Mechanisms for Intersegmental Leg Coordination in Walking Stick Insects

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Zusammenfassung

Zur effizienten Fortbewegung müssen Bewegungen einzelner Beine während dem Laufen koordiniert werden, was in einem Schrittmuster oder einer Gangart resultiert. Diese Dissertation untersucht die neuronalen Mechanismen, die der Bildung einer solchen Gangart unterliegen, besonders die neuronalen Grundlagen der Koppelung von ipsilateralen Beinbewegungen.
In einer semi-intakten Laufpräparation der Stabhauschrecke *Carausius morosus* wurde die Korrelation von Aktivität ipsilateraler, mesothorakaler Motoneurone und Laufbewegungen eines Vorderbeines beschrieben. Extrazelluläre Ableitungen zeigten eine ausgeprägte Ankoppelung mesothorakaler protractor coxae und extensor tibiae Motoneuronen. Depressor trochanteris Motoneurone zeigten eine variablere Ankoppelung. Mesothorakale retractor coxae und levator trochanteris Motoneurone waren antizyklisch zur ihren jeweiligen Antagonisten aktiv.
Intrazelluläre Ableitungen zeigten zwei unterschiedliche Arten der Modulation des Membranpotentials mesothorakaler Motoneurone: Eine tonische Modulation, die während der Laufaktivität des Vorderbeins anhielt, und eine rhythmische Modulation, die mit einzelnen Schritten des Vorderbeins korreliert war. Es wurden Hinweise auf tonisch erregende und hemmende, wie auch auf rhythmisch erregende und hemmende Eingänge auf verschiedenen Motoneurone gefunden.
Intrazelluläre Ableitungen von mesothorakalen nicht-spikenden Interneuronen, des den Motoneuronen vorgeschalteten Netzwerks, zeigten, daß auch diese Neuronen intersegmental koordinierende Signale erhalten. Tonische wie auch rhythmische Modulationen des Membranpotentials, die mit der Laufaktivität des Vorderbeins korreliert waren, wurden gefunden. Die nicht-spikenden Interneuronen wurden zum Teil morphologisch identifiziert und es ist bekannt, daß sie lokale sensorische Information verarbeiten. Sie stellen daher eine Grundlage zur Integration von Information lokaler Sinnesorgane und intersegmentaler Signale dar.
Weiterhin wurden Versuche durchgeführt um die Herkunft intersegmentaler Signale aufzuklären. In Experimenten mit einer isolierten Ganglienkette, die pharmakologisch mittels Pilocarpine aktiviert wurde, wurde die Interaktionen von zentralen, Rhythmus generierenden Netzwerken untersucht. Sensorische Eingänge waren in dieser Präparation ausgeschlossen. Es wurden keine Hinweise auf eine starke Koppelung zentraler Mustergeneratoren zwischen mesothorakalen und metathorakalen, sowie prothorakalen und mesothorakalen Segmenten gefunden.
Zwei weitere Versuchsreihen beschäftigten sich mit der Rolle von sensorischen Signalen bei der intersegmentalen Koordination. Signale vom mesothorakalen femoralen Chordotonalorgan, welches die Position und Bewegung im Femur-Tibia Gelenk mißt, zeigten im 'aktiven' Tier keinen deutlichen Einfluß auf die Aktivität metathorakaler Motorneurone. Signale der Campaniformen Sensilla, welche die Belastung eines

Beines messen, zeigten nur einen schwachen intersegmentalen Einfluß auf die Aktivität mesothorakaler Motoneurone. Letzteres wurde im ruhenden Tier und mit umgekehrtem Effekt im 'aktiven' Tier, wie auch während durch Pilocarpine induzierter rhythmischer Aktivität, gezeigt.

Abstract

For efficient locomotion, the movements of single legs need to be coordinated during walking, which results in a stepping pattern or gait. This dissertation explores the neural mechanisms underlying the formation of a gait, in particular the neural basis of coupling of ipsilateral leg movements.
In a semi-intact walking preparation of the stick insect *Carausius morosus*, correlations between ipsilateral mesothoracic motoneuron activity and walking movements of a front leg were described. Extracellular recordings showed a dedicated coupling of activity for mesothoracic protractor coxae and extensor tibiae motoneurons. Depressor trochanteris motoneurons showed a more flexible coupling. Mesothoracic retractor coxae and levator trochanteris motoneurons were active in anti-phase with their respective antagonists.
Intracellular recordings revealed two different modulations of membrane potentials of mesothoracic motoneurons: a tonic modulation, lasting during the stepping activity of the front leg, and a rhythmic modulation, correlated with individual steps of the front leg. Evidence for tonic excitatory and inhibitory, as well as for rhythmic excitatory and inhibitory inputs were found for different motoneurons.
Intracellular recordings of mesothoracic non-spiking interneurons of the pre-motor network revealed that these interneurons receive intersegmental coordinating signals. A tonic as well as a rhythmic modulation of their membrane potential, correlated with the walking activity of the ipsilateral front leg, were found. The non-spiking interneurons were in part morphologically identified and are known to process local sensory information. Hence, they could provide the basis for integration of local sensory and intersegmental signals.
Additionally experiments were performed to investigate the origin of intersegmental signals. In experiments with an isolated chain of ganglia which was pharmacologically activated with pilocarpine, interaction between the central rhythm generating networks were studied. Sensory input was excluded in this preparation. No evidence was found for strong coupling of central pattern generators in mesothoracic and metathoracic segments, nor in prothoracic and mesothoracic segments.
Two more sets of experiments focused on the role of sensory signals for intersegmental coordination. Signals from the mesothoracic femoral chordotonal organ, measuring position and movement of the femur-tibia joint, showed no clear influence on the activity of metathoracic motoneurons in the 'active' animal. Sensory signals from the metathoracic campaniform sensilla, measuring load on the leg, showed only a weak intersegmental influence on mesothoracic motoneuron activity, but a clear influence on local protractor and retractor motoneuron activity. The latter was found in the resting animal and with reversed effects in the 'active' animal, as well as during rhythmic activity evoked by application of pilocarpine.

Chapter 1

Introduction

1.1 Intersegmental Coordination

Locomotion, whether in the form of swimming, crawling, flying, or walking, requires two simultaneous actions. First, the part of the body responsible for locomotion must be moved in an appropriate way to produce propulsion. A wing must rotate around its longitudinal axis while it is moving upward and downward to advance the animal. A leg must perform stance and swing phases, which means it must be on the ground while the body is pushed forward (stance) and lifted off while it does not support the body and is moved forward (swing). Second, multiple body parts responsible for locomotion must act in a coordinated way. Left and right wings need to move upwards and downwards in a synchronized way to assure stable flight. Left and right legs need to alternate in their cycles to guarantee a biped, slowly walking individual that at least one leg is on the ground all the time, so the individual does not fall. If an animal has more than two legs, additional coordination is required: the multiple pairs of legs, e.g. front, middle, and hind legs of an insect have to be coordinated. This coordination is termed intersegmental coordination as one segment of an animal usually contains the circuitry to control one pair of legs. In a multi legged organism this coordination between segments leads to so called stepping patterns. Examples for a horse would be trot or gallop, for an insect tripod or tetrapod gait. In animals with other forms of locomotion, for example snakes or lampreys, this coordination creates a wave of body undulation that propels the animal. The neural basis of intersegmental coordination of walking is the topic of this thesis.

1.2 Possible mechanisms

How is intersegmental coordination achieved? Of several proposed mechanisms, some have been demonstrated to exist in various animals.
A **central command unit**, located in the central nervous system, could activate local circuitry in the segments. The local circuitry could then execute the motor program to perform a step. The higher level central command unit 'supervises' the segments, thus ensuring coordination (reviewed by Pearson, 1993).

Such a central command unit is not necessarily required, as the segmental circuitries could exchange information between each other. A segment commonly contains central oscillators that generate rhythmic activity underlying locomotion. These oscillators are referred to as central pattern generators (Delcomyn, 1980; Pearson, 2000; Marder, 2001; MacKay-Lyons, 2002). A mechanism ensuring intersegmental coordination is the **coupling of central pattern generators**, that is maintaining a stable phase relationship between their oscillators. This can be achieved by specific connections and suitable intrinsic frequencies (Marder and Calabrese, 1996; Friesen and Cang, 2001).
A disadvantage of these central coupling mechanisms becomes clear for locomotion on irregular terrain. For an insect climbing in shrub, it might not be possible to place a leg at the predetermined location. Walking on rock, a leg might not find the support needed. Feedback from sense organs is required to adjust the movements to the given environment. In most systems a variety of such sense organs, measuring position, movement, and load, are found. Their information is processed locally in the segments and intersegmentally in neighboring segments. Sense organs are often components of reflex loops or they interact with central pattern generators. The **sensory information can also be shared** with other segments and used for intersegmental coordination. This can be done directly through projections of sensory cells in adjacent segments (Bräunig et al., 1981; Pflüger et al., 1981) or via intersegmental interneurons (Laurent, 1986; Büschges, 1989; Brunn and Dean, 1994; Matheson, 2002).
Coordinating information does not necessarily have to be transmitted electrically (via neurons) between the segments. A **mechanical coupling** exists and if for example one leg of a multi legged organism is lifted off the ground, the load on the other legs increases. This is sensed by local sensory organs such as campaniform sensilla in an insect leg or muscle spindles in a vertebrate muscle and influences the local control (Cruse et al., 1998; Friesen and Cang, 2001). A similar coupling exists if the active movement of one limb passively moves another limb. This passive movement may trigger reflexes and thereby cause active movements ('reflex chain theory', reviewed by Grillner (1981)). In both cases coordination of movements of both segments results.

1.3 Evidence for mechanisms used by invertebrates

In the last few decades several invertebrate systems have been used to study the question of how intersegmental coordination is achieved and which of the mechanisms mentioned above are realized.

1.3.1 Coordination of leech swimming movements

A well studied system is the leech. Besides mechanisms for intersegmental coordination of heartbeat and crawling behavior, swimming especially has been extensively studied. Leeches swim by alternating contractions of the dorsal and ventral muscles of their body segments. The timing of these contractions is controlled by a chain of

18-21 segmental ganglia containing the swim network. Each ganglion contains local interneurons that drive motoneurons to produce the dorsal-ventral alteration. It also contains intersegmental interneurons that project to approximately five ganglia in either direction of the chain. These intersegmental connections are asymmetric: correlated with its activity phase, a neuron either projects caudally or rostrally; sole excitatory neurons only project caudally (Brodfuehrer et al., 1995).
If the nerve cord is isolated and lateral nerve roots are briefly stimulated electrically, it responds with a fictive motor pattern similar to the one produced by a swimming animal. This suggests that coupling can be achieved in the absence of sensory inputs, solely by central mechanisms (Kristan and Calabrese, 1976). The timing and phase relations of the motor pattern are thought to result from the asymmetry of intersegmental connections mentioned, and from a rostrocaudally-decreasing intrinsic cycle period of the segmental central pattern generators (Friesen and Pearce, 1993). However, the motor pattern of the isolated nerve cord shows a longer period and a smaller phase lag when compared to an intact preparation. This suggests that sensory information, e.g. from the ventral stretch receptors, which are known to influence the phase lag, is required for appropriate coordination (Cang and Friesen, 2000; Friesen and Cang, 2001).
By severing the nerve cord Yu et al. (1999) showed that proper coordination can be achieved by local sensory feedback alone. They suggest that six to eight pairs of segmental stretch receptors provide the information used for this coordination. As these stretch receptors do not project to other segments (Blackshaw and Thompson, 1988; Huang et al., 1998), information has to be transmitted mechanically and perceived locally by the sense organ. Thus coordination results from mechanical coupling.

In summary, intersegmental coordination in the leech seems to be achieved by both, central coupling of segmental pattern generators and mechanical coupling via sensory organs - the ventral stretch receptors.

1.3.2 Coordination of locomotion in crustaceans

Another group of animals intensively studied with regard to the coordination of locomotion movements is crustacea, especially crayfish and lobsters.

The crayfish swimmeret system

The swimmerets of crayfish are paired, jointed limbs on four or five abdominal segments, used for swimming. They rhythmically perform stereotyped power- and return-strokes and thereby produce forward thrust. Swimmerets of neighboring segments move with the same period and constant phase relation (reviewed by Cattaert et al., 2001).
Ikeda and Wiersma (1964) showed that a deafferented and isolated chain of abdominal ganglia spontaneously produces an activity pattern which parallels the motor pattern of a swimming animal. Thus no sensory input or 'single master center' is required. It was proposed that the coordination between the ganglia could derive from

a excitability-gradient - that is the neurons driving the more posterior swimmerets are more excitable and reach thresholds before their more anterior counterparts (Wiersma and Ikeda, 1964). But Mulloney (1997) showed that the excitability is identical for all segments and thus proved this hypothesis incorrect. Instead, the coordination between the segments is achieved by the actions of three types of intersegmental interneurons (Stein, 1971; Namba and Mulloney, 1999).
The observation that the normal motor pattern keeps existing even after shortening the chain of ganglia to only two ganglia, led to the proposal that only nearest neighbors are coupled (Paul and Mulloney, 1986). This idea was incorporated in mathematical models which produced the characteristic phase differences and relative durations of the swimmeret system (Skinner et al., 1997; Mulloney et al., 1998). Although the coupling of only nearest neighbors seems to be sufficient to explain the intersegmental coordination in the swimmeret system, Tschuluun et al. (2001) showed that interneurons project beyond the neighboring ganglion and maintain coordination even if the synaptic transmission is blocked in the nearest neighboring ganglion.

In the crayfish swimmeret system intersegmental coordination is achieved by central coupling of segmental pattern generators. The coordinating interneurons link not only nearest neighbors, but also more distant ganglia. Sensory feedback might be unnecessary for basic coordination.

Walking of lobsters and crayfish

Both, lobster and crayfish, were used for studying terrestrial locomotion. For walking, decapods perform periodical stepping movements with all or only a subset of their ten walking legs. Each single leg alternates between a stance and a swing phase. On a smooth, flat surface, the left and right legs of a pair move in antiphase, while the phase lag of adjacent ipsilateral legs is approximately one-quarter of a cycle (Clarac and Chasserat, 1983; Chasserat and Clarac, 1980). This coordination produces the specific gait. Experiments in which left and right legs walked on separate treadmill belts, driven with different speeds, revealed that left and right legs were able to step at different frequencies. Thus separate leg controllers must exist (Clarac and Chasserat, 1986).
Chrachri and Clarac (1987) showed that bath application of cholinergic agonists, such as pilocarpine or oxotremorine, evoked rhythmic activity of motoneuron pools in an isolated thoracic ganglion preparation of crayfish. This rhythmic activity resembled the motor pattern seen in an intact, walking animal. Even a single isolated ganglion produced a rhythmic activity pattern after application of cholinergic agonists. Therefore, segmental central pattern generators are responsible for the generation of basic motor output patterns, and interactions between them lead to an intersegmental coordination of motor activity (Chrachri and Clarac, 1990).
Although coordinated patterns of activity are found in the isolated chain of ganglia, sensory signals strongly affect the inter-leg coordination (reviewed by Clarac, 1985; Cattaert et al., 2001). An 'in phase' coordination with the next anterior intact leg is observed when legs are not able to generate propulsive forces. This is the case when

legs are held up off the ground ('waving behavior'), or when legs are autotomized and only the stumps move. The observations suggest that substrate contact and/or power stroke forces are necessary for the production of an alternating pattern in the intact animal. Studies by Cruse et al. (1983) showed that the position of one leg influences the coordination of the remaining legs. While a lobster was walking on a driven treadmill, they isolated one leg from the walking legs by placing it on a platform. Moving the platform, and thereby changing the position of the leg, they could alter the stepping pattern of the walking legs. In other experiments Cruse and Müller (1986) analyzed the effects of disturbing the stance phase of individual legs of a walking crayfish. Both studies led to the description of two coordinating influences: a forward directed influence, facilitating a swing phase of the anterior leg while the posterior leg is in stance phase, and a backward directed influence, facilitating a stance phase in the posterior leg at the transition from stance to swing phase of the anterior leg (Cruse and Müller, 1986).

In summary, it appears that a central coupling mechanism, as well as information from sensory organs, are involved in the coordination of leg movements of decapods. Both seems to be important for the production of the gait seen in the intact, walking animal.

1.3.3 Coordination of walking in insects

Many studies of motor systems and locomotion have been performed with insects - namely stick insects, locusts, and cockroaches. Their six legs are coordinated into a tripod gait, a tetrapod gait, or an intermediate form - depending on species, age and speed of locomotion of the animal tested (Graham, 1985). Most experimental designs to unravel mechanisms responsible for this intersegmental coordination rely on describing changes of stepping patterns caused by alteration or disruption of sensory feedback.
Experiments in which one leg was amputated, led to slight changes of the spatial and temporal pattern of movements of the remaining legs. Apparently no learning period is required to establish the new coordination and it was shown that the changes are not due to a change of walking speed or alteration of the load distribution. Interestingly, normal coordination was reestablished if a prothesis was glued to the stump of the amputated leg. If a middle leg was not amputated, but just immobilized, coordination between the legs was completely destroyed and movements became disorganized. These findings suggest that available sensory information, especially from proximal sensory organs, is used for intersegmental coordination, but that the system generating the stepping pattern does not rely upon a complete set of sensory information to produce a stable pattern of movements (stick insect: von Buddenbrock (1921); Wendler (1964); Bässler (1972); Graham (1977, 1985), cockroach: Hughes (1957); Pearson (1972); Delcomyn (1991a,b), locust: Macmillan and Kien (1983)).
To investigate if mechanical coupling is used to coordinate walking movements in stick insects, Graham and Cruse (1981) analyzed the stepping pattern of a tethered stick insect walking on a mercury surface. The surface tension provided force feed-

back for every individual leg, while isolating it from the forces applied by other legs - ruling out mechanical coupling of the legs. Epstein and Graham (1983) conducted similar experiments using a slippery oiled glass surface instead of the mercury surface to exclude mechanical coupling. Under these conditions the animal walked with fully coordinated leg movements. The only difference, compared to the stepping pattern of an animal walking on solid substrate, was a shorter stance phase, which was accredited to the lower friction between tarsus and substrate. Mechanical coupling appears not to be a relevant mechanisms for coordination of walking movements of stick insects.
Additional evidence comes from experiments in which one of the paired thoracic connectives was cut. After the operation, severe changes in step coordination occurred. A coordination of leg movements as it is seen in the intact animal, apparently requires exchange of information between the thoracic ganglia (mantis: Roeder (1937), stick insect: Dean (1989), cockroach: Greene and Spirito (1979).
A comprehensive set of 'rules' for the coordination of leg movements in a walking arthropods was proposed by Cruse (1990). He investigated coupling between ipsilateral legs by briefly interrupting the stance phase of an animal walking on a slippery surface. Observing how the legs return to normal coordination following this disturbance, he deduced three 'rules' (Cruse and Schwarze, 1988; Cruse and Müller, 1986):

1. A forward directed influence inhibits the start of the swing phase as long as the posterior leg is in swing phase.
2. A forward directed influence excites the start of the swing phase when the posterior leg starts the stance phase.
3. A backward directed influence excites the start of a swing phase, the influence being stronger the further the anterior leg has moved backwards.

Three more proposed 'rules' are based on older observations:

4. During walking, the tarsus of the posterior leg is placed directly behind the middle leg tarsus. (Cruse, 1979; Dean and Wendler, 1983)
5. An increase in the motor output of a leg immediately leads to an increase in the motor output of neighboring legs during their stance phase. (Bässler, 1979; Cruse, 1985)
6. If at the end of a stance phase a tarsus is placed onto the tarsus of the anterior leg, the posterior leg is lifted again and placed slightly to the rear (treading-on-tarsus reflex). (Graham, 1979a,b)

Although little is known about a neuronal basis for these 'rules', it was shown with hardware and software simulations that they are sufficient to produce a pattern of leg movement similar to the one seen in walking stick insects (Cruse et al., 1995, 1996, 1998).
With their experiments Bässler et al. (1985) demonstrated the existence of a pathway from the prothorax to the metathorax which, together with the suboesophageal ganglion, induces the hind legs to walk in a forward direction. They found that in decapitated animals mounted above a slippery surface, the front legs appear to

walk forward (retraction during stance phase), while the hind legs appear to walk backwards (protraction during stance phase). In contrast, decerebrated insects still possessing the suboesophageal ganglion did produce a normal stepping pattern. Decapitated animals on solid substrate showed no walking-like behavior, which is explained by the forward pull of the front legs being cancelled by the backward pull of the hind legs. Bässler et al. (1985) suggested that signals from prothoracic sense organs could reach the metathoracic ganglion directly or via the suboesophageal ganglion. In the first case the suboesophageal ganglion could gate the signals of a direct pathway. These signals could determine the walking direction of the metathoracic legs. Details about the neuronal basis of this pathway are not known.
Two other coordinating channels were described by Foth and Bässler (1985a,b). They used an experimental setup in which a stick insect walked on a 'double treadwheel' with five legs, while the remaining hind leg walked on a motor driven belt. Analyzing the coordination of movements of the legs, they described one posterior and one anterior directed influence between the ipsilateral middle and hind leg that inhibit the transition from stance to swing phase of a leg.
The experiments mentioned so far were behavioral studies, most of them describing the effects of manipulation of sensory input. To study the interaction between central rhythm generators, preparations with an isolated thoracic nerve cord were used (locust: Ryckenbusch and Laurent (1994), stick insect: Büschges et al. (1995a), hawkmoth: Johnston and Levine (1996a, 2002)). Sensory feedback was completely removed in these preparations. Rhythmic activity of leg motoneurons was evoked by superfusion with the muscarinic agonist pilocarpine, and monitored with extracellular electrodes. In locusts activity of levator motoneurons, on the same side of different segments, appeared to be uncorrelated in most experiments (Ryckenbusch and Laurent, 1994). In the experiments where a correlation was seen, levator activity in one segment immediately preceded or followed levator activity in an adjacent segment. Analyzing the correlation of protractor motoneuron activity on the same side of different segments in the stick insect, Büschges et al. (1995a) found no stereotyped or fixed coupling, although in some preparations they observed long periods of in-phase activity. In juvenile as well as in adult hawkmoth, Johnston and Levine (1996a, 2002) found patterns of activity that resembled the patterns seen in intact animals. When activated with pilocarpine, leg motoneurons of the isolated nerve cord of adult *Manduca sexta* produced activity that parallels the coordination of a tripod gait. Although the patterns differed in some aspects, the experiments demonstrate that in *Manduca sexta* coupling of central pattern generators exists, and is sufficient to produce a basic tripod-like activity pattern.

A neural basis for the transmission of sensory information between segments was described by Bräunig et al. (1981) and Pflüger et al. (1981) for locusts. With morphological studies they showed that exteroceptive tactile hairs on the thorax form intersegmental projections. The same is true for receptor cells in chordotonal organs of ventral thoracic parts and proximal leg joints.
More evidence for neurons that could mediate the neural information used for intersegmental coordination comes from experiments performed by Laurent (1986, 1987). He recorded intracellularly from interneurons in the mesothoracic ganglion of locusts

which, as he could show by dye filling, project into the metathoracic segment. Conversely, for interneurons in the metathoracic ganglion, he could show projections into the mesothoracic ganglion. These intersegmental interneurons receive presumably direct inputs from specific local mechanosensors, like exteroceptive hairs or the femoral chordotonal organ, a proprioceptor in the femur of the leg (Laurent and Burrows, 1988). Further studies, using simultaneous recordings with two intracellular electrodes, showed that they make direct synaptic connections with motoneurons and non-spiking interneurons in the adjacent segment. The latter integrate local and intersegmental information and could modify the effectiveness of the intersegmental pathway (Laurent and Burrows, 1989a,b). The described population of intersegmental interneurons thus provides a neural basis for the transfer of information used to coordinate leg movements.
In the stick insect, Büschges (1989) found a different population of spiking interneurons that show intersegmental processes and respond to stimulation of the femoral chordotonal organ. He could show that these interneurons respond to very specific aspects, like position, velocity, and acceleration of the tibia in respect to the femur of a leg. This population also provides a pathway for coordination of leg movements.
Another effort to clarify the neuronal basis of intersegmental coordinating pathways was done by Brunn and Dean (1994). They intracellularly recorded from interneurons in the metathoracic ganglion of stick insects, while systematically moving the ipsilateral mesothoracic leg. Several interneurons were found which code for the position and movement of joints of the mesothoracic leg. Dye injection revealed that all but one of the recorded interneurons span several ganglia. These intersegmental interneurons could be part of the mechanism for targeting behavior which assures that the tarsus of the hind leg of a walking stick insect is placed directly behind the tarsus of the middle leg.

In summary, coordination between insect leg pairs has been well studied on the behavioral level. Experiments suggest that a mechanical coupling mechanism is of minor importance. Coupling of segmental oscillators seems to exist in the hawkmoth *Manduca sexta*, and is able to generate a basic stepping pattern. Studies of other insect systems did not reveal an important role of central mechanisms for intersegmental coordination. Sensory input appears to be a major source of signals for intersegmental coordination, and studies have shown that neural pathways for the exchange between the segments exist.

1.4 Studies of vertebrate systems

Vertebrates face the same problem of coordination of body segments or limbs as invertebrates; and they presumably use the same mechanisms to solve it. As vertebrate nervous systems are often more complex and their behavior less stereotyped, it is more difficult to study these mechanisms. Nevertheless experiments, especially with lampreys and cats, have advanced the understanding of vertebrate locomotion.

1.4.1 Intersegmental coordination of swimming in lamprey

The lamprey, a lower vertebrate, swims by alternately contracting muscles on left and right side of its body segments. An intersegmental phase lag of about one percent of the cycle period, from anterior to posterior, creates the undulation needed to produce forward thrust (Grillner et al., 1995). How this phase lag is achieved and controlled was the topic of several studies.
Neuronal connectivity was described with E and CC-type interneurons (reviewed by Grillner and Wallén, 2002). The first ideas for a possible mechanism came from a mathematical model, simulating phase-coupled oscillators (Cohen et al., 1982; Kopell and Ermentrout, 1988). In this model, an oscillator in each segment, controlling the alternating contractions, was coupled in both directions to its nearest neighbor oscillators. The neighbor oscillators had identical properties, e.g. the same intrinsic frequency, but the coupling was asymmetric in that the ascending coupling dominated in setting the phase lags. The model produced an output similar to the motor output observed in pharmacologically activated *in vitro* spinal cord preparations. But experiments in which rostral and caudal segments were differentially activated showed that the assumption of a dominating ascending coupling was not correct for the lamprey (Sigvardt and Williams, 1996). Furthermore long range coupling, which was not implemented in the model, was found to contribute to coordination (McClellan and Hagevik, 1998; Miller and Sigvardt, 2000). Another theory was proposed with the 'trailing oscillator hypothesis' (Matsushima and Grillner, 1990). It was based on the assumption of symmetric coupling and different intrinsic frequencies of the segments' oscillators. The phase lag was explained by ascending intrinsic frequencies in the caudal direction.
One aspect not included in these theories was the role of sensory feedback. Earlier experiments showed that stimulation of stretch receptive cells, located at the lateral margin of the spinal cord (edge cells), had an influence on the rhythmic activity (reviewed by Wallén and Williams, 1986). Thus it seems likely that sensory information assists intersegmental coordination in the intact animal. However, a simulation study using a neuro-mechanical model that considers the visco-elastic properties of the muscles as well as the viscous properties of water, suggested that sensory feedback has no apparent influence on the pattern of movement. Yet, sensory feedback was found to be of critical importance, as it enabled the model lamprey to compensate for perturbations (reviewed by Grillner and Wallén, 2002).

Mainly simulation studies have shown that central coupling could be the major mechanism used by lampreys to coordinate their segments' movements. The models reflect many physiological aspects found in the animal and produce similar coordination patterns.

1.4.2 Intersegmental coordination of leg movements of cats

In tetrapod locomotion a variety of different coordination patterns between fore and hind limbs can be observed. They can be divided into alternating gaits and non alternating gaits. In the former case, left and right legs show a clear phase differ-

ence and the left leg touches the ground while the right is lifted. Examples include walk, pace and trot. In non alternating gaits, left and right leg touch the ground approximately simultaneously. Examples include gallop, half bound and leaping. In general, animals can switch between different gaits, and different gaits are often used for different speeds of locomotion. This, and the need to keep in balance, require a flexible or selective control of coordination and can not be explained by a 'hard wired' network (reviewed by Grillner, 1981).
Lesion experiments in which the dorsal column of the spinal cord, containing ascending axons that carry somatic sensory information, was cut in the lower thoracic region, caused changes in the pattern of fore-hind limb coordination. This suggests that propriospinal pathways are used to transmit coordinating signals (English, 1980).
An effect of sensory input was shown for a leg pair. In experiments with decerebrated and awake cats walking on a treadmill, perturbations (mechanical taps) were applied to the paw. This stimulus changed the coordination of leg muscles in the walking leg, as well as in the contralateral leg (Matsukawa et al., 1982). Indirect evidence for influence of sensory information on homolateral coordination comes from experiments in which such information was removed by deafferentation. Wetzel et al. (1976) used cats, in which they removed the dorsal root ganglia carrying the sensory information from the left hind leg. Analyzing the stepping pattern of these animals on a motor driven treadmill, they described the observed inter-limb coordination as blurred, although distinct patterns were still seen. Orsal et al. (1990) used a thalamic cat preparation, in which all higher centers of the brain were removed. They recorded extracellularly from stumps of cut motor nerves of all four limbs during periods of spontaneous activity - described as fictive locomotion. Any phasic sensory input could be ruled out. They found distinct patterns of activity such as strict alternation between left and right front leg nerves. A small number of patterns involved nerves of all four limbs and corresponded to walking or trotting gaits of the intact cat. The experiments demonstrate that different forms of inter-limb coordination, including coupling between front- and hind legs, exist during fictive locomotion and do not depend on sensory feedback.
Though coordinated leg movements can be observed in high spinal cats, in which the spinal cord is transected in the cervical region (Miller and Van der Meche, 1976), supraspinal structures may have a crucial role in fore-hind limb coordination. An example includes neurons of the medullary reticular formation. Their activity is correlated with activity of motoneurons controlling muscles of different limbs. Stimulation of the medullary reticular formation may evoke simultaneous responses of such motoneurons (Rossignol et al., 1993; Armstrong, 1986).

In summary, the experiments show that sensory information has a strong influence on coordination of the leg pairs, as a lack of such strongly affects the stepping pattern. Yet, some coupling must be achieved by central mechanisms to explain the coordination seen in fictive locomotion. Supraspinal structures could play a stronger role for inter-limb coordination in cats than higher brain structures in lower vertebrates or invertebrates.

1.5 Question and experiments

The intention of this thesis is to further investigate the neural basis of mechanisms used by animals to coordinate walking movements of different segments. It describes influences of walking movements on the activity of ipsilateral motoneurons in the adjacent segment in a semi-intact walking preparation of the stick insect (3.1). Evidence for how the motoneurons are modulated is presented (3.2). Non-spiking interneurons of the pre-motor network are studied as possible mediators of intersegmental signals (3.3). To assess the origin of the coordinating signals described, coupling of central pattern generators in adjacent segments is examined with a pharmacologically activated preparation (3.4), and the role of signals from two sensory organs for intersegmental coordination is investigated on the neuronal level (3.5 and 3.6).

Chapter 2

Materials and Methods

2.1 Animals and experimental procedures

All experiments were performed on adult, female Indian stick insects (*Carausius morosus* Brunner 1908, syn.n. *Dixippus morosus*) from the breeding colony at the University Cologne. For most experiments the animals were fixed dorsal side up on a foam platform using dental cement (Protemp II, 3M ESPE, Seefeld) and placed on a vibration isolating table (MICRO-g, TMC, Peabody, MA, USA) in a Faraday cage (custom made). The thorax of the animal was opened by cutting along the midline of the tergum. Both sides were folded apart and fixed with insect pins. The gut was lifted out of the animal and placed parallel to the body. Connective tissue was removed to expose the nerve cord. Care was taken to do as little damage as possible, especially with regard to tracheae. The body cavity was filled with saline (pH 7.2) composed according to Weidler and Diecke (1969), for some experiments (partly sections 3.2 and 3.4.2) 10 mM HEPES instead of TRIS buffer was used. Leg nerves were identified according to Marquardt (1940) and Graham (1985). All experiments were carried out under daylight conditions and at temperatures between 18 °C and 24 °C.

2.2 Experimental conditions

Some experiments (sections 3.4, 3.5, and 3.6) were carried out with animals in different behavioral states: resting, in an 'active' state after the animal was tactilely activated by touching the abdomen with a paint brush, or in a state of pharmacologically evoked rhythmic activity. The latter was achieved by application of the muscarinic agonist pilocarpine hydrochloride (Sigma-Aldrich Chemie GmbH, Steinheim). Drops of stock solution with a concentration of $10^{-3}M$ were delivered into the bath, to reach a final concentration in the range of $10^{-4}M$ to $5 * 10^{-3}M$. This concentration evokes in stick insects rhythmic activity of antagonistic motoneuron pools (Büschges et al., 1995a).

2.3 Extracellular recordings

Activity of motoneurons was monitored by recording from nerves containing their axons. Nerves used for this purpose were nl2 (protractor), nl5 (retractor), C1 (levator), C2 (depressor), nl3 or F2 (extensor), and nCr (flexor). A monopolar hook electrode (custom built, modified after Schmitz et al. (1988, 1991)) was brought close to the nerve and electrically isolated from the surrounding medium with vaseline ('Baysilone-Paste hochviskos', Bayer AG, Leverkusen). The nerve was either cut or crushed distal to the electrode. The signals were amplified and filtered (highpass: approx. 400 Hz, lowpass approx. 1 KHz) with a custom built pre- and filter-amplifier.

2.4 Intracellular recordings

For intracellular recordings from the neuropilar processes of motoneurons, the ganglion was fixed on a small wax coated platform with cut minutien pins or fine cactus spines. The ganglion sheath was treated with Pronase E (Merck, Darmstadt) for 45 to 90 seconds to allow electrode penetration. Microelectrodes were pulled from glass capillaries (GB100TF-8P, Science Products GmbH, Hofheim) and filled with 0,05 M KCl and 3 M KAc, which resulted in an electrode resistance of 15 to 25 MΩ. Recordings were made in bridge and discontinuous current clamp (DCC) mode with a SEC-10L, SEC-5L, or SEC-1L/H intracellular amplifier (npi electronic GmbH, Tamm). Motoneurons were identified by a one to one correlation with spikes in an extracellular recording of nerves nl2 (protractor), nl5 (retractor), C1 (levator), C2 (depressor), nl3 (extensor), or nCr (flexor). Non-spiking interneurons were identified by their effect on extensor motoneuron activity during hyperpolarization or depolarization. Furthermore, dextran tetramethylrhodamine (3000 MN, lysine fixable, Molecular Probes Inc., Eugene, OR, USA) was injected at the end of the experiment and the interneurons were viewed with an UV-microscope, after fixation with 4% paraformaldehyde in 0.1M phosphate buffer, dehydration with an alcohol series, and clearing in methylsalycilate. A catalog with camera lucida drawings of known interneurons was available for identification (catalog compiled by A. Büschges, in part published in Büschges (1990)).

2.5 Electromyograms (EMG)

To record activity of the flexor muscle, two small holes with a distance of approximately 2 mm were drilled in the femur right above the flexor muscle. Two 65 μm copper wires, insulated except for the tip, were inserted and fixed with dental cement. The signals were amplified and filtered (highpass: approx. 400 Hz, lowpass approx. 1 KHz) with a custom built pre- and filter-amplifier.

2.6 Experiments using a treadmill

Some experiments (sections 3.1, 3.2, and 3.3) required the animal to perform walking like movements with a single front leg on a treadmill (Fig. 2.1). Here, a custom made treadmill with two lightweight drums (Ø 40 mm x 28 mm, shaft distance 51 mm) covered with crepe paper was used. Inside each drum a motor was installed: one to measure speed, the other one to slightly drive the treadmill to reduce friction. The signal from the tachometer was amplified and filtered (lowpass approx. 20 Hz). For a more detailed description see Gabriel (2002). The left front leg, middle and hind legs were cut. The animal was then fixed on the edge of a foam platform, in a way that the remaining right front leg could move mostly unrestricted - in contrast to the single leg preparation described eg. by Fischer et al. (2001). The height of the treadmill was adjusted, so that with horizontal femur, the angle of femur - tibia and tibia - tarsus were both 90°. Wires for a EMG recording were fixed on the front leg and the mesothoracic ganglion was exposed as described in section 2.1. All leg nerves in the mesothoracic segment were either cut or crushed to exclude local sensory input.

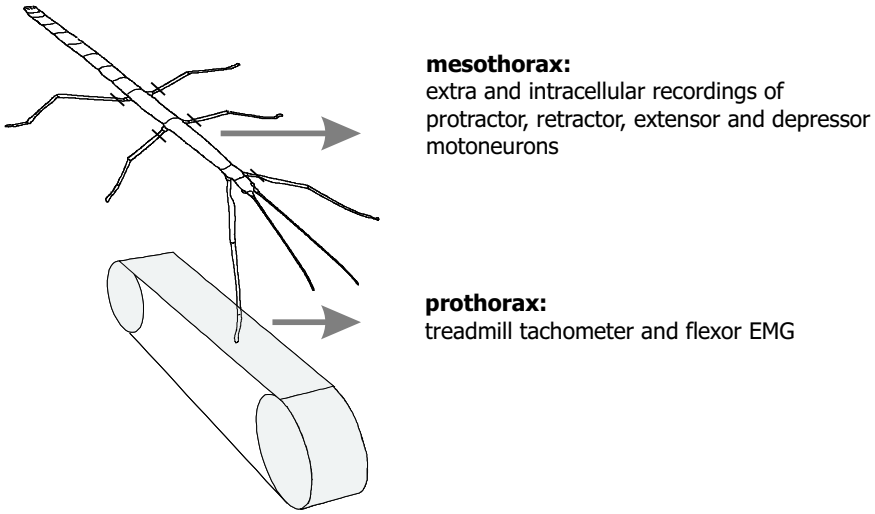

Figure 2.1: The animal walked on the treadmill with the mostly unrestricted right front leg. All other legs were cut. Inside the two drums of the treadmill motors were installed: one was used as tachometer the other one slightly drove the treadmill to reduce friction.

2.7 Pharmacological stimulation in 'split bath' experiments

To investigate central coupling mechanisms (section 3.4), a 'split bath' preparation was developed that allowed pharmacological stimulation of a single ganglion and monitoring the activity of motoneurons in an adjacent ganglion, while excluding all sensory input.

2.7.1 Meso- and metathoracic ganglia

After fixing the animal on a foam platform, decapitating it and opening the thorax, the connectives between pro- and mesothoracic ganglia and between metathoracic and second abdominal ganglion were cut (the first abdominal ganglion is fused with the metathoracic ganglion in *Carausius morosus* and in future I will refer to it simply as *metathoracic ganglion*). All nerves of the meso- and metathoracic ganglia were cut, leaving an isolated chain of two ganglia. Next, a stripe of cuticula with a width of approximately 2 mm was removed between meso- and metathoracic ganglion. All tissue in this region was removed, but care was taken not to damage the connective, so that the front and rear part of the animal was now only connected by the connective. Around this connective a barrier from purified vaseline (Engelhard Arzneimittel GmbH & Co. KG, Niederdorfelden)was modelled, to obtain two separate baths - one for the mesothoracic and one for the metathoracic ganglion (Fig. 2.2A). In most experiments the dye Janus Green B (Eastman Chemical Company, Rochester, NY, USA) was delivered into either one of the baths to verify that the fluids were not connected.
Pharmacological stimulation was achieved by application of pilocarpine as described in section 2.2. The success of the stimulation was controlled with two extracellular recordings from the stumps of nerve C1 and C2 - containing the axons of levator and depressor motoneurons. Preparations were only used when a stable rhythm with clear alternation between levator and depressor motoneuron activity was established. The activity of motoneurons in the ganglion not stimulated was monitored by extracellular recordings of the stumps of nerve nl2 (protractor), nl3 (extensor) or C1 (levator).

2.7.2 Pro- and mesothoracic ganglia

Because of the more complex internal morphology of the prothoracic segment, and therefore worse accessibility of the nerves, a different preparation had to be used. The body of the animal was cut posterior to the head and between the meso- and metathoracic segment. The gut was removed and the body cavity flushed with saline to remove unintentionally spilled gut content. After removal of the tergum, the remaining part was pinned out on a foam platform. The two ganglia were exposed by removing the overlaying tissue and some trachae. Cuticula and tissue were carefully cut posterior to the prothoracic ganglion and anterior to mesothoracic ganglion, without damaging the connective. This resulted in two pieces of cuticula, each containing some muscles, trachae, and a ganglion connected via the connective

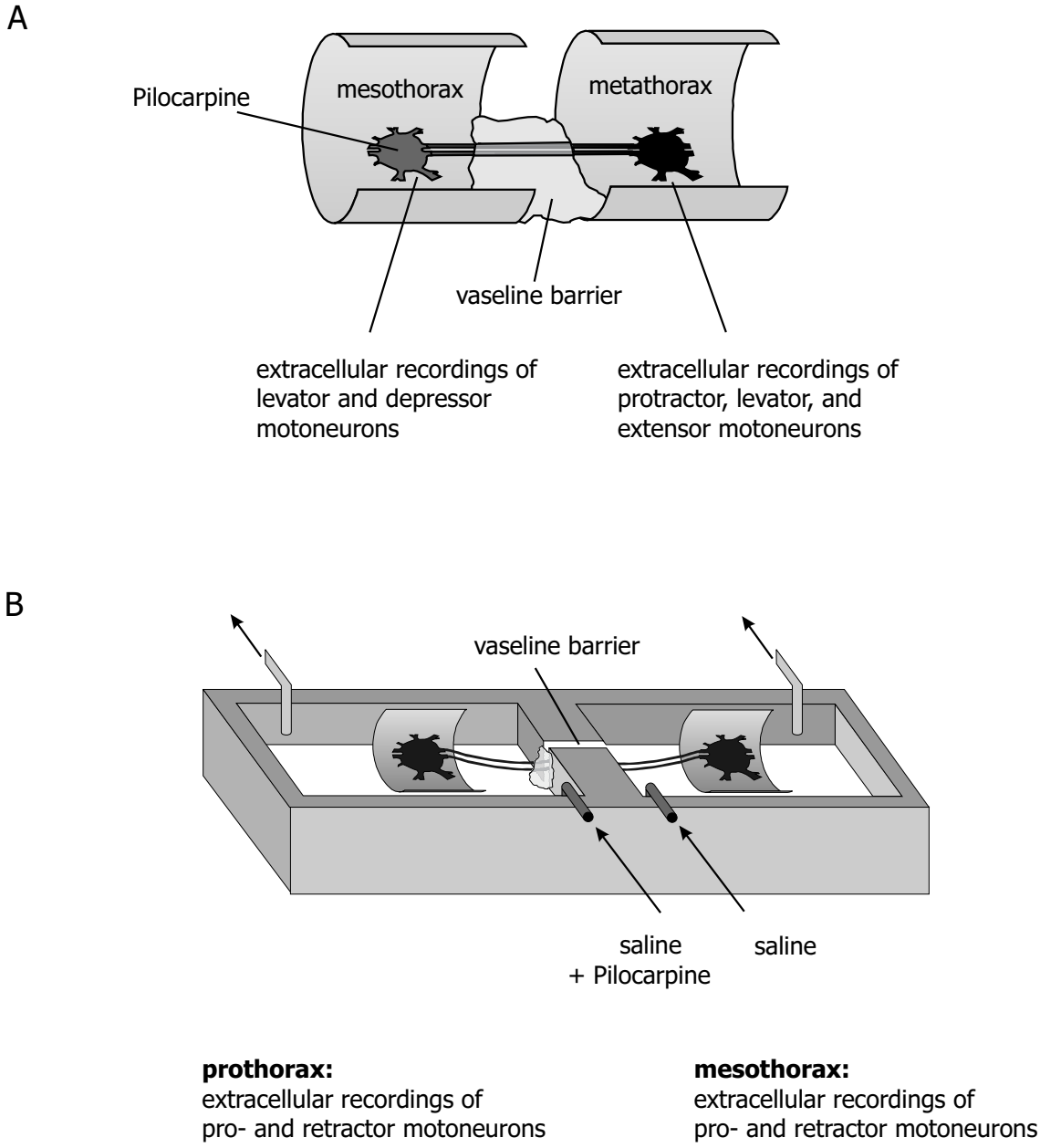

Figure 2.2: To study central coupling mechanisms a 'split bath' preparation was used. (A) The meso- and metathoracic ganglia were isolated in the animal and separated with a vaseline barrier. After the mesothoracic ganglion was made rhythmically active by application of pilocarpine, activity of motoneurons was recorded extracellular from the nerve stumps. (B) To study coupling of pro- and mesothoracic ganglia, they were taken out of the animal together with some tissue and cuticular structures. They were placed in a special dish and constantly superfused. Rhythmic activity was induced in the prothoracic ganglion by superfusion of pilocarpine dissolved in saline.

to the other ganglion. This preparation was now transferred to a special dish (Fig. 2.2B).
The dish consisted of two compartments separated by a wall. A gap located in the wall was filled with vaseline so that no exchange of fluids between the two compartments was possible. Separate superfusion devices allowed a constant flow of fresh saline through each compartment.
Each piece of cuticula was pinned out in one compartment, and the connective put in the vaseline filled gap. After removal of some more tissue, all nerves except for nl2 and nl5 were cut at both ganglia, so that no sensory input was possible. Extracellular electrodes were placed at the latter nerves, which were then crushed distal to the electrodes. This allowed monitoring of the activity of protractor and retractor motoneurons in both the pro- and mesothoracic ganglion.
Rhythmic activity of motoneuron pools was evoked by superfusion of pilocarpine at a concentration of approximately $5 * 10^{-4} M$ (see also section 2.2) on the prothoracic ganglion. Preparations were only used when a stable rhythm, with clear alternation between protractor and retractor motoneuron activity, established.

2.8 Stimulation of the femoral chordotonal organ

In a set of experiments (section 3.5) the femoral chordotonal organ, a sensory organ measuring position, velocity and acceleration of the femur-tibia joint, of the right middle leg was stimulated. For that, the receptor apodeme was exposed by cutting a small window into the distal part of the femur, fixed in a clamp, and cut distally (Fig. 2.3A). The clamp was moved with a stimulation device as described by Hofmann and Koch (1985a); Hofmann et al. (1985b). The zero position corresponded to an angle between femur and tibia of approximately 120°. A movement of 300 μm simulated a flexion of approximately 60°, that is, to a femur-tibia angle of 60° (Weiland et al., 1986). Typical velocities used were between 140°/s and 300°/s. An extracellular recording of nerve F2 - carrying the axons of the extensor motoneurons - was used as a control. It showed a resistance reflex response during stimulation of the resting animal as described by e.g. by Bässler and Storrer (1980).
Front legs were fixed with insect pins, the left middle leg and hind legs were cut. The meso- and metathoracic ganglia were exposed as described in section 2.1 and all leg nerves, except for the right mesothoracic nCr carrying the afferents of the chordotonal organ, were cut or crushed to restrict sensory input. The experiments were carried out in resting animals as well as in 'active' animals (see section 2.2).

2.9 Stimulation of the campaniform sensilla

In other experiments (section 3.6), campaniform sensilla, strain sensors located in three groups (anterior, posterior and dorsal) on the trochanter and one group on the femur of the right hind leg, were stimulated. The leg was cut at the proximal tibia and positioned perpendicular to the body axis. The coxa was embedded in dental cement to impede pro- and retraction of the leg. Care was taken not to cover the campaniform sensilla. A piezoelectrical element (Physik Instrumente GmbH &

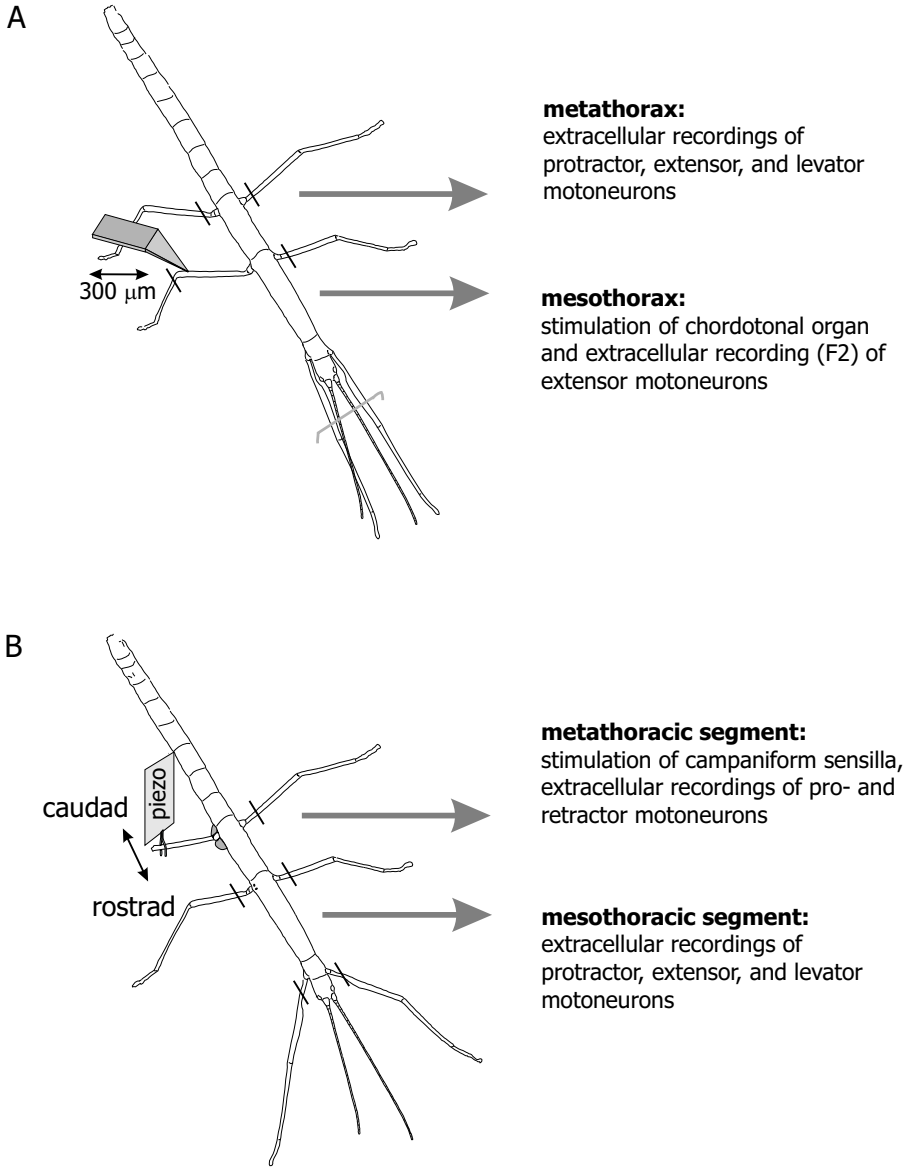

Figure 2.3: To study intersegmental influences of sensory organs, (A) the femoral chordotonal organ of the right middle leg and (B) campaniform sensilla of the right hind leg were stimulated, while the activity of motoneurons in the adjacent segment was monitored with extracellular electrodes.

Co. KG, Karlsruhe) was attached to the distal end of the femur and, by horizontal deflection of the femur by approximately 200 μm, strain was applied to the cuticula and thus the campaniform sensilla stimulated (Fig.2.3B, see also Schmitz (1993). Front, middle, and left hind legs were cut. The meso- and metathoracic ganglia were exposed as described in section 2.1. All leg nerves, except the right metathoracic nCr which carries the afferents of the campaniform sensilla, were either cut or crushed to restrict sensory input. Extracellular recordings of nerves nl2 and nl5 - carrying axons of protractor and retractor motoneurons respectively - were used to monitor a local reflex response similar to the one described for the mesothoracic segment by Schmitz (1993). The experiments were carried out with resting animals, 'active' animals, and under pharmacologically evoked rhythmic conditions (see section 2.2).

2.10 Data acquisition and processing

Electrophysiological and stimulus data from the semi-intact walking preparation (sections 3.1, 3.2, and 3.3) were viewed online on an oscilloscope (Tektronix 5103n series, Beaverton, OR, USA), and A/D converted and stored on a computer (Micro 1401 hardware and Spike II software version 3.13 - 4.12, Cambridge Electronic Design Limited, Cambridge, England). The same data acquisition was used for experiments investigating central interaction between pro- and mesothoracic ganglia (section 3.4.2). In experiments that studied the central interaction between meso- and metathoracic ganglia (section 3.4.1), data were additionally viewed online on a digital scope (DL 708E, Yokogawa Electric Corporation, Tokyo, Japan), recorded on video tape (StorePlus VL, Racal Elektronik System GmbH, Bergisch-Gladbach) and A/D converted online or offline. Data on the intersegmental influences of the chordotonal organ (section 3.5) were recorded on 1/2" magnetic tape (Store 7DS, Racal) and A/D converted offline (1401 plus hardware and Spike II software, Cambridge Electronic Design Limited, Cambridge, England). During the experiments studying the influence of campaniform sensilla (section 3.6), data were recorded on digital audio tape (DAT 1800, Bio-Logic - Science Instruments SA, Claix, France) and A/D converted online (again Micro 1401).
For the A/D conversion of electrophysiological data, a sampling rate close to 12.5 KHz was used for extracellular data and close to 6.25 KHz for intracellular data. At these sample rates no loss of information compared to the signal displayed on the oscilloscope could be detected. The evaluation was done with custom written scripts within the Spike 2 software and through printouts (DL2300 or OR1400 thermal printer, Yokogawa Electric Corporation, Tokyo, Japan or with Spike II on a laser printer).
Data plotted in the form of a histogram was processed with the Spike 2 software, and plotted with Grapher version 4 (Golden Software Inc., Golden, CO, USA). Changes described in 'per cent' relate to the ratio between the most and the least filled class. Prior to averaging data from intracellular recordings of spiking neurons (section 3.2), action potentials were removed from the recording. For this, the typical durations of depolarization (1-2 ms) and repolarization (1-3 ms) during an action potential in the recording were determined. A Spike 2 script was used to substitute the data of each

action potential with a linear interpolation during the determined time windows.
In sections 3.2 and 3.3, a tonic depolarization of membrane potentials during walking activity is described. To access its amplitude, a simultaneously occurring modulation of the membrane potential was eliminated by averaging the amplitudes during the period of walking activity. This average value was then compared to the resting potential of the neuron prior to the start of the walking activity. Time constants were used to describe the decay of the tonic depolarization. The time constant describes the time until the depolarization decays to approximately 37 % of its initial amplitude. For the calculation, data were exported to Excel 2002 (Microsoft Corporation, Redmond, WA, USA) and an exponential function was fitted. The time constant equals $|exponent\ of\ the\ fitted\ function|^{-1}$.
In text and figures, a capital N denotes the number of animals used for an experiment, and a lowercase n denotes the number of trials with one animal.

Chapter 3

Results

3.1 Semi-intact walking preparation: extracellular recordings

Investigating the neural basis of ipsilateral coupling of walking, a correlation between front leg movements and activity of mesothoracic motoneurons was described in a semi-intact preparation. In this preparation the right front leg performed stepping movements on a treadmill. The other legs were cut. At ipsilateral nerve stumps of the deafferented mesothoracic ganglion, motoneuron activity was recorded extracellularly.
Usually a tactile stimulus, given by briefly touching the abdomen with a paintbrush, was necessary to elicit walking movements. Most animals responded with a sequence of eight to fifteen steps. Exceptional animals spontaneously started with walking movements, or performed sequences of 50 and more steps. More often it was necessary to give a continuous tactile stimulus during the walking sequence: after a first brushing, the paintbrush rested steadily on the abdomen. No difference could be found between data from such walking sequences and spontaneous sequences.
Some animals performed extensive search-movements when tactilely activated. This could often be reduced by slightly limiting the possible protraction of the leg. It was achieved by placing a pin close to the anterior side of the coxae. This mechanical barrier did not affect walking movements on the treadmill, but allowed the animal to more quickly locate the treadmill, once it had started with search-movements. No difference was found in data from such experiments.

Activity of mesothoracic motoneurons was analyzed with respect to the start of the stance phase of the front leg. A rising tachometer trace, which indicates acceleration of the treadmill, was regarded as stance phase for this analysis, as here the leg clearly has ground contact. A maximum of the tachometer trace was regarded as end of the stance phase. No statement can be made about the swing phase of the leg, as a falling tachometer trace only reflects the physical properties of the treadmill - the leg is lifted off.
During walking sequences of the right front leg, activity of several mesothoracic motoneurons was recorded extracellularly. Local sensory input was excluded in the

mesothoracic segment by cutting the leg nerves distal to the site of recording. Upon tactile activation the activity of all motoneurons increased. As EMG recordings of the front leg's flexor muscle showed, flexor activity started shortly before the treadmill accelerated (for example Fig. 3.1A; mean advance: 0.10 s; SD ± 0.05 s) and in some experiments continued after the treadmill reached its maximum velocity. That is, the front leg's flexor muscles started earlier than and sometime outlasted the defined stance phase.

3.1.1 Protractor coxae motoneurons

In nine of ten animals, activity of mesothoracic protractor motoneurons was clearly modulated during stepping activity (Fig. 3.1A). To analyze this modulation with respect to the front leg's activity, individual spikes were plotted as dots for a time window from 0.5 seconds before and after the start of a front leg's stance phase. The spikes of each step were plotted in one row and several rows were aligned with the start of stance phase as reference (time 0; Fig. 3.1B). This plot shows the distribution of mesothoracic protractor spikes with respect to the front leg's stance phase; the average end of stance phase is marked by a vertical line at 0.29 seconds (SD ± 0.06 s). 117 steps of an animal are plotted. A decrease of protractor activity was seen for all steps during the stance phase of the front leg, although in some steps (e.g. step 10 to 15) this is more pronounced than in other steps (e.g. step 45 to 50). To quantify the modulation, these data were summed in time bins of 0.033 seconds to obtain a histogram (Fig. 3.1C). It shows a distinct decrease of activity by 30 %, approximately 0.1 seconds before the start of stance phase of the front leg. This decrease persists until approximately 0.3 seconds after the start of stance phase, which coincides with the average end of the stance phase (0.29 s, SD ± 0.06 s, marked again by a vertical line). The experiment plotted in Fig. 3.1 is representative for the modulation of mesothoracic protractor motoneurons seen in eight of the ten animals tested. They all showed a decrease of mesothoracic protractor activity in the range of 17 % to 88 % (average: 55 %) at time of the stance phase of the front leg. One out of ten animals showed a different modulation, to the effect that mesothoracic protractor activity clearly increased around the start of the front leg's stance phase. Another animal out of the ten showed no distinct modulation of protractor activity at all.
In addition to the modulation described, the histogram shows the general increase of activity after tactile activation, when compared to the spontaneous activity of protractor motoneurons during periods in which the front leg rested. For the animal analyzed for Fig. 3.1, this spontaneous activity was 2.1 spikes per second, calculated from a total of 899 seconds recorded between step sequences. This spike rate plotted in the histogram (0.033 s bin width) would result in a bar with a height of 0.07.

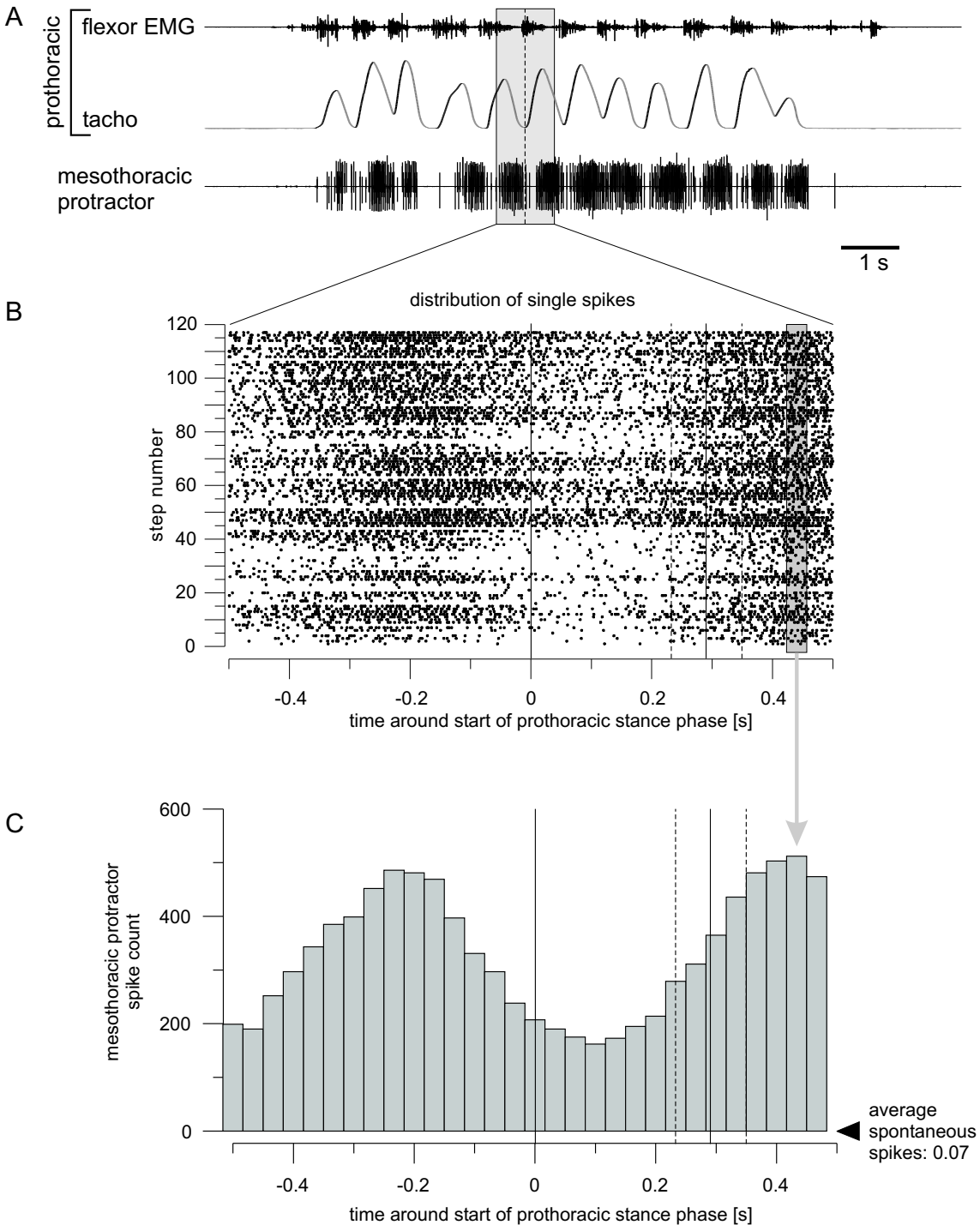

Figure 3.1: Activity of mesothoracic protractor motoneurons during walking movements of the ipsilateral front leg. A similar correlation of activity was observed in 8 of 10 animals tested. A: Original recording of a walking sequence. A rising tachometer trace indicates acceleration of the treadmill, which was regarded as stance phase of a step (starts marked by vertical lines). B: Mesothoracic protractor spikes plotted as dots for a time window around the start of the stance phase in the front leg. Each line represents one step; 117 steps of an animal are shown. Additional vertical lines mark the average end of stance phase and its standard deviation. C: Protractor spike activity shown in B summed up and plotted as a histogram.

3.1.2 Depressor trochanteris motoneurons

Activity of mesothoracic depressor trochanteris motoneurons showed in seven of nine animals tested a modulation that could be related to walking movements of the ipsilateral front leg. In general, the correlation appeared to be more variable between animals than the correlations observed for activity of protractor or extensor motoneurons with stepping activity of the front leg. Two different types of correlation were discriminated: response type I (Fig. 3.2), seen in three animals, and type II (Fig. 3.3), seen in four animals.
Fig 3.2B shows that for almost all steps analyzed, activity of slow and fast depressor motoneurons decreases during a time window of approximately 0.7 seconds around the start of the front leg's stance phase (response type I). Quantitative analysis of 84 steps of this animal reveals a decrease of activity of approximately 49 % (slow depressor motoneuron), and 84 % (fast depressor motoneuron) respectively. This decrease starts approximately 0.35 seconds before, and persists until 0.35 seconds after the start of front leg's stance phase (Fig. 3.2C). Two more animals showed a similar modulation. It started slightly later, 0.15 - 0.2 seconds before start of a stance phase, and persisted for more than 0.5 seconds after a start of stance phase, which had an average duration of 0.36 seconds (SD $\pm$ 0.07s). In the latter two animals activity of depressor motoneurons almost ceased during the front leg's stance phase.
Four out of nine animals showed a different correlation between mesothoracic depressor motoneuron activity and the walking movements of the front leg (response type II, Fig. 3.3B). In 52 steps of one animal, the activity of the slow depressor increased during the stance phase of the front leg. In this animal, the fast depressor motoneuron spiked only occasionally and was therefore not evaluated. Quantitative analysis (Fig. 3.3C) showed an increase by 142 % of slow depressor activity, starting 0.3 seconds before and persisting until 0.35 seconds after the start of the front leg's stance phase, which had an average duration of 0.29 seconds (SD $\pm$ 0.08 s). This is representative for a total of four out of nine animals, all showing an increase of depressor motoneuron activity in the range of 70 % to 200 % in the same time window with respect to the stance phase of the front leg.

The slow depressor motoneurons plotted in Fig. 3.2 and Fig. 3.3 showed a spontaneous spike rate outside step sequences of 0.03 spikes/s and 0.07 spikes/s respectively (averaged from 150 s and 99 s recorded). Fast motoneurons showed a spike rate of 0.05 spikes/s (averaged from 154 s recorded). All values equal 0.00 spikes/bin in the histograms.

In summary, depressor motoneurons show either a decrease or an increase of activity during the stance phase of the front leg. This modulation is more variable between animals than the modulation of activity seen for protractor or extensor motoneurons. In common with the other motoneurons is a general increase of activity during walking sequences, compared to periods with resting front leg.

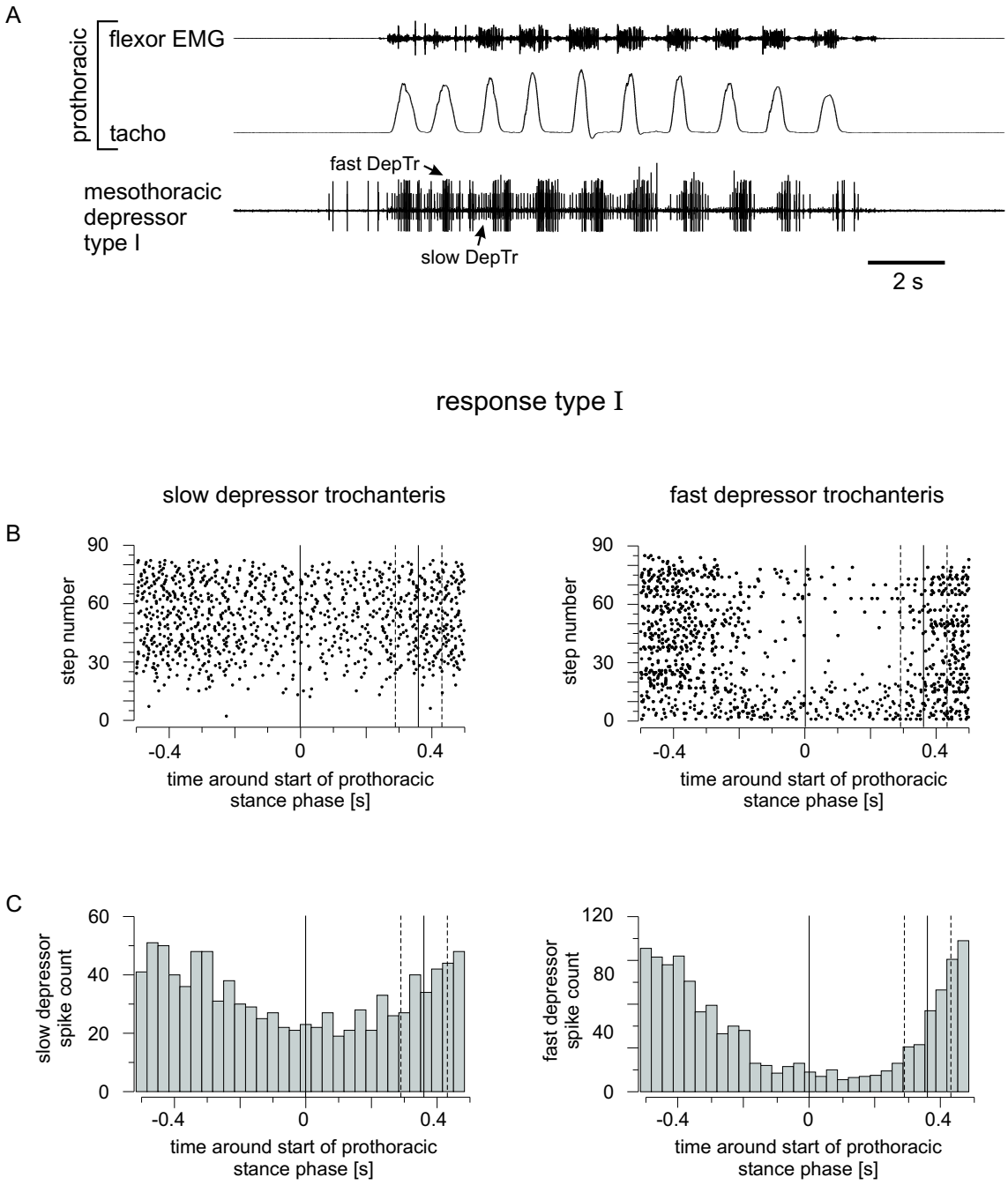

Figure 3.2: Activity of mesothoracic depressor trochanteris motoneurons during walking movements of the ipsilateral front leg. This figure shows one (response type I) of two different correlations found. It was seen in 3 of 9 animals. A: Original recording. B: Spike distributions of slow and fast unit around start of front leg's stance phase. 84 steps of one animal are shown. C: Summed spiking activity of depressor motoneurons plotted as a histogram.

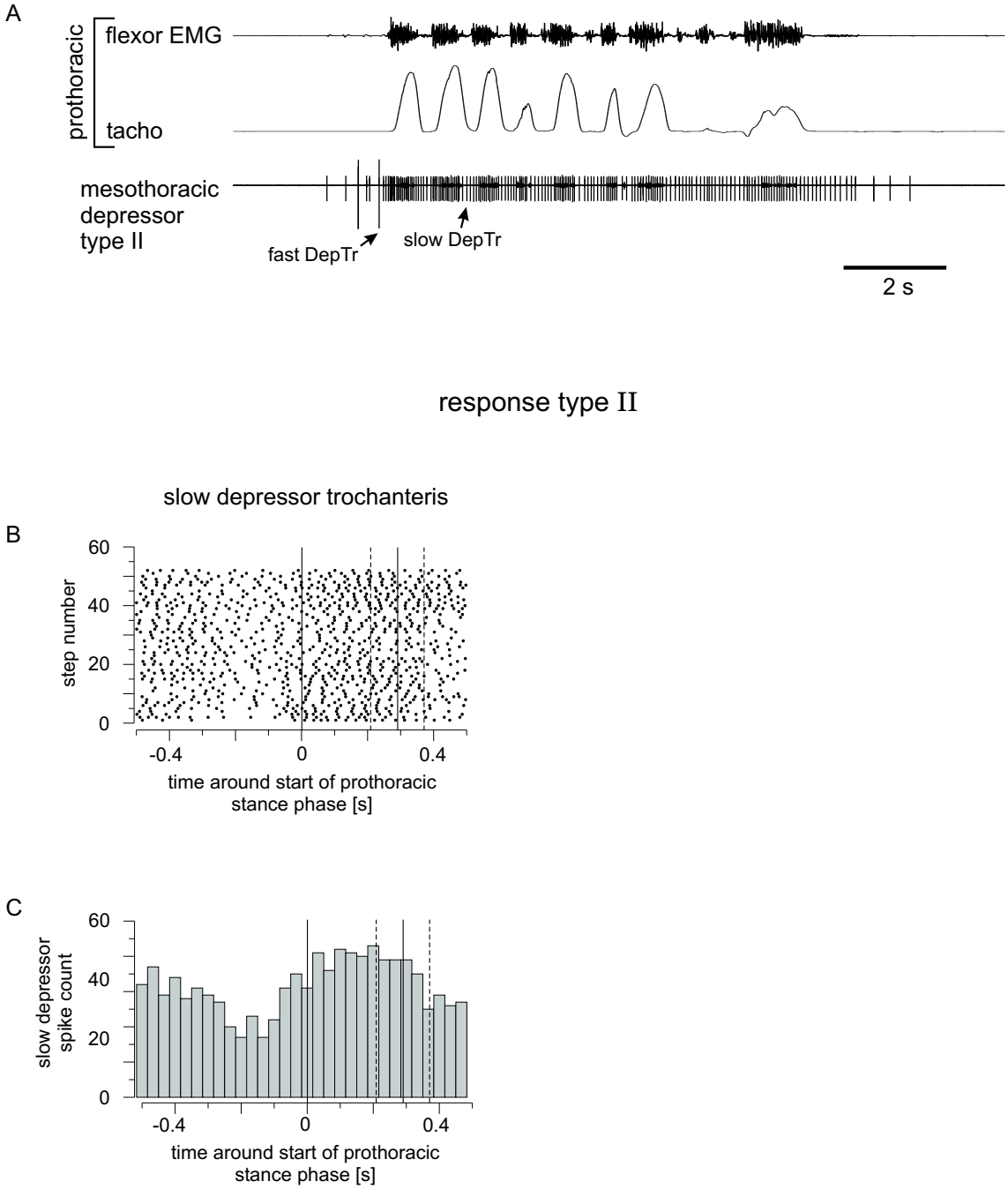

Figure 3.3: Activity of mesothoracic depressor trochanteris motoneurons during walking movements of the ipsilateral front leg. This figure shows one (response type II) of two different correlations found. It was seen in 4 of 9 animals. A: Original recording. B: Spike distributions of the slow unit around start of front leg's stance phase. 52 steps of an animal are shown. C: Summed spiking activity of depressor motoneurons plotted as a histogram.

3.1.3 Extensor tibiae motoneurons

Activity of the mesothoracic extensor tibiae motoneurons during stepping activity of the front leg showed a correlated modulation in seven of eight animals tested (Fig. 3.4A). It was analyzed separately for the fast extensor motoneuron (FETi) and the slow extensor motoneuron (SETi). In general the SETi had a higher firing rate than FETi, protractor, and depressor motoneurons, which is shown by a higher density of dots in Fig. 3.4B. In most of the 62 steps analyzed in this animal, there was a further increase of activity during the stance phase of the front leg. The latter is also true for the FETi, which clearly shows a higher activity during the font leg's stance phase for almost all steps.
Quantitative analysis shows an increase of mesothoracic SETi activity by approximately 55 %, starting 0.1 seconds before the start of the front leg's stance phase. The increase outlasts the stance phase (average duration: 0.27 s $\pm$ 0.09 s) by more than 0.2 seconds (Fig. 3.4C). The modulation of SETi activity shown is representative for three of five animals analyzed, showing an increase of activity of 30 % to 55 %. One animal out of five showed an increase of about 48 %, already starting 0.3 seconds before and lasting approximately until 0.15 seconds after the front leg's stance phase. Another animal did not show any correlated modulation of the mesothoracic SETi activity; in this animal, the FETi only occasionally showed action potentials. Analysis of FETi activity in Fig. 3.4C shows a strong increase by 212 %, occurring at the same time as the increase of SETi activity. This is representative for all four animals tested. The increase ranged from 135 % to FETi spikes only occurring during the front leg's stance phase. In all four animals the increased FETi activity outlasted the front leg's stance phase.

In summary, mesothoracic extensor activity is mostly increased during and at least 0.2 seconds after the front leg's stance phase. FETi activity shows a stronger modulation than SETi activity. The spontaneous activity during periods with a resting front leg was 18.9 spikes per second for the SETi (averaged from 123 s recorded) and 0.48 spikes per second for the FETi (averaged from 140 s recorded). In the histogram shown in Fig. 3.4C, this would equal (0.033s bin width) values of 0.63 and 0.02 respectively.

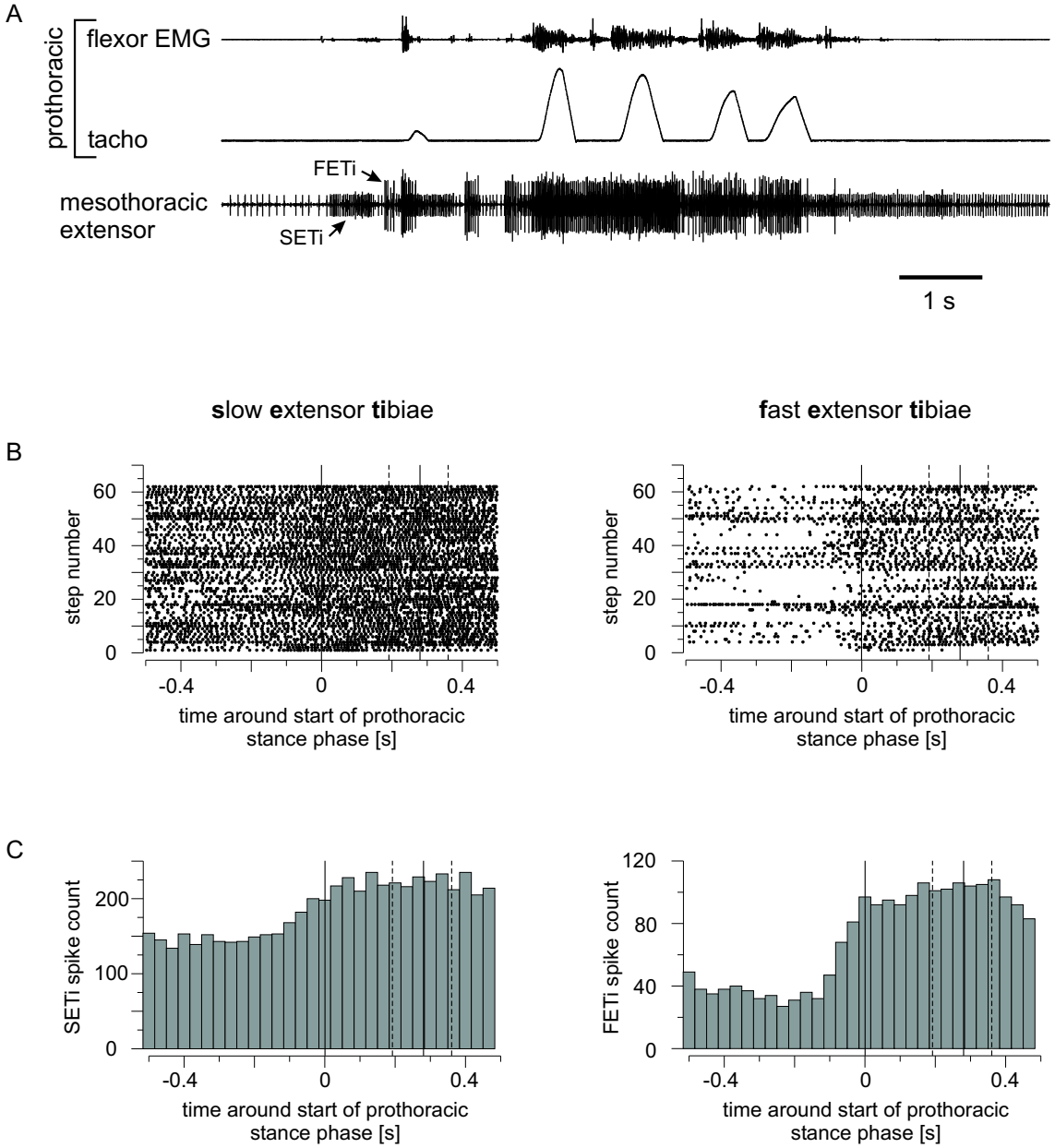

Figure 3.4: Activity of mesothoracic extensor tibiae motoneurons (FETi and SETi) during walking movements of the ipsilateral front leg. A similar coordination of SETi activity was found in 3 of 5 animals, respectively 4 of 4 animals for FETi activity. A: Original recording. B: Spike distributions around start of the front leg's stance phase for 62 steps of an animal. C: Summed spiking activity of extensor motoneurons plotted as a histogram.

3.1.4 Antagonistic motoneuron pools

To study the activity of motoneuron pools antagonistic to the ones described, retractor coxae and levator trochanteris motoneurons were recorded. Retractor motoneurons showed an increase of activity starting at approximately 0.07 seconds before and persisting until 0.45 seconds after the start of the stance front leg's stance phase (N=4). In this time window protractor activity was decreased. The simultaneous recording of retractor and protractor motoneuron pools during front leg walking activity showed clearly alternating activity (Fig. 3.5A).
Levator motoneuron activity increased in three of four animals at the starting time of the front leg's stance phase. In one animal, activity already increased 0.3 seconds before this point. The increase persisted longer than 0.5 seconds after the start of stance phase. The depressor motoneurons of response type I showed decreased activity in this time window, but increased activity 0.35 seconds after the end of the stance phase. The simultaneous recording of both, levator and depressor motoneuron pools during front leg walking movements again showed alternating activity (Fig. 3.5B).

In summary, in all mesothoracic motoneurons extracellularly recorded, the activity was increased upon tactile activation of the animal. When the front leg started walking movements on the treadmill, all investigated ipsilateral motoneurons pools showed a modulation of their activity. Protractor coxae activity was decreased during a stance phase of the ipsilateral front leg. Depressor trochanteris activity in some animals was decreased (response type I) and in other increased (response type II). Extensor tibia activity increased during the front leg's stance phase. Activity of retractor motoneurons alternated with that of protractor coxae motoneurons. Activity of levator motoneurons alternated with that of depressor trochanteris motoneurons of response type I.

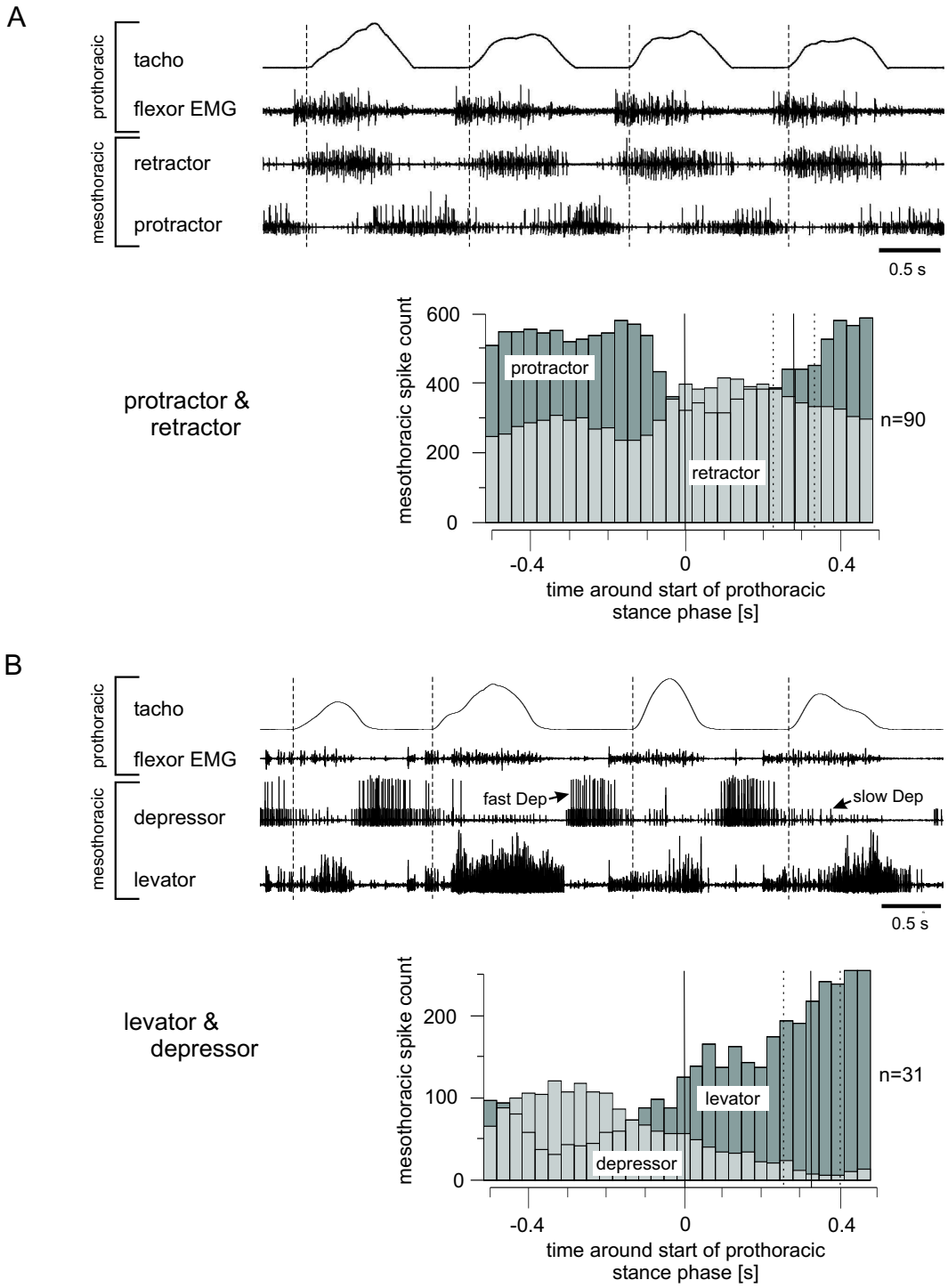

Figure 3.5: A: Simultaneous recordings from mesothoracic protractor and retractor coxae motoneurons during walking movements of the ipsilateral front leg. The histogram shows the summed activity during 90 steps in a time window around the start of the front leg's stance phase. Protractor and retractor motoneurons show clear alternating activity. B: Activity of mesothoracic depressor (response type I) and levator trochanteris during 31 steps of an animal. Levator and depressor motoneurons show alternating activity, too.

3.2 Semi-intact walking preparation: intracellular recordings

For a closer investigation of how mesothoracic motoneurons are modulated during stepping activity of the ipsilateral front leg, intracellular recordings of single motoneurons were performed. As in the previous experiments, the front leg was walking on a treadmill and motoneuron activity was analyzed with respect to the start of the stance phase of the ipsilateral front leg. Again, an acceleration of the treadmill, indicated by a rising tachometer trace, was regarded as the stance phase. Two different activity related changes of the membrane potential were found in mesothoracic motoneurons. In most motoneurons the membrane potential shifted with the beginning of the walking activity of the front leg. This shift lasted until the walking movements stopped or even beyond. I will refer to this longer lasting change of membrane potential as *tonic* change. The second change was a *rhythmic modulation* of the membrane potential. This modulation was mostly coupled to individual steps of the front leg. For the description of the modulation, the terms 'hyperpolarization' and 'depolarization' are used. These terms only relate to the change of membrane potential towards more negative or positive values, and are not meant to imply a mechanism, such as inhibition or excitation.

3.2.1 Protractor coxae motoneurons

A mesothoracic protractor coxae motoneuron was recorded intracellularly in three animals. All recordings showed a tonic depolarization with an amplitude of 2.3 to 4.3 mV, measured as average potential (see section 2.10) during a walking sequence. This tonic depolarization started 0.3 to 0.5 seconds prior to flexor activity of the front leg, recorded with an EMG. It repolarized slowly after the walking sequence, with a time constant (see section 2.10) in the range of 0.1 to 0.6 seconds. Additionally, the membrane potential of protractor motoneurons showed a rhythmic modulation, which correlated with the start of a front leg's stance phase (Fig. 3.6). In some experiments the rhythmic modulation continued at the end of a walking sequence, despite the leg not performing visible walking movements.

For closer analysis, traces of the membrane potential during individual steps were aligned, with the start of the front leg's stance phase as reference, and plotted one upon another. All steps of a walking sequence, with the exception of the first, were used. During all 11 steps, the rhythmic modulation of the mesothoracic protractor motoneuron's membrane potential had a similar time course (Fig.3.7A). Next, action potentials were removed from the recording (see section 2.10) and the 11 traces were averaged. The average modulation had the form of a hyperpolarization, started 0.15 seconds prior and persisted until 0.4 seconds after the start of the front leg's stance phase. The front leg's stance phase had an average duration of 0.30 seconds (SD: 0.07 s). The average peak-to-peak amplitude was 1.7 mV (Fig. 3.7B). Additionally, the same data were plotted after normalizing each trace to the duration of the front leg's stance phase. This phase dependent analysis shows the same effect as the one seen in the analysis of non-normalized data (Fig. 3.7C). Recordings of all three

animals tested, showed a rhythmic membrane potential modulation of mesothoracic protractor motoneurons. Their time courses with respect to the front leg's stance phase were similar. The peak-to-peak amplitude was in the range of 0.5 mV to 2 mV.

In two animals, experiments were performed to measure the cell's input resistance by injection of short hyperpolarizing current pulses of constant amplitude via the amplifier. The expectation is that the tonic depolarization, as well as the rhythmic modulation of the membrane potential, results from changes of the membrane's ion conductance. These changes should be reflected in the resistance measurement by changes in amplitude of the potential steps, caused by the current pulses. In the experiments performed, no such changes in amplitude of the potential steps could be detected (Fig. 3.8).

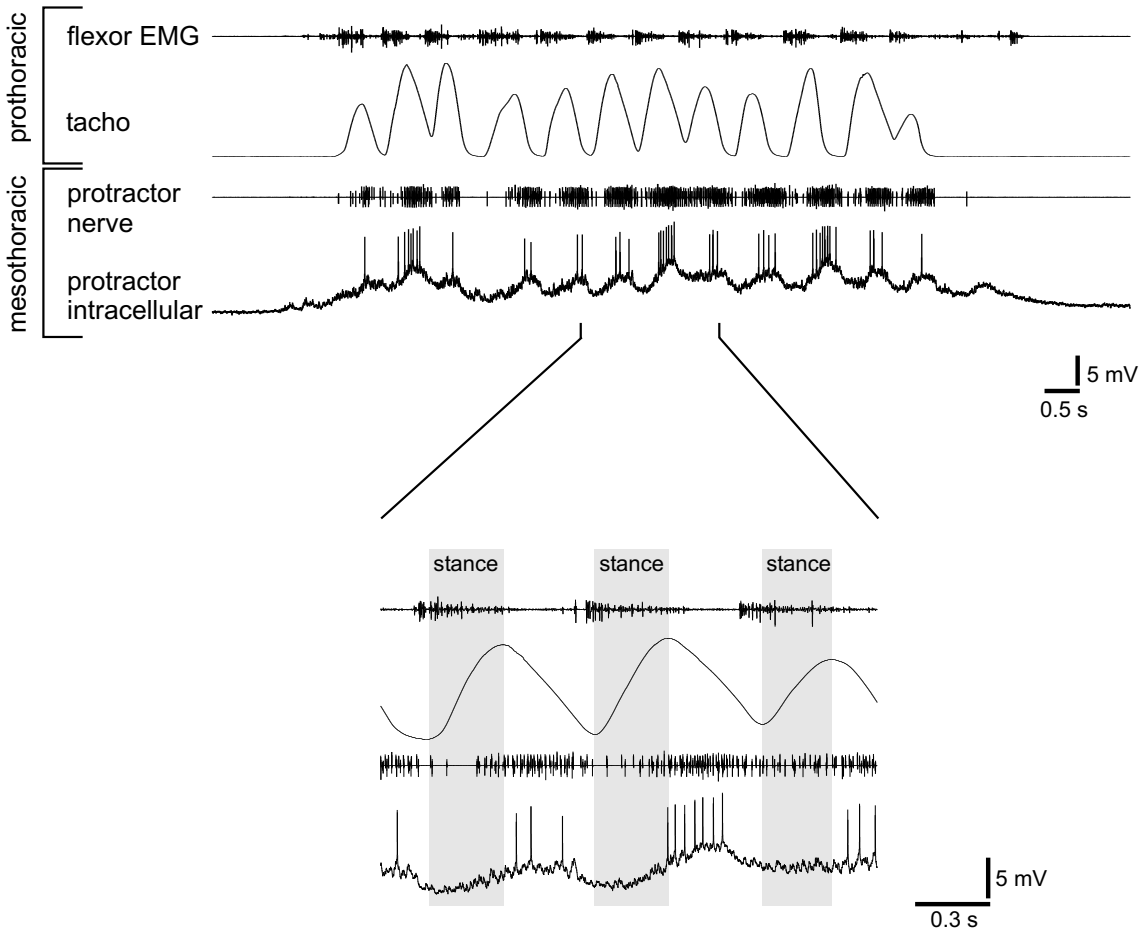

Figure 3.6: Recording of mesothoracic protractor coxae motoneuron activity during walking movements of the ipsilateral front leg on a treadmill. A rising tachometer trace indicated an acceleration of the treadmill, which was regarded as the stance phase of a step. The intracellular recording and one unit in the extracellular recording showed a one to one correlation of spikes. This identifies the recorded unit as a protractor motoneuron. During the walking sequence, it was tonically depolarized and rhythmically modulated with the walking activity of the front leg. The resting potential (RP) was -58 mV.

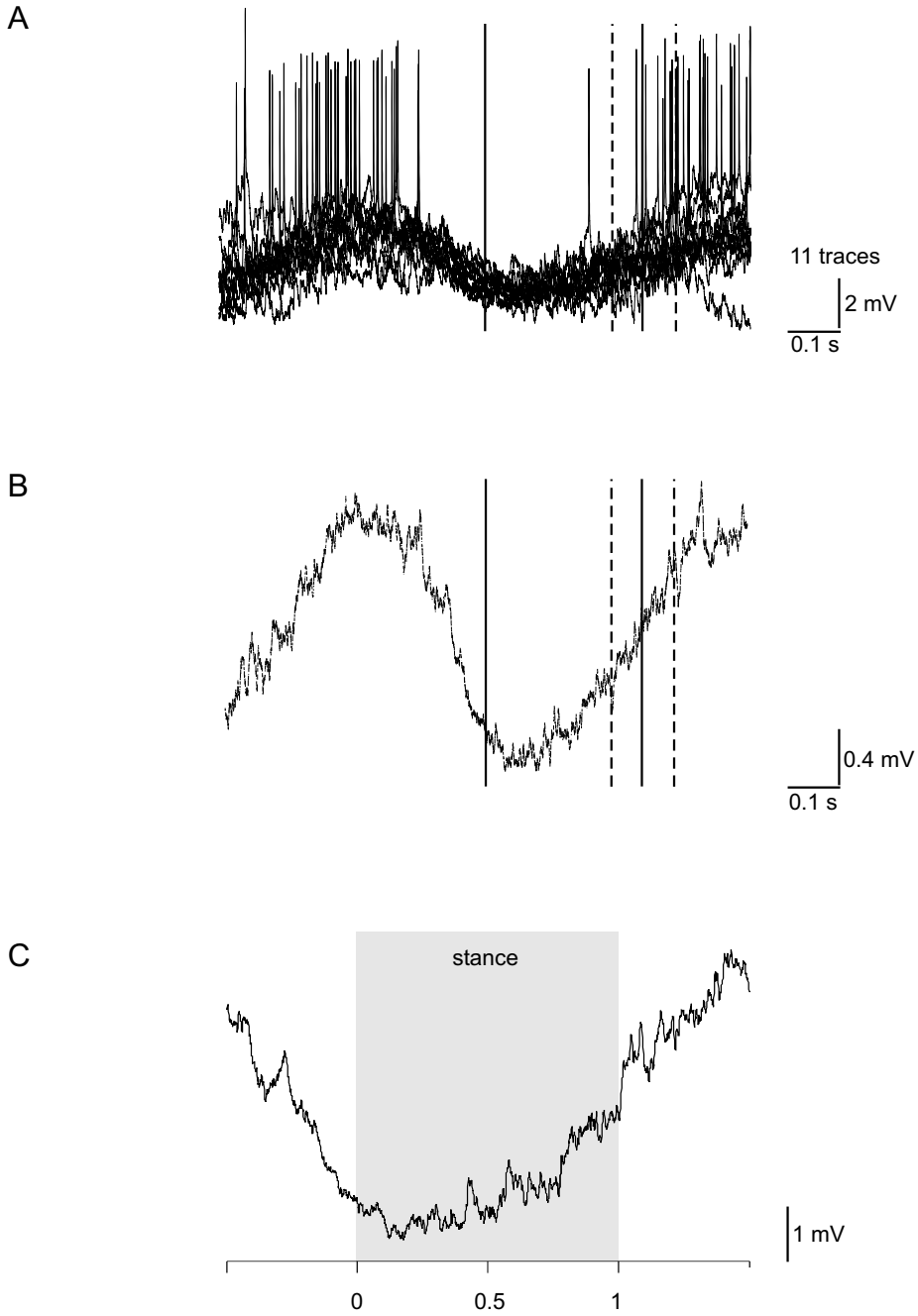

Figure 3.7: A: Intracellular recording of a mesothoracic protractor coxae motoneuron. Traces of 11 steps of a walking sequence were aligned with the start of the front leg's stance phase as reference (left vertical line). The end of the stance phase and its standard deviation are given by the vertical lines on the right. During all steps, the protractor motoneuron hyperpolarized at the start of the front leg's stance phase. B: Average of the traces shown in A, after removal of action potentials. The front leg's stance phase is again marked by vertical lines. C: Same data after normalizing each single trace with the durations of the associated stance phase. This phase dependent analysis shows the same effects as the time dependent analysis in B.

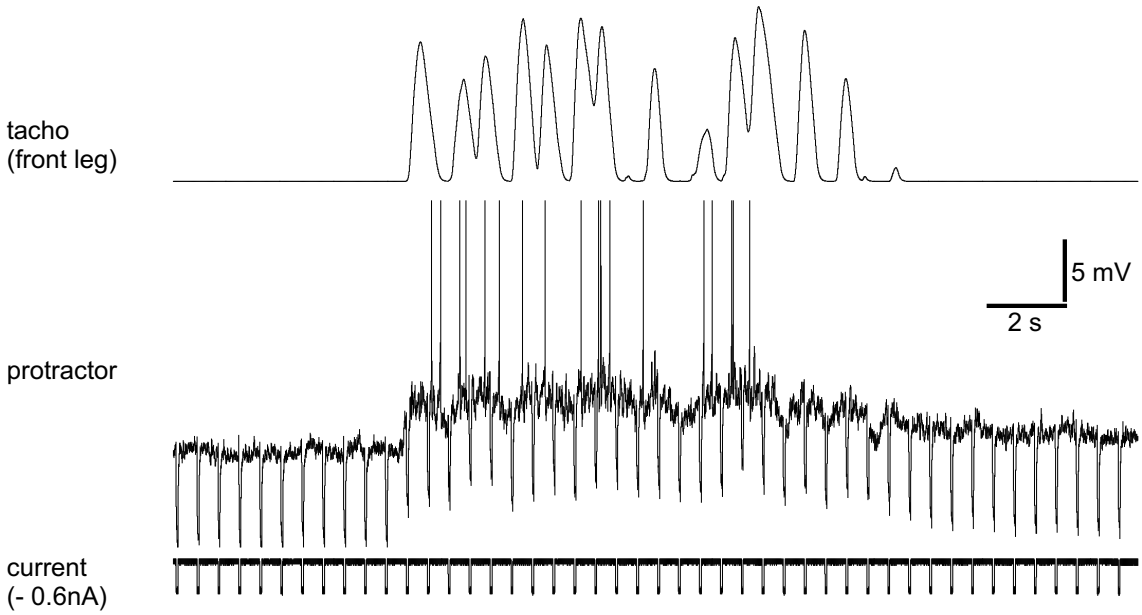

Figure 3.8: Membrane resistance was measured by injection of short hyperpolarizing current pulses of 0.6 nA (action potentials truncated). Changes of the amplitude of the evoked potential steps reflect the membrane resistance according to Ohm's law. During the stepping sequence of the front leg, no changes of the resistance could be detected.

3.2.2 Depressor trochanteris motoneurons

During walking movements of the front leg, the activity of mesothoracic fast depressor trochanteris motoneurons was recorded in four animals, that of slow depressor trochanteris motoneurons in three animals. No systematic difference was found between slow and fast motoneurons. The responses were in general more variable than in the other motoneurons examined. A correlation between the stepping activity of the front leg and of the activity of the depressor motoneurons was less obvious.
During a stepping sequence the membrane potential showed either a tonic depolarization (Fig. 3.9A; seen in 2 fast and 1 slow motoneuron), a tonic hyperpolarization (Fig. 3.10A1; seen in 1 fast and 1 slow motoneuron), or no clear tonic change (seen in 1 fast and 1 slow motoneuron). If constant hyperpolarizing current was injected via the electrode, and the potential of the motoneuron thereby was changed, the amplitude of a tonic depolarization increased for more negative potentials (Fig. 3.9A; -2 nA). The amplitude of a tonic hyperpolarization was decreased, and at more negative potentials reversed (Fig. 3.10A2). This indicates that excitatory and inhibitory inputs, respectively, onto the motoneuron are the source of the tonic changes of membrane potential during a walking sequence. Resistance measurements by injection of short hyperpolarizing current pulses with constant amplitudes, revealed in two experiments a clear decrease of membrane resistance during the walking sequence (slow depressor: Fig. 3.11; fast depressor not shown), due to the opening of ion channels.
In addition to the tonic change of membrane potential, the depressor motoneuron's potential showed a rhythmic modulation. Without experimental alteration of the neuron's membrane potential, these rhythmic changes could in most experiments not be clearly correlated with stepping movements of the front leg. At more negative membrane potentials, achieved by the injection of a constant current via the electrode, the modulations gained amplitude (Fig. 3.9A, Fig. 3.10A2). This indicates that excitatory inputs are their source. To analyze a correlation between the modulation of membrane potential and the stepping movements of the front leg, recordings with experimentally hyperpolarized membrane potential were used (Fig. 3.9B, Fig. 3.10B). Plotting the course of the membrane potential during several steps, aligned with the start of the front leg's stance phase as reference, revealed a rhythmic hyperpolarization shortly before and during the front leg's stance phase (Fig. 3.9C, Fig. 3.10C). On average, this hyperpolarization (amplitudes of 1 to 2.5 mV) started 0.24 to 0.45 seconds prior to the stance phase. The slow repolarization occurred during the front leg's stance phase (Fig. 3.9D, Fig. 3.10D).

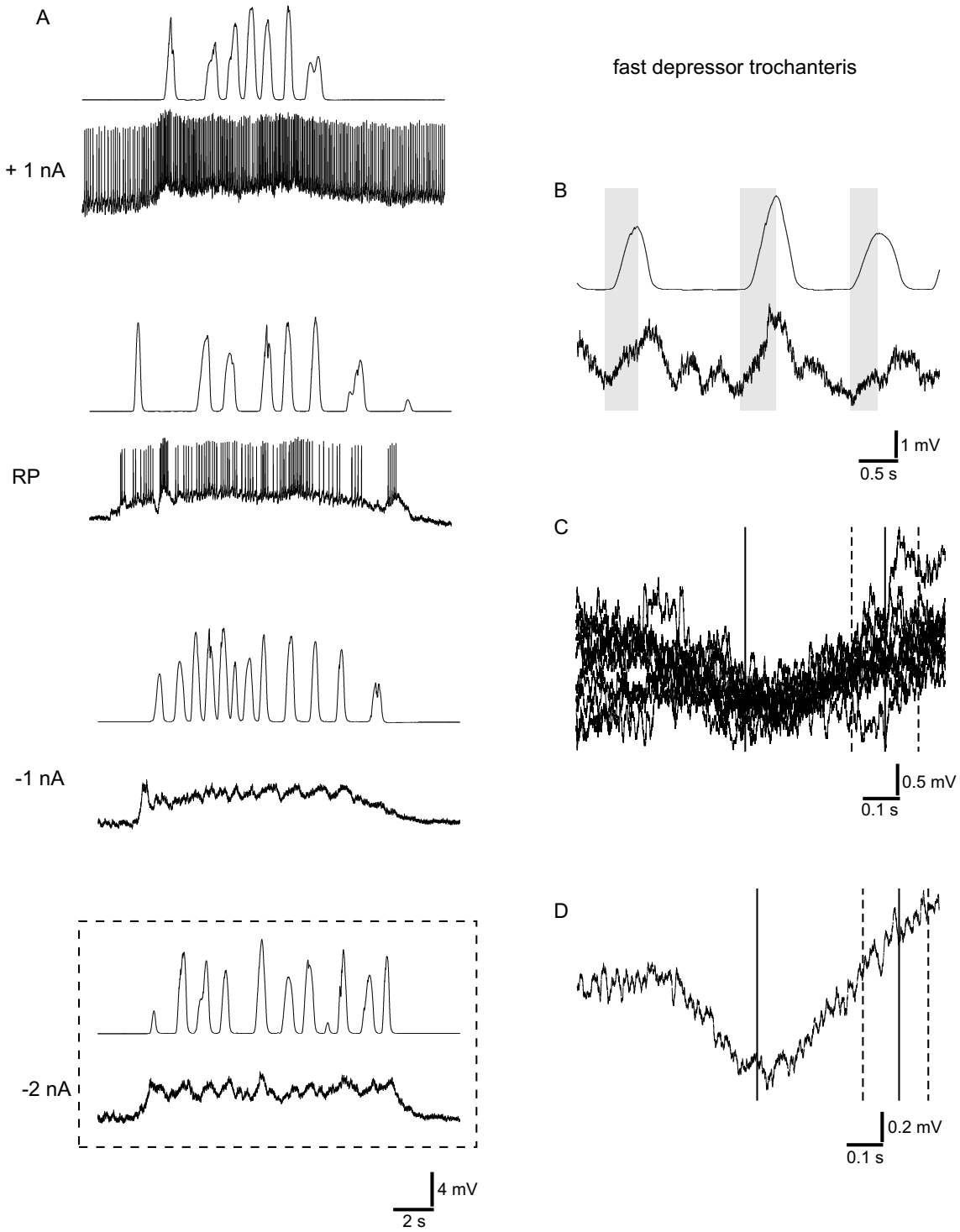

Figure 3.9: Example of an intracellular recording of a fast mesothoracic depressor trochanteris motoneuron during walking movements of the ipsilateral front leg. A: At resting potential (RP: -47 mV), the membrane potential of the motoneuron showed a tonic depolarization during the whole walking sequence. At more negative membrane potentials (-1 nA and -2 nA current injected) a distinct rhythmic modulation of the potential was visible. B: Enlarged view of a recording with constant injection of -2nA current. The depressor membrane potential was modulated during individual steps. C: Intracellular traces during 10 steps of this recording overlaid with the start of stance phase as reference. All traces showed a hyperpolarization at start of the front leg's stance phase. D: An average of all 7 traces.

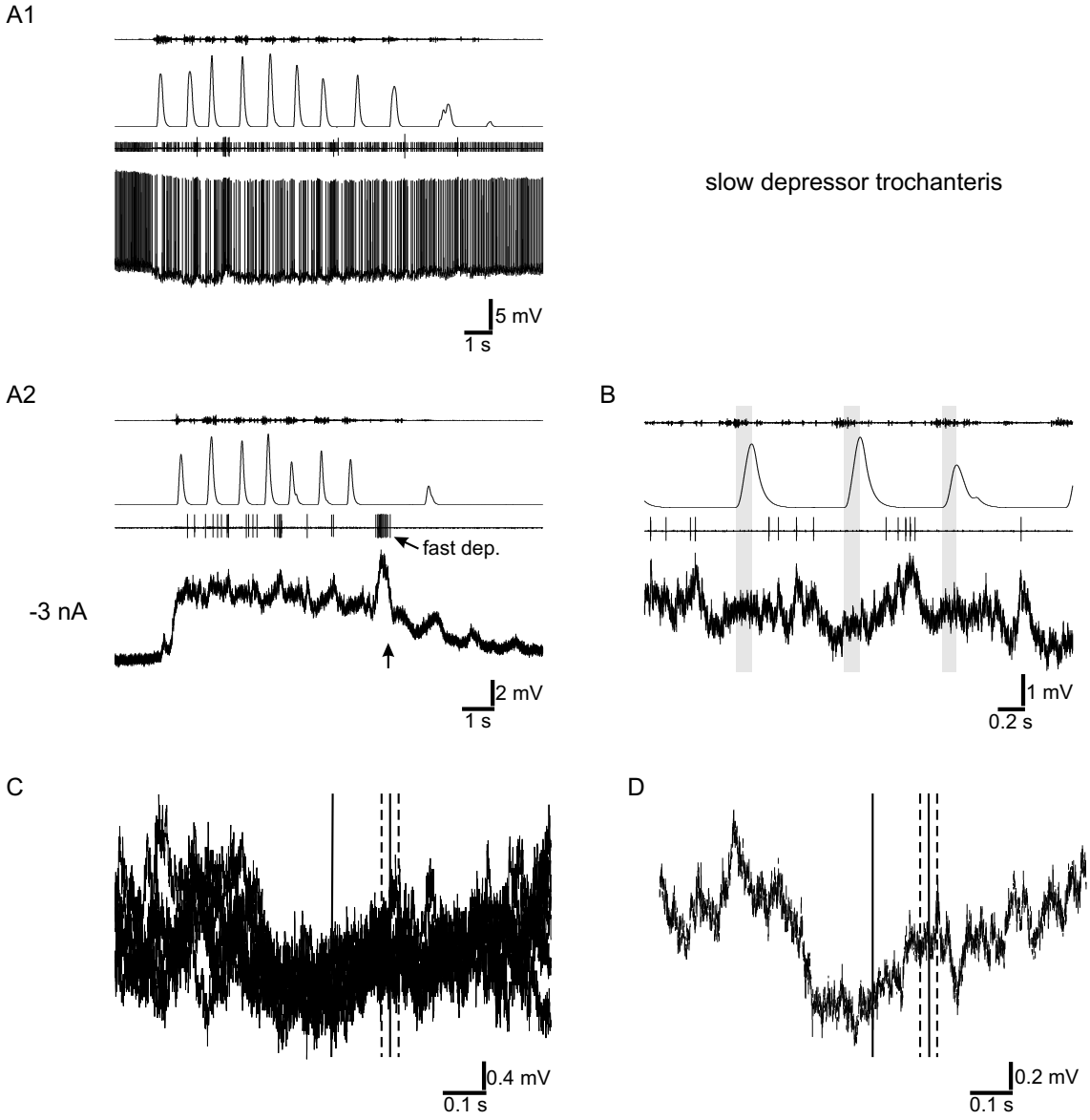

Figure 3.10: Example of an intracellular recording of a slow mesothoracic depressor trochanteris motoneuron during walking movements of the ipsilateral front leg. A1: At resting potential (RP: -45 mV), the membrane potential of the motoneuron showed a tonic hyperpolarization during the whole walking sequence. A2: When the motoneuron was hyperpolarized by constant injection of -3 nA current via the electrode, the membrane potential of the motoneuron showed a tonic hyperpolarization and a clear rhythmic modulation became visible. The arrow marks a spontaneous burst of depressor motoneurons at the end of the walking sequence; the motoneuron recorded intracellularly is strongly depolarized (fast dep.: fast depressor). B: Enlarged view of recording with constant injection of -3nA current. The depressor membrane potential was modulated during individual steps. C: Intracellular traces during 7 steps of this recording overlaid with the start of stance phase as reference. All traces showed a hyperpolarization prior to the start of the front leg's stance phase. D: An average of all 7 traces.

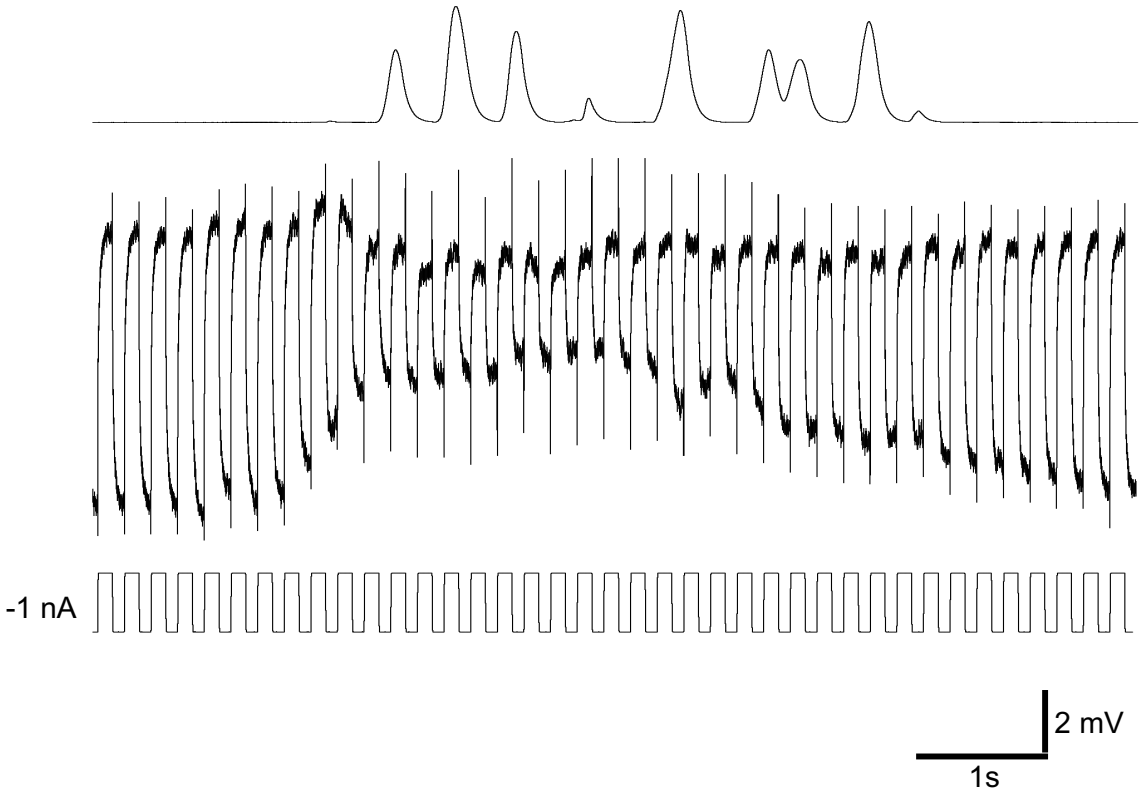

Figure 3.11: Membrane resistance of a mesothoracic slow depressor motoneuron was measured by injection of short hyperpolarizing current pulses of 1 nA. The spikes visible are due to capacitive artifacts. Changes of the amplitude of the evoked potential steps reflect the membrane resistance according to Ohm's law. During the stepping sequence of the front leg the membrane's resistance clearly decreases, reflecting the opening of ion channels.

3.2.3 Levator trochanteris motoneurons

In two animals, a levator trochanteris motoneuron, which is an antagonist to depressor trochanteris motoneurons, was recorded. Both recordings showed a tonic depolarization as well as a rhythmic modulation of membrane potential. The modulation had in both experiments the form of a hyperpolarization prior to the start of the front leg's stance phase. A steep repolarization occurred during, or at the end of the stance phase (Fig. 3.12). Compared to depressor motoneurons, levator motoneurons reached most depolarized membrane potentials earlier after the start of the front leg's stance phase.

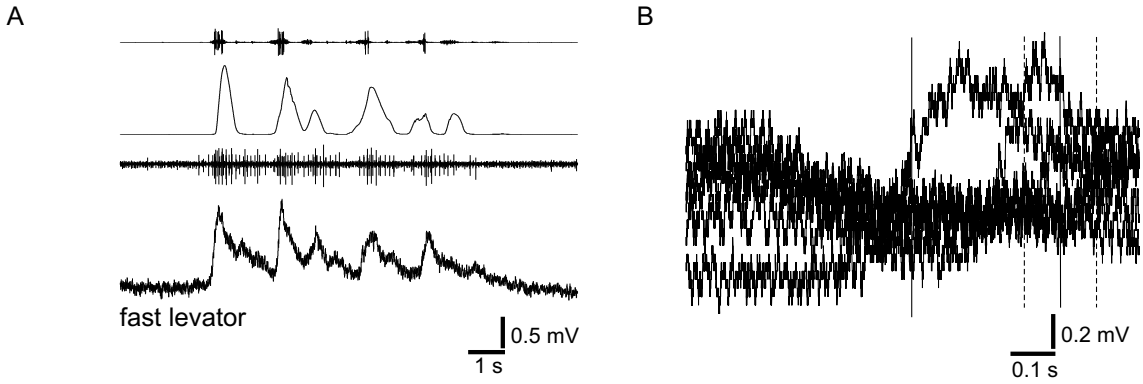

Figure 3.12: Example of an intracellular recording of a fast mesothoracic levator trochanteris motoneuron during walking movements of the ipsilateral front leg. A: At resting potential (RP: -51 mV), the membrane potential of the motoneuron showed a tonic depolarization during the whole walking sequence. The depressor membrane potential was modulated during individual steps. B: Intracellular traces during 5 steps of this recording overlaid with the start of stance phase as reference. All traces showed a hyperpolarization prior to the start of the front leg's stance phase and a steep repolarization during or at the end of the stance phase.

3.2.4 Extensor tibiae motoneurons

During walking activity of the ipsilateral front leg, the membrane potential of four slow (SETi) and three fast (FETi) extensor tibia motoneurons was recorded intracellularly.
Slow extensor tibiae motoneurons showed a tonic depolarization with an amplitude of 0.3 to 5 mV (average potential) during the walking sequence. It started 0.02 to 0.1 seconds before flexor activity in the front leg (measured from EMG activity) and its amplitude declined with a time constant of 0.1 to 0.3 seconds after the end of the sequence (Fig. 3.13A).
Additionally, SETi motoneurons showed a rhythmic modulation of their membrane potential. Hyperpolarizations occurred prior to the start of the front leg's stance phase, although it was not always possible to determine the precise phase relation in all recordings, due to the small amplitude of 0.5 to 5 mV (Fig. 3.13A). Aligning traces of individual steps of a walking sequence, with the start of the front leg's stance phase as reference revealed similar time courses of the rhythmic modulations (Fig. 3.13B). Averaging the 12 steps after removal of action potentials from the recording showed that the hyperpolarization (average amplitude 1.3 mV) started on average 0.38 seconds before and persisted until 0.08 seconds after the start of the front leg's stance phase (Fig. 3.13C).

Fast extensor tibia motoneurons also showed a tonic depolarization with an amplitude of 4 to 12 mV (average potential) during the walking sequence. It started 0.2 to 0.5 seconds before flexor activity in the front leg (measured from EMG activity) and its amplitude declined with a time constant of 0.1 to 0.7 seconds after the end of the sequence (Fig. 3.14A). The membrane potential of FETi motoneurons showed a rhythmic modulation, coupled to the start of the front leg's stance phase. Interestingly, it showed additional modulations that could not be associated with steps of the front leg (Fig. 3.14A). When the courses of membrane potentials during eight front leg steps are plotted on top of each other (aligned with start of stance phase), they are not as uniform as in a plot from data recorded from a SETi motoneuron. However, during all steps the traces superimposed well around the start of the stance phase (Fig. 3.14B). An average of all traces, after removal of action potentials from the recording, showed that the membrane potential was hyperpolarized 0.22 seconds prior to the start of the stance phase (average amplitude 2.5 mV), stayed constant during the stance phase, and was depolarized at the end of the stance phase (Fig. 3.14C). In one animal the recording showed distinct inhibitory post synaptic potentials (IPSPs) starting about 0.25 seconds before the start of the stance phase, and becoming less frequent during the stance phase (Fig. 3.15). To investigate the nature of tonic depolarization and rhythmic modulation of the FETi membrane potential during walking activity of the front leg, recordings at altered membrane potentials were made. The motoneuron was experimentally depolarized and hyperpolarized by injection of constant current via the electrode. At more negative membrane potentials, the amplitude of the tonic depolarization increased (Fig. 3.16; +1.8 nA: 5 mV; RP: 7 mV; -1.5 nA: 7 mV). This suggests that an excitatory input to the FETi is responsible for the tonic depolarization.

The amplitude of rhythmic hyperpolarizations remained constant or even slightly decreased (+1.8 nA: 6±1 mV; RP: 6±1 mV; -1.5 nA: 5 ±2 mV). Experiments to measure the membrane resistance by injection of short pulses of constant current via the electrode did not reveal a change in resistance (Fig. 3.17).

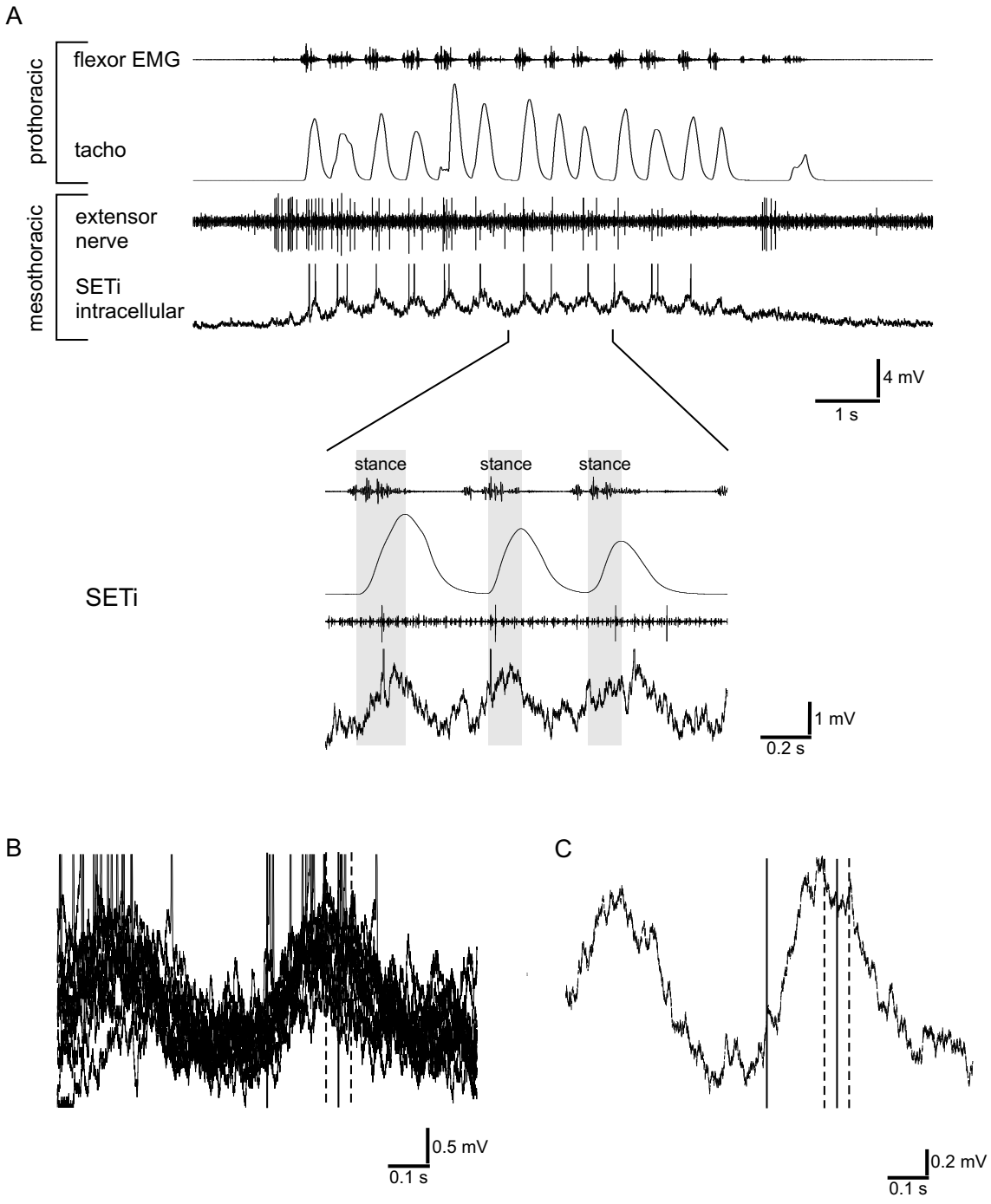

Figure 3.13: A: Intracellular recording of mesothoracic slow extensor tibiae motoneuron (SETi) during walking movements of the ipsilateral front leg (RP: -51 mV; action potentials truncated). The membrane potential showed a tonic depolarization during the whole walking sequence and a rhythmic modulation coupled to the start of a stance phase in the front leg. B: Traces of 12 steps of a walking sequence (first and last step not analyzed) aligned with the stance phase (marked by the vertical lines) as reference. The rhythmic modulations had a similar time course during all steps of the sequence. C: The same 12 traces averaged, after removal of the action potentials from the recording.

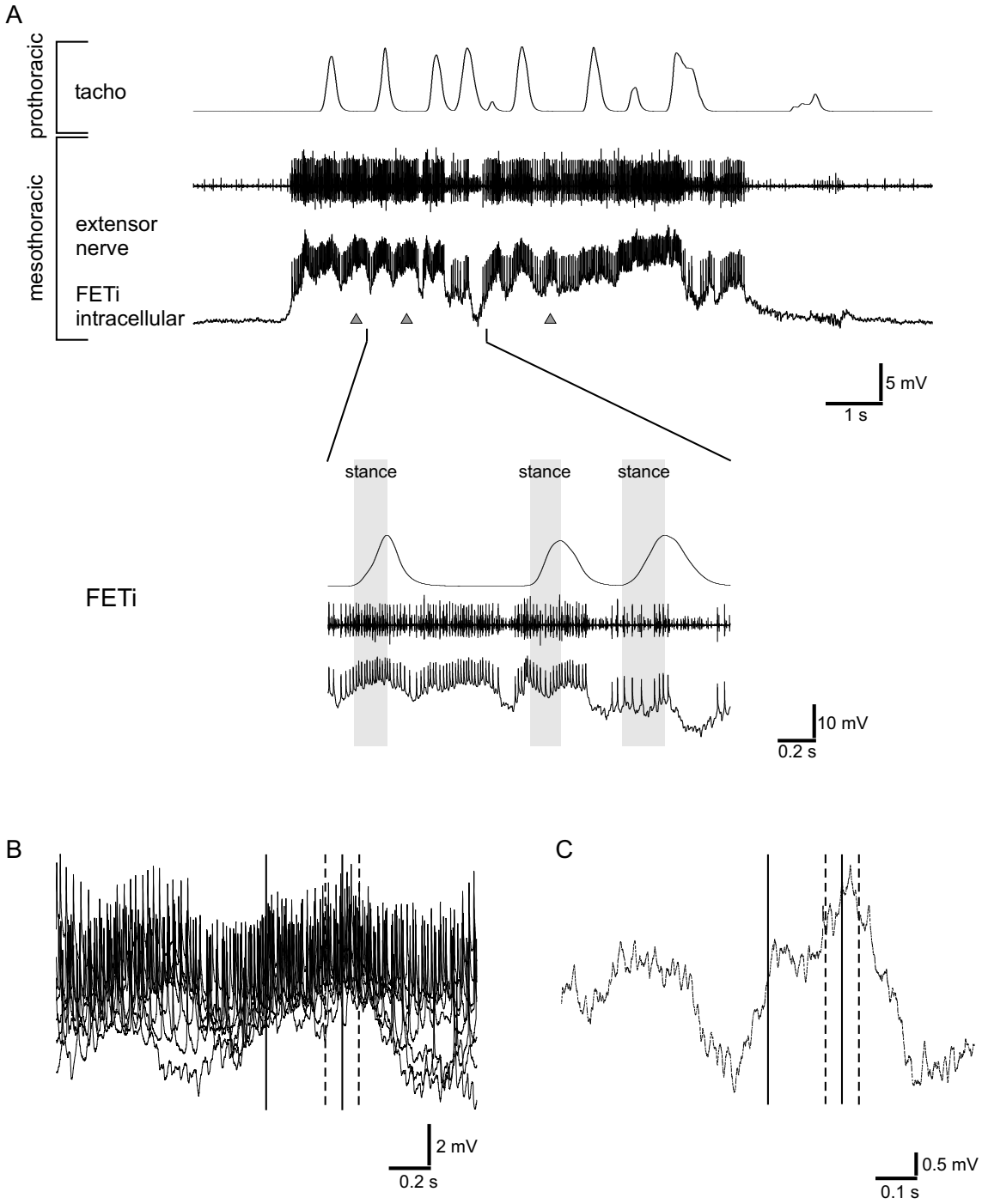

Figure 3.14: Intracellular recording of mesothoracic fast extensor tibiae motoneuron (FETi) during walking movements of the ipsilateral front leg (RP: -60 mV). The membrane potential showed a tonic depolarization during the whole walking sequence and rhythmic hyperpolarizations before the start of a stance phase in the front leg. Note additional modulation, not associated with a step (marked by gray arrows). B: Traces of 8 steps of a walking sequence (first and last step not analyzed) aligned with the stance phase as reference. The rhythmic modulations had a similar time course during all steps of the sequence. C: The same 8 traces averaged, after removal of the action potentials from the recording.

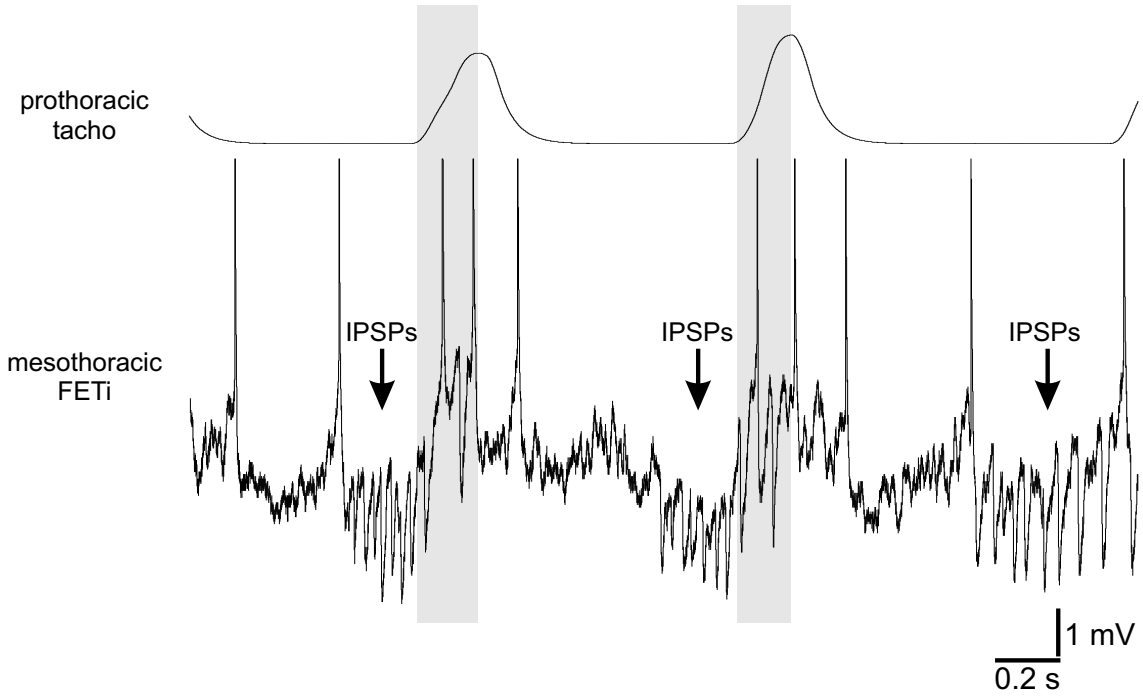

Figure 3.15: The recording of FETi membrane potential in one animal revealed clear inhibitory post synaptic potentials (IPSPs) before and less frequently during the stance phase of the ipsilateral front leg. The recording suggests that an inhibitory input from a spiking neuron contributes to the rhythmic modulation of FETi membrane potential.

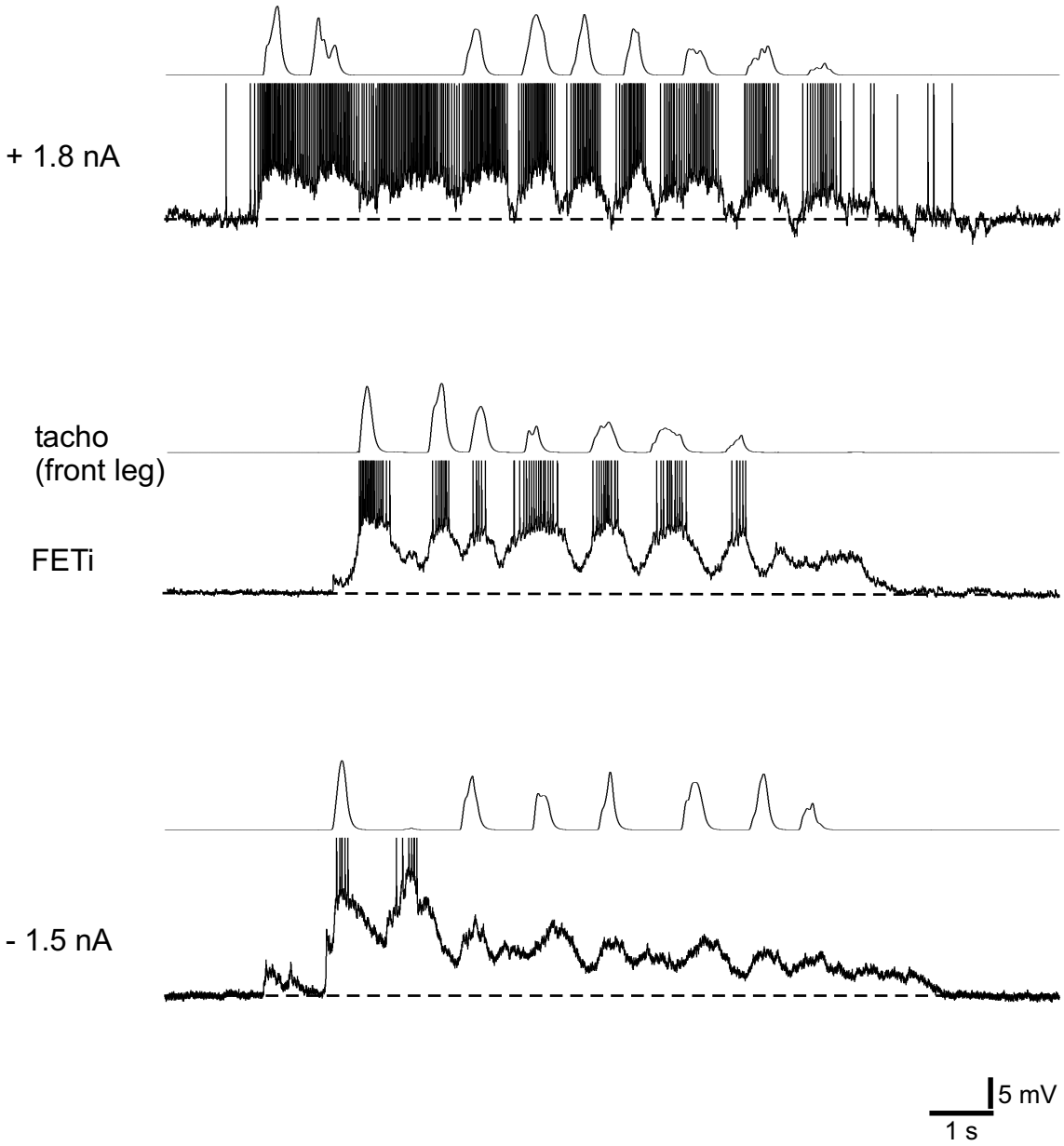

Figure 3.16: Intracellular recording of a mesothoracic fast extensor tibiae motoneuron (FETi) during front leg walking activity (action potentials truncated). The FETi was experimentally depolarized and hyperpolarized to different membrane potentials by injection of current via the electrode. At more negative membrane potentials, the tonic depolarization seen during the whole walking sequence became larger in amplitude. This suggests that an excitatory input to the FETi is responsible for the tonic depolarization. The amplitude of the rhythmic modulation remained constant or became slightly smaller.

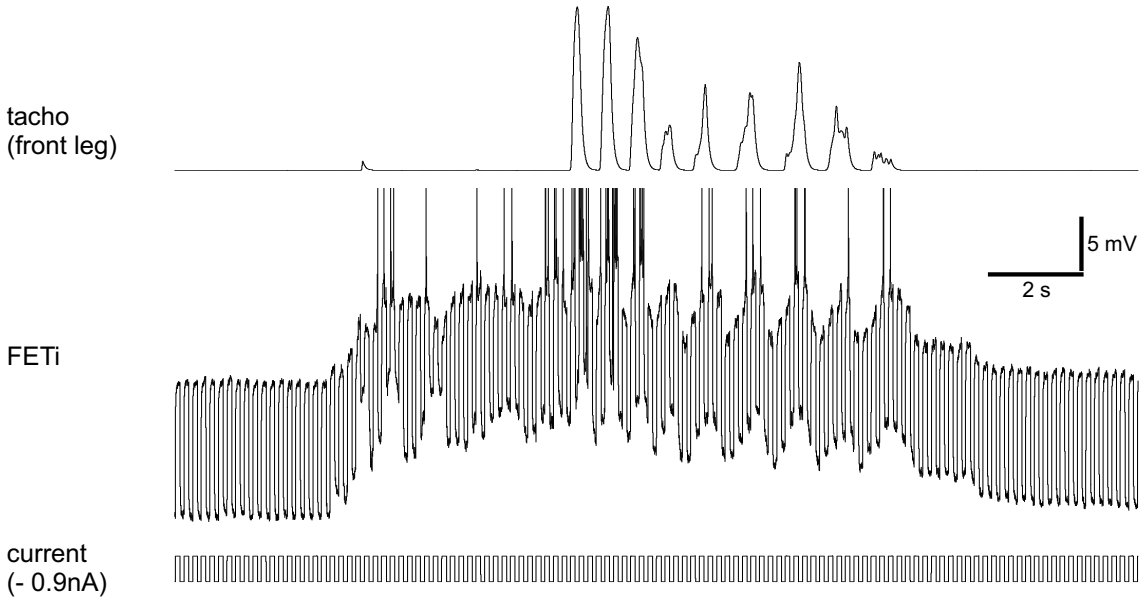

Figure 3.17: Membrane resistance was measured by injection of short hyperpolarizing current pulses of 0.9 nA (action potentials truncated). Changes of the amplitude of the evoked potential steps reflect the membrane resistance according to Ohm's law. During the stepping sequence of the front leg, no changes of the resistance could be detected.

3.2.5 Flexor tibiae motoneurons

In four animals activity of mesothoracic fast flexor motoneurons, and in one animal activity of a semi-fast flexor motoneuron, were recorded intracellularly, while the front leg performed walking movements on a treadmill. All flexor motoneurons showed a tonic depolarization during the whole walking sequence, although in some animals its amplitude was very small (range: 0.4 to 7.8 mV). The depolarization started approximately 0.1 to 0.2 seconds before flexor activity (recorded with an EMG) in the front leg. Its amplitude declined with a time constant of 0.4 to 0.6 seconds after the end of walking activity (Fig. 3.18A). The membrane potentials of the flexor motoneurons also showed a rhythmic modulation. As in recordings of fast extensor motoneurons, sometimes additional modulation was seen that could not be associated with steps of the front leg (not shown). Overlaying traces of the membrane potentials during 11 steps of a walking sequence revealed a long period of hyperpolarized membrane potential during all steps. It lasted for approximately 0.6 seconds, which is about 70 % of a step with an average duration of 0.84 seconds (SD: 0.16 s) (Fig. 3.18B). On average a hyperpolarization started 0.18 seconds before the start of the stance phase of the front leg. The average maximum amplitude of approximately 1.8 mV was reached at the start of the stance phase. A slow repolarization occurred during the front leg's stance phase.
Comparing the courses of membrane potentials of the antagonistic mesothoracic extensor and flexor motoneurons, both showed a maximal hyperpolarization shortly before the start of the front leg's stance phase. Slow and fast extensor motoneurons were quickly repolarized during the early stance phase, while flexor motoneurons only slowly reached more positive potentials. This resulted in the most positive membrane potentials occurring at the end of the stance phase in extensor motoneurons and after the end of the stance phase in flexor motoneurons.

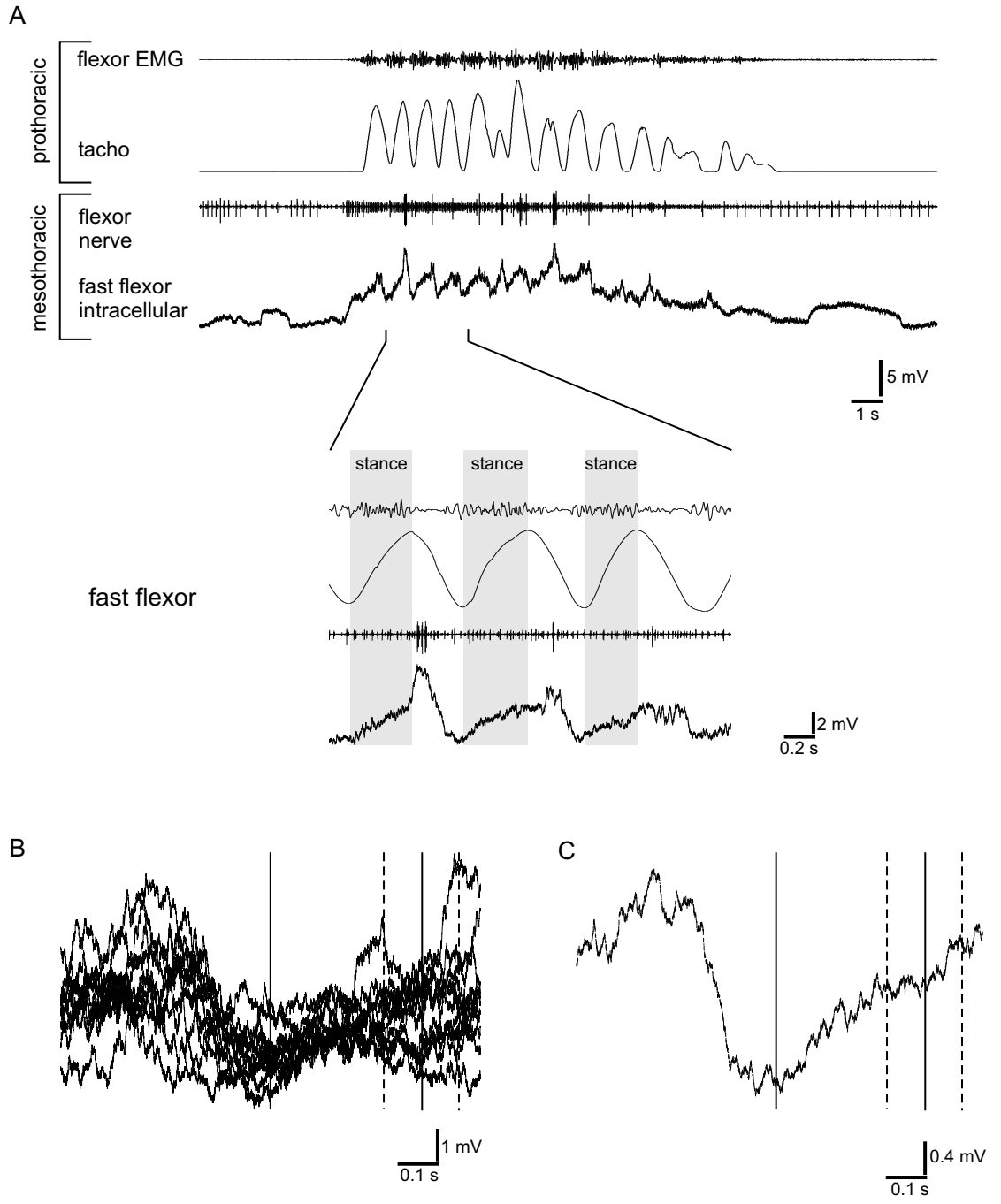

Figure 3.18: A: Intracellular recording of mesothoracic fast flexor motoneuron during walking movements of the ipsilateral front leg (RP: -55 mV). The membrane potential showed a tonic depolarization during the whole walking sequence and rhythmic hyperpolarization starting shortly before and persisting during the front leg's stance phase. In some steps, the membrane potential showed an additional depolarization at the end of the stance phase. B: Traces of 11 steps of a walking sequence (first and last three steps not analyzed) aligned with the stance phase as reference. All traces show a hyperpolarization shortly before and during the stance phase of the front leg. C: Average of the 11 steps.

3.3 Semi-intact walking preparation: recordings of non-spiking interneurons

Intracellular recordings of motoneurons showed different tonic and rhythmic inputs. To investigate the origin of these inputs, intracellular recordings from non-spiking interneurons were performed. Non-spiking interneurons are known to be part of the pre-motor network and to have excitatory or inhibitory effects on motoneurons (Büschges, 1995a). They are involved in the state dependent processing of sensory information for posture control and movement of leg joints (Büschges, 1995b). Several of them can be identified in stick insects by their physiology and morphology (Büschges, 1990).

A total of 19 mesothoracic non-spiking interneurons, all ipsilateral to the front leg performing walking movements on a treadmill, were recorded. Fourteen interneurons had an excitatory effect on extensor motoneurons; one excited FETi, but inhibited SETi. Four interneurons had an inhibitory effect.
Non-spiking interneurons with an excitatory effect on extensor motoneurons showed either a tonic depolarization (9 neurons, e.g. Fig. 3.20A) or a tonic hyperpolarization (5 neurons, e.g. Fig. 3.19C) in the range of 0.5 to 6 mV during the walking sequence of the front leg. All interneurons showed to some extend a rhythmic modulation of their membrane potential with amplitudes ranging from 0.2 mV to 6 mV. This rhythmic modulation was mostly coupled to the stepping activity of the front leg: prior to the start of the front leg's stance phase, some interneurons were hyperpolarized (e.g. Fig. 3.20A) and others were depolarized (e.g. Fig. 3.21A). During some recordings, often at the end of a walking sequence, a spontaneous burst of extensor activity occurred (e.g. Fig. 3.19B). This burst was accompanied by a strong depolarization of the non-spiking interneuron. The depolarization had a larger amplitude than the amplitudes of the rhythmic or tonic changes seen in conjunction with the walking activity of the front leg.

The interneuron with an excitatory effect on FETi and inhibitory effect on SETi, was tonically depolarized during the walking sequence and also showed a weak rhythmic modulation by approximately 0.5 mV(Fig. 3.21B).

Among the non-spiking interneurons with an inhibitory effect on extensor motoneurons, three were tonically depolarized (e.g. Fig. 3.22C) and one was tonically hyperpolarized (Fig. 3.22A) during the walking sequence of the front leg. The amplitudes of these tonic shifts of membrane potentials were in the range of 1 mV to 6 mV. All interneurons also showed, at least a weak, rhythmic modulation of their membrane potential. It had the form of a hyperpolarization prior to the start of the front leg's stance phase, with amplitudes in the range of 0.4 to 6 mV (e.g. Fig. 3.23). In some recordings the membrane potential showed oscillations after the walking sequence stopped (Fig. 3.22B). From time of occurrence, frequency, and decline of their amplitudes, these oscillations resemble a behavior described as 'rocking' (Pflüger, 1977).

A subset of the non-spiking interneurons recorded, was successfully morphologically identified. Interneuron E4 was identified in four animals (Fig. 3.24A). One interneuron showed a morphology similar to interneurons E3 and E2. The precise identity could not be determined and I will refer to it as E3/E2 (Fig. 3.24B). Another interneuron was identified as E5 or E6 (Fig. 3.24C) and one interneuron (Fig. 3.25) had a similar morphology to a non-spiking interneuron described by Büschges and Wolf (1995b, page 1852, Fig. 7E) in *Locusta migratoria*. The identified non-spiking interneurons showed either a tonic depolarization (E4, approx. 3.7 mV), a tonic hyperpolarization (E3/E2, approx. 2.3 mV), or no clear tonic change of membrane potential (E5/E6) during the walking sequence of the ipsilateral front leg. All three interneurons showed an additional rhythmic modulation of their membrane potential, which was correlated with individual steps of the front leg. Prior to the start of the front leg's stance phase, E4 was hyperpolarized (by approx. 1.6 mV), while E3/E2 was depolarized (by approx. 2.8 mV). Interneuron E5/6 appears to be only weakly modulated.

In Figure 3.26 the membrane potential modulations of all described mesothoracic non-spiking interneurons are plotted around the start of the front leg's stance phase. For this plot, the membrane potential of each individual neuron was averaged over several steps. The modulation of membrane potentials seen in SETi and FETi motoneurons (lower part of Fig. 3.26) results in part from excitatory (black traces) and inhibitory (gray traces) inputs from the non-spiking interneurons.

In summary, all mesothoracic non-spiking interneurons recorded, showed either a tonic depolarization or hyperpolarization of their membrane potential. The membrane potential also showed a modulation that appears to be related to the activity of the front leg, although this modulation was in some recordings only of small amplitude.

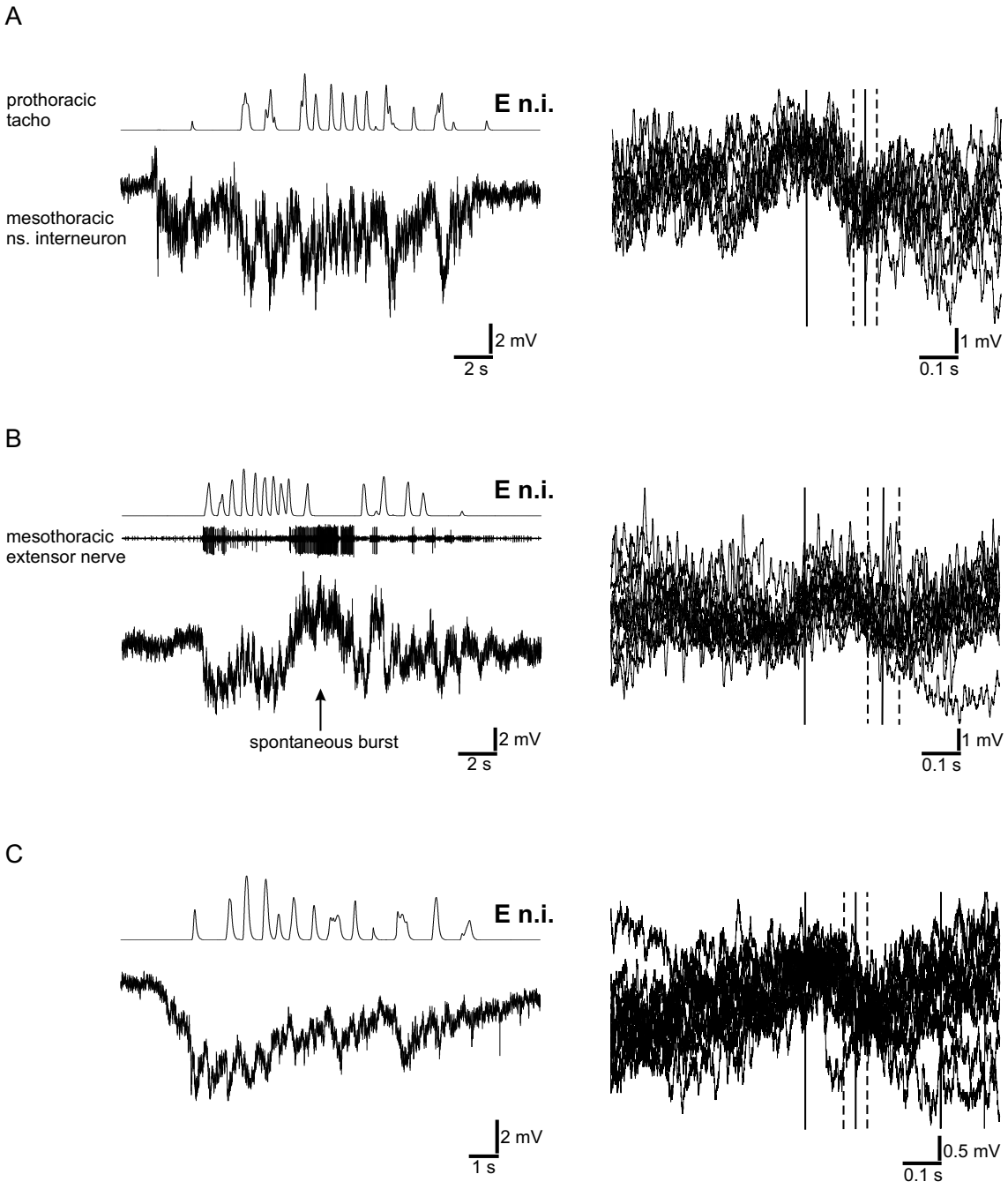

Figure 3.19: Mesothoracic non-spiking interneurons with an excitatory effect on extensor motoneurons. Original recording during walking activity of the ipsilateral front leg (left) and traces overlaid with the start of front leg's stance phase as reference (right). Additional vertical lines indicate end of stance phase and SD. Note the spontaneous burst of activity of extensor motoneurons which is accompanied by a strong depolarization of the interneuron in B. (RPs: -39 mV (A), -54 mV (B), -62 mV (C))

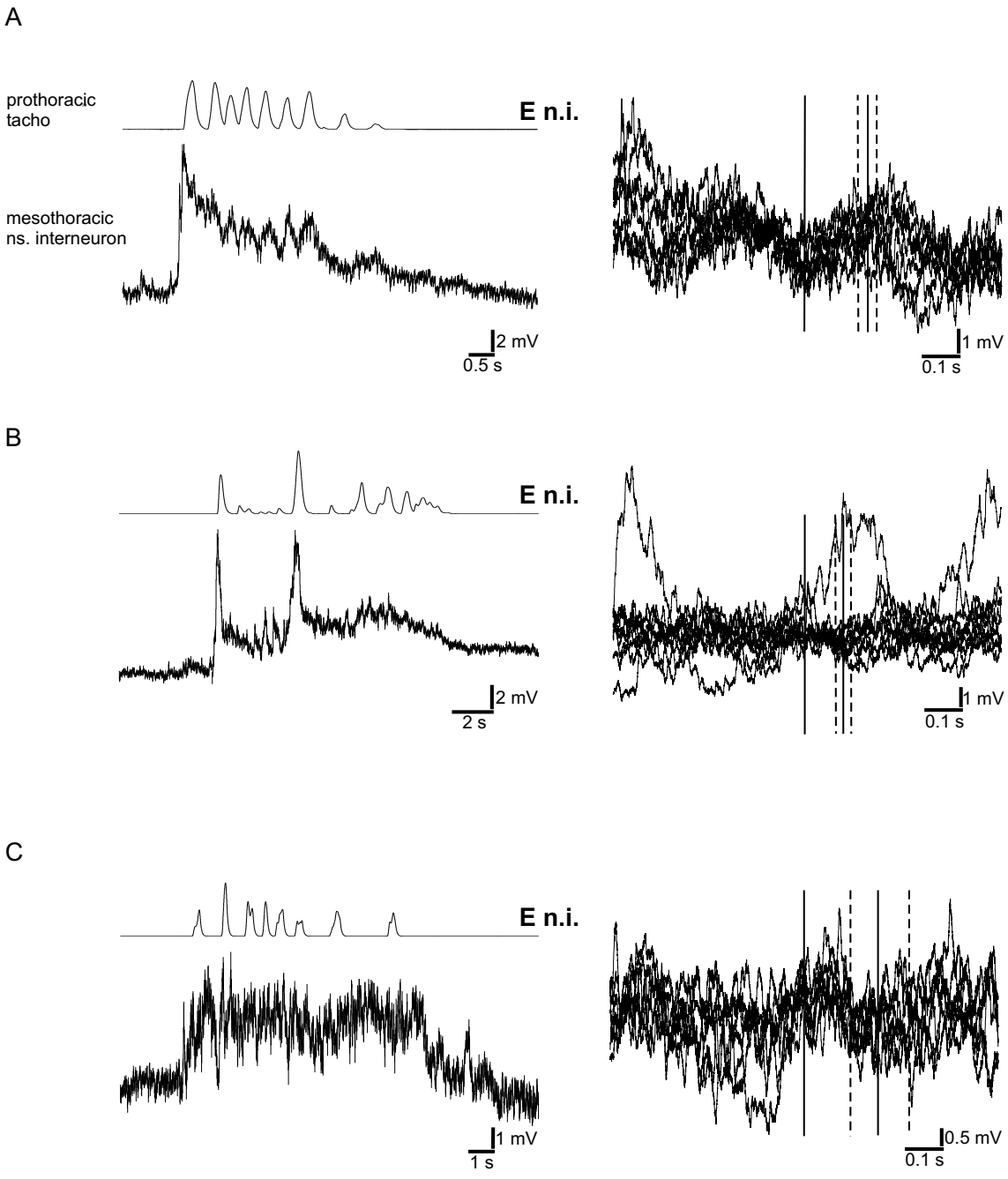

Figure 3.20: Mesothoracic non-spiking interneurons with an excitatory effect on extensor motoneurons. Original recording during walking activity of the front leg (left) and traces overlaid with the start of front leg's stance phase as reference (right). (RPs: -60 mV (A), -62 mV (B), -60 mV (C))

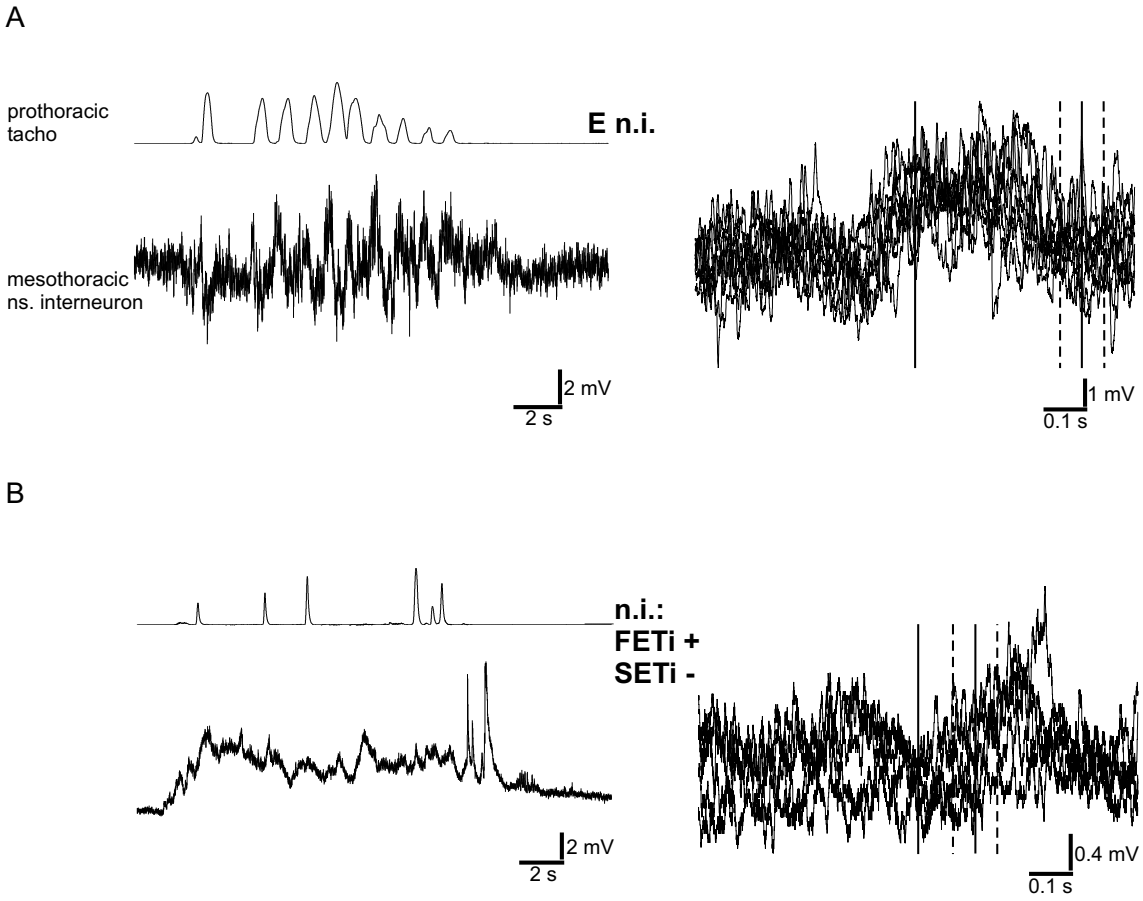

Figure 3.21: A: Mesothoracic non-spiking interneuron with an excitatory effect on extensor motoneurons(RP: -67 mV) B: Mesothoracic non-spiking interneuron with an excitatory effect on FETi and inhibitory effect on SETi (RP: -66 mV).

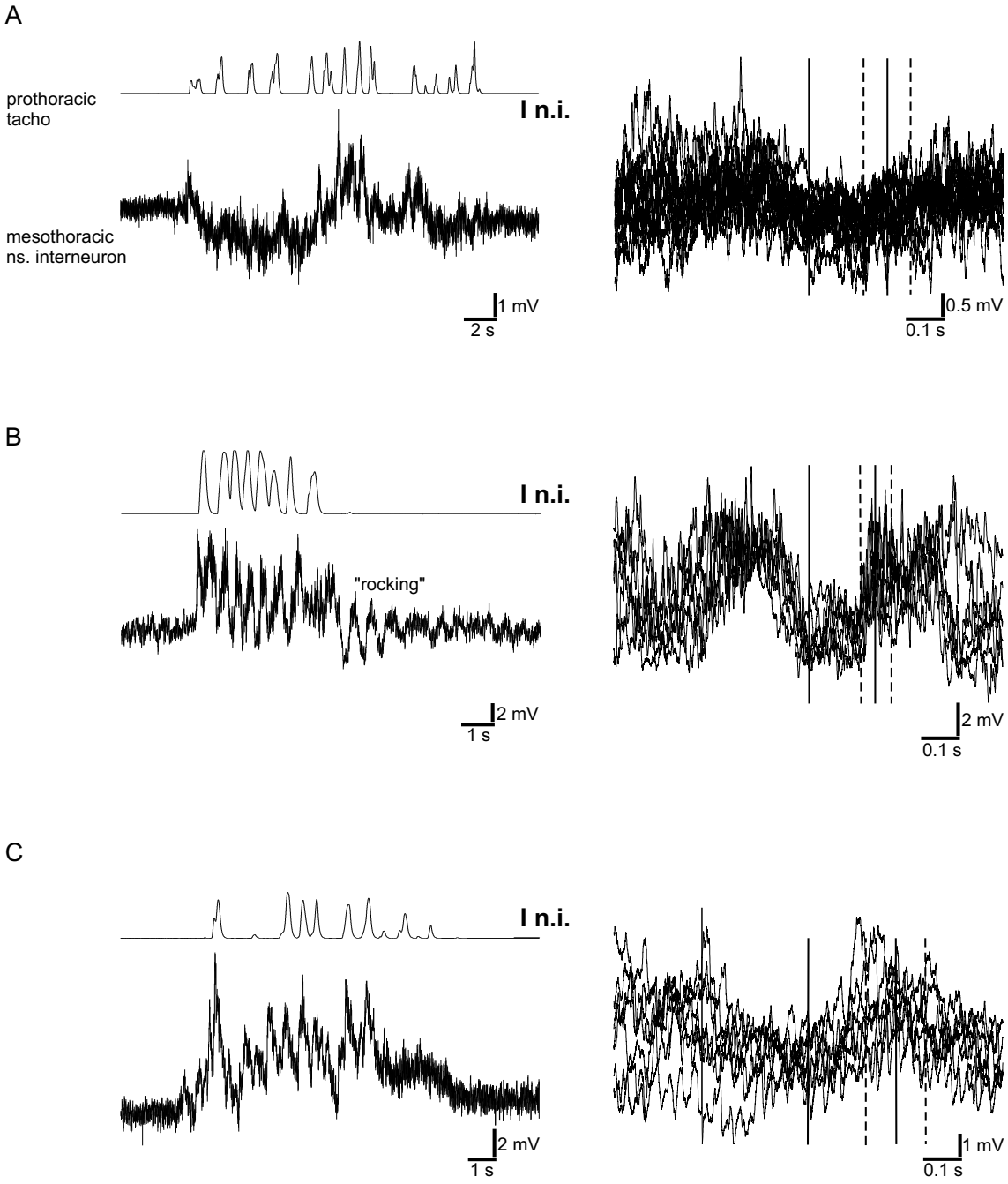

Figure 3.22: Mesothoracic non-spiking interneurons with inhibitory effects on extensor motoneurons. Original recording during walking activity of the front leg (left) and traces overlaid with the start of front leg's stance phase as reference (right). Additional vertical lines indicate end of stance phase and SD. (RPs: -54 mV (A), -70 mV (B), -58 mV (C))

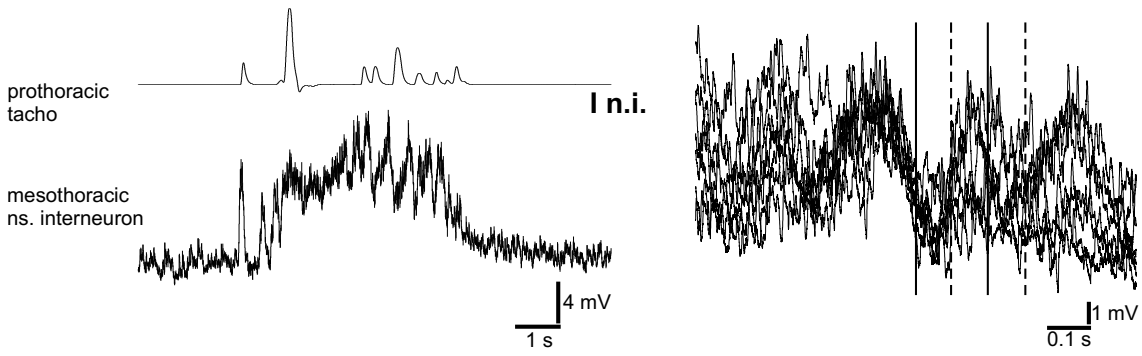

Figure 3.23: Mesothoracic non-spiking interneuron with inhibitory effects on extensor motoneurons. Original recording during walking activity of the front leg (left) and traces overlaid with the start of front leg's stance phase as reference (right). (RP: -62 mV)

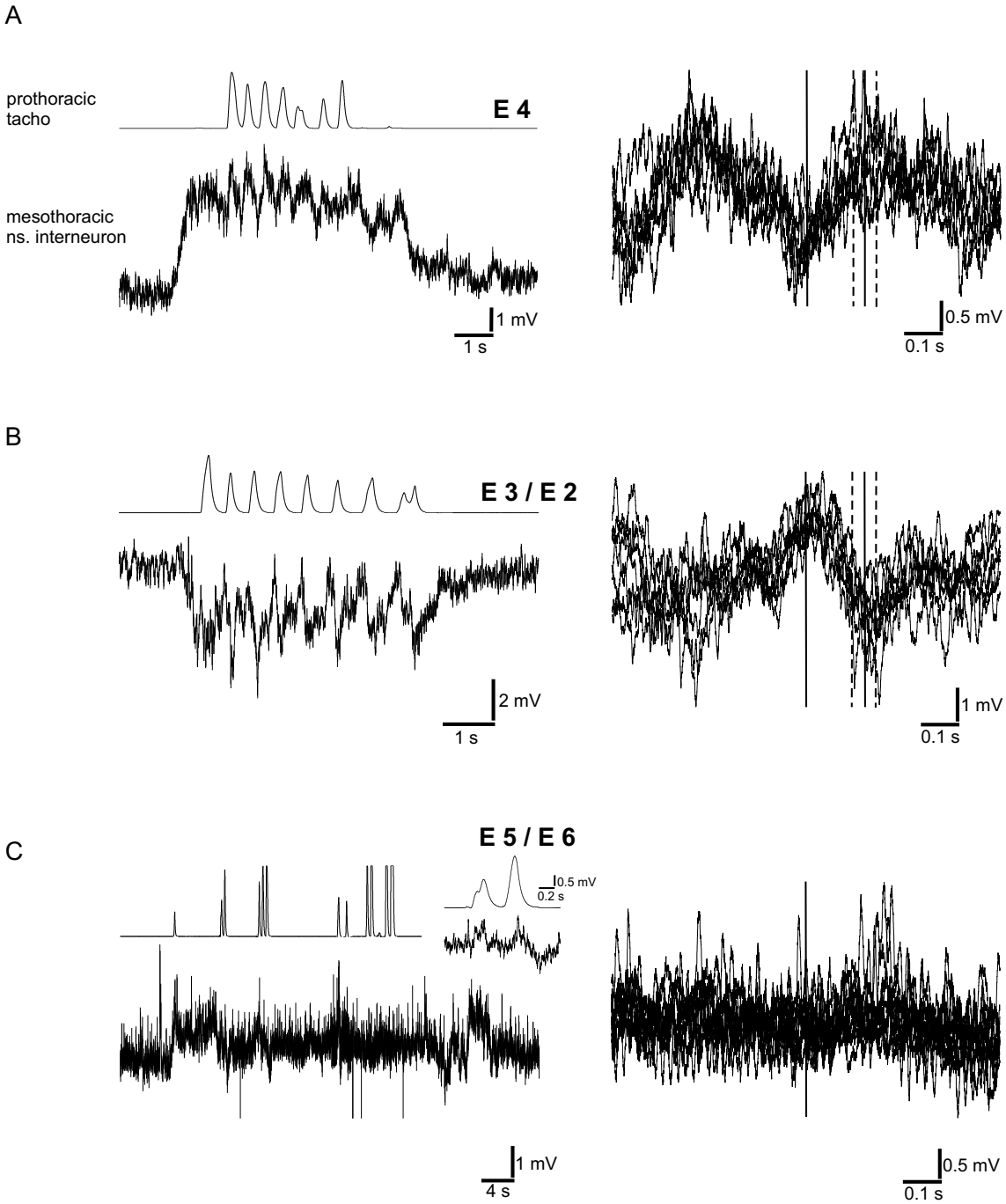

Figure 3.24: Activity of identified mesothoracic non-spiking interneurons during walking activity of the ipsilateral front leg. Original recording (left) and traces overlaid with the start of front leg's stance phase as reference (right). The neurons were identified as E4 (A, RP: -51 mV; recorded in 3 more animals), E3/E2 (B, RP: -43 mV), and E5/E6 (C, RP: -47 mV) by their morphology after dye injection. The inset in C shows an enlarged section.

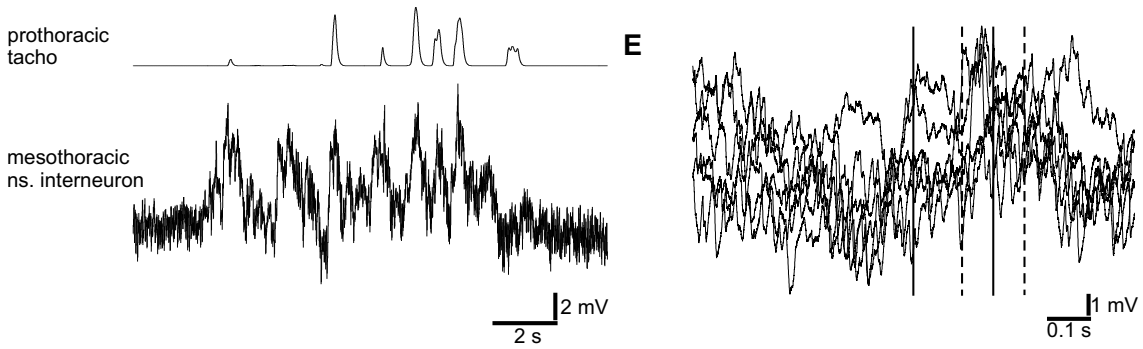

Figure 3.25: Activity of mesothoracic non-spiking interneuron with an excitatory effect on extensor motoneurons. Original recording during walking activity of the ipsilateral front leg (left) and traces overlaid with the start of front leg's stance phase as reference (right). The neuron had a similar morphology to a non-spiking motoneuron described by Büschges and Wolf (1995b, page 1852, Fig. 7E) in *Locusta migratoria*. (RP: -57 mV)

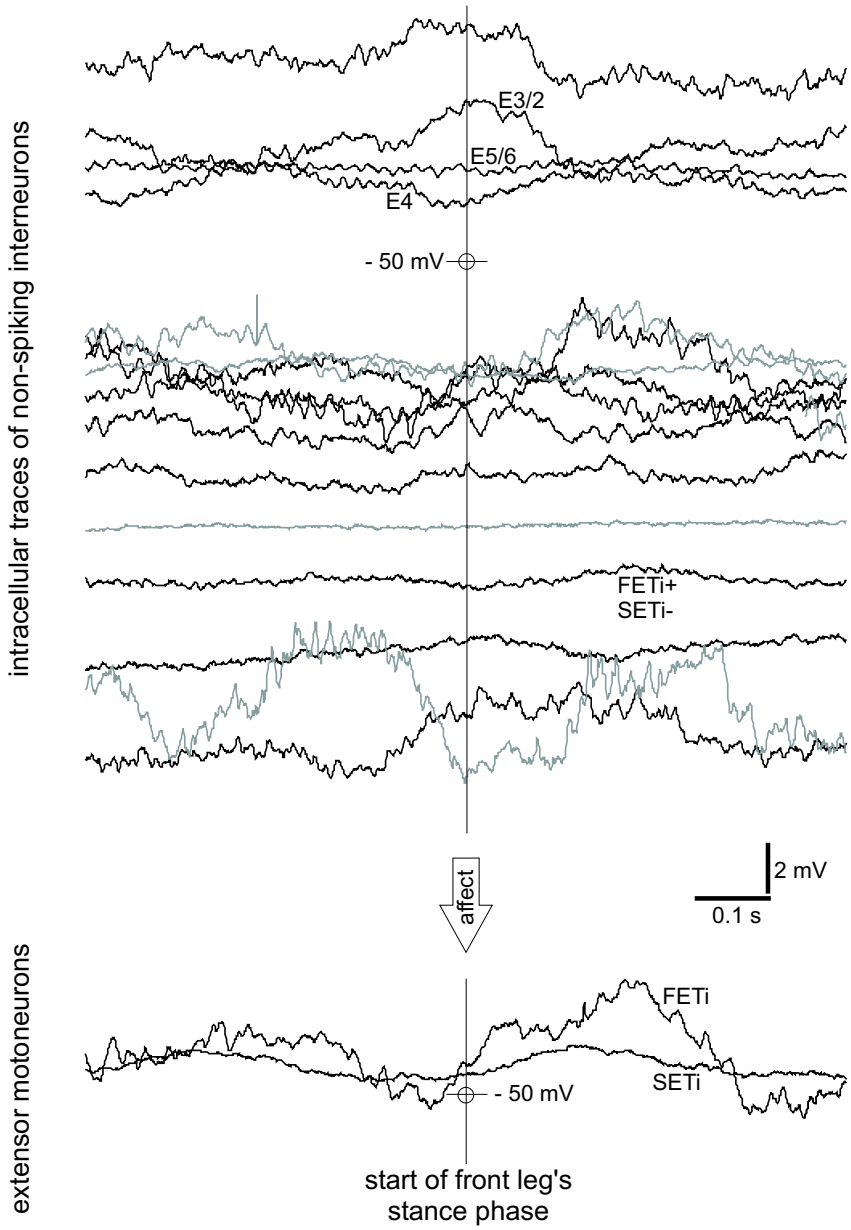

Figure 3.26: Summarizing plot of membrane potential modulations of all mesothoracic non-spiking interneurons previously described. The traces show membrane potentials, averaged over several steps, around the start of front leg's stance phase (vertical line). Interneurons with excitatory effect on extensor tibiae motoneurons are plotted black, such with inhibitory effect gray. The lower part shows the modulation of the membrane potential of extensor tibiae motoneurons.

3.4 Mutual influences of segmental central pattern generators

The role of central mechanisms in intersegmental coordination was investigated with a preparation in which sensory input was completely removed by cutting all side nerves. One ganglion was pharmacologically activated by use of pilocarpine. Pilocarpine evokes rhythmic activity of antagonistic motoneuron pools (Büschges et al., 1995a). Any coupled modulation of motoneuron activity seen in the adjacent, not activated, ganglion must therefore be due to central coordinating mechanisms. One set of experiments used the meso- and metathoracic ganglia, a second set used pro- and mesothoracic ganglia.

3.4.1 Interaction between meso- and metathoracic ganglia

The mesothoracic ganglion was pharmacologically activated, which was monitored with extracellular recordings of ipsilateral depressor and levator trochanteris motoneurons. Simultaneously, recordings at nerve stumps of the adjacent metathoracic ganglion were made of extensor tibiae (N=5), protractor coxae (N=3), retractor coxae (N=3), levator trochanteris (N=2), or depressor trochanteris (N=3) motoneurons. In all experiments slow units of both ganglia showed spontaneous activity. After the application of pilocarpine on the mesothoracic ganglion, rhythmic activity established in about 58 % of the experiments conducted (N=74). Only experiments with periods of a stable alternating activity of mesothoracic levator and depressor motoneurons were used. In no experiment an obvious modulation of metathoracic motoneuron activity, that could be related to mesothoracic activity, was visible (Fig. 3.27).

To detect a subtle modulation, the start of a mesothoracic levator burst was taken as a reference point, and metathoracic motoneuron activity in a time window around this point plotted in the form of a histogram. This was repeated for several points, and the motoneuron activity summed. This adding exposes small changes of activity, occurring with a constant delay to the reference point. No clear modulation was evident in data from individual animals (Fig. 3.28A). The same is true for starts of levator burst as points of reference (Fig. 3.28B), and also if the activity of the metathoracic motoneuron was plotted over phase in mesothoracic levator cycles. The latter exposes small changes of activity occurring with a constant phase delay to the rhythmic levator activity (Fig. 3.28C).
For statistical analysis, it was tested if the spike count in individual bins equals the mean spike count calculated from all bins (χ^2-test, Zar (1999)). This would be expected, if no modulation of activity occurs. For the data analyzed with the starts of levator bursts as points of reference (Fig. 3.28A), this hypothesis could not be rejected. Thus the variations between the spike counts of different bins can be attributed to chance.

Nonetheless, pooling averaged data from several animals revealed a weak correlation of mesothoracic depressor bursts and metathoracic motoneuron activity. Figure 3.29A shows the extensor motoneuron activity around starts of mesothoracic depres-

sor bursts. Data were pooled from five animals. The statistical analysis shows that spike counts in individual bins are significantly ($0.001 < P$) different from the mean spike count. Extensor activity is decreased in a time window from 0.6 to 0.3 seconds before the start of the mesothoracic depressor burst by approximately 19 %, compared to the mean activity. Also for the protractor data pooled from three animals, spike counts in individual bins are significantly ($0.01 < P < 0.035$) different from the mean spike count (Fig. 3.29B). Protractor activity is increased by approximately 20 % approximately 0.8 seconds after the start of the mesothoracic depressor burst. In contrast, for levator data pooled from two animals, spike counts in individual bins were not significantly different from the mean spike count. Variations of spike counts in individual bins can be attributed to chance (Fig. 3.29C).

In a second set of experiments, the coordinating signals were examined in the reverse direction. The metathoracic ganglion was stimulated with pilocarpine, while spontaneous activity of motoneurons in the mesothoracic ganglion was monitored and analyzed. No correlation between start of metathoracic depressor or levator bursts and mesothoracic activity of extensor (N=4), protractor (N=3), or levator (N=4) motoneurons could be found in the analysis of data from individual animals (data not shown).

Central mechanisms might play only a minor role for the coordination of mesothoracic levator and depressor activity with activity of metathoracic motoneurons. The same is true for the reverse direction. A correlation could only be found after adding many data from several animals, indicating a very weak nature of the coupling.

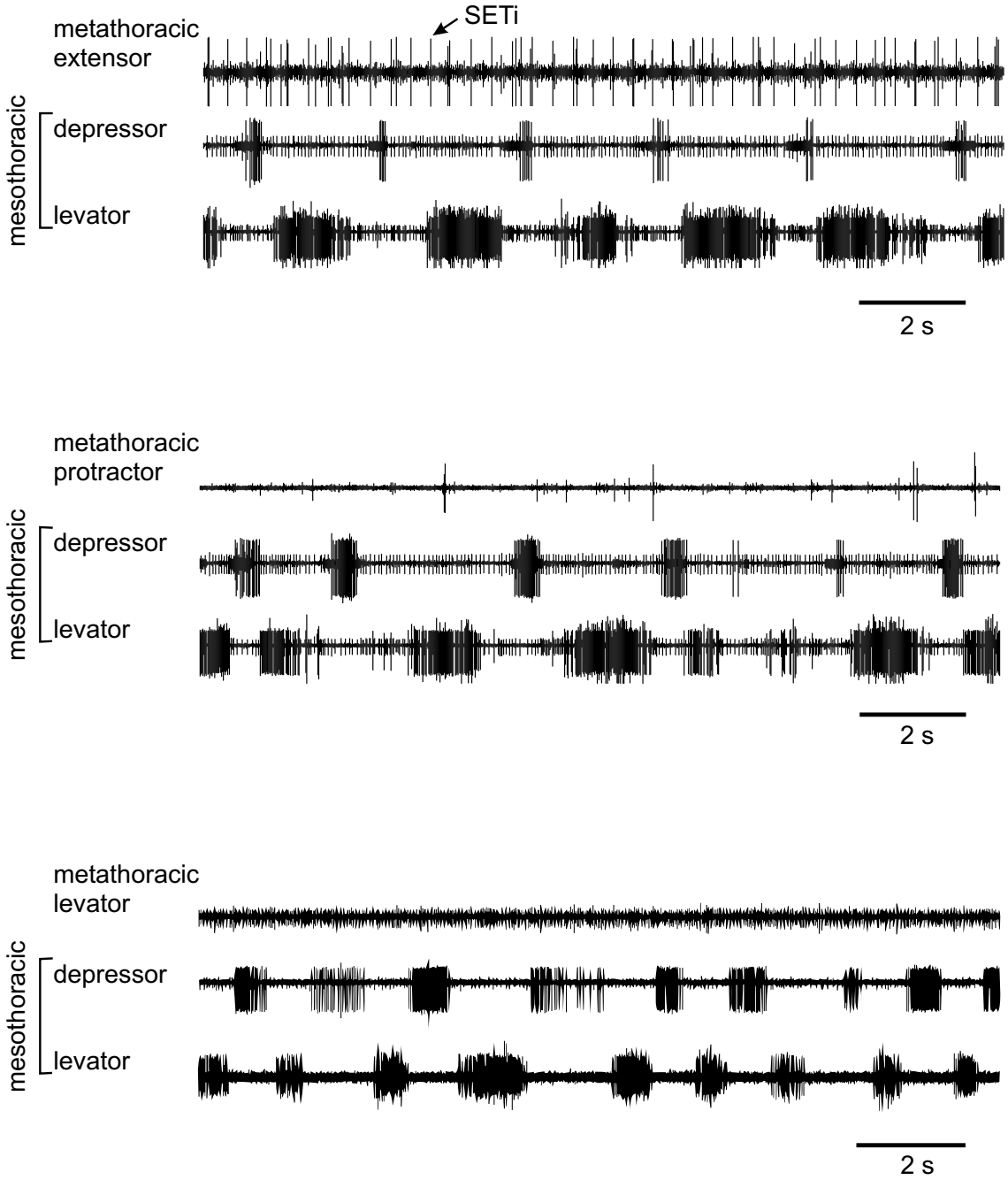

Figure 3.27: Recordings of metathoracic extensor tibiae, protractor coxae, and levator trochanteris motoneurons, during rhythmic activity in the mesothoracic segment (SETi: slow extensor tibiae). The rhythmic activity was induced by application of pilocarpine on only the mesothoracic ganglion and monitored by recordings of depressor and levator trochanteris motoneurons. All afferent input was excluded by cutting the side nerves of both ganglia.

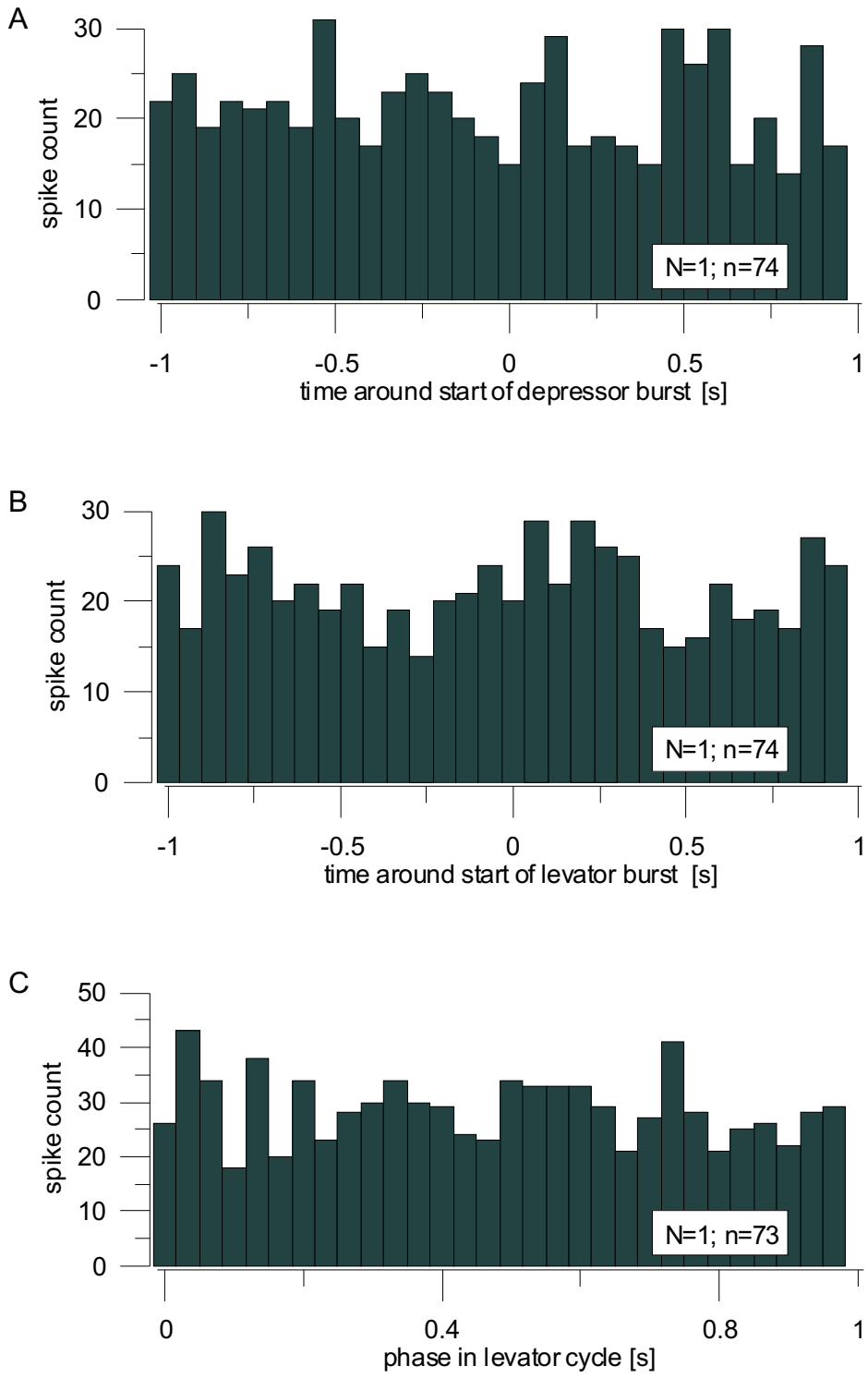

Figure 3.28: Average activity of a metathoracic extensor tibia motoneuron (SETi) around mesothoracic starts of depressor bursts (A), starts of levator bursts (B), and plotted over the phase of levator cycles (C). All data plotted were derived from one experiment. Different methods of analysis were tried to detect subtle intersegmental coupling.

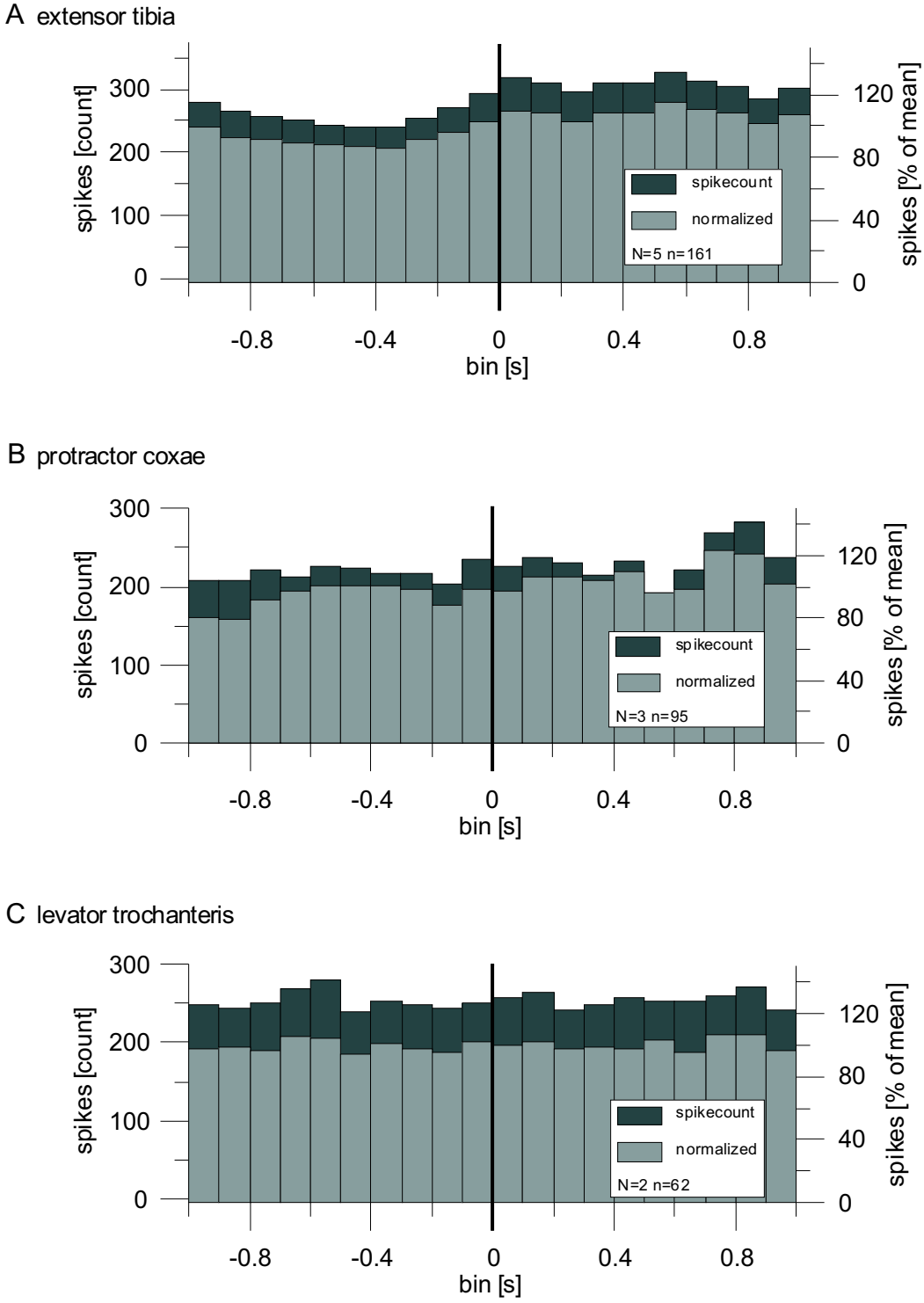

Figure 3.29: Summed activity of metathoracic motoneurons extensor tibiae, protractor coxae, and levator trochanteris, during rhythmic activity of mesothoracic depressor and levator trochanteris motoneurons. Data from several experiments were pooled to obtain a higher sample size (A: N=5; B: N=3; C: N=2). At time zero, a mesothoracic depressor burst starts. Dark bars show absolute spike counts, light bar show same data, but with each experiment normalized with its mean activity.

3.4.2 Interaction between pro- and mesothoracic ganglia

Using a slightly different preparation that allowed recording from nerve stumps in the prothoracic ganglion, central coordinating mechanisms between prothoracic and mesothoracic motoneurons were examined. Ipsilateral levator and depressor trochanteris motoneurons were recorded simultaneously from both segments, while rhythmic activity was induced with pilocarpine in only the prothoracic segment. The rhythmicity was induced less reliably: while in about 58% of the animals (N=74) sequences of rhythmicity could be induced in the mesothoracic ganglion, this was achieved in only 19% of the animals in the prothoracic ganglion (N=43). In a total of eight experiments with rhythmic activity of prothoracic protractor and retractor coxae motoneurons, no related modulation of spontaneous activity could be found in mesothoracic protractor or retractor coxae motoneurons (Fig. 3.30).

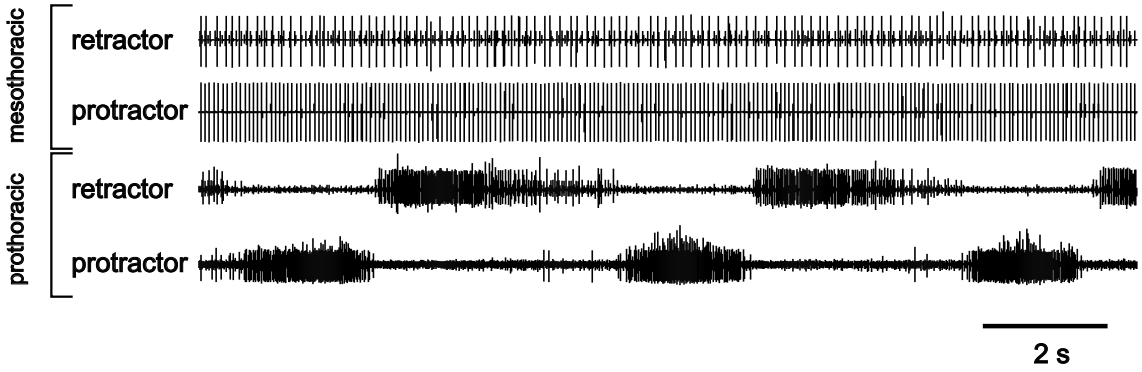

Figure 3.30: Extracellular recordings of mesothoracic protractor and retractor coxae motoneurons in the prothoracic and mesothoracic segments. In the prothoracic segment rhythmic activity was induced by application of pilocarpine. The mesothoracic ganglion was not in contact with pilocarpine. All side nerves were cut distal to the site of recording.

Summing motoneuron activity as described in 3.4.1 revealed no correlation of prothoracic and mesothoracic protractor and retractor activity. Starts of prothoracic protractor bursts were taken as reference points and spontaneous activity of mesothoracic protractor (N=4, n=166) and retractor motoneurons (N=3, n=118) were plotted in a time window around these points (Fig. 3.31A and B). The same is true for starts of prothoracic retractor bursts as reference points (mesothoracic protractor: N=4, n=161; retractor: N=3, n=118; Fig. 3.31C and D).

In summary, no evidence for a central mechanism ensuring coordination of mesothoracic protractor and retractor motoneuron activity with prothoracic protractor and retractor activity could be found.

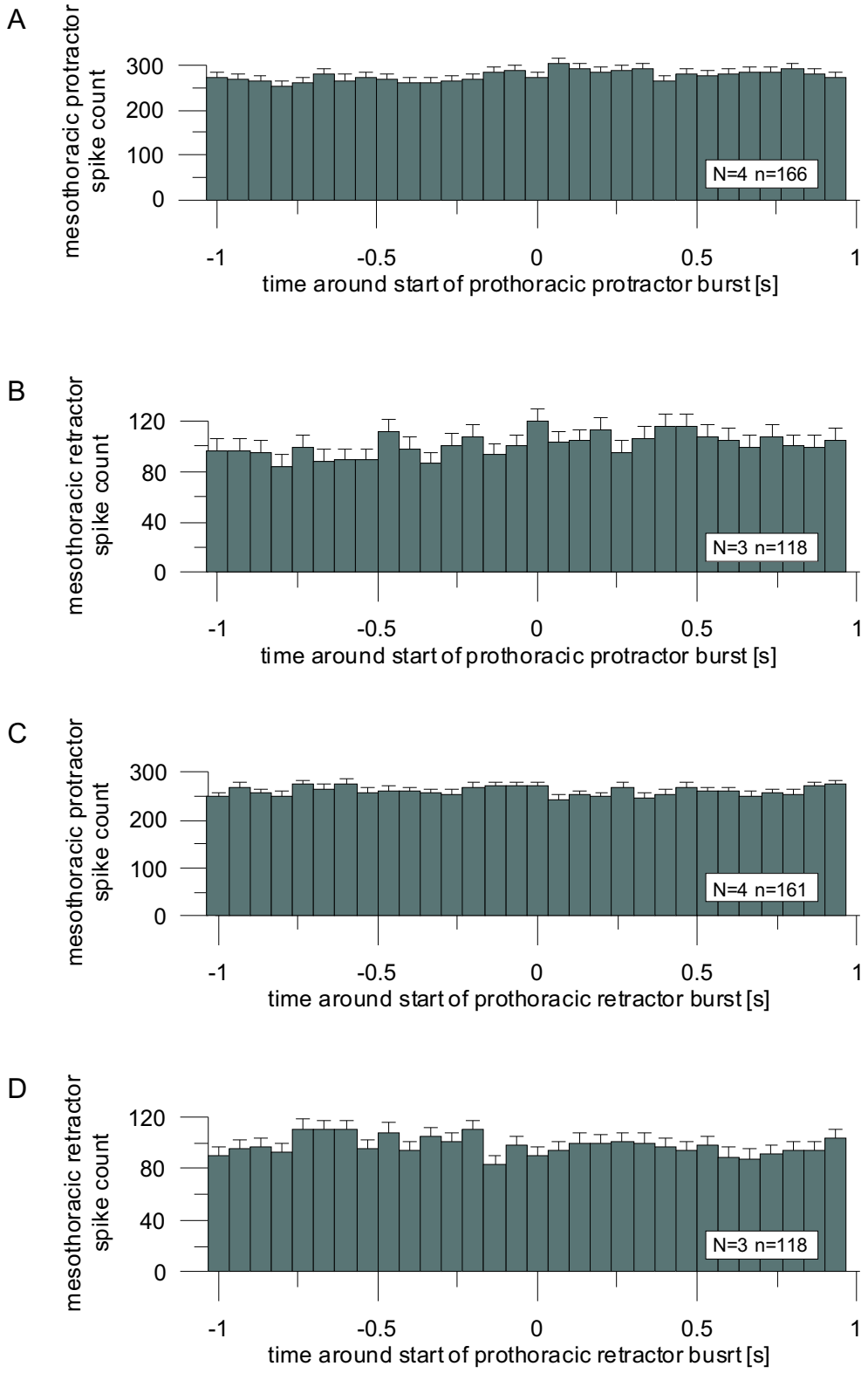

Figure 3.31: Average activity of mesothoracic protractor and retractor coxae motoneurons during rhythmic activity of prothoracic protractor and retractor coxae motoneurons. At time zero a prothoracic protractor (A, B) or retractor (C, D) burst starts. Error bars denote the standard deviation.

3.5 Intersegmental influences of the chordotonal organ

The femoral chordotonal organ is a sensory organ measuring position, velocity, and acceleration of the leg's tibia with respect to the femur (Hofmann and Koch, 1985a; Hofmann et al., 1985b). It is involved in the state dependant control of the femur-tibia joint, as well as in inter-joint coordination, that is, signals from the femoral chordotonal organ influence for example the activity of motoneurons controlling the coxa-trochanter joint (reviewed by Bässler and Büschges, 1998). To study its role in intersegmental coordination of leg movements, it was specifically stimulated in the middle leg. Activity of protractor coxae motoneurons, levator trochanteris motoneurons, and extensor tibiae motoneurons was recorded extracellularly in the adjacent metathoracic segment. The animals were 'activated' by touching the abdomen with a paint brush. The 'active state' is characterized by a higher spontaneous activity of motoneurons and a raised likelihood of an 'active reaction'. The latter is a reversal of a resistance reflex to an assisting reflex in the femur tibia joint, which only occurs in the 'active' animal (Bässler and Büschges, 1998). To detect this 'active reaction', an additional extracellular recording was made of extensor tibia motoneurons (nerve F2) in the stimulated middle leg.
Slow protractor motoneurons showed no clear stimulus-correlated change of activity in seven animals (Fig. 3.32A). Quantitative analysis with peri-stimulus-histograms of 31 trials, in which an 'active reaction' was observed in the femur-tibia joint of the stimulated leg, also showed no distinct influence on metathoracic protractor activity. Slow levator motoneurons showed no stimulus correlated change of activity in 18 animals and 109 trials analyzed with peri-stimulus-histograms (Fig. 3.32B). Similarly, slow extensor motoneurons showed no stimulus correlated change of activity in 7 animals and 73 trials analyzed (Fig. 3.32C).

In summary, no clear influence of signals from the middle leg's chordotonal organ on the activity of hind leg protractor coxae, levator trochanteris, or extensor tibiae motoneurons was found.

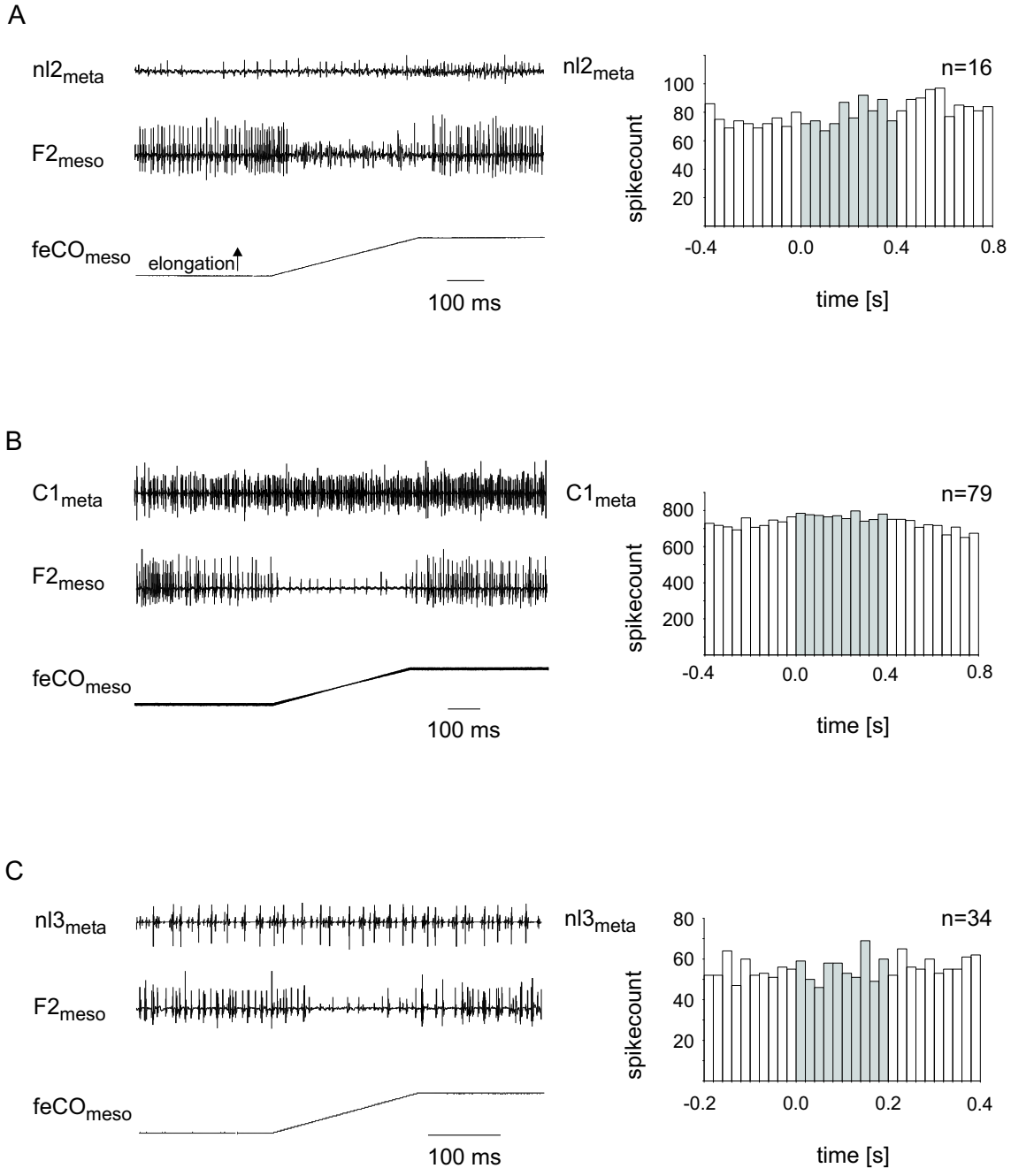

Figure 3.32: Activity of hind leg nerves nl2 (A; slow protractor coxae), C1 (B; slow levator trochanteris), and nl3 (C; large unit: SETi; small unit: CI) during stimulation of the femoral chordotonal organ (feCO) of the ipsilateral middle leg. The ramp-wise stimulation simulates a flexion of the tibia. The animal was in an 'active' state, as indicated by the reduction of extensor motoneuron activity in nerve F2 (large unit: FETi; middle unit: SETi; small unit: CI, not visible in A) in the stimulated leg after the onset of stimulation ('active reaction'). The peri-stimulus-histograms show accumulated activity of nerves investigated for several stimulations; the ramp is denoted by gray shading.

3.6 Local and intersegmental influences of campaniform sensilla

A second sensory organ studied with respect to its role for intersegmental coordination were the campaniform sensilla. The campaniform sensilla are a sensory organ composed of three groups located on the femur and trochanter of a leg. They measure cuticular strain and thereby the load on a leg (Schmitz, 1993). This load information affects the anterior and posterior extreme position of a leg, as well as the duration of the stance phase of a leg (Duysens et al., 2000). It is known that signals from the campaniform sensilla induce a switch from protractor coxae activity to retractor coxae activity of a stick insect's middle leg. They apparently play an important role for inter-joint coordination (Akay, 2002).
In a set of experiments, the local effect of campaniform sensilla on activity of protractor and retractor coxae motoneurons in the hind leg was studied. Additionally, simultaneous extracellular recordings of ipsilateral protractor and retractor motoneurons in the adjacent mesothoracic ganglion were performed, to study an intersegmental effect.

3.6.1 Local effect of campaniform sensilla signals in the hind leg

Stimulation of the campaniform sensilla, by bending the leg caudad, led to inactivation of spontaneously active protractor motoneurons and initiated activity of retractor motoneurons (N=21; Fig. 3.33A). The reverse is seen by stimulation through rostrad bending. This is in accordance with the effect known in the middle leg (Akay, 2002).
If the animal was 'activated' by touching the abdomen with a paintbrush, the effect was almost reversed: caudad bending led in 43 % of the stimuli during retractor activity to a switch to protractor activity (N=5; n=84, Fig. 3.33B). Stimulation during protractor activity induced only in 8 % of the stimuli a switch to retractor activity (N=5; n=51). If the stimulation was applied rostrad during retractor activity, 16 % of the stimuli induced a switch, while 4 % of the stimuli caused the retractor to cease activity, but the protractor showed no change in activity (N=5; n=57). Stimulation during protractor activity induced in 6 % of the stimuli a switch and in 11 % a cessation (N=5; n=73).
Under rhythmic conditions, that is rhythmic activity induced by pilocarpine, the influence of the campaniform sensilla was more pronounced (Fig. 3.33C). 73 % of the caudad stimuli during retractor activity induced a switch to protractor activity and 10 % led to cessation of retractor activity (N=5; n=190). Stimulation during protractor activity induced a switch in only 15 % of the stimuli (N=5; n=59). Stimulation in rostral direction led in 22 % of the stimuli to a switch and in 12 % only to cessation of retractor activity (N=5; n=127). If stimulation took place during protractor activity, 22 % of stimuli induced a switch, while 12 % induced cessation (N=5; n=127). Rostrad bending during protractor activity induced in 55 % of stimuli a switch and in 2 % the protractor only to cease activity (N=5; n=126).

Stimulation of the campaniform sensilla of the hind leg induced a local effect opposite to the one described for the middle leg: in most caudad stimulations, a switch from retractor- to protractor activity occurred, while a switch mostly from protractor to retractor is described for the middle leg (Akay, 2002).

To investigate whether signals from the campaniform sensilla have access to central rhythm generating networks, controlling protractor- and retractor coxae, data from the rhythmic preparation was further analyzed (Fig. 3.34). It was found that the duration of a burst was strongly correlated with the time from retractor burst onset to the onset of the stimuli. Hence, the stimulus leads to cessation of the bursts. The time from the end of the burst to the onset of the succeeding burst, the inter-burst interval, is not correlated with the stimulus onset. The cycle period is again correlated with the stimulus onset, excluding a possible negative correlation of burst duration and inter-burst interval. This suggests that signals from the campaniform sensilla have a resetting influence on rhythmic retractor motoneuron activity, which requires access to central rhythm generating networks.

A reset plot of data with randomly timed stimulation shows the influence of stimulus phasing (Fig. 3.35). It plots the influence on rhythmicity ($(cycle\ period / mean cycle period) - 1$) over the stimulus phase ($stimulus onset / mean cycle period$). Stimuli early in the cycle period lead to a shortening of the cycle period, whereas stimuli in the last 40 % of the cycle, thereby mostly during protractor activity, cause no shortening, but even a slight prolongation of the cycle period (N=3; n=39).

3.6.2 Intersegmental effect of campaniform sensilla signals

To investigate if signals from the metathoracic campaniform sensilla have an intersegmental effect, activity of pro- and retractor motoneurons in the adjacent mesothoracic segment were recorded.
In 'active' animals, a switch from mesothoracic retractor activity to protractor activity occurred in 6 % of caudad, metathoracic stimuli (N=4; n=33; Fig. 3.36). If the protractor was active during stimulation, 22 % of the stimuli induced a switch to retractor activity and 3 % induced cessation of protractor activity (N=4; n=36). Rostrally directed stimulation during retractor activity induced in 4 % of the stimuli a switch and in 4 % cessation (N=4; n=48). During protractor activity in 18 % of stimuli a switch occurred, while cessation occurred in 12 % (N=4; n=17).

Under rhythmic conditions induced by pilocarpine, caudally directed stimulation in the metathorax induced in 10 % of stimuli a switch from mesothoracic retractor to protractor activity and retractor activity only ceased in 18 % (N=4; n=61; Fig. 3.37). If the mesothoracic protractor was active, 9 % of the stimuli induced a switch, while 2 % caused cessation (N=4; n=45). Rostrad stimulation caused a switch from retractor activity in 11 % of stimuli and cessation in 9 % (N=4; n=47). Protractor activity was switched to retractor activity in 7 % of stimuli and it ceased activity in 6 %.

For a further analysis of the experiments, only stimuli that evoked a local effect in the metathoracic segment were analyzed with respect to their effect on mesothoracic retractor and protractor activity. Under both conditions, in the 'active' animal as well as under pharmacologically evoked rhythmic conditions, no qualitative differences to the effects described, were found.

In summary, the metathoracic campaniform sensilla exert only a very weak influence on mesothoracic protractor and retractor motoneuron activity in the active animal. The data suggest that their signals mildly facilitate a switch from protractor to retractor activity. No influence could be detected in the rhythmic preparation.

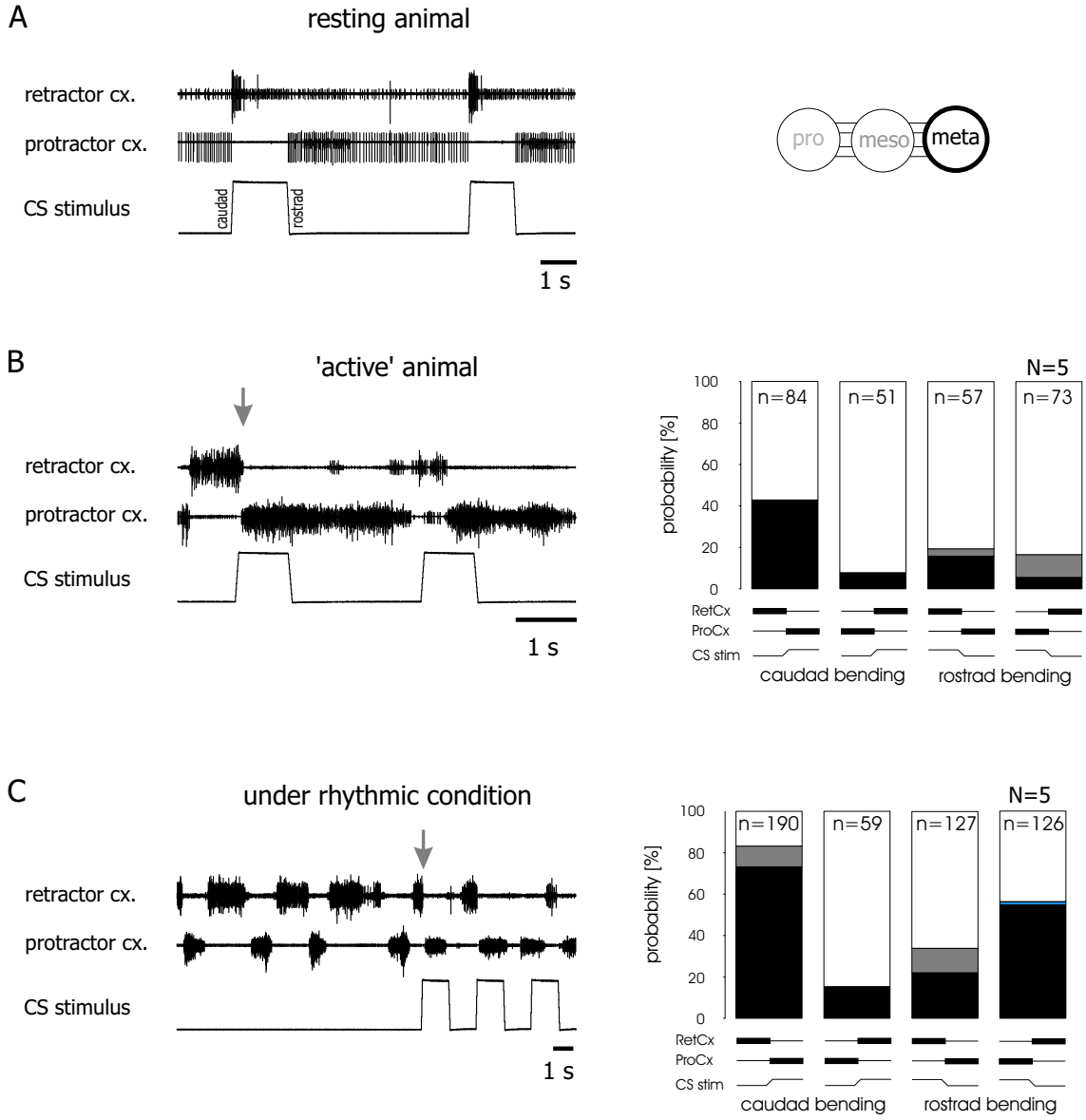

Figure 3.33: Influence of stimulation of the campaniform sensilla on activity of protractor coxae and retractor coxae motoneurons in the same segment. Data shown for the resting animal (A), the 'active' animal (B), and under rhythmic condition, induced by bath application of pilocarpine. Arrows point out a switch from retractor to protractor activity, shortly after stimulus onset. Histograms show the probability for a switch (black) or truncation (gray) to occur shortly after stimulus onset. The stimulation occurred by caudad (left half) or rostrad (right half) bending of the femur and either during a retractor (left) or a protractor (right) burst.

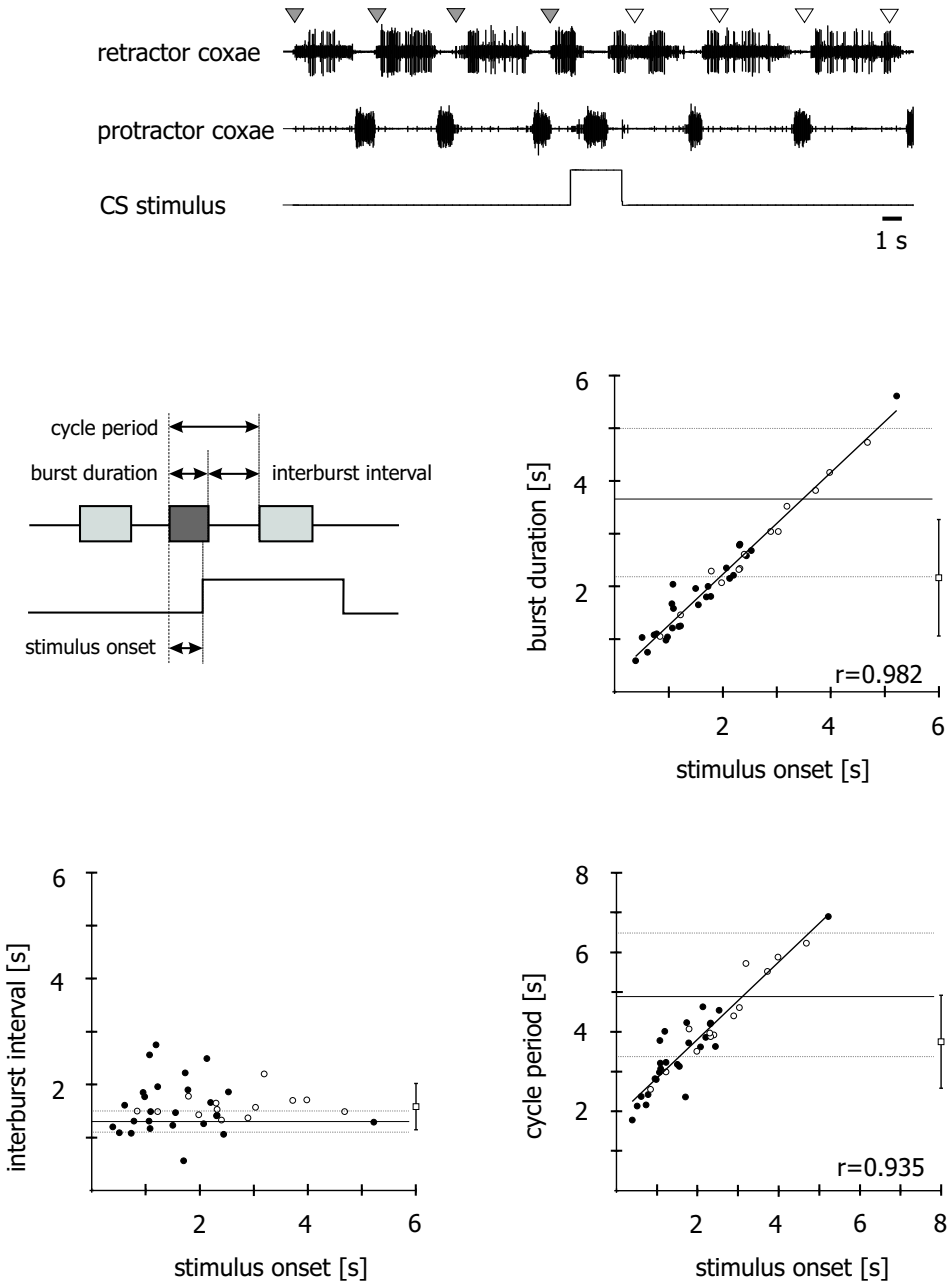

Figure 3.34: Influences from the campaniform sensilla can reset rhythmic protractor and retractor activity in the same segment. Original recording of a preparation made rhythmically active by application of pilocarpine. Gray arrows denote the start of retractor bursts (small unit: slow retractor coxae); white arrows denote the expected start if no reset occurs. Stimulus onset describes the latency between retractor burst onset and stimulus. Burst-duration and cycle period show a significant dependency on stimulus onset, while the inter-burst interval shows no such dependency (N=3; n=39; square symbol: mean ± SD; horizontal line: mean value of control cycles ± SD; hollow symbols indicate data from one animal).

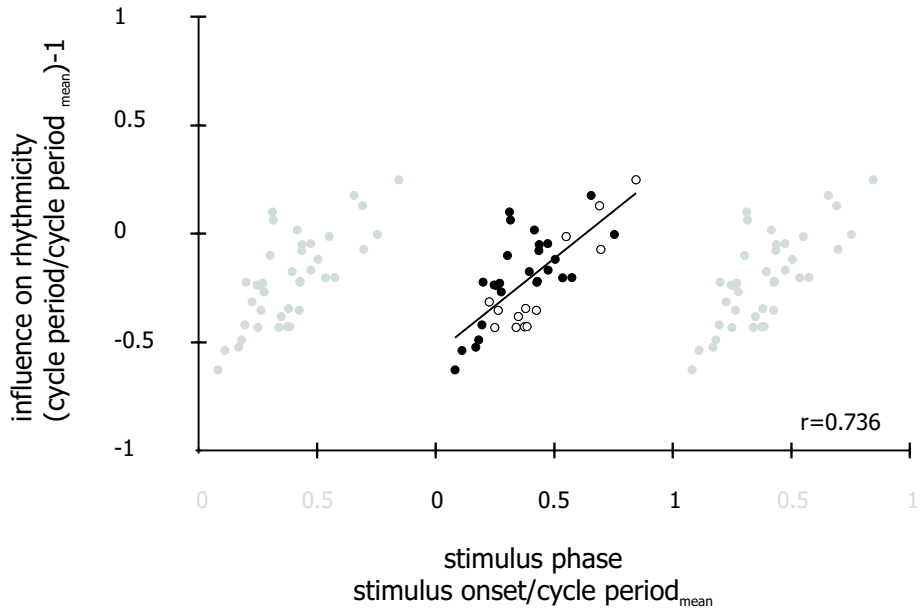

Figure 3.35: Reset-plot showing data normalized with mean cycle period from three control cycles preceding the cycle influenced by the stimulus. A correlation exists between the phase of stimulation and the influence on rhythmicity (N=3; n=39).

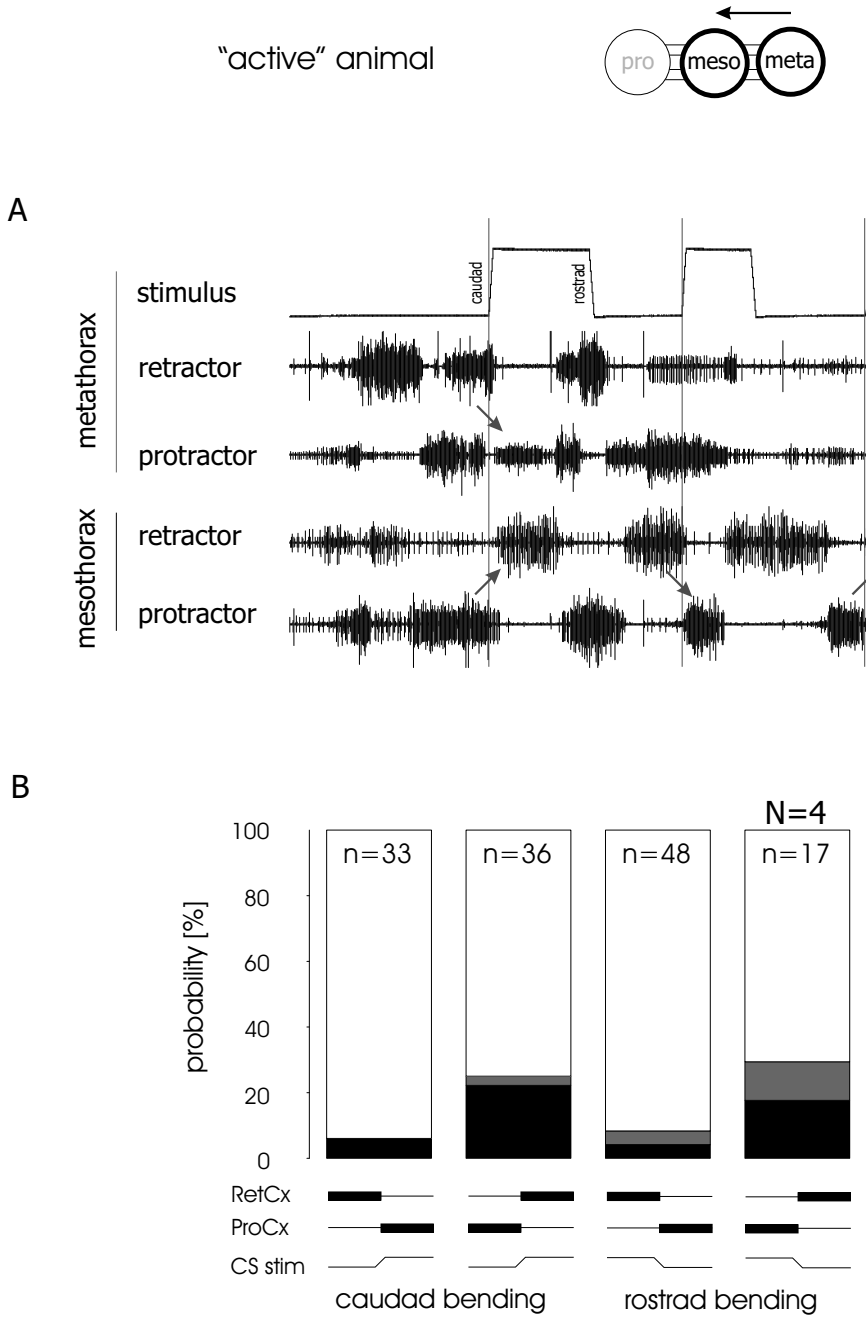

Figure 3.36: Intersegmental influence of stimulation of metathoracic campaniform sensilla on activity of protractor and retractor coxae motoneurons in the metathorax (upper two traces) and the mesothorax (lower two traces) in the 'active' animal. Arrows point out transitions in activity between antagonists shortly after stimulus onset. The histogram shows the probability for a switch (black) or truncation (gray) to occur shortly after stimulus onset. The stimulation occurred by caudad (left half) or rostrad (right half) bending of the femur and either during a retractor (left) or a protractor (right) burst.

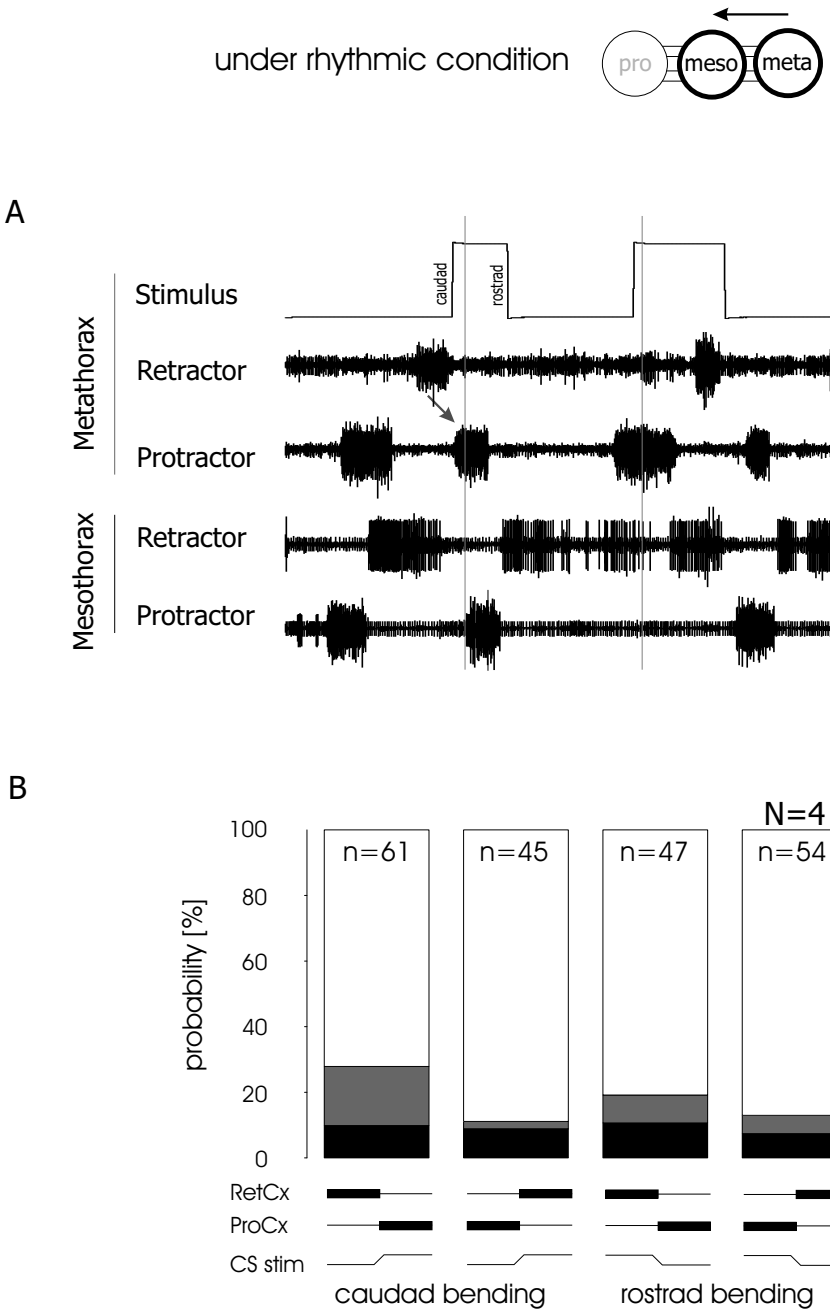

Figure 3.37: Intersegmental influence of stimulation of metathoracic campaniform sensilla on activity of protractor and retractor coxae motoneurons in the metathorax (upper two traces) and the mesothorax (lower two traces) under rhythmic condition, induced by pilocarpine. Arrows point out transitions in activity between antagonists, shortly after stimulus onset. The histogram shows the probability for a switch (black) or truncation (gray) to occur shortly after stimulus onset. The stimulation occurred by caudad (left half) or rostrad (right half) bending of the femur and either during a retractor (left) or a protractor (right) burst.

Chapter 4

Discussion

4.1 Correlation of mesothoracic motoneuron activity and front leg walking

Extracellular recordings of mesothoracic motoneuron activity in the semi-intact walking preparation revealed a correlation of motoneuron activity and front leg walking activity. With a stance phase of the front leg, protractor coxae activity decreased, while extensor tibiae (FETi and SETi) activity increased. Activity of depressor trochanteris motoneurons was modulated, but coupling with the front leg appears to be more variable: activity was either low (response type I) or high (response type II) during a front leg's stance phase (chapter 3.1).
As extracellular recordings only show the summed activity of a motoneuron pool, the activity of individual motoneurons could differ. All individual motoneurons, intracellularly recorded from the protractor pool, which consists of 9-12 motoneurons, showed a qualitatively identical modulation of activity (chapter 3.2). Data on SETi and FETi motoneurons were consistent for extracellular and intracellular recordings. The intracellular recordings of depressor motoneurons showed modulations of membrane potential that were smaller in amplitude and more variable than the modulations seen in other motoneurons. This is in agreement with the results from the extracellular recordings, where two different response types were classified. These response types were not specific for fast or slow depressor motoneurons. The coupling of depressor motoneurons might require additional information not present in the semi-intact walking preparation, such as local sensory information. Depressor motoneurons control the movement of the coxa-trochanteral leg joint. The control of this leg joint is of particular importance for the support of the animal's body weight (Cruse, 1976). It is conceivable that therefore local sensory signals are of higher importance, than for the control of the thoraco-coxal joint and the femur tibia joint, which are controlled by protractor and extensor motoneurons respectively. This important function of depressor motoneurons could also be accounted for by a separate intersegmental coordinating pathway, which could be reflected in the higher degree of variability of the rhythmic modulation.

A discrepancy between the semi-intact walking preparation and a freely walking animal becomes clear when the activity patterns of motoneurons observed are compared to the activity patterns expected for a tetrapod gait. The latter is the predominant gait of an adult stick insect walking with moderate speed (Graham, 1972). At time of the start of the front leg's stance phase, the middle leg's stance phase should be in its second quarter (Cruse et al., 1995). This means that the protractor and extensor motoneurons should be inactive, while depressor motoneurons should still be active (Fischer et al., 2001). This expectation was roughly fulfilled only for the activity of the protractor and single depressor motoneurons recorded. Other depressor motoneurons and extensor motoneurons showed an activity pattern not matching the expectations for a tetrapod gait. Additional local sensory information or coordinating signals from contralateral legs and/or hind legs, both not available in the reduced walking preparation, might be required to produce the coordination pattern of a tetrapod gait.

Cruse (1990) derived a set of 'rules' from behavioral experiments that are sufficient to explain a walking pattern as seen in walking stick insects. 'Rule' number three describes a backward directed influence, facilitating the start of a swing phase with the anterior leg moving backwards. That is, motoneurons active during the swing phase should increase activity with the progress of the stance phase of the front leg. Motoneurons active during the beginning swing phase of the middle leg are protractor coxae, levator trochanteris, and extensor tibia motoneurons (Graham, 1985). Protractor and levator motoneurons showed the expected increase of activity. Also extensor motoneurons showed an increase of activity, although it occurred very early in, or even shortly before the start of, the front leg's stance phase.

The basic alteration of activity in antagonistic motoneuron pools of each leg joint, is thought to be produced by a central rhythm generator for each joint (Bässler and Büschges, 1998). For example for extensor and flexor motoneurons, its action leads to alternating depolarizations of extensor and flexor membrane potentials (Schmidt et al., 2000). Such a clear alteration was not found in the semi-intact walking preparation. The intracellular recordings of both, extensor and antagonistic flexor motoneurons, showed hyperpolarized membrane potentials before the start of a front leg's stance phase. The antagonistic action arises from a following quick depolarization of extensor motoneurons, while flexor motoneurons are only slowly depolarized. Flexor motoneurons reach most positive potentials clearly later in a step cycle than extensor motoneurons. The lack of clearly alternating activity suggests that intersegmental influences rather act in a modulatory way. Local control mechanisms, that would lead to a sharper transition of activity of antagonistic motoneurons pools, might not be active in the mesothoracic segment of the semi-intact walking preparation.

4.2 Origins of coordinating signals

4.2.1 Intersegmental effects of sensory organs

Göritz (2003) performed experiments which point to a possible origin of intersegmental signals. She simulated a flexion of the front leg by specifically stimulating the femoral chordotonal organ, a sense organ measuring position and movement of the femur-tibia joint. Such a flexion occurs at the transition from swing to stance phase during walking.
Göritz (2003) found a stimulus correlated reduction of protractor motoneuron activity in the mesothoracic segment of 'active' animals. Activity of mesothoracic retractor motoneurons increased. Thus, signals from the front leg's chordotonal organ could contribute to the reduction of protractor and the increase of retractor activity seen in the semi-intact walking preparation.
The slight increase of depressor motoneuron activity observed by Göritz (2003), parallels the increased activity of depressor motoneurons of response type II in the semi-intact walking preparation. But signals from the femoral chordotonal organ are most likely not its only cause, as depressor activity already started to increase before a transition from swing to stance phase of the front leg.
In 'active' animals, Göritz (2003) found an increase of mesothoracic flexor tibiae activity upon stimulation of the front leg's femoral chordotonal organ. In general no such change was observed for extensor tibiae motoneuron activity, though in some experiments it also increased after stimulation (Göritz, personal communication). Such an increase of extensor motoneuron activity matches the data of the semi-intact walking preparation. The increase of flexor activity occurred very rapidly after stimulation, while flexor motoneurons were only slowly depolarized after the font leg's transition from swing to stance phase in the semi-intact walking preparation. Hence, signals from the femoral chordotonal organ may contribute to the modulation of the extensor and flexor motoneuron activity observed in the semi-intact walking preparation, but are unlikely to be their major source.

Stein (1998) investigated the intersegmental distribution of signals from the femoral chordotonal organ in the resting stick insect. Stimulating the femoral chordotonal organ of a middle leg, he showed that information about position and movement of the femur-tibia joint affects the activity of extensor motoneurons of all other legs. This influence was only visible after bath application of picrotoxin, a non-competitive inhibitor of GABA action. Stein argues that in the resting animal coordinating pathways are shut off by the influence of GABA-activated chloride channels. In the semi-intact walking preparation these pathways presumably were functional, as the animal clearly was in an 'active' state. The release from chloride channel mediated synaptic inhibition might be reflected in the tonic depolarization seen in many intracellular recordings of neurons.

While signals from the front leg's chordotonal organ could contribute to the modulation of mesothoracic protractor and retractor motoneurons, and maybe to a smaller degree also to the modulation of depressor, flexor, and extensor motoneu-

rons, the role of such signals might be different in other segments. Experiments with stimulation of the middle leg's chordotonal organ showed no clear effect on metathoracic motoneuron activity (chapter 3.5). Thus, for the coordination of movements of the middle and hind leg of an animal, other mechanisms than signals of the adjacent ipsilateral chordotonal organ might be of greater importance.

Another sensory organ studied were the campaniform sensilla. Comparing their local effects on protractor and retractor activity between segments revealed segment specific differences. While in the resting animal stimulation of mesothoracic (Akay, 2002) as well as metathoracic (chapter 3.6) campaniform sensilla promoted a switch from protractor to retractor motoneuron activity, the effect was different in the 'active' or pilocarpine activated animal: stimulation of metathoracic, but not mesothoracic, campaniform sensilla showed a reversal of the effect and promoted a switch from retractor activity to protractor activity. This reversal might be linked to the different 'intrinsic walking directions' described by Bässler et al. (1985).

Regarding the intersegmental effect of signals from the metathoracic campaniform sensilla, it seems unlikely that they play an important role for the generation of a stepping pattern (chapter 3.6). Nevertheless, campaniform sensilla of the prothoracic or mesothoracic segments might be of importance for intersegmental coordination and still need to be investigated.

In addition to the sensory organs studied, several more are found in stick insects. Examples are hair plates on the leg's coxa and trochanter, multipolar sense cells on the femur-tibia joint, or tension receptors (Wendler, 1964; Bässler, 1977). They all possibly contribute to intersegmental coordination and therefore need to be further studied in this context.

4.2.2 Coupling of segmental central pattern generators

To study the role of coupling of segmental central pattern generators, sensory information needs to be experimentally removed. This generates the problem of evoking the behavioral state of locomotion. An approach is pharmacological stimulation with pilocarpine, as it was used e.g. in experiments with crayfish or insects (Chrachri and Clarac, 1987; Ryckebusch and Laurent, 1993; Büschges et al., 1995a; Johnston and Levine, 1996a, 2002). The cholinergic agonist pilocarpine evokes rhythmic, alternating activity of antagonistic motoneuron pools. Therefore it activates at least parts of the neural network used for locomotion. In experiments, the activities of motoneuron pools in adjacent ganglia were studied, while only one ganglion was pharmacologically activated (chapter 3.4).
No evidence for strong coupling of motoneuron activity in different segments by central coupling mechanisms, could be found. Nevertheless, pooling data from five animals revealed a very weak modulation of metathoracic extensor tibiae motoneuron activity. Activity slightly increased prior to the start of a mesothoracic depressor burst. A transition from levator to depressor activity occurs in the intact walking animal at the transition from a swing to a stance phase. Activity of mesothoracic

extensor motoneurons was analyzed in the semi-intact walking preparation around the transition from swing to stance phase of the front leg. It also showed an increase. No such parallels were found for the second influence observed in the preparation of the isolated chain of ganglia, a slight increase of activity of protractor coxae motoneurons approximately 0.8 seconds after the start of a mesothoracic depressor burst.
Despite the weak modulation of metathoracic motoneuron activity found, it should be kept in mind that both effects could only be revealed after pooling data from several experiments. Central coupling appears to be very weak, and is unlikely to play an important role for the intersegmental coordination of walking movements in the stick insect. This is in agreement with observations by Ryckenbusch and Laurent (1994) in locusts and by Büschges et al. (1995a) in stick insects.
Nevertheless, Johnston and Levine (2002) report from similar experiments with the hawkmoth *Manduca sexta*, in which they found coordinated activity of leg motoneurons in all three thoracic ganglia. The patterns observed parallel the patterns for a tripod gait. Central coupling mechanisms appear to be sufficient for a basic tripod-like coordination pattern, although this basic pattern must be modified by additional influences, as a much more complex stepping pattern is observed in intact walking animals (Johnston and Levine, 1996b).
Coupling of central pattern generators seems to be of different importance for ensuring intersegmental coordination in hawkmoth and at least some orthopteroidea (locusts and stick insects). Especially for stick insects a more flexible control of leg movements, with stronger consideration of sensory input, might be of importance, as slow walking and climbing on irregular terrain is their primary mode of locomotion. A rather inflexible central coupling of leg movements would be clearly disadvantageous in this environment. Hawkmoths mostly fly, and walking is only rarely used. It is therefore possible that a primarily central coordinating mechanism with modifications by sensory signals is an evolutionary favorable solution.
An uncertainty remains that the different results of experiments with hawkmoth and orthopteroidea are due to different effects of pilocarpine in these animals. Due to differences in the nervous system, pilocarpine might not activate networks necessary for the inter-ganglionic exchange of information in orthopteroidea. Additionally, pilocarpine had better access to the neurons in the experiments with *Manduca sexta*, as Johnston and Levine (2002) desheathed the ganglia, while using comparable concentrations in the bath.

4.2.3 Peripheral vs. central coordinating mechanisms

In the semi-intact walking preparation the front leg provided a most complete set of sensory signals from one leg, but local sensory information and information from other legs were not available to the mesothoracic segment. The prothoracic motor network was in a state to produce walking activity. This was sufficient to induce a modulation of mesothoracic motoneuron activity, although the modulation did not fully resemble the activity patterns expected from an intact, walking animal. A clear intersegmental coordinating effect of signals from single sensory organs is only shown for influence from the prothoracic chordotonal organ on ipsilateral mesotho-

racic protractor, retractor, and flexor motoneurons (Göritz, 2003). No clear central coordinating mechanisms could be found and mechanical coupling appears to be not a relevant mechanism (Graham and Cruse, 1981; Epstein and Graham, 1983). This leads to the conclusion that the coordinated stepping pattern of a walking stick insect might result from the integration of intersegmental and local sensory information from several sensory organs, a dominant role of a sensory organ not yet studied, not excluded.

4.3 Inputs on motoneurons

Intracellular recordings from the semi-intact walking preparation not only revealed the correlation of front leg walking and modulation of mesothoracic motoneurons, but also mechanisms by which this modulation occurs. They showed two different influences on the motoneurons studied: a tonic shift of membrane potential that persisted during the whole walking sequence of the front leg, and a rhythmic modulation of the membrane potential that mostly was directly correlated with individual steps of the front leg.

The tonic change had the form of a depolarization in protractor and extensor motoneurons. Depressor motoneurons did not always show a tonic depolarization, but some neurons recorded, showed a tonic hyperpolarization. In recordings of extensor and some depressor motoneurons, the amplitude of the tonic depolarization increased when the neuron was experimentally hyperpolarized by injection of constant current via the electrode. In depressor motoneurons showing a tonic hyperpolarization, the latter decreased in amplitude, and was reversed for more negative potentials. This suggests that the tonic shifts originate from excitatory and inhibitory inputs from the pre-motor network or intersegmental interneurons. Resistance measurements by injection of short hyperpolarizing current pulses of known amplitude were performed in many experiments with different motoneurons. Only in a few recordings of depressor motoneurons a clear reduction of membrane resistance was seen. This reduction is most likely caused by the opening of ion channels and its time course matches the time course of the tonic depolarizations seen in these recordings. Thus opening of ion channels, in response to an excitatory input, could cause the tonic depolarization. The lack of similar observations in other motoneurons could be due to technical limitations. The site of recording/injection might have had a too large electrotonic distance from the site of inputs, responsible for the tonic shifts of membrane potential, to affect them.
As the tonic depolarization had a rather slow time course, with the repolarization after the end of the walking sequence showing time constants in the range of 0.1 to 0.6 seconds, it could also result from neuromodulation without direct synaptic action. A depolarizing effect of the neuromodulator octopamine on flexor tibiae motoneurons was described by Parker (1996) in locusts.
Depressor motoneurons did not always show a tonic depolarization, but one slow and one fast motoneuron recorded showed a tonic hyperpolarization. Experimental hyperpolarization led to a decrease of its amplitude and even reversal. This suggests

the existence of an additional tonic inhibitory input depressor motoneurons.

In general, the tonic depolarization could reflect a state of arousal that is initiated with the activation of the animal. Excitatory, and in some cases inhibitory, inputs on the motoneurons, change their membrane potential during activity. By bringing it closer to the spike threshold, the excitability of a motoneuron could be increased, making contractions of the associated muscle more likely.

The second influence on motoneurons was a rhythmic modulation of the membrane potential. It was usually correlated with individual steps of the front leg, although single changes of membrane potential occurred without visible movements of the front leg. These changes could arise from additional rhythmic inhibitory or excitatory inputs, or from discontinuation of a tonic influence.
An exceptional recording of a FETi motoneuron showed distinct inhibitory post synaptic potentials (IPSPs) prior to and during the stance phase of the front leg. This suggests that an inhibitory input from a spiking neuron is involved in the formation of the modulation seen in FETi, although an interposed non-spiking interneuron can not completely be ruled out (Laurent and Burrows, 1989b). In locusts Laurent and Burrows (1989a,b) showed direct synaptic connections from mesothoracic spiking intersegmental interneurons and afferents onto motoneurons of the coxo-trochanteral, femoro-tibial, and tibio-tarsal joint of the hindleg. Similar synaptic connections could be responsible for the IPSPs seen in FETi.
In recordings of depressor motoneurons, experimental hyperpolarization led to an increase of amplitude of rhythmic changes. This suggests excitatory inputs as another basis of the modulation. Recordings of FETi motoneurons did not show such an increase of amplitude during constant hyperpolarization; resistance measurements again showed no change of resistance. This might be due to a large electrotonic distance between the site of recording/injection and site of inputs on the cells responsible for the rhythmic modulation. Laurent and Burrows (1989b) described intersegmental inputs from hind leg afferents onto mesothoracic non-spiking local interneurons. Stimulation of the hind leg's tarsus evoked excitatory post synaptic potentials (EPSPs) in the interneuron, but similar to the results discussed, resistance measurements often did not show an underlying conductance change. Laurent and Burrows (1989b) propose a compartmentalization, with synaptic inputs and their associated conductance changes restricted to particular branches of the interneuron. Possibly the motoneurons studied are likewise compartmentalized, and inputs causing the rhythmic modulation of membrane potential are only limited to certain branches.

4.4 The role of the pre-motor network

Several studies described intersegmental interneurons that could provide the information needed for the inputs described. Laurent and Burrows (1989a,b) found in locusts a population of intersegmental interneurons that make direct synaptic con-

nections with non-spiking local interneurons and leg motoneurons in the neighboring ganglion. Büschges (1989) described a population of interneurons with intersegmental processes in the stick insect. These interneurons respond to stimulation of the chordotonal organ and can mediate this sensory information between different ganglia. Their target neurons in the adjacent ganglia are not known. Brunn and Dean (1994) presented several interneurons which code for the position of the adjacent leg and possess intersegmental processes. Local projections of these interneurons are also unknown.
As the local sensory input is of high importance for the control of leg muscles in stick insects (Bässler and Büschges, 1998), local and intersegmental information must be integrated. Laurent and Burrows (1989a,b) showed that local non-spiking interneurons in locusts are a point of convergence of local and intersegmental pathways. In stick insects, a well researched group of non-spiking interneurons, which is involved in the femur-tibia control loop, could adopt this function. Providing synaptic drive onto motoneurons, these interneurons themselves receive strong synaptic drive from central rhythm-generating networks, play an important role in the generation of adaptive reflexes, and some are known to respond to tactile stimuli to the tarsi of ipsilateral legs in the standing animal (Büschges et al., 1994; Büschges, 1995a; Kittmann and Büschges, 1996). A series of experiments was performed to explore whether non-spiking interneurons could be responsible for the inputs to motoneurons observed in the semi-intact walking preparation (Chapter 3.3).

All of the interneurons recorded, including the morphological identified ones, showed a tonic and at least a weak rhythmic modulation of their membrane potential - similar to the effects described for motoneurons. Thus, non-spiking interneurons mediate the intersegmental information, although a recording of a FETi motoneuron suggested an additional pathway for intersegmental information, which can also not be excluded for other motoneurons (Fig. 4.1).

Tonic, as well as rhythmic modulation was various among the non-spiking interneurons. Tonic depolarization and tonic hyperpolarization was found in both, interneurons with excitatory and inhibitory effect on extensor motoneurons. The phasing of modulations in the stepping cycle of the front leg were diverse. These different activity patterns most likely result from different connectivity and intrinsic properties. Further modification of activity is added by local sensory input in the intact animal. In some experiments spontaneous bursts of extensor motoneurons were seen in the extracellular recording. Simultaneously, a strong depolarization of the membrane potential of the interneuron recorded occurred. The amplitudes of these spontaneous depolarizations were bigger than the amplitudes of the modulation observed in connection with front leg's stepping activity. They might reflect strong influences of local signals on the non-spiking interneuron.
Activity patterns of motoneurons result from the integration of excitatory and inhibitory inputs from several interneurons. Such a form of information processing was described as *distributed processing* (Sauer et al., 1996) or *parliamentary principle* (Bässler, 1993; Büschges et al., 1999) for the stick insect's femur-tibia control system. With a computer simulation and physiological experiments, Sauer et al.

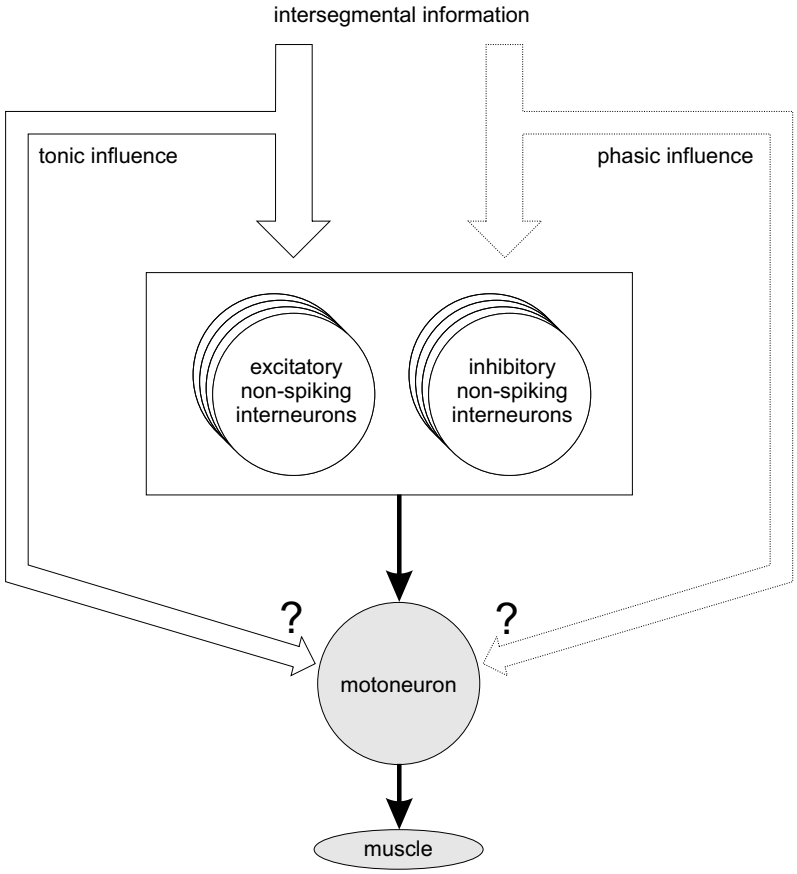

Figure 4.1: Intersegmental coordinating signals persist during walking activity of the adjacent leg (tonic) or correlate with individual steps (rhythmic modulation). They influence non-spiking interneurons of the pre-motor network, which in turn alter the activity of motoneurons. An additional direct pathway for intersegmental signals on motoneurons can not be excluded.

(1996) could show that posture control of the femur-tibia joint can be explained by the action of parallel and antagonistic pathways, formed by a network of identified non-spiking interneurons. Similarly for the processing of intersegmental information, several interneurons with different phasing and amplitudes of modulations in the stepping cycle of the front leg project on a single motoneuron, with different synaptic strength providing a weighting of the inputs. By integration of these inputs, the activity of the motoneuron is determined and the corresponding muscle controlled.

The identified interneuron E4 is a good example to illustrate the function of non-spiking interneurons for the processing of intersegmental coordinating information. The membrane potential of interneuron E4 is depolarized in response to elongation and, with smaller amplitude, relaxation stimuli of the local femoral chordotonal organ (Büschges, 1990; Sauer et al., 1995). Stimulation of the local campaniform sensilla is known to evoke a hyperpolarization of E4's membrane potential (Akay, 2002). In the semi-intact walking preparation, interneuron E4 showed a clear tonic

as well as a rhythmic modulation, correlated with the activity of the ipsilateral front leg; that is, it has access to intersegmental coordinating information. Thus in the intact animal, E4 is an interneuron that provides the integration of local sensory information with intersegmental information.
Beside the excitatory effect on extensor motoneurons, E4 is also known to excite protractor and levator motoneurons (all mainly active during the swing phase of a leg) and to inhibit retractor and depressor motoneurons (both mainly active during the stance phase) (Büschges, 1995a). In the semi-intact walking preparation E4's membrane potential was depolarized during a front leg's stance phase. As E4 has an excitatory effect on motoneurons mainly active during the swing phase of a leg, it would facilitate a swing phase in the ipsilateral middle leg. The motoneurons integrate this input with inputs from other non-spiking and possibly spiking interneurons, to produce the activity patterns which determine movements of the leg joints necessary for walking. (Fig. 4.2).

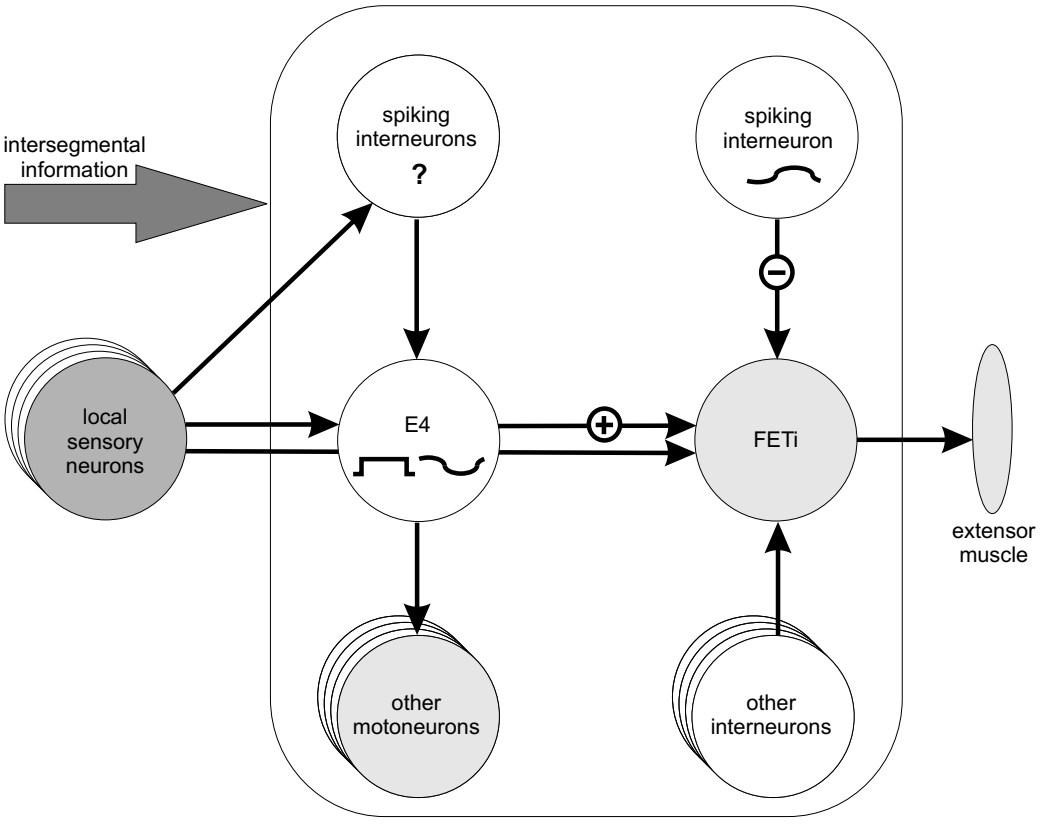

Figure 4.2: Non-spiking interneuron E4 is known to receive direct and indirect inputs from local sensory organs. It also has access to intersegmental coordinating signals and is therefore a point of integration for local and intersegmental information. E4 influences the activity of the fast extensor tibiae (FETi) and other motoneurons, some controlling movements of other leg joints. The FETi motoneuron integrates inputs from E4 and other non-spiking and spiking interneurons to determine the activity of the extensor muscle.

4.5 Conclusions

It was shown that activity of motoneurons controlling movements of the middle leg, is modulated in a manner correlated with movements of the walking, ipsilateral front leg. Details of how this modulation is realized were described. Non-spiking interneurons of the pre-motor network were shown to mediate the intersegmental information required for the coordination and to provide the basis for integration of local sensory and intersegmental signals. No evidence was found that coupling of central pattern generators is used for intersegmental coordination in stick insects. In the segments of middle and hind leg, signals from the femoral chordotonal organ and the campaniform sensilla do not contribute to intersegmental coordination.
Although some aspects of the question "What is the neural basis of intersegmental coordination of walking movements?" could be answered, the results raise many new questions. The experiments concentrated mainly on the coordination between a front leg and the ipsilateral middle leg. The coordinating mechanisms of the four remaining legs are currently under investigation by Borgman (2003). Little is known about the origin of coordinating signals in the walking segment and the intersegmental interneurons distributing them to the other ganglia. The origin of the tonic effects described needs to be investigated. Experiments need to clarify the interaction of local with intersegmental information.

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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. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. A. Büschges betreut worden.

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