, the averages over all stimuli show a change in the FETi membrane potential similar to that present in the resistance reflex. However, two fCO stimulations generated reflex reversals in the outer leg and the average of the FETi MN membrane potential reflects an inhibition during fCO elongation (Fig.3.23 C). In general, in all four intracellular recorded *extensor tibiae* MNs a clear hyperpolarisation in the inner leg due to fCO elongation was found.

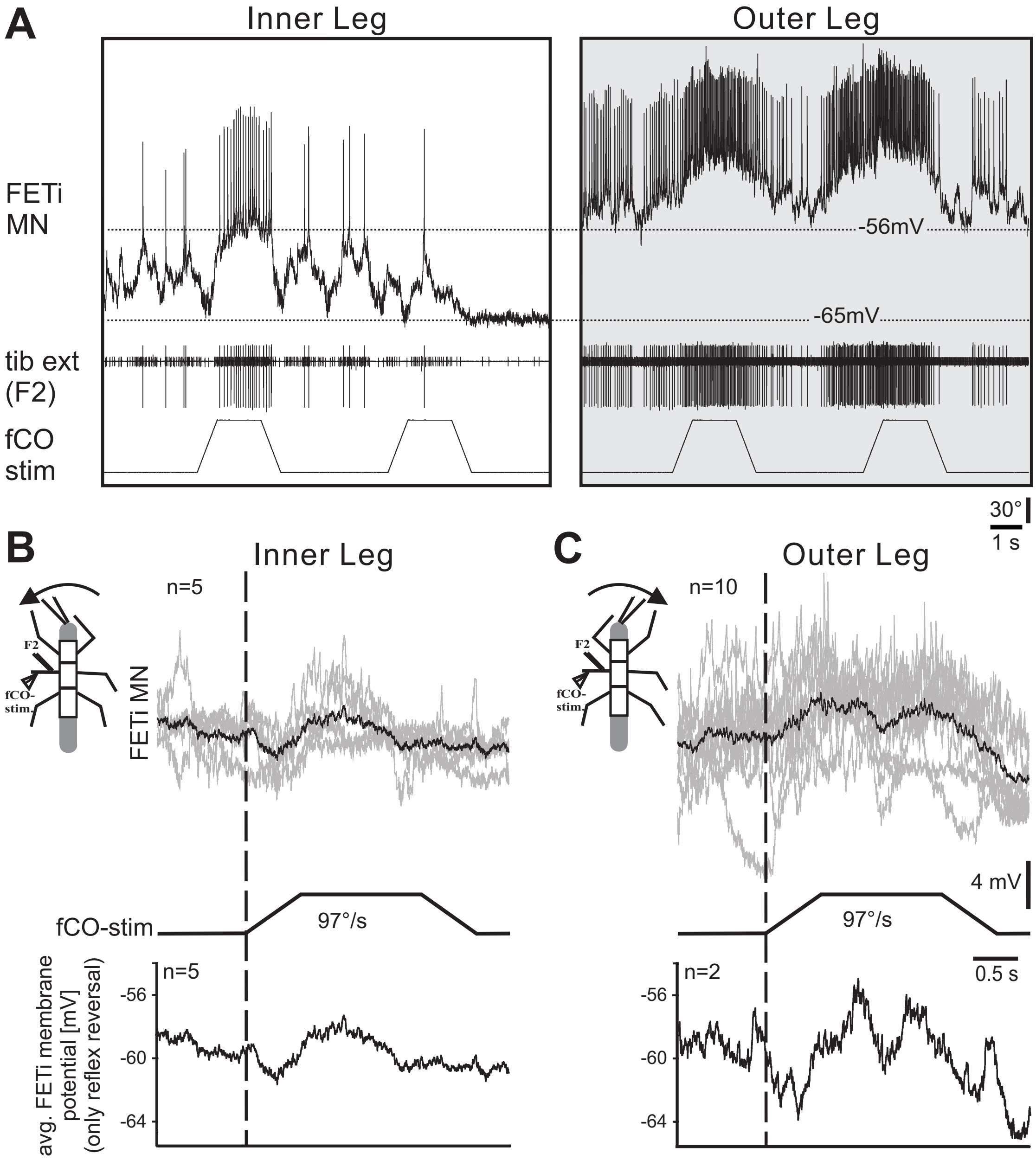

Figure 3.23: (A) Influence of fCO signals during turning in a fast *extensor tibiae* (FETi MN) in one exemplary animal. The femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor nerve activity (tib ext (F2)) in that leg. Motoneuronal activity during fCO elongation in the inner leg (left panel) and in the outer leg (right panel). (B) and (C) Reflex reversals occurred more frequently in the inner leg than in the outer leg. B) In the overlays of the FETi MN membrane potential during all fCO stimulations in the inner leg ($n = 5$) as well as in the averaged FETi MN potential only during fCO stimulations with reflex reversal (B bottom, $n = 5$) a small depolarisation at the start of fCO elongation, followed by a hyperpolarisation is shown. In the outer leg, fCO stimulation did not elicit a clear response in FETi MN membrane potential. However, the average (black trace) shows a depolarisation due to fCO elongation (C, $n = 22$). Nevertheless, in the FETi MN, averages of two stimulations with only reflex reversals revealed a hyperpolarisation in FETi MN potential during fCO elongation (C).

3.2.4 Influence of curve walking on the processing of fCO-mediated signals in nonspiking interneurons

The interneuronal basis of proprioceptive reflexes in the FTi joint control system is well-studied in the locust (for review see Burrows, 1996) and in the stick insect (for review see: Bässler, 1993; Bässler and Büschges, 1998). It is known that sensory afferents of the fCO receive presynaptic inhibition from inputs arising from afferents of the same sensory type of fCO afferents (Sauer et al., 1997). Furthermore, sensory afferents can project directly or indirectly via nonspiking interneurons (NSI) or spiking interneurons, monitoring the same movement parameters, onto MNs (Büschges, 1990; Sauer et al., 1996, 1995). Several of the identified NSIs inhibit or excite MNs. Previous studies have identified nonspiking interneurons, which either excite (excitatory (E) NSIs) or inhibit (inhibitory (I) NSIs) extensor MNs that are involved in the processing of fCO signals of the FTi joint (Akay, 2002; Büschges, 1990; Stein and Sauer, 1998). In order to understand the information processing of fCO signals in the curve walking stick insect, intracellular recordings of nonspiking interneurons of the FTi joint premotor network were performed. In the following section, I will present initial results of intracellular recordings of nonspiking INs involved in the FTi joint control during curve walking. Several nonspiking interneurons were identified by their influence on the extensor activity due to current injection and their change in membrane potential caused by fCO stimulation (cf. Akay, 2002; Büschges, 1990; Stein and Sauer, 1998). Subsequently, the physiological properties of the NSIs were investigated during curve walking. For this purpose, the membrane potential modulations in the inner and outer leg were characterised for all stimulations and only for stimulations, which generated reflex reversals.

Büschges (1990) identified the nonspiking interneurons type E2 and E3, which both were depolarised in response to elongation and hyperpolarised during relaxation of the fCO. Apart from small differences in their morphology, E3 showed a slight, yet variable, position-dependent depolarisation (Büschges, 1990; Sauer et al., 1997). Therefore, in this thesis the interneurons of type E2 and E3 were classified as one group (NSI type E2/3). First, in order to characterise the nature of the interneuron, depolarising and hyperpo-

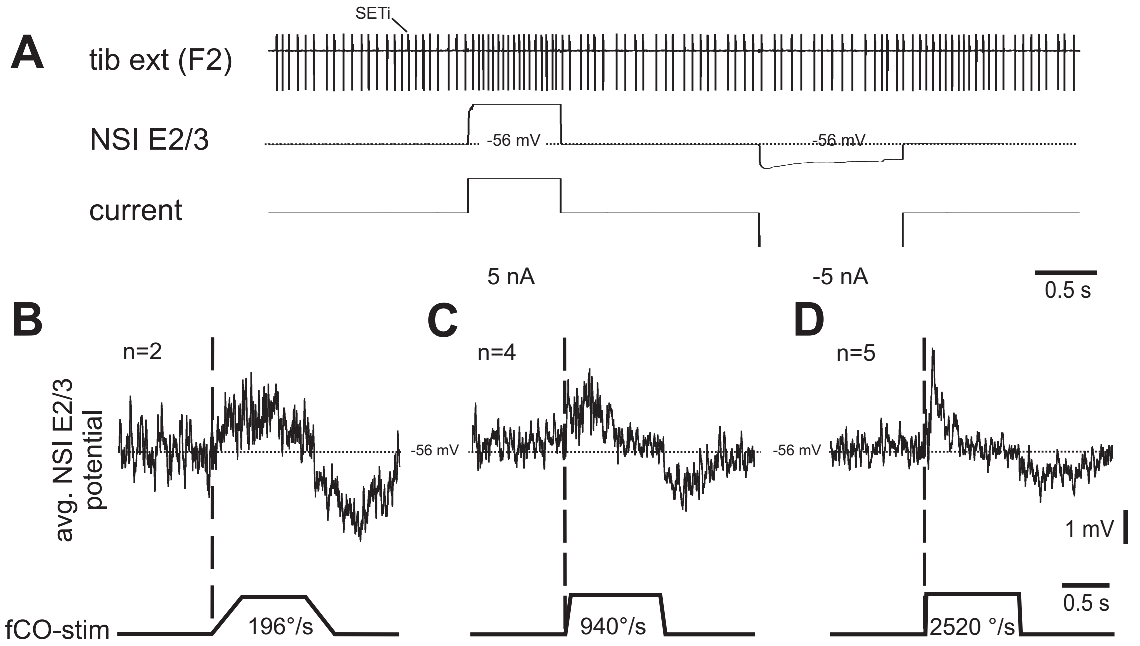

Figure 3.24: (A) Physiological properties of NSI type E 2/3 in a resting animal. Change in *extensor tibiae* motoneuronal activity (tib ext (F2)) in response to depolarising and hyperpolarising current injection into nonspiking interneuron E2/3. The spontaneous SETi firing frequency increased due to depolarising current injection and decreased during hyperpolarising current injection. B)-D) Influence of fCO stimulus velocity ($B = 195\,°/s$, $C = 940\,°/s, D = 2520\,°/s$) on the membrane potential of NSI E2/3 (B, $n = 2$; C, $n = 5$; D, $n = 5$).

larising currents were injected. The interneuron was identified as excitatory based on the fact that depolarisation increased the firing frequency of the SETi and hyperpolarising current injection decreased SETi spiking frequency (Fig.3.24 A, cf. Büschges, 1990). In resting animals, the response to fCO stimulation in the membrane potential of this excitatory interneuron showed a depolarisation with fCO elongation. With higher stimulus velocity the depolarisation slightly increased (Fig. 3.24 B-D, cf. Büschges, 1990). Relaxation of the fCO induced a hyperpolarisation, which was reduced with higher stimulus velocities (Fig. 3.24 B-D).

During curve walking, the membrane potential of the NSI E2/3 was strongly hyperpolarised in response to fCO elongation in the inner leg (Fig. 3.25 A). In contrast, in the outer leg only slight changes in the membrane potential independent of fCO stimulation become obvious (Fig. 3.25 A). In the inner leg, the prominent inhibition caused by fCO elongation is displayed in the overlays of the membrane potential of 19 stimulations (Fig. 3.25 B, top). The membrane potential of these NSI E 2/3 was on average also hyperpolarised during the stimulations with reflex reversals (Fig. 3.25 B, bottom; $n = 11$).

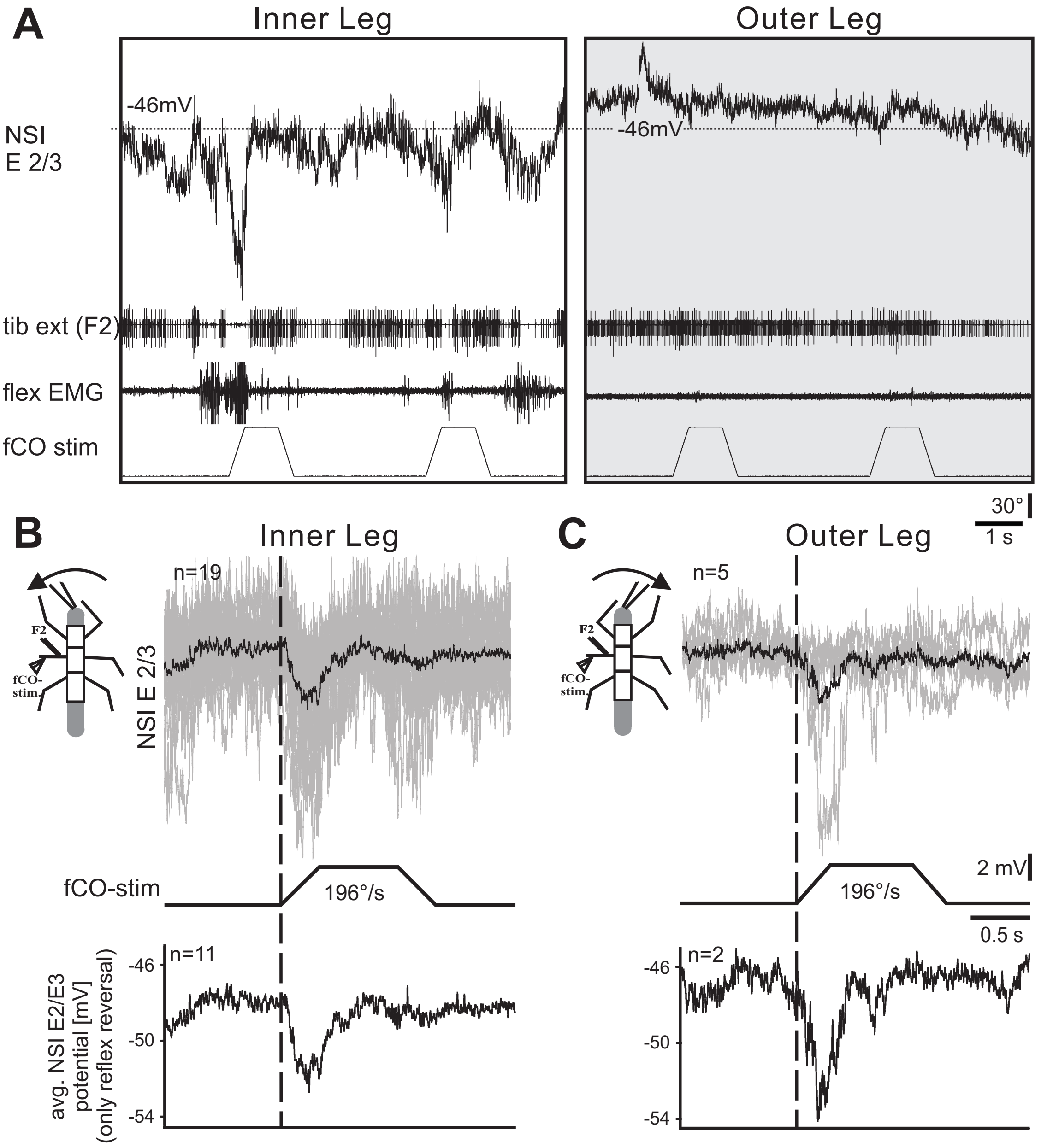

Figure 3.25: (A) Influence of fCO signals during turning on a mesothoracic nonspiking interneuron of type E2/3 (NSI E2/3). The femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor nerve activity (tib ext (F2)) and flexor muscle activities (flex EMG) in the same leg of one exemplary animal. During walking, the investigated leg was either a functional inner leg (left panel) or an outer leg (right panel). (B) and (C) Averages of the membrane potential of NSI E2/3. Top panels: overlays of the membrane potential of NSI type E2/3 during all fCO stimulations in the inner ($n = 19$) and outer leg ($n = 5$). Bottom panels: averages of NSI E2/3 membrane potential only during reflex reversals caused by fCO stimulation in the inner ($n = 11$) and outer leg ($n = 2$).

However, the inhibition was on average lower in the outer leg (Fig. 3.25 C), and mainly based on the occurrence of two reflex reversals when that leg was on the outside of the curve (Fig. 3.25, bottom). Thus, in the NSI type E2/3 the change in membrane potential caused by fCO elongation was reversed between resting and walking animals (cf. Bässler and Büschges, 1990; Driesang and Büschges, 1996). Interestingly, the noticeable inhibition during fCO elongation in the inner leg was reduced in the outer leg, which indicates a difference in the processing of fCO signals between the inner and outer leg.

Next, the nonspiking interneuron E4 was identified in the resting stick insect by the following criteria: first depolarising current was injected into the nonspiking interneuron and an excitatory influence on the SETi activity was shown. The firing frequency of the SETi MN increased with increasing depolarised current injection (Fig. 3.26 A, cf. Büschges, 1990). Second, this excitatory interneuron responded to elongation and relaxation of the fCO with a phasic depolarisation of the membrane potential. The de-

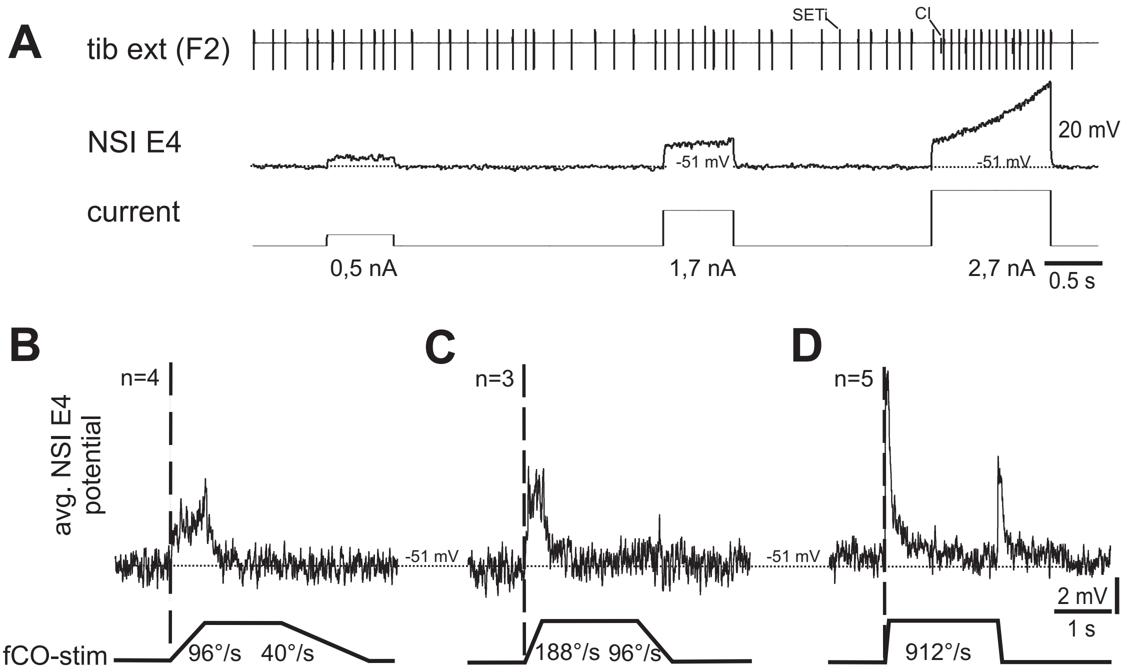

Figure 3.26: (A) Physiological properties of NSI type E4. Change in *extensor tibiae* motoneuronal activity (ext tib (F2)) in response to depolarising current injection into nonspiking interneuron E4. The spontaneous SETi firing frequency and the membrane potential increased due to rising depolarised current injection. Here, the slow increase in the membrane potential was caused by a badly balanced electrode. B)-D) Influence of the fCO stimulus velocity (B = 96 °/s (elongation); 40 °/s (relaxation), C = 188 °/s, D = 912 °/s) on the membrane potential of NSI E4 in the resting animal. Note the increase in depolarisation with higher stimulus velocity due to elongation and relaxation of the fCO. n denotes number of fCO stimuli.

polarisation increased with higher stimulus velocities.

During fCO elongation, the depolarisation of the membrane potential in the NSI E4 was always larger than during relaxation (Fig. 3.26 B-D, Büschges, 1990) In general, the animals in which the intracellular recordings of the nonspiking interneuron E4 were performed, only rarely turned in both curve directions in the same experiment. Therefore, only a qualitative representation of the membrane potential for the inner and outer leg is given in Fig. 3.27 A. During curve walking, the membrane potential of NSI E4 in the inner leg was slightly phasic modulated compared to the outer leg, which in general showed a more depolarised membrane potential (Fig. 3.27 A). The membrane potential during curve walking is shown in Fig. 3.27 B, C for both walking directions. In the overlays of the E4 membrane potential for the inner and outer leg (each five fCO stimuli) a clear depolarisation in response to fCO elongation was identified (Fig. 3.27 B, C; cf. Driesang and Büschges, 1996). Furthermore, during reflex reversals elicited by fCO stimulations in the inner (n=2) and outer leg (n=6) of two different animals, NSI E4 was depolarised at the beginning of stimulus onset. About 200 *ms* after the onset of the stimulus the amplitude of the depolarisation decreased. After the decrease the membrane potential of NSI E4 becomes more variable (Fig. 3.27 D). In summary, during curve walking, the membrane potential of the NSI E4 depolarised at the onset of fCO elongation in the inner and outer leg. This depolarisation was also found during reflex reversals in the inner and outer leg. However, it is not established how the NSI E4, in general, is involved in the processing of fCO signal during curve walking, with regard to variability between the different animals. In addition, the results are limited by a small sample size.

Büschges (1990) was able to show that the excitatory NSIs E5 and E6 were depolarised due to elongation and, even more, due to relaxation of the fCO. Despite their morphological and physiological similarities, NSI E6 showed a prominent position-dependent tonical depolarisation and, thus, was classified separately (Büschges, 1990). However, this tonic component was hardly identifiable in the intracellular recordings performed in the present thesis. Therefore, NSI E5 and E6 were grouped together as NSI E5/6.

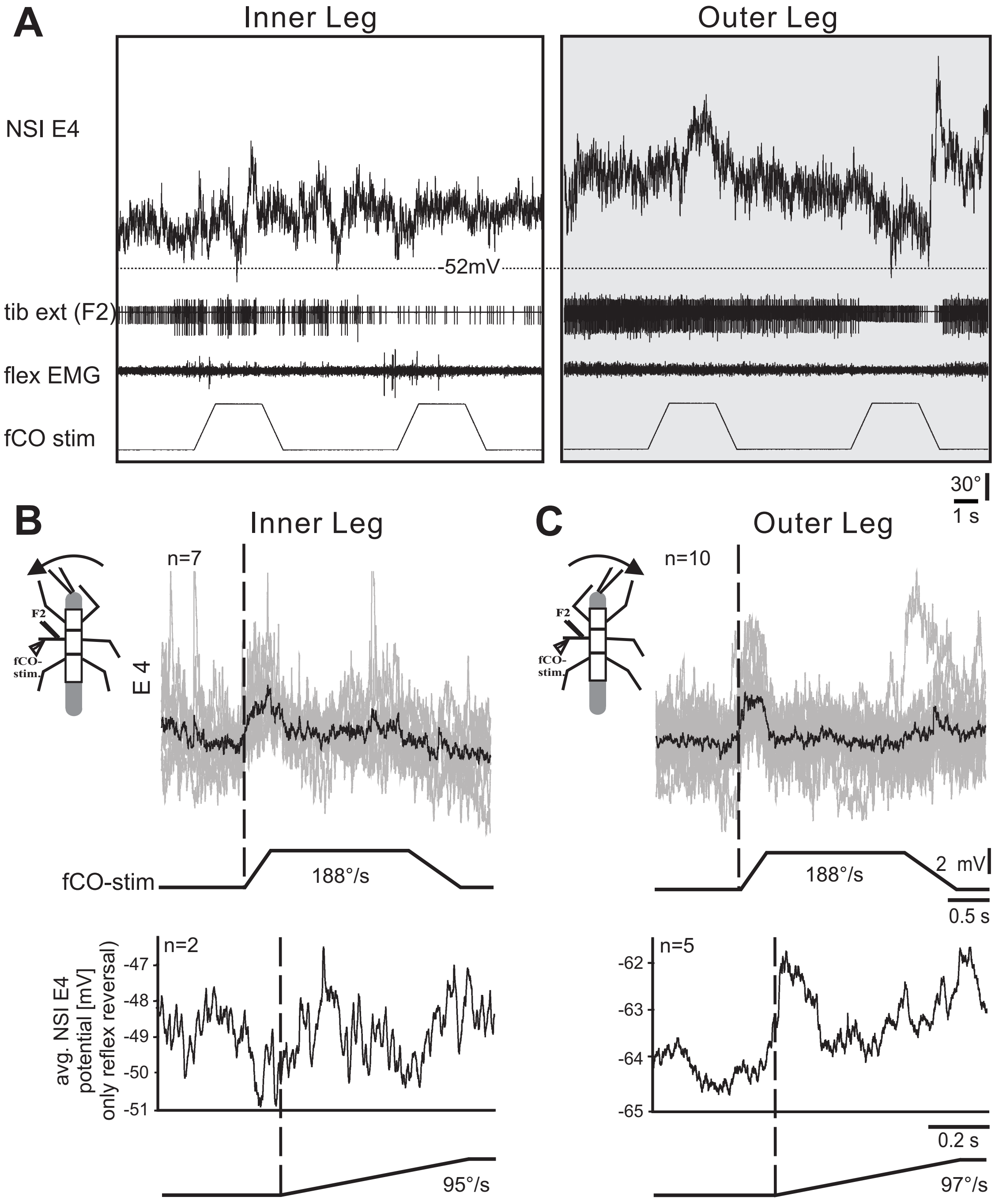

Figure 3.27: (A) Influence of fCO signals during turning on a mesothoracic nonspiking interneuron of type E4 (NSI E4). The femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor nerve activity (tib ext (F2)) and flexor muscle activities (flex EMG) in the same leg of one exemplary animal. During walking, the investigated leg was either a functional inner leg (left panel) or an outer leg (right panel). (B) and (C) Averages of the membrane potential of NSI E4. Top panels: overlays of the membrane potential of NSI type E4 during all fCO stimulations in the inner ($n = 7$) and outer leg ($n = 10$). Bottom panels: averages of NSI E4 membrane potential only during reflex reversals caused by fCO stimulation in the inner ($n = 2$) and outer leg ($n = 5$). n denotes number of stimuli.

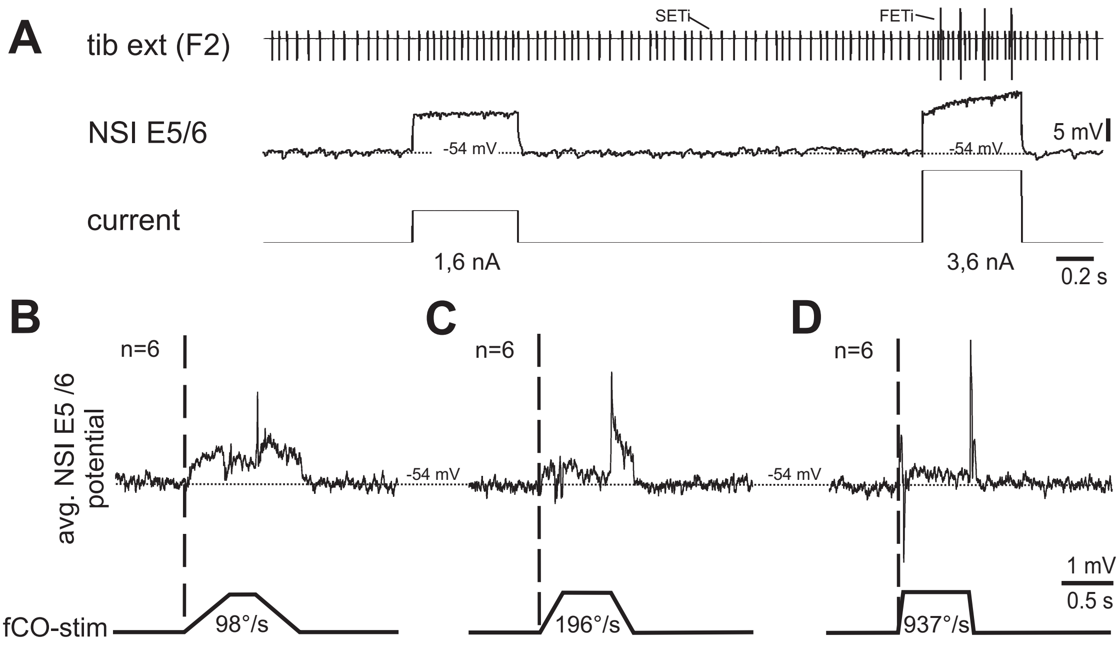

Figure 3.28: (A) Physiological properties of NSI type E5/6. Change in *extensor tibiae* motoneuronal activity (ext tib (F2)) in response to depolarising current injection into nonspiking interneuron E5/6. The spontaneous SETi firing frequency increased due to rising depolarised current injection; high current injection also elicited spike generation in the FETi MN. B)-D) Influence of fCO stimulus velocity ($B = 96\,°/s$, $C = 196\,°/s$, $D = 937\,°/s$) on the membrane potential of NSI E5/6 in the resting animal. Note the increase in depolarisation with higher stimulus velocity due to elongation and relaxation of the fCO and in particular the increase in inhibitory synaptic inputs during fCO elongation. n denotes number of fCO stimuli.

One of the neurons classified as NSI E5/6 is shown in Figure 3.28 A. In the resting stick insect, depolarisation of this interneuron increased the discharge rate of SETi activity and induced spikes in the FETi MN (cf. Büschges, 1990). During fCO elongation and relaxation the membrane potential of this excitatory nonspiking interneuron was depolarised. The depolarisation during relaxation increased with increasing stimulus velocities and showed to be always larger than during fCO elongation (Fig. 3.28 B-D, cf. Büschges, 1990).

In one exemplary sequence of the recordings of this NSI E5/6 in the resting animal, the membrane potential of the NSI was depolarised due to fCO elongation and relaxation. Simultaneously, the activities of the *extensor* and *flexor tibiae* reflected the activity pattern of a resistance reflex (Fig. 3.29 A). When the animal started walking and the investigated leg was the functional outer leg, a tonic depolarisation becomes apparent, which increased in the inner leg. In the inner leg, the overlaid membrane potentials of all stimulations ($n = 19$) varied in this time courses. However, a slight hyperpolarisation during fCO elongation and a depolarisation in response to fCO relaxation was observed on average (Fig. 3.29 B). In contrast, in the outer leg, fCO elongation depolarised the membrane potential of this NSI E5/6. Similar to the inner leg, relaxation of the fCO apodeme induced a depolarisation (Fig. 3.29 Ci). Interestingly, in the recordings of NSI E5/6 of a different animal, in which also reflex reversals occurred, fCO elongation hyperpolarised this NSI E5/6 in the outer leg (Fig. 3.29 Cii). During reflex reversals in the inner leg a prominent inhibition was found (Fig. 3.29 B, bottom). Identical, during reflex reversals in the second animal fCO elongation hyperpolarised the membrane potential of the NSI E5/6 (Fig. 3.29 Cii, bottom). These results differ from the finding that during the first part of the AR in active animals almost no changes in membrane potential could be seen (Driesang and Büschges, 1996). It was further shown, that after the inhibition the membrane potential depolarised and therefore assists the second part of the AR, as was shown by Driesang and Büschges (1996). To sum up, in the NSI E5/6, the influence of the processing of fCO signals in resting and curve walking animals during fCO elongation is reversed, with exception of the response in the outer leg of one of the animals shown (Fig. 3.29 Ci). In the outer leg, fCO elongation induced depolarisation as well as hyperpolarisation in a different animal. During reflex reversals in both the inner and outer legs, a strong inhibition was found during elongation.

Only a qualitative characterisation of the excitatory NSI type E8 was performed. NSI E8 was identified by depolarising current injection, which increased the activity of spontaneously firing SETi MN (Fig. 3.30 A, Stein and Sauer, 1998). In resting stick insects, hyperpolarisation in response to fCO relaxation and elongation was found (Fig. 3.30 B-D), Stein and Sauer, 1998). Both phasic hyperpolarisations increased with higher stimulus velocities (Fig. 3.30 D). During one exemplary fCO stimulation in the inner leg a reflex reversal was generated and the membrane potential of NSI E8 remained depolarised during fCO elongation (Fig. 3.30 E). In contrast, in the outer leg, the membrane potential of the NSI E8 was hyperpolarised during one exemplary fCO elongation and simultaneously in the extensor nerve an activity pattern similar to a resistance reflex becomes apparent (Fig. 3.30 F). Regarding the number of fCO stimulations during curve

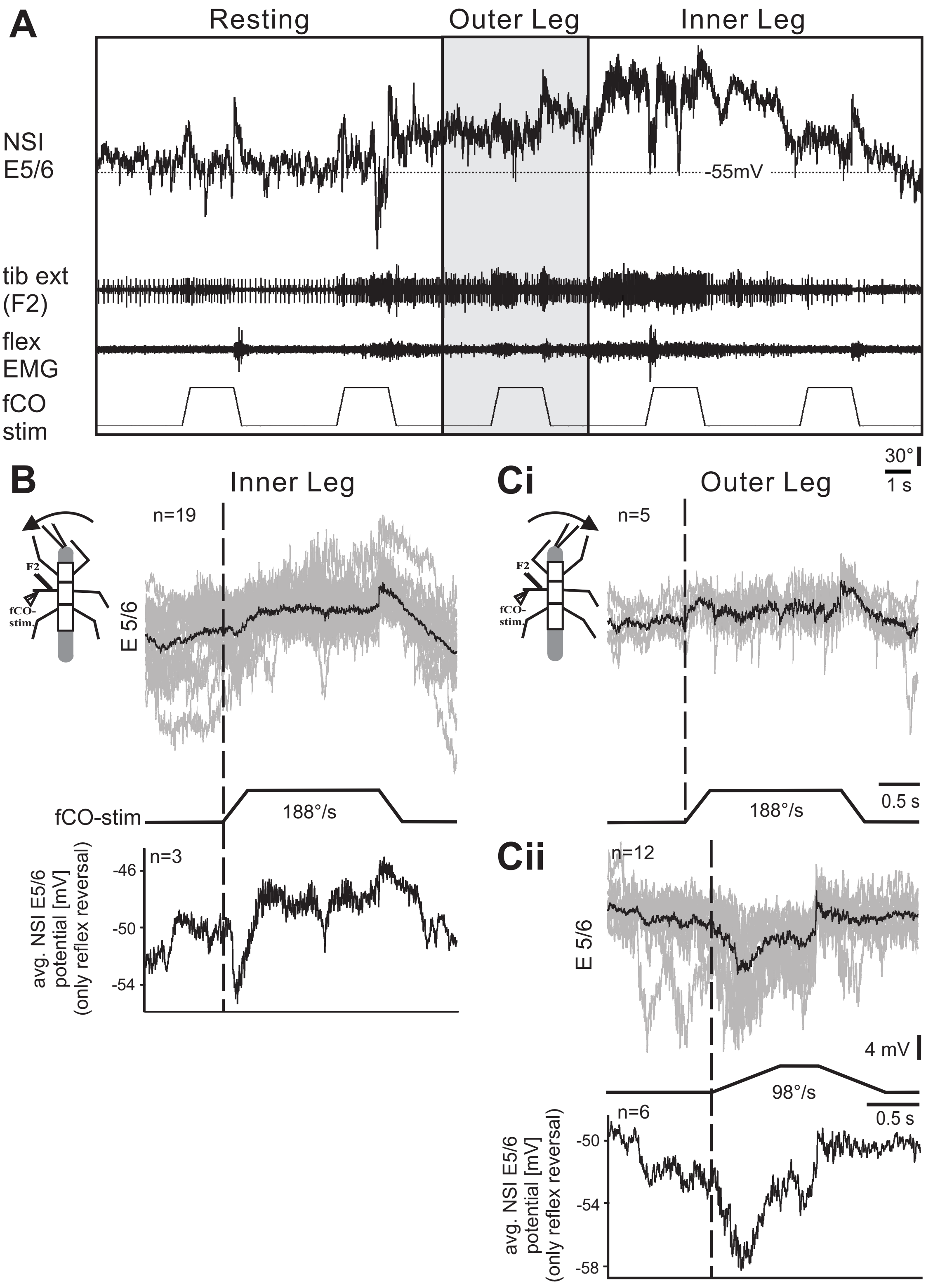

Figure 3.29: (A) Influence of fCO signals during turning on mesothoracic NSI of type E5/6 (NSI E5/6). The femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor nerve activity (tib ext (F2)) and flexor muscle activities (flex EMG) in the same leg of one exemplary animal. During walking the investigated leg was either a functional inner leg (left panel) or an outer leg (right panel). (B) and (C) Averages of the membrane potential of NSI E5/6. Top panels: overlays of the membrane potential of NSI type E5/6 during all fCO stimulations in the inner ($n = 19$) and in the outer leg of two different animals (Ci $n = 5$; Cii $n = 12$). Bottom panels: averages of NSI E5/6 membrane potential only during reflex reversals caused by fCO stimulation in the inner ($n = 3$) and outer leg ($n = 6$; Cii, bottom). n denotes number of stimuli.

walking, it remains unclear how NSI E8 is involved in the processing of fCO signals in the inner and outer leg.

The last classified excitatory nonspiking interneurons were interneurons of the type E9 and E10. They were also grouped together as NSI E9/10. Akay (2002) showed for both NSI types a tonic depolarisation in response to fCO elongation. The physiological difference between these two NSIs is the change in membrane potential caused by fCO relaxation. NSI E9 showed a slight hyperpolarisation caused by fCO relaxation, whereas no change in NSI E10 was found (Akay, 2002). The identification of these NSIs by this differentiation in the two recorded interneurons shown here was not feasible. Therefore, they were grouped as NSI E9/10. The NSI E9/10 were identified by their responses to current injection and fCO stimulation in the resting animal. Depolarisation of NSI E9/10 increased the spontaneous activity of SETi MN (Fig. 3.31 A, cf. Akay, 2002). Furthermore, fCO elongation induced a strong tonic depolarisation and, additionally, during fast elongation, a phasic depolarisation (Fig. 3.31 D). Relaxation of the fCO terminated the depolarisation and caused a slight hyperpolarisation (Fig. 3.31 B-D, cf. Akay, 2002). As shown in the resting animal, during curve walking, fCO elongation leads to a strong tonic depolarisation in the inner as well as in the outer leg (Fig. 3.32 A). This becomes in particular obvious in the overlays of the membrane potential for both walking directions. On average, in the inner (Fig. 3.32 B, top) as well as in the outer leg (Fig. 3.32 C, top), the membrane potential was position-dependently depolarised and the amplitude decreased in response to fCO relaxation. During reflex reversals, similar time courses were found for both walking directions (3.32 B, C bottom). Taken together, fCO stimulation in the resting, as well as in the curve walking animal, leads always to a position-dependent depolarisation in the membrane potential of NSI E9/10.

In addition to the excitatory nonspiking interneurons, also the presence of inhibitory NSIs is known in the FTi joint premotor network (Büschges, 1990). Here, inhibitory interneuron I2 was recorded during curve walking. It was characterised as inhibitory by its property to inhibit extensor firing during and after positive current injection (Fig. 3.33

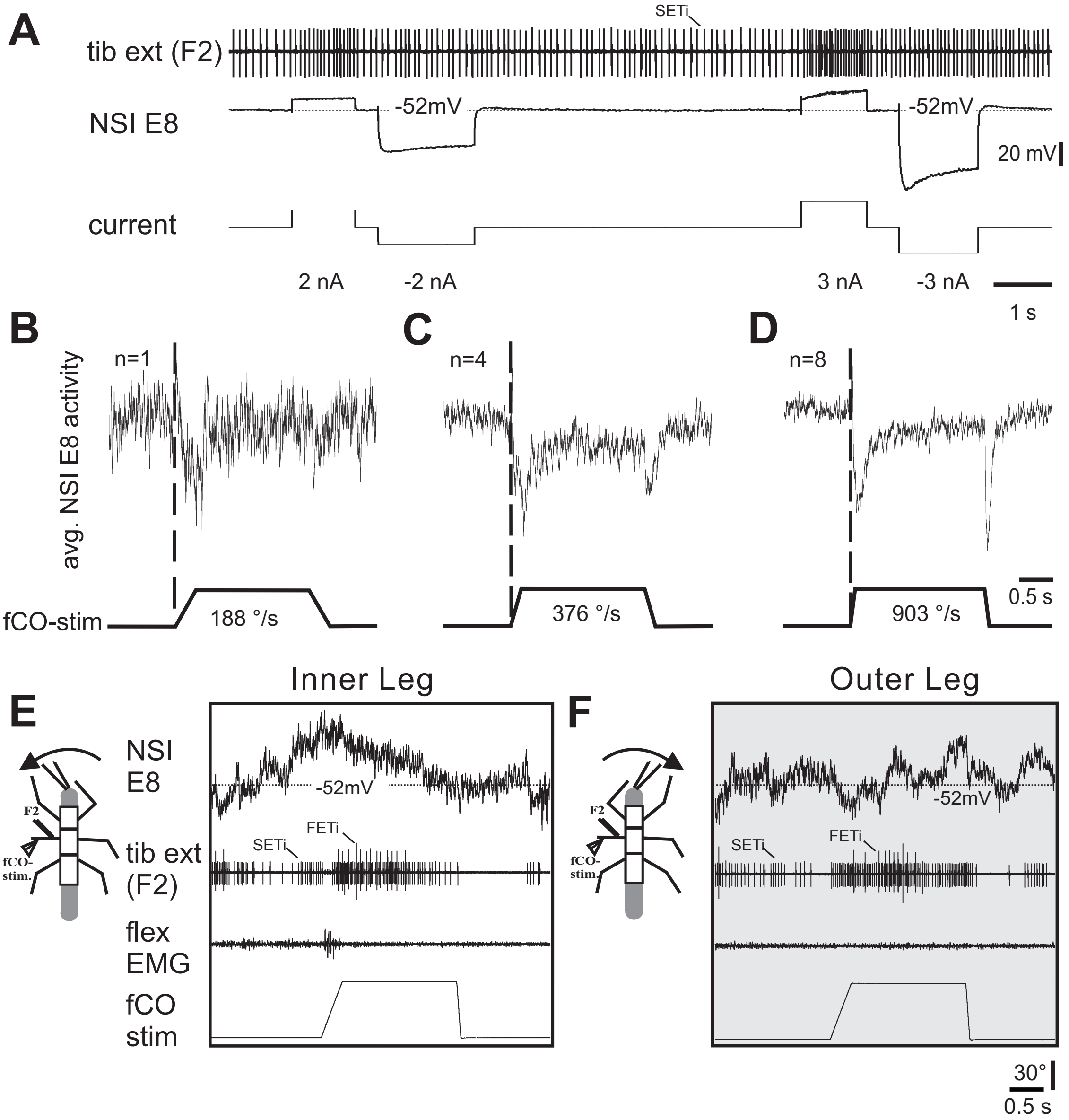

Figure 3.30: (A) Physiological properties and influence of fCO signals during turning in NSI type E8 in one exemplary animal. Change in *extensor tibiae* motoneuronal activity (ext tib (F2)) in response to depolarising and hyperpolarising current injection into nonspiking interneuron E8. The spontaneous SETi firing frequency and the membrane potential increased due to rising depolarised current injection. B)-D) Influence of fCO stimulus velocity ($B = 188\,°/s$, $C = 376\,°/s$, $D = 903\,°/s$) on the membrane potential of NSI E8 in the resting animal. Note the increase in hyperpolarisation caused by increasing stimulus velocity. (E) and (F) Influence of fCO signals during turning on a mesothoracic nonspiking interneuron of type E8 (NSI E8). The femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor nerve activity (tib ext (F2)) and flexor muscle activities (flex EMG) in the same leg of one exemplary animal. During walking, the investigated leg was either a functional inner leg (E) or an outer leg (F). n denotes number of fCO stimuli.

A, cf. Büschges, 1990). In resting animals, fCO elongation and relaxation caused a depolarisation in the membrane potential of NSI I2. (Fig. 3.33 B-D, Büschges, 1990). The amplitude of the depolarisation in response to fCO elongation, as well as, relaxation increased with increasing stimulus velocities.

During curve walking, only a slight influence of fCO stimulation was found (Fig. 3.34 A). In the inner leg, as well as, in the outer leg the membrane potential of the NSI I2 was slightly depolarised in response to fCO elongation and relaxation (Fig. 3.34 B, C; top). During reflex reversals, the same effect was found, which is shown in the averages of the membrane potential of NSI I2 in the inner (Fig. 3.34 B, bottom) and in the outer leg (Fig. 3.34 B, D). In general, in the resting and walking stick insect, fCO elongation, as well as, relaxation caused a depolarisation. Furthermore, in the inner and outer leg and in both legs during reflex reversals, the membrane potential of NSI I2 was depolarised in response to fCO elongation and relaxation.

Finally, an unknown inhibitory nonspiking interneuron (NSI 'I') of the FTi joint premo-

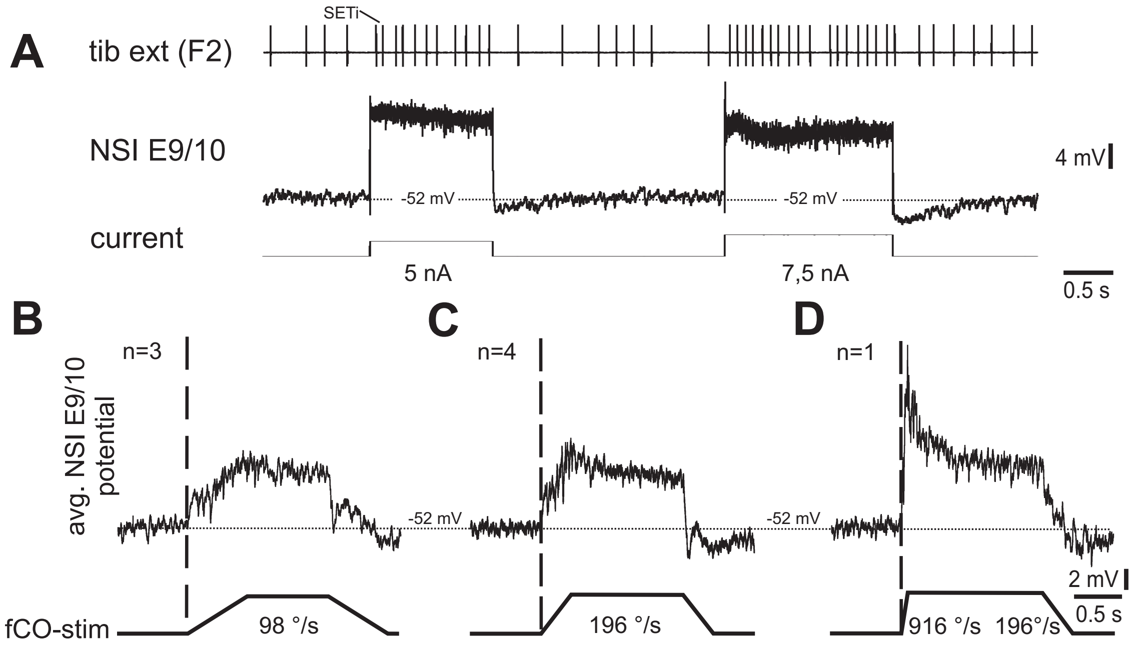

Figure 3.31: (A) Physiological properties of NSI type E9/10. Change in *extensor tibiae* motoneuronal activity (ext tib (F2)) in response to depolarising and hyperpolarising current injection into nonspiking interneuron E9/10. The spontaneous SETi firing frequency and the membrane potential increased due to depolarised current injection and decreased during hyperpolarised current injection. B)-D) Influence of fCO stimulus velocity ($B = 98\,°/s$, $C = 196\,°/s$, $D = 916\,°/s$) on the membrane potential of NSI E9/10 in the resting animal. Note the increase in the phasic component of the depolarisation due to higher elongation velocity (D). n denotes number of fCO stimuli.

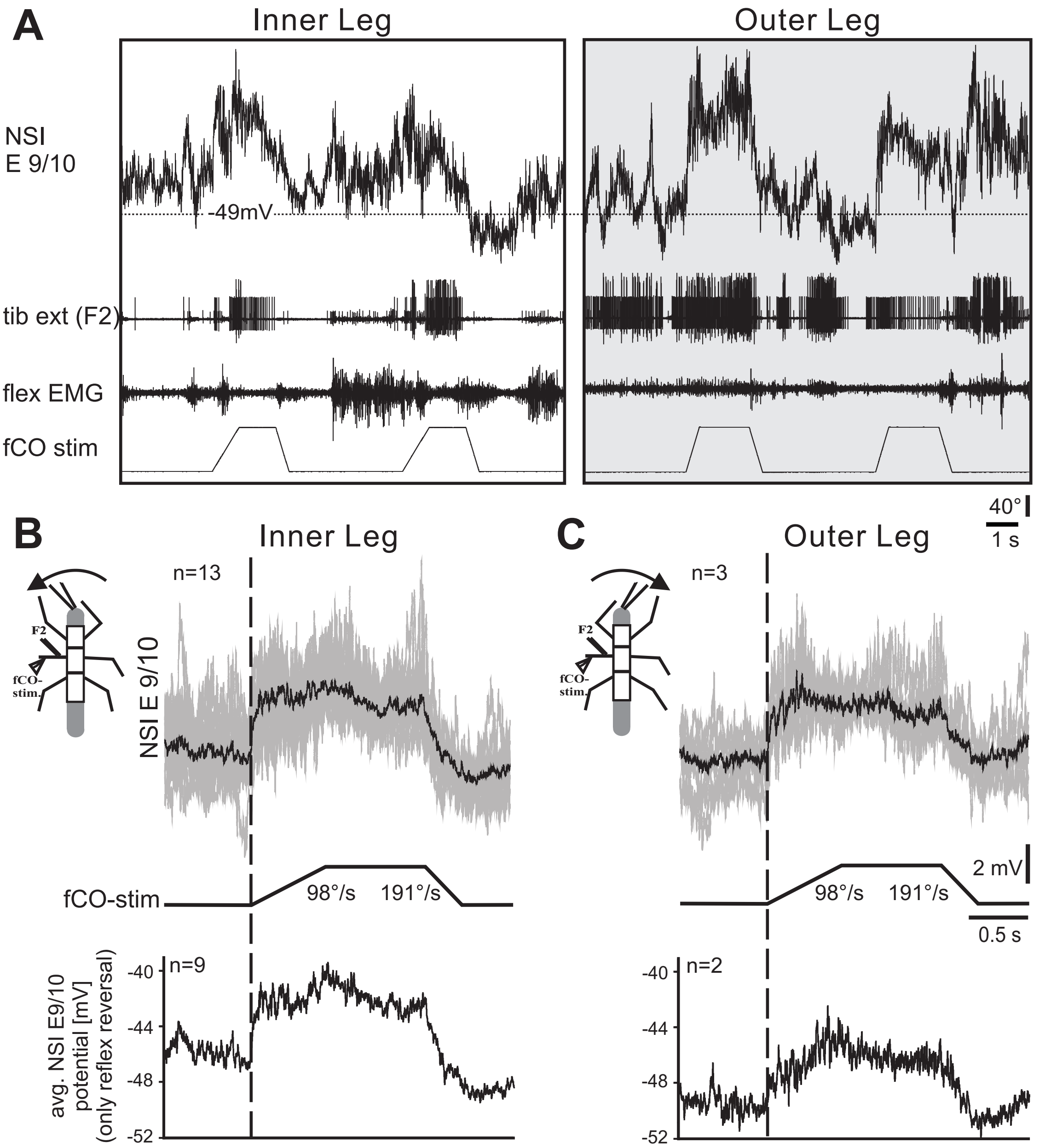

Figure 3.32: (A) Influence of fCO signals during turning on a mesothoracic nonspiking interneuron of type E9/10 (NSI E9/10). The femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor nerve activity (tib ext (F2)) and flexor muscle activities (flex EMG) in the same leg of one exemplary animal. During walking, the investigated leg was either a functional inner leg (left panel) or an outer leg (right panel). (B) and (C) Averages of the membrane potential of NSI E9/10. Top panels: overlays of the membrane potential of NSI type E9/10 during all fCO stimulations in the inner ($n = 13$) and outer leg ($n = 3$). Bottom panels: averages of NSI E9/10 membrane potential only during reflex reversals caused by fCO stimulation in the inner ($n = 9$) and outer leg ($n = 2$). n denotes number of stimuli.

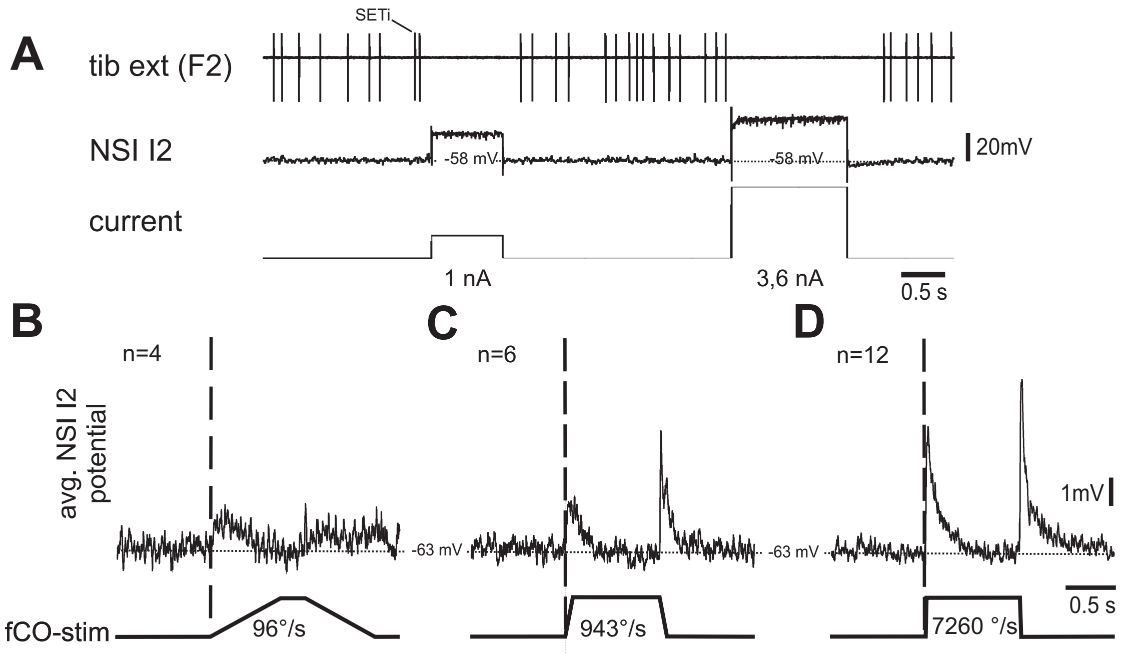

Figure 3.33: (A) Physiological properties and reflex reversal during turning in NSI type I2. Change in *extensor tibiae* motoneuronal activity (ext tib (F2)) in response to depolarising current injection into nonspiking interneuron I2. The spontaneous SETi firing frequency was completely blocked by depolarising current injection. B) - D) Influence of fCO stimulus velocity ($B = 96\,°/s$, $C = 943\,°/s$, $D = 7260\,°/s$) on the membrane potential of NSI I2 in the resting animal. Note the increase in the phasic components of the depolarisation due to higher elongation and relaxation velocity (D). n denotes the number of fCO stimuli.

tor network was characterised (Fig. 3.35 A). In the resting animal, during depolarised current injection this interneuron stopped the spontaneous SETi MN spiking activity (Fig. 3.35 A). To characterise the physiological properties of this interneuron, in the resting animal fCO stimulations with different stimulus velocities were applied. The membrane potential of this inhibitory NSI was tonically hyperpolarised by fCO elongation. Relaxation of the fCO generated a phasic depolarisation, which increased with increasing stimulus velocities (Fig. 3.35 B-D).

During curve walking, the membrane potential of this inhibitory nonspiking interneuron is phasic modulated and, in general, more depolarised in the outer leg (Fig. 3.36 A). In the inner and the outer leg, the influence of fCO stimulation was similar to the time course of the membrane potential in the resting animal. Elongation of the fCO induced a position-dependent hyperpolarisation in this neuron and relaxation causes a depolarisation (Fig. 3.36 B, C, top).

However, during reflex reversals in the inner leg, the hyperpolarisation caused by fCO elongation occurred slightly later (Fig. 3.36 B, bottom). Interestingly, in the outer leg dur-

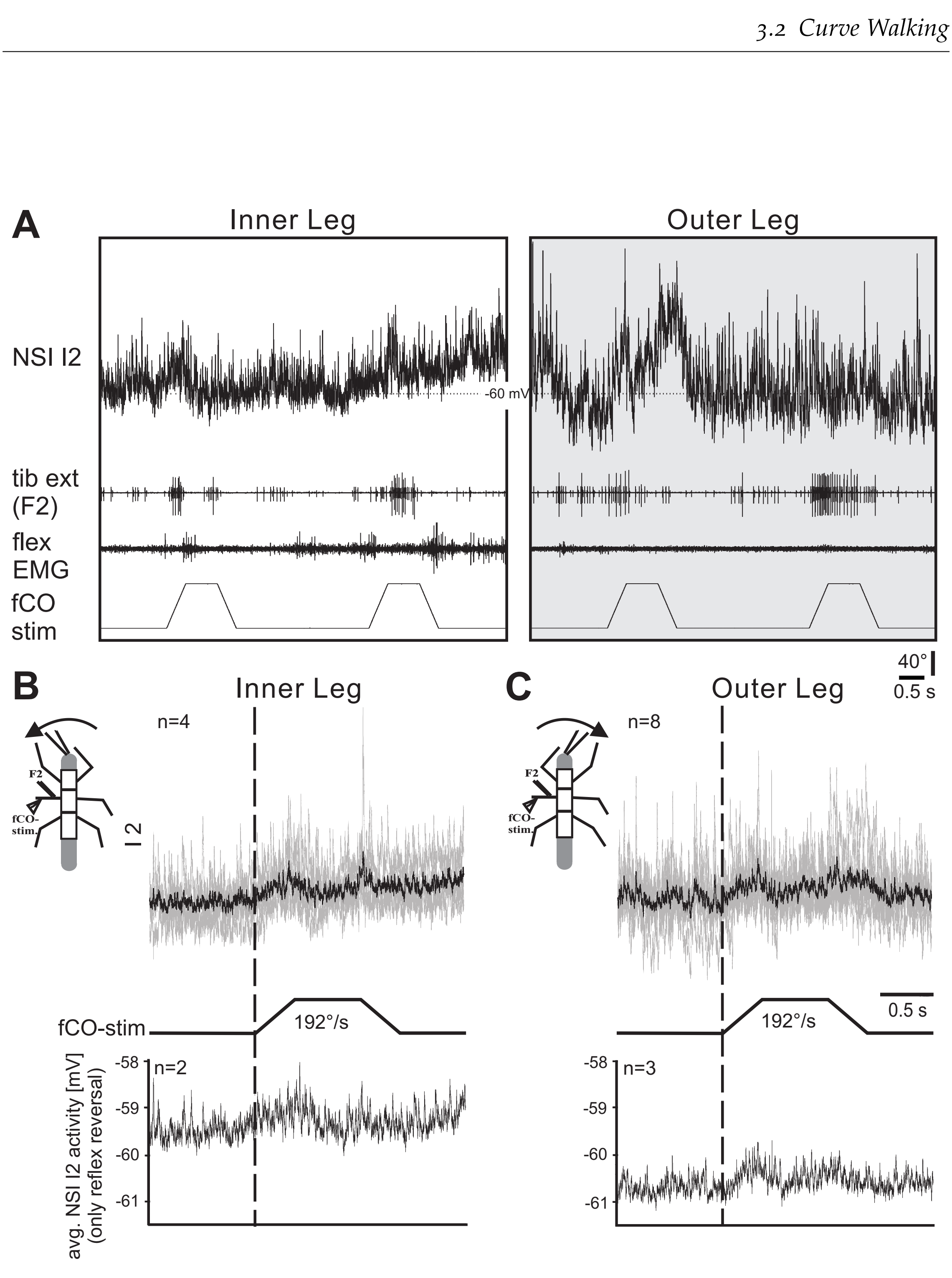

Figure 3.34: (A) Influence of fCO signals during turning on a mesothoracic nonspiking interneuron of type I2 (NSI I2). The femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor nerve activity (tib ext (F2)) and flexor muscle activities (flex EMG) in the same leg of one exemplary animal. During walking, the investigated leg was either a functional inner leg (left panel) or an outer leg (right panel). (B) and (C) Averages of the membrane potential of NSI I2. Top panels: overlays of the membrane potential of NSI type I2 during all fCO stimulations in the inner ($n = 4$) and outer leg ($n = 8$). Bottom panels: averages of NSI I2 membrane potential only during reflex reversals caused by fCO stimulation in the inner ($n = 2$) and outer leg ($n = 3$). n denotes number of stimuli.

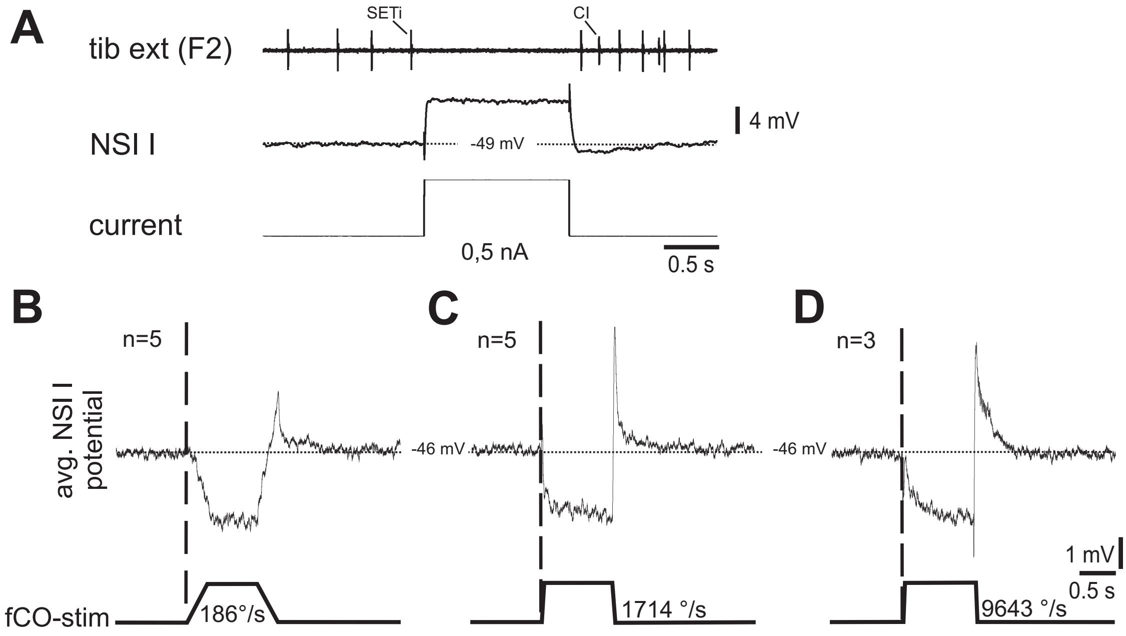

Figure 3.35: (A) Physiological properties and reflex reversal during turning in NSI 'I' type. Change in *extensor tibiae* motoneuronal activity (ext tib (F2)) in response to depolarising current injection into nonspiking interneuron 'I'. The spontaneous SETi firing frequency was completely blocked by depolarising current injection. B) - D) Influence of fCO stimulus velocity ($B = 186\,°/s$, $C = 1714\,°/s$, $D = 9643\,°/s$) on the membrane potential of NSI 'I' in the resting animal. Note the strong tonic hyperpolarisation during elongation and the increase in the phasic depolarisation due to higher relaxation velocity (C, D). n denotes number of fCO stimuli.

ing one reflex reversal a depolarisation caused by fCO elongation was found (Fig. 3.36 C, bottom). In conclusion, in resting and curve walking animals the time course of this inhibitory interneuron was similar, seen as a position-dependent inhibition caused by elongation and a phasic depolarisation during relaxation. However, during reflex reversal, particularly in the outer leg a depolarisation, induced by fCO elongation, was found.

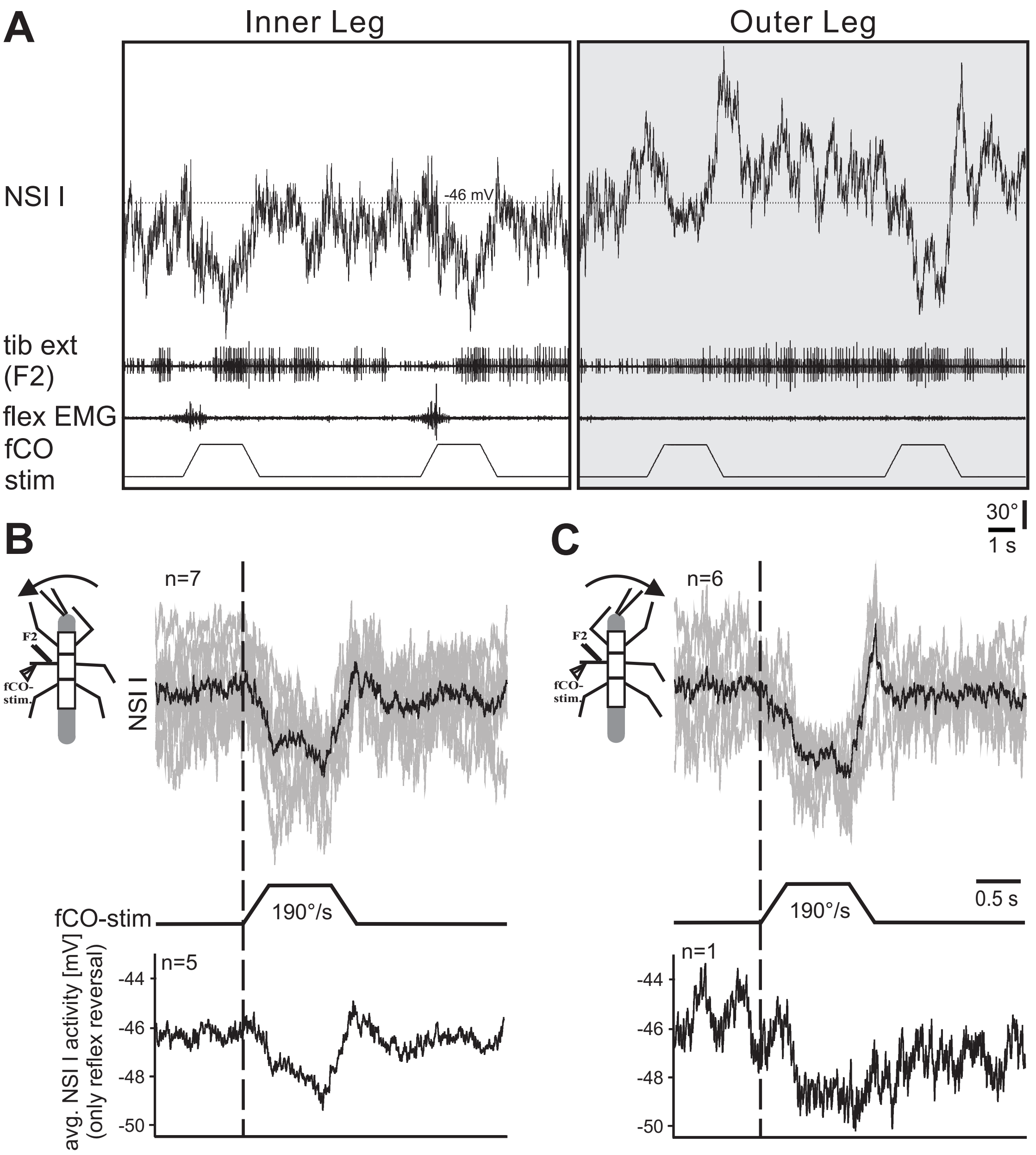

Figure 3.36: (A) Influence of fCO signals during turning on a mesothoracic nonspiking interneuron of type 'I' (NSI 'I'). The femoral chordotonal organ was displaced (fCO stim) in the middle leg while monitoring the tibial extensor nerve activity (tib ext (F2)) and flexor muscle activities (flex EMG) in the same leg of one exemplary animal. During walking the investigated leg was either a functional inner leg (left panel) or an outer leg (right panel). (B) and (C) Averages of the membrane potential of NSI 'I'. Top panels: overlays of the membrane potential of NSI type 'I' during all fCO stimulations in the inner ($n = 4$) and outer leg ($n = 8$). Bottom panels: averages of NSI 'I' membrane potential only during reflex reversals caused by fCO stimulation in the inner ($n = 2$) and outer leg ($n = 3$). n denotes number of stimuli.

3.2.5 Summary: Influence of curve walking on the NSIs

The exemplified intracellular recordings of nonspiking interneurons during curve walking reveal first insights into the neuronal processing of fCO signals during adaptive locomotor behaviour. All of them, known to be part of the FTi joint premotor control network and participate in the generation of resistance reflexes and reflex reversals (cf. Akay, 2002; Büschges, 1990; Driesang and Büschges, 1996; Stein and Sauer, 1998), were also involved in the processing of fCO signals during curve walking. According to their change in the membrane potential, caused by fCO elongation in the inner and outer leg, they were classified in three groups. First, NSIs that have shown differences in their change in membrane potential between fCO elongation in the inner and outer leg, such as NSI E2/3 and E5/6. Second, NSIs that responded similar to fCO elongation in the inner and outer leg as well as during reflex reversals in the inner and outer leg. The following interneurons showed these response characteristics: NSI E4, I2, E9/10 and the here identified inhibitory NSI 'I'. Finally the physiological properties of NSI E8 during the processing of fCO signals in curve walking stick insects were hardly identifiable due to the small number of fCO stimulations during curve walking.

In conclusion, all investigated NSIs known so far, showed very similar physiological changes in the course of the membrane potential caused by fCO stimulation during curve walking, which was reported for the active animal (Driesang and Büschges, 1996). For a better understanding of the physiological properties of the processing of fCO signals during curve walking, further studies should be underwent to investigate, in particular, the course of membrane potential in the different NSIs during curve walking and the occurrence of reflex reversals.

4 Discussion

How does sensorimotor processing of movement related sensory feedback contribute to adaptive motor behaviour? The results of this dissertation demonstrate that modifications in the influence and processing of movement related feedback from leg proprioceptors are segment and task specific modulated for generating adaptive locomotor movements dependent on the insect's present behavioural state. Flexion signals of the front leg fCO generated reflex reversals more frequently during forward walking than in backward walking. In contrast, in the hind leg a higher frequency of reflex reversals was elicited in backward walking. These results reveal a segment-specific and direction-dependent processing of fCO signals mediating reflexes in forward as well as in backward walking. It was also found that during optomotor-induced curve walking, fCO signals in the inner leg generated reflex reversals more frequently than in the outer leg. These findings support the idea of task-dependent processing of fCO signals arising from FTi joint movement, here in the functional context of the leg walking on the inner or outer side of a curve.

Furthermore, the results of the kinematics analyses demonstrate that the sensory signals of the fCO consistently reinforced and enhanced the activities of the muscles that generate force during stance. In forward and backward walking, front and hind legs stance phases are mediated by FTi joint flexion both in the forward walking front leg and in the backward walking hind leg. In contrast, during backward walking the front leg stance phases are generated by flexion as well as extension of the FTi joint. In the forward walking hind leg, it is also shown that the stance phases are performed either by FTi joint flexion or extension.

Movement and position signals from the legs also affect the activity of interjoint coordination, in which signals from the fCO detecting FTi joint movements influence motoneurons controlling the CTr joint. Experiments performed in this thesis confirm the existence of these fCO-mediated interjoint reflexes during forward and backward walking in the middle leg CTr joint.

Finally, it has been shown that nonspiking interneurons, known to be involved in the premotor network of the FTi joint, contribute to the reflex responses in both the inner and outer middle leg during curve walking. In general, my thesis has shown that sensorimotor processing is modulated in a task and segment-specific fashion thereby assisting the generation of adaptive motor behaviour.

4.1 Movement related reflex reversal in stick insect walking

The first studies on reflex reversals in invertebrates were done during the 1970s, where it was shown that reflexes mediated by the stick insect's femoral chordotonal organ can be reversed by changes in the behavioural state (Bässler, 1976). To date, similar effects have been demonstrated in a number of vertebrate and invertebrate locomotor systems (for summaries see Büschges and El Manira, 1998; Clarac et al., 2000). In resting stick insects, for example elongation of the fCO signalling flexion of the FTi joint has been shown to produce resistance reflexes. These activate tibial extensor motoneurons and inhibit tibial flexor motoneurons. Resistance reflexes commonly occur in legged motor systems and constitute compensatory reactions following passive displacements, thus ensuring postural stability (summaries in Bässler, 1993; Pearson et al., 1993). Bässler first reported that in active animals (i.e. that showed motoneuron firing associated with leg movements), fCO elongation, which corresponds to joint flexion, produces excitation in tibial flexor motoneurons and inhibition in tibial extensor motoneurons (Bässler, 1973, 1976, 1988). This means that when the animal is actively moving, e.g., during walking, sensory signals indicating a change in FTi joint angle, do not elicit motor activity to resist the apparent joint movement yet, instead, assist and reinforce activity in the muscle that would produce the joint movement. Bässler called this reflex reversal part I of the *active reaction* (AR), as it was associated with a change from a resting to an active locomotory state. This active reaction consists of two parts. During the first part, as described above, flexion signals of the FTi joint reinforce flexor muscle activity. In the second part, extensor MNs are position-dependently activated. The present notion is that the active reaction assists the ongoing stance movement (part I) and then contributes to the stance-swing transition (part II) during walking. Flexor motoneurons are active in walking during the stance phases of front and middle legs. Assistance reflexes could, therefore, serve to amplify flexor muscle tension and aid in generating support and propulsion. Signals from the fCO could also be important in phase transitions, such as initiation and termination of the stance phase. This idea was supported by the finding that specific patterns of motor activity occur in a number of leg muscles during *active reactions* that are similar to those seen in phase transitions during walking (Bässler, 1986a, 1988). This implies that sensory signals from the chordotonal organ are processed by premotor networks controlling tibial MNs. This notion has been further supported by studies on sensory processing in local nonspiking interneurons (Bässler and Büschges, 1990; Driesang and Büschges, 1996, see section 4.3.2).

However, by now, only little was known about which signals contribute to the generation of the active locomotor state in the segmental ganglia of a walking animal (Bässler, 1993). In earlier studies, the active state was typically elicited by prolonged and repetitive touching of the experimental animal with a paintbrush on the abdomen, head or antennae (Bässler, 1976, 1986a, 1988). As a result, slow and fast motoneurons innervating the leg muscles were activated and generated sequences of bursts of alternating activity in their associated antagonistic muscles. In addition, movement was observed in the neighbouring legs or leg stumps which were free to move, as well as in the antennae (Bässler, 1986a, 1988; Bässler et al., 1974). It is also important to note that many of the previous studies on reflex reversal were performed in animals that were largely restrained or in preparations in which the legs were either immobilized or partially removed (e.g. Akay and Büschges, 2006; Bässler, 1976, 1986a, 1988; Bässler and Büschges, 1990; Driesang and Büschges, 1993). By now, one study directly demonstrated that the AR was associated with movements at the FTi joint in the same leg (Weiland et al., 1986; compare with Bässler, 1972, 1973; Bässler et al., 1974). In these experiments, an electronically closed FTi joint control loop was used and the animal was restrained with the exception that the tibia was free to move. The results showed that the reflex reversal was indeed generated when the animal executed active movements of the tibia of that segmental leg.

The experiments performed in this thesis and in a recently published study (Hellekes et al., 2012) expand previous experimental approaches insofar that other legs performed walking movements and that long-lasting tactile stimulation of the animal was omitted. Additionally, changes in walking direction of the animal could easily be induced by the use of optomotor-stimulation. In the present thesis, the frequency of occurrence of reflex reversals was investigated in behaving animals to determine the mechanisms controlling and modulating reflex reversals. The occurrence of a reflex reversal might be differentially affected as part of the general mechanisms that regulate stepping in single legs, such as those controlling the rate of walking. On the other hand, reflex reversals might also be specifically modulated by mechanisms that are active in the functional adaptation of leg kinematics to specific walking patterns, such as changes in direction or turning. In the following section, I will discuss the modulation of reflex reversals in walking stick insects with regard to the functional needs of the system for control and adaptation of walking.

4.2 Task-dependent modulation of proprioceptive reflexes

4.2.1 Influence of fCO signals on the tibial MN activity during forward and backward walking

The results of this thesis demonstrate that reflex reversals upon flexion signals from the fCO could be elicited in front legs during forward walking. However, reflex reversals were only rarely observed during backward walking (Fig. 3.1, 3.2, 3.11 experimental condition 1). This finding clearly shows that fCO signals in the front leg are processed differentially depending on the walking direction. A possible explanation for this might be that the difference in processing of fCO signals in the prothoracic segment during forward and backward walking entails different leg movements for these two behaviours. Cruse and Bartling (1995) have described that during forward walking the FTi joint angle in the front leg decreases during stance phase. To confirm this result in the experimental conditions used here and in order to understand the stance kinematics of the front leg during backward walking, the stance directions during forward and backward walking on the slippery surface were analysed (see results 3.1.8). In 90% of the investigated forward steps in the front leg, the stance phase was accompanied by flexion of the FTi joint (Fig.3.14). This results from the fact that a forward walking front leg generates forces during the stance phase by flexing the tibia, thereby pulling the animal forward. In contrast, in the backward walking condition, front leg stance movement was, in addition to flexion of the FTi joint, also performed by FTi joint extension (Fig.3.14). In backward walking, the tibia of the front leg is often extended, pushing the body of the animal backwards. The difference in reflex effects of fCO elongation can, therefore, assist the function of supporting muscles that are active during the stance phase.

During forward walking, the high frequency of occurrence of reflex reversals in the front leg revealed that signals of joint flexion increased the activity of the tibial flexor muscle (Fig. 3.1, 3.2). This could assist activity of the flexor muscle. In contrast, during backward walking, the activity in the tibial extensor motoneuron was not inhibited yet, instead, showed a modulation similar to a resistance reflex generated at rest (Fig. 3.1, 3.2). In both of these cases, the changes in joint angle enhance the activities of those muscles that are active during stance. Thus, the sensory signals of the chordotonal organ consistently reinforced and enhanced the activities of the muscles that generate force during stance.

At first glance, the findings presented in this study do not seem to correspond the experiments obtained by Nothof and Bässler (1990). These authors suggested that the neural system generating the AR is a functional element of the pattern generator for forward and backward walking. However, in the experiments presented here no reflex reversal was found during backward walking in the front legs, which suggests a difference in the neural mechanisms underlying the generation of forward versus backward walking. On closer examination, however, Nothof and Bässler (1990) stated that "the neural system producing the AR in hind legs is a functional element of the pattern generator for forward walking (during swing phase) as well as of the pattern generator for backward walking (during stance phase)". In their experiments, they were able to show that in the hind legs of forward walking animals reflex reversals were generated, albeit less often than in front legs (Bässler, 1986b). Furthermore, they found an activation of extensor MNs due to fCO elongation in forward walking hind legs, which is similar to the activity pattern of a resistance reflex. Consequently, these results are consistent with the findings of this thesis, as I demonstrated that reflex reversals in the hind leg during forward walking could be generated by fCO elongation, which occurred, however, less frequently than in forward walking front legs (see results 3.1.6, Fig. 3.11). It is, therefore, plausible to assume that the sensory control of the stance phase in the forward walking hind leg differs from that in the forward walking front leg and also from the backward walking hind leg. Cruse and Bartling (1995) reported an increase in the FTi joint angle during stance phase of the forward walking hind leg. In the experiments of this thesis, these findings showed to be only partially reproducible when analysing hind leg stance kinematic on the slippery surface (see results 3.1.8, Fig. 3.15). In fact, in the current experiments, the stance kinematics of the forward walking hind leg showed to be quite variable. It was shown, that in approximately 40% of the steps the stance phase was mediated by flexion of the FTi joint and, in contrast, in further 40% of the cases the FTi joint was extended. This suggests that the stick insect uses two different strategies for its forward walking hind leg, given the experimental conditions used here. Either it pulls the body forward by flexion of the FTi joint or it generates propulsion by extending the FTi joint and hence pushes the body forward. These interpretations are further supported by experiments of Bässler and Wegner (1983), in which animals were fixated above a treadwheel and fCO stimulation in the hind leg elicited resistance reflexes while the other legs walked forward.

Furthermore, Nothof and Bässler (1990) reported that in the hind leg of animals lacking the front legs (which is known to result in backward walking, see Bässler et al., 1985), fCO elongation elicited reflex reversals in up to 80% of the stimulations and less frequently increases in extensor activity, similar to activity pattern of the resistance reflex. The presented data of experiments performed in the hind leg (see results 3.1.3,3.1.4,3.1.5) from my thesis also reveal that the frequency of reflex reversal in hind legs was higher during backward walking than in forward walking. In general, however, the reflex reversal was less often generated in the experiments conducted here than in the experiments of Nothof and Bässler (1990). Several interpretations might be valid to account for this difference. One possible explanation might be the difference in the way backward walking was initiated. Nothof and Bässler (1990) removed the front and middle legs to obtain backward walking in the hind legs. In the experiments performed here the front and hind legs were intact and moved backwards. Consequently, the different number of walking legs and hence the absence of intersegmental signals between the study of Nothof and Bässler (1990) and the experiments of this thesis could influence the occurrence of reflex reversal in the hind leg (further discussed in section 4.5). Another possible explanation for this could be the role of signals from further leg sense organs being relevant for assisting or facilitating the generation of reflex reversal mediated by fCO signals (further discussed in section 4.4). However, interestingly, in the hind leg walking backwards, the animal pulls the body backwards via flexion of the FTi joint in almost all hind leg steps (see results 3.1.8, Fig. 3.15). This result shows that there is a weak connection between the flexion movements in the backward walking hind leg and the stance-assisting component of the reflex reversal mediated by the fCO, with regard to the irregular occurrence of reflex reversal in the hind leg (see results 3.1.3, Fig. 3.6, 3.11). This inconsistency may be due to the fact that additional sensory organs of the leg are involved in the neuronal processing of stance phase generation (further discussed in section 4.4). Nevertheless, the fact that reflex reversals in the hind leg are found more frequently during backward walking in hind legs than during forward walking (Fig. 3.11, Nothof and Bässler, 1990) suggests that the fCO-mediated reflex reversal, at least partially, assists the stance phase during backward walking in the hind leg. In contrast, during forward walking, processing of fCO signals seems to function in a different way. Interestingly, in the middle leg, there was no significant difference of the processing of fCO signals during forward and backward walking (see results 3.1.2; Fig. 3.3, 3.4, 3.11). This also accords with recent observations, which showed that the muscle activity of the *flexor tibiae* and *extensor tibiae* in the middle leg is similar during forward and backward walking on the slippery surface (Rosenbaum, Wosnitza et al., 2010).

4.2.2 Influence of fCO signals on the tibial MN activity during curve walking

Furthermore, the effect of curve walking on the generation of reflex reversal was studied (see results 3.2). For this, experiments were performed in which the middle leg fCO was stimulated. The neuronal activity of the extensor and the muscle activity of the flexor were measured and all other legs exhibited curve walking. It was found, that fCO elongation during curve walking more frequently elicited reflex reversal when the stimulated leg was on the inside of the curve than on the outside of the curve (see results 3.2, Fig. 3.22, 3.23). The front and middle leg on the inner side are directed towards the curve direction and pull the body into the curve, while the outer legs more extended in a posterior direction pushing the body forward. This indicates that the processing of proprioceptive signals is altered between the inner and outer legs, which could be explained by several differences between the generated movements of legs when either walking on the inside or the outside of a curve. Furthermore, the difference in the frequency of occurrence of reflex reversals found in the inner and outer middle leg (Fig. 3.2) may be explained by the fact that in inner legs always an outside-in movement and a shortening in the step length between the anterior extreme position (AEP) and the posterior extreme position (PEP) was found. These mechanisms are thought to pull the body into the curve (Gruhn et al., 2009). These pulling movements of the inner leg could very well be established by reinforcement of flexor muscle activity as generated during the reflex reversal. In contrast, in the outer middle and hind legs no (or merely marginal changes) in the step length between AEP and PEP were shown. However, the placement of the outer leg changes, which results in leg movements that push the body around the curve. Interestingly, Hoffmann (2010) could show that in the restrained middle leg flexion signals from the fCO did not modify tibial motoneuron activity in the outer leg at all, not even expressing the time course of the activity pattern of the resistance reflex. In general, tonic motoneuronal activity in the extensor nerve of the outer leg was observed (Hoffmann, 2010). However, these results only partially support findings of

Rosenbaum (2008) who showed in intact stepping that there is only a slight difference in tibial muscles activity between the inner and outer legs while curve walking.

Another interesting study by Dürr and Ebeling (2005) demonstrated that, at the beginning of curve walking, leg movements on the outer side of the curve differed drastically from the legs walking on the inner side of the curve. This could be interpreted as prolonged stance phases in the inner middle and hind legs. Although in the present results of curve walking animals (sec. 3.2), no differentiation between the start of turning and the long-lasting curve walking was considered, the prolonged stance phases reported by Dürr and Ebeling (2005) could suggest the existence of stance assistance mechanisms like those occurring during reflex reversals. In general, Dürr and Ebeling (2005) demonstrated that in animals walking on an air-supported styrofoam ball, the transition from straight to curve walking was generated by an initial change in stance direction of both front legs followed by subsequent changes of all other legs. This leading role of the front leg in stick insects turning movements suggested by Dürr and Ebeling (2005) is further supported by experiments on the slippery surface (Gruhn et al., 2009) and body trajectory analysis (Rosano and Webb, 2007). Therefore, it is also conceivable that the change of sensory information from the front legs in curve walking correlates with the change of the efficacy of the coordination rules proposed by Cruse (1990). Consequently, it was reported that the specifics of the coordination rules is modulated context-dependent for straight or curve walking (Dürr and Ebeling, 2005; Ebeling and Dürr, 2006). It is possible that these conditions are likely to occur under the control of local positive feedback, which was already successfully tested in kinematics simulations and in the local joint controllers of six-legged walking robots (Kindermann and Cruse, 2002; Schmitz et al., 2008; Schneider et al., 2006). Thus, two mechanisms known from curve walking stick insects could be involved in the differential processing of fCO information between the outer and inner leg reported in the presented thesis. First, each leg is driven by a specific motor program that depends on turning direction. This notion is supported by experiments, in which animals, walking with only one or two legs, were capable of producing leg movements similar to those in intact curve walking stick insects (Gruhn et al., 2009).

Second, the differences of proprioceptive signalling could be due to influences of the neighbouring walking legs (Dürr and Ebeling, 2005; Ebeling and Dürr, 2006).

In conclusion, the results of the present thesis clearly demonstrate that local information processing of fCO signals correlates with the actual movement to be generated in specific motor behavioural states. Furthermore, the results as well as results of Akay and co-workers (2007) and Hellekes and co-workers (2012) demonstrate that sensory feedback in the control of leg movements is task-specific modified to generate adaptive leg movements required in complex walking behaviours like curve walking or forward and backward walking. The specific adaptations in the generation of leg movements executed in distinct walking behaviours could be explained by modifications of the activity in the local neuronal networks affected by local sensorimotor, intersegmental and descending signals.

4.3 The femur-tibia control network

Position and movement signals of the FTi joint measured by the fCO are processed in a distributed fashion within the neuronal network generating the resistance reflex and the reflex reversal (Bässler, 1993). Bässler (1993) termed this specific distributed processing **parliamentary principle** (cf. Morton and Chiel, 1994, see also Kristan, 2000). It occurs on five different levels of parallel and antagonistic neuronal interactions. First, sensory cells of the fCO measure either separately position, velocity and acceleration of the FTi joint or combinations of these parameters (Büschges, 1994b; Hofmann and Koch, 1985; Hofmann et al., 1985). Sauer and co-workers have shown that the excitatory fCO-afferents were modulated by presynaptic inhibition from inputs arising from affferents of the same typ of sensitively. Second, NSIs receive either direct excitatory or delayed inhibitory inputs from fCO afferents monitoring the same movement parameters (Büschges, 1990; Sauer et al., 1996, 1995). Third, the NSIs provide in parallel excitatory or inhibitory drive onto extensor MNs (Büschges, 1990; Driesang and Büschges, 1996; Sauer et al., 1996). Fourth, the extensor muscle force is produced by simultaneous inhibitory (CI1) and excitatory (FETi, SETi) tibial motoneuronal activities (Bässler and Storrer, 1980). Finally, the tibia movement is the net outcome of the antagonistic activities of the *flexor* and *extensor tibiae* muscles (Bässler and Stein, 1996).

4.3.1 Tibial motoneurons generating the reflex reversal

Studies of the motoneuronal basis of the femur tibiae control loop focused mainly on the characteristics of the extensor motoneurons (e.g. Bässler, 1983a). Due to the fact that the innervation of the *flexor tibiae* is highly complex its function in the FTi joint control was only partially studied (Debrodt and Bässler, 1989, 1990).

In the present investigations in resting animals 12 of 22 flexors were found to depolarise in response to fCO elongation and relaxation (Fig.3.21). Furthermore, eight flexor MNs were hyperpolarised by fCO elongation and depolarised by relaxation. In two further flexor MNs no change of fCO elongation was found. These findings are supported by results of Debrodt and Bässler (1990) in resting stick insects. In their studies, it was shown that elongation of the fCO elicited in different flexor MNs depolarisation as well as hyperpolarisation in the membrane potential. Furthermore, Pfeiffer (1991) also reported different responses of flexor MNs due to fCO stimulation in the resting stick insect, but similar responses of slow and fast flexor MNs in the active animal. This was also suggested by studies in inactive (Field and Burrows, 1982; Siegler, 1981) and active locusts (Zill, 1985), in which the flexor MNs membrane potential was depolarised in response to fCO elongation. It was further suggested that flexor MNs have specific roles during movement (Theophilidis and Burns, 1983, Zill and Moran, 1982). Duch and Pflüger (1995) showed different activity patterns of flexor MNs dependent on the performed behaviour. They found differences in the activity of flexor MNs between horizontal walking, vertical climbing and upside-down walking. These notions indicate a specific role of different flexor MNs dependent on the animal's behavioural states. Therefore, further research should be underwent to investigate the role of individual flexor MNs in different behavioural states. Regarding this question, in the seven intercellular recordings performed during curve walking the effect of the different flexor MNs could not be observed. In the majority of flexor MNs recorded, the membrane potential was depolarised during fCO elongation. In these seven flexor MNs fCO elongation caused in 53% of the fCO stimulations a reflex reversal in the inner leg ($n = 104$) and 14% in the outer leg ($n = 73$). This observation supports the results shown in Fig. 3.20 and in previously reported studies by Hoffmann (2010) and by Hellekes and co-workers (2012). In conclusion, the difference in the processing of fCO signals in the inner and outer leg could be verified in the flexor MNs.

The intracellular recordings in resting animals of either the *slow extensor tibiae* (SETi) or the *fast extensor tibiae* (FETi) revealed the typical depolarisation caused by fCO elongation. During relaxation of the fCO, the membrane potential was hyperpolarised, congruent with the findings from Bässler (1983a). Depending on their resting membrane potential, fCO elongation elicits higher spike frequencies in SETi than in FETi (Gabriel, 2005). During curve walking in the outer leg, the membrane potential of the *extensor tibiae* is frequently tonical depolarised and the spiking frequency is increased compared to the inner leg (Fig.3.23 A). This was also reported by Hoffmann (2010). In the present investigations of the inner leg, fCO elongation caused a hyperpolarisation in the extensor MNs and simultaneously an inactivation of motor units in the extensor nerve (Fig.3.23 A, left). This response is known as the first part of the *active reaction* (cf. Bässler, 1973, 1976, 1986a). In the second part of the AR, a position-dependent reactivation of the extensor MNs was observed (cf. Bässler, 1973, 1976, 1986a). During curve walking in 4 intracellular recorded *extensor tibiae* MNs, the frequency of reflex reversal was higher in the inner leg (33%) compared to the outer leg (16%). These results are also supported by extracellular recordings of tibial MNs during curve walking (Hellekes et al., 2012; Hoffmann, 2010).

4.3.2 Nonspiking interneurons involved in the generation of reflex reversal

In the following section, I will present initial results of a small set of experiments of intracellular recordings of nonspiking interneurons (NSI) involved in the FTi joint control during curve walking. The data reveal first insights about the processing of fCO signals during curve walking, however, more experiments are needed to demonstrate the physiological properties of the NSIs in the processing of fCO signals during curve walking. The interneuronal basis of proprioceptive reflexes in the FTi joint control system is well-studied in the locust (for review see Burrows, 1996) and in the stick insect (for review see: Bässler, 1993; Bässler and Büschges, 1998). Three different levels of information processing are described. First, sensory afferents of the fCO receive presynaptic inhibition from inputs arising from afferents of the same type of fCO afferents (Sauer et al., 1997). Second, sensory afferent can project directly onto MNs or indirectly via nonspiking interneurons or spiking interneurons monitoring the same movement parameters. (Büschges, 1990; Sauer et al., 1996, 1995). Finally, a part of the NSIs inhibit or excite several MNs. Previous studies have identified nonspiking interneurons, which either excite or inhibit extensor MNs and are involved in the processing of fCO signals of the FTi joint (Akay, 2002; Büschges, 1990; Stein and Sauer, 1998). Nonspiking interneurons that excite the extensor are termed excitatory (E) NSIs, while those inhibiting the extensor are known as inhibitory (I) NSIs (Büschges, 1990).

In the present thesis it is demonstrated that NSIs, which are part of the FTi joint premotor control network and known to participate in the generation of resistance reflexes and reflex reversals (Akay, 2002; Büschges, 1990; Driesang and Büschges, 1996; Stein and Sauer, 1998), are involved in the processing of fCO signals during curve walking. In the following section, the change of the NSIs membrane potential in response to fCO elongation during curve walking shall be discussed. According to their responses to fCO elongation in the inner and outer leg they can be classified in three groups. First, NSIs that have shown differences in their change in membrane potential caused by fCO elongation in the inner and outer leg. Nonspiking interneurons of the classes NSI E2/3 and E5/6 belong to this group. Second, NSIs that responded similar to fCO elongation in the inner and outer leg as well as during reflex reversals in the inner and outer leg. These characteristics are found in the responses of NSI E4, NSI I2, E9/10 and the here identified inhibitory NSI 'I'. Finally, in interneuron E8 the responses in the inner and outer leg were hardly identifiable. This was mainly caused by a small number of fCO stimulations during curve walking.

In the present study, it was found that the change in membrane potential of NSI type E2/3 during fCO elongation in resting and curve walking animals was oppositional (cf. Bässler and Büschges, 1990; Driesang and Büschges, 1996). Providing excitatory drive to extensor tibiae MNs, NSI E2/3 contributed to the reflex activation of the resistance reflex in the resting stick insect (cf. Bässler and Büschges, 1990; Driesang and Büschges, 1996). During curve walking in the inner leg, a strong inhibition in NSI E2/3 in response to fCO elongation was found. Additionally, this strong inhibition occurred during reflex reversal in the inner and outer leg (Fig. 3.25, sect. 3.2.4). This finding confirms that NSI E2/3 supported the first part of the AR (Bässler and Büschges, 1990; Driesang and Büschges, 1996). In this study a strong hyperpolarisation was shown, which occurred in parallel with the inactivation of extensor MNs during the first part of the fCO elongation in the active animal. In contrast, during all stimulations in the outer leg this inhibition was strongly reduced. It seems possible that the slight inhibition, which is still observable in the outer leg, is caused by two reflex reversals occurring in this leg (Fig. 3.25 C). Driesang and Büschges (1996) further reported that during the second part of the AR, the membrane potential was depolarised simultaneously to the reactivation of the extensor. This was also found in the inner leg (cf. Driesang and Büschges, 1996). In their study they also demonstrated that the time course of the E2/3 membrane potential was correlated with the time course of extensor activity (Driesang and Büschges, 1996). This could be a possible explanation for the observed difference in the inner and outer leg in the change of membrane potential of NSI E2/3 caused by fCO stimulation. In the outer leg, reflex reversals were found only rarely. Additionally, an increase in tonical motoneuronal activity was observed (Fig. 3.23 A, C top). This could explain the reduction of the inhibition in the NSI E2/3 in the outer leg. Furthermore, during leg stepping NSI E2/3 is hyperpolarised during stance phase and depolarised during swing phase (von Uckermann and Büschges, 2009). Therefore, different response properties of the NSI E2/3 in the inner and outer leg correlate with differences in the stance kinematics between the inner and outer leg during curve walking (see section 4.2.2).

In resting animals, NSI E5/6 recorded in this thesis contribute to the resistance reflex during fCO elongation and oppose the extensor inactivation during fCO relaxation (Fig. 3.28, cf. Büschges, 1990). This is seen as depolarisation during elongation and relaxation of the fCO in the resting animal. During curve walking, fCO elongation elicited different responses in the membrane potential of NSI E5/6 (Fig. 3.29). In the inner leg, fCO elongation elicited a slight hyperpolarisation followed by a depolarisation (Fig. 3.29 B). In contrast, in the outer leg, recordings revealed that fCO elongation caused a depolarisation and on the other hand a hyperpolarisation (Fig. 3.29 Ci, Cii). This difference in the course of membrane potential between the inner and outer leg is probably related to the generation of reflex reversals in the outer leg of the second animal (Fig. 3.29 Cii, bottom). During reflex reversal in the inner as well as in the outer leg, a strong inhibition caused by fCO elongation was found (Fig. 3.29 B, bottom; Cii, bottom). This observation differs from the results of Driesang and Büschges (1996), who reported almost no change in the membrane potential of NSI E5/6 during the first part of the active reaction. It is further known that in the membrane potential of NSI E5/6 depolarising and hyperpolarising inputs are superimposed during the first part of the active reaction (Driesang and Büschges, 1996). The physiological properties could be involved in the tuning of the membrane potential caused by fCO elongation in the inner and outer legs. Furthermore, NSI E5/6 are known to receive direct excitatory and polysynaptic inhibitory signals (Sauer et al., 1995), which can be assumed to modify the reflex responses to fCO stimulation in different behavioural states.

During stepping, the individual membrane potentials of NSI E5 and E6 were shown to be different (von Uckermann and Büschges, 2009). NSI E5 was depolarised during stance phase and hyperpolarised during swing phase. In contrast, in NSI E6 a hyperpolarisation during stance phase and a depolarisation during swing phase was found (von Uckermann and Büschges, 2009).

Therefore, the differences in the responses of the group NSI E5/6 between the inner and outer leg could also result from the variations shown in the membrane potential modulation during stepping. Despite compatible with empirical findings, this explanation remains speculative, as NSI E5 and NSI E6 were not observed individually in the present study. Therefore, future studies should consider neuronal staining of the investigated NSIs to distinguish between similar NSI groups.

In resting stick insects, NSI E4 depolarise during fCO elongation and relaxation (Fig. 3.26, cf. Büschges, 1990). During curve walking, the membrane potential of the NSI E4 depolarised at the onset of fCO elongation in the inner and outer leg (Fig. 3.27). Furthermore, this initial depolarisation was also found during reflex reversals in the inner and outer leg. The present findings are consistent with the study of Driesang and Büschges (1996), who found three different types of time courses of the membrane potential of NSI E4 during an active reaction. All three types show a depolarisation caused by fCO elongation, however, their time courses possessed a high degree of variability. This result was also replicated in the experiments of this thesis (Fig. 3.27 B bottom, C bottom). Furthermore, it is reported that NSI E4 receives direct excitatory and polysynaptic inhibitory signals from the fCO. Their efficacy is probably responsible for the variability in the response to fCO stimulation in the active and also in the walking animal. In single leg stepping, E4 is strongly depolarised during swing phase. With the onset of the stance phase the depolarisation declined (von Uckermann and Büschges, 2009). In conclusion, it is not justified to assume a difference in the processing of fCO signals between the inner and outer leg.

The interneurons of group NSI E9/10, NSI I2 and the new identified NSI 'I' showed in all observed situation the same physiological properties. The membrane potential of NSI E9/10 was depolarised during fCO elongation and during relaxation the course of membrane potential decreased (Fig. 3.31, 3.32; cf. Akay, 2002). This change in membrane potential was found in resting and curve walking animals. During curve walking, this response of the membrane potential was found during all fCO stimulations and during the stimulation with reflex reversals in the inner and outer leg. To date, during reflex reversal, only the time course of the membrane potential for NSI E9 is known (Akay, 2002). It was shown that the position-dependent depolarisation caused by fCO elongation in the resting animal vanished during the active reaction (Akay, 2002). However, in the experiments performed here, interneurons of the group E9/10 showed this depolarisation dependent on stimulus velocity (Fig. 3.31). Therefore, it is not distinguishable, if the position-dependent depolarisation vanished as a consequence of the stimulus velocity or by the actual reflex reversal. In general, neurons of the type NSI E9/10 have presumably no contribution to the different activities of tibial motoneuron activity found during fCO stimulation in the inner and outer leg.

During fCO elongation in the resting animal, I2 received depolarising signals from the fCO (Fig. 3.33, cf. Büschges, 1990). NSI I2 is known to provide inhibitory drive to extensor motoneurons and, therefore, oppose the generation of the resistance reflex (Fig. 3.33, cf. Büschges, 1990). During curve walking of a stick insect, the same time course of the membrane potentials as in the resting animal, were found (Fig. 3.34). In the inner and outer leg and in both legs, during reflex reversal, the membrane potential of NSI I2 was depolarised in response to fCO elongation. These findings confirm that NSI I2 supported both parts of the active reaction (cf. Bässler and Büschges, 1990; Büschges, 1990). During curve walking, no evidence of a different contribution of NSI I2 to the processing of fCO signals in the inner and outer leg was found.

In the present studies one previously unknown inhibitory nonspiking interneuron was found. In the resting animal, fCO elongation caused a depolarisation and, during relaxation, the membrane potential was depolarised (Fig. 3.35). In curve walking animals, no difference in the response to fCO stimulation in the inner and outer leg were found (Fig. 3.36). The course of the membrane potential was similar to that observed in the resting animal. Similarly, during fCO elongations in the inner leg, which causes reflex reversal, the membrane potential was hyperpolarised and, during relaxation, depolarised. In the outer leg, only one reflex reversal was generated. During this reflex reversal, the inhibition started shortly later than in the inner leg. In general, however, the course of the membrane potential was similar. In short, this neuron responds to fCO stimulation in the inner and outer leg in the same way.

Finally, interneuron E8 could only be characterised qualitatively. In resting animals, fCO elongation, as well as, relaxation leads to a hyperpolarisation of the membrane potential (Fig. 3.30, Stein and Sauer, 1998). During one fCO stimulation in the inner leg, the inhibition during fCO elongation in the resting animal has vanished. In the outer leg, during one fCO elongation, the inhibition is still visible. This could indicate a difference in fCO processing during curve walking. However, regarding the number of fCO stimulations during curve walking, it remains unclear, how NSI E8 is involved in the processing of fCO stimulations in the inner and outer leg.

In conclusion, the alteration in membrane potential modulation of several NSIs, which are part of the FTi joint premotor control network and known to participate in the generation of resistance reflexes and reflex reversals (Akay, 2002; Büschges, 1990; Driesang and Büschges, 1996; Stein et al., 1998), reveal different contributions of the NSIs to the changes in FTi joint control during curve walking. In the interneuron types E2/3 and E5/6 first evidences for a different processing of proprioceptive signals during curve walking was found. The findings, while preliminary, suggest further that the contribution of the NSI I2, E9/10 and 'I' in the processing of fCO signals during curve walking is similar. In future studies, it should be tested how the alterations in the NSIs membrane potential are correlated with the actual extensor and flexor activity. This will also reveal the contribution of the different NSIs to the reflex reversal and respectively to the active reaction in a walking animal. The mechanisms underlying the differences in processing of fCO signals during curve walking might be possible to determine in further investigations. The modulation could be first mediated by presynaptic inhibition of fCO afferents (Burrows and Laurent, 1993; Sauer et al., 1997). Secondly, presynaptic inhibition mediated by other sense organs (Stein and Schmitz, 1999) and additional influences on tibial MNs from other leg sensors (Schmitz and Stein, 2000) (see further discussion section 4.4). Finally, the influence of intersegmental and descending signals on the modulation of the FTi joint motor output (see further discussion section 4.5. The latter issue could also be studied by investigating the response of NSIs to optomotor-stimulation.

4.4 Influence of other leg sense organs

Another finding of the present study was that in hind legs a change in ThC position as well as ablation of the campaniform sensilla (CS) altered the occurrence of reflex reversal and, thus, the processing of fCO signals in forward and backward walking (see results 3.1.4, 3.1.5, Fig. 3.12, 3.13). It was found that during forward walking the frequency of reflex reversal is decreased in experiments with ablated CS compared to the situation, where the CS are intact and the hind leg position is either 45° posterior or anterior (Fig. 3.12). In backward walking, the highest frequency of reflex reversals in the hind leg occurred in the 45° anterior-directed hind leg, which differs from the condition found in the posterior-directed hind leg as well as to the anterior directed hind leg with ablated CS (Fig. 3.13). Although these results clearly suggest that position information of the ThC joint and load information of the CS could influence the frequency of the fCO-mediated reflex reversal in the hind leg during forward and backward walking, these data need to be interpreted cautiously with regard to the high experimental variability. This variability can be explained as a consequence of the unspecific sensory stimulation and the inherent inaccuracy of the applied method. However, the observed difference between the forward walking hind leg with intact CS and with ablated CS corroborates the results of Akay and Büschges (2006), who demonstrated that an increase in load information signalling by the fCS leads to higher frequency of occurrence of fCO mediated AR. First evidence that the processing of load information is associated with the processing of position information was provided by Cruse (1985). In this study, it was shown that the transition between swing and stance phase is generated with regard to the leg's anterior extreme position and an increase of load signals. A very interesting study of Akay and co-workers (2007), reported a segment-specific processing of load signals during forward and backward walking. In this study, an increase in load signalling promoted the stance phase activity of the ThC joint, dependent on the walking direction. During the stance phase in forward walking, the *retractor coxae* activity was increased and in backward walking the *protractor coxae* exhibited increased activity. Therefore, it is plausible that load signals during the generation of stance phase movement during forward and backward walking also contribute to the tibial motoneuronal activity mediated by the processing of fCO position signals.

The results obtained with different ThC joint positions in the hind leg, show that during forward and backward walking the frequency of reflex reversal is higher in the 45° anterior-directed hind leg compared to the same position with ablated CS and the 45° posterior-directed hind leg (see results 3.1.4,3.1.5,Fig. 3.12, 3.13). Due to the fact that there are only slight differences in the frequency of reflex reversal with regard to the change in the ThC joint position, these findings might not be transferable to a ThC joint position-dependent processing of fCO signals in forward and backward walking. However, it is also important to mention that movements of the ThC joint are detected by the ventral coxal hairfield (vcxHP) (Dean and Schmitz, 1992). In a very interesting study, Bässler (1977) showed that by manipulating the ThC joint such that a constant leg protraction was measured, the manipulated leg moves during stance phase fully backwards and in some experiments remains in retraction during walking of the other legs, not switching to leg swing any more. This also accords with the here present results. In the forward directed leg, which corresponds to a protracted leg position, the highest frequency of reflex reversal was found, which showed that stance phase mechanisms are supported (Fig. 3.11). Regarding further leg sense organs, several limitations due to the experimental design need to be considered. First, the investigated leg is fixed in a certain position, which leads to the absence of phasic sensory leg signals that would be present during an undisturbed step cycle. Secondly, the animal was fixated, which diminished or even eliminated load signals that are normally present when the animal has to carry its own weight during walking. Third, the influences of tibial CS (Zill et al., 2011) are non-existent due to the fact that the tibia is removed. Finally, the influence of tarsal tactile hairs, which are known to influence the stance and swing cycle in the locust *Schistocerca gregaria* are also non-existing. These sensors are known to initiate reflexes that elevate the tarsus, with involvement of nonspiking interneurons by sending signals directly to the tarsal levator MNs (Laurent and Burrows, 1988; Laurent and Hustert, 1988). We can conclude that new experimental approaches are needed to provide a more integrative account on the undisputedly important role of sensory contributions in locomotion.

4.5 Intersegmental and descending control of local processing of proprioceptive signals

The findings of the present study indicate a segment-dependent difference of sensory processing of fCO signals during forward and backward walking (Fig. 3.12, 3.13). In particular, the processing of fCO mediated sensory signals in the front leg differs from the processing in middle and hind legs. During forward walking, the highest frequency of reflex reversal was detected in the front leg compared to the middle and hind leg (Fig. 3.12), and vice versa during backward walking (Fig. 3.13). These findings are consistent with previous studies that have shown that the prothoracic segment and the front leg play an important role in determining the locomotor state of the caudal segmental ganglia. Ludwar and co-workers (2005a, b) demonstrated that stepping of the front leg as well as stimulation of the prothoracic fCO produce activation of MN pools in the deafferented, ipsilateral middle leg. The motoneuronal activity in the mesothoracic motoneurons was found to be modulated in phase with stepping of the front leg. In a subsequent study, Borgmann and co-workers (2009) showed that stepping of the front leg activates and entrains the CPG driving the ThC joint motoneurons in the ipsilateral hemi-segment of the mesothoracic ganglion. Front leg stepping was, however, only accompanied by a general tonic activation of leg motoneurons in all other segmental ganglia of the walking system. Furthermore, recent results provide evidence that stepping in neighbouring legs, in particular in the ipsilateral front leg, facilitates to the generation of reflex reversal in the middle leg (Hellekes et al., 2012). However, it is presently not clear whether the effects of the stepping front leg via intersegmental pathways affect processing of sensory inputs in local sensorimotor pathways of the respective leg or if they affect sensorimotor processing by acting on the local walking pattern generating networks (cf. Büschges and El Manira, 1998.

The specific modulation of the fCO-mediated reflex reversal during changes in the direction of walking (forwards vs. backwards) and in optomotor-induced curve walking (Fig. 3.1, 3.2) could also be related to further influences of intersegmental and contralateral signals. Intersegmental transmission of position- and velocity dependent fCO signals between different legs was shown by pharmacological reduction of GABA-mediated inhibition by picrotoxin (Stein et al., 2006) Contralateral influences are, for example, shown in the hind legs, where signals between the hind legs are involved in the mutual coordination (Graham and Wendler, 1981). Consequently, it was shown that forward and backward movements of the hind leg altered the leg movement of the contralateral hind leg (Wendler, 1964). Furthermore, activities in contralateral legs may have other effects such as inhibiting swing movements in order to ensure proper alternation of legs in walking (e.g. Cruse, 1990).

At present, it is not known which specific neuronal signals contribute to the underlying segmental modifications in the processing of local sensory signals from the fCO in different behavioural states. However, several previous studies have shown that descending signals from the brain can modulate local reflexes. In locusts, Knop and coworkers (2001) demonstrated that lesioning axons of descending neurons in the ipsilateral connective strongly affected the reflex effects of fCO signals on mesothoracic tibial motoneurons. The effects of signals of FTi joint flexion were found to change from posture control to a movement control mode. Mu and Ritzmann (2008a) also found that lesions of the rostral connections in cockroaches produce pronounced changes in the reflex motor activity induced by proprioceptive signals in the mesothoracic leg. These studies suggest that descending signals from the brain (the supraoesophageal and/or the suboesophageal ganglion) can play a decisive role in determining the locomotor state and processing of sensory inputs in the thoracic ganglia. Furthermore, the central body complex (CBC) in the cerebral ganglion of fruit flies and cockroach is plausibly involved in transmitting descending signals contributing to turning (Mu and Ritzmann, 2008a,b; Ridgel et al., 2007; Strauss and Heisenberg, 1993). Future studies on this topic are definitely needed for clarification.

4.6 Functions of reflex reversals: reinforcement of movement in the control of walking

What specific functions do reflex reversals serve during locomotor behaviors? One function that has been postulated for reflex reversal (during ARs) is the enhancement of motor activities (reviews in Büschges and El Manira, 1998; Duysens et al., 2000; Pearson et al., 1993). When the fCO reflexes reverse, signals indicating joint flexion inhibit extensor firing and can activate and reinforce activity in the tibial flexor muscle. During forward walking, this effect would enhance activity in the muscle that provides propulsion. In addition, during curve walking, the flexor muscle acts to pull the animal in the direction of the turn. The increased probability of reflex reversal in the leg walking on the inside of the curve may reflect enhancements of the mechanisms that generate force during the turn. Thus, in both situations, the modulation of chordotonal reflexes may reflect the utilization of sensory inputs to amplify muscle contractions and movements. Previous accounts on the neural mechanisms underlying sensory control of leg stepping in animals have indicated that reinforcement of movement is a significant mechanism in the generation of terrestrial locomotion (for review see Bässler and Büschges, 1998; Cattaert and Ray, 2001; Clarac et al., 2000; Pearson, 1995b, 2008; Yang and Gorassini, 2006; e.g. *insects*: Bässler, 1976, 1988, 1992; *crustaceans*: El Manira et al., 1991; Leibrock et al., 1996; Skorupski and Sillar, 1986; *cats*: Gossard et al., 1994; McCrea et al., 1995; Pearson et al., 1993; *humans*: Grey et al., 2007). These studies demonstrated that reinforcement of movement by sensory feedback is state-dependent and only occurs when the locomotor system is active (e.g. Bässler, 1988; Skorupski and Sillar, 1986). In some cases, movement reinforcement is also phase-dependent and linked to the activity of CPGs that generate rhythmic locomotor actions (e.g. El Manira et al., 1991; McCrea et al., 1995; Skorupski, 1992). Studies on the cat and the crayfish provided compelling evidence for specific and detailed alterations in the activity of pathways processing sensory information underlying the generation of reinforcement of movement during walking (for review see Cattaert and Ray, 2001 (crayfish); Pearson, 1995b, 2008 (cats)). In the cat hind leg, movement and force feedback reinforce stance motor output in a phase-dependent manner, when the central neural networks, i.e. the hind leg CPGs, are active (e.g. Pearson, 1995b, 2008). In crayfish, the mechanisms underlying reflex reversal and reinforcement of movement have been documented in preparations expressing rhythmic motor activity ("fictive locomotion") in one or both of the proximal leg joints (e.g. Chrachri and Clarac, 1990; Skorupski and Sillar, 1986). In cats, rhythmic activity was initiated and maintained either by perineum stimulation, by pharmacologically treatment or by electrical stimulation of the mesencephalic locomotor region (e.g. McCrea et al., 1995; Pearson et al., 1993), while in the present study walking in stick insects was experimentally initiated but mainly maintained by the animal. In both preparations, in the crayfish and cat, the pattern of motor activity generated was considered to represent forward walking. However, it is still unclear whether the generation of reinforcement of movement is also affected by varying and adapting the specific motor behaviour (cf. Pearson, 2008).

The present study provides evidence that processing of proprioceptive information not only depends on the behavioural state but also upon the specific locomotor task, e.g., the control of the movement of a leg walking on the inside of a curve as compared to walking on the outside of a curve. The alterations in the control of the FTi joint movement during forward and backward walking depends both on the walking directions and on segmental differences between the legs. Futhermore, the investigations of NSIs known to be part of the FTi joint control network demonstrate that these neurons are involved in the processing of fCO signals in the curve walking stick insect. In summary, the results of this thesis demonstrate that sensory feedback in the control of leg movements is task-specifically modified to generate adaptive leg movements required in complex walking behaviours. Future studies are needed to identify how these task-specific modifications in the generation of leg movements during adaptative locomotor movements are affected by local sensorimotor, intersegmental and descending signals on the level of local neuronal networks.

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List of Figures

Abbreviations

AR	active reaction
AEP	anterior extreme position
C1	coxa branch 1
CI	common inhibitor
CPG	central pattern generator
CS	campaniform sensilla
CTr	coxa-trochanter
EMG	electromyogram
F2	femoral nerve F2
fCO	femoral chordotonal organ
fCS	femoral campaniform sensilla
fps	frames per second
FTi	femur-tibia
GTO	golgi tendon organ
L-dopa	L-3,4-dihydroxyphenylalanine
MN	motoneuron
NSI	non-spiking-interneuron
PEP	posterior extreme position
PSTH	peristimulus time histogram
rHP	rhombic hairplate
RR	resistance reflex
vcxHP	ventral coxal hairplate
tib ext	tibial extensor

trCS	trochanteral campaniform sensilla
trHP	trochanteral hairplate
ThC	thorax-coxa

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Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen –, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie – abgesehen von unten angegebenen Teilpublikationen -noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.
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