State-dependent long lasting modulation of leg motoneuron membrane potential during stick insect walking.

Inaugural - Dissertation

zur

Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität zu Köln

vorgelegt von

Sandra Westmark

aus Emden

Köln

Mai 2007

Berichterstatter:
PD Dr. Joachim Schmidt
Prof. Dr. Peter Kloppenburg

Tag der mündlichen Prüfung:

28.06.2007

Abstract

The basis of locomotion is the rhythmic activity of locomotor organs. During walking, rhythmic alternating bursts occur in leg motoneurons which result from the integration of signals from central pattern generators, sense organs and coordinating signals from neighboring segments. The rhythmic bursting pattern is shaped by rhythmic excitatory and inhibitory drive, as well as a long lasting (tonic) depolarizing modulation.

This dissertation investigates the mechanisms that underlie the tonic modulation of neuronal activity, which is the basis of the rhythmic activity. A single-legged preparation of the stick insect was used, that allows the analysis of neuronal activity in mesothoracic motoneurons during front leg stepping, without the influence of local sense organs. Intracellular recordings of the membrane potential in flexor motoneurons ipsilateral and contralateral to the stepping front leg revealed a mean tonic membrane depolarization of 1.8 ±1.1 mV that could outlast the stepping sequence by several seconds. Furthermore, a phasic modulation of membrane potential occurred on top of the tonic depolarization, which was variably coupled to front leg steps. The tonic depolarization was associated with a decrease in input resistance, its amplitude depended on membrane potential and its mean reversal potential was found to be -41 mV. These properties of the tonic depolarization indicate that it is based on a nonselective cation conductance or a mixed inward and outward current through different channels. Furthermore, the tonic depolarization increased the excitability of the membrane to depolarizing input and was found to have long repolarization time constants ($\bar{\tau}$=800 ms).

Pharmacological experiments were performed to identify the participating transmitter(s) and to test for a possible involvement of second messengers. Flexor motoneurons were recorded intracellularly while superfusing pharmacological agents, which were restricted to the mesothoracic ganglion. The muscarinic antagonist atropine decreased the tonic depolarization amplitude, thus indicating a role for acetylcholine in mediating the tonic depolarization via metabotropic receptors. Other transmitters/receptors might be involved too, as octopamine increased the tonic depolarization amplitude. This was supported by the reduction of the tonic depolarization amplitude by an octopaminergic antagonist (mianserin). Serotonin had an opposing effect on the tonic depolarization amplitude. It was shown that serotonin increased, but also decreased the tonic depolarization amplitude in different experiments.

Several second messenger pathways might be involved in mediating the tonic depolarization, one of which seems to include calcium in the flexor motoneurons. Furthermore, the increase of the tonic depolarization amplitude by 8-Br-cAMP suggests a role for cAMP in mediating the tonic depolarization. An involvement of an IP_3/DAG pathway was indicated by the increase in tonic depolarization in the presence of neomycin and U-73122.

Additional experiments addressed the role of the brain (supraesophageal ganglion) in controlling neuronal activity in the mesothoracic ganglion induced by front leg stepping. Especially the relevance of the brain for generating the tonic depolarization was tested in lesion experiments. Brain removal decreased the tonic depolarization amplitude, thus indicating a role for descending pathways in influencing the tonic depolarization. Another preparation was used to investigate the neuronal activity in neck connectives during front leg stepping. The neuronal activity increased and was correlated to front leg stepping velocity. In brainless animals, however, the increased activity in neck connectives was diminished.

Zusammenfassung

Fortbewegung beruht auf der rhythmischen Aktivität der Fortbewegungsorgane. Während des Laufens treten rhythmisch alternierende Aktionspotentialsalven in Beinmotoneuronen auf, die aus der Integration von Signalen zentraler Rhythmusgeneratoren, Sinnesorganen und koordinierenden Signalen aus benachbarten Segmenten resultieren. Das rhythmische Muster von Aktionspotentialsalven wird durch rhythmisch erregende und hemmende Eingänge, sowie durch eine lang anhaltende (tonische) depolarisierende Modulation strukturiert.

Die vorliegende Dissertation untersucht die der tonischen Modulation neuronaler Aktivität unterliegenden Mechanismen, welche die Basis der rhythmischen Aktivität sind. Es wurde ein Einbein-Präparat der Stabheuschrecke verwendet, welches die Analyse neuronaler Aktivität in mesothorakalen Motoneuronen ohne die Einflüsse der lokalen Sinnesorgane während des Vorderbein-Laufens erlaubt. Intrazelluläre Ableitungen des Membranpotentials von Flexor Motoneuronen ipsi- und kontralateral zum laufenden Vorderbein zeigten eine mittlere tonische Depolarisation der Membran von 1.8 ±1.1 mV, welche die Schrittsequenz um mehrere Sekunden überdauern konnte. Außerdem trat eine phasische Modulation des Membranpotentials auf, die variabel an Vorderbeinschritte gekoppelt war. Die tonische Depolarisation ging mit einer Abnahme des Eingangswiderstands einher, ihre Amplitude hing vom Membranpotential ab und das Umkehrpotential lag bei -41 mV. Diese Eigenschaften der tonischen Depolarisation weisen darauf hin, dass sie entweder auf einer unselektiven Kationen-Leitfähigkeit oder einem gemischten

Einwärts- und Auswärtsstrom durch verschiedene Kanäle basiert. Außerdem steigerte die tonische Depolarisation die Erregbarkeit der Membran für depolarisierende Eingänge und zeigte lange Repolarisations-Zeitkonstanten ($\bar{\tau}$= 800 ms).

Es wurden pharmakologische Experimente durchgeführt um die beteiligten Transmitter zu identifizieren und die mögliche Mitwirkung von second messengern zu testen. Während einer auf das mesothorakale Ganglion beschränkten Applikation von Pharmaka wurde intrazellulär von Flexor-Motoneuronen abgeleitet. Der muskarinerge Antagonist Atropin reduzierte die Amplitude der tonischen Depolarisation und deutet auf eine Rolle von Acetylcholin bei der Übertragung der tonischen Depolarisation über metabotrope Rezeptoren hin. Es scheinen aber auch andere Transmitter bzw. Rezeptoren beteiligt zu sein, da Oktopamin die Amplitude der tonischen Depolarisation vergrößerte. Die Reduktion der Amplitude der tonischen Depolarisation durch den oktopaminergen Antagonisten Mianserin unterstützte diesen Befund. Serotonin hatte gegensätzliche Wirkung auf die Amplitude der tonischen Depolarisation. Es wurde gezeigt, dass Serotonin die Amplitude der tonischen Depolarisation in verschiedenen Experimenten vergrößerte oder reduzierte.

Verschiedene second messenger Wege könnten an der Übertragung der tonischen Depolarisation beteiligt sein, möglicherweise unter Nutzung von Calcium in den Motoneuronen. Eine Erhöhung der tonischen Depolarisation durch 8-Br-cAMP weist zudem auf eine Beteiligung von cAMP bei der Übertragung der tonischen Depolarisation hin. Eine Beteiligung eines IP_3/DAG Weges lässt der Anstieg der tonischen Depolarisation in Gegenwart von Neomycin und U-73122 vermuten.

Ergänzende Experimente behandelten die Rolle des Gehirns (Oberschlundganglion) bei der Kontrolle neuronaler Aktivität im mesothorakalen Ganglion, die durch Schreiten des Vorderbeins induziert wurde. Speziell die Relevanz des Gehirns für die Erzeugung der tonischen Depolarisation wurde in Läsionsexperimenten untersucht. Das Entfernen des Gehirns reduzierte die Amplitude der tonischen Depolarisation, und deutet damit auf eine Rolle deszendierender Bahnen bei der Beeinflussung der tonischen Depolarisation hin. In einer weiteren Präparation wurde die neuronale Aktivität in Halskonnektiven während Schreitens des Vorderbeins untersucht. Die neuronale Aktivität zeigte eine mit

der Schreitgeschwindigkeit des Vorderbeins korrelierte Zunahme. In gehirnlosen Tieren war der Aktivitätsanstieg in den Halskonnektiven vermindert.

Contents

CHAPTER 1

Introduction

Throughout the animal kingdom, movement through the environment - locomotion - is a necessary part of behaviors such as foraging, escape or reproduction. Different forms of locomotion have evolved, such as walking, running, flying, swimming and crawling. Rhythmic muscle contractions lead to movements of limbs or appendages respectively, and/or the body itself. In vertebrates and invertebrates, these movements are controlled by central pattern generator networks (CPGs), that lead to coordinated, repetitive contractions of antagonistic muscles (Pearson 1993; Grillner 2003; Selverston 2005; Büschges 2005; Marder et al. 2005). Motor rhythms are the expression of oscillatory discharges from an ensemble of neurons wired together to generate a coherent motor output.

Rhythmic motor patterns play also a crucial role in nonlocomotory systems as respiration, heartbeat, chewing or saccadic eye movements (Pearson 1993; Calabrese et al. 1995; Grillner 2006). Interaction of the centrally generated activity and sensory information from various sense organs leads to a functional motor output (e.g., reviewed in Grillner and Wallen 2002; Fouad et al. 2003; Pearson 2004; Zill et al. 2004; Büschges 2005). Sense

organs ensure a functionally, coordinated and efficient behavior by monitoring and adjusting it to the actual environment. The degree of sensory information needed depends on the complexity of the locomotor organs and on the properties of the environment through which the animal moves (Orlovsky et al. 1999). Furthermore, central commands and neuromodulators influence the patterns produced by CPGs.

1.1 Descending control of motor activity

Motor activity can be influenced in its initiation, maintenance and adaptation by descending signals from the brain. In the lamprey, the transition from the quiescent to the active state is induced by activation of the reticulospinal system (RS) via mesencephalic (MLR) and diencephalic locomotor regions (Deliagina et al. 2002; Grillner et al. 1998; Grillner and Wallen 2002). The RS is the main descending system and activates glutamatergic receptors on both excitatory and inhibitory interneurons and the output motoneurons (MNs) in the spinal cord. Furthermore, a range of behaviors that include coordinated motor activity, can be elicited by stimulation of discrete brain regions in vertebrates and invertebrates. Stimulation of the MLR in cats activates RS neurons and evokes locomotion (Mori et al. 1998; Jordan 1998), and wing flapping in birds can be induced by stimulating brainstem regions (Steeves et al. 1987).

Stimulation of single neurons was shown to evoke motor activity in invertebrates, such as the activation of swimmerets in the crayfish (Hughes and Wiersma 1960; Wiersma and Ikeda 1964). Those neurons were termed "command neurons" by Wiersma and Ikeda (1964), and Kupfermann and Weiss (1978) defined them to be both, necessary and sufficient for evoking a motor act. Only few examples are known up to now that meet both criteria, for example the dorsal ramp interneuron in *Tritonia* that elicits the swim motor program due to stimulation above a certain frequency (Frost and Katz 1996). In general, these neurons usually act parallel with other elements of the network, thus distributing the command properties. Leech swimming can be initiated and terminated/suppressed by trigger neurons located in the subesophageal ganglion (SEG) (Tr1 and Tr2, respectively; Kristan and Weeks 1983; Brodfuehrer et al. 1995; Taylor et al. 2003). Evidence for descending influences on motor activity in insects was obtained from several species

(e.g., cockroach walking, flight: Gal and Libersat 2006; Ridgel and Ritzmann 2005; cricket walking, mating and stridulation: Böhm and Schildberger 1992; Matsumoto and Sakai 2000; Hedwig 2000; locust walking: Kien 1983). In stick insects, the influence of higher brain centers on walking activity was analyzed by the means of lesion experiments. According to these experiments, transection of head-connectives suggests an inhibitory influence of the supraesophageal ganglion (brain) on spontaneous walking activity and an opposing effect of the SEG (Graham 1979a;b; Bässler 1983; Graham 1985). Up to now, nothing is known about the activity of single descending interneurons (DINs) in stick insects, but Altman and Kien (1979) showed that locusts' DINs in the SEG are active during leg movements with varying response properties.

1.2 Modulatory influences

Neuronal networks have to produce flexible outputs for behaviors adapted to varying environments. There are several ways of altering the motor output, for example by sensory and descending influences (see above) or neuromodulatory action. Furthermore, the output of the motor network depends on the interplay of all involved neurons (synaptic interaction) and of course on intrinsic membrane properties of single neurons. The latter is determined by channel function, and involves the generation of plateau potentials, post-inhibitory rebound spiking and spike frequency adaptation. All these aspects can be subject to modulation by several neuroactive substances.

1.2.1 Intrinsic properties

Neurons with plateau potentials make rapid transitions between two relatively stable membrane potentials (Marder 1991). The ability to produce a plateau potential depends on the respective status of the neuron, but usually it can be induced by brief excitation (from an electrode respectively synaptic input) and terminated by a short hyperpolarization. Once a plateau potential is initiated, the neuron can fire action potentials without continuous excitation. Modulation of plateaus can influence for example specific phase relationships in a rhythmic motor pattern or influence frequencies. Several neuroactive

substances promote plateau potentials in MNs, for example serotonin (crab: Zhang and Harris-Warrick 1995, snail: Arshavsky et al. 1998, turtle: Hounsgaard and Kiehn 1989, cat: Hounsgaard et al. 1988), and influencing actions of glutamate, ACh and GABA were shown for spinal MNs (Alaburda et al. 2002). In locusts, octopamine causes plateau potentials in flight interneurons (Ramirez and Pearson 1991a). Plateau properties were also shown for neurons in the cockroach and moth (Hancox and Pitman 1991; Mills and Pitman 1997; Mercer et al. 2005). Although up to now, there is no evidence for plateau potentials in stick insect neurons, sustained depolarizations were observed in motor- and interneurons associated with motor activity (Büschges et al. 2004; Ludwar et al. 2005b).

One mechanism by which plateau potentials are generated is a persistent inward current (PIC). PICs are depolarizing currents that are generated by voltage-sensitive channels, which stay open as long as the membrane potential is above the threshold for their activation (Heckmann et al. 2005). The threshold for a PIC is usually close to the voltage for initiating action potentials and most of the PIC is generated in dendritic regions. In vertebrate MNs, sodium or calcium ions play an important role in generating the PIC (e.g., turtle MNs: Hounsgaard et al. 1984). Evidence for PICs in invertebrates comes from studies on leech interneurons, and from MNs in the stomatogastric ganglion (STG) of the lobster (Lu et al. 1999; Elson and Selverston 1997). Furthermore, PICs are subject to neuromodulation, as shown for serotonergic modulation of spinal MNs by DINs in cats (Hyngstrom et al. 2007).

Neurons with post-inhibitory rebound (PIR) properties show increased excitability after inhibition. It can be produced by several mechanisms, such as activation of hyperpolarization-activated depolarizing conductances (I_h, e.g., snail MNs: Straub and Benjamin 2001, mice brainstem neurons: Sekirnjak and du Lac 2002) or deinactivation of depolarization-activated inward currents. For leech swim MNs PIR is the product of a combination of low-threshold sodium and calcium currents (Angstadt et al. 2005). PIR plays an important role in locomotor rhythms, e.g., in the spinal cord (lamprey: Matsushima et al. 1993, xenopus: Roberts and Tunstall 1990, the crustacean STG: Hooper and DiCaprio 2004, or leech heartbeat and swimming: Calabrese et al. 1995; Angstadt and Friesen 1993a;b). Evidence for PIR in insects comes from studies on locusts, *Manduca*

sexta and stick insects (dorsal unpaired median (DUM) neurons: Heidel and Pflüger 2006, antennal lobe neurons: Mercer et al. 2005; sag potential in fast flexor MNs: Schmidt et al. 2001). Serotonin modulates PIR responses in leech swim neurons (Angstadt et al. 2005) and dopamine enhances the PIR property in pyloric neurons of lobsters (Harris-Warrick et al. 1995). Spike frequency adaptation (SFA) is known to influence the final output of many neurons, for example by determination of burst duration in MNs of vertebrates (lamprey: el Manira et al. 1994, rat: Sawczuk et al. 1995; reviewed in Hultborn et al. 2004) and invertebrates (crab: Krans and Chapple 2005, stick insect: Schmidt et al. 2001). Interneurons involved in motor acts can also exhibit SFA and are subject to modulatory action (e.g., interneuron C2 in *Tritonia*: Katz and Frost 1997). SFA can result from an outward current that develops during the spike train and causes spike frequency to decrease. These outward currents are often expressed as postspike afterhyperpolarization (AHP) occurring after stimulus termination, but there is also evidence for AHP-independent SFA in other neuronal types (rat: Melnick et al. 2004; mouse: Fleidervish et al. 1996).

1.2.2 Neuroactive substances

Most neuroactive substances can exert their effects via ionotropic and metabotropic receptors, which are divided into two distinct superfamilies. Ionotropic receptors are ligand-gated ion channels that are responsible for fast neurotransmission (milliseconds), whereas metabotropic receptors are linked with ion-channels and exert their effect via signal transduction cascades (seconds to minutes).

The major neurotransmitter in the nervous system of vertebrates and invertebrates is **acetylcholine** (ACh). It acts on distinct receptors, which are named according to their activation by the respective agonist, nicotinic and muscarinic ACh receptors (n- and mAChRs). A third receptor type shows mixed pharmacological properties (mixed nicotinic/muscarinic receptor). nAChRs usually mediate rapid and short-lived depolarizations, whereas mAChR activation exerts slow and prolonged effects (Gundelfinger and Schulz 2000; Felder 1995). ACh is an excitatory transmitter in insects, and is not involved in neuromuscular transmission (Sattelle 1980). Several studies revealed ACh as a trans-

mitter between sensory- and interneurons, or sensory- and motoneurons respectively (e.g., cricket: Meyer and Reddy 1985, locust: Parker and Newland 1995, *Manduca sexta*: Trimmer and Weeks 1989). There is also evidence that ACh is released by DINs, thereby effecting interneurons in the locust (Baines and Bacon 1994). In the insect nervous system, nAChRs are found in high density and far exceed the number of mAChRs (Breer 1981; Orr et al. 1991), whereas for both, different receptor subtypes were identified (Benke and Breer 1989). A functional role for nAChRs in synaptic transmission was found in several neuron types (cockroach: David and Sattelle 1984; Grolleau et al. 1996, *Manduca sexta*: Waldrop and Hildebrand 1989, cricket: Cayre et al. 1999, honey bee: Goldberg et al. 1999). A postsynaptic effect for nAChRs was found for the fast coxal depressor MN in cockroaches (Butt and Pitman 2005). In insects, mAChRs exert their effect pre- or postsynaptically. Presynaptic mAChRs can act as negative feedback autoreceptors (in Breer and Sattelle 1987; Osborne 1996). Postsynaptic mAChRs mainly regulate spike threshold and excitability of moto- and interneurons (Trimmer 1995). Evidence for coupling of mAChRs to second messenger pathways is found in a range of different species. Muscarinic stimulation can lead to increased excitability and membrane depolarizations (Trimmer 1994; David and Pitman 1996), which could be mediated by activation of phospholipase C (PLC). An inhibitory action of mAChRs was found in locust synaptosomes, where they presynaptically inhibited transmitter release by reducing the cyclic adenosine monophosphate (cAMP) level (Knipper and Breer 1988).

The function of insect mAChRs might be in the rhythm-generating networks, since application of the muscarinic agonist pilocarpine induces rhythmic activity in MNs in stick insects (Büschges et al. 1995), locusts (Ryckebusch and Laurent 1993) and *Manduca sexta* (Johnston and Levine 1996). Rhythmic activity influenced by pilocarpine was also shown in some crustacean preparations, for example in the STG (Marder and Hooper 1985). Trimmer and Weeks (1993) and Wenzel et al. (2002) suggest a functional role for mAChRs as being the basis for specific arousal.
In some studies, receptors were found which were equally affected by nicotinic and muscarinic agonists, e.g., in cockroach DUM neurons (Lapied et al. 1990) or in housefly brains (Eldefrawi and O'Brien 1970).

Another important neuroactive substance in invertebrates is **octopamine**, which was first described in the salivary glands of *Octopus* (Erspamer and Boretti 1951). Due to the similar chemical structure and effects, it is thought that the octopaminergic system is homologous to the noradrenergic system of vertebrates. The respective receptors are G-protein coupled. Actions of octopamine are versatile, as it plays a role as neurotransmitter, neurohormone and neuromodulator (Orchard 1982; Roeder 1999).

In the insect central nervous system octopamine is involved in flight and walking initiation (locust: Sombati and Hoyle 1984) and in memory processes (honey bee: Menzel et al. 1990). In the periphery it modulates muscle contraction and functions of the oviduct and fatbody. In the locust extensor tibiae muscle preparation, octopamine presynaptically modulates the release of transmitters from MNs (Evans and O'Shea 1977), whereas octopamine affects muscle fibers postsynaptically. Octopamine release into the hemolymph due to stress exerts neurohormonal effects, for example in the locust, cockroach and honey bee (Davenport and Evans 1984; Hirashimai and Eto 1993; Harris and Woodring 1992) and an aggression related role was described by Stevenson et al. (2005) in crickets. In the thoraco-abdominal nervous system, octopamine is synthesized and released by DUM and VUM (ventral unpaired median) neurons, which for example supply leg and flight muscles in locusts (Hoyle and Barker 1975; Morton and Evans 1984). Mentel et al. (2003) showed that locust flight muscles are poised for flight activity by the release of octopamine from DUM neurons, and Ramirez and Pearson (1991a;b) described octopamine induced bursting and plateau potentials. The only known effect of octopamine in stick insects so far is a suppression of the pathways involved in the resistance reflex of the femur-tibiae control loop (Ramirez et al. 1993; Büschges et al. 1993). Responses induced by octopamine last from milliseconds to seconds and the effect of octopamine is known to be mainly mediated by activation of adenylate cyclase (AC), but in some systems also other second messenger pathways play a role. Rhythmic motor behavior can be influenced by octopamine, as shown for eliciting complete flight motor output in an isolated metathoracic ganglion in locusts (Stevenson and Kutsch 1986) or the mesothoracic ganglion in *Manduca sexta* (Claassen and Kammer 1986).

The neuroactive substance **serotonin** (5-hydroxytryptamine, 5-HT) is known to play an

important role in modulating feeding, postural and aggressive behaviors, and generally arousal levels in vertebrates and invertebrates (Weiger 1997). The effects of serotonin, as those of octopamine, include that of a neurotransmitter, neuromodulator and neurohormone. Modulatory action of neuronal activity was shown in context with learning and memory in *Aplysia* (Kandel 2001). Serotonin influences rhythmic motor behaviors, e.g., the expression of swim activity in the leech is facilitated amongst others by serotonin (Brodfuehrer et al. 1995), although also an inhibitory influence was described by Crisp and Mesce (2003) when applied only to the brain. In the swim CPG of *Tritonia* a role for serotonergic receptors is suggested (Clemens and Katz 2001). With the exception of one receptor type (5-HT_3, which is known to induce quick opening of an ion channel), all serotonin receptors are G-protein coupled and mediate their effects by second messengers (Hoyer et al. 1994; Barnes and Sharp 1999).

In insects, serotonin affects neurons in the central nervous system, the periphery and neuromuscular junction. Brain structures, such as the optic and antennal lobes, mushroom bodies and the central body, show intense serotonin immunoreactivity (see review by Osborne 1996). Within the central nervous system it antagonizes the effects of other transmitters in the control of rhythmic behaviors, e.g., suppression of the dopamine induced production of flight motor output in *Manduca sexta* (Claassen and Kammer 1986). Application of serotonin to the nerve cord of *Drosophila* stimulates locomotion (Yellman et al. 1997), and Lundell and Hirsh (1998) could show that mutants with reduced numbers of serotonin cells in the ventral nerve cord are inactive. Isolated neuronal somata of locusts respond with three different membrane currents to serotonin application, thus indicating different receptor subtypes (Bermudez et al. 1992). Furthermore, Parker (1995) showed that properties of leg MNs in the locust are modulated by serotonin, which leads to a potentiation of synaptic transmission between MNs.

1.2.3 Second messenger pathways

The activation of a second messenger system leads to a cascade of intracellular events that induce delayed effects on neurotransmission. The involved receptors usually activate guanosine nucleotide-binding proteins (G-proteins), which causes the synthesis of

second messengers, such as cAMP, inositol trisphosphate (IP_3) and diacylglycerol (DAG) or arachidonic acid metabolites. Besides channel modification, second messengers influence enzyme production, protein synthesis and regulate genes (Kandel et al. 1996). NO and CO are membrane permeable second messengers and influence guanylyl cyclase in the cytoplasm.

In second messenger systems, the binding of a first messenger (neurotransmitter) to the receptor induces a range of events. In most cases, the receptor interacts with the G-protein and this binds to its effector enzyme:

1) In the cAMP cascade, AC is activated (or inhibited) due to interaction with the G-protein. The function of AC is to convert adenosine triphosphate (ATP) to adenosine diphosphate (ADP). The latter is metabolized to cAMP, and activation of another enzyme or protein kinase occurs. This, in turn, can lead to phosphorylation of ion channels or transcription factors. The involvement of cAMP pathways was shown for many systems, as for the other second messengers. One example is the modulation of respiratory MNs in rats by cAMP-dependent protein kinase A (Bocchiaro et al. 2003), another comes from insects, where mAChRs mediate excitation in the grasshopper brain by an AC/cAMP/PKA dependent pathway (Wenzel et al. 2002).

Two other second messenger pathways involve the hydrolysis of phospholipids in the inner leaflet of the plasma membrane. The hydrolysis is catalyzed by phospholipase C and A_2.

2) Phospholipase C produces IP_3 or DAG. IP_3 serves as another second messenger and induces the release of calcium from internal stores (endoplasmatic reticulum). DAG on the other hand remains within the plasma membrane and activates protein kinase C (PKC). Furthermore, the activation of protein kinase C also requires phospholipids. The active form of PKC is translocated to the membrane and forms an active complex with DAG, thus phosphorylation of proteins in the cell is possible. Genetic studies from vertebrate and invertebrate model systems suggest that coordinated rhythmic motor functions are most susceptible to changes in intracellular Ca^{2+}, released from internal stores (endoplasmatic reticulum) by IP_3 receptors (in Banerjee and Hasan 2005). Levi and Selverston (2006) showed that lobster gastric mill neurons are excited by metabotropic glutamate

receptors acting via phospholipase and IP_3, and Qazi and Trimmer (1999) investigated the role of inositol signaling in the central nervous system of larval *Manduca sexta*.
3) Receptors that activate phospholipase A_2 induce the release of arachidonic acid from the cell membrane, which is rapidly converted into *eicosanoid* metabolites.

The different second messengers can interact with each other, their synthetic pathways are intertwined. Thus a combination of different modulators can act together, the level of one second messenger may for example influence the response mediated by another one, which gives rise to a large range of behavioral possibilities.

1.3 Stick insect walking

The muscles of the three leg pairs of stick insects are innervated by MNs from the respective thoracic ganglia. The stick insect leg is divided into five segments, whereas only three proximal leg joints are crucial for walking movements. A single step consists of the so called stance and swing phase. During stance phase the animal is pushed forward by developing force with the leg towards the ground. The leg is then lifted during swing phase and moves forward to the starting position for the next step. A characteristic motoneuronal and muscle activity pattern can be attributed to each phase (Graham 1985; Fischer et al. 2001; Büschges 2005). There is strong evidence that each leg joint is associated with an individual CPG (Büschges et al. 1995). Sensory information from several sources is involved in inter- and intra-leg coordination, e.g., femoral chordotonal organ (fCO) and campaniform sensilla (CS) (Akay et al. 2004; Akay and Büschges 2006). The fCO is a stretch receptor that provides information about angle and movement of the femur tibia joint, whereas CS are strain sensors that report signals about the load of the leg.
The rhythmic bursting pattern observed in leg MNs during walking is due to an alternating rhythmic (phasic) excitation and inhibition (Schmidt et al. 2001; Gabriel 2005), and an additional long-lasting (tonic) depolarization (Ludwar 2003; Büschges et al. 2004; Gabriel 2005; Ludwar et al. 2005b). Phasic excitatory input seems to originate from sensory organs in the leg (Akay et al. 2001). It is suggested, that the phasic inhibition as well as

tonic excitation can be attributed to the activity of CPGs. In the deafferented thoracic nerve chain rhythmic activity in tibial MNs could be evoked by mechanical stimulation of head or abdomen, and was based on phasic inhibition with an underlying tonic excitation (Büschges et al. 2004). Furthermore, the pilocarpine induced rhythmicity in MNs is also based on a phasic inhibitory and tonic excitatory input (Büschges 1998).

Previous studies using the single-legged preparation showed, that the activity in mesothoracic MNs increased during stepping sequences of the ipsilateral front leg (Ludwar 2003). Furthermore, intracellular recordings revealed that this increased activity consists of a phasic and tonic modulation (depolarization). The phasic modulation was usually coupled to front leg steps and the tonic depolarization outlasted the complete stepping sequence. Recently, Borgmann (2006) could show that the activity in ipsi- and contralateral coxae MNs of all thoracic ganglia is increased during stepping sequences of a single leg.

1.3.1 Objectives

The focus of this thesis was on the state-dependent, long-lasting tonic depolarization observed in leg MNs during walking in stick insects. In the light of recent studies, the tonic depolarization became of particular interest, because it might be ubiquitous during locomotion.

All experiments were conducted on reduced preparations. The single-legged preparation of the stick insect allowed an analysis of activity in the mesothoracic segment induced by front leg stepping, without sensory influence from other segments. The experiments were conducted solely on flexor MNs and should shed light onto the following issues:

1. Characterization of the membrane potential modulation in **contralateral** mesothoracic flexor MNs during front leg stepping and analysis of the properties of the tonic depolarization.
2. Pharmacological analysis of the tonic depolarization to answer the following questions:
    a) Which are the involved transmitters / receptors, that participate in the tonic depolarization?

b) Are second messengers involved in mediating the tonic depolarization?

3. Analysis of the influence of lesions (of the brain or connectives) on neuronal activity, especially on the tonic depolarization.

CHAPTER 2

Materials and Methods

2.1 Experimental animal

The experiments were carried out on adult female stick insects of the species *Carausius morosus* (syn. *Dixippus morosus*) and *Cuniculina impigra* (syn. *Baculum extradentatum*) from a colony maintained at the University of Cologne. The colonies were kept at temperatures between 20°C and 25°C and at an artificial light/dark rhythm. The forage plant for the colony was blackberry (*Rubus fructiosus*).

Carausius morosus and *Cuniculina impigra* belong to the phylum: arthropoda, class: insecta, subclass: pterygota, superorder: orthopteroidea, order: phasmida, family: phasmatidae, subfamily: phasmatinae. Both are wingless, nocturnal animals. The native place of *Carausius morosus* is southern India, adults grow up to 8 cm and the reproduction is generally parthenogenetic. The origin of *Cuniculina impigra* is Vietnam, females grow up to 10 cm, males up to 7 cm (but they are seldom). The lifetime is 12 to 14 months. The

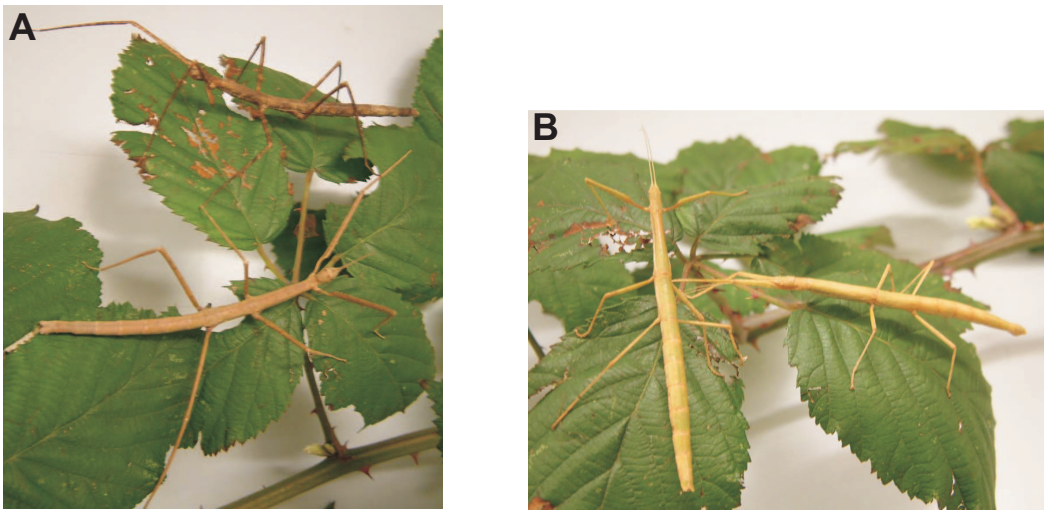


Figure 2.1: *Stick insects on blackberry leafs.* ***A:*** *Cuniculina impigra and* ***B:*** *Carausius morosus*

body is divided into three main parts - head, thorax and abdomen, whereas the head consists of six segments and carries the brain, sense organs, antennae and mouthparts. The thorax is divided into pro-, meso- and metathorax, and each segment carries a leg pair. The abdomen serves for reproduction and digestion and consists of 11 segments. The central nervous system (CNS) is composed of the ventral nerve cord (including the subesophageal ganglion = SEG) and the dorsally positioned brain (supraesophageal ganglion). The brain is a fused ganglion composed of the protocerebrum, deutocerebrum and tritocerebrum. The anterior three pairs of ganglia in the ventral chain are also fused, forming the cephalic SEG. The brain and the SEG are linked via the circumoesophageal connectives, whereas the SEG is linked to the prothoracic ganglion via the neck connectives.

2.2 Preparation and experimental setup

This chapter describes first the general preparation which had to be performed for all experiments. Then slight differences for the individual experiments are listed.

2.2.1 General preparation

The experiments were carried out under dimmed light conditions at room temperature (20-25°C). A semi-intact preparation was used with one intact front leg walking, while all other legs were amputated at the level of the mid-coxa. The thorax was fixed ventrally on a platform using dental cement (PROTEMP, 3M ESPE, Seefeld, Germany). The cuticule was cut dorsally and removed from the middle of the mesothorax to the middle of the metathorax. The gut was left intact, moved aside and the cavity was filled with saline (composition according to Weidler and Diecke (1969), see chapter 2.4, table 2.1). Connective tissue and fat were removed to allow access to the mesothoracic ganglion and leg nerves. Care was taken to leave as much as possible trachea intact, and cut main trachea were exposed to air. All lateral nerves but the *nervus cruris* (ncr, nomenclature according to Graham 1985; Marquardt 1940) ipsi- or contralateral to the remaining leg were cut near the mesothoracic ganglion to exclude sensory input in this segment. In most cases the tissue between the two connectives had to be carefully cut with fine scissors to allow lifting of the mesothoracic ganglion on a movable waxed platform. The surrounding connective tissue was pinned down with small cactus spines (*Nopalea dejecta*) to stabilize the ganglion for penetration with sharp microelectrodes.

2.2.2 Non-pharmacological experiments

For recording of mesothoracic MNs the ganglion sheath had to be softened to allow penetration of intracellular electrodes. Therefore saline was quickly removed from the cavity and the ganglion sheath was treated with crystals of a proteolytic enzyme (PRONASE E, MERCH, Darmstadt, Germany) for 60-90 s. The enzyme was thoroughly washed out and saline was applied again.

2.2.3 Pharmacological experiments

The ganglion sheath in stick insects serves as a blood-brain barrier and displays therefore a diffusion barrier for ions (Schofield 1979; Dörr et al. 1996) and pharmacological agents. Due to that, the first step to be able to perform pharmacological experiments in

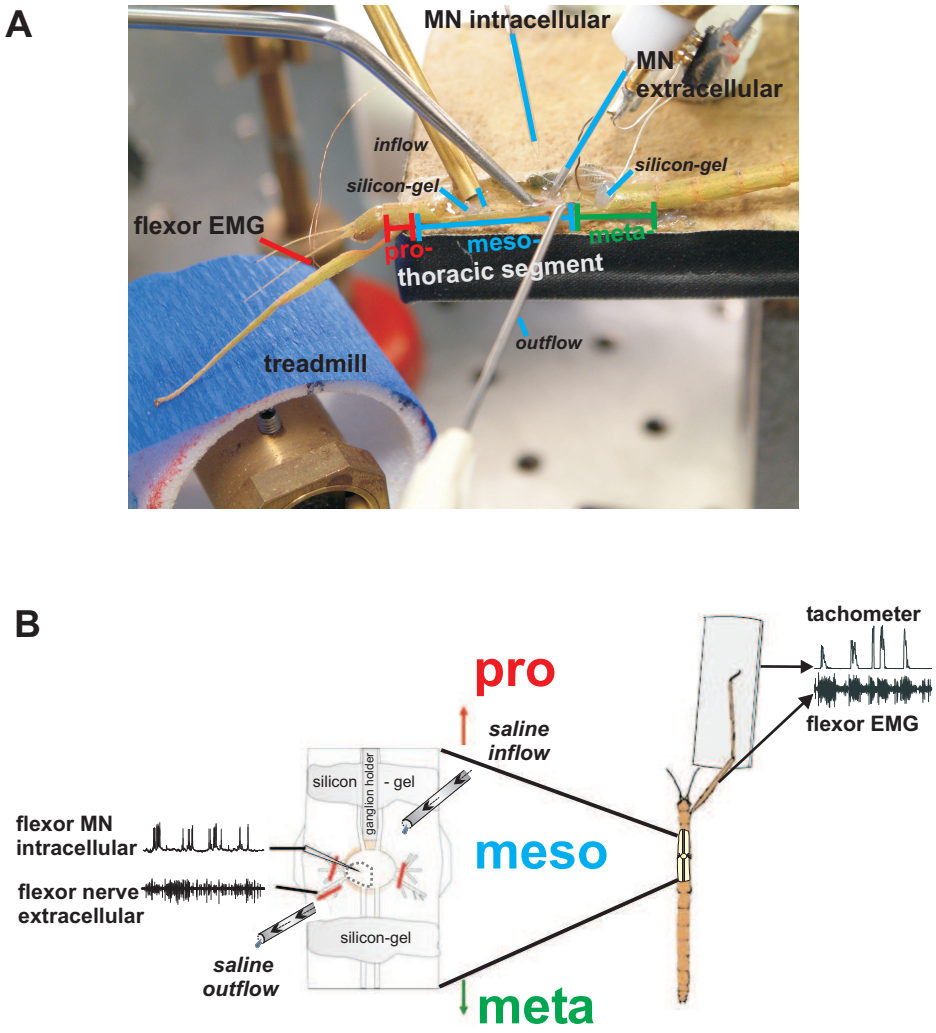


Figure 2.2: *Split-bath preparation of the experimental animal.* ***A:*** *Picture of the split-bath preparation.* ***B:*** *Schematic of a split-bath setup.* ***A-B:*** *Rostral and caudal of the mesothoracic (meso) ganglion silicon-gel barriers are built to allow superfusion with saline containing drugs of only the mesothoracic ganglion.*

behaving stick insects was to establish a preparation in which the ganglion sheath of the segment in focus could be removed without destroying nerve cells or losing stability for intracellular recordings. Tissue and fat along the connectives were removed toward the pro-and metathoracic ganglion. Saline was removed from the cavity and silicon-gel barriers (BAYSILONE-PASTE, hochviskos, Bayer AG, Leverkusen) were quickly built with a modified syringe (Fig. 2.2). Three separate compartments were formed, one rostral and caudal to the mesothoracic ganglion and one around the ganglion. This allowed a separate superfusion of drugs only to the mesothoracic ganglion. Then saline was added again (chapter 2.4, table 2.1), and the superfusion system was adjusted and started. Before desheathing the ganglion it was made sure that the system worked and the ganglion was completely covered with saline because exposure of the desheathed ganglion to air would damage the nerve cells immediately. By using very fine scissors (SuperFine VANNAS SCISSORS straight, Harry Fein WORLD PRECISION INSTRUMENTS, Berlin, Germany) a first cut was made along the midline from posterior to anterior of the ganglion, then the sheath was cut either towards the ipsi- or contralateral side. The loosened sheath was fixed with fine forceps and finally removed by a third cut. In this way at least one third of the ganglion was desheathed. The solutions were exchanged by a gravity driven perfusion system, positioned 50 cm above the platform and conducted via a tube system toward the cavity (0.75 mm inner diameter of the inflow tube). The flow rate was 2 ml/min, and it took 20 s for a new solution to enter the cavity. The volume of the cavity was 100 µL, the volume was therefore exchanged 20 times per minute. A continuous flow rate was achieved by an adequate positioning of the outflow.

2.2.4 Lesion experiments

In one set of experiments, it was necessary to have access to the supraesophageal ganglion (brain) during recording from mesothoracic flexor MNs. Therefore the head was stabilized with plasticine while the cuticule of the head capsule was carefully cut with a sharp razor blade and removed to expose the brain. The cuts ran between the eyes along the front rim and bilateral toward the posterior end of the head. During the experiment, care was taken to add saline into the cut regularly. Referring to Graham (1979a), immobile

antennae indicated a successful preparation. The animal was then prepared according to chapter 2.2.3. As soon as the intracellular recording of a flexor MN was stable, modulation in its membrane potential was recorded during a few stepping sequences of the front leg. Then the brain was removed by holding it with fine forceps and cutting the connectives with sharp scissors. Care was taken to induce as less perturbations as possible and not to lose the intracellular recording. If the lesion was successful and the quality of the intracellular recording did not change, again the membrane potential was recorded during stepping sequences of the front leg. Successful experiments were analyzed according to chapter 2.6.1.

2.2.5 Connective recordings

To perform extracellular recordings from both, the ipsi- and contralateral neck connectives (connectives between the prothoracic ganglion and the SEG) the general preparation was extended. The cuticule was cut dorsally and removed from the middle of the mesothorax towards the headcapsule. The gut was moved aside and the cavity was filled with saline. One front leg performed stepping sequences on a treadmill.

2.3 Treadmill

A lightweight low-friction treadmill (Gabriel et al. 2003) was used, which consisted of two styrofoam drums (∅ 40 mm; width 28 mm), each mounted on a micro DC-motor (*DC1516*, FAULHABER, Schönaich, Germany), that had a center distance of 50 mm (Figure 2.3 A). A belt made of light crêpe paper was placed around the styrofoam drums. The tangential force that had to be applied to overcome belt friction was 4.0 ± 0.3 mN. The moment of inertia of the system, which is determined by the effective mass of the treadmill was 1.1 g and thus approximately equal to the mass of an adult animal (Gabriel et al. 2003). One DC-motor served as a tachometer, with the other belt friction could be varied. The tachometer signal was digitized with a MICRO1401 A/D converter (sampling rate: 6.5 kHz) and recorded with SPIKE2 software (both CAMBRIDGE ELECTRONIC DESIGN, Cambridge, UK) on a personal computer. The treadmill was positioned 45° be-

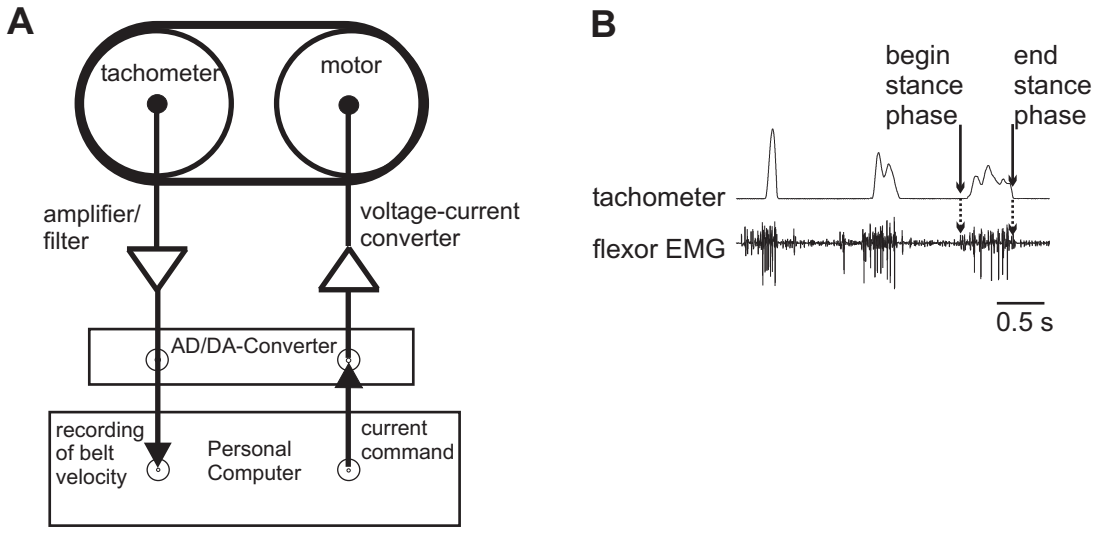


Figure 2.3: *Treadmill.* ***A:*** *Tachometer. The signal from the tachometer was filtered and digitized prior to recording. A current could be applied to the other motor to decrease or increase belt friction. (Picture modified after Gabriel et al. 2003).* ***B:*** *Tachometer trace.*

low the intact leg, and the height was adjusted so that the animal could easily pull the belt and performed walking like movements. The tachometer trace in combination with the flexor EMG (see chapter 2.5.1) allowed determining the stance phase of a step (Fig. 2.3 B). The beginning of stance was defined as the beginning of activity in the flexor EMG. The end of stance was defined as the last maximum of the trace before it decreases back to zero. The falling edge is just determined by the inertia of the treadmill and does not contain any information about the status of the step. In all experiments, not single steps were used for analysis but stepping sequences. A stepping sequence was defined as a minimum of three consecutive steps that had a maximum temporal distance of 3.5 s.

2.4 Solutions

The composition of the physiological saline is listed in table 2.1. Different saline compositions were used in non-pharmacological (chapter 2.2.3) and pharmacological experiments, both with a pH of 7.2 (chapter 2.2.2). A different saline composition was necessary in those experiments where the ganglion was desheathed.

A

Substance	**[mM]**
NaCl	178.5
KCl	17.6
$CaCl_2$ * 2 H_2O	7.3
$MgCl_2$ * 6 H_2O	25
HEPES	10

B

Substance	**[mM]**
NaCl	180
KCl	4
$CaCl_2$ * 2 H_2O	5
$MgCl_2$ * 6 H_2O	1
Saccharose	30
HEPES	10

Table 2.1: *Saline composition used in **A:** non-pharmacological experiments according to Weidler and Diecke (1969), and **B:** pharmacological experiments; saline modified after Schmidt.*

Drugs for pharmacological experiments were dissolved in saline prior to the experiment or added as aliquots, stored at $-20°$ C and thawed prior to the experiment. H-89 and U-73122 were dissolved in DMSO, mianserin was dissolved in ethanol and verapamil in methanol, whereas the solvents had a final concentration in the experiment of less than 1 %. This solvent concentration in saline alone had no effect on membrane potential of flexor MNs. All drugs were obtained from SIGMA (Sigma-Aldrich, Schnelldorf, Germany) with the exception of verapamil (FLUKA, Switzerland). The chemical structures of the drugs are shown in figure 2.4.

2.5 Electrophysiology

2.5.1 Extracellular recordings and electromyograms

Extracellular recordings were made from a lateral nerve or from connectives with monopolar hook electrodes (custom built, modified after Schmitz et al. (1988; 1991)) filled with silicon-gel (BAYSILONE-PASTE, hochviskos, Bayer AG, Leverkusen). Recordings were made from the mesothoracic leg nerve ncr (*nervus cruris*), ipsi- or contralateral to the intact front leg. The ncr contains the axons of flexor motoneurons (MNs). In another set of experiments, different connectives were recorded.

Electromyograms (EMGs) were recorded with two twirled copper wires (∅ 50 µm), which

short term	**chemical name/source**
8-Br-cAMP	8-Bromoadenosine 3′,5′-cyclic monophosphate sodium salt
Atropine	Atropine sulfate salt monohydrate
BAPTA	1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrapotassium salt
H-89	N-[2-(p-Bromocinnamylamino)ethyl]-5-iso-quinolinesulfonamide dihydrochloride
Mianserin	1,2,3,4,10,14b-Hexahydro-2-methyl-dibenzo[c,f]pyrazino[1,2-a]azepine hydrochloride
Neomycin	Neomycin trisulfate salt hydrate
±Octopamine	(±)-1-(4-Hydroxyphenyl)-2-aminoethanol hydrochloride
Riluzole	2-Amino-6-(trifluoromethoxy)benzothiazole
Serotonin	5-Hydroxytryptamine hydrochloride
SQ22,536	9-(Tetrahydro-2-furanyl)-9H-purin-6-amine
U-73122	1-[6-[((17ß)-3-Methoxyestra-1,3,5[10]-trien-17-yl)amino]hexyl]-1H-pyrrole-2,5-dione
±Verapamil	5-[N-(3,4-Dimethoxyphenylethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride (Fluka)

Table 2.2: *Drugs used in the experiments. All drugs were obtained from Sigma if not indicated differently.*

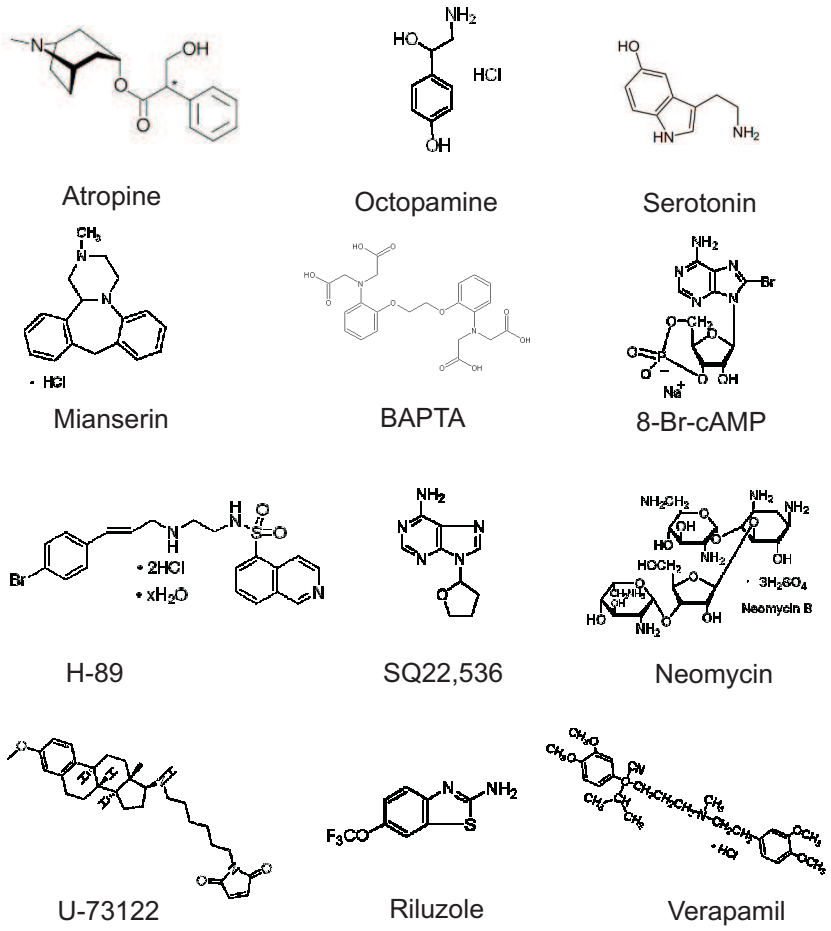


Figure 2.4: *Chemical structures of the used drugs. The molecular weight varied between 190 and 909.*

were insulated except for the tips. The cuticule above the proximal prothoracic flexor muscle was perforated in two locations closely together with the tip of a small insect pin (∅ 0.1 mm). The copper wires were inserted and fixed with dental cement.
Both the extracellular recordings and the EMG recordings were amplified and filtered (250 Hz - 5 kHz, 50 Hz - 1 kHz).

2.5.2 Intracellular recordings

Intracellular recordings from flexor MNs were made using thin-walled glass microelectrodes (GC100TF-10, HARVARD APPARATUS ltd, Edenbridge, Kent, UK), which were pulled on a P-97 filament puller (SUTTER INSTRUMENTS, Novato, USA). Microelectrodes were filled with a solution of 3 M potassium acetate (KAc) and 0.1 M potassium chloride (KCl) or 1.5 M KAc and 1.5 M KCl. The electrode resistances were 15-25 MΩ. The recordings were made from the aborizations of the flexor MNs in the neuropilar region of the mesothoracic ganglion (ipsi- or contralateral to the walking front leg). An SEC-10L amplifier (NPI, Tamm, Germany) was used to amplify the signals in bridge or discontinuous current clamp (DCC) mode (switching frequency 12-25 kHz). The DCC mode was used during current injections. The MNs were identified by a one-to-one relationship of intracellularly recorded spikes with spikes in the appropriate extracellular nerve recording. Recordings were made from fast, semi-fast and slow flexor MNs, whereas no discrimination were made for data analysis.
Recordings where no stable membrane potential was reached or where the input resistance decreased without external stimulus were discarded. A total of 77 flexor MNs for non-pharmacological experiments and 67 flexor MNs for pharmacological experiments were recorded in 144 animals.

2.5.3 Experimental setup

The equipment (platform, electrodes etc.) was placed on an air table (MICRO-g, TMC, Peabody, MA, USA), which was surrounded by a Faraday cage. Thus vibrations and movements of the recording electrodes relative to the animal were minimized. Depending on the experimental conditions, next to the platform where the animal was fixed the

following devices were positioned: three micromanipulators for the extracellular electrode, the ganglion holder and the outflow, a flexible magnetic stand for the inflow, and a micromanipulator for the intracellular electrode (LEICA Micromanipulators, Wetzlar, Germany). A lamp with optical fibers illuminated the setup during the preparation. A treadmill was positioned next to the platform under the front leg of the animal in an approximately 40° angle to the animal as this corresponds to the mean position of the front leg in a standing animal (Cruse 1976).

2.6 Data recording and evaluation

The voltage output of the treadmill and the electrophysiological data were recorded using a MICRO 1401 A/D converter and SPIKE 2 data-acquisition/analysis software (versions 3.13 - 4.12, CAMBRIDGE ELECTRONIC DESIGN, Cambridge, UK) and a personal computer. The second DC-motor of the treadmill was connected to a voltage-current converter. A SPIKE 2 sequencer script was written to apply continuous current to the motor. For the A/D conversion of electrophysiological data, a sampling rate of 12.5 kHz was used for extracellular, and of 6.25 kHz for intracellular data.

The extracellular recordings and the tachometer trace were preprocessed in SPIKE2 for further data evaluation. Neuronal activity of the extracellular recordings (only for connective recordings) and beginning and end of a stance phase in the tachometer trace were displayed as event channels.

Some data were analyzed with respect to the start of the front leg stance phase, to describe the modulation in membrane potential in mesothoracic flexor MNs. A time window of 1 s before and after the beginning of each stance phase was analyzed. The animals sometimes performed spontaneous stepping sequences, but generally they had to be elicited by gently touching the abdomen with a paintbrush. The paintbrush was removed as soon as the animal started a sequence of stepping movements. Steps that were elicited due to stimulation with a paintbrush were discarded for the analysis of the phasic modulation.

In order to estimate the gross activity of a connective recording, it was rectified and smoothed (first order low pass filter, time constant τ = 0.07 s). Custom SPIKE2 script programs were written to analyze the recordings.

Layout editing was achieved using Corel-Draw 11 (COREL CORPORATION, Ottawa, ON, Canada), and statistical analysis and plots were rendered using Excel (MICROSOFT) and Microcal Origin (version 6.0, ORIGINLAB CORPORATION Northampton, MA, USA).

2.6.1 Analysis of intracellular recordings

The analysis in this thesis focuses mainly on the tonic membrane potential modulation in mesothoracic MNs, namely the tonic depolarization. This tonic depolarization is only induced by a behavior of the animal itself and did not occur in resting animals nor could it be induced artificial by current injection or the like. Flexor MNs exhibited the tonic depolarization either during stepping sequences of the front leg on a treadmill, or it occurred during searching movements (Büschges et al. 2004) of the front leg. The latter was not included in the analysis of this thesis, because without reference to steps, there was no evidence of comparing the amplitudes of the tonic depolarization in different sequences. If possible, the amplitude of the tonic depolarization was measured and compared during similar stepping sequences. Once a stable intracellular recording could be established, the success of an experiment depended on the behavior of the animal.

The determination of the amplitude of the tonic depolarization is shown in figure 2.5. It was measured as the offset between the resting membrane potential before onset of a stepping sequence and the lower edge of the phasic modulation during a stepping sequence. One could argue, that this is an underestimation of the real amplitude of the tonic depolarization. It is possible that the tonic depolarization amplitudes are higher than determined in this thesis, but I wanted to exclude a contamination by any phasic modulation. This was achieved in all probability by using this analysis (see also discussion, chapter 4.1.1).

Each data point in the graphics (see e.g., Fig. 3.10) represents the amplitude of the tonic depolarization in membrane potential induced by one complete stepping sequence (3 to 20 steps) of the front leg at certain times. For the pharmacological experiments, it was

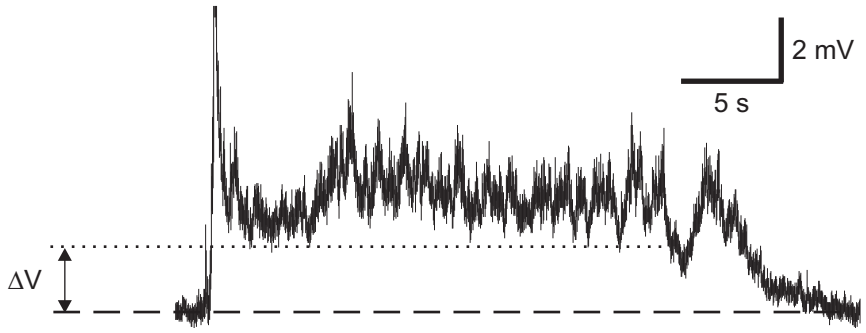


Figure 2.5: *Determination of the tonic depolarization amplitude. The tonic depolarization shift (ΔV) was measured as the offset between the voltage baseline (dashed line: resting membrane potential) before onset of a stepping sequence and the lower edge of the phasic modulation (dotted line) during a stepping sequence.*

not possible to analyze and compare the amplitudes in different experiments at a particular time: for the above described reasons a stepping sequence of the animal could not be induced at any time. To analyze if a certain drug influenced the tonic depolarization, the mean amplitude before superfusion of the drug was measured. Then each tonic depolarization amplitude induced by a stepping sequence during superfusion of the drug was plotted against time. These data were fitted using linear regression and the levels of significance were analyzed according to chapter 2.6.2.

2.6.2 Statistics

In text and figures, N is the number of experiments and n is the sample size (number of stepping sequences). Values were compared using a t-test, shown as mean ±SD. Means and samples were regarded as significantly different at $p < 0.05$. The level of statistical significance was indicated by the following symbols: (n.s.) not significant; (*) $0.01 \leq p < 0.05$; (**) $0.001 \leq p < 0.01$; (***) $p < 0.001$. For significance tests of coefficients of correlation, the significance test for Pearson's r was used that is computed as follows:

$$\hat{t} = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

r is Pearson's correlation, and n is the number of pairs of scores that went into the computation of r, which were tested for significance with the Fisher test (Sachs 1974).

For significance test of regression coefficients, the coefficients were compared as follows:

$$\hat{t} = \frac{|b_1 - b_2|}{\sqrt{\frac{s^2_{y1x1}(n_1-2)+s^2_{y2x2}(n_2-2)}{n_1+n_2-4}\left[\frac{1}{Q_{x1}} + \frac{1}{Q_{x2}}\right]}}$$

With b_1, b_2 as regression coefficients, s standard deviation, Q sum of the squared differences ($Q_x = \sum(x - \bar{x})^2$). For unequal remaining variances i.e.: $\frac{s^2_{y1x1}}{s^2_{y2x2}} > F_{(n1-2;n2-2;0.10)}$ the calculation was performed approximatively with:

$$\hat{z} = \frac{|b_1 - b_2|}{\sqrt{\frac{s^2_{y1x1}}{Q_{x1}} + \frac{s^2_{y2x2}}{Q_{x2}}}}$$

CHAPTER 3

Results

3.1 Activity in mesothoracic flexor MNs during walking of the contralateral front leg

In previous studies, it was shown that stepping sequences of a single front leg influence the activity of ipsilateral mesothoracic motoneurons (MNs) (Ludwar 2003). Ipsilateral mesothoracic MNs of antagonists of all three joints show an increase in spike activity and a clear coupling of activity to front leg stepping (Ludwar et al. 2005a). Analysis of intracellular recordings revealed that the membrane potential of ipsilateral mesothoracic MNs is phasically modulated and tonically depolarized by 0.5 to 5 mV (Ludwar 2003; Ludwar et al. 2005b). Borgmann (2006) showed in extracellular recordings that activity in pro- and retractor MNs increased in all thoracic segments during stepping of a single front, middle or hind leg. These observations lead to the question of whether the observed membrane potential modulation in ipsilateral mesothoracic MNs is a general phenomenon associated with walking movements. To tackle this question, in the first

part of my thesis the activity in mesothoracic flexor MNs was analyzed during stepping sequences of the contralateral front leg.

In this part, a semi-intact preparation was used according to the procedures in chapter 2.2, where the right front leg performed stepping movements on a treadmill and all other legs were cut off. The activity of contralateral flexor MNs was recorded extracellularly from the lateral nerve ncr (*nervus cruris*), that contains the axons of flexor MNs, retractor unguis MNs as well as sensory axons.

Intracellular recordings from neuropilar regions of contralateral flexor MNs were performed according to chapter 2.2.2. Stepping sequences of the front leg were evoked by briefly touching the animal's abdomen with a paintbrush. The stepping sequences consisted of typically 5 to 20 consecutive steps. Occasionally spontaneous stepping sequences occurred without external stimulation. No differences between tactile elicited or spontaneous stepping sequences were detected, this was also reported by Ludwar (2003) and Ludwar et al. (2005b).

Figure 3.1 shows the typical activity in a contralateral flexor MN recorded during a stepping sequence of the front leg. Stance phase of the front leg was determined by a flexor EMG and acceleration of the treadmill, which corresponds to an increase in velocity in the tachometer trace. With the beginning of the stepping sequence, activity in the mesothoracic flexor nerve increased. The increase in activity lasted during the stepping sequence and ceased 2 s after the last step of the front leg. The simultaneous intracellular recording of a flexor MN showed two distinct changes in membrane potential with onset of the stepping sequence. Contralateral flexor MNs showed a tonic depolarization and a rhythmic modulation in membrane potential. This was also shown for ipsilateral MNs. The characteristics of these two types of changes in membrane potential are analyzed in the following (chapter 3.1.1, 3.1.2).

3.1.1 Tonic depolarization in membrane potential in contralateral flexor MNs

As shown in figure 3.1, contralateral flexor MNs exhibited a tonic depolarization in membrane potential with onset of walking movements of the front leg. The amplitudes of the tonic depolarization were determined by obtaining the voltage difference between the

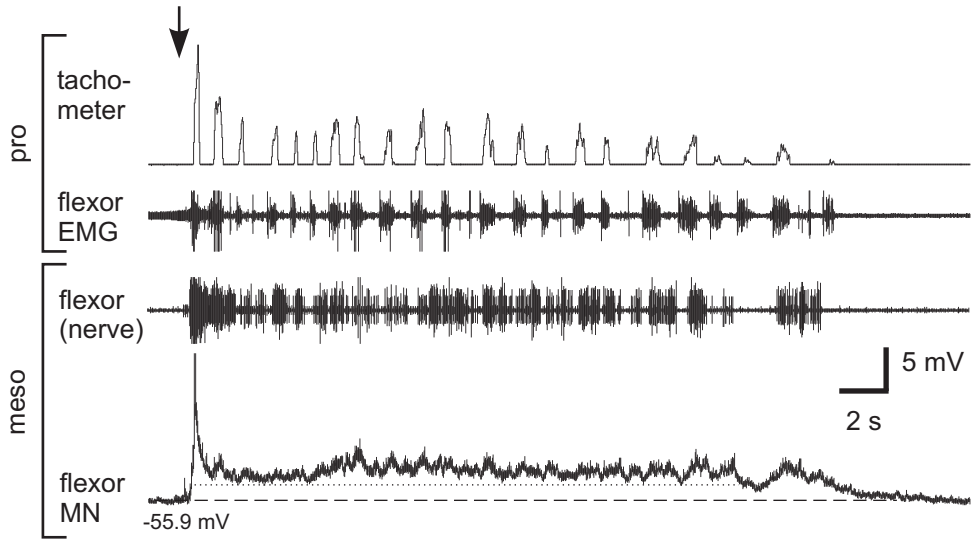


Figure 3.1: *Contralateral mesothoracic flexor MN depolarized tonically and showed phasic membrane potential modulation during a front leg stepping sequence.* ***1st trace****: Treadmill velocity (tachometer). A rising tachometer trace, indicating treadmill acceleration, was used to define the stance phase of the leg.* ***2nd trace****: Electromyographic (EMG) recording of the front leg flexor tibiae muscle.* ***3rd trace****: Extracellular recording from nerve ncr that contains the axons of flexor MNs.* ***4th trace****: Intracellular recording of a flexor MN. Action potentials truncated. The dashed line indicates the resting membrane potential as given by numbers, the dotted line indicates the lower edge of the phasic modulation. Arrow indicates tactile stimulation.*

resting potential (chapter 2.6.1, Fig. 2.5) and the lower edge of the phasic modulation.

From a mean resting potential of -57.5 ± 4.6 mV the membranes tonically depolarized by 1.7 ± 1.2 mV (range: 0.6 to 4.3 mV, N=21/24). In three contralateral flexor MNs no tonic depolarization in membrane potential was obvious at their resting membrane potential. The amplitude and sign of the tonic voltage shift was dependent on membrane potential. In figure 3.2 the membrane potential of a contralateral flexor MN was manipulated by constant current injection. The tonic shift was hyperpolarizing at a potential of -42 mV, depolarizing at its resting potential of -58 mV (0.8 mV), and at a membrane potential of -78 mV the depolarization was increased (5.4 mV).

The dependence of the tonic shift on membrane potential is shown in figure 3.3. Measurements of tonic voltage shifts were taken at different membrane potentials in current-clamp mode. The measurements at different membrane potentials were pooled together

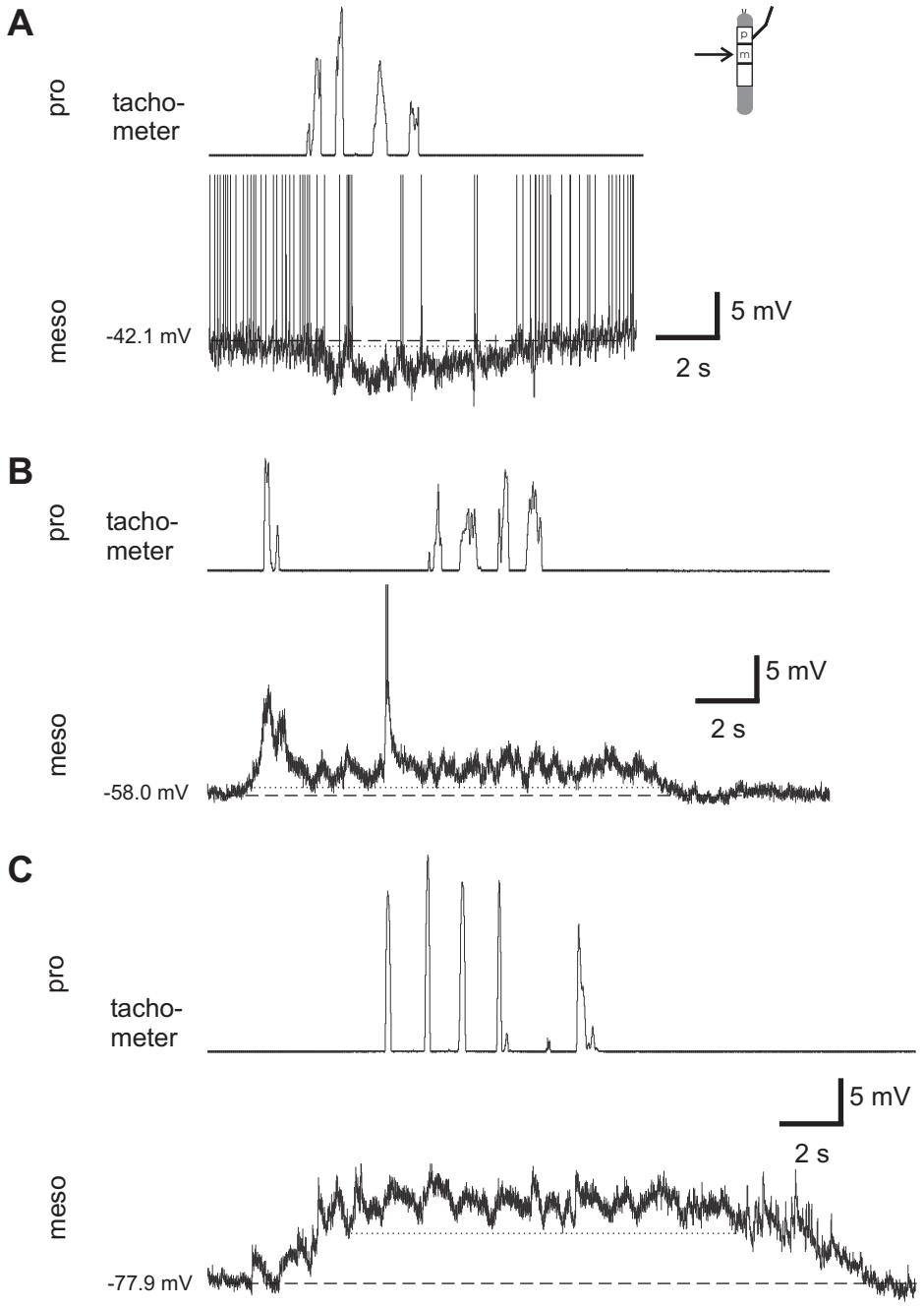


Figure 3.2: *Reversal of the tonic depolarization in a contralateral flexor MN.* ***A:*** *A stepping sequence of the front leg (tachometer) induced a slight tonic hyperpolarization at a depolarized membrane potential of -42 mV (constant current injection).* ***B:*** *At its resting membrane potential of -58 mV, the tonic shift was depolarizing during front leg stepping.* ***C:*** *The amplitude of the tonic depolarization was increased during constant hyperpolarization.*

and data were fitted using linear regression. The correlation coefficient (r=−0.648) was significant at the 0.1 % level for contralateral flexor MNs. The reversal potential of the tonic depolarization was calculated from graph fitted equation and was found to be −40.7 mV. Thus the calculated reversal potential is 17 mV more positive than the mean resting potential.

As in ipsilateral MNs, the tonic depolarization was associated with a decrease in input resistance (Fig. 3.4). Changes in input resistance (membrane resistance) reflect a change in membrane conductance due to the opening/closing of ion channels in the membrane. The input resistance was measured by injection of constant hyperpolarizing current pulses of 300 ms duration. The amplitude of the voltage deflection upon current injection is a measure of the membrane resistance. Thus if the voltage deflection upon current injection decreases, the input resistance decreases due to the opening of ion channels (excitatory or inhibitory). In seven contralateral flexor MNs, the input resistance was measured during stepping activity of the front leg. The mean decrease in input resistance was 12.4 ±3.5 %. Measurements were taken between actual stepping movements or at the end of the stepping sequence to avoid contamination by phasic synaptic input. Figure 3.4 B shows a reduction in current induced voltage deflection that is equal to a reduction from 8.5 M Ω (value at rest) to 6.7 MΩ at the end of the stepping sequence.

The reduction in input resistance that is generally observed in MNs throughout a stepping sequence of an ipsi- or contralateral front leg might somewhat reduce the responsiveness of the neurons to incoming depolarizing input. This assumption was tested in flexor MNs contralateral to the walking front leg by injecting depolarizing current pulses of 300 ms duration that evoked one or two spikes before onset of walking movements. In 73 % of the flexor MNs that were tested (N=11), the number of spikes per pulse more than doubled at comparable membrane potentials, indicating an increased responsiveness (Fig. 3.5).

In two of these recordings, increased responsiveness outlasted the stepping sequence by tens of seconds. In two of eleven MNs, the responsiveness was reduced, possibly by current shunt, because in both neurons the input resistance decrease was exceptionally high (30 %) during walking sequences. One MN did not exhibit a consistent activity

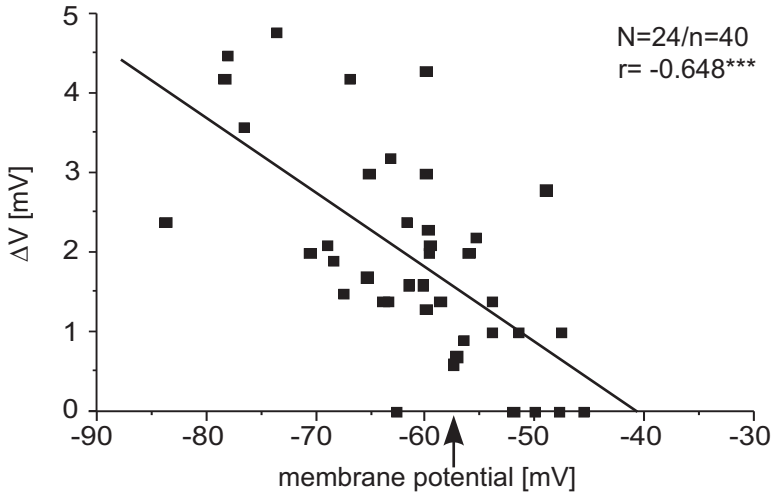


Figure 3.3: *Dependence of the tonic membrane potential shift that was induced by front leg stepping on membrane potential in contralateral mesothoracic flexor MNs (N=24, n=40; r=-0,648***). The arrow indicates the mean resting membrane potential of −57.5 ±4.6 mV.*

pattern on current injection. During four stepping sequences, the responsiveness was reduced and no tonic depolarization was observed; during one stepping sequence, the responsiveness was enhanced while the membrane potential was tonically depolarized by 1.3 mV.

In figure 3.6, two recordings of contralateral flexor MNs are shown, which demonstrate different shaping of their activity during front leg stepping. Although both showed a clear tonic depolarization in membrane potential during a stepping sequence of the front leg (3.4 and 2.3 mV), only in the first (Fig. 3.6 A) a distinct phasic membrane potential modulation was visible, while the membrane potential in the second (Fig. 3.6 B) did not hyperpolarize between steps. Coupling of phasic modulation in membrane potential to front leg stepping will be discussed later in this chapter (see 3.1.2). The repolarization to resting membrane potential values took 550 ms in this recording (Fig. 3.6 A; τ=290 ms respectively). In figure 3.6 B the repolarization to resting values took 3 s (respectively 6 s after the last step was performed; with τ=1.6 s). Long repolarization time constants were found in several recordings, where repolarization to resting values took up to 30 s.

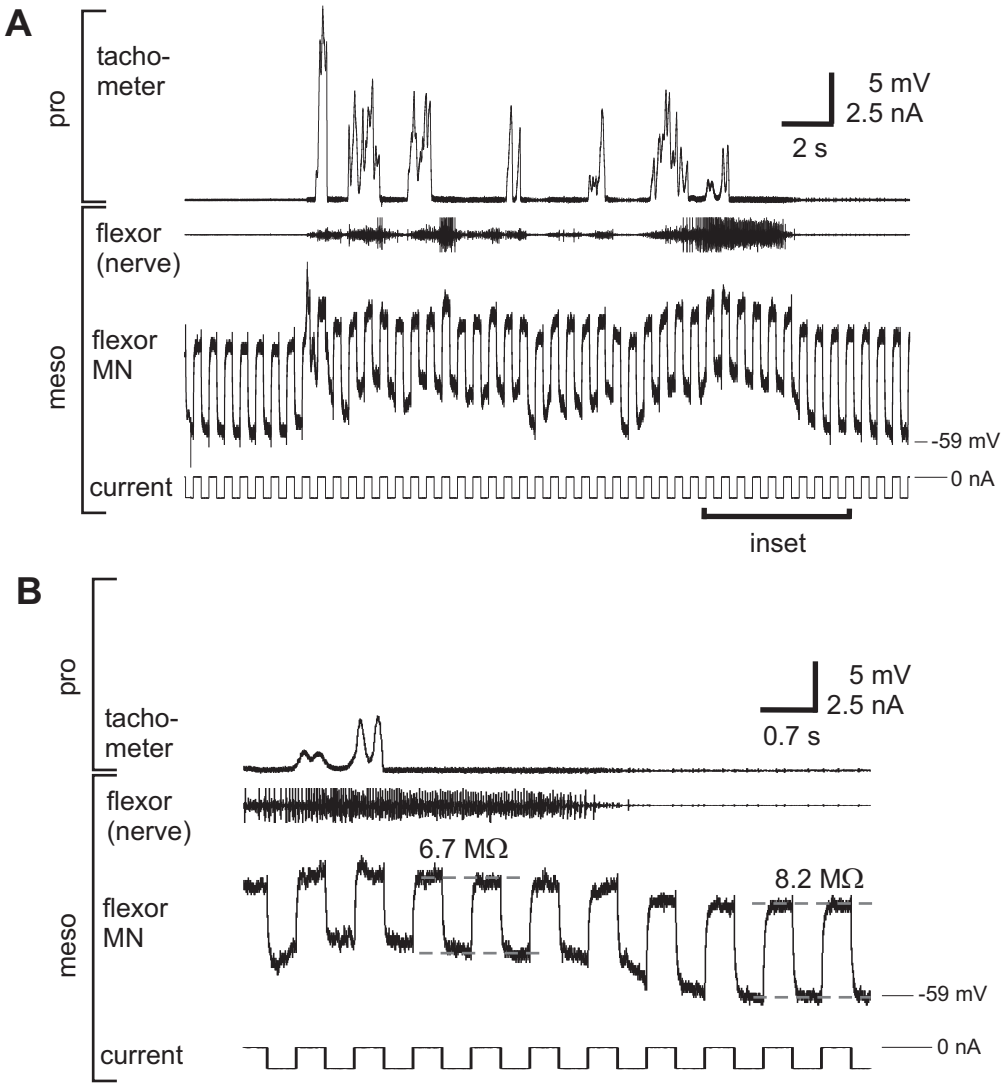


Figure 3.4: *Input resistance in a mesothoracic flexor MN was reduced during a stepping sequence of the contralateral front leg.* ***A:*** *Stepping sequence of 7 steps (**1st trace**) evoked increased motor activity recorded extracellularly (**2nd trace**). Current pulses of −1 nA (**4th trace**) were injected into a flexor MN (**3rd trace**) that was recorded intracellularly.* ***B:*** *Clipping from **A** with an extended time scale as indicated. Voltage deflections on current injection were decreased even when no steps were performed (horizontal dashed lines). Thus the tonic response is based on a decrease in input resistance.*

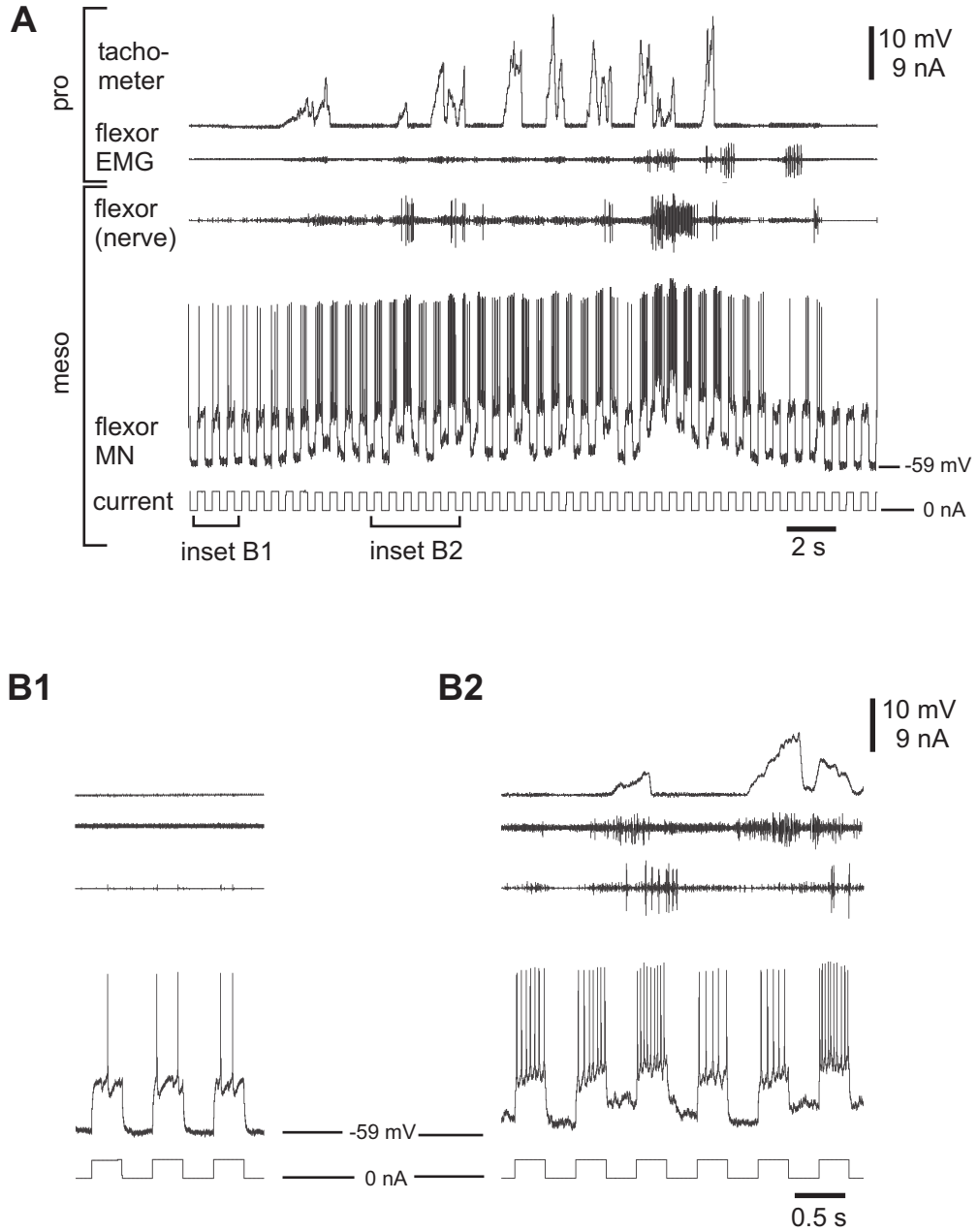


Figure 3.5: *Increased membrane responsiveness during tonic depolarization of contralateral mesothoracic flexor MNs. Short current pulses (**5th trace**) that depolarized the flexor MNs (**4th trace**) just above threshold were injected. **A:** Before and after a front leg stepping sequence (**1st trace**) each current pulse induced 1 or 2 spikes in a flexor MN (see clipping **B1**). During a stepping sequence of the front leg, the number of current induced spikes increased (see clipping **B2**).*

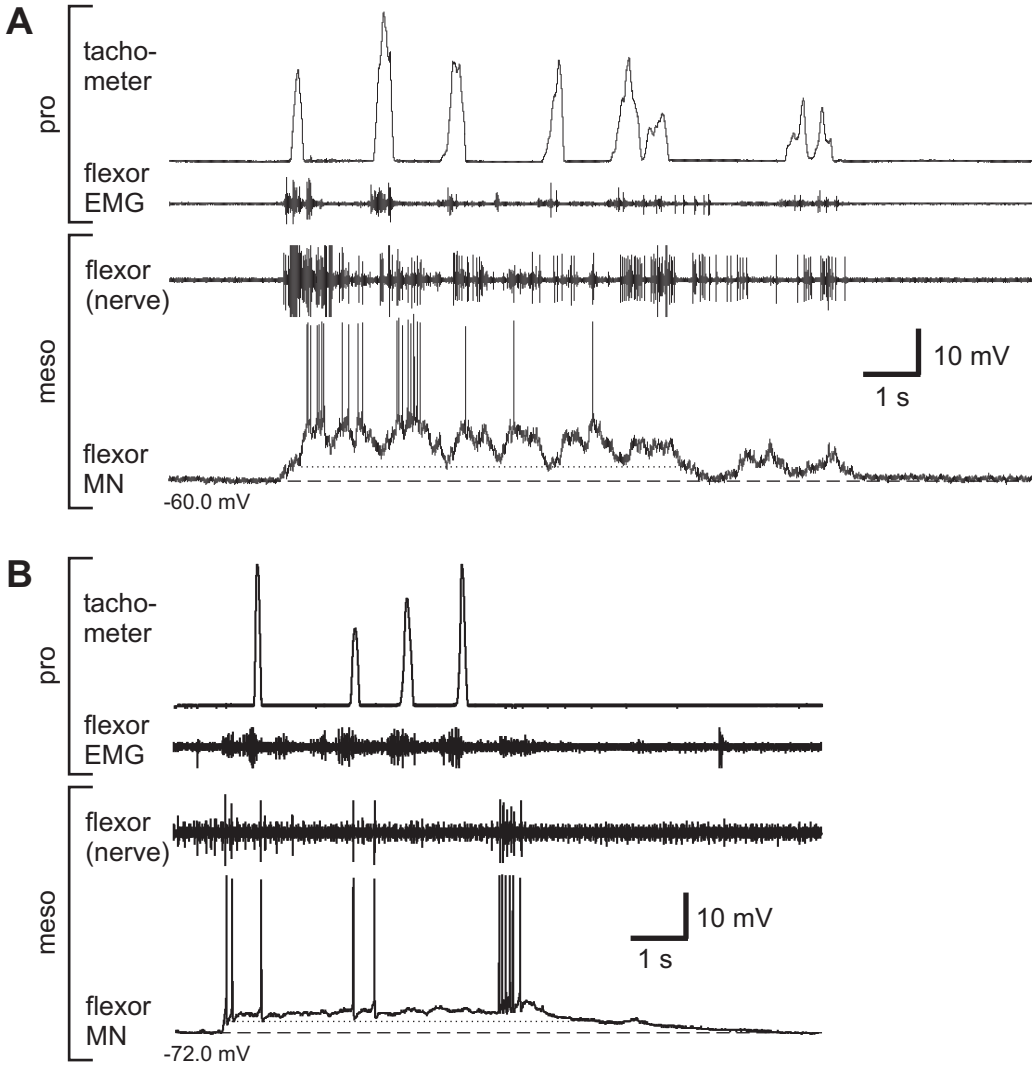


Figure 3.6: *Different shapings of activity in mesothoracic flexor MNs contralateral to the stepping front leg.* ***1st and 2nd trace****: Treadmill velocity (tachometer) and EMG recording of the stepping leg's flexor tibiae muscle.* ***3rd trace****: Extracellular recording from the contralateral mesothoracic ncr.* ***A: 4th trace****: A flexor MN showed a tonic depolarization as well as pronounced membrane potential modulation during front leg stepping. The tonic depolarization repolarized to resting values after 550 ms.* ***B: 4th trace****: Flexor MN showed a tonic depolarization but no apparent membrane potential modulation during front leg stepping. Thus, the tonic depolarization appears to be largely independent of the actual stepping movement. The repolarization in membrane potential took ~3 s. Dashed lines indicate resting membrane potentials, dotted lines indicate the lower edge of the phasic modulation.*

3.1.2 Rhythmic modulation in mesothoracic flexor MNs

Phasic membrane potential changes in contralateral flexor MNs during front leg stepping sequences were coupled to movements of the front leg in 16 of 24 recordings. Two different coupling patterns were observed in these 16 flexor MNs: in 12 flexor MNs a depolarization in membrane potential with a mean peak-to-peak amplitude of 2.8 ±1.5 mV was observed, which began approximately 200 ms before onset of front leg stance phase (Fig. 3.7 A). The depolarization reached a maximum during stance phase and began to decline at the end of stance phase. Figure 3.7 B shows a different coupling pattern with similar peak-to-peak amplitudes that was observed in four flexor MNs. The membrane potential began to decline from a maximum some 100 ms before onset of stance and reached a minimum during the first half of the stance phase. In the other 33 % of the recordings (N=8/24; Fig. 3.7 C), no phasic modulation of the membrane potential that was coupled to front leg steps was observed.

Ludwar et al. (2005b) described the phasic modulation of **ipsilateral** MNs, including the phasic modulation of flexor MNs. They described that in four out of five ipsilateral flexor MNs from the most hyperpolarized potential around onset of front leg stance the membrane potential depolarized (peak-to-peak amplitudes ~4 mV, peak depolarization after stance phase). This resembles the phasic modulation observed in 17 % of contralateral flexor MNs (Fig. 3.7 B). In another ipsilateral flexor MN no step coupled phasic modulation could be detected. Due to the fact that contralateral flexor MNs exhibited three distinct types of phasic modulation, the question arose whether this holds true also for ipsilateral flexor MNs if a comparable population is analyzed. Therefore a population of 27 ipsilateral flexor MNs was analyzed and figure 3.8 shows that three types of phasic modulation occurred. In 15 % of the recordings (N=4/27), the onset of a membrane depolarization was ~0.05 s before onset of front leg stance phase. The depolarization reached a maximum at the end of stance phase (Fig. 3.8 A). Mean peak-to-peak amplitudes were 2.2 ±1.0 mV. The most prominent type (52 %, N=14/27) is shown in figure 3.8 B. The membrane potential began to decline from a maximum some 200 ms before onset of stance and reached a minimum during the first 100 ms of the stance phase. During stance, the membrane potential repolarized. Mean peak-to-peak amplitude was

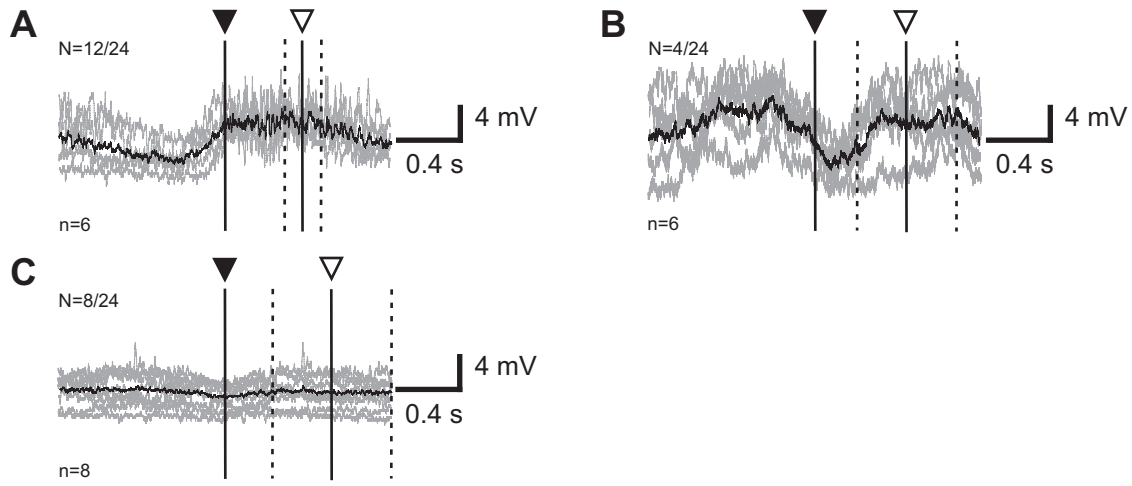


Figure 3.7: *Phasic modulation in membrane potential in contralateral flexor MNs during front leg stepping.* ***A*-*C:*** *overlays (gray area) and averages (solid lines) of 6 to 8 sweeps triggered by the onset of stance phase (1st vertical solid lines and black arrowheads). Mean end of stance phase is indicated by open arrowheads and 2nd solid line, dashed lines indicate SD.* ***A:*** *Depolarization of the membrane in a flexor MN started ~200 ms before onset of front leg stance phase and reached its peak depolarization with onset of stance (observed in 12 of 24 experiments). The membrane remained depolarized during stance.* ***B:*** *Hyperpolarization of the membrane in a different flexor MN started ~200 ms before onset of front leg stance phase and reached its minimum ~100 ms after onset of stance (N = 4/24). During stance, the membrane repolarized.* ***C:*** *In a different flexor MN, there is no obvious coupling of membrane potential modulation to front leg steps (N = 8/24).*

2.6 ±0.6 mV. Similar to contralateral flexor MNs, the other 33 % of the ipsilateral recordings showed no phasic modulation of the membrane potential coupled to front leg steps (Fig. 3.8 C).

3.1.3 Summary

In this chapter, I analyzed the activity of mesothoracic flexor MNs during front leg stepping sequences. The motoneuronal activity in the extracellular nerve recordings was increased during contralateral front leg stepping and intracellular recordings revealed two distinct modulations in membrane potential: a tonic depolarization and a phasic modulation that was mostly coupled to front leg steps. The tonic depolarization was associated with a decrease in input resistance. The amplitudes were dependent on membrane potential, revealing a reversal potential of -40.7 mV. These properties indicate that either the tonic depolarization is based on nonselective cation conductance (e.g., for Na^+ and K^+) or it is a heterogeneous product of currents through different channels. The responsiveness of contralateral flexor MNs was enhanced during front leg stepping sequences, and the tonic depolarization could outlast the stepping sequence up to 30 s. The properties of the tonic depolarization lead to the question of which transmitters/receptors might be involved in mediating this effect. The prolonged hyperexcitability indicates a role for metabotropic receptors in mediating the tonic depolarization. Involvement of second messengers seems also to be possible, due to the long repolarization time constants. These assumptions are partly tested and discussed in chapter 3.2 and 3.3.
Three distinct types of phasic modulation in membrane potential in flexor MNs were revealed, both for ipsi- and contralateral flexor MNs. Two types showed a phasic modulation that was coupled to front leg steps: 1) 15 % of **ipsilateral** flexor MNs showed a depolarization of membrane potential that started ~50 ms before onset of stance phase and reached its peak at the end of stance. A similar phasic modulation with a slightly shifted time course was observed in 50 % of **contralateral** flexor MNs. The onset of membrane potential depolarization was ~200 ms before onset of front leg stance phase and reached its peak with the onset of stance. 2) In 52 % of **ipsilateral** and 17 % of **contralateral** flexor MNs a hyperpolarization of membrane potential started ~200 ms before onset

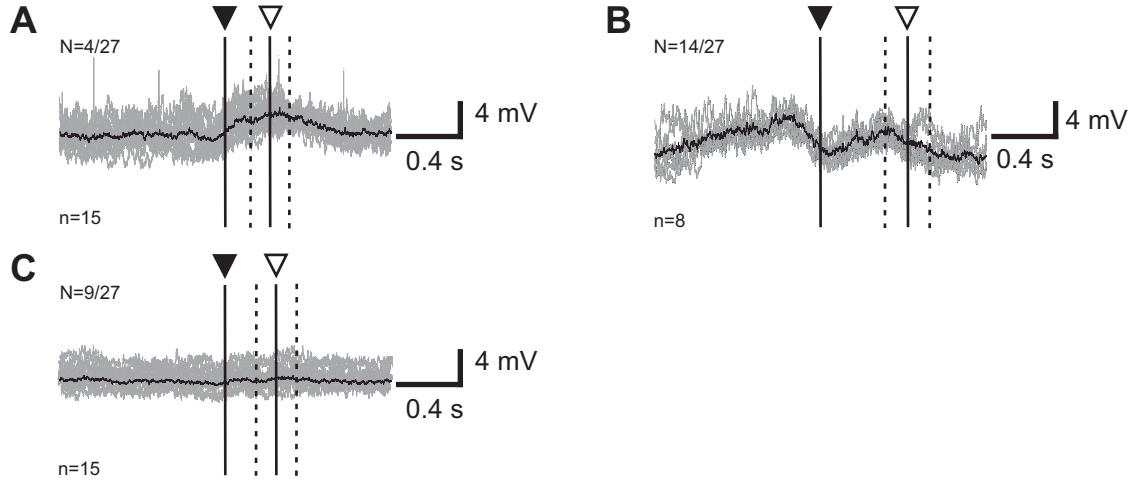


Figure 3.8: *Phasic modulation in membrane potential in ipsilateral flexor MNs during front leg stepping.* ***A-C:*** *Overlays (gray area) and averages (solid lines) of 8 to 15 sweeps triggered by onset of stance phase (1st vertical solid lines and black arrowheads). Mean end of stance phase is indicated by open arrowheads and 2nd solid line, dashed lines indicate SD.* ***A:*** *Depolarization of the membrane of a flexor MN started ~50 ms before onset of front leg stance phase and reached its peak during stance (observed in 4 of 27 experiments). The membrane potential declined after the end of stance.* ***B:*** *Hyperpolarization of the membrane in a different flexor MN started ~200 ms before onset of front leg stance phase and reached its minimum ~100 ms after onset of stance (N = 14/27). During stance, the membrane repolarized.* ***C:*** *In a different flexor MN, there was no obvious coupling of membrane potential modulation to front leg steps (N = 9/27).*

of front leg stance phase and reached its minimum approximately 100 ms after onset of stance. 3) No obvious coupling of phasic modulation to front leg steps was observed in 33 % of **ipsi**- and **contralateral** flexor MNs.

3.2 Transmitters

The properties of the tonic depolarization in mesothoracic flexor MNs that are observed during front leg stepping indicate that it is based on a nonselective cation conductance and possibly involves metabotropic receptors and second messengers (chapter 3.1). The presence of several neuroactive substances was shown for insect nervous systems. These are for example acetylcholine, glutamate, GABA (γ-amino butyric acid), biogenic monoamines (octopamine and serotonin) and peptides as proctolin (see review by Pichon and Treherne (1972) and references therein). By the use of pharmacological means the role of certain neurotransmitters and modulators in mediating the tonic depolarization of mesothoracic flexor MNs was investigated while the front leg performed stepping sequences on a treadmill. In stick insects, the ganglion sheath is a diffusion barrier (Pichon and Treherne 1972). Diffusion of pharmacological agents is blocked to the same degree as for ions. This problem is bypassed by desheathing the ganglion, and thus allowing the diffusion of substances. Based on the single-legged preparation, the mesothoracic ganglion was desheathed and superfused separately (due to silicon-gel barriers) with drug containing solutions (see materials and methods, chapter 2.2.3).

3.2.1 Role of Acetylcholine

Acetylcholine (ACh) appears to be the dominant neurotransmitter in the central nervous system of insects (Osborne 1996; Breer and Sattelle 1987). The action of ACh in insects is mediated by either nicotinic or muscarinic receptors (Breer 1981; Breer and Sattelle 1987). With few exceptions muscarinic ACh receptors (mAChRs) are present only in a low density in the insect nervous system compared to nicotinic ACh receptors (nAChRs) (Knipper and Breer 1988; Trimmer and Weeks 1993). This is in contrast to vertebrates, where the action of ACh is mainly transduced by mAChRs (Birdsall and Hulme 1976). Furthermore, no evidence was found for insect mAChRs in mediating effects at the neuromuscular synapse (Sattelle 1980). Evidence for mAChRs mediating effects at other synapses was found for several insect species: *Locusta migratoria*, *Manduca sexta*, *Periplaneta americana*, *Drosophila melanogaster*, honey bee and housefly (detailed references in Trimmer and Weeks 1993; Bai and Sattelle 1994).

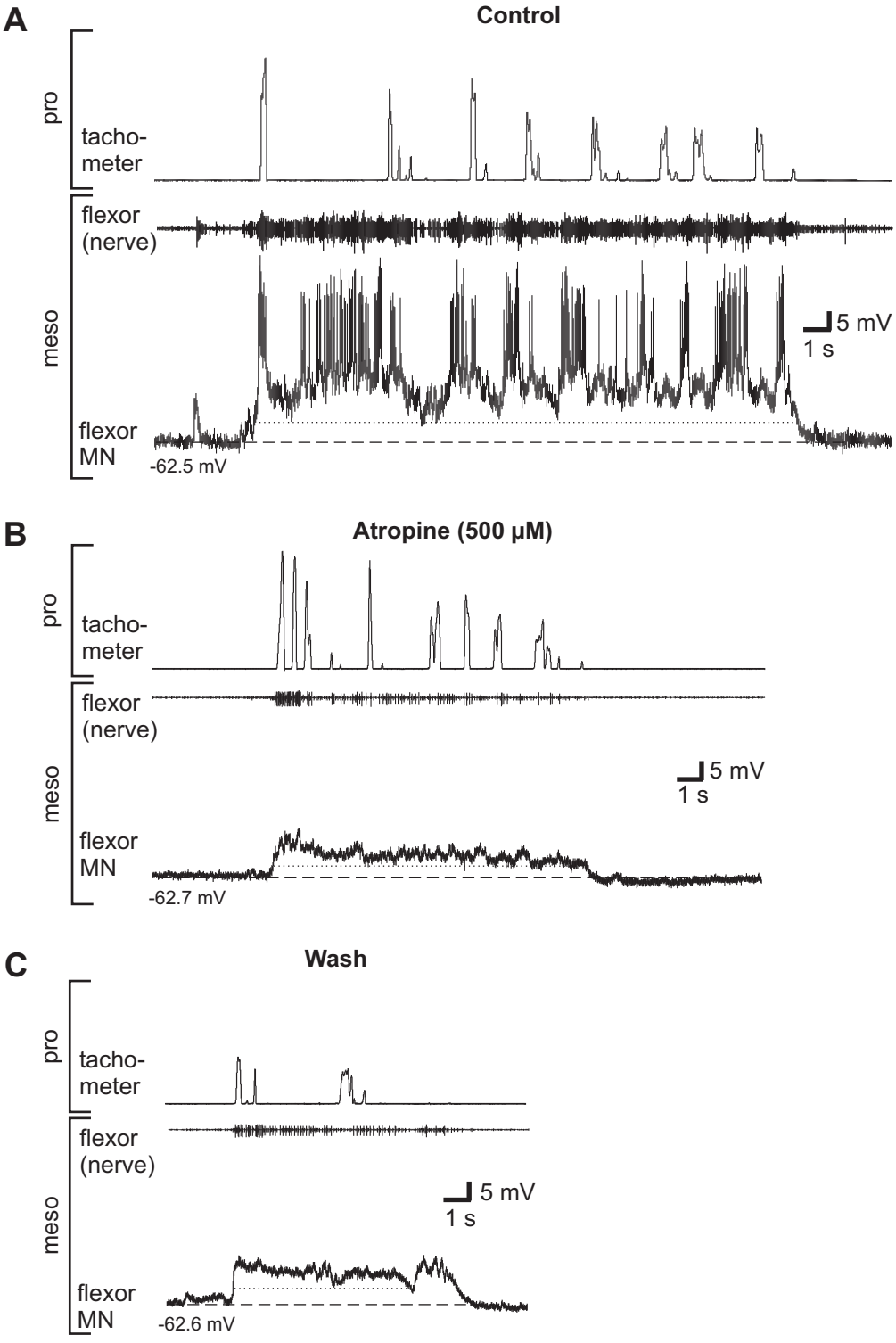

A
Control
pro
tacho-
meter
flexor
(nerve)
meso
5 mV
1 s
flexor
MN
-62.5 mV
B
Atropine (500 µM)
pro
tacho-
meter
flexor
(nerve)
meso
5 mV
1 s
flexor
MN
-62.7 mV
C
Wash
pro
tacho-
meter
flexor
(nerve)
meso
5 mV
1 s
flexor
MN
-62.6 mV

Figure 3.9: *Atropine decreased the tonic depolarization in mesothoracic flexor MNs during front leg stepping.* ***A:*** *Saline: With front leg stepping the activity in the ipsilateral mesothoracic nerve recording (ncr) was increased and the intracellular recording of a flexor MN showed a distinct tonic depolarization (~5 mV).* ***B:*** *Nerve activity as well as the amplitude of the tonic depolarization was decreased to 2.1 mV during superfusion of atropine (7 min)* ***C:*** *Control (15 min normal saline) showed a partial recovery of the amplitude of the tonic depolarization (2.5 mV).*

Metabotropic receptors might be involved in mediating the tonic depolarization in membrane potential. This is based on the observation that the responsiveness of the membrane is enhanced despite the decrease in input resistance (chapter 3.1, Fig. 3.5), and that this increased responsiveness could outlast the stepping sequence for tens of seconds. Trimmer and Weeks (1989) showed for *Manduca* MNs increased excitability that was mediated by mAChRs, however, the voltage sensitivity of this current differs from the tonic depolarization in stick insects.

Evidence for a role of mAChRs in stick insect motor acts comes from experiments using the muscarinic agonist pilocarpine which induces in MNs a rhythmic bursting pattern on top of a tonic depolarization (Büschges 1998). The antagonistic effect of atropine on mAChRs in insects was shown for the fast coxal depressor MN and a ventral giant interneuron in cockroaches (Bai and Sattelle 1994; le Corronc and Hue 1993) and in stick insects, atropine inhibited the pilocarpine induced rhythm in pro- and retractor MNs (Büschges et al. 1995). The effect of atropine on the tonic depolarization was analyzed, to test whether mAChRs might play a role in mediation of the tonic depolarization.

Figure 3.9 shows the effect of atropine on flexor spike activity, as recorded from *nervus cruris* and the membrane potential of a middle leg flexor MN during ipsilateral front leg stepping. In saline (control, Fig. 3.9 A) the activity in the nerve recording (ncr) was increased and the intracellular recording of a flexor MN showed a tonic depolarization of approximately 5 mV during a stepping sequence of eight front leg steps. On top of the tonic depolarization this flexor MN showed an additional phasic modulation with spike activity. The activity in the nerve recording was reduced during superfusion with 500 µM atropine (7 min, Fig. 3.9 B). Furthermore, the amplitude of the tonic depolarization was

decreased (by ~2 mV) during the stepping sequence. After rinsing 15 minutes with saline the recording showed a partial recovery in the tonic depolarization amplitude, to ~2.5 mV (Fig. 3.9 C).

In eight of nine recorded flexor MNs, the tonic depolarization amplitude was decreased after 5 min superfusion with atropine by 39.9 ±16.5 % (range −16.7 to −61.5%, values determined from linear fits in figure 3.10 B). A detailed analysis for a single experiment is shown in figure 3.10 A. The amplitude of the tonic depolarization (ΔV) is plotted against time. In saline (control, ○), the tonic depolarization amplitude was 4.1 ±0.2 mV (n=3) and decreased during superfusion of atropine (●) to 2.1 mV (9 min). During wash with saline (○), the tonic depolarization recovered to a value of 2.5 mV after 9 minutes. The correlation coefficient for the linear fit in this experiment was significant at the 0.1 % level. In figure 3.10 B all nine experiments are shown, each experiment represented by a different symbol. For the sake of clarity data points are not shown during atropine superfusion (n=3 to 8). Four flexor MNs were successfully recorded during wash and the individual amplitudes of these tonic depolarizations during wash are shown as data points. A partial recovery of the tonic depolarization amplitude was observed. In five of nine experiments the correlation coefficient was significant (at least at the 5 % level), in four experiments no significance could be detected. The degree of reduction in the tonic depolarization amplitude during atropine superfusion could be dependent on the value of the amplitude under control conditions (in saline). The slopes of linear fits (see Fig. 3.10 B) were plotted against the mean amplitudes of the tonic depolarization obtained in saline. The linear fit of this relationship was significant at the 5 % level (Fig. 3.10 C). Thus, there could be a dependency of the height of the control value of the tonic depolarization amplitude and the decrease caused by atropine. A combined analysis of all data points from all experiments would therefore implicit first to normalize the data to the control values. The normalized data points from all experiments showed a high correlation for the reduction of the tonic depolarization amplitude over time (data not shown). The correlation coefficient differed only slightly from that obtained from the unnormalized plot (r=0.529 and 0.501), therefore the original data are shown in figure 3.10 D.

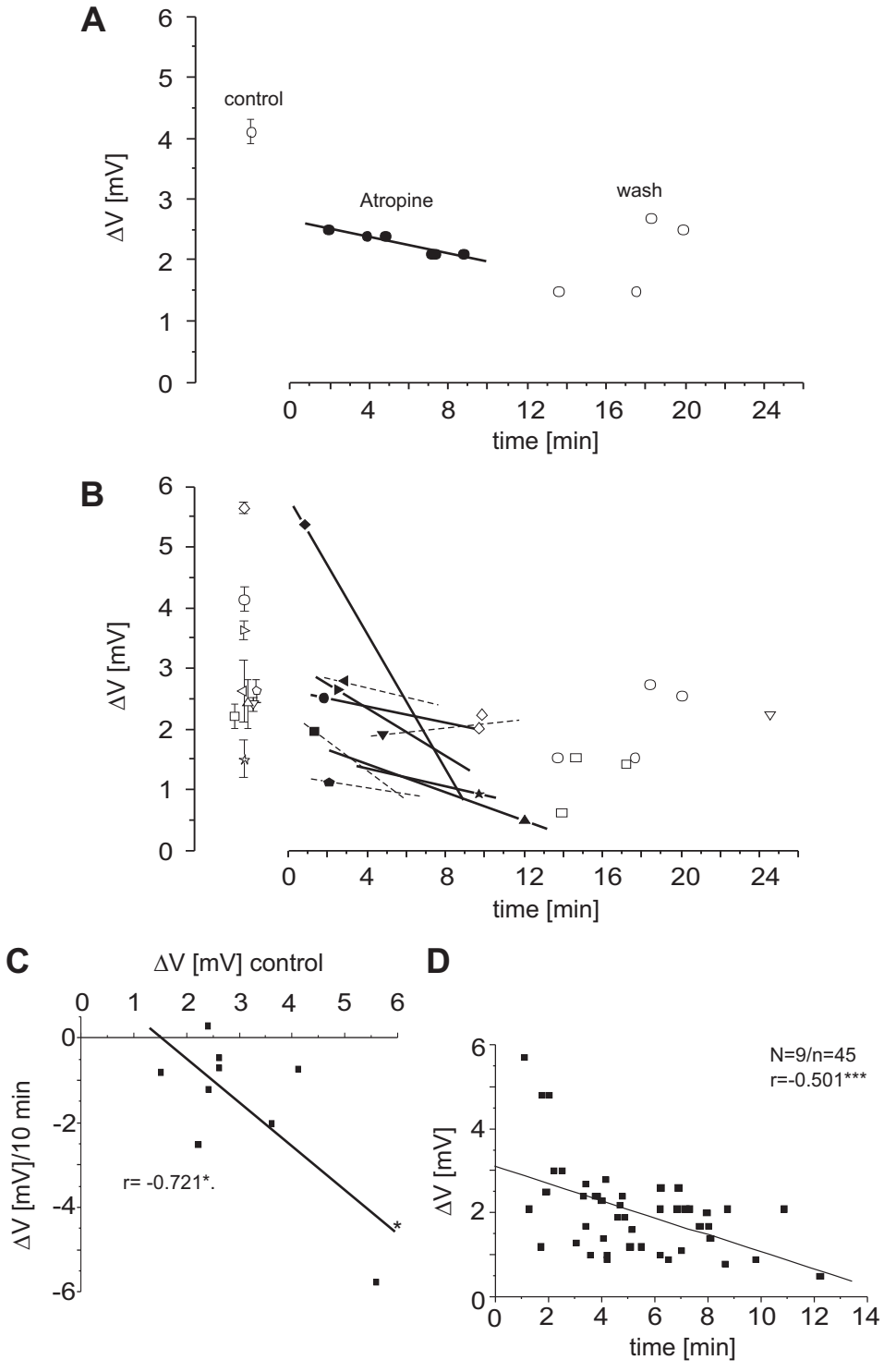

A
control
Atropine
wash
ΔV [mV]
time [min]
B
ΔV [mV]
time [min]
C
ΔV [mV] control
ΔV [mV]/10 min
r= -0.721*.
D
N=9/n=45
r=-0.501***
ΔV [mV]
time [min]

Figure 3.10: *Tonic depolarization amplitude over time during atropine superfusion.* ***A:*** *Detailed analysis for a single experiment. From a control value of 4.1 ±0.2 mV (n=3, ○) the tonic depolarization amplitude (ΔV) decreased to 2.1 mV after 9 min superfusion with 500 µM atropine (●). During wash (○) ΔV recovered to a value of 2.5 mV. Data points are fitted by linear regression (n=6).* ***B:*** *Time course for all experiments (N=9). Only linear fits are shown for ΔV during atropine superfusion (n=3 to 8). Solid lines: level of significance at least 5 %, dashed lines: no significance. Same symbol is one experiment. Open symbols indicate control values (from n=2 to 8 stepping sequences).* ***C:*** *To analyze if there is a dependency between the height of the control value of the tonic depolarization amplitude and the degree of decrease during superfusion with atropine, regression coefficients of the linear fits were plotted against ΔV in control condition. The plot shows a linear relationship (*).* ***D:*** *Plot of ΔV obtained from all stepping sequences during atropine superfusion showing a high correlation (***) level of significance P < 0.001 (N=9, n=45)*

3.2.2 Effect of Octopamine

Although in chapter 3.2.1 a possible role for ACh as a transmitter was shown (or at least a role for mAChRs in mediating the tonic depolarization), this does not eliminate the possibility that other transmitters and neuromodulators are also involved. Cotransmission is known in many systems. In rats glycine and GABA cotransmission occurs in brain stem MNs and spinal interneurons (Wu et al. 2002). In the stomatogastric nervous system of the crab, *Cancer borealis*, proctolin and GABA are found in the modulatory proctolin neuron (Blitz and Nusbaum 1999). In locusts, proctolin acts as a cotransmitter with octopamine and probably glutamate in nerves supplying the oviduct (Lange 2002). Until now, nothing is known about cotransmission in stick insects, but an independent interaction of transmitters seems possible, if one bears in mind that the tonic depolarization could be the product of the activity of different channels.

There is no detailed knowledge about the role of octopamine in stick insects, but previous studies have shown that octopamine injection into the hemolymph of intact, inactive stick insects caused an activation of the animal and suppressed pathways involved in the resistance reflex (Büschges et al. 1993; Ramirez et al. 1993). Generally, in insects, octopamine can act as a neurotransmitter, neurohormone and neuromodulator (Orchard 1982). It is the invertebrate counterpart of noradrenaline. Octopamine has several physiologi-

cal roles in insects, such as modulating the activities of sense organs, or neurons within the brain (Roeder 2002). These effects are usually mediated by metabotropic receptors. Sombati and Hoyle (1984) showed that injection of octopamine into the locust nervous system elicited behaviors such as flight or running.

In this set of experiments it was analyzed if octopamine influences the tonic depolarization in mesothoracic flexor MNs. Different concentrations of octopamine were used, ranging from 0.25 to 1 mM. In summary, ten of eleven flexor MNs exhibited an increased tonic depolarization during superfusion of octopamine (0.25 to 1 mM). In one experiment, octopamine (1 mM) reduced the tonic depolarization in one flexor MN, and showed a decrease in the amplitude. A representative experiment for the increase of the tonic depolarization amplitude is shown in figure 3.11. In saline, the activity in the nerve recording was increased and a clear tonic depolarization (2.3 mV, Fig. 3.11 A) was visible in a flexor MN during a stepping sequence of the ipsilateral front leg (10 steps). The flexor MN generated spikes throughout the stepping sequence. After 9 min superfusion of 500 µM octopamine, the tonic depolarization amplitude increased to 4.3 mV during a sequence of 8 steps (Fig. 3.11 B). The spike activity in the flexor MN as well as the activity in the nerve recording was slightly increased. Phasic inhibition as seen in this flexor MN in saline (Fig. 3.11 A) seemed to be less pronounced in the presence of octopamine.

Figure 3.12 A shows a detailed analysis for a single experiment. The amplitude of the tonic depolarization (ΔV) is plotted against time. In control conditions, ΔV was 2.5 ±0.4 mV (n=8) and increased during superfusion of octopamine (500 µM) to 4.3 mV (9 min). The amplitude of the tonic depolarization increased further during wash with saline. The correlation coefficient for the linear fit in this experiment was significant at the 0.1 % level (n=11). The effect of 500 µM octopamine was tested in four of eleven experiments. The analysis for these experiments together with a single experiment using 250 µM octopamine is shown in figure 3.12 B. The positive slopes of all the linear fits indicate an increase of the tonic depolarization by octopamine. One flexor MN was recorded successfully during wash and the individual amplitudes are shown as data points. The correlation coefficient was significant in three of four experiments (n=5 to 13). In one experiment

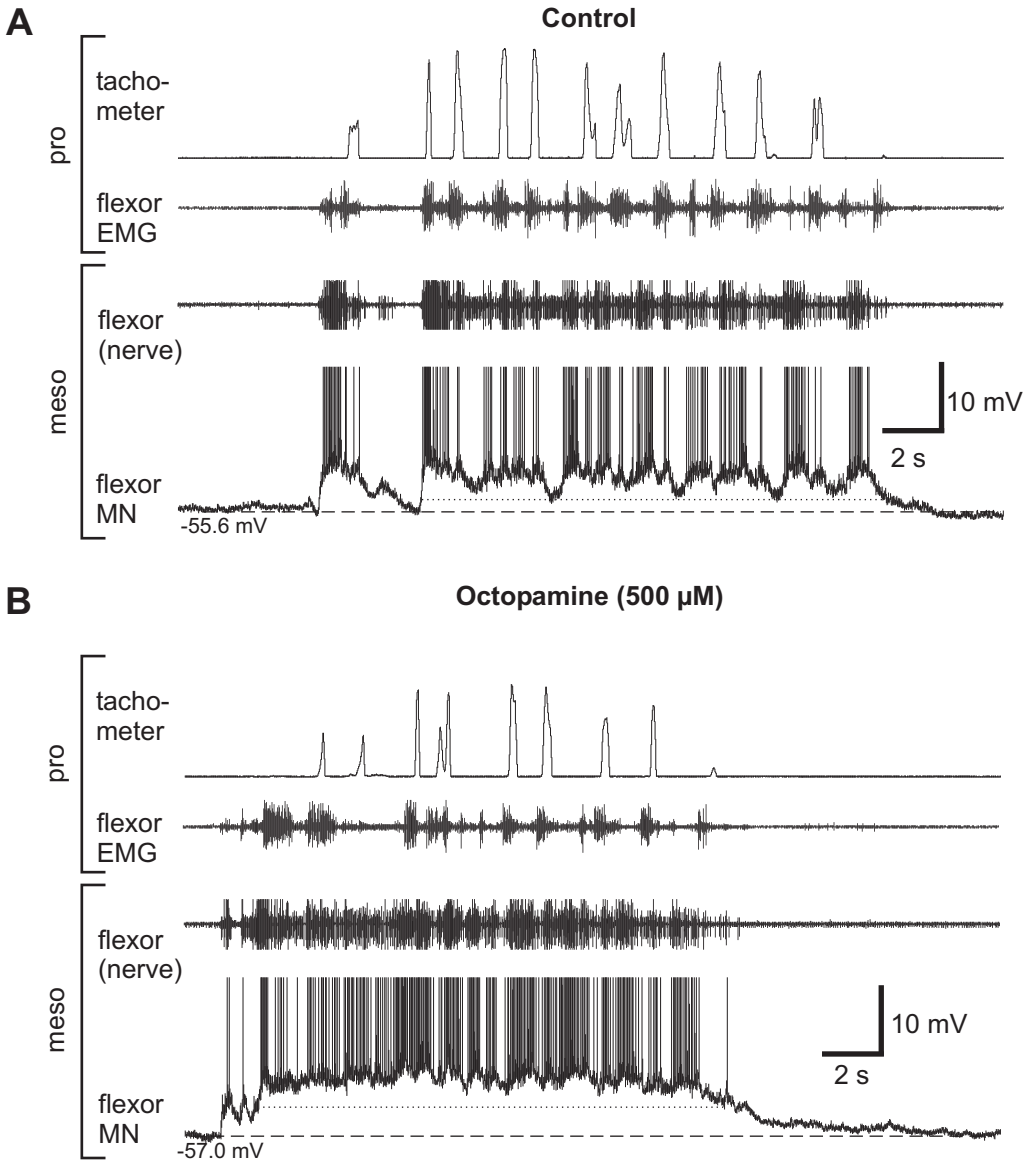
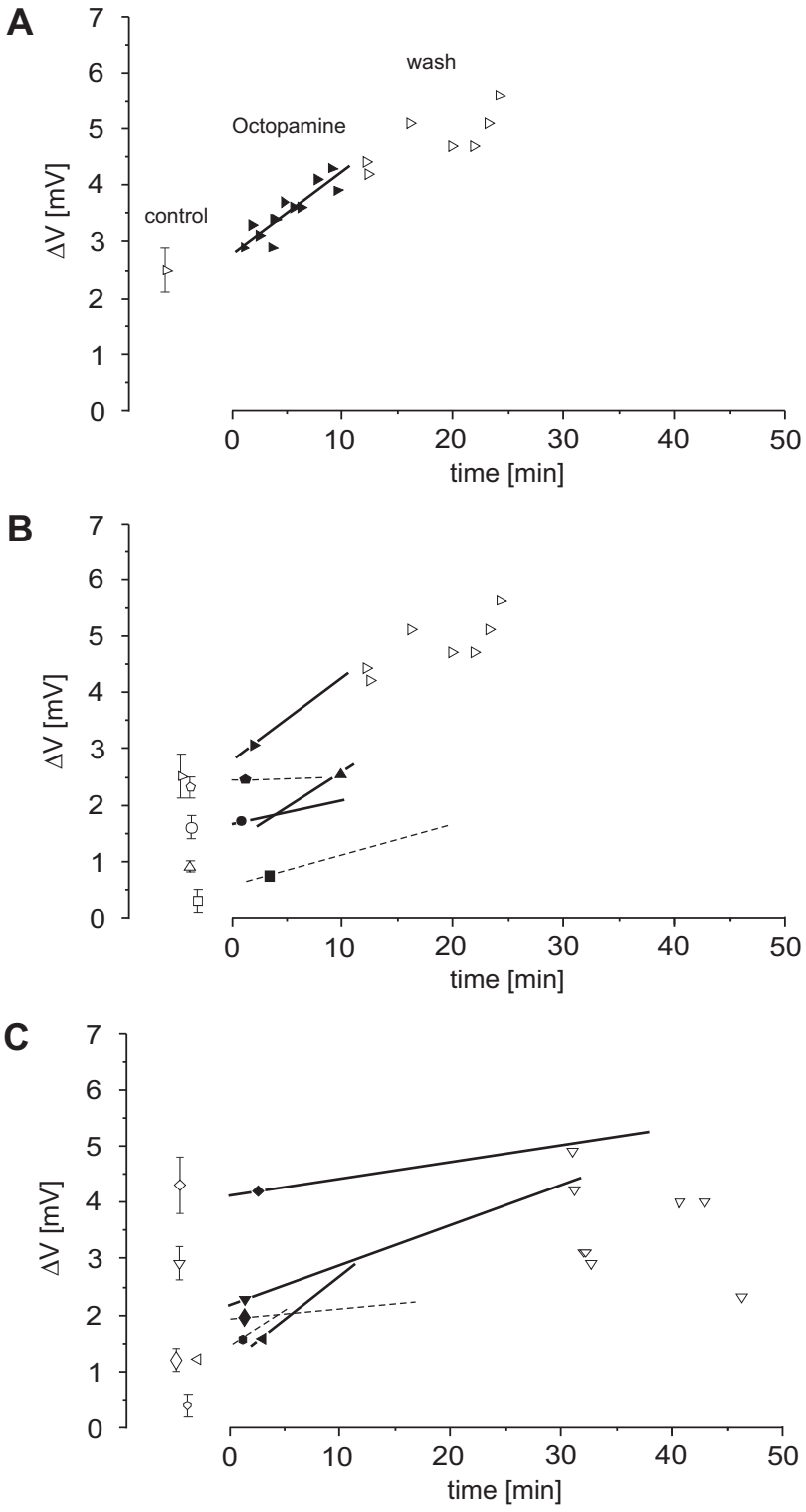


Figure 3.11: *Octopamine increased the tonic depolarization in mesothoracic flexor MNs during front leg stepping.* ***A:*** *Saline: A sequence of 10 steps led to an increased activity in the ipsilateral mesothoracic nerve recording (ncr), as well as a clear tonic depolarization (~2.3 mV) in a flexor MN. This flexor MN showed spike activity (action potentials truncated) throughout the stepping sequence.* ***B:*** *The tonic depolarization amplitude was increased during superfusion of 500 µM octopamine (9 min, 4.3 mV). Nerve activity as well as spike activity in the flexor MN was slightly increased.*


A
B
C
ΔV [mV]
time [min]
control
Octopamine
wash
0
1
2
3
4
5
6
7
0
10
20
30
40
50

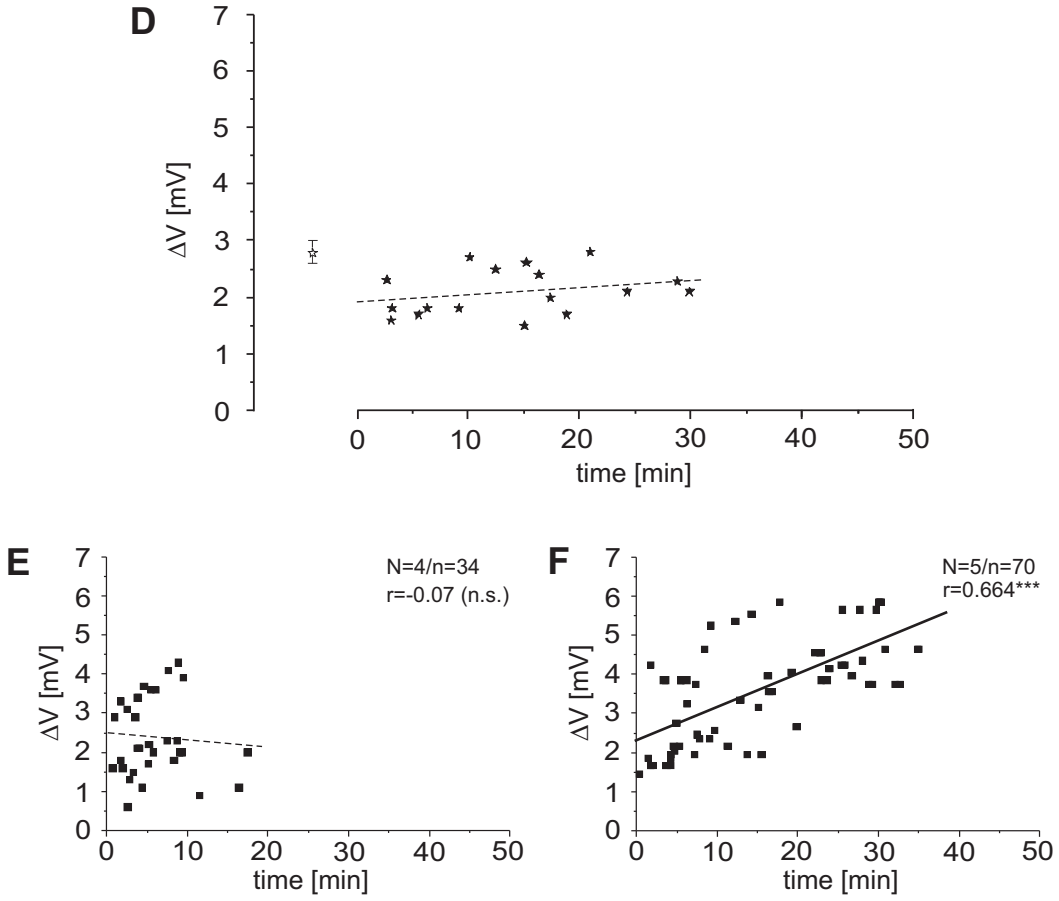


Figure 3.12: *Tonic depolarization amplitude over time during octopamine superfusion.* ***A:*** *Detailed analysis for a single experiment. From a control value of 2.5 ±0.4 (n=6, ▷) ΔV increased to 4.3 mV after 9 min superfusion with 500 µM octopamine (►). During wash (▷), ΔV increased further (5.6 mV, 15 min wash). Data points are fitted by linear regression (n=11).* ***B-D:*** *Same symbol is one experiment. Solid lines: level of significance at least 5 %, dashed lines: no significance. Only linear fits are shown for ΔV during octopamine superfusion.* ***B:*** *Time course for experiments during superfusion of 500 µM octopamine (N=4, n=5-13), and for one experiment using 250 µM octopamine (⬠, n=8).* ***C:*** *Time course for experiments during superfusion of 1 mM octopamine with increased ΔV (N=5/6, n=4-32).* ***D:*** *Time course for one experiment with decreased ΔV during superfusion of 1 mM octopamine (N=1/6, n=17).* ***E:*** *Plot of ΔV obtained from all stepping sequences during superfusion of 500 µM octopamine showing no correlation (N=4/n=34; r=−0.07).* ***F:*** *Plot of ΔV obtained from all stepping sequences during superfusion of 1 mM octopamine showing a high correlation (r=0.664***), level of significance p < 0.001 (N=5, n=70).*

it was not significant, as well as in the experiment using 250 µM octopamine (n=8). A concentration of 1 mM octopamine induced in five of six experiments an increase in the amplitude of the tonic depolarization (n=4 to 32; Fig. 3.12 C), whereas the correlation coefficient was significant in three experiments. In one experiment, the same concentration led to a decrease in the tonic depolarization (n=17; Fig. 3.12 D), which was not significant.

Slopes of linear fits from figures 3.12 B and 3.12 C were plotted against the mean amplitudes obtained in saline (data not shown). No linear correlation was observed. Thus, all tonic depolarization amplitudes obtained during octopamine superfusion were plotted over time, for 500 µM and 1 mM octopamine respectively (Figs. 3.12 E, 3.12 F). Although for some experiments, the correlation coefficient was significant during superfusion of 500 µM octopamine (*, ***), the plot for all amplitudes showed no significant correlation (Fig. 3.12 E). This is different from experiments where 1 mM octopamine was superfused. A high correlation for the increase in the amplitude of the tonic depolarization over time was observed (r=0.664***).

In summary, the tonic depolarization amplitude increased during superfusion of 500 µM octopamine by 88.9 ±73.9 %, range 19 to 180 % (0.7 ±0.4 mV, range 0.3 to 1.1 mV; N=4) and during superfusion of 1 mM octopamine by 108.6 ±179.9 %, range −6.9 to +425 % (0.6 ±0.8 mV, range −0.2 to+ 1.7 mV; N=5).

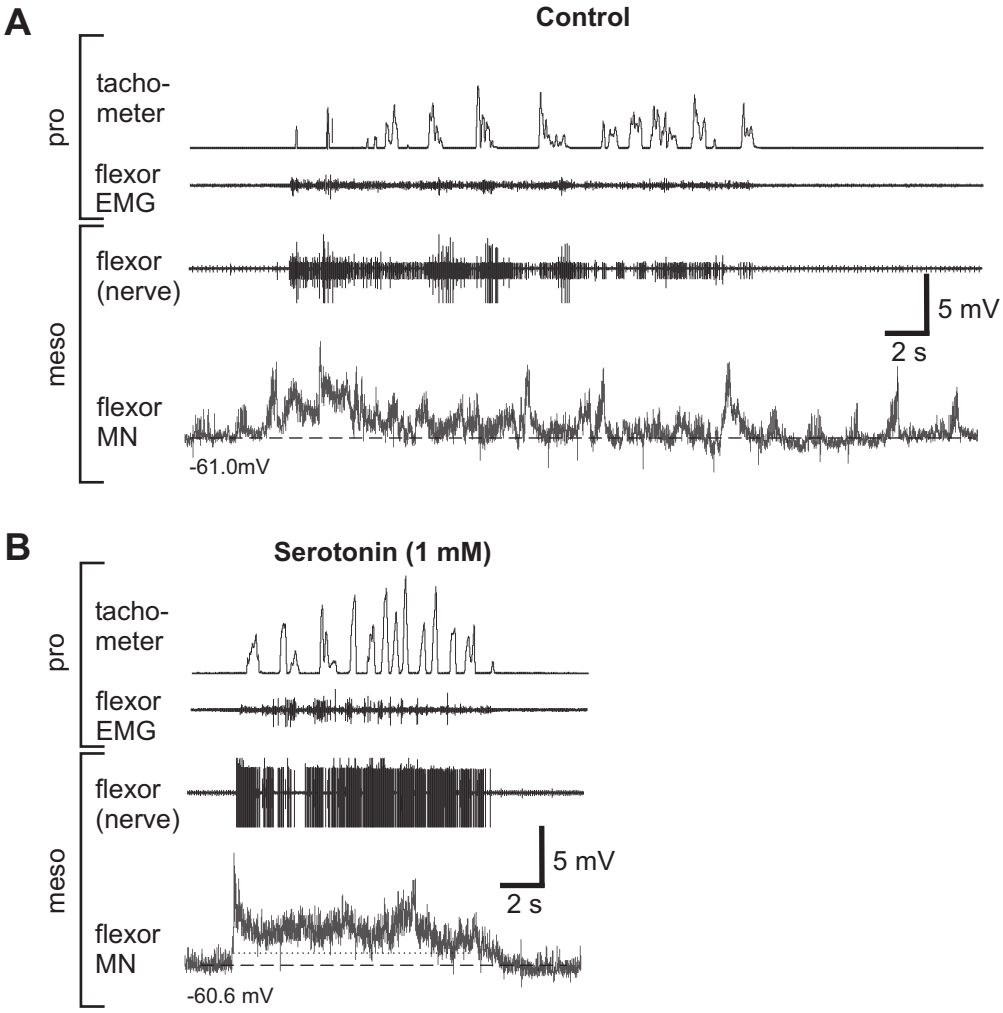
3.2.3 Effect of Serotonin

The presence of serotonin (5-hydroxytryptamine, 5-HT) in the CNS has been shown for several insect species, including the locust, the cockroach and the honey bee (see Nässel (1988) and references therein). Serotonin functions as a neurotransmitter and neuromodulator (Roeder 1994). Immunoreactivity was shown for locust ascending interneurons (Tyrer et al. 1984) and a modulatory role was shown for tibial MNs in the metathoracic ganglion (Parker 1995). The only described effect of serotonin in stick insects is that of the control of salivary glands secretion (Ali 1997).

The effect of serotonin on the tonic depolarization in mesothoracic flexor MNs during front leg stepping was analyzed in six animals. Concentrations ranging from 0.5 to 4 mM were tested. Serotonin showed contrary effects: in four of six experiments, the amplitude of the tonic depolarization was increased. Figure 3.13 shows a representative experiment. Under control conditions in saline, the activity in the nerve recording was increased but no tonic depolarization was visible in this flexor MN during a stepping sequence (10 steps, Fig. 3.13 A). A tonic depolarization of 1.3 mV during a sequence of 12 steps was apparent in the presence of 1 mM serotonin (Fig. 3.13 B). The spike activity in the nerve recording was largely increased. The effect was reversible, no tonic depolarization was detectable after 9 min wash with saline (Fig. 3.13 C). In this experiment, serotonin was superfused a second time (4 mM, Fig. 3.13 D) and again showed an increased tonic depolarization (1.2 mV). This flexor MN exhibited pronounced IPSPs in saline (Fig. 3.13 A, C), which were less obvious during superfusion of serotonin (Fig. 3.13 B, D).

The time course for this single experiment is shown in figure 3.14 A. The tonic depolarization amplitude (ΔV) is plotted against time. In this experiment, ΔV in saline was 0 mV (n=7). A tonic depolarization was not visible until superfusion of 1 mM serotonin. The amplitude was 1.3 mV after 37 min (n=16). During wash, ΔV decreased to control values and a second superfusion of serotonin induced a tonic depolarization during stepping sequences of up to 1.6 mV. The correlation coefficient for the linear fits in this experiment is significant at the 1 % (1 mM) and 5 % (4 mM) level.

A range of different serotonin concentrations was used in six experiments. The following concentrations were used: 0.5 mM (N=1, n=4), 1 mM (N=3, n=12 to 16) and 4 mM (N=3,


A
Control
pro
tacho-
meter
flexor
EMG
meso
flexor
(nerve)
5 mV
2 s
flexor
MN
-61.0mV
B
Serotonin (1 mM)
pro
tacho-
meter
flexor
EMG
meso
flexor
(nerve)
5 mV
2 s
flexor
MN
-60.6 mV

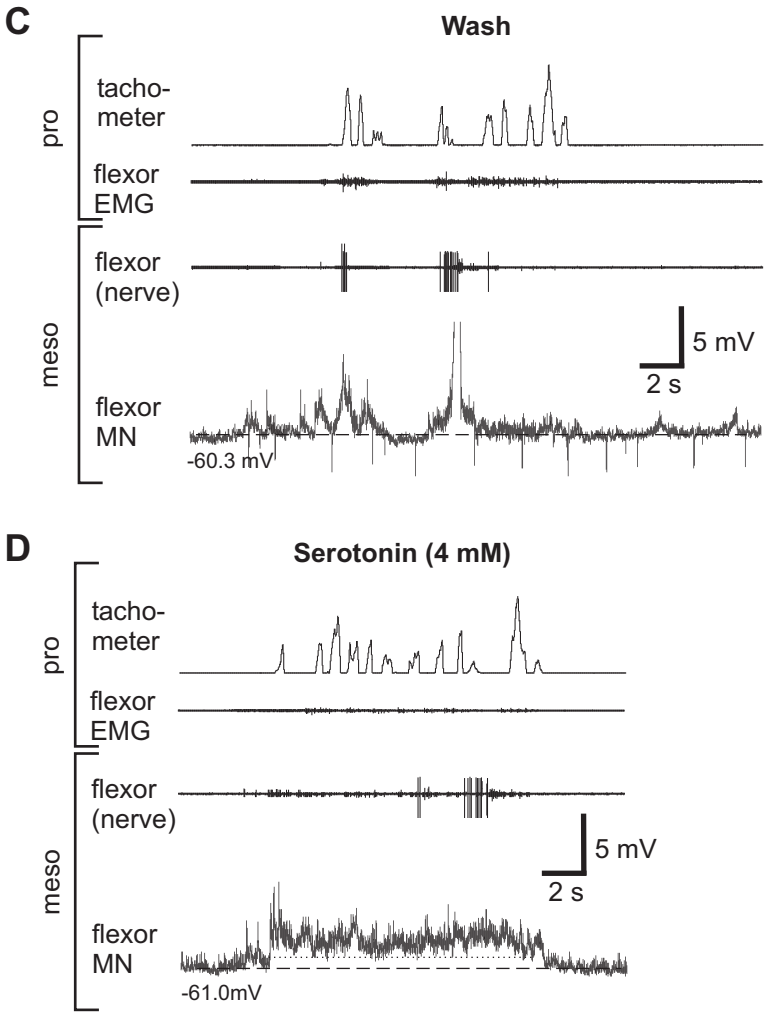


Figure 3.13: *Serotonin increased the tonic depolarization in mesothoracic flexor MNs during front leg stepping.* ***A:*** *During a stepping sequence of the front leg (10 steps), no tonic depolarization was visible in an ipsilateral mesothoracic flexor MN (control in saline). Nerve activity throughout the stepping sequence was increased.* ***B:*** *A distinct tonic depolarization was visible during superfusion of 1 mM serotonin (12 steps; 20 min, 1.3 mV), as well as pronounced increased nerve activity.* ***C:*** *During wash, the tonic depolarization recovered to control value (9 steps; 9 min).* ***D:*** *Superfusion of 4 mM serotonin induced a pronounced tonic depolarization during a sequence of 10 steps (5 min, 1.2 mV).*

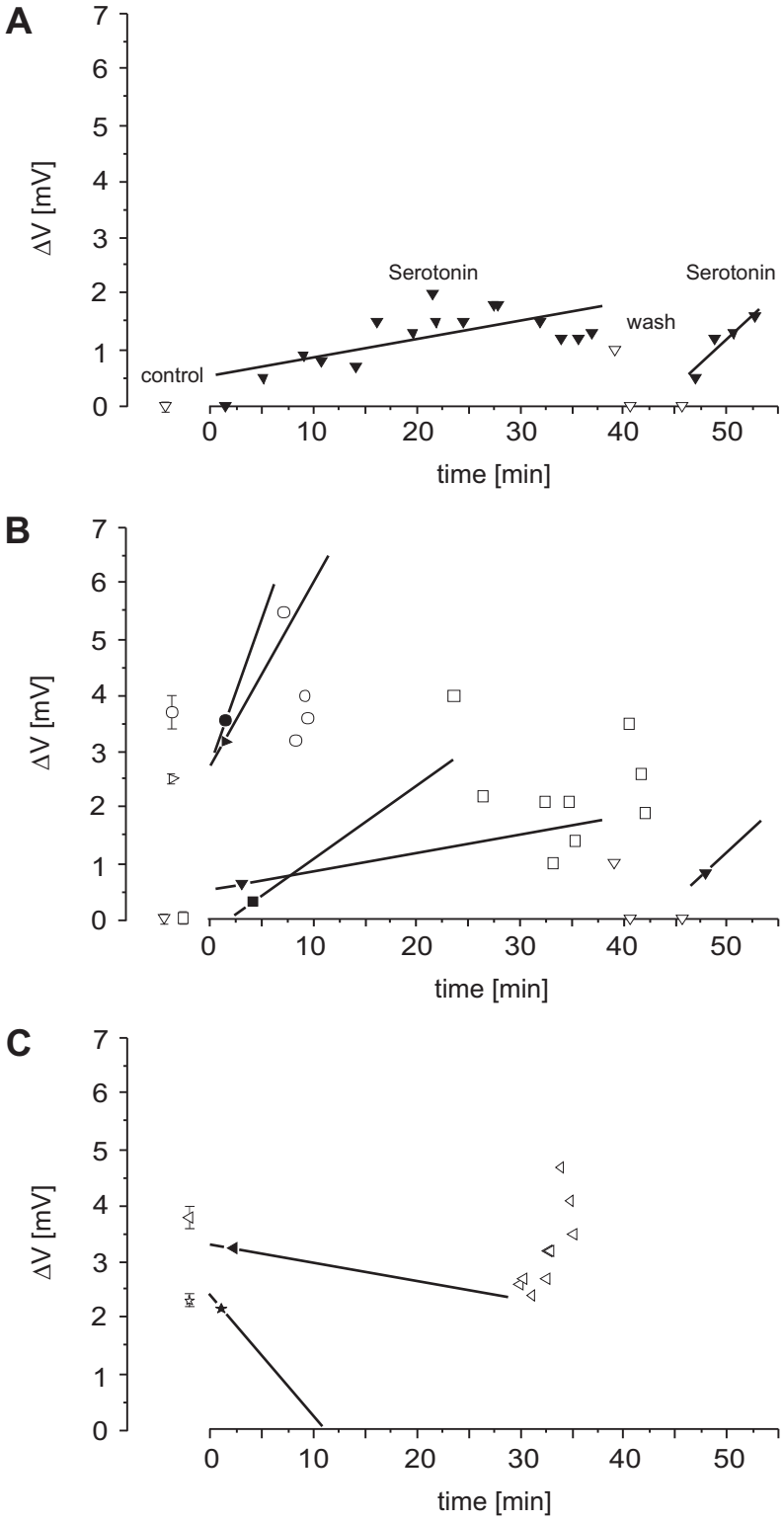

A
B
C
ΔV [mV]
time [min]
control
Serotonin
wash
Serotonin
0
1
2
3
4
5
6
7
0
10
20
30
40
50

Figure 3.14: *Tonic depolarization amplitude over time during serotonin superfusion.* ***A:*** *Detailed analysis for a single experiment. 1 mM serotonin increased the tonic depolarization from a control value of 0 mV (n=7, ▽) to 1.3 mV (20 min, ▼). During wash (▽) the amplitude of the tonic depolarization recovered to 0.3 ±0.6 mV (n=3). Superfusion of 4 mM serotonin induced an increase in the tonic depolarization in the same flexor MN to 1.6 mV (7 min). Data points are fitted by linear regression (1 mM serotonin: n=16; 4 mM serotonin: n=4).* ***B-C:*** *Same symbol is one experiment. Solid lines: level of significance at least 5 %. Only linear fits are shown for ΔV during serotonin superfusion.* ***B:*** *Time course for experiments during superfusion of serotonin with increased ΔV (n=4-16). The concentrations are labeled as follows: 0.5 mM: ●; 1 mM: ▼, ■; 4 mM: ►. In four experiments serotonin increased the tonic depolarization (linear fits, filled symbols) compared to control (open symbols). Three flexor MNs were successfully recorded during wash, two showed a partial recovery in the tonic depolarization amplitude.* ***C:*** *Time course for experiments during superfusion of serotonin with decreased ΔV (n=8-13). Serotonin (1-4 mM) decreased the tonic depolarization in two experiments. Wash with saline was successfully performed in one experiment and showed a recovery.*

n=4 to 10). In general, a specific concentration was superfused only once in one animal or during the recording of a single flexor MN respectively. An exception is shown in figures 3.13 D and 3.14 A, where 1 mM and 4 mM serotonin were superfused successively during the same recording. Serotonin showed two opposing effects on the tonic depolarization amplitude. Therefore, the analysis was performed separately for both. Serotonin increased the tonic depolarization amplitude in four of six experiments (Fig. 3.14 B). In all these different concentrations serotonin increased the amplitude significantly. Figure 3.14 C shows that serotonin induced the opposite effect in two experiments (1 and 4 mM), the correlation coefficient was significant at least at the 1 % level (n=8-13). In summary, the increase in the tonic depolarization amplitude by serotonin was 0.4 to 1.9 mV (without consideration of the used concentrations), the decrease was 0.7 and 1.0 mV.

3.2.4 Effect of Mianserin

In vertebrates, mianserin acts as a serotonergic antagonist. Invertebrate studies revealed that mianserin acts as an inhibitor of **octopamine receptors** in the locust extensor tibiae neuromuscular preparation (Evans 1981), and Nickisch-Rosenegk von et al. (1996) showed an antagonistic action in the moth. However, there is also evidence that mianserin might antagonize **serotonergic receptors** (Barrett and Orchard 1990; Baines and Downer 1991).

In six of seven animals, mianserin (100 µM) decreased the tonic depolarization observed in contralateral flexor MNs during stepping sequences of the front leg. Figure 3.15 shows a representative experiment. In saline (Fig. 3.15 A), a sequence of seven front leg steps led to an increased nerve activity and a tonic depolarization in a flexor MN of 2.9 mV. In a comparable stepping sequence (six steps), a pronounced decrease in the tonic depolarization was observed during superfusion of mianserin (Fig. 3.15 B) to a value of 0.5 mV after 12 min. In addition, the nerve recording showed also decreased spike activity. This flexor MN showed a recovery of 80 % after a 10 min wash (Fig. 3.15 C).

A detailed analysis for a single experiment is shown in figure 3.16 A. The amplitude of the tonic depolarization (ΔV) is plotted against time. In saline ΔV was 3.1 ±0.1 mV and decreased during superfusion of mianserin to 0.9 mV (12 min). During wash with saline, the tonic depolarization recovered to a value of 1.4 mV (25 min). The correlation coefficient for the linear fit in this experiment is significant at the 0.1 % level. In figure 3.16 B, all seven experiments are shown, each experiment represented by a different symbol. Five flexor MNs were successfully recorded during wash, and the amplitudes during wash are shown as data points. In six of seven experiments the correlation coefficient was significant (at least at the 5 % level, n=4 to 10).

Again, it was tested whether the amount of decrease in the tonic depolarization amplitude depended on the respective control value. Therefore, the slopes of the linear fits were plotted against the amplitudes in saline. This relationship is shown in figure 3.16 C, the slopes of regression increased with increased control values. Thus, a normalization of the data points was necessary to plot values from all seven experiments (data not shown). The normalized plot, as well as the plot shown in figure 3.16 D comprises the

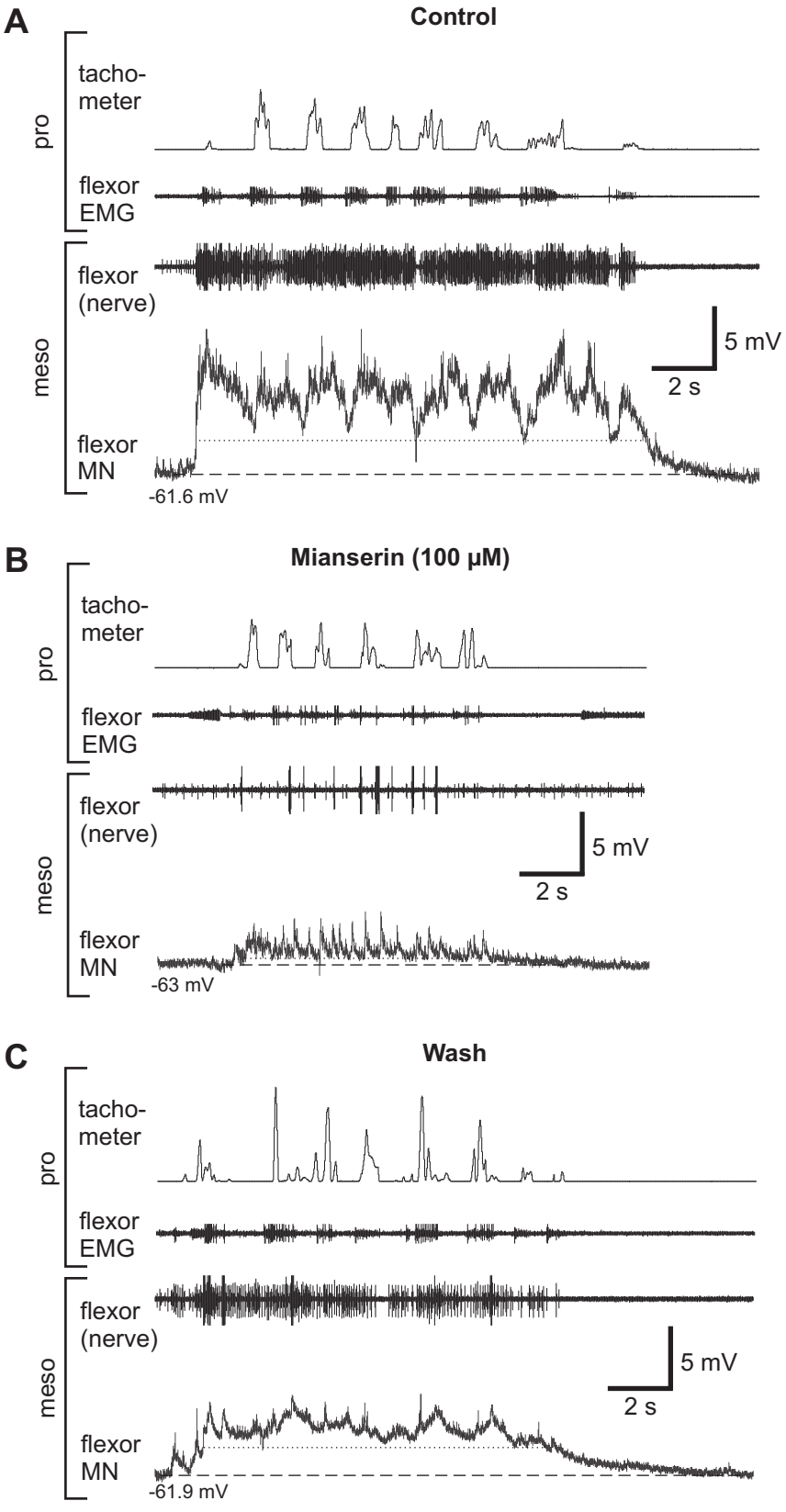
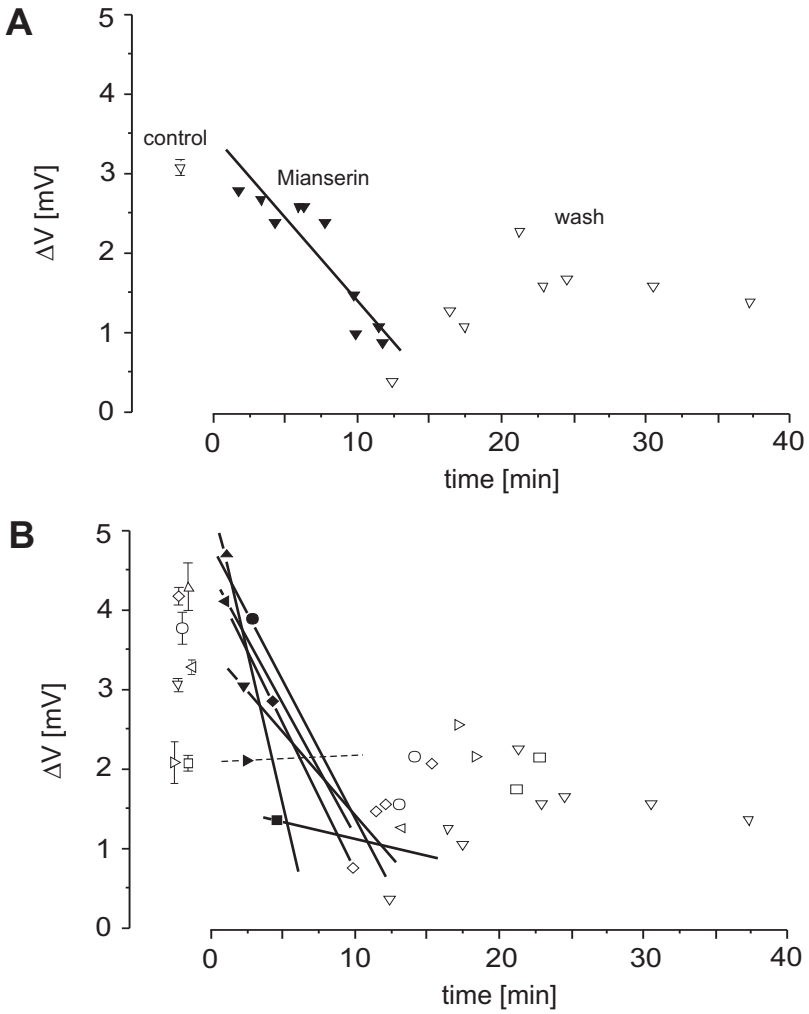

A
Control
tacho-
meter
pro
flexor
EMG
flexor
(nerve)
meso
5 mV
2 s
flexor
MN
-61.6 mV
B
Mianserin (100 µM)
tacho-
meter
pro
flexor
EMG
flexor
(nerve)
meso
5 mV
2 s
flexor
MN
-63 mV
C
Wash
tacho-
meter
pro
flexor
EMG
flexor
(nerve)
meso
5 mV
2 s
flexor
MN
-61.9 mV

Figure 3.15: *Mianserin decreased the tonic depolarization in mesothoracic flexor MNs during front leg stepping.* ***A:*** *A sequence of 7 steps induced a tonic depolarization of 2.9 mV in a contralateral mesothoracic flexor MN (control in saline). Nerve activity throughout the stepping sequence was increased.* ***B:*** *A pronounced decrease in the tonic depolarization was visible during superfusion of 100 µM mianserin (12 min, 0.5 mV), as well as a decrease in nerve activity.* ***C:*** *During wash, the tonic depolarization and the nerve activity partially recovered (2.3 mV, 10 min).*



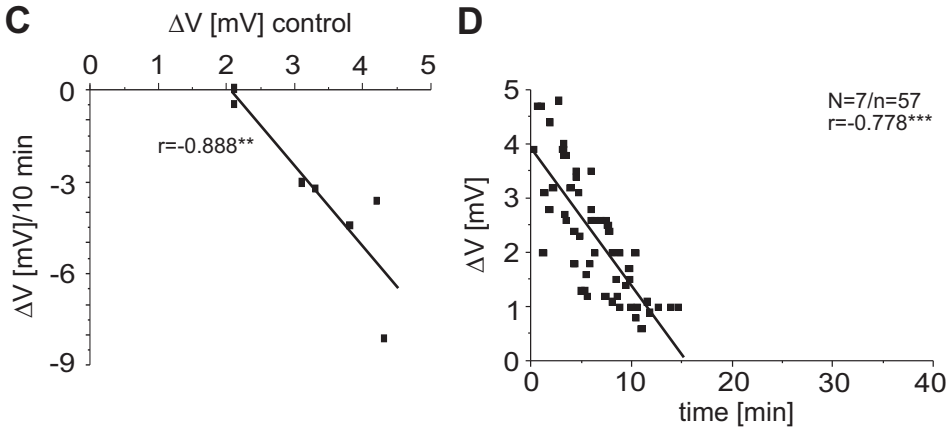


Figure 3.16: *Tonic depolarization amplitude over time during mianserin superfusion.* ***A:*** *Detailed analysis for a single experiment. The amplitude of the tonic depolarization (ΔV) decreased to 0.9 mV after 12 min superfusion of mianserin (▲, n=10). (Control in saline: 3.1 ±0.1 (n=8, △). During wash ΔV recovered to a value of 1.4 mV after 25 min. Data points are fitted by linear regression (n=10).* ***B:*** *Time course for all experiments (N=7, n=4-10). Only linear fits are shown for ΔV during mianserin superfusion. Same symbol is one experiment. Solid lines: level of significance at least 5 %, dashed line: no significance.* ***C:*** *Slopes of linear fits plotted against ΔV in control condition show a linear relationship.* ***D:*** *Plot of ΔV obtained from all stepping sequences during mianserin superfusion showing a high correlation (r=−0.778***; N=7, n=57).*

data points from all experiments and showed a high correlation for the reduction of the amplitude of the tonic depolarization over time (r=−0.778 and −0,654).

In summary, the amplitude of the tonic depolarization was decreased by 31.2 ± 18.0 % after 5 min superfusion of mianserin (N=6/7, values calculated from linear fits in figure 3.16 B, range −15.2 to −62.8 %).

3.2.5 Summary

In this chapter the influence of a variety of transmitters and a receptor antagonist on the tonic depolarization amplitude was investigated. A role for metabotropic receptors was already indicated by prolonged hyperexcitability during the tonic depolarization (see chapter 3.1.1 and Ludwar et al. 2005b). The mAChR antagonist atropine decreased the tonic depolarization in mesothoracic flexor MNs by ~40 % in eight of nine experiments, indicating that ACh might acts as a transmitter in mediating the tonic depolarization.
The increase in tonic depolarization amplitude by octopamine (500 µM: ~89 % and 1 mM: 109 % respectively) indicates that other transmitters/modulators might be involved as well. The effect of octopamine was not consistent, as in one experiment a slight decrease in the tonic depolarization amplitude was observed. Additional evidence for a role of octopaminergic receptors comes from experiments where mianserin induced a pronounced decrease in tonic depolarization amplitude by ~31 % in six of seven experiments.
The effect of serotonin was dual and opposing, as it increased the tonic depolarization amplitude in four of six experiments (up to 1.9 mV) and decreased it in two experiments (up to 1.0 mV) independent of concentration.
An interaction of more than one transmitter/neuromodulator seems possible, if one bears in mind that cotransmission occurs in most systems. Furthermore, it is not known whether any of these neuroactive substances act directly on the flexor MNs, or if their effect is mediated via pre-motor interneurons, whereas intersegmental signals might affect MNs directly or indirectly as well.

3.3 Second messengers

The long repolarization time constants (τ ranging from 120 ms to 3.7 s, e.g., Fig. 3.6) of the tonic depolarization indicate, that second messengers might be involved in mediating the tonic depolarization in mesothoracic flexor MNs during front leg stepping. Furthermore, neuroactive substances like ACh, octopamine and serotonin seem to have an effect on the tonic depolarization (see chapter 3.2). These substances mostly act via metabotropic receptors and therefore can involve a range of second messenger pathways. This chapter analyses the influence of different drugs that act on second messenger pathways that might be utilized in forming the tonic depolarization in flexor MNs.

3.3.1 Role of Calcium

Elevation of intracellular calcium in cells is induced for example by acetylcholine receptor activation as shown for a cloned mAChR in a *Drosophila* cell line (Millar et al. 1995), but also other receptor types can exhibit their effects via calcium (Ca^{2+}) dependent pathways. Although most known octopamine receptors (OARs) are coupled to the activation of adenylate cyclase (AC), one class of OARs mediates its action via the IP_3-system and activates Ca^{2+}-release from internal stores (Roeder 1999). The same is true for one type of serotonin receptors, e.g., in salivary glands of the blowfly (Berridge and Heslop 1981; Berridge 1981).

A possible involvement of Ca^{2+} as a second messenger mediating the tonic depolarization was analyzed by supplementation of BAPTA to the electrode electrolyte solution. BAPTA is a fast Ca^{2+}-chelator. Chelating of intracellular Ca^{2+} may affect the amplitude of the tonic depolarization. Effective chelation of Ca^{2+} by BAPTA was shown for cockroach MN somata (Mills and Pitman 1997).

For analysis of the effect of BAPTA on the tonic depolarization, 200 mM BAPTA was applied through the electrolyte and eight flexor MNs were recorded. Different from the other sets of experiments, BAPTA was present in the electrode from the beginning, so it was not possible to obtain control recordings without BAPTA. Therefore the 'control' value used was the amplitude of the tonic depolarization during the first stepping sequence that could be elicited and it was assumed that the effect of BAPTA was rather

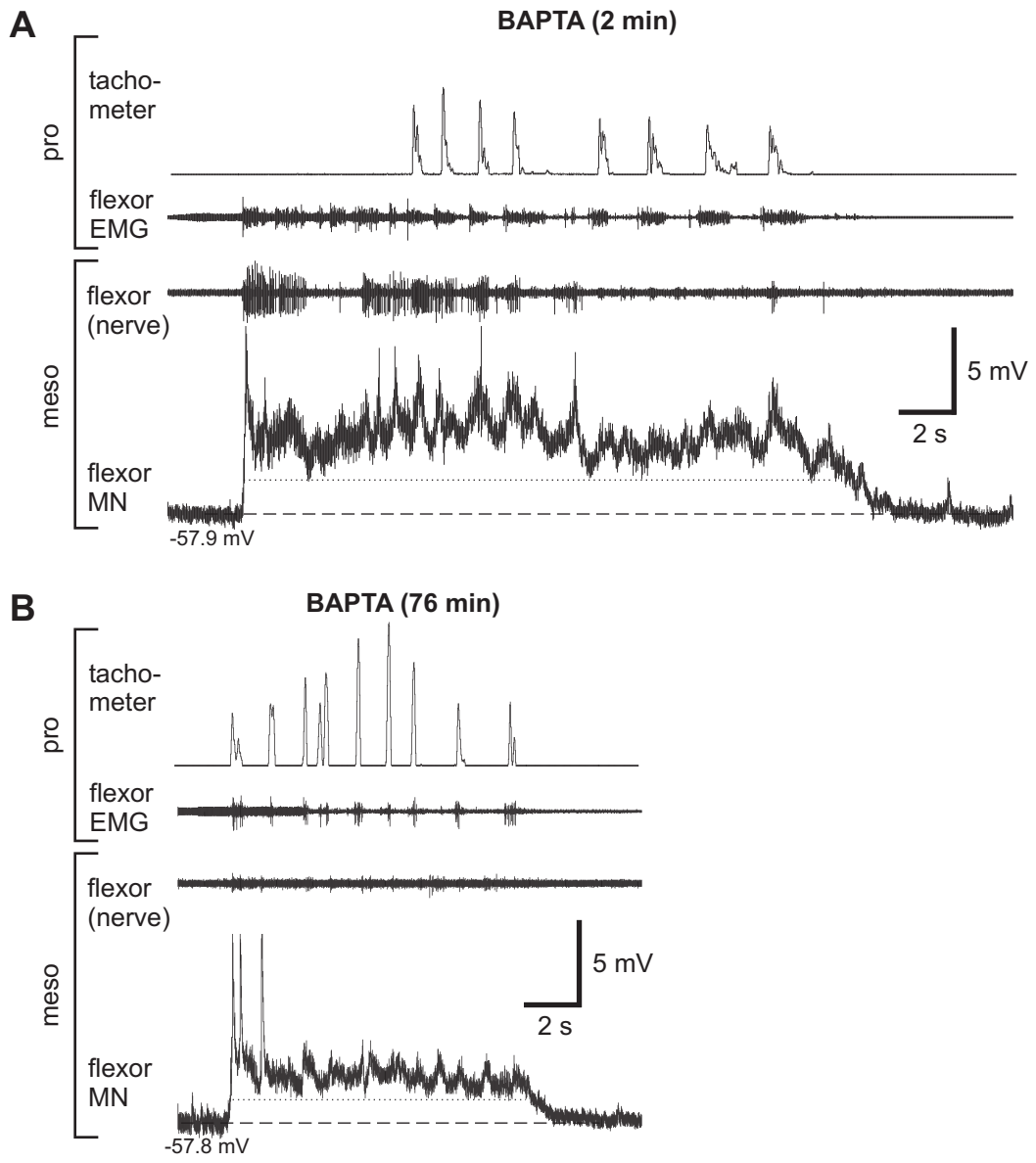
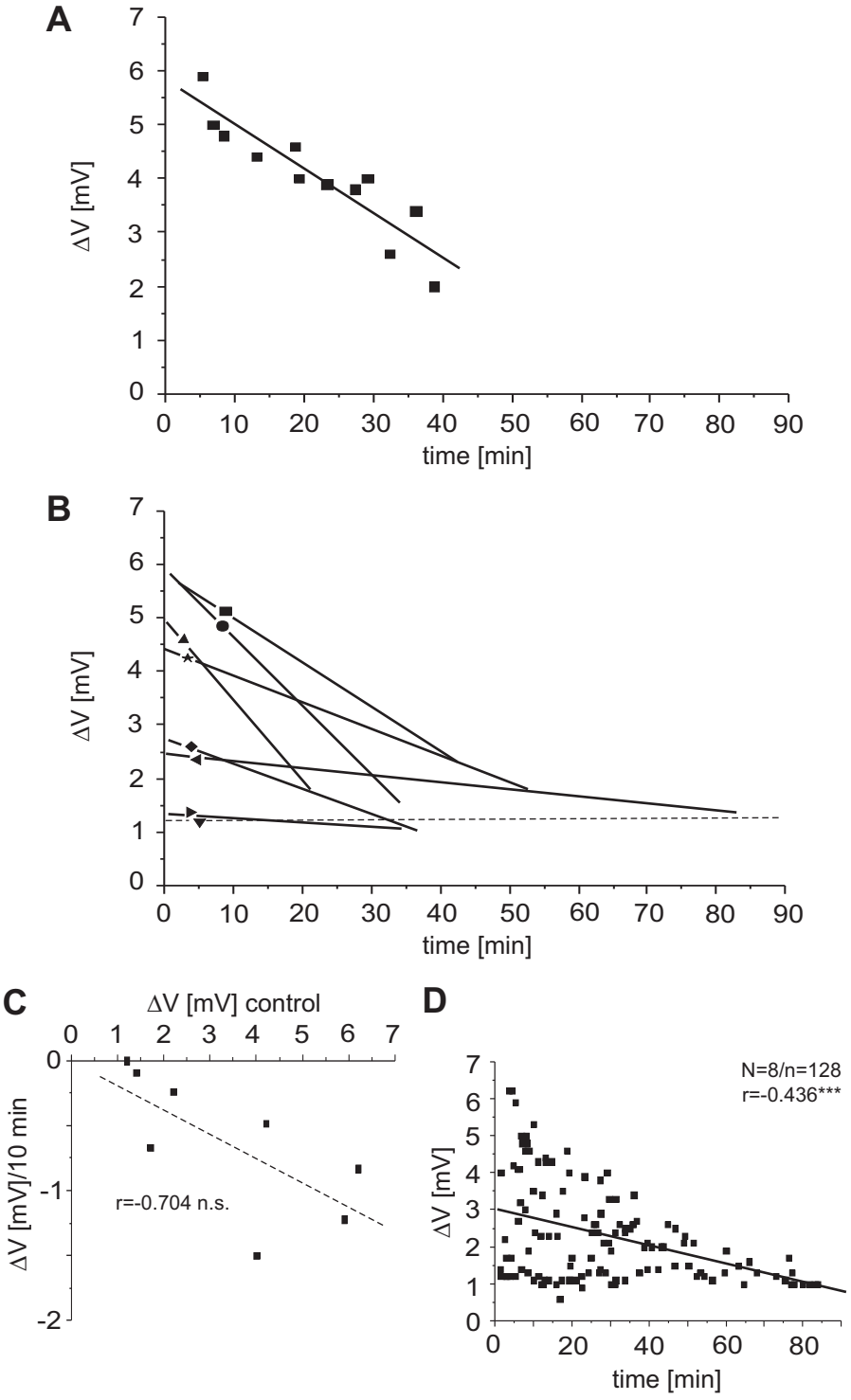


Figure 3.17: *BAPTA decreased the tonic depolarization in mesothoracic flexor MNs during front leg stepping.* ***A:*** *A sequence of 8 steps induced a tonic depolarization of 2.2 mV in an ipsilateral mesothoracic flexor MN (2 min recording with 200 mM BAPTA containing electrode).* ***B:*** *The tonic depolarization decreased during recording with the BAPTA containing electrode (1.4 mV, 76 min).*


A
ΔV [mV]
time [min]
B
ΔV [mV]
time [min]
C
ΔV [mV] control
ΔV [mV]/10 min
r=-0.704 n.s.
D
N=8/n=128
r=-0.436***
ΔV [mV]
time [min]

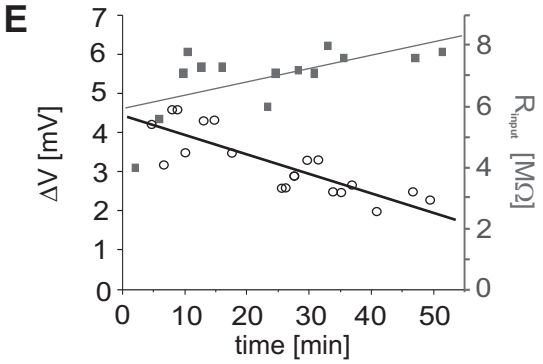


Figure 3.18: *Tonic depolarization amplitude over time during recording with BAPTA containing electrodes.* ***A:*** *Detailed analysis for a single experiment. The tonic depolarization amplitude (ΔV) decreased during recording with 200 mM BAPTA containing electrodes (n=12).* ***B:*** *Time course for all experiments (N=8, n=6-34). Only linear fits for ΔV are shown. Solid lines: level of significance at least 5 %, dashed line: no significance.* ***C:*** *Slopes of linear fits plotted against ΔV in 'control' condition show no significant linear relationship.* ***D:*** *Plot of ΔV obtained from all stepping sequences during recording with BAPTA containing electrodes showing a high correlation ($r=-0.436^{***}$, $p < 0.001$; N=8, n=128).* ***E:*** *Input resistance increases over time during recording with BAPTA containing electrodes.*

small. For the same reason no wash could be performed in this set of experiments. A representative experiment is shown in figure 3.17. The 'control' condition shows that after 2 min recording with 200 mM BAPTA containing electrodes, the tonic depolarization amplitude was 2.2 mV during a stepping sequence of 8 steps (Fig. 3.17 A). After 76 min, the amplitude was decreased to 1.4 mV during a sequence of nine steps (Fig. 3.17 B).

The time course for one experiment is shown in figure 3.18 A. The amplitude of the tonic depolarization (ΔV) is plotted against time. At the beginning of the recording in figure 3.18 A ΔV was 5.9 mV and decreased during recording with a BAPTA containing electrode to 2.0 mV (38 min). The correlation coefficient for the linear fit in this experiment was significant at the 0.1 % level (n=12). In figure 3.18 B, all eight experiments are shown, each experiment represented by a different symbol. In all experiments the tonic depolarization amplitude decreased with time. In seven of eight experiments the correlation coefficient was significant (at least at the 5 % level, n=6 to 34). The decrease in tonic depolarization amplitude does not appear to depend on the 'control' value as shown in figure 3.18 C. Slopes of the linear fits were plotted against the amplitude in 'control'. Fig-

ure 3.18 D shows a summarizing plot of all obtained values (N=8, n=128) with BAPTA containing electrodes. It shows a high correlation (r=−0.436***) for the reduction of the amplitude of the tonic depolarization over time.

The question was if the observed decrease in amplitude was due to the effect of BAPTA or to an increasing leakiness of the cell membrane. Therefore the input resistance was measured between the stepping sequences at resting potential and plotted against time (Fig. 3.18 E). With decreasing tonic depolarization amplitudes, the input resistance increased over time. Thus the decrease in amplitude was at least not due to a decrease in input resistance.
In summary, the tonic depolarization amplitude was decreased by 7.8 ±3.9 % (N=7/8, range −4 to −14.3 %) after 5 min recording with BAPTA containing electrodes (values calculated from linear fits in figure 3.18 B).

3.3.2 Role of cAMP

The possible involvement of Ca^{2+} as a second messenger in mediating the tonic depolarization amplitude described in chapter 3.3.1 does not rule out an additional action via other second messenger pathways, for example involving cAMP. Complex interaction between different pathways in arthropods are hypothesized, e.g., for the modulatory effect of serotonin in crayfish command neurons (Teshiba et al. 2001) or on pheromone sensitivity in silkmoths (Gatellier et al. 2004). cAMP triggers a variety of responses in insect tissue, and mAChRs for example are usually coupled to AC/cAMP-signaling pathways, in which cAMP levels are typically reduced (locust: Knipper and Breer 1989), but also excitatory responses due to activation of AC, increased levels of cAMP and stimulation of PKA (protein kinase A) are possible (Wenzel et al. 2002). Furthermore, responses induced by serotonin (Baines et al. 1990; Parker 1995) or octopamine (Han et al. 1998; Walther and Zittlau 1998) involve cAMP-pathways. In this chapter, the effects of a range of neuroactive drugs on the tonic depolarization amplitude are analyzed, which act on different steps in a cAMP signaling cascade.

3.3.2.1 Effect of 8-Br-cAMP

Elevation of cAMP by 8-Br-cAMP (a membrane permeable cAMP analog) was shown in several invertebrate preparations, as in the lobster stomatogastric ganglion (STG) (Flamm et al. 1987) but also in insects (grasshopper brain: Heinrich et al. 2001, locust DUM (dorsal unpaired median) neurons: Lundquist and Nässel 1997). To examine the possible involvement of cAMP in mediating the tonic depolarization, the effect of 8-Br-cAMP was tested. A representative experiment is shown in figure 3.19. The tonic depolarization in saline was 1.2 mV in an ipsilateral flexor MN during a stepping sequence of five steps (Fig. 3.19 A). Superfusion of 500 µM 8-Br-cAMP increased the amplitude of the tonic depolarization to a value of 2.2 mV (8 min, Fig. 3.19 B).

The analysis for a single experiment is shown in figure 3.20 A. The amplitude of the tonic depolarization (ΔV) is plotted over time. In this experiment, ΔV in saline was 2.0 ±0.1 mV (n=6) and increased during superfusion of 500 µM 8-Br-cAMP to 4.9 mV (26 min). During wash with saline, the tonic depolarization recovered only slightly to a value of 4.5 mV (9 min). The correlation coefficient for the linear fit in this experiment is significant at the 0.1% level (n=22). In figure 3.20 B all seven experiments are shown, each experiment represented by a different symbol, with control values obtained from two to six stepping sequences. The results from six experiments confirm that 8-Br-cAMP increases the tonic depolarization amplitude. In three of seven experiments the correlation coefficient was significant (at least at the 5 % level, n=5 to 22 for a given experiment). Four flexor MNs were successfully recorded during wash, and the individual amplitudes of tonic depolarizations are shown as data points. Wash with saline led to a partial recovery in two of four experiments. In one experiment, the slope of regression was negative (▲ in figure 3.20 B). This is due to the fact that during the first three minutes of the experiment, the tonic depolarization increased from 1.4 mV to values of 2.5 mV but then declined to control values of 1.6 mV after 7 min. Thus, the tonic depolarization increased in this experiment as well. In another experiment, no linear fit could be calculated due to too few data points (●, n=2), which showed an increase in the tonic depolarization. The increase in the tonic depolarization amplitude during superfusion of 8-Br-cAMP was independent of the control value (Fig. 3.20 C), shown by plotting the slopes of linear fits against

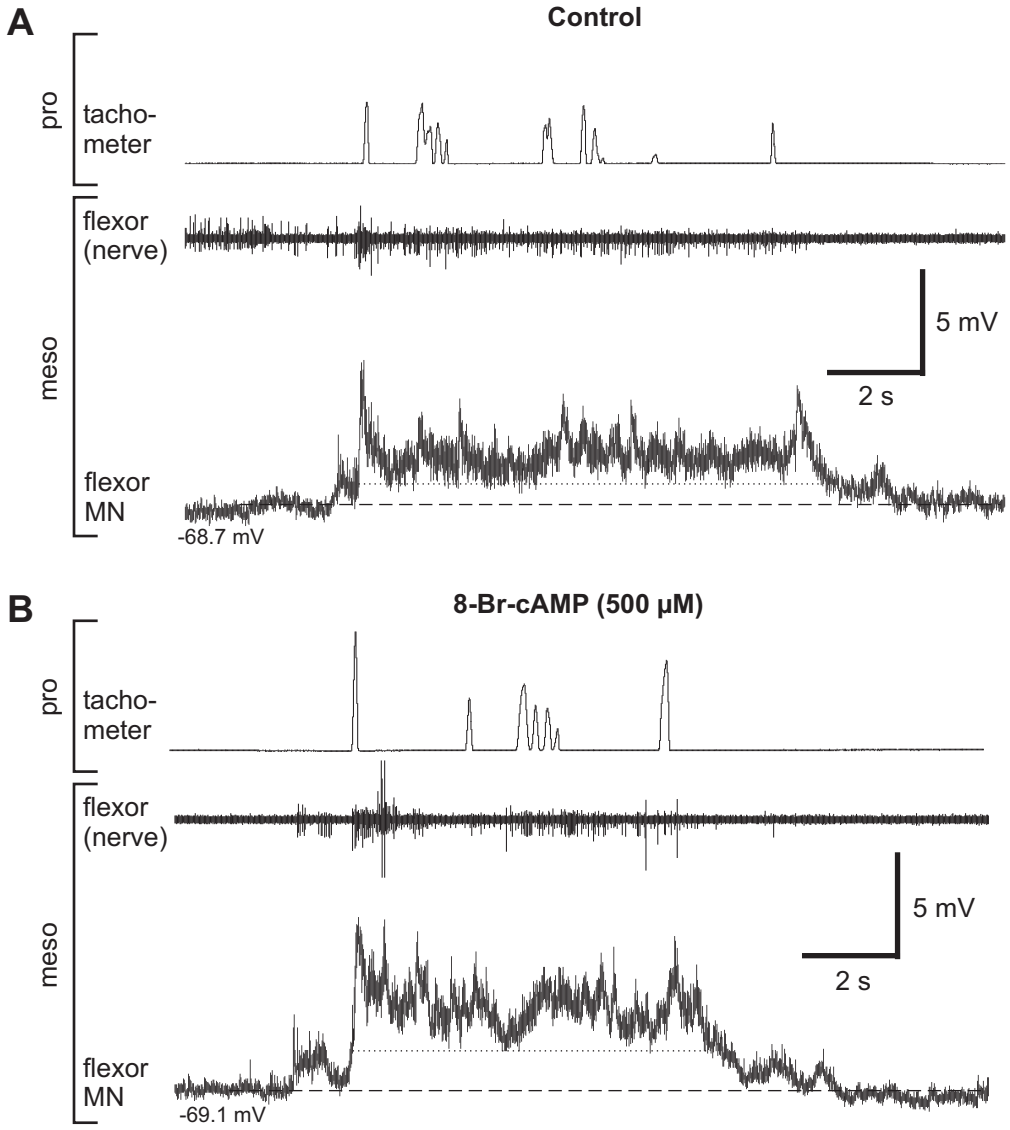


Figure 3.19: *8-Br-cAMP increased the tonic depolarization in mesothoracic flexor MNs during front leg stepping.* ***A:*** *Control in saline. Throughout a sequence of 5 steps, the ipsilateral mesothoracic flexor MN shown, exhibited a tonic depolarization of 1.2 mV.* ***B:*** *During superfusion of 500 µM 8-Br-cAMP the amplitude of the tonic depolarization during a stepping sequence of the front leg (4 steps) increased (8 min, 2.2 mV).*

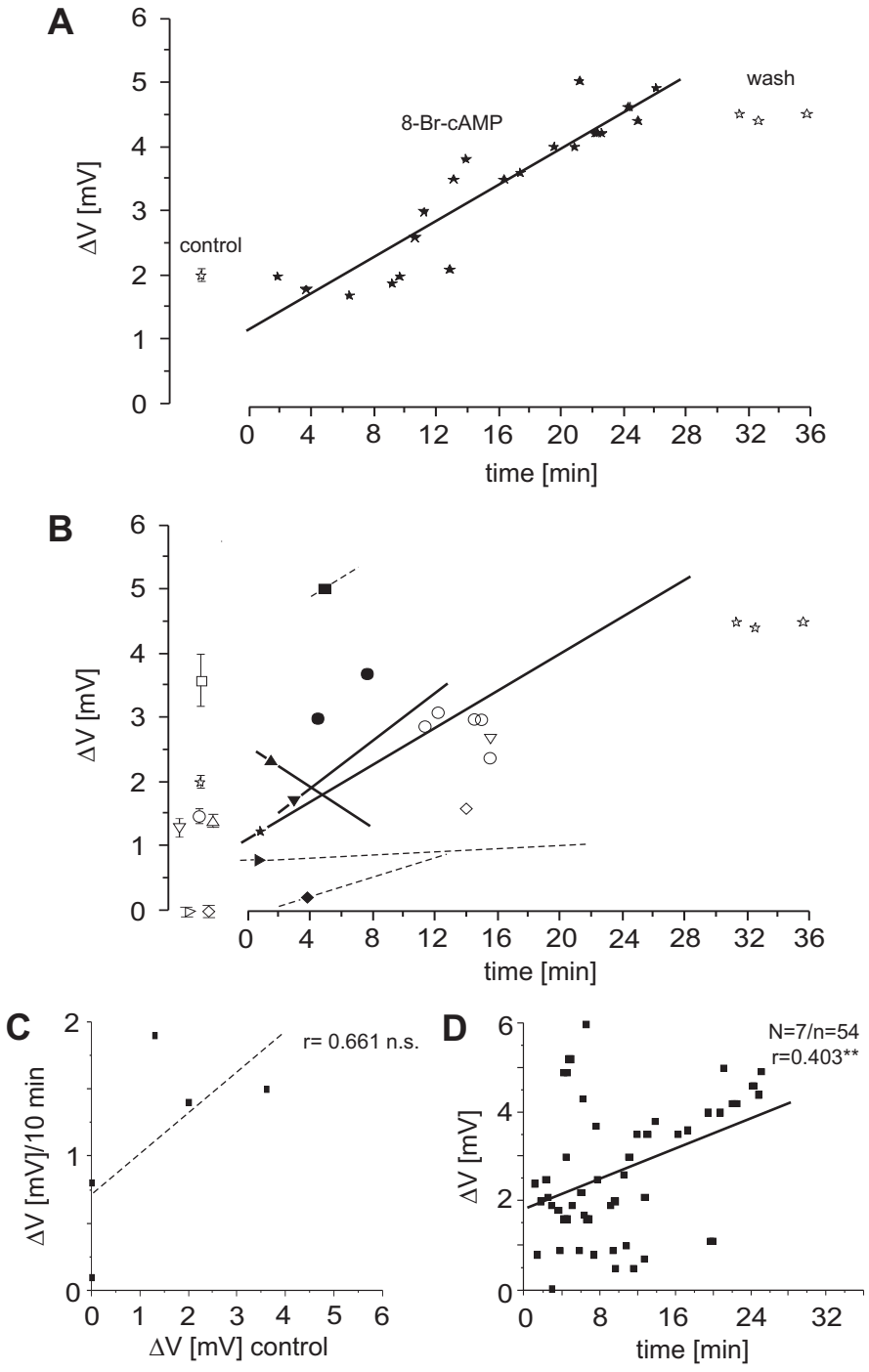

A
ΔV [mV]
control
8-Br-cAMP
wash
time [min]
B
ΔV [mV]
time [min]
C
ΔV [mV]/10 min
r= 0.661 n.s.
ΔV [mV] control
D
ΔV [mV]
N=7/n=54
r=0.403**
time [min]

Figure 3.20: *Tonic depolarization amplitude over time during 8-Br-cAMP (500 µM) superfusion.* ***A:*** *Detailed analysis for a single experiment. Control in saline: the amplitude of the tonic depolarization (ΔV) was 2.0 ±0.1 mV (open stars; n=6) and increased to 4.9 mV after 26 min superfusion of 8-Br-cAMP (★). During wash with saline, the tonic depolarization recovered slightly to a value of 4.5 mV (9 min). Data points are fitted by linear regression (n=22).* ***B:*** *Time course for all experiments (N=7). Control (open symbols) obtained from n=2 to 6 stepping sequences. Only linear fits are shown for ΔV during 8-Br-cAMP superfusion (n=5 to 22). Same symbol is one experiment. Solid lines: level of significance at least 5 %, dashed line: no significance.* ***C:*** *Slopes of linear fits plotted against ΔV in control condition show no significant linear relationship. Plot includes slopes from five of seven experiments. In one experiment no linear fit was possible, in another, the slope was negative (see text).* ***D:*** *Plot of ΔV obtained from all stepping sequences during 8-Br-cAMP superfusion showing a significant correlation (r=0.403**, level of significance 0.1 %; N=7, n=54).*

the amplitude in saline (five of seven experiments). Values obtained from the other two experiments were excluded due to the above mentioned reasons. A summarizing plot of the obtained tonic depolarization amplitudes during superfusion of 8-Br-cAMP is shown in figure 3.20 D. The plot shows a high correlation (r=0.403**) for the increase in tonic depolarization over time.

In summary, the amplitude of the tonic depolarization increased after 5 min of superfusion with 8-Br-cAMP by 0.8 ±0.5 mV (range 0.3 to 1.5 mV; data calculated from linear fits, N=5/7, see also Fig. 3.20 B). A percentaged increase could not be calculated for each single experiment, as the tonic depolarization in saline (control) was zero in two experiments. Therefore, the percentaged increase was calculated from the mean increase after five minutes in relation to the mean amplitude in control condition. The percentaged increase calculated in this way was about 56 %.

3.3.2.2 Effect of H-89 and SQ22,536

The experiments using 8-Br-cAMP indicated that cAMP might play a role in mediating the tonic depolarization. This finding should be clarified by some preliminary experiments, in which the effect of substances influencing the cAMP pathway at different steps

were investigated (Fig. 3.21). Two substances were tested, H-89 and SQ22,536.

In general, it is thought that the signaling pathway of cAMP is mediated through the activation of PKA. H-89 is a well-known inhibitor of PKA, and its effectiveness in insects was shown by its inhibitory effect in the grasshopper brain (Wenzel et al. 2002), further evidence comes from the locust muscle (Walther and Zittlau 1998).

In two experiments, the effect of H-89 on the tonic depolarization in mesothoracic flexor MNs was analyzed. One of which is shown in figure 3.22. From a control value of 3.0 mV, the tonic depolarization amplitude decreased to 0.9 mV after 16 min superfusion of 100 µM H-89 (Fig. 3.22 A, 3.22 B). The mesothoracic ncr recording during superfusion of H-89 also showed a decreased activity during front leg stepping. In this flexor MN large EPSPs occurred in saline and vanished in the presence of H-89. Figure 3.22 C shows no recovery in the amplitude of the tonic depolarization, but the mesothoracic nerve activity was slightly increased.

The analysis for both experiments is shown in figure 3.23. In one ipsilateral flexor MN the amplitude of the tonic depolarization in saline was 3.2 ±0.2 mV and decreased to 2.5 mV after ~5 min in H-89. Details for the other flexor MN recording were described in the text above and in figure legends 3.22 and 3.23.

SQ22,536 is known to inhibit adenylate cyclase (AC) which catalyzes the synthesis of cAMP from ATP (Fig. 3.21). In some invertebrate preparations, SQ22,536 was successfully used to reduce cAMP-dependent effects (e.g., leech: Britz et al. 2004, grasshopper: Heinrich et al. 2001; Wenzel et al. 2002, crayfish: Araki et al. 2005).

The effect of SQ22,536 on the tonic depolarization amplitude in ipsilateral mesothoracic flexor MNs was analyzed in two experiments. One experiment is shown in figure 3.24. The amplitude of the tonic depolarization during front leg stepping increased to a value of 1.3 mV after 11 min superfusion of 500 µM SQ22,536 (Fig. 3.24 B), whereas under control conditions in saline, no tonic depolarization was visible (Fig. 3.24 A). The mesothoracic nerve recording showed no significant difference in activity during superfusion of SQ22,536.

Figure 3.25 shows the analysis for both experiments. In one ipsilateral flexor MN, the

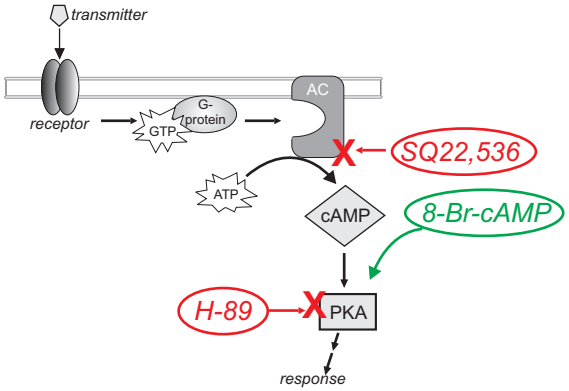


Figure 3.21: *cAMP pathway. Activation of a G-protein coupled receptor activates adenylate cyclase (AC), which catalyses the synthesis of cAMP. The membrane permeable cAMP analog 8-Br-cAMP increases the intracellular cAMP-level. The AC inhibitor SQ22,536 leads to a decreased cAMP-level. H-89 inhibits protein kinase (PKA). Crosses mark an inhibitory effect. Schematic altered after textbook figures.*

amplitude of the tonic depolarization in saline was 1.3 ±0.1 mV and increased only slightly to 1.5 mV after 14 min superfusion of SQ22,536. Details for the other flexor MN were described in the text above and in figure legends 3.24 and 3.25.

In summary, 8-Br-cAMP was shown to increase the tonic depolarization amplitude, thus indicating a role for cAMP in mediating the tonic depolarization. The experiments using H-89 and SQ22,536, although preliminary, might support this finding. H-89 decreased the tonic depolarization amplitude by 33 and 22 % (data calculated from linear fits after 5 min, respectively mean value, see figure 3.23). The amplitude of the tonic depolarization increased in the presence of SQ22,536 by 0.2 and 0.8 mV (data calculated from linear fits after 5 min, see figure 3.25).

3.3.3 Role of IP_3/DAG

For several transmitters a link to the IP_3-system has been described (see references in Berridge 1984). In the locust, a linkage of muscarinic-like receptors to the phosphatidylinositol metabolism was proposed by Trimmer and Berridge (1985). Another example comes from the blowfly, where serotonin receptors on the salivary gland are coupled amongst others to the IP_3-pathway (Berridge and Heslop 1981). IP_3 also acts in parallel to cAMP in affecting molting in *Drosophila* (Venkatesh et al. 2001). In this chapter the ef-

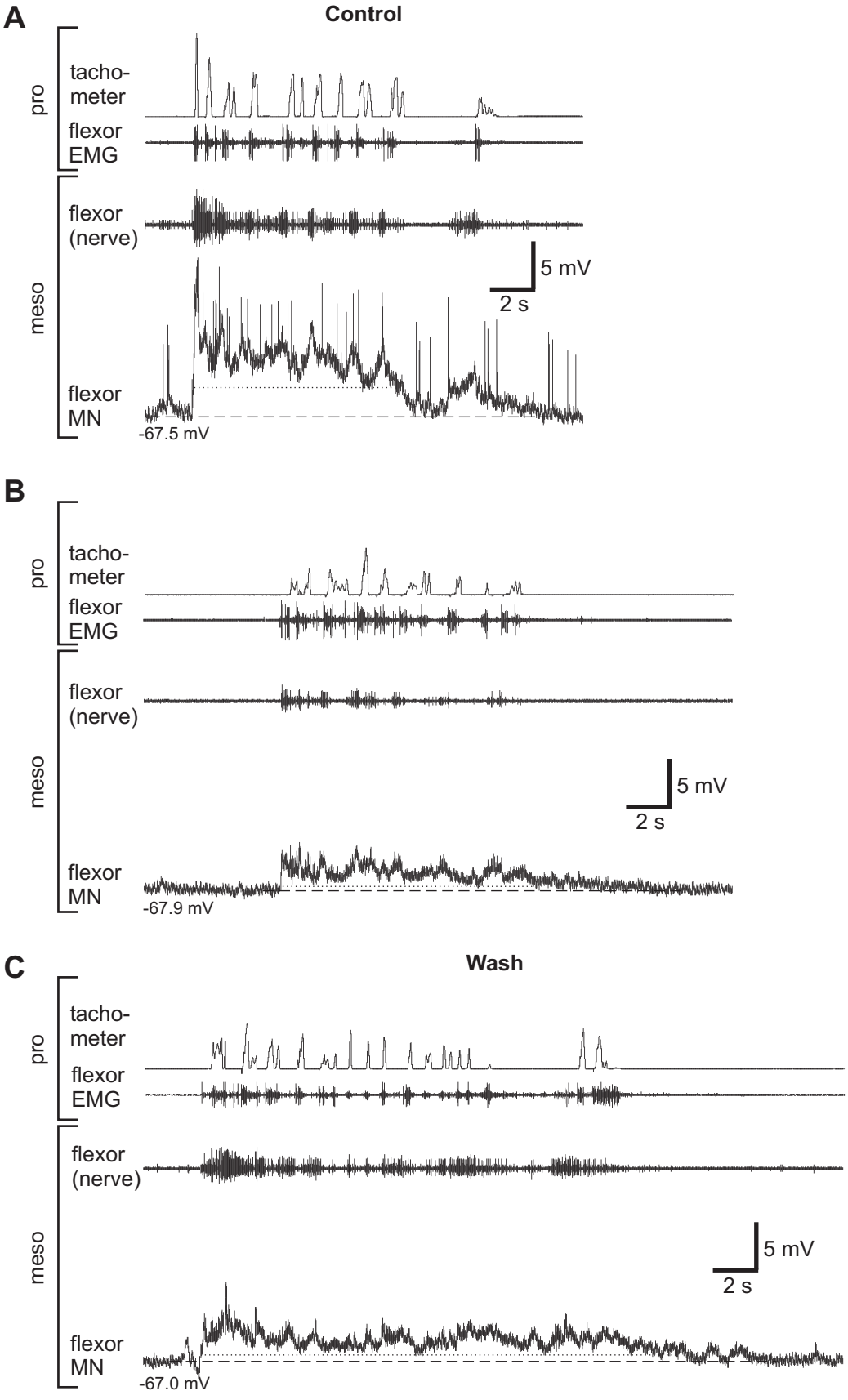

A
Control
pro
tacho-
meter
flexor
EMG
meso
flexor
(nerve)
5 mV
2 s
flexor
MN
-67.5 mV
B
pro
tacho-
meter
flexor
EMG
meso
flexor
(nerve)
5 mV
2 s
flexor
MN
-67.9 mV
C
Wash
pro
tacho-
meter
flexor
EMG
meso
flexor
(nerve)
5 mV
2 s
flexor
MN
-67.0 mV

Figure 3.22: *H-89 decreased the tonic depolarization in an ipsilateral mesothoracic flexor MN during front leg stepping.* ***A:*** *The amplitude of the tonic depolarization in an ipsilateral mesothoracic flexor MN was 3.0 mV (10 steps, control in saline).* ***B:*** *During superfusion of 100 µM H-89, the amplitude of the tonic depolarization during a stepping sequence of the front leg (9 steps) decreased (16 min, 0.9 mV).* ***C:*** *During wash with saline, the tonic depolarization amplitude remains decreased (17 steps; 6 min, 1 mV).*

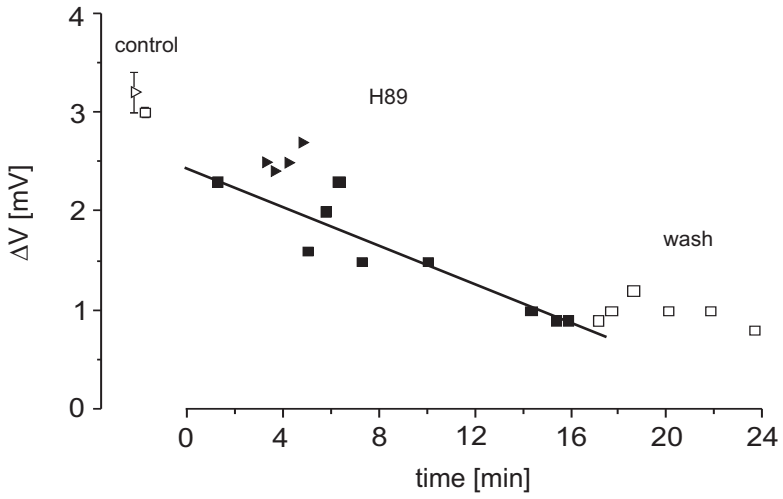


Figure 3.23: *Tonic depolarization amplitude over time during H-89 (100 µM) superfusion. The details for both performed experiments are shown. Controls in saline: the amplitudes of the tonic depolarization were 3.0 mV (□, n=2) and 3.2 ±0.2 mV (▷, n=5). The tonic depolarization (ΔV) decreased to 0.9 mV (16 min, ■) and to 2.5 mV ±0.1 mV (▶) during superfusion of H-89. Data points are fitted by linear regression (n=9). Same symbol is one experiment. Solid line: level of significance 0.1 %. During wash the amplitude of the tonic depolarization remains decreased (□, n=6).*

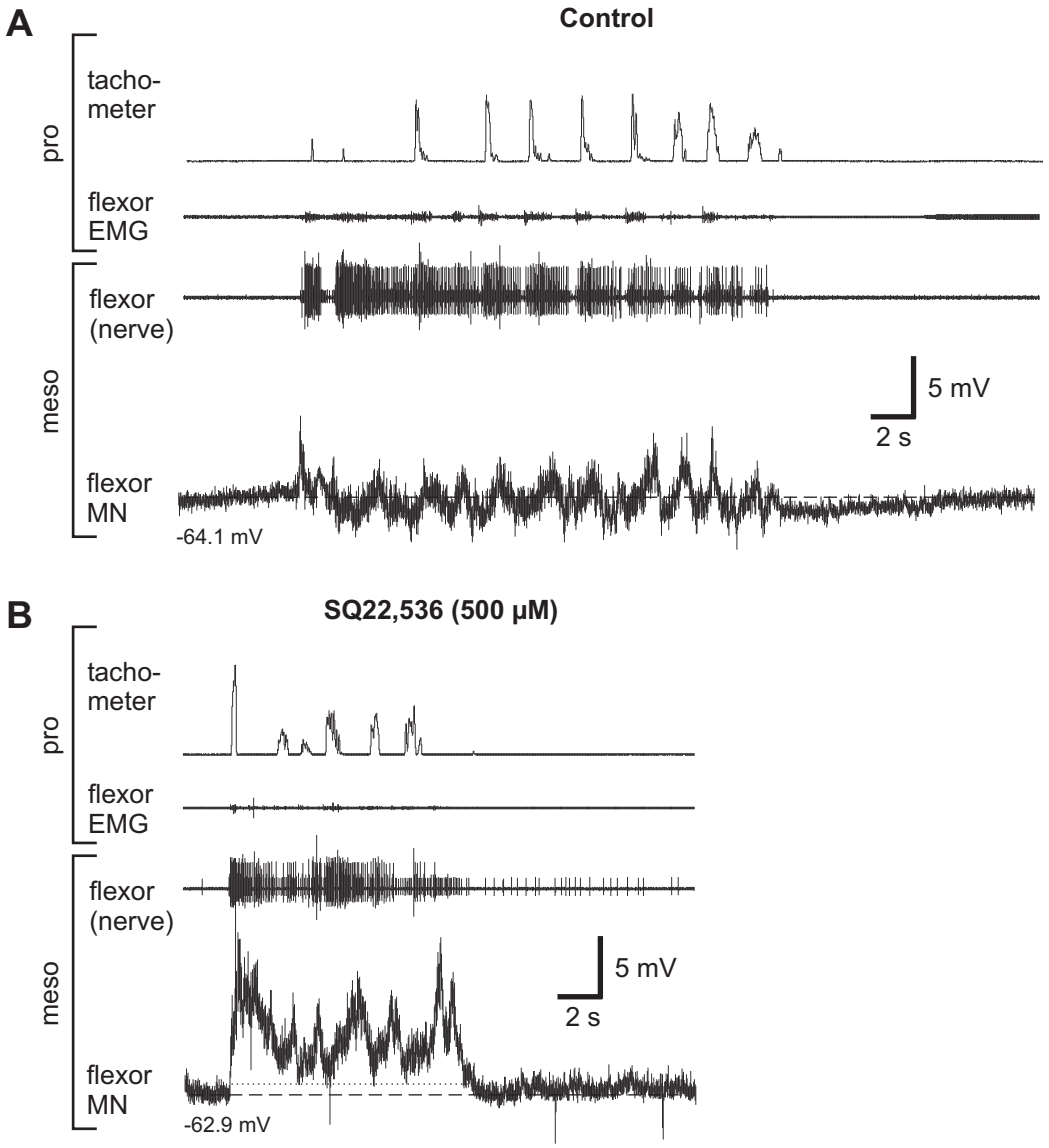


Figure 3.24: *SQ22,536 increased the tonic depolarization in an ipsilateral mesothoracic flexor MN during front leg stepping.* ***A:*** *During a stepping sequence in saline no tonic depolarization was visible.* ***B:*** *During superfusion of 500 µM SQ22,536 (11 min) the flexor MN exhibited a tonic depolarization of 1.3 mV during a sequence of 6 steps of the front leg.*

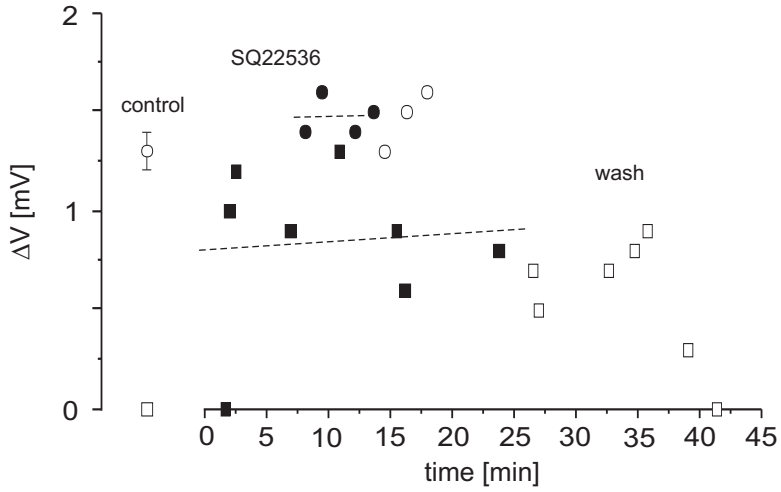


Figure 3.25: *Tonic depolarization amplitude over time during SQ22,536 superfusion. Details for both performed experiments are shown. Controls in saline 1.3 ±0.1 mV (○, n=2) and 0 mV (□, n=3): the amplitudes of the tonic depolarization increased to 1.5 mV after 14 min (1 mM SQ22,536, ●) and to 0.8 mV after 24 min (500 µM SQ22,536, ■). Data points are fitted by linear regression (n=4 and 8). Same symbol is one experiment. Dashed lines indicate no significance in the correlation coefficients.*

fects of neomycin and U-73122, both known to inhibit PLC (phospholipase C) synthesis (Fig. 3.26), on the tonic depolarization were tested.

3.3.3.1 Effect of Neomycin

Suarez-Kurtz (1974) showed that the aminoglycoside antibiotic neomycin reduced the amplitude of graded membrane responses in crab muscle fibres and in the crayfish stretch receptor neuron it depressed cell discharge activity (Nation and Roth 1988). Neomycin reduces PLC activity by binding to the enzyme's substrate, PIP_2, and was shown to reduce the muscarine-stimulated stridulation in the grasshopper brain (Wenzel et al. 2002). To investigate a possible involvement of IP_3 in mediating the tonic depolarization in a first step neomycin was superfused. The effect of neomycin on the tonic depolarization amplitude in mesothoracic flexor MNs was tested in five animals. Concentrations of neomycin ranged between 0.8 and 2 mM. In four out of five experiments the amplitude of the tonic depolarization was increased. Figure 3.27 shows a representative experiment. Under control conditions in saline the tonic depolarization in this flexor MN was 2.5 mV

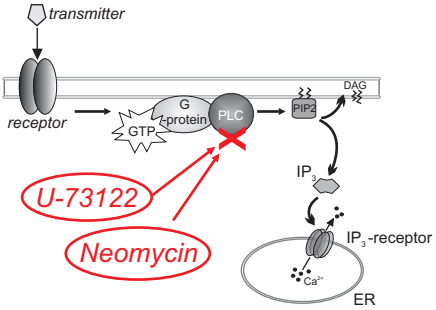


Figure 3.26: *IP_3-pathway. Activation of a G-protein coupled receptor activates phospholipase C (PLC), which leads to the synthesis of IP_3 and DAG via PIP_2. IP_3 induces calcium release from internal stores (ER = endoplasmatic reticulum). Neomycin and U-73122 inhibit phospholipase C. The cross marks an inhibitory effect. Schematic altered after textbook figures.*

during a stepping sequence of the front leg (7 steps, Fig. 3.27 A). During superfusion of 800 µM neomycin the amplitude of the tonic depolarization increased to 4.7 mV (Fig. 3.27 B).

The time course for a single experiment is shown in figure 3.28. The amplitude of the tonic depolarization (ΔV) is plotted against time and was 2.2 ±0.3 mV (control, Fig. 3.28 A). During superfusion of 2 mM neomycin the amplitude increased to 3.4 mV (after 17 min). The correlation coefficient for the linear fit in this experiment was significant at the 5 % level. A range of different neomycin concentrations was used in the experiments (Fig. 3.28 B). The following concentrations were used: 0.8 mM (N=1), 1 mM (N=3) and 2 mM (N=1). In all these different concentrations neomycin increased the amplitude of the tonic depolarization in four of five experiments. One experiment showed a significant increase in the tonic depolarization amplitude. Three more experiments support this finding, however, their correlation coefficient was not significant. In one experiment no change in the amplitude of the tonic depolarization was observed during superfusion of neomycin.

In summary, the tonic depolarization amplitude increased by up to 84 % (range 4 to 84 %, data calculated from linear fits after 5 min, see figure 3.28 B).

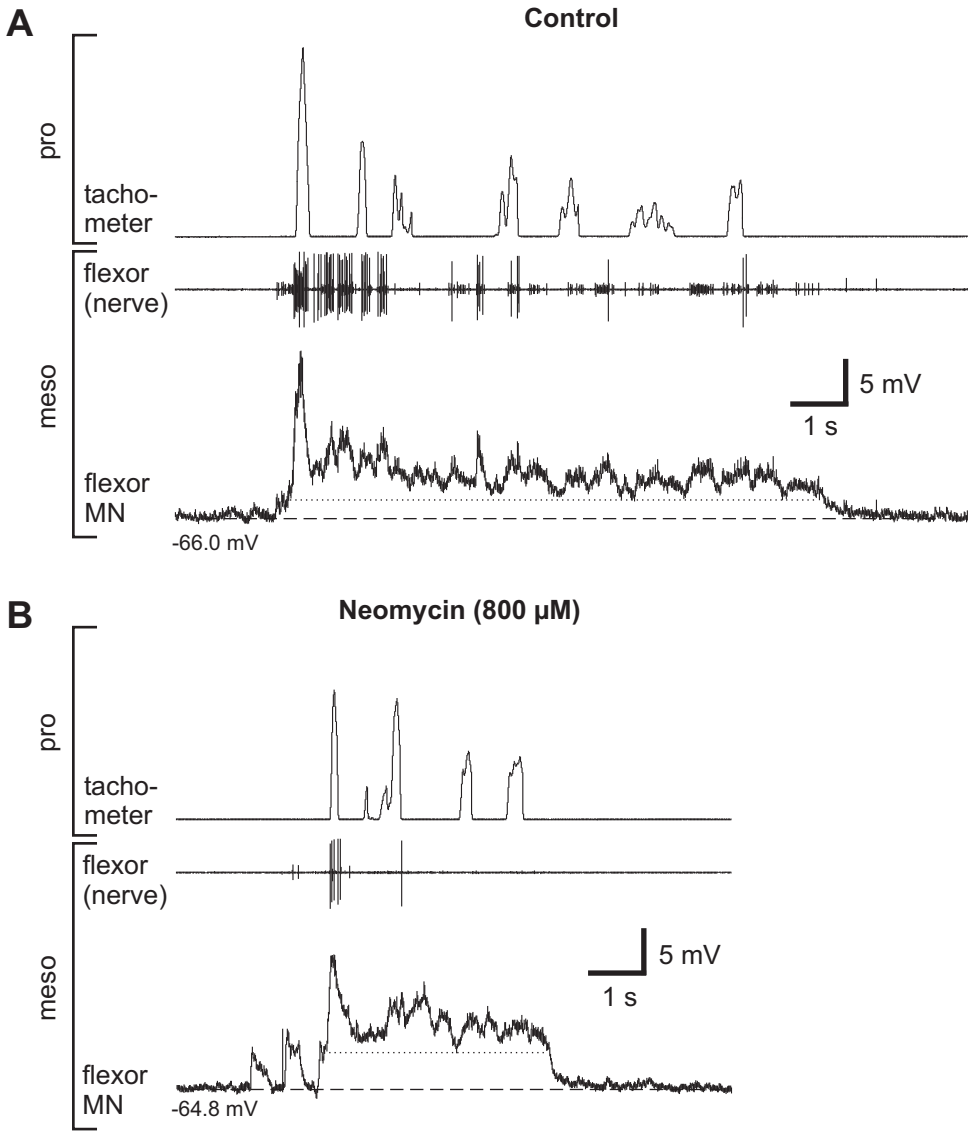


Figure 3.27: *Neomycin increased the tonic depolarization in ipsilateral mesothoracic flexor MNs during front leg stepping.* ***A:*** *Control in saline. Throughout a sequence of 7 steps an ipsilateral mesothoracic flexor MN exhibited a tonic depolarization of 2.5 mV.* ***B:*** *During superfusion of 800 µM neomycin the amplitude of the tonic depolarization during a stepping sequence of the front leg (5 steps) increased (23 min, 4.7 mV).*

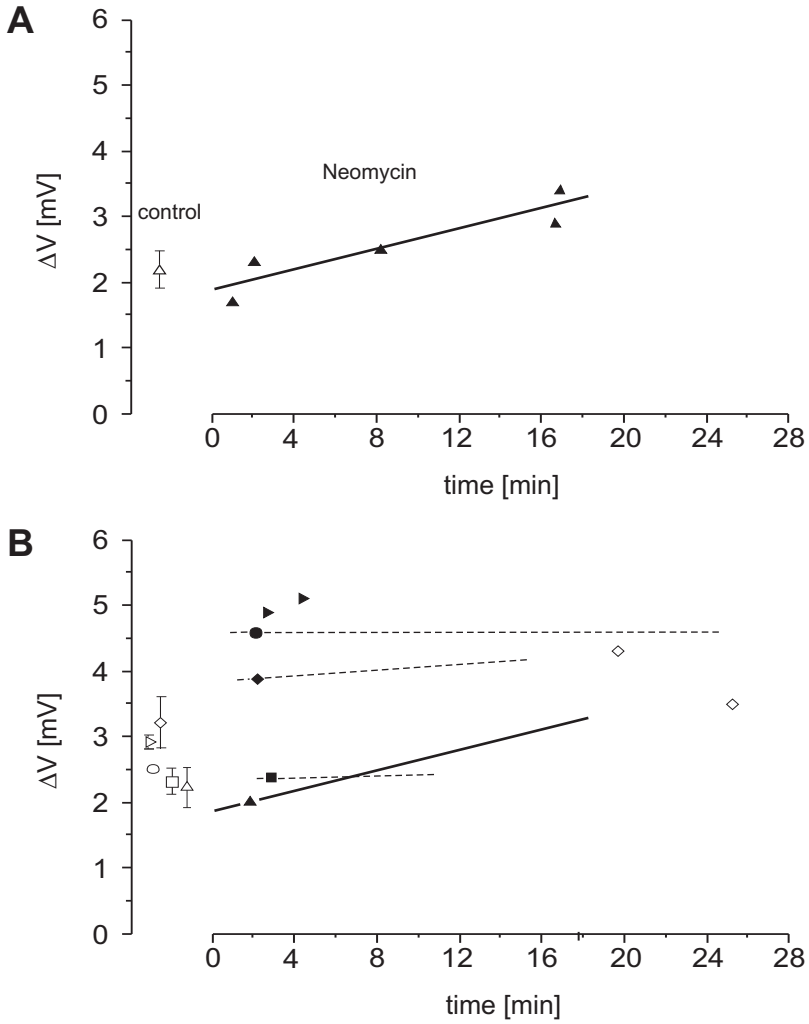


Figure 3.28: *Tonic depolarization amplitude over time during neomycin superfusion.* ***A:*** *Detailed analysis for one experiment. Control in saline: 2.2 ±0.3 mV (△, n=2). The amplitudes of the tonic depolarization increased to 3.4 mV after 17 min (2 mM neomycin, ▲). Data points are fitted by linear regression (n=5).* ***B:*** *Time course for all experiments (N=5). The concentrations are labeled as follows: 0.8 mM: ●; 1 mM: ►, ♦, ■; 2 mM: ▲. Only linear fits are shown for ΔV during neomycin superfusion for four experiments (n=4-11). In the fifth experiment the two obtained data points are shown (►). Same symbol is one experiment. Solid line: level of significance 5 %, dashed lines: no significance.*

3.3.3.2 Effect of U-73122

The involvement of IP_3 in mediating the tonic depolarization was also analyzed by superfusion of U-73122, which inhibits (like neomycin) triggering of stridulation in the grasshopper brain by application of muscarine (Wenzel et al. 2002). In three animals the tonic depolarization was analyzed during superfusion of U-73122. The concentration of U-73122 ranged between 75 µM and 250 µM. In all experiments the amplitude of the tonic depolarization was increased during superfusion of U-73122. In these experiments, no flexor MN could be recorded successfully during wash with saline. Figure 3.29 shows one representative experiment. Under control condition in saline, the tonic depolarization in this flexor MN was 2.4 mV during a stepping sequence of the front leg (6 steps, Fig. 3.29 A). During superfusion of 250 µM U-73122 the tonic depolarization amplitude increased to 3.7 mV (6 steps, Fig. 3.29 B).

Figure 3.30 shows the analysis for all three experiments. The amplitudes of the tonic depolarization increased by 25 % during superfusion of 75 µM U-73122 and by 58 % during superfusion of 125 and 250 µM. The data were calculated from linear fits after 5 min.

3.3.4 Summary

This chapter deals with the question of which second messengers play a role in mediating the tonic depolarization. A participation of second messengers was indicated by long repolarization time constants of the tonic depolarization (see chapter 3.1, Ludwar et al. 2005b). Experiments using BAPTA as Ca^{2+}-chelator showed a decrease in the tonic depolarization amplitude by approximately 8 %. Thus, Ca^{2+} might act as a second messenger in mediating the tonic depolarization. Furthermore, the cAMP analog 8-Br-cAMP induced an increase in the tonic depolarization amplitude by approximately 0.8 mV, indicating a role for cAMP in mediating the tonic depolarization. This result is supported by preliminary results showing that H-89, which inhibits PKA, decreased the tonic depolarization.

The type of the mediation remains unclear, as preliminary results show that the AC inhibitor SQ22,536 seems to increase the tonic depolarization amplitude (see discussion

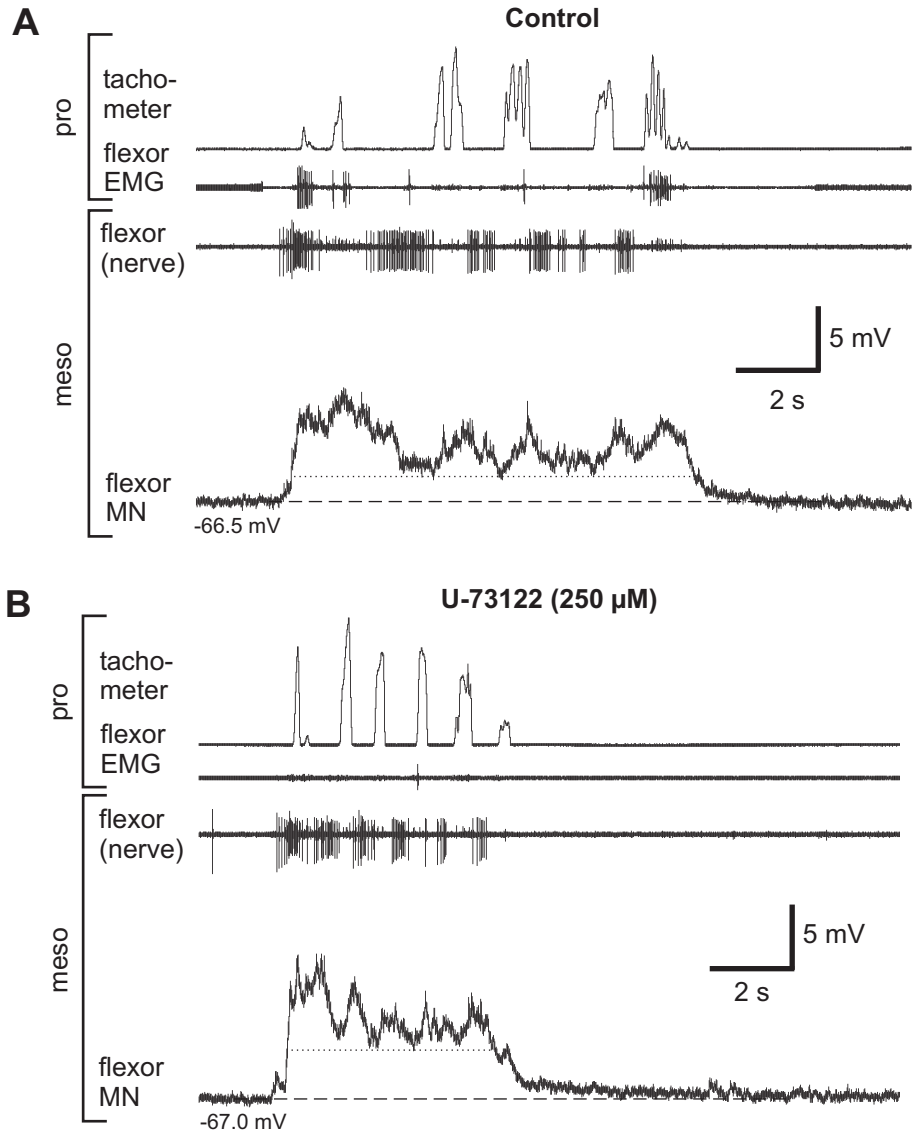


Figure 3.29: *U-73122 increased the tonic depolarization in a mesothoracic flexor MN during front leg stepping.* ***A:*** *The tonic depolarization in saline was 2.4 mV during a stepping sequence of the front leg (6 steps).* ***B:*** *During superfusion of 250 µM U-73122 the amplitude of the tonic depolarization during a sequence of 6 steps increased (10 min, 3.7 mV).*

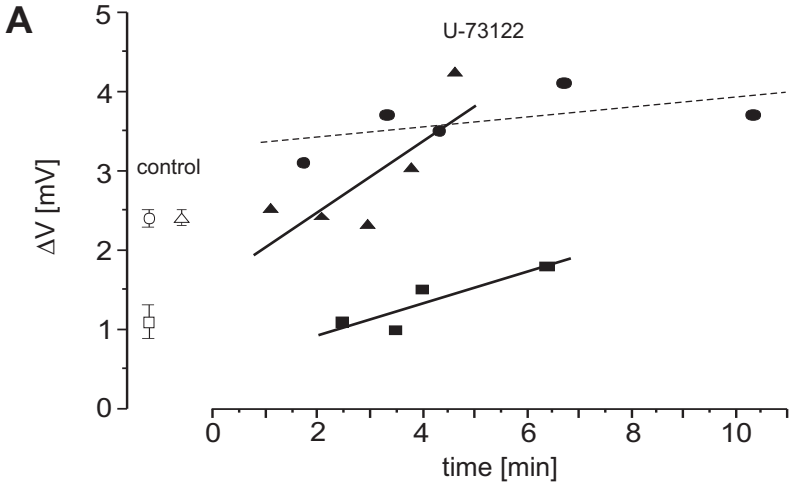


Figure 3.30: *Tonic depolarization amplitude over time during U-73122 superfusion. Details for all experiments are shown. Controls in saline: 1.2 ±0.1 mV (□, n=6); 2.4 ±0.1 mV (△, n=4); 2.4 ±0.1 mV (○, n=3). The tonic depolarization amplitudes increased to 1.8 mV after 6 min (75 µM, ■), to 4.2 mV after 5 min (125 µM, ▲) and to 3.7 mV after 10 min (250 µM, ●). Data points are fitted by linear regression (n=5-6). Same symbol is one experiment. Solid lines: level of significance at least 5 %, dashed line: no significance.*

4.2.3, Fig. 4.2). A reduction in the amplitude would have been expected because SQ22,536 catalyzes the synthesis of cAMP (Fig. 3.21).

A role of an IP_3-pathway cannot be excluded either, as the PLC inhibitors neomycin and U-73122 increased the tonic depolarization amplitude by up to 84 % and 58 %. The enhancement of the tonic depolarization amplitude by these substances points to an indirect influence on mesothoracic motoneurons, possibly by a reduction of an inhibitory input mediated by interneurons that employ PLC in a second messenger pathway (see discussion chapter 4.2.3, Fig. 4.2). An opposing effect of these neuroactive drugs would have been expected if they act directly on flexor MNs (Fig. 3.26).

The results indicate a role for calcium, cAMP and IP_3 in mediating the tonic depolarization, whereas no distinction can be made if some of the actions of cAMP and IP_3 might be due to indirect influences (via pre-motor or intersegmental interneurons). In addition, an interaction between different second messenger pathways seems possible.

Summary for pharmacological experiments

Table 3.3.4 summarizes the results of the pharmacological experiments, described in chapter 3.2 and 3.3. The general effect for each substance as well as the effect on the tonic depolarization amplitude is listed. The column labeled 'N' indicates the number of cells, that showed the effect (calculated after 5 minutes) in relation to the total number of cells tested. For these values no difference was made between a significant or no significant effect.

Drug	Effect	[mM]	Tonic depolarization	N
Atropine	Muscarinic acetylcholine receptor antagonist	0.5	↓	8/9
Serotonin	Neurotransmitter	0.5 - 4	↑	4/6
		1 - 4	↓	2/6
Octopamine	Neurotransmitter	0.25 - 1	↑	10/11
		1	↓	1/11
Mianserin	Octopaminergic/Serotonergic Antagonist	0.1	↓	6/7
8-Br-cAMP	cAMP analog	0.5	↑	6/7
H-89	Inhibitor of cAMP-dependent protein kinase	0.2	↓	2/2
SQ22,536	Adenylyl cyclase inhibitor	0.2 - 1	↑	2/2
Neomycin	Inhibitor of phospholipase C	0.4 - 1.6	↑	4/5
U-73122	Inhibitor of phospholipase C	0.2	↑	3/3
BAPTA	Calcium chelating reagent	200	↓	7/8
Riluzole	Inhibitor of persistent sodium channels	0.025-0.2	↑	3/4
			↓	1/4

Figure 3.31: *Effect of the used drugs on the tonic depolarization amplitude in mesothoracic flexor MNs during front leg stepping. All drugs but BAPTA were bath applied. BAPTA was added to the electrolyte solution in the recording electrode. The arrows indicate an increase or decrease of the tonic depolarization amplitude. N indicates the number of cells that showed the corresponding effect without consideration of significance (number of cells that showed the effect/total number). Note that the effect of riluzole is described only in the appendix.*

3.4 Neuronal activity in lesioned animals

Lesion experiments have ever since been a favored tool in investigating the influence of higher brain centers on the initiation of locomotion, such as flight, swimming and walking. For example, the flight performance in locusts is still possible in decerebrated animals (Wilson 1961). The initiation of swimming in leeches is controlled by pathways emanating from the head ganglion (Brodfuehrer et al. 1995) and ablation of these ganglia revealed their role in setting the level of arousal and general activity (see Cornford et al. (2006) and references therein). The initiation of walking in insects may involve a brain command system, and recent results indicate such a role for descending interneurons (DINs) in crickets, called CNW (Zorovic and Hedwig 2007). In locusts, there are about 440 neurons DINs that project from head to thoracic ganglia, and lesion studies revealed that some of them may be involved in initiation of walking (Kien 1983; 1990). Walking and righting behavior in brainless stick insects was described by Graham (1979a;b), and even decerebrated animals are capable of walking-like movements after an appropriate stimulation (Bässler 1986).

The tonic depolarization in mesothoracic MNs described in this thesis is at least correlated to stepping movements of the front leg, but nothing is known about how this information is transferred or if other sources (e.g., head ganglia) play a role in influencing this membrane potential modulation.

The first part of this chapter investigates, if and how brain (supraesophageal ganglion) removal influences the tonic depolarization in mesothoracic flexor MNs. Then a few preliminary experiments are presented, in which connectives were transected to see in a first attempt, whether the neuronal information is redundant in both connectives and if the tonic depolarization is affected. The second part analyzes the neuronal activity extracellularly in neck connectives during front leg stepping to unravel a possible correlation. Furthermore, these results are compared to those of brainless animals.

3.4.1 Tonic depolarization in lesioned animals

In a first set of experiments the effect of brain removal on the tonic depolarization amplitude was analyzed. Therefore, the activity in flexor MNs was recorded in four animals

before ('intact') and after removal of the brain (brainless).
The preparation of the animal was performed according to chapter 2.2.3 with an addition in which the brain was exposed before recording intracellularly from flexor MNs (see details in chapter 2.2.4). For each experiment a stable intracellular recording of a contralateral flexor MN was established and the amplitude of the tonic depolarization was recorded during at least two stepping sequences of the front leg. During the respective recording, the brain was carefully removed and the tonic depolarization was again recorded during stepping sequences.

In general, brain removal led to an increased stepping activity in most animals (N=5/7, Fig. 3.32). Figure 3.32 A shows a stepping sequence of 12 steps that led to increased spike activity in the mesothoracic flexor nerve recording. After removal of the brain, the same animal performed 37 steps after tactile stimulation (Fig. 3.32 B). Although the activity in the nerve recording increased compared to the level at rest, it was decreased compared to the situation in the 'intact' animal. 'Intact' animals performed on average 8 steps per stepping sequence of the front leg, after brain removal an increase to 16 steps (mean) was observed. For 'intact' and brainless animals five stepping sequences at a time were analyzed. This increase was significant in 5 of 7 animals (Fig. 3.33). Stance duration as well as the step cycle period (begin stance to begin stance) were determined for these experiments (data not shown). In five of seven experiments, duration of stance phase was decreased in brainless animals, whereas this decrease was significant in three experiments. A significant increase was observed in a sixth experiment, and the seventh experiment showed no changed in stance duration. The cycle period increased in five of seven experiments after brain removal, which was significant in two experiments. Two animals showed a decreased cycle period, but this change was not significant.

Figure 3.34 shows the influence of brain removal on the tonic depolarization. During a stepping sequence of the front leg, a tonic depolarization of 5.5 mV was visible in a contralateral flexor MN and the nerve activity increased (Fig. 3.34 A). Figure 3.34 B shows the same recordings after brain removal. The nerve activity decreased, and the tonic depolarization amplitude showed a pronounced decrease as well (1.4 mV, 4 min

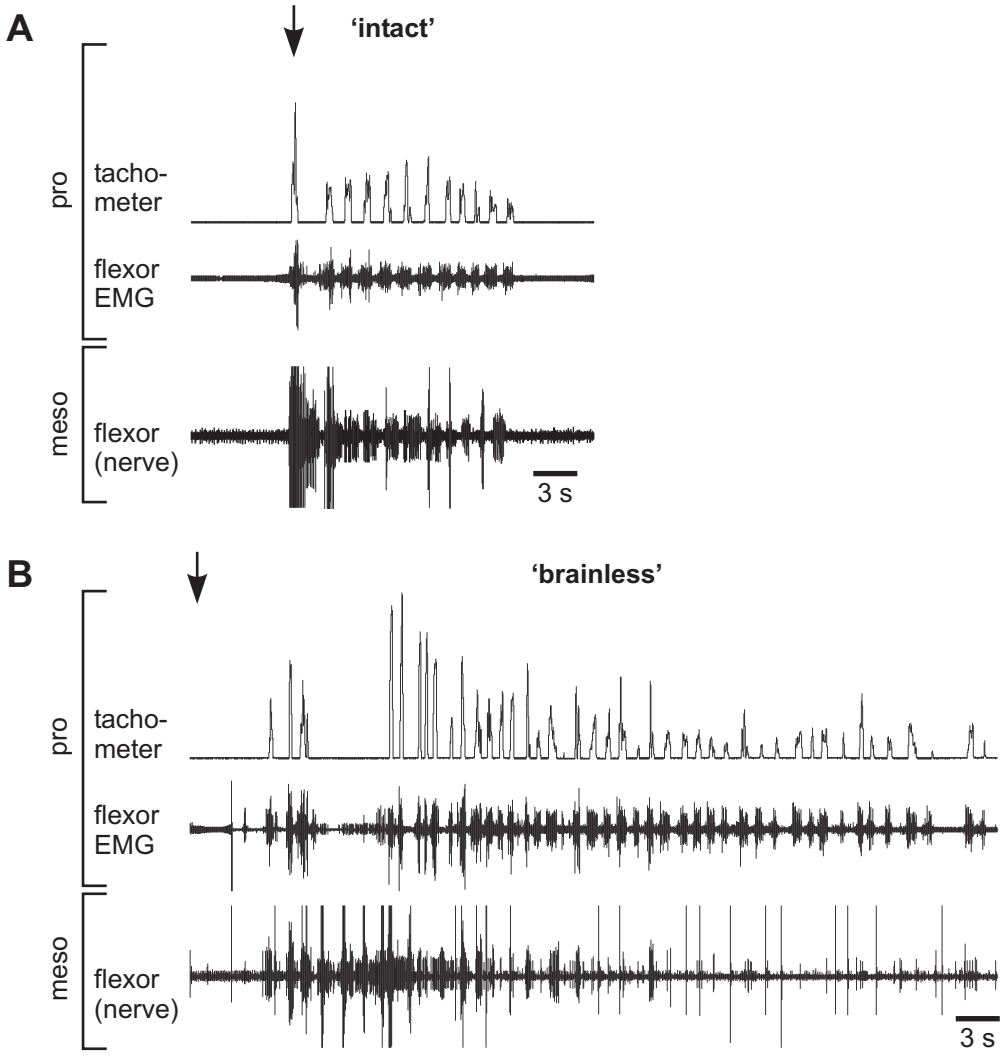


Figure 3.32: *Brain removal increased stepping activity* ***A:*** *A stepping sequence of the front leg (12 steps) after tactile stimulation showed increased activity in the contralateral mesothoracic nerve recording.* ***B:*** *After brain removal the animal performed 37 consecutive steps due to a comparable tactile stimulation. The activity in the nerve recording increased compared to rest, but was less pronounced compared to the 'intact' animal (**A**). Arrows indicate tactile stimulation.*

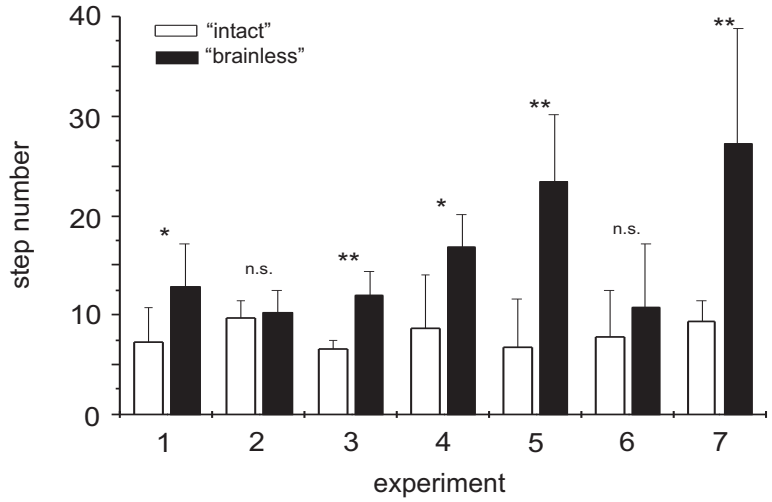


Figure 3.33: *Step number per stepping sequence in 'intact' and brainless animals. In five of seven experiments, the animal performed significantly more steps after brain removal (n=5 stepping sequences were analyzed in 'intact' and brainless animals). Asterisks on top mark the level of significance: (n.s.) no significance; (*) 0.01 $\leq p <$ 0.05; (**) 0.001 $\leq p <$ 0.01; (***) $p <$ 0.001.*

after brain removal). The tonic depolarization was significantly decreased in all experiments (at least at the 1 % level, Fig. 3.35 A). The maximum tachometer trace showed no difference in three of four experiments, in one experiment the values were significantly decreased after brain removal.

To analyse if the decrease in the tonic depolarization amplitude after brain removal was only a short-term effect, the amplitude was plotted over time, shown in figures 3.35 B, 3.35 C. At least during a period of 16 min after brain removal no significant change in the amplitude of the tonic depolarization was observed.

In two experiments the effect of cutting the connective ipsilateral to the stepping front leg (between the pro- and mesothoracic ganglion) on the tonic depolarization amplitude was tested. The result for one experiment is shown in figure 3.36. In the 'intact' condition the tonic depolarization amplitude was 1.3 mV (Fig. 3.36 A) and decreased to 0.8 mV after cutting the ipsilateral connective (Fig. 3.36 B). The activity decrease was also visible in the nerve recording. The tonic depolarization amplitude decreased by 39 % and 64 % and this decrease was significant for both experiments ($p < 0.001$).

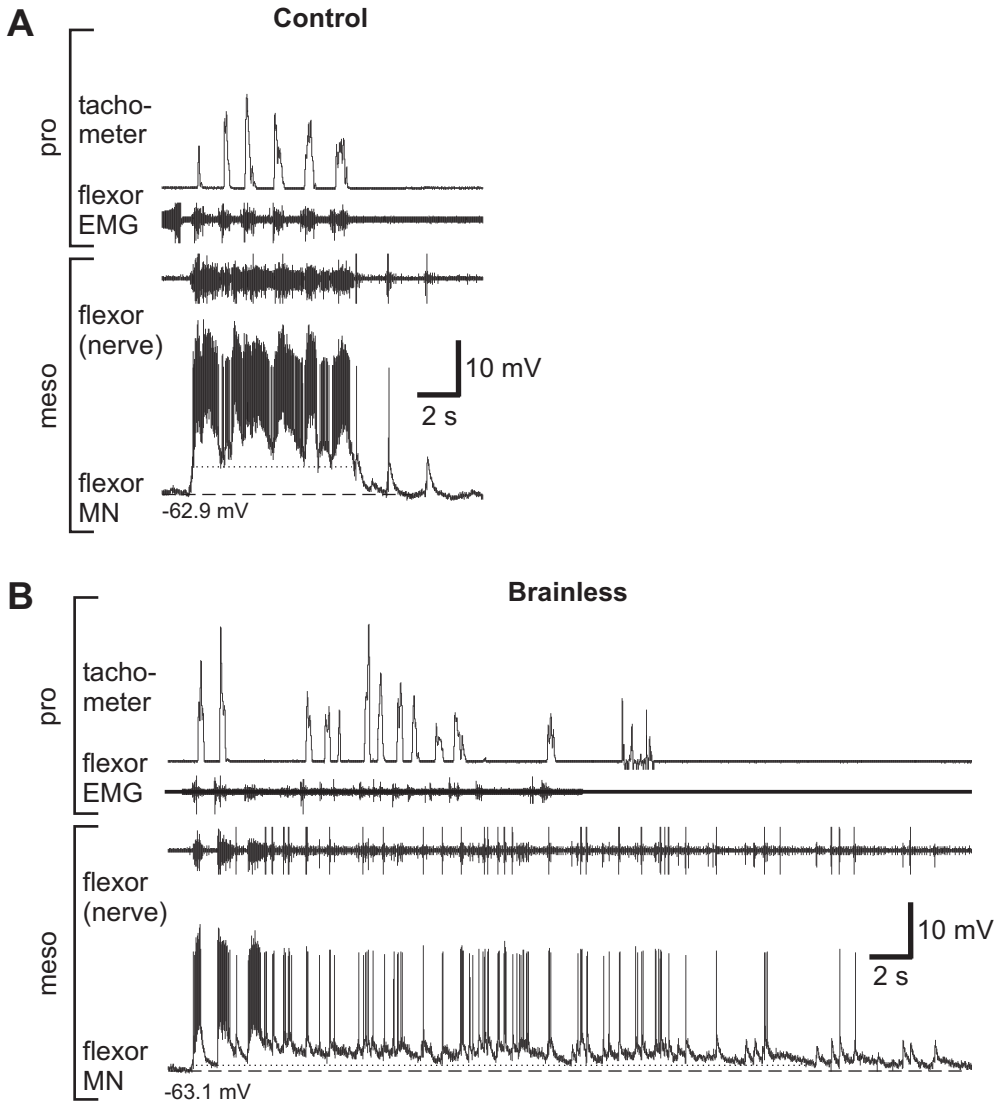


Figure 3.34: *Tonic depolarization in brainless animals.* ***A:*** *During a stepping sequence of the front leg (6 steps), the activity in the contralateral mesothoracic nerve recording increased. Throughout the stepping sequence a pronounced tonic depolarization was visible in a flexor MN (5.5 mV).* ***B:*** *After brain removal the same flexor MN showed a decreased tonic depolarization amplitude (1.4 mV) during a sequence of 12 steps of the front leg. The nerve recording showed also decreased activity.*

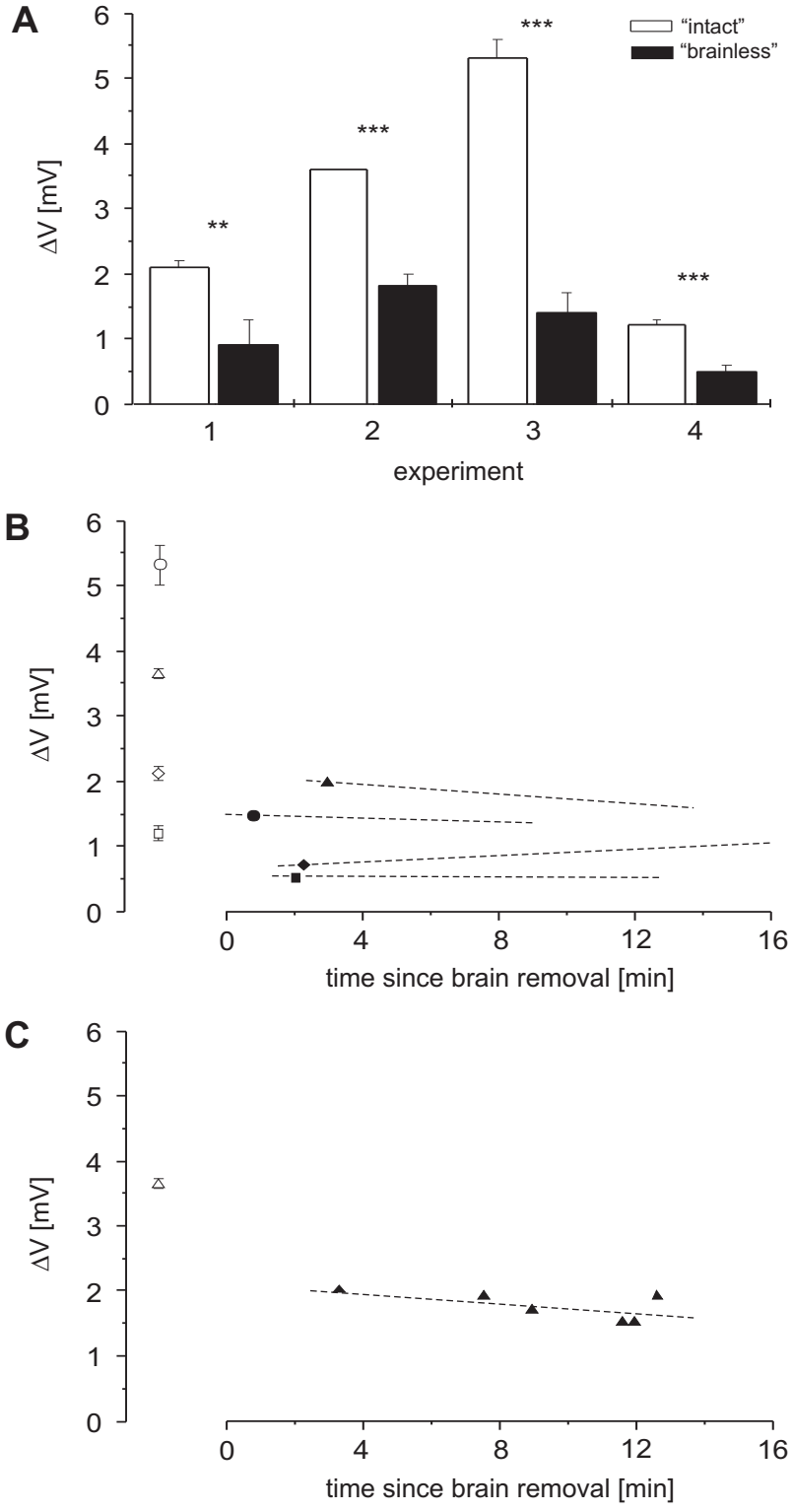

A
"intact"
"brainless"
ΔV [mV]
experiment
**
***
***
***
B
ΔV [mV]
time since brain removal [min]
C
ΔV [mV]
time since brain removal [min]

Figure 3.35: *The tonic depolarization amplitude was decreased in brainless animals.* ***A:*** *Comparison of the amplitude of the tonic depolarization (ΔV) in 'intact' (white bars) and brainless (black bars) animals. Asterisks on top mark the level of significance: (**) $0.001 \leq p < 0.01$; (***) $p < 0.001$.* ***B:*** *Detailed analysis for one experiment. The amplitude of the tonic depolarization is plotted over time. In this experiment the amplitude of the tonic depolarization in the 'intact' animal was 3.6 mV (n=2, △) and decreased to 1.9 mV after removal of the brain (▲). No significant change in the amplitude over time could be detected (dashed line).* ***C:*** *Analysis for all four experiments. The amplitude of the tonic depolarization decreased after brain removal and showed no significant change over time.*

Similar experiments were performed by cutting the ipsilateral neck connective. In one of two experiments the animal performed no stepping sequences after the lesion, the second experiment is shown in figure 3.37. The tonic depolarization amplitude in the 'intact' animal was 2.9 mV and decreased to 0.7 mV after cutting the connective between the pro- and subesophageal ganglion (Fig. 3.37 B). The decrease in the tonic depolarization amplitude was significant at the 0.1 % level (68 %). Strong phasic depolarizations were observed after brain removal, that might resemble twitching observed in brainless animals (Graham 1979a).

In summary, brain (supraesophageal ganglion) removal decreased the tonic depolarization significantly by 59.8 ±9.9 %. A decrease in the tonic depolarization amplitude was also observed by transecting the connective between pro- and mesothoracic ganglion or the neck connective. The decrease ranged between 39 % and 68 %.

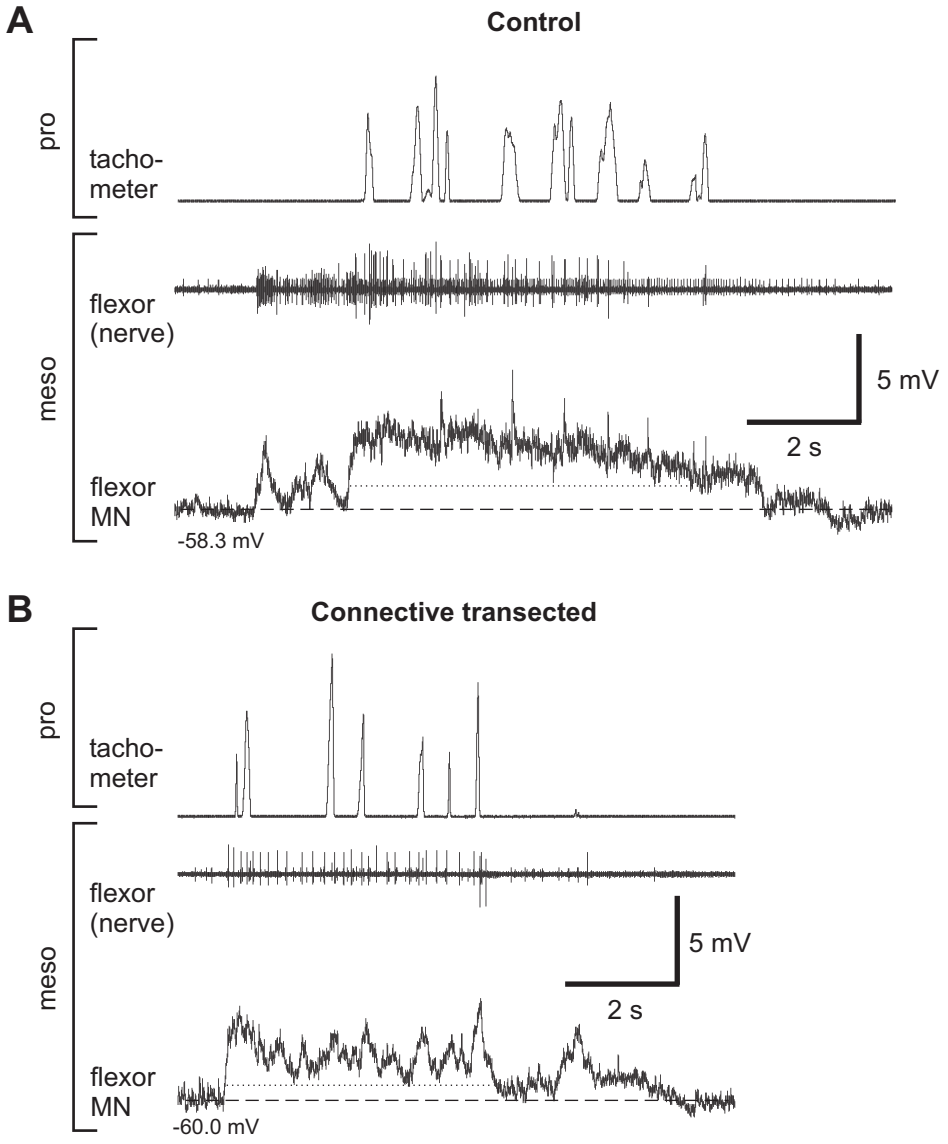


Figure 3.36: *Effect of ipsilateral connective lesion between the pro- and mesothoracic ganglion on the tonic depolarization.* ***A:*** *During a stepping sequence of the front leg (10 steps) the activity in the ipsilateral mesothoracic nerve recording increased. Throughout the stepping sequence a tonic depolarization was visible in a flexor MN (1.3 mV).* ***B:*** *Cutting the ipsilateral connective between the pro- and mesothoracic ganglion showed a decrease in the nerve recording and in the tonic depolarization amplitude to 0.8 mV during a front leg stepping sequence (7 steps).*

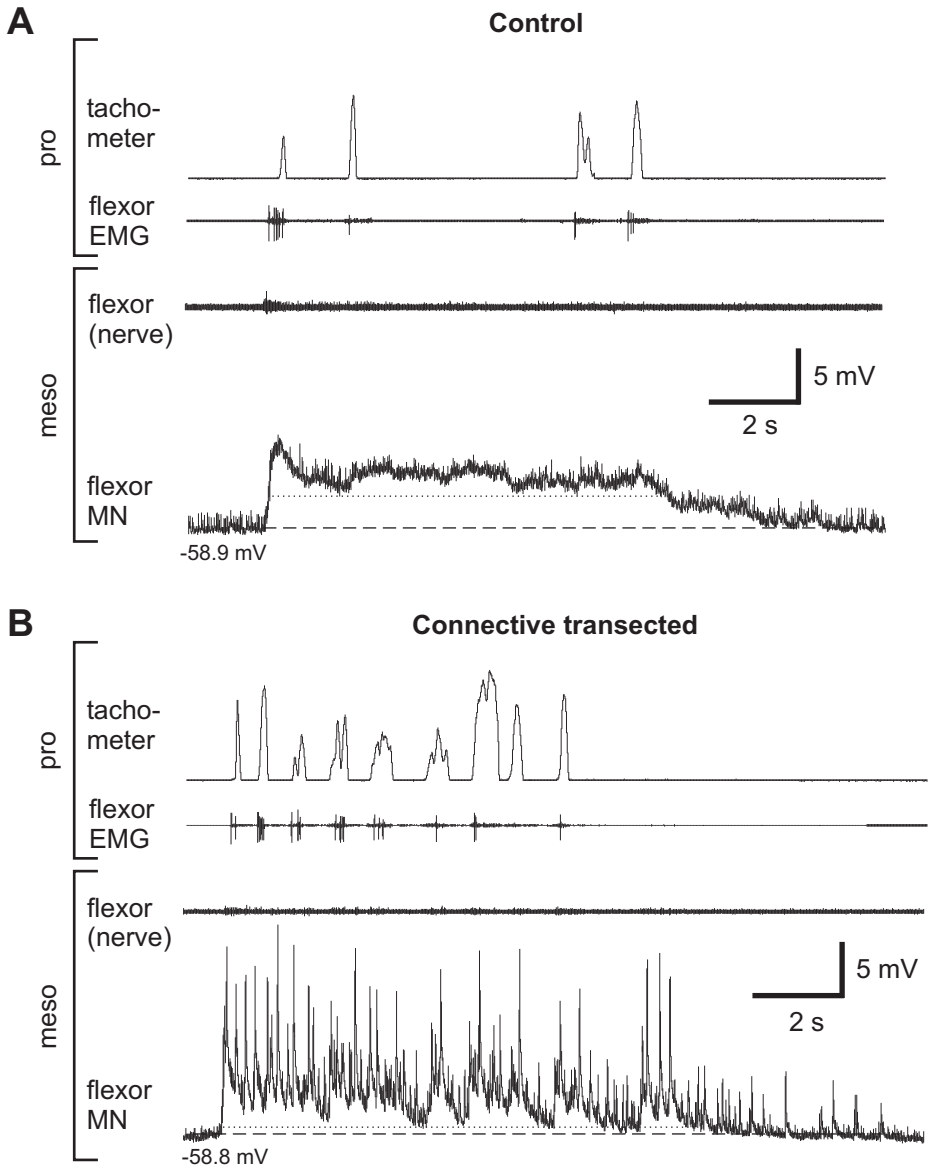


Figure 3.37: *Effect of ipsilateral neck connective lesion on the tonic depolarization.* ***A:*** *During a stepping sequence of the front leg (4 steps), the tonic depolarization in a flexor MN was 2.9 mV.* ***B:*** *Cutting the ipsilateral connective between the pro- and subesophageal ganglion showed a pronounced decrease in the tonic depolarization amplitude to 0.7 mV during a sequence of 9 steps. Throughout the stepping sequence the flexor MN showed strong phasic depolarization in membrane potential.*

3.4.2 Activity in neck connectives

Borgmann (2006) showed that the neuronal activity in ipsilateral connectives between thoracic ganglia is dependent on front leg stepping. The activity not only increased but appeared to be phasically modulated. It was shown, that the stepping velocity was in most cases related to the neuronal activity in the connectives.

This raised the question, if this dependency is also represented at a higher level, for example in the neck connectives. Therefore, in a first attempt the ipsi- and contralateral neck connectives were recorded extracellularly during front leg stepping. Figure 3.38 shows a representative experiment with recordings of both connectives. Under resting conditions the connective recordings showed a basic level of neuronal activity, that increased with the onset of a stepping sequence of the front leg (Fig. 3.38 A). According to the experiments performed by Borgmann (2006) not single action potentials were of interest but the course of the mean neuronal activity, therefore the rectified and smoothed (τ = 0.07 s) traces of the respective extracellular recordings are also presented. In this recording, the activity increase and time course in the connectives was almost identical for the ipsi- and contralateral neck connective (Fig. 3.38 B). The rectified and smoothed extracellular recordings showed two components (Fig. 3.39), a phasic and a tonic component, which were also shown in thoracic connectives in experiments performed by Borgmann (2006). The analysis of the activity increase in neck connectives during front leg stepping was performed in reference to the tonic component (Fig. 3.39). The increase was determined for each stepping sequence in four experiments (18 to 45 sequences). The activity increase in ipsilateral neck connectives was between 40.8 % and 68.9 %, in contralateral neck connectives between 39.2 % and 55.1 % (Fig. 3.40 A, 3.40 B). The increase was significantly ($p < 0.001$) different from zero for all four experiments. A comparison of the activity increase in ipsi- and contralateral connectives showed a difference in one of four experiments (Fig. 3.40 C). In this experiment, the activity increase was higher in the ipsilateral neck connective. In the experiment shown in figure 3.38, the activity in the neck connectives increased by 40.8 ±11.6 % (ipsilateral) and by 39.2 ±13.6 % (contralateral) and was not significantly different between the connectives (23 stepping sequences).

The performed experiments were further analysed to show if there is a dependency of

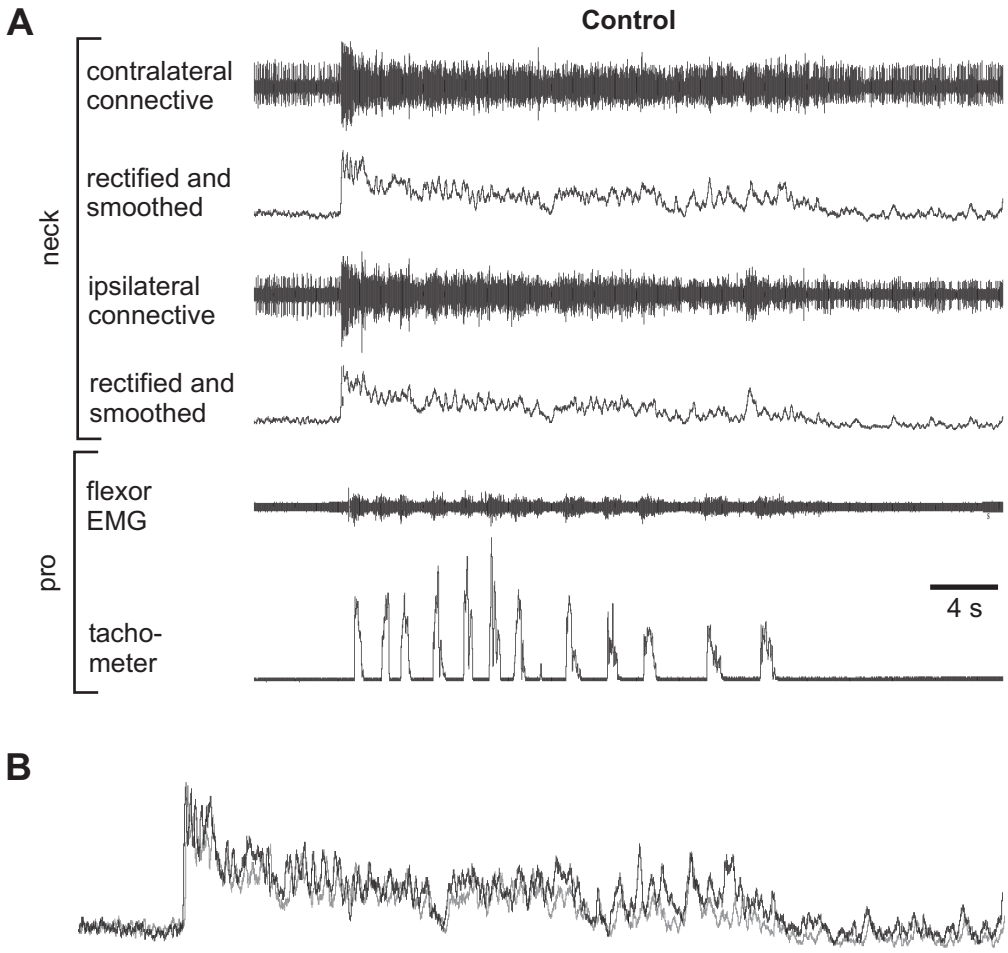


Figure 3.38: *Extracellular recordings of the ipsi- and contralateral neck connectives during front leg stepping. The neuronal activity in ipsi- and contralateral neck connectives increased during a front leg stepping sequence. The rectified and smoothed (τ = 0.07 s) extracellular recording is presented below the respective original recording.* ***B:*** *Overlay of both rectified and smoothed extracellular neck connective recordings. The time course of the activity is almost identical in both connectives.*

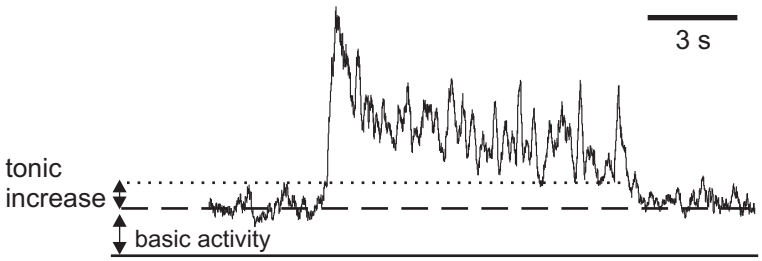


Figure 3.39: *Determination of the activity increase in the rectified and smoothed connective recordings. The relative increase in tonic activity in neck connectives was measured as the offset between the baseline of the basic activity (dashed line) before onset of a stepping sequence and the lower edge of the phasic activity (dotted line) during a stepping sequence.*

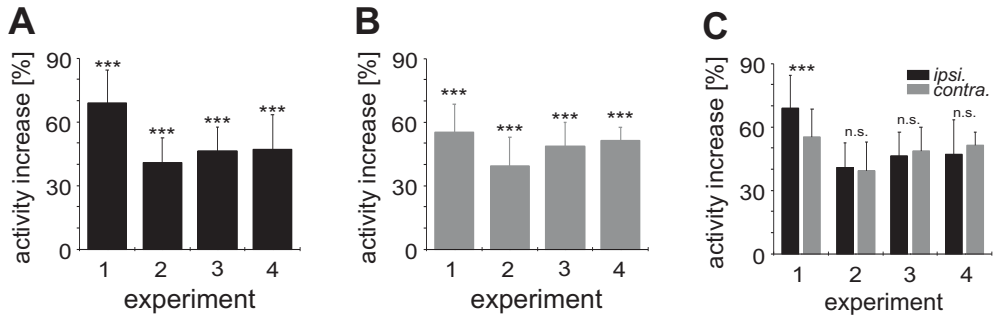


Figure 3.40: *Activity increase in neck connectives.* ***A:*** *Relative tonic activity increase during front leg stepping in* ***A:*** *ipsilateral neck connectives and* ***B:*** *contralateral neck connectives.* ***C:*** *Comparison of activity increase in ipsi- and contralateral neck connectives. Asterisks on top mark the level of significance: (n.s) no significance, (***) $p < 0.001$.*

activity in neck connectives and front leg stepping, e.g. stepping velocity. The detailed analysis for one experiment is shown in figure 3.41 A. The mean neuronal activity in the connectives, determined as integral under the rectified and smoothed extracellular recording for each step cycle, was normalized by respective step cycle period.

The normalized integral was plotted against the mean treadmill velocity, which was determined as the integral under the tachometer trace during stance phase and normalized by stance duration. The experiment showed that a linear dependency existed for both, the ipsilateral and contralateral neck connective (data obtained from 23 stepping sequences including 260 steps). This dependency was observed in all four experiments, as shown in figure 3.41 B, where a high correlation was detected for both, the ipsi- and contralateral connectives (18-38 stepping sequences including 131-274 steps, table 3.1). Analysis and comparison of the regression coefficients for the ipsilateral and contralateral neck connectives revealed a significant higher coefficient in three of four experiments: the coefficient was significantly higher in the ipsilateral connective in two experiments, in the third the coefficient for the contralateral connective was significantly higher (table 3.2).

Furthermore, the influence of brain removal on the activity in neck connectives was analyzed. Neck connective recordings in a brainless animal are shown in figure 3.42. These recordings were obtained from the same animal shown 'intact' in figure 3.38. An increase in the neuronal activity of the ipsi- and contralateral connective was induced during a front leg stepping sequence, which was less pronounced in comparison to the intact animal (see below). The activity increase and its time course in the connectives was similar for the ipsi- and contralateral recording (Fig. 3.42 B). One of four animals performed no stepping movements after removal of the brain, thus only three experiments were analyzed. The activity increase after brain removal is shown in figure 3.43. Ipsilateral neck connectives showed an increase of 21.0 % to 35.0 % (Fig. 3.43 A), in contralateral connectives it was 18.0 % to 47.1 % (Fig. 3.43 B).

These values were significantly (***) different from zero in all experiments (data obtained from 15 to 22 stepping sequences). The tonic activity increase in the experiment shown in figure 3.42 was 21.0 ±4.8 % in the ipsilateral, and 18.0 ±4.4 % in the contralateral neck connective (15 stepping sequences). A comparison of the tonic activity increase in both

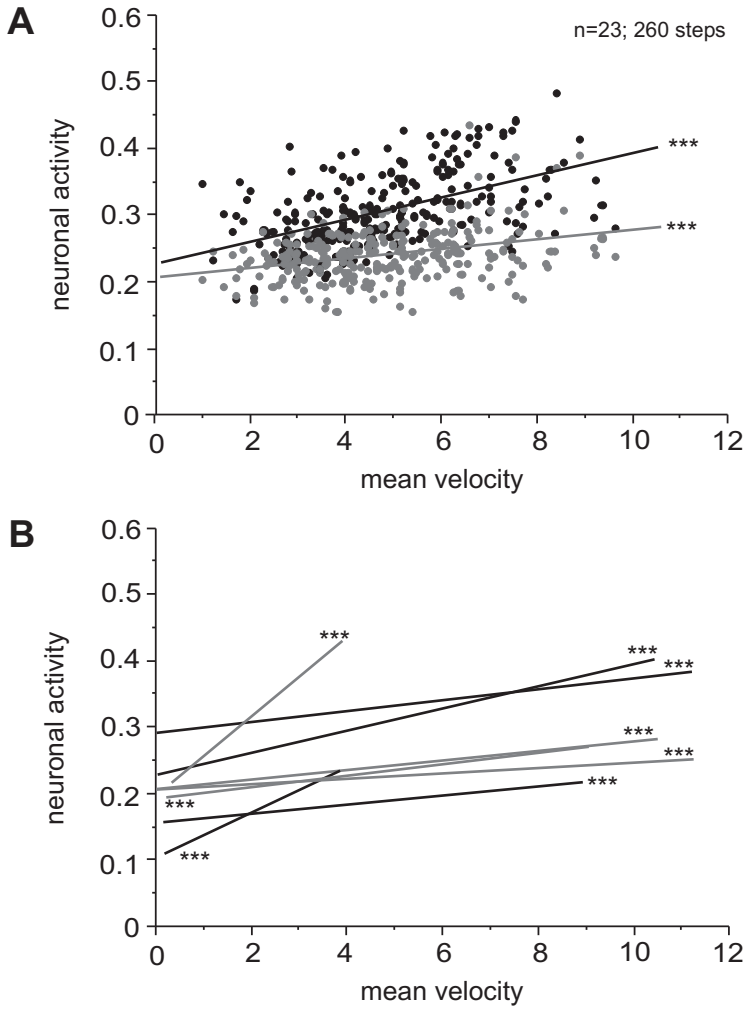


Figure 3.41: *Correlation of the mean activity in neck connectives and the mean velocity of the treadmill.* ***A:*** *Plot of the mean activity (integral/step duration) per step of the ipsi- and contralateral neck connective vs. the mean velocity of the treadmill (both in arbitrary units) for one experiment. Data fitted by linear regression.* ***B:*** *Linear fits of the mean activity (integral/step duration) per step of the ipsi- and contralateral connective vs. the mean velocity of the treadmill (both in arbitrary units). Black lines: ipsilateral connectives, grey lines: contralateral connectives. n= 18-38 stepping sequences, including 131-274 steps. The asterisks mark the level of significance:(***) $p < 0.001$.*

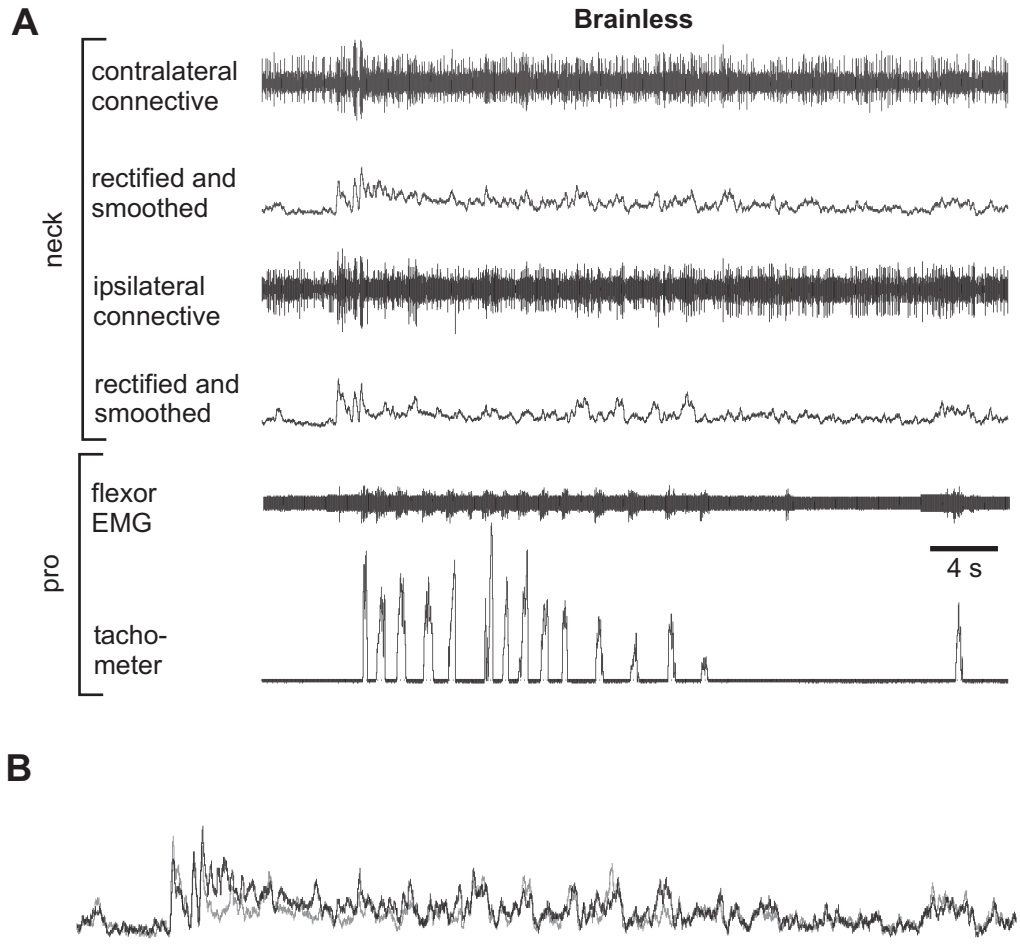


Figure 3.42: *Extracellular recordings of the ipsi- and contralateral neck connectives during front leg stepping in a brainless animal. The neuronal activity in the ipsi- and contralateral neck connectives increased slightly during a front leg stepping sequence, but was decreased in comparison to 'intact' animals (see Fig. 3.38). The rectified and smoothed (τ = 0.07 s) extracellular recording is presented below the original recording.* ***B:*** *Overlay of the rectified and smoothed extracellular recordings of the ipsi- and contralateral neck connectives. The time course of the activity is almost identical in both connectives.*

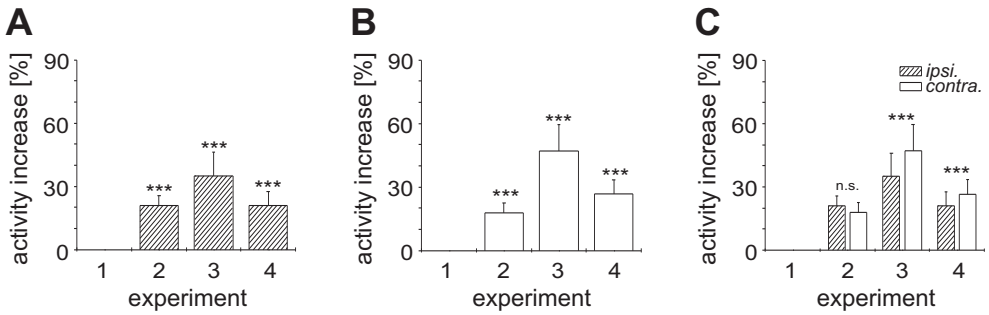


Figure 3.43: *Activity increase in neck connectives in brainless animals. Relative tonic activity increase during front leg stepping in* ***A:*** *ipsilateral neck connectives and* ***B:*** *contralateral neck connectives.* ***C:*** *Comparison of activity increase in ipsi- and contralateral neck connectives. Asterisks on top mark the level of significance: (n.s) no significance, (***) $p < 0.001$.*

	'intact'	
experiment	ipsilateral	contralateral
1	0.256	0.216
2	0.513	0.324
3	0.508	0.671
4	0.363	0.290

Table 3.1: *Correlation coefficients for the dependence of mean neuronal activity and stepping velocity in 'intact' animals, calculated for ipsi- and contralateral neck connectives.*

	'intact'	
experiment	ipsilateral	contralateral
1	0.00826	0.00385
2	0.01703	0.00715
3	0.03438	0.05913
4	0.00725	0.00883

Table 3.2: *Regression coefficients for the dependence of mean neuronal activity and stepping velocity in 'intact' animals, calculated for ipsi- and contralateral neck connectives.*

connectives showed a significant higher increase in contralateral neck connectives in two of three experiments (Fig. 3.43 C).

The tonic activity increase in neck connectives was compared between 'intact' and brainless animals in all experiments (Fig. 3.44). After brain removal the activity increase was significantly less pronounced in ipsilateral neck connectives (Fig. 3.44 A). The analysis for the contralateral neck connectives showed this significant difference in two of three experiments (Fig. 3.44 B).

As in 'intact' animals, it was analyzed whether a correlation between stepping velocity of the front leg and the neuronal activity in neck connectives exists. Figure 3.45 shows a linear dependency between the mean velocity and mean activity in ipsi- and contralateral connectives in two of three experiments (15 to 22 stepping sequences, 109 to 228 steps, see also correlation coefficients in table 3.3). In the third experiment there was no significant relationship in the ipsilateral neck connective. Comparison of the regression coefficients for ipsi- and contralateral neck connectives revealed no significant difference (table 3.4).

The regression coefficients were also compared between 'intact' and brainless animals (tables 3.2, 3.4): coefficients obtained for ipsilateral neck connectives showed a significant difference in all three experiments (level of significance at least 0.1 %). The regression coefficient obtained for the contralateral neck connective was significantly different in one of three experiments (level of significance 0.1 %). Thus in 'intact' animals the neuronal activity increase seemed to be higher during comparable stepping velocities.

3.4.3 Summary

In this chapter the neuronal activity in 'intact' and lesioned animals was analyzed and compared. The first part analyzed the influence of lesions on the tonic depolarization, which was described in detail in 'intact' animals in chapter 3.1.1. Brain (supraesophageal ganglion) removal decreased the tonic depolarization amplitude significantly by ~60 %. Thus, the brain might be important for induction of the tonic depolarization. A decrease was also observed by transection of the ipsilateral connective between the pro- and mesothoracic ganglion (up to 64 %) and by transection of the ipsilateral neck connective (68 %). Furthermore, these experiments showed an additional activity decrease in the extracel-

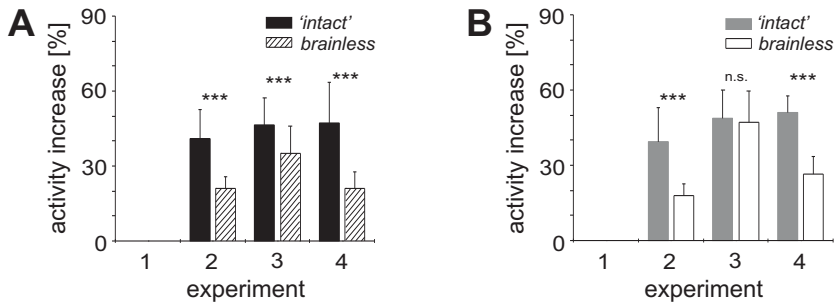


Figure 3.44: *Comparison of activity increase in neck connectives in 'intact' and brainless animals. Relative tonic activity increase during front leg stepping before and after brain removal in* ***A:*** *ipsilateral neck connectives and* ***B:*** *contralateral neck connectives. Asterisks on top mark the level of significance: (n.s) no significance, (***) $p < 0.001$.*

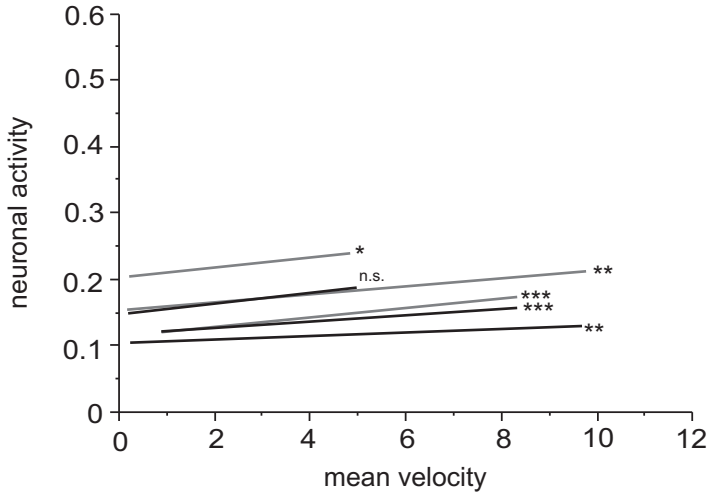


Figure 3.45: *Correlation of the mean activity in neck connectives and the mean velocity of the treadmill in brainless animals. Linear fits of the mean activity (integral/step duration) per step of the ipsi- and contralateral connective vs. the mean velocity of the treadmill (both in arbitrary units). Black lines: ipsilateral connectives, grey lines: contralateral connectives. n= 15-22 stepping sequences including 109-228 steps. The asterisks mark the level of significance: (n.s.) no significance, (*) $0.05 < p < 0.01$, (**) $0.01 < p < 0.001$, (***) $p < 0.001$*

	brainless	
experiment	ipsilateral	contralateral
1	-	-
2	0.280	0.415
3	0.173	0.189
4	0.258	0.250

Table 3.3: *Correlation coefficients for the dependence of mean neuronal activity and stepping velocity in brainless animals, calculated for ipsi- and contralateral neck connectives. In the first experiment the animal performed no stepping sequence after brain removal.*

	brainless	
experiment	ipsilateral	contralateral
1	-	-
2	0.00515	0.00706
3	0.00820	0.00754
4	0.00259	0.00565

Table 3.4: *Regression coefficients for the dependence of mean neuronal activity and stepping velocity in brainless animals, calculated for ipsi- and contralateral neck connectives. In the first experiment the animal performed no stepping sequence after brain removal.*

lular nerve recording. This indicates that the tonic depolarization might be transmitted by parallel pathways from the adjacent segments and that it is influenced by information from one or both head ganglia.

In the second part of this chapter the neuronal activity in neck connectives during front leg stepping was analyzed in 'intact' and brainless animals. In 'intact' animals, the tonic neuronal activity was significantly increased by 39.2 % to 68.9 %. This increase was not significantly different between the ipsi- and contralateral connectives. A dependency of neuronal activity in neck connectives and front leg stepping velocity was observed. Although a significant difference in the regression coefficients was observed between ipsi- and contralateral connectives, no preference for a stronger activity increase was detectable for the one or the other connective.

In brainless animals, the neuronal activity increased significantly during front leg stepping as well. The tonic increase ranged between 18.0 % and 47.1 % and was significantly higher in contralateral neck connectives.

It was shown, that the tonic neuronal activity increase was significantly higher in 'intact' animals. The correlation of neuronal activity in neck connectives and stepping velocity observed in 'intact' animals persisted after brain removal. The regression coefficients were significantly higher in 'intact' animals, thus brain removal seems to reduce the observed neuronal activity during respective velocities.

CHAPTER 4

Discussion

Membrane potential modulation in stick insect mesothoracic flexor MNs during single front leg stepping has been investigated in this thesis. Properties of a tonic depolarization associated with stepping behavior were described in detail, as well as effects of a range of drugs acting on different receptors and second messenger pathways. Furthermore, the influences of lesions (for example brain removal) on neuronal activity were investigated.

4.1 Activity in mesothoracic flexor MNs during walking of the contralateral front leg

Activity in the main leg nerve (*nervus cruris*, ncr) increased in the middle leg contralateral to the stepping front leg. The activity increase is indicative for spike activity in flexor MNs because their axons run through ncr. Intracellular recordings from these flexor MNs revealed, that the membrane potential shows two distinct changes during front leg stepping (see e.g., Fig. 3.6): 1) A tonic depolarization, that began with the onset of stepping

activity of the front leg and repolarized after the last step was performed. The repolarization to resting membrane potential could outlast the stepping sequence by up to several seconds. 2) A phasic membrane potential modulation that was coupled to front leg stance phase.

4.1.1 Tonic depolarization

The membrane potential in contralateral flexor MNs depolarized tonically by 1.7 mV ±1.2 mV (N=24) during front leg stepping. In few cases flexor MNs showed no tonic depolarization at resting membrane potential, but it was revealed by injection of hyperpolarizing current. This supports the hypothesis that the tonic depolarization is a general phenomenon. Previous studies showed a tonic membrane potential modulation in all mesothoracic MNs ipsilateral to the walking front leg, as well as in MNs recorded in the single middle leg preparation (Gabriel 2005; Ludwar et al. 2005b). Furthermore, in a recent study by Borgmann (2006), extracellular recordings showed an increase in MN spike activity in all segments during stepping sequences of a front, middle or hind leg.

Determination of the tonic depolarization amplitude

The tonic depolarization was defined as the difference between the resting membrane potential and the lower edge of the phasic modulation (Fig. 4.1, see also chapter 2.6.1). The tonic depolarization might be sculpted by phasic **inhibitory** input, in this case the "real" amplitude would be underestimated by the chosen method ('B' in Fig. 4.1). But if the tonic depolarization is sculpted by phasic **excitatory** input then the lower edge of the

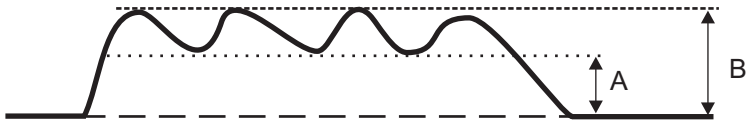


Figure 4.1: *Determination of the tonic depolarization amplitude. The tonic depolarization amplitude (ΔV) can be measured as the offset between the voltage baseline (lower dashed line: resting membrane potential) and the **lower** edge of the phasic modulation (dotted line, 'A') or the upper edge of the phasic modulation (upper dashed line, 'B'). Amplitudes calculated according to 'A' exclude shaping of the tonic depolarization by possible phasic excitatory input, whereas it would be an underestimation if phasic inhibitory input shapes the tonic depolarization.*

modulation would give the right amplitude ('A'). Thus the chosen method seems justified and was confirmed by the fact that only slight variations in control values occurred (shown throughout the results).

Reversal potential

The amplitude of the tonic depolarization was dependent on membrane potential, with increasing amplitudes at more hyperpolarized potentials, whereas it reversed at more depolarized potentials. The reversal potential was approximately −41 mV (Fig. 3.3). The tonic depolarization amplitude in all ipsilateral leg MNs described by Ludwar et al. (2005b) ranged from 0.5 to 5 mV and the mean reversal potential was found to be −38 mV. In my experiments, the mean amplitude of the tonic depolarization in **ipsilateral flexor** MNs was 1.9 mV ±1.2 mV (N=27) and these values were not significantly different from those obtained from **contralateral flexor** MNs (see above). Thus, the obtained values for contralateral flexor MNs are comparable to ipsilateral MNs. The variability in reversal potential is likely to result from the variability in depolarization amplitudes that in turn is likely due to the circumstance that tonic responses were not evoked by a standardized stimulation protocol but by the execution of a highly variable locomotor behavior. Different amplitudes in tonic depolarization in different recordings/animals might occur through a variable activation of the underlying current(s), which in turn depends on the amount of released transmitter. Nevertheless, tonic depolarization amplitudes within one experiment were relatively stable, usually with standard deviations of 0.1 to 0.2 mV.

Furthermore, the tonic depolarization was associated with a decrease in input resistance by about 12 %, which was in the same range as for ipsilateral MNs. The decrease in input resistance, respectively the increase in conductance, as well as the reversal potential suggest that the tonic depolarization could be caused by an excitatory, nonselective cation conductance. This might be a mixed conductance for example for sodium and potassium, although also a conductance through separate channels for sodium and potassium seems possible.

The reversal potential of the tonic depolarization is more depolarized than the value for spike threshold in flexor MNs (−51 mV to −47 mV (Gabriel et al. 2003)). A possible function of the tonic depolarization could be to bring the membrane potential closer to spike

threshold and thereby increase the sensibility of the membrane for excitatory inputs.

Enhanced responsiveness

In general, a decrease in input resistance would hinder spike generation by shunt-inhibition and thus counteract the suggested role for the tonic depolarization (see above). I could show, that injection of depolarizing current evoked one to two spikes in control conditions at rest, whereas the number of spikes was more than doubled throughout a stepping sequence. Thus the responsiveness was enhanced (in 73 % of the recordings, see also Fig. 3.5), which indicates that the current shunt due to increased conductance is more than compensated by the tonic depolarization.

Although these results indicate a mechanism that serves the enhancement of excitability of MNs, this seems to be a mechanism that allows fine tuning of MN activity. This assumption is based on two experiments, in which the responsiveness was decreased. In these experiments decrease in input resistance was exceptionally high (30 %). The different adjustment of this mechanism was particular prominent in an experiment, where no enhanced responsiveness was obvious throughout four stepping sequences, in which no tonic depolarization was visible. In the same flexor MN, throughout a fifth stepping sequence, the membrane tonically depolarized and responsiveness to depolarizing pulses was enhanced. Different channels are known to affect the excitability of membranes. The hyperpolarization activated I_h current plays a role in several vertebrate and invertebrate preparations. It contributes to pacemaker activity of the heart or modulates rhythmic activity in respiratory circuits in the mice (DiFrancesco 1993; Thoby-Brisson et al. 2000). Interestingly, I_h selectively conducts both Na^+ and K^+ ions and the reversal potential for I_h currents can be -25 to -40 mV, depending on the ratio for Na^+ and K^+ permeabilities (Robinson and Siegelbaum 2003). Although the tonic depolarization seems not to be a voltage dependent mechanism, a mechanism similar to I_h mediated depolarization cannot be excluded, because increased excitability could be due to a presynaptic I_h mediated effect. Furthermore, I_h is subject to amine modulation in the neurons of the lobster STG, that leads to an increase of I_h at less-hyperpolarized voltages (Peck et al. 2006). A possible influence of amines is also indicated by the effect of serotonin on the tonic depolarization in stick insect MNs (see discussion in chapter 4.2). Another exam-

ple for increased excitability during rhythmic activity comes from turtle MNs, which are part of the scratch-like motor network (Alaburda and Hounsgaard 2003). The underlying current is mediated by metabotropic glutamate receptors whose activity facilitates L-type Ca^{2+}-channels. The increased excitability could outlast the motor activity for tens of seconds, similar to the effect observed in stick insect MNs. Increased excitability was also shown for the proleg retractor MNs in the tobacco hornworm *Manduca sexta*, where it is due to a long-lasting depolarization from mechanosensory planta hairs (Trimmer 1994). In this system, mAChRs play a role in mediating the increased excitability and the responsible inward current increases with depolarization, thus exhibiting regenerative properties (Trimmer and Weeks 1993). This inward current is predominantly carried by Na^+, but possible additional changes in K^+ or Cl^- were not excluded (Trimmer 1994, see discussion "*Metabotropic acetylcholine receptors*"). The author suggested, that this current affects the time-course and strength of the response in the MN to all incoming inputs, thus acting as a form of motor arousal.

The increased excitability observed during stepping activity in stick insect MNs, as well as the finding that the muscarinic agonist pilocarpine induced a tonic depolarization (Büschges 1998), indicates a role for metabotropic receptors in mediating the tonic depolarization. A possible involvement of mAChRs was investigated by using the antagonist atropine (see chapter 3.2.1), which partly reduced the tonic depolarization amplitude (see discussion below; chapter 4.2.1).

Due to the shown properties of the tonic depolarization, the function of the tonic depolarization might be to bring a neuron closer to spike threshold, and thereby increasing the effectiveness of phasic depolarizing inputs in eliciting spikes. This suggests, that the tonic depolarization represents a state of arousal that reflects activation of the animal (this thesis, Ludwar et al. 2005b).

4.2 Pharmacological experiments

The influence of several neuroactive substances on the tonic depolarization in flexor MNs was investigated. The experiments were performed in the semi-intact preparation with

one intact front leg, and recordings were made from either ipsi- or contralateral mesothoracic flexor MNs. This preparation enabled an easier and in-depth analysis of the tonic depolarization, as the phasic modulation in membrane potential was rather small compared to that of MNs innervating the walking leg (see discussion 4.3 and Gabriel 2005; Fischer et al. 2001; Schmidt et al. 2001). Furthermore, the activity observed in mesothoracic MNs was evoked by neurons that are involved in the control of walking, and was not induced pharmacologically, e.g., by pilocarpine. In the chosen preparation, the use of pharmacological agents did not affect the stepping leg, as the superfusion was restricted to the mesothoracic segment by silicone-gel barriers. The removal of the ganglion sheath allowed a better diffusion of the superfused substances, and seemed to have no effect on the vitality and activity of the recorded flexor MNs.

4.2.1 Involved transmitters/receptors

By using a range of neuroactive substances, it was investigated which transmitters, respectively receptors might be involved in mediating the tonic depolarization in MNs. Due to the shown properties of the tonic depolarization, for example the long lasting excitability, the focus was on substances that mainly exert their effects through metabotropic receptors.

Metabotropic acetylcholine receptors

Experiments using the mAChR agonist pilocarpine in stick insects indicated a role for mAChRs in locomotor networks, because it induced a rhythmic bursting pattern in MNs that was inhibited by atropine (Büschges et al. 1995; Büschges 1998). A role for ACh as a transmitter mediating the tonic depolarization was shown indirectly by the decrease in tonic depolarization amplitude in the presence of atropine, which is an antagonist of ACh on mAChRs. The tonic depolarization amplitude decreased after 5 minutes by around 40 % in eight of nine experiments.

It is not known whether the inhibiting effect of atropine on mAChRs is exerted pre- or postsynaptically. In insects, mAChRs are found pre- and postsynaptically, whereas the latter regulate mainly the excitability of neurons (Trimmer 1995). The presynaptic function of a mAChR is usually that of an autoreceptor and inhibits transmitter release (Breer

and Sattelle 1987; Trimmer 1995; Osborne 1996). Trimmer (1994) described an increased excitability of proleg retractor MNs in *Manduca sexta* induced by the muscarinic agonist oxotremorine-M (oxo-M), which is thought to mimic an afferent-induced long-lasting depolarization (see discussion "*Enhanced responsiveness*"). The action of oxo-M is mediated by mAChRs and mainly carried by Na^+. The author suggested a postsynaptic localization of the responsible mAChRs. Although the underlying current showed some differences, for example its voltage-sensitive properties, it seems possible that the tonic depolarization is mediated by mAChRs similar to the described ones in *Manduca*, and thus localization on flexor MNs seems possible. On the other hand changes in excitability of neurons mediated by mAChRs can be achieved by activation or inactivation of several other currents. For example an inhibition of the so-called M-current (I_M) is known to increase excitability in vertebrates (frog sympathetic neurons: Brown and Adams 1980). I_M is a voltage-dependent K^+ current which is activated with depolarizing potentials and induces thereby a decrease in responsiveness to depolarizing input (Brown and Adams 1980; McCormick 1992). Interestingly, stimulation of serotonin or muscarinic receptors can lead to a suppression of I_M and these receptor types seem also to play a role in mediating the tonic depolarization in flexor MNs (see also discussion "*Serotonin*"). But an inhibition of I_M is accompanied by an increase in input resistance in the respective cell, which is in contrast to the observed decrease in input resistance during the tonic depolarization in flexor MNs. At least no I_M current in the flexor MNs seems to be involved in increasing the excitability observed during the tonic depolarization.

The concentration of 500 µM atropine used in my experiments might appear rather high, but even higher concentrations of atropine (1 mM) were used by David and Sattelle (1984) to analyze its effect on ionotophoretically injected ACh on the fast coxal depressor MN of the cockroach. Similar to the experiments in my thesis, they also bath applied atropine to the desheathed ganglion. The effectiveness of 500 µM atropine was also shown for the proleg retractor MN of *Manduca*, where it inhibited at least 50 % of the amplitude of an afferent induced EPSP, which is therefore thought to be mediated by mAChRs (Trimmer and Weeks 1993). In the mentioned study, the use of a quite high concentration of another substance, a muscarinic agonist (McN-A-343) is discussed. The latter was

effective only at very high concentrations, which is explained by possible secondary effects such as metabolic inactivation or sequestration. In general, besides atropine, also other substances were used at rather high concentrations. Although the ganglion sheath, known as a diffusion barrier in insects (Pichon and Treherne 1972), was removed and should have eased diffusion, the substances still had to diffuse through the neuropile to their sites of action. It is not known how permeable the neuropile is for the different substances. Reflecting the experiments performed in this thesis, the partial inhibition of the tonic depolarization by atropine (40 %) could be due to a concentration that was not high enough for complete suppression, or that atropine is not a potent inhibitor of the involved mAChR type, that mediates the tonic depolarization in this system. On the other hand, in none of the performed recordings a steady-state of the tonic depolarization amplitude was reached during atropine superfusion, which leads to the suggestion, that the used concentration might have had a more potent effect over time. Furthermore, it is not known which concentration of atropine or of any other used substance is present at the location where it exerts its effect.

The degree of reduction in tonic depolarization amplitude (40 %) was calculated from the linear fits (see figure 3.10 B) after 5 minutes superfusion with atropine. This analysis was chosen due to the fact, that it was difficult to elicit stepping sequences to a defined point in time in the individual recordings. The last value for the tonic depolarization in each experiment was not statistically analyzed and not further mentioned in the results, because intracellular recordings varied in duration and thus a statistical comparison of these values was not feasible. Nevertheless, the decrease in the tonic depolarization amplitude in the presence of atropine was up to 80 % if calculated from the latest value measured in each recording, indicating a more potent effect of atropine over time.

Although atropine is known as an mAChR antagonist, there is some evidence that it competitively inhibits currents through nAChR in insects (e.g., Kenyon cells, honey bee: Wüstenberg and Grünewald 2004). Benson (1992) reported a concentration dependent inhibition of different receptor types by atropine in isolated locust neuronal somata. At low concentrations atropine was a potent inhibitor of the induced muscarinic response ($EC_{50} \sim 10^{-8}$ mol/l), but showed moderate inhibition of the nicotinic response at higher

concentrations ($EC_{50} \sim 10^{-5}$ mol/l). On the one hand, it cannot be excluded, that atropine exerted its effect by acting on nicotinic **and** muscarinic ACh receptors in stick insect MNs. But then a possible action of atropine on nicotinic receptors should influence rather fast effects, such as the phasic modulation in membrane potential. The action on muscarinic receptors should influence slower effects, such as the tonic depolarization in flexor MNs. On the other hand, other studies of insect preparations showed that nicotinic currents are **not** atropine sensitive, e.g., in cockroach sensory- and interneurons (Blagburn and Sattelle 1987; Buckingham et al. 1997).

Furthermore, the existence of 'mixed nicotinic/muscarinic' receptors was discussed in several insect species, where the pharmacological profile showed sensitivity for agonists/antagonists of both receptor types (review by Osborne 1996, for example cockroach DUM neurons: Grolleau et al. 1996).

Octopamine

Octopamine was shown to increase the tonic depolarization amplitude in ten of eleven experiments, whereas the tonic depolarization amplitude was almost doubled (see chapter 3.2.2). In a range of invertebrate preparations, octopamine exerts a multitude of effects, by acting as a neurotransmitter, -modulator or -hormone (Orchard 1982; Roeder 1999). In the locust, central and peripheral modulatory effects of octopamine have been shown. A prominent example for the peripheral action of octopamine is the locust extensor tibiae muscle, where octopamine modifies the contraction properties of the muscle (Evans and O'Shea 1977). In general, octopamine is known to interfere with the initiation and maintenance of various rhythmic behaviors in the insect CNS. Injection of octopamine into specific areas of thoracic ganglia leads to an initiation or suppression of different rhythmic behaviors (Sombati and Hoyle 1984).

Metathoracic flexor tibiae MNs in locust depolarized by a few millivolts and showed increased excitability in the presence of octopamine, whereas the membrane potential of the fast extensor tibiae (FETi) MN was not influenced (Parker 1996). The efficacy of synaptic inputs was affected differently in fast and slow flexor MNs by octopamine. The FETi evoked EPSP depended furthermore on the used concentration of octopamine. Bath application of 1 mM octopamine decreased the EPSP amplitude in slow flexors and caused

an increase of the EPSP in the fast flexor MNs. A concentration of 10 mM octopamine also decreased the EPSP amplitude in slow, but had variable effects on the EPSP in fast flexor MNs.

In the experiments performed for my thesis, octopamine seemed to have no obvious effect on spike activity in the *nervus cruris* and flexor MNs in the resting animal (this was not analyzed statistically), and seemed to exert its effect only during stepping activity of the front leg, namely on the tonic depolarization. Despite the well known influence of octopamine in other insect species, very little is known about its effects in stick insects. Ramirez et al. (1993) and Büschges et al. (1993) described a role in the resistance reflex of the femur-tibiae control loop and an octopaminergic modulation of the fCO. Injection of octopamine into the hemolymph of intact stick insects led to an initial activation, followed by a phase where the animals were inactive (Büschges et al. 1993). Interestingly, octopamine modulates intrinsic properties in locust interneurons that are involved in the flight system (Ramirez and Pearson 1991a;b), where it induced bursting and plateau potentials. The tonic depolarization in stick insect MNs is less likely caused by intrinsic properties. So far, injection of a depolarizing current into stick insect neurons was never followed by plateau potentials. Furthermore, plateau potentials can be generated by persistent inward currents (PICs), and the persistent sodium channel blocker riluzole increased the tonic depolarization amplitude (see appendix A). This effect was in contrast to the expected decrease in the tonic depolarization amplitude in flexor MNs, if PICs carried by sodium were involved.

In insects, dorsal unpaired median (DUM) and ventral unpaired median (VUM) neurons are known to contain and release octopamine and are believed to be the most important sources for octopamine in the thoracic and abdominal nervous system (Roeder 1999). Periphal tissues such as flight and leg muscles are densely innervated by octopaminergic DUM neurons. Although the actions of specific sets of DUM neurons seem to be coupled to a given behavior, by now there is no evidence of a direct activation of different motor systems via thoracic DUM neurons, but a parallel activation seems possible (Burrows and Pflüger 1995). Discernible output structures of the segmental efferent DUM neurons within the metathoracic ganglion of the locust are missing (Watson 1984). Furthermore,

somata of DUM/VUM neurons are also found in the subesophageal ganglion, which innervates all major neuropils in the brain (Bräunig 1991), whereas a different group of DUM neurons has descending axons that project to the ventral nerve cord (Bräunig et al. 2004). These descending DUM neurons are putatively octopaminergic and have no direct peripheral targets. Although it is not known if those DUM neurons are also existent in the stick insect they could be possible candidates for the release of octopamine, which might be involved in the modulation of synaptic transmission in thoracic ganglia, and thereby influencing the tonic depolarization.

A role of octopamine receptors in mediating the tonic depolarization may be supported by the finding, that the octopamine receptor antagonist mianserin decreased the tonic depolarization amplitude in six of seven experiments by about 31 % (Fig. 3.16). Again, the values were calculated after 5 minutes superfusion of the substance. A comparison of the respective last value of the tonic depolarization in each experiment was not possible due to the above mentioned reasons (see discussion in "Metabotropic acetylcholine receptors"). But these values showed that the decrease in tonic depolarization amplitude could be even higher over time (up to 69 %). In the literature, the reference to mianserin is in most cases that of an octopaminergic antagonist, and in several papers it was even used to pharmacologically discriminate different octopaminergic receptors (e.g., Evans 1981; Roeder 1991; 1994). A concentration of 100 µM mianserin was sufficient to antagonize octopaminergic autoreceptors on an identified DUM neuron in the locust (Howell and Evans 1998). The possibility of mianserin acting as a serotonergic receptor antagonist cannot be neglected, as it was also described as an antagonist of these receptors in some vertebrate and invertebrate preparations (Schmidt and Jordan 2000; Dringenberg 2000; Tierney 2001).

High concentrations of neuroactive substances can induce unselective effects of the respective substance on other receptors. The effect of octopamine (up to 1 mM) seemed quite consistent and might exclude an unselective action of octopamine. Furthermore, Parker (1996) pointed out, that he used relatively high concentrations of octopamine (1 mM) in a preparation of the locust metathoracic ganglion and argued, that the ganglion sheath limits the entry of drugs (the ganglion was only softened with an enzyme and not

desheathed), and that specific uptake and degradation mechanisms reduce the concentration in the neuropil. Furthermore, he cites a result described in Stevenson (1989), where bath application of 100 mM octopamine was needed to mimic the effect of 1 µM released into the neuropil. Thus, in the light of these observations and the rather distinct effect of octopamine on the tonic depolarization, the used concentrations of octopamine seem justified.

Serotonin

Serotonin showed two opposing effects on the tonic depolarization, as it increased the tonic depolarization amplitude in four of six experiments, and decreased it in two experiments (see Fig. 3.14). Multiple effects of serotonin were also described in other invertebrate systems, where the excitatory or inhibitory effect depended for example on the concentration, rate and time course of exposure or even on the social status of the animal (Teshiba et al. 2001; Yeh et al. 1996). The opposing effect of serotonin in the experiments shown in this thesis depended less likely on the applied concentration. At least no correlation between the concentration in the superfusate and the sign of the effect was observed, as concentrations of 1 mM and 4 mM were able to induce opposing effects in different experiments. Teshiba et al. (2001) argued that an inhibitory effect of serotonin on the lateral giant neurons in the crayfish occurs when high concentrations are reached rapidly, and contrarily, excitatory effects of serotonin are observed when the final concentration (low or high) is reached gradually. The conditions in my experiments were very similar, concerning perfusion rate, the volume in the split bath configuration and so forth. Therefore the different effects of serotonin on flexor MNs might not be due to these 'external' conditions. Nevertheless, slight differences in diffusion of serotonin through the neuropil might have influenced how fast and concentrated serotonin could exert its effect. Teshiba et al. (2001) suggested furthermore, that serotonin might activate two parallel intracellular pathways, which in turn are activated either by different levels of a common initial second messenger and/or different receptor types (see also Bear 1995; Gatellier et al. 2004). Thus, the opposing effects of serotonin in stick insect MNs could also be due to an activation of parallel second messenger pathways. Depending on the strength of activation of either second messenger pathway, different effects of serotonin

could be induced.

Le Bon-Jego et al. (2004) performed experiments analyzing the effect of serotonin on the resistance reflex in crayfish legs. They found a variability in the serotonin induced responses in intracellular recorded depressor MNs. On the one hand, they try to explain these differences with inter-individual sources, as either all recorded depressor MNs in one animal displayed an increase of the resistance reflex or none were affected. Edwards et al. (2002) also described differences of serotonin induced effects depending on the social status of the animal. On the other hand Le Bon-Jego et al. (2004) suggest an inter-motoneuron variability. An inter-individual dependence of the serotonin induced effect on the tonic depolarization in flexor MNs in stick insects is less likely, as social status in stick insects is probably irrelevant. The inter-motoneuron variability might be excluded as an interpretation, as all recorded flexor MNs expressed tonic **depolarization** at resting membrane potential (with few exceptions where the tonic depolarization was only visible during injection of constant hyperpolarizing current). In the above mentioned paper, the authors found a serotonin induced tonic depolarization in depressor MNs, the function of which is thought to bring the membrane potential closer to spike threshold and causing an increased firing rate. Although a similar function of the tonic depolarization in stick insects is suggested (Ludwar 2003; Ludwar et al. 2005b; Gabriel 2005), the mechanisms underlying the tonic depolarization seem only partially comparable. First, in contrast to the effect on crayfish MNs, serotonin alone induced no tonic depolarization in stick insect MNs. Second, the serotonin induced tonic depolarization in crayfish depressor MNs was accompanied by an increase in input resistance, which is in contrast to the properties of the tonic depolarization in stick insect flexor MNs. The differences in input resistance can only be compared with reservations, as it is not known how the input resistance in flexor MNs is influenced during a stepping sequence in the **presence of serotonin**.

Co-transmission and neuromodulation

A role for metabotropic ACh receptors in mediating the tonic depolarization was proposed due to experiments using pilocarpine (previous studies) and the decrease induced

by atropine (this thesis, chapter 3.2.1). But also octopamine and serotonin seemed to influence the tonic depolarization, although the effect of serotonin was ambiguous. This raised the question, how can the tonic depolarization be influenced by several neuroactive substances? Up to now, nothing is known about co-transmission in stick insects, but speculation about such a mechanism causing the observed effect on the tonic depolarization might be possible. Co-transmission was shown for a range of vertebrate and invertebrate systems (Lundberg 1996; Nusbaum et al. 2001; Burnstock 2004). In most cases, classical neurotransmitters are co-released with neuropeptides, ATP and other substances (e.g., octopamine and proctolin in the locust oviduct or GABA and proctolin in the crab STG (Lange 2002; Blitz and Nusbaum 1999)). In general, little is known about co-transmission in insects. At least there are some studies of co-localization of several neuroactive substances in the locust brain (e.g., Würden and Homberg 1995; Homberg et al. 1999), although that is not necessarily proof for co-transmission. Co-transmission can occur through several mechanisms. First, two transmitters can be released from all presynaptic terminals of one neuron, and all postsynaptic sites of another neuron can contain receptors for both. Second, a presynaptic neuron releases two transmitters, and each postsynaptic terminal expresses receptors for only one transmitter. Third, the presynaptic neuron releases co-transmitters, but only one transmitter is released at different processes (e.g., in Marder et al. (1995); Marder (1999)). One of these mechanisms of co-transmission might lead to the different, and in some cases opposing effects of neuroactive substances in stick insect MNs.

The tonic depolarization in flexor MNs might be induced by an action of ACh **and** octopamine, although that does not necessarily imply a co-release from the same presynaptic neuron. Another possibility includes the release of these neuroactive substances from different presynaptic neurons, acting directly or indirectly on the flexor MNs.

As for co-transmission, neuromodulation in stick insects has not been described up to now. In the cockroach, octopamine as well as serotonin are known to have neuromodulatory properties, for example in the fast coxal depressor MN, where they reversibly suppressed the ACh induced responses (Butt and Pitman 2002). The authors suggest that the analyzed amines (including dopamine) share a common mechanism, in relation

either to receptors or second messenger pathways. Although this implies an opposing effect of ACh and the amines, it is at least an indication for a possible interaction of these neuroactive substances in insect MNs.

In the light of previous studies on different invertebrates, the observed effects of octopamine and serotonin on the tonic depolarization described in my thesis do not exclude each other. Octopamine and serotonin can have antagonistic, but also agonistic effects. Antagonistic action relates to aggression behavior in crustaceans, where serotonin increases the willingness to fight and octopamine causes a submissive posture (Harris-Warrick and Kravitz 1984; Harris-Warrick 1985). Similar effects of octopamine and serotonin were found in *Drosophila*, where both stimulated locomotion and grooming (Yellman et al. 1997), and in crayfish both substances increased the excitability of lateral giant interneurons to sensory stimulation (Araki et al. 2005). Furthermore, there is some evidence that serotonin might affect octopaminergic receptors, as it had affinity to a cloned cockroach octopamine receptor (Bischof and Enan 2004). Thus it seems possible, that both substances can have a similar, that is, an enhancing effect on the tonic depolarization amplitude in stick insect MNs (shown for octopamine in 91 %, and for serotonin in 66 % of the experiments). The increase induced by both substances might be explained for example by an action of serotonin on octopaminergic receptors (see discussion above). But the opposing effect of serotonin in 33 % of the experiments should also be taken into account for interpretation. The opposing effect of serotonin might be induced by a state dependent action.

In summary, a modulatory influence of octopamine and/or serotonin on the tonic depolarization seems possible, because it only increased or decreased the amplitude and exerted no obvious effect on membrane potential. This is in contrast to the effect of pilocarpine, which elicited rhythmic membrane potential modulations on top of a tonic depolarization. Octopamine and/or serotonin may modulate ACh receptors, as a possible role for mAChRs was indicated by the action of pilocarpine and the decrease by the antagonist atropine.

Further experiments are needed to clarify and to confirm the results obtained in this thesis, especially the role of octopamine and serotonin in mediating the tonic depolarization

should be investigated in more detail, for example by simultaneous superfusion of both substances to see whether the effect is additive.

4.2.2 Second messenger pathways

In several sets of experiments, neuroactive substances that interact with different second messenger pathways were analyzed to investigate which second messengers might be involved in mediating the tonic depolarization (chapter 3.3). A participation of second messengers was indicated by quite long repolarization time constants, lasting up to several seconds (chapter 3.1.1, Fig. 3.6).

Calcium

Calcium plays an important role in several intracellular signaling cascades and is known to influence a range of cellular functions. It acts over a wide dynamic range, exerting effects in microseconds or even hours (Berridge et al. 2003). The influence of calcium on the tonic depolarization was tested by using BAPTA in the electrode electrolyte solution to chelate intracellular calcium. The amplitude of the tonic depolarization decreased in these experiments by 8 % after 5 minutes (Fig. 3.18 B). This appears to be a rather small effect, taking into account that BAPTA is a fast calcium chelator. The concentration of 200 mM should be high enough, as even 100 mM BAPTA were sufficient to decrease Ca^{2+}-induced responses for example in the horseshoe crab or the lamprey (Frank and Fein 1991; Di Prisco et al. 2000). It is not known, whether the recording site was near the location where synaptic inputs generate the tonic depolarization in flexor MNs, thus diffusion of BAPTA might have not reached these locations. At least the experiments indicate that part of the tonic depolarization amplitude might be due to calcium dependent pathways.

cAMP

Evidence that the tonic depolarization is mediated by a second messenger pathway that involves cAMP comes from the experiments shown in chapter 3.3.2.1, where the superfusion with the membrane permeable cAMP analog 8-Br-cAMP led to an increase of the tonic depolarization amplitude of about 56 % (chapter 3.3.2.1, Fig. 3.20 B). A role for cAMP

in mediating the tonic depolarization is supported by the results of preliminary experiments using H-89, an inhibitor of PKA (shown in Walther and Zittlau 1998 and Wenzel et al. 2002), that reduced the tonic depolarization amplitude (Figs. 3.24, 3.22). Thus, a pathway involving cAMP/PKA might mediate the tonic depolarization. As discussed in chapter 4.2.1, mAChRs are possible candidates for mediating the tonic depolarization in flexor MNs (indicating that ACh might act as transmitter), and furthermore an effect of octopamine and serotonin was shown. All these transmitters can exert their effects by activating a second messenger pathway that involves cAMP, for example octopamine receptors were shown to activate AC and increase cAMP concentration, leading to the activation of PKA in the wandering spider *Cupiennius salei* (Widmer et al. 2005). Elevation of cAMP was also shown to be induced by serotonergic receptors (Parker 1995), and finally, Wenzel et al. (2002) could show a positive coupling of mAChRs to AC, leading to an increased cAMP level in the grasshopper brain.

However, the preliminary results using the AC inhibitor SQ22,536 (see e.g., Heinrich et al. 2001, Araki et al. 2005) are controversial, as they show an increase in the tonic depolarization amplitude (Fig. 3.24, chapter 3.3.2.2). Assuming that SQ22,536 inhibits AC, and that the tonic depolarization amplitude is mediated via an AC/cAMP pathway, this should have led to a decrease in amplitude (Fig. 3.21). The opposing effect of SQ22,536 on the tonic depolarization is hard to explain. In the literature, SQ22,536 is solely referred to as an inhibitor of AC, thus an activation by this neuroactive substance in flexor MNs seems unlikely. A possible explanation for the observed effect is that SQ22,536 inhibits AC in a presynaptic neuron that usually has an inhibitory effect on the tonic depolarization which involves AC (Fig. 4.2). An explanation, why SQ22,536 exerts an effect on AC in some other neuron but is ineffective with AC in flexor MNs might be, that a cAMP pathway is not involved in mediating an effect in these neurons. Further experiments are needed to see whether these preliminary effects of SQ22,536 will be confirmed.

IP_3/DAG

A possible involvement of an IP_3-pathway was investigated by inhibiting phospholipase C (PLC) with either neomycin or U-73122 (Fig. 3.26). The effectiveness of these substances on neurons was shown for example in the grasshopper brain and for a central snail neu-

ron (Wenzel et al. 2002; Lin et al. 2005). With the assumption that the tonic depolarization might be mediated by a second messenger pathway that involves IP_3, the expected effect on the tonic depolarization in flexor MNs was a decrease in amplitude (chapter 3.3.3, Fig. 3.26). But both substances induced an opposing effect. Neomycin increased the tonic depolarization amplitude by up to 84 % (Fig. 3.28 B), whereas U-73122 induced an increase by up to 58 % (Fig. 3.30). The enhancement of the tonic depolarization seems to be explainable, if one assumes that in some other neuron an IP_3-pathway is inhibited by neomycin and U-73122 acting on PLC, which usually exerts an inhibitory effect on flexor MNs. This inhibitory input might be reduced to some extent and could have led to the observed increase (Fig. 4.2, see also discussion in chapter 4.2.3).

Interaction of second messenger pathways

The results showed, that participation of more than one second messenger pathway is possible. This is not remarkable, as it is known for example from STG neurons, that one neuroactive substance can induce activation of different second messengers and ion channels (Selverston et al. 1998). Furthermore, several neuroactive substances, as well as second messenger pathways can interact. Thus, a role for more than one second messenger in mediating the tonic depolarization seems possible, this might be an interaction of second messenger pathways in one cell or by separate action in different cells.

4.2.3 Direct or indirect influences?

For none of the investigated neuroactive substances (transmitters or second messengers) it is known, whether they act directly on the flexor MNs (Fig. 4.2). Some of the obtained effects rather point to an indirect influence, but which cells might be possible candidates for mediating such an indirect effect? In the locust, some intersegmental interneurons may play a role in transmitting **coordinating** information, or at least information from sense organs such as the fCO, from one segment to a neighbouring one. These neurons make direct synaptic connections with MNs and interneurons in the adjacent segment (Laurent and Burrows 1989a;b). Büschges (1989) showed that spiking interneurons respond to stimulation of the fCO in the stick insect and Brunn and Dean (1994) recorded from metathoracic interneurons that code for position and movement of joints of the

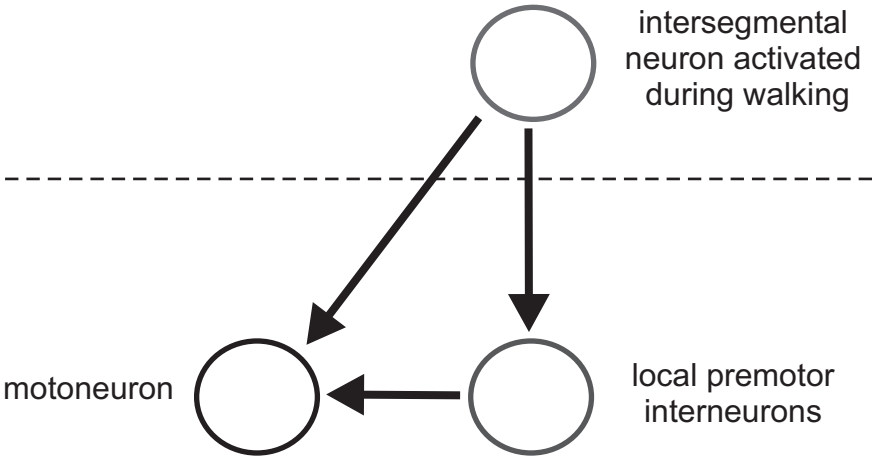


Figure 4.2: *Schematic diagram of possible pathways influencing the tonic depolarization. Motoneurons might be affected directly by the drugs or via pre-motor interneurons. Correspondingly, an intersegmental signal could affect MNs directly and/or via pre-motor interneurons. Dashed line indicates segmental border.*

mesothoracic segment. A transmission of the tonic depolarization by graded transmitter release seems possible, as spiking neurons may not be able to achieve a high enough spike frequency to sustain such a tonic signal. Graded synaptic transmission allows the required, increased transmission rate, as it was shown for photoreceptors and interneurons of the fly compound eye (Laughlin et al. 1998).

At the **segmental** level, nonspiking interneurons are known to process sensory signals from the middle leg in local reflex pathways (Büschges 1995; Stein et al. 2006). Interestingly, spike frequency alteration of front leg extensor MNs were reflected in membrane potential oscillation in an identified nonspiking interneuron (E4) in the mesothorax (Ludwar et al. 2005b). This nonspiking interneuron showed also a tonic depolarization in membrane potential throughout the stepping sequence.

The connectivity of nonspiking interneurons indicates that they may serve the integration of local and **intersegmental** signals and play a role in transmitting informations to MNs, as it was shown for the locust (Laurent and Burrows 1989a;b). One problem might be, that Wildman et al. (2002) describe that the main transmitter in locust nonspiking interneurons seems to be GABA. But the authors discuss that some neurons may use an excitatory transmitter, as both excitatory and inhibitory effects of these neurons are known. An interesting effect of GABA was shown in some STG neurons of the crab, *Can-*

cer borealis. In this system GABA can have both, an excitatory and inhibitory effect on these neurons by activating permeabilities for different ions (Swensen et al. 2000). Furthermore, Nagayama (2002) showed that nonspiking local interneurons in the crayfish are either depolarized or hyperpolarized by serotonin, with amplitudes of 10 to 30 mV. These interneurons might contribute to the level of tonic excitation of the described uropod MNs. Thus, at least from arthropod studies several alternatives can be derived on if and how nonspiking neurons might contribute to the tonic depolarization.
Tonic signals may also originate from descending brain neurons, which were shown to be tonically active during initiation and generation of locomotor activity in locusts (Kien 1990).

4.3 Rhythmic modulation

In addition to the tonic depolarization, the membrane potential of mesothoracic flexor MNs was phasically modulated, which was usually coupled to front leg steps.

Contralateral flexor MNs

Phasic membrane potential changes in contralateral flexor MNs showed three distinct types of coupling to front leg stepping (N=16/24, Fig. 3.7). In most experiments (50 %), flexor MNs exhibited a phasic modulation pattern that showed maximum activity with the onset of the front leg stance phase. In 17 % of the recordings, contralateral flexor MNs reached the maximum depolarization after the end of front leg stance phase, whereas in 33 % the observed phasic modulation was not coupled to front leg stepping at all.

Ipsilateral flexor MNs

Although the phasic membrane potential modulation in ipsilateral flexor MNs during front leg stepping was already described by Ludwar et al. (2005b), the variability found for **contralateral** flexor MNs led to the assumption, that this might be similar in **ipsilateral** flexors. In the previous study, a rather small sample size was analyzed and only two different types of phasic modulation were found for ipsilateral flexor MNs. A population of five ipsilateral flexor MNs was investigated and revealed, that in four flexors

a maximum depolarization occurred after stance phase, and the fifth flexor showed no step coupled phasic modulation.

In this thesis, 27 ipsilateral flexor MNs were recorded and revealed three distinct types of phasic modulation in membrane potential (Fig. 3.8), that were already described for contralateral flexor MNs. In 52 % of the recordings, ipsilateral flexor MNs showed a maximum depolarization after the end of front leg stance phase, whereas in 15 % the maximum activity was reached during stance. As in contralateral flexor MNs, no obvious coupling of the phasic modulation to front leg stepping was observed in 33 % of the recordings. A difference between the types of phasic modulation in ipsi- and contralateral flexor MNs was found only in the reversed frequency of percental occurrence of the other two types of modulation. The finding of three instead of two different types is not inconsistent with the data from Ludwar et al. (2005b), rather the higher sample size analyzed in my thesis brought up coupling patterns that do not often occur.

Amplitude of the phasic modulation

In general, the observed phasic modulation amplitude was rather small (around 3 mV) compared to amplitude estimated from single-leg preparations with intact middle legs (Schmidt et al. 2001; Gabriel 2005), where usually amplitudes of up to 17 mV were observed. This might be also due to missing sensory input (see above), which sculpted the activity pattern in the above mentioned preparations.

Coordinated walking pattern

In my thesis, the most prominent type (50 %) of phasic modulation in **contralateral** flexor MNs showed maximum activity during front leg stance phase, this pattern of activity was also described for ipsilateral **extensor** MNs (see Ludwar et al. 2005b). In 52 % of the recordings, **ipsilateral** flexor MNs did not show a maximum depolarization until the end of front leg stance phase. This indicates, that in most cases (~50 %) an activity pattern of ipsilateral and contralateral flexor MNs occurred that excluded in-phase coupled activity, which resembles alternating activity. Furthermore, 17 % of contralateral flexor MNs did not show a maximum depolarization until the end of front leg stance phase, while 15 % of ipsilateral flexor MNs showed maximum activity during front leg stance. Thus,

although this pattern implies antagonistic movements of both middle legs in presumably 67 % of the experiments, a co-activation of flexor MNs that innervate the left and right leg appears possible. Nevertheless, simultaneous intracellular recordings from ipsi- and contralateral flexor MNs would be necessary to confirm these interpretations. Ludwar et al. (2005a;b) already described that the patterning of extra- and intracellular recorded activity in mesothoracic MNs is inconsistent with a functional inter-leg coordination during walking. Up to now it is not known how MNs are coordinated between the ipsi- and contralateral side. Possible mechanisms include an influence by sensory organs, as for example in active stick insects, fCO (femoral chordotonal organ) stimulation in one middle leg induces transitions from flexor to extensor activity and vice versa in the contralateral leg (Stein et al. 2006). Local sensory information was excluded in my preparation, as it was completely deafferented. Thus, any sensory information that might serve as coordinating information between both hemi-ganglia was absent and might explain the variability in the observed phasic modulation patterns (see also below).

In general, sensory signals are important for intra-leg coordination, which was shown in behavioral and amputation studies on stick insects (see e.g., Bässler and Büschges 1998, Büschges 2005). Campaniform sensilla (CS) are known to influence the local control of leg movements in stick insects and cockroaches (Ridgel et al. 2000; Akay et al. 2004; 2007).

Intersegmental influences of CS were recently described by Borgmann (2006). She showed in her thesis, that stimulation of middle leg CS, which signal cuticular strain as induced by load on the leg, induced an increase in front leg depressor MNs, and a decrease in front leg levator MN activity during front leg stepping. Similar results were obtained for protractor (decreased) and retractor (increased) MN activity.

In addition, the fCO is known to influence inter-leg coordination. Stimulation of the fCO mimics flexion (e.g., of the front leg), which occurs at the start of the front leg stance phase during walking. Signals from the fCO did not affect motoneuronal activity in neighboring legs in **resting** animals. In contrast, fCO stimulation in **active** animals induced increased activity in mesothoracic flexor MNs, as well as in retractor MNs, but decreased the activity in protractor MNs (Ludwar et al. 2005a). During front leg **stepping**, mesothoracic retractor and protractor MNs showed a comparable activity pattern

as during stimulation of the fCO of the front leg. The observed increase in flexor MN activity during fCO stimulation was opposing. Thus, at least part of the sensory information needed for a coordinated walking pattern is due to the activity of the fCO, but additional information from other sensory sources or the integration of intersegmental and local information might be necessary to ensure a functional coordination. The observed influence of the fCO seemed to be more specific for information transfer from the front to the middle leg, as no comparable effect was found in hind leg MNs by stimulation of the middle leg fCO (Ludwar 2003).

A difference in gating of intra- or intersegmental information might be another explanation for the observed variations in the phasic modulation in flexor MNs, which implicates an inconsistent coordination pattern. It might depend on the activity state of the animal if local or intersegmental sensory information is sufficient to influence MNs. A recent study by Stein et al. (2006) revealed that picrotoxin, a blocker of GABA-ergic inhibition, led to responses of tibial MNs of all legs after stimulation of the middle leg fCO. Furthermore, in the presence of picrotoxin, stimulation of the fCO elicited tibial forces that would lead to an alternating activity of ipsi- and contralateral legs (Stein et al. 2006). In the same study, nonspiking interneurons that are part of the pre-motor network, were shown to integrate sensory signals from local proprioceptors (fCO) and those of other legs.

Evidence that phasic modulation of mesothoracic MNs is not due to activity of prothoracic CPGs comes from experiments in which the latter were activated by superfusion with pilocarpine. Activity in mesothoracic MNs was not coupled to rhythmic activity in prothoracic pro- and retractor MNs (Ludwar et al. 2005a). Further evidence that intersegmental coordination does not depend on central coupling between legs comes from experiments, in which five legs of a stick insect walked on one treadmill, while one leg walked on another. The treadmills differed in velocity, but all six legs were still coordinated and adapted to the respective treadmill velocity (Foth and Bässler 1985a;b).

One could argue, that the different types of phasic modulation relate to the different MNs within the flexor MN pool (slow, fast, semi-fast), but this is not very likely for the following reasons: The mean resting membrane potential of the three flexor MNs types differs (fast MNs: -67 mV, semi-fast: -51 mV, slow MNs: -62 mV; mean values in the

single middle-leg preparation analyzed by Schmidt et al. 2001; Gabriel 2005). But flexor MNs receive **common synaptic drive** during walking and are recruited differently due to the differences in resting membrane potential (Gabriel 2005; Gabriel and Büschges 2007). Furthermore, my results showed no clear correlation of the three types of phasic modulation to the different MN types. In general, the identification of a flexor MN occurred by a one-to-one correlation of spikes in the extracellular ncr recording, which were induced by intracellular current injection. A clear discrimination of the three flexor MN types is more difficult in the intersegmental preparation, because no reference to movements of the leg due to current injection as in the middle leg preparation was possible. But at least the resting membrane potential and the action potential size in the nerve recording allowed the distinction of fast and slow MNs. An additional argument comes from Ludwar (2003), who already described two types of modulation patterns for ipsilateral mesothoracic **depressor** MNs, that were not specific for fast or slow MNs from the same MN pool.

4.4 Neuronal activity in lesioned animals

In several sets of experiments the influence of lesions on the neuronal activity in stick insects was investigated. These experiments could contribute to clarify, e.g., the influence of the brain on neuronal activity in neck connectives and especially on the tonic depolarization.

4.4.1 Influence of lesions on the tonic depolarization

The experiments performed in this thesis showed, that removal of the brain had a pronounced effect on the tonic depolarization amplitude in flexor MNs. The tonic depolarization amplitude decreased by approximately 60 % immediately after brain removal (chapter 3.4.1, Figs. 3.34, 3.35). This suggests, that the mechanisms leading to the tonic depolarization in mesothoracic flexor MNs during front leg stepping include descending signals from the brain. The tonic depolarization was not completely absent after brain removal, so maybe the SEG also contributes to the tonic depolarization amplitude (see

below). The projections from descending brain neurons run through or terminate in the SEG (Heinrich 2002; Gal and Libersat 2006). Thus, it is possible that the tonic depolarization is influenced indirectly by descending brain neurons that shape activity of neurons in the SEG, which in turn descend to thoracic ganglia.

The reduced amplitude does not imply that the tonic depolarization is generated by descending information from the brain, a feedback mechanism might be disturbed that is processed in the brain that in turn influences the tonic depolarization. My suggestion for such a feedback mechanism is, that descending neurons from the SEG are involved in the generation of the tonic depolarization, thus information from the SEG descends to the thoracic MNs. Then ascending neurons in the thoracic ganglion send information about the initiated tonic depolarization to the brain, which in turn is able and possibly even necessary to modulate (for example by increasing the amplitude) the tonic depolarization.

Removal of the brain clearly reduces signals from head sense organs, for example from the antennae or eyes. Can this reduction of sensory information be related to the decrease in tonic depolarization amplitude? The answer might be no, because the tonic depolarization seems not to rely mainly on sensory information, but is generated by central mechanisms. For example in this thesis, local sensory information was excluded by deafferentation. Nevertheless, if one argues that information of sense organs from the front leg might contribute to the tonic depolarization, then there is the argument that the stepping front leg usually did not exert less force, as shown by the EMG activity and treadmill velocity in figure 3.32. In five of seven analyzed experiments, the maximum treadmill velocity was unchanged in 'intact' and brainless animals. Thus, the transmitted information from sense organs should be in the same range. Furthermore, Kien (1983) described that brainless locusts show changes in walking speed, which could also not be related to loss in sensory input.

Despite the decrease in tonic depolarization amplitude, brain removal led to an increased front leg stepping activity (Figs. 3.32, 3.33). This is in agreement with previous studies. Roeder (1937) described that mantids were rather 'restless' after brain removal, and locomotion was long-lasting after stimulation. Such an enhanced ability to produce motor

activity was also reported for decerebrated locusts, cockroaches and stick insects (Kien 1983; Ridgel and Ritzmann 2005; Graham 1979a;b). Furthermore, decapitated insects (brain and SEG removed) did not walk well and initiated no spontaneous locomotor activity. It was suggested, that the brain has a rather inhibitory influence on locomotor activity, whereas the SEG expresses excitatory influences (Roeder 1937; Kien 1983; Ridgel and Ritzmann 2005; Graham 1979a;b).

It might appear contradictory, that removal of the brain leads to a decrease in tonic depolarization amplitude, that is, less responsive MNs, and to an increase in locomotor activity at the same time. It was suggested, that the tonic depolarization in stick insect MNs reflects a state of arousal of the animal (this thesis and Ludwar et al. 2005b; Gabriel 2005). The initial hypothesis was, that during an increased stepping activity after brain removal the tonic depolarization might be increased, or at least no change in amplitude should occur in comparison to 'intact' animals. In my experiments, brainless animals seemed to be in a state of increased responsiveness concerning their willingness to perform stepping sequences. I hypothesize, that the decrease in the tonic depolarization amplitude and the increase in stepping activity after brain removal are based on two independent influences. One involves the removal of an inhibitory influence on the initiation of locomotor activity from the brain. Behavioral studies on stick insects indicated a role for the SEG in initiation and termination of a stepping sequence, as well as on the walking direction (Bässler et al. 1985). Thus, the SEG was able to display its excitatory influence after brain removal. The other influence involves the removal of a suggested excitatory, descending influence on the tonic depolarization. It is possible, that neurons in the brain generate the tonic depolarization or contribute to it in the 'intact' animal (see also discussion 4.2.3), these neurons would be absent due to brain removal. Thus, quite different sets of descending neurons could be responsible for the observed effects on the tonic depolarization and the willingness to perform stepping sequences.

These different sets of descending neurons would therefore serve for an explanation of the apparent contradiction. Gronenberg and Strausfeld (1990) described three major functional classes of descending neurons: 'trigger', 'driver' and 'modulator' neurons. 'Trigger' neurons initiate or release motor activity, and respond to distinctive stimulus

combinations that dictate their tuning. Such trigger neurons are for example found in the locust or crickets: the descending contralateral movement detector (DCMD) in locusts triggers the extension of hind legs resulting in a jump (e.g., in O'shea and Rowell 1976; Heitler and Burrows 1977), and in crickets, a descending interneuron called CNW seems to be involved in the initiation of walking (Zorovic and Hedwig 2007). The 'driver' class of descending neurons contributes to a distributed network, which generates and maintains motor activity (Gronenberg and Strausfeld 1990). The activity of these descending neurons is similar to motor activity and accompanies it. Pheromone-sensitive multimodal descending neurons start and maintain wingbeat oscillations in moths, which play an important role in courtship (Kanzaki and Shibuya 1986). Both, wing flapping frequency and descending neurons show the same dose-response relationship and interestingly, both outlast the pheromone stimulus by several seconds. 'Modulator' descending neurons can modulate ongoing motor activity, they change for example the strength, directional or temporal properties of the motor output (Gronenberg and Strausfeld 1990). The tritocerebral commisure giant (TCG) in the locust is an example for a 'modulator' descending neuron. TCG feeds back integrated information about air currents perceived by sensory organs (antennae and head hairs) and contributes to flight stabilization (Bacon and Möhl 1983). For example wing depressor MN activity can be advanced or reset by TCG activity, the role of TCG might be that of a "course-deviation-detector" (Möhl and Bacon 1983). In summary, the hypothesized descending interneuron(s) that might be responsible for or generate(s) the tonic depolarization in leg MNs, could act as a 'driver' or 'modulator' neuron.

Another possibility to decrease the tonic depolarization amplitude is that some gating dependent mechanism is hindered which in the 'intact' animal leads to the generation of the tonic depolarization. Similar suggestions derive from experiments with locusts and crickets, where the influence of descending neurons might be limited by a gating mechanism that is differently expressed during separate behavioral contexts (Steeves and Pearson 1982; Staudacher and Schildberger 1998). State dependent changes in response behavior are also known from the resistance reflex in stick insects (Bässler 1993; Driesang and Büschges 1996; Stein et al. 2006).

Preliminary results support the idea of a descending influence on the tonic depolarization. Transection of an ipsilateral connective between the pro- and mesothoracic ganglion or one neck connective during front leg stepping decreased the tonic depolarization amplitude by up to 68 % (Figs. 3.36, 3.37). Furthermore, signals that contribute to the tonic depolarization seem to be at least partially redundant in both connectives, because the tonic depolarization was not completely abolished. Similar results were obtained from locust flight, where transection of one connective between the meso- and metathoracic ganglion was not sufficient to abolish flight capability (Ronacher et al. 1988).

4.4.2 Correlation of neuronal and stepping activity

Borgmann (2006) showed in her thesis, that information about activity of front leg stepping is transferred in thoracic connectives. She used the rectified and smoothed connective recordings to analyze the information transfer during front leg stepping, because single action potentials were not of interest but the course of the mean neuronal activity. In these rectified and smoothed recordings she found that the neuronal activity increased tonically and was phasically modulated. In general, the activity increase was stronger and was correlated to front leg stepping velocity in the connective between the pro- and mesothoracic ganglion (Borgmann 2006). Activity in the connectives is thus representative of walking activity of the front leg. Furthermore, Borgmann (2006) showed that the tonic increase in neuronal activity could partially be related to the activity of pattern generating networks, whereas the phasic modulation seemed to be shaped by sensory signals of the front leg. In my thesis, I analyzed the neuronal activity in **neck** connectives during front leg stepping to see whether activity of the front leg is represented in a similar way in these connectives.

Smoothed recordings surely could mask distinct signals, depending on the chosen time constant (see chapter 2.6). Thus it is possible, that an apparently tonic signal occurs, but the underlying activity was phasic activity with a rather high cycle period. Furthermore, action potentials that occur at the same time sum up in the extracellular recording and mask relevant signals, but nevertheless, the results in my thesis as well as in experiments from Borgmann (2006) were very consistent and showed a similar recurring behavior to

comparable stimuli.

Activity in neck connectives in 'intact' animals

Extracellular recordings from neck connectives revealed an increased neuronal activity during front leg stepping (chapter 3.4.2, Fig. 3.38). The activity increase showed two components, a tonic and a phasic component, which was also described for connectives between thoracic ganglia by Borgmann (2006, see above).

There might be no causal relationship between the tonic activity increase in connectives and the intracellular recorded tonic depolarization, but it is another example for the occurrence of a tonic increase associated with stepping activity. That supports the idea, that the tonic increase might be a general phenomenon (this thesis and Büschges et al. 2004; Ludwar et al. 2005b; Gabriel 2005; Borgmann 2006).

I could show in my experiments, that the tonic activity increase (measured in the rectified and smoothed recording) was similar in ipsi- and contralateral neck connectives and reached values up to 69 %. The simultaneous increase in neuronal activity might suggest that ascending and descending signals are transferred in parallel in both connectives. A correlation of front leg velocity and the activity increase was observed in both connectives, that means a higher mean velocity of the front leg was associated with a stronger neuronal activity (integral under the rectified and smoothed extracellular recording for each step cycle) in the connectives. It was analyzed, if this correlation is more obvious for ipsilateral neck connectives by comparison of the regression coefficients. The assumption was, that this correlation might be stronger in the connective ipsilateral to the stepping leg. This was only partially supported by a comparison of the regression coefficients, which revealed a significant higher correlation in the ipsilateral neck connective (in two of four experiments). Thus, the correlation between stepping velocity and neuronal activity seems to be equally represented in both connectives. Due to the equal representation, information about the actual speed might be at least also provided to the contralateral leg, which in turn might serve the adaptation of legs to an equal stepping velocity.

Activity in neck connectives in brainless animals

In brainless animals, the activity in neck connectives increased during front leg stepping, but analysis of the tonic activity increase revealed that it was less pronounced compared to 'intact' animals. The tonic activity increase ranged between 18.0 and 47.1 %. This suggests, that part of the increase observed during stepping in the 'intact' animals is due to information descending from both head ganglia. The increase observed in brainless animals might be therefore attributed to information descending from the SEG and/or information ascending from the thoracic ganglia. Although a correlation of stepping velocity of the front leg and neuronal activity was also observed in brainless animals, this correlation was stronger in 'intact' animals (the regression coefficients had significant higher values). Thus, during comparable velocities less activity was observed in brainless animals. This supports the idea, that the general activity increase in neuronal activity was less pronounced in brainless animals (see above).

The results further indicate, that the influence of the brain might not be necessary for the animal to induce different stepping velocities, because a correlation between neuronal activity and stepping velocity was still present in brainless animals (see also discussion below). A less prominent role for the brain in influencing velocity is supported by suggestions derived from studies on lesioned cockroaches (Ridgel and Ritzmann 2005). The authors suggest that the **SEG** in cockroaches could provide tonic signals that control stepping speed. These tonic signals might be generated by tonically firing neurons in the SEG, which could not be detected in the rectified and smoothed connective recording analyzed in my experiments, as it is the sum of a presumably rather high amount of neurons (around 2000 axons in each connective (Leslie 1973)). Ridgel and Ritzmann (2005) also stated that the SEG may have an important role in maintaining intersegmental coordination and that the latter could be a secondary effect of speed control. That only the SEG plays a role in the control of velocity and not the brain is somewhat challenged by findings in the cricket, where descending brain interneurons might control direction and velocity and many descending neurons act in concert to enable a variable motor output (Böhm and Schildberger 1992; Staudacher and Schildberger 1998; Heinrich 2002; Zorovic and Hedwig 2007).

Concerning the single leg, influences on stepping velocity can be achieved by changing the cycle period or the step length; a combination of both seems also possible. In stick insects, it seems to be primarily the decrease in cycle period that increases stepping velocity (Wendler 1964; Graham 1972; Gabriel and Büschges 2007). In general, the descending signals rather determine the strength of muscle contractions (Shik et al. 1966) and do only indirectly control the cycle period, which suggests that an **afferent** feedback from the limb might be responsible for changes in cycle period (Orlovsky et al. 1999). Descending tonic excitation might be responsible for generating changes in stepping velocity in stick insects, and recent studies showed with intracellular recordings from MNs, that extensors and flexors are not affected in a similar way during changes in stepping velocity (Gabriel 2005; Gabriel and Büschges 2007).

4.5 Conclusions

My results show that not only ipsilateral mesothoracic MNs but also mesothoracic flexor MNs **contralateral** to the stepping front leg generate a tonic depolarization of the membrane. The results are in line with previous conclusions, which suggested that the tonic depolarization might be a general phenomenon in stick insect neurons involved in locomotion (Ludwar 2003; Ludwar et al. 2005b; Gabriel 2005). The shown properties of the tonic depolarization suggest that it is based on a nonselective cation conductance or a mixed inward and outward current through different channels, and that the tonic depolarization might represent a state of arousal that is generated with activation of the animal. The tonic depolarization is sculpted by phasic membrane potential changes, which are variably coupled to front leg stepping.

Pharmacological experiments indicate a role for acetylcholine in mediating the tonic depolarization via metabotropic receptors. Further experiments show an additional influence of octopamine and serotonin, which suggests a role for co-transmission or neuromodulation. My results indicate an influence of several second messenger pathways, including an involvement of calcium, cAMP and phospholipase C.

In my experiments, removal of the brain (supraesophageal ganglion) decreased the tonic depolarization, indicating a role of the brain in either the generation or maintenance of

the tonic depolarization.

Although the question "Which are the involved transmitters or receptors?" could be partially answered, my results raise new questions, for example if the analyzed substances act directly on flexor MNs or not? Furthermore, the finding that second messenger **are** involved in mediating the tonic depolarization has to be analyzed in more detail, as some of the observed effects are contradicting and point to an indirect influence. Experiments need furthermore to clarify the origin of the tonic depolarization, because a role of other sources than the brain cannot be excluded.

Bibliography

Akay, T., Bässler, U., Gerharz, P., and Büschges, A. (2001). The role of sensory signals from the insect coxa-trochanteral joint in controlling motor activity of the femur-tibia joint. *J Neurophysiol*, 85(2):594–604. [22]

Akay, T. and Büschges, A. (2006). Load signals assist the generation of movement-dependent reflex reversal in the femur-tibia joint of stick insects. *J Neurophysiol*, 96(6):3532–3537. [22]

Akay, T., Haehn, S., Schmitz, J., and Büschges, A. (2004). Signals from load sensors underlie interjoint coordination during stepping movements of the stick insect leg. *J Neurophysiol*, 92(1):42–51. [22, 140]

Akay, T., Ludwar, B. C., Göritz, M. L., Schmitz, J., and Büschges, A. (2007). Segment specificity of load signal processing depends on walking direction in the stick insect leg muscle control system. *J Neurosci*, 27(12):3285–3294. [140]

Alaburda, A. and Hounsgaard, J. (2003). Metabotropic modulation of motoneurons by scratch-like spinal network activity. *J Neurosci*, 23(25):8625–8629. [123]

Alaburda, A., Perrier, J.-F., and Hounsgaard, J. (2002). Mechanisms causing plateau potentials in spinal motoneurones. *Adv Exp Med Biol*, 508:219–226. [16]

Ali, D. (1997). The aminergic and peptidergic innervation of insect salivary glands. *J Exp Biol*, 200(14):1941–1949. [65]

Altman, J. S. and Kien, J. (1979). Subesophageal neurons involved in head movements and feeding in locusts. *Proc R Soc Lond B Biol Sci*, 205(1159):209–227. [15]

Angstadt, J. D. and Friesen, W. O. (1993a). Modulation of swimming behavior in the medicinal leech. I. Effects of serotonin on the electrical properties of swim-gating cell 204. *J Comp Physiol [A]*, 172(2):223–234. [16]

Angstadt, J. D. and Friesen, W. O. (1993b). Modulation of swimming behavior in the medicinal leech. II. Ionic conductances underlying serotonergic modulation of swim-gating cell 204. *J Comp Physiol [A]*, 172(2):235–248. [16]

Angstadt, J. D., Grassmann, J. L., Theriault, K. M., and Levasseur, S. M. (2005). Mechanisms of postinhibitory rebound and its modulation by serotonin in excitatory swim motor neurons of the medicinal leech. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*, 191(8):715–732. [16, 17]

Araki, M., Nagayama, T., and Sprayberry, J. (2005). Cyclic AMP mediates serotonin-induced synaptic enhancement of lateral giant interneuron of the crayfish. *J Neurophysiol*, 94(4):2644–2652. [84, 133, 135]

Arshavsky, Y., Deliagina, T., Orlovsky, G., Panchin, Y., Popov, A., and Sadreev, R. (1998). Analysis of the central pattern generator for swimming in the mollusk *Clione*. In Kiehn, O., Harris-Warrick, R., Jordan, L., Hultborn, H., and Kudo, N., editors, *Annals of the New York Academy of Sciences*, pages 51–69. The New York Academy of Sciences, New York. [16]

Bacon, J. and Möhl, B. (1983). The tritocerebral comissure giant (TCG) wind-sensitive interneurone in the locust. I. Its activity in straight flight. *J Comp Physiol [A]*, 150:439–542. [145]

Bai, D. and Sattelle, D. B. (1994). Muscarinic acetylcholine receptors on an identified motor neurone in the cockroach, *Periplaneta americana*. *Neurosci Lett*, 175(1-2):161–165. [54, 56]

Baines, R. and Bacon, J. (1994). Pharmacological analysis of the cholinergic input to the locust VPLI neuron from an extraocular photoreceptor system. *J Neurophysiol*, 72:2864–2874. [18]

Baines, R. A. and Downer, R. G. (1991). Pharmacological characterization of a 5-hydroxytryptamine-sensitive receptor/adenylate cyclase complex in the mandibular closer muscles of the cricket, *Gryllus domestica*. *Arch Insect Biochem Physiol*, 16(3):153–163. [70]

Baines, R. A., Tyrer, N. M., and Downer, R. G. (1990). Serotonergic innervation of the locust mandibular closer muscle modulates contractions through the elevation of cyclic adenosine monophosphate. *J Comp Neurol*, 294(4):623–632. [79]

Banerjee, S. and Hasan, G. (2005). The InsP3 receptor: its role in neuronal physiology and neurodegeneration. *Bioessays*, 27(10):1035–1047. [21]

Barnes, N. M. and Sharp, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacol*, 38(8):1083–1152. [20]

Barrett, M. and Orchard, I. (1990). Serotonin-induced elevation of cAMP levels in the epidermis of the blood-sucking bug, *Rhodnius prolixus*. *J Insect Physiol*, 36:625–633. [70]

Bässler, U. (1983). *Neural Basis of Elementary Behavior in Stick Insects*, volume 10 of *Studies of Brain Function*. Springer-Verlag Berlin Heidelberg New York 1983. [15]

Bässler, U. (1986). Afferent control of walking movements in the stick insect *Cuniculina impigra*. I. Decerebrated animals on a treadband. *J Comp Physiol A*, 158:345–349. [98]

Bässler, U. (1993). The femur-tibia control system of stick insects - a model system for the study of the neural basis of joint control. *Brain Res Rev*, 18:207–226. [145]

Bässler, U. and Büschges, A. (1998). Pattern generation for stick insect walking movements -multisensory control of a locomotor program. *Brain Res*, 27:65–88. [140]

Bässler, U., Foth, E., and Breutel, G. (1985). The inherent walking direction differs for the prothoracic and metathoracic legs of stick insects. *J exp Biol*, 116:301–311. [144]

Bear, M. F. (1995). Mechanism for a sliding synaptic modification threshold. *Neuron*, 15(1):1–4. [130]

Benke, D. and Breer, H. (1989). Comparison of acetylcholine and alpha-bungarotoxin binding sites in insects and vertebrates. *Comp Biochem Physiol C*, 94(1):71–80. [18]

Benquet, P., Guen, J. L., Dayanithi, G., Pichon, Y., and Tiaho, F. (1999). Omega-AgaIVA-sensitive (P/Q-type) and -resistant (R-type) high-voltage-activated Ba^{2+} currents in embryonic cockroach brain neurons. *J Neurophysiol*, 82(5):2284–2293. [175]

Benson, J. (1992). Electrophysiological pharmacology of the nicotinic and muscarinic cholinergic responses of isolated neuronal somata from locust thoracic ganglia. *J exp Biol*, 170:203–233. [126]

Bermudez, I., Beadle, D. J., and Benson, J. (1992). Multiple serotonin-activated currents in isolated, neuronal somata from locust thoracic ganglia. *J exp Biol*, 165(1-2):43–60. [20]

Berridge, M. J. (1981). Electrophysiological evidence for the existence of separate receptor mechanisms mediating the action of 5-hydroxytryptamine. *Mol Cell Endocrinol*, 23(1):91–104. [75]

Berridge, M. J. (1984). Inositol trisphosphate and diacylglycerol as second messengers. *Biochem J*, 220(2):345–360. [85]

Berridge, M. J., Bootman, M. D., and Roderick, H. L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol*, 4(7):517–529. [134]

Berridge, M. J. and Heslop, J. P. (1981). Separate 5-hydroxytryptamine receptors on the salivary gland of the blowfly are linked to the generation of either cyclic adenosine 3',5'-monophosphate or calcium signals. *Br J Pharmacol*, 73(3):729–738. [75, 85]

Böhm, H. and Schildberger, K. (1992). Brain neurones involved in the control of walking in the cricket *Gryllus bimaculatus*. *J exp Biol*, 166:113–130. [15, 148]

Birdsall, N. J. and Hulme, E. C. (1976). Biochemical studies on muscarinic acetylcholine receptors. *J Neurochem*, 27(1):7–16. [54]

Bischof, L. J. and Enan, E. E. (2004). Cloning, expression and functional analysis of an octopamine receptor from *Periplaneta americana*. *Insect Biochem Mol Biol*, 34(6):511–521. [133]

Blagburn, J. M. and Sattelle, D. B. (1987). Nicotinic acetylcholine receptors on a cholinergic nerve terminal in the cockroach, *Periplaneta americana*. *J Comp Physiol [A]*, 161(2):215–225. [127]

Blitz, D. M. and Nusbaum, M. P. (1999). Distinct functions for cotransmitters mediating motor pattern selection. *J Neurosci*, 19(16):6774–6783. [59, 132]

Bocchiaro, C. M., Saywell, S. A., and Feldman, J. L. (2003). Dynamic modulation of inspiratory drive currents by protein kinase A and protein phosphatases in functionally active motoneurons. *J Neurosci*, 23(4):1099–1103. [21]

Borgmann, A. (2006). *Intersegmental influences contributing to coordination in a walking insect*. PhD thesis, University of Cologne. [23, 41, 107, 120, 140, 146, 147]

Bräunig, P. (1991). Suboesophageal DUM neurons innervate the principal neuropiles of the locust brain. *Phil Trans R Soc Lond [B]*, 332:221–240. [129]

Bräunig, P., Burrows, M., and Morris, O. T. (2004). Properties of descending dorsal unpaired median (DUM) neurons of the locust suboesophageal ganglion. *Acta Biol Hung*, 55(1-4):13–19. [129]

Breer, H. (1981). Properties of putative nicotinic and muscarinic cholinergic receptors in the central nervous system of *Locusta migratoria*. *Neurochemistry International*, 3:43–52. [18, 54]

Breer, H. and Sattelle, D. B. (1987). Molecular properties and functions of insect acetylcholine receptors. *J Insect Physiol*, 33:771–790. [18, 54, 124]

Britz, F. C., Hirth, I. C., and Deitmer, J. W. (2004). Second messenger cascade of glial responses evoked by interneuron activity and by a myomodulin peptide in the leech central nervous system. *Eur J Neurosci*, 19(4):983–992. [84]

Brodfuehrer, P., Debski, E., O'Gara, B., and Friesen, W. (1995). Neuronal control of leech swimming. *J Neurobiol*, 27:403–418. [14, 20, 98]

Brown, D. A. and Adams, P. R. (1980). Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neurone. *Nature*, 283(5748):673–676. [125]

Brunn, D. and Dean, J. (1994). Intersegmental and local interneurons in the metathorax of the stick insect *Carausius morosus* that monitor leg position. *J Neurophysiol*, 72:1208–1219. [136]

Buckingham, S., Lapied, B., le Corronc, H., and Sattelle, D. (1997). Imidacloprid actions on insect neuronal acetylcholine receptors. *J Exp Biol*, 200(21):2685–2692. [127]

Burnstock, G. (2004). Cotransmission. *Curr Opin Pharmacol*, 4(1):47–52. [132]

Burrows, M. and Pflüger, H. (1995). Action of locust neuromodulatory neurons is coupled to specific motor patterns. *J Neurophysiol*, 74:347–357. [128]

Büschges, A. (1989). Processing of sensory input from the femoral chordotonal by spiking interneurons of stick insects. *J exp Biol*, 144:81–111. [136]

Büschges, A. (1995). Role of local nonspiking interneurons in the generation of rhythmic motor activity in the stick insect. *J Neurobiol*, 27(4):488–512. [137]

Büschges, A. (1998). Inhibitory synaptic drive patterns motoneuronal activity in rhythmic preparations of isolated thoracic ganglia in the stick insect. *Brain Res*, 783(2):262–271. [23, 56, 123, 124]

Büschges, A. (2005). Sensory control and organization of neural networks mediating coordination of multisegmental organs for locomotion. *J Neurophysiol*, 93(3):1127–1135. [13, 22, 140]

Büschges, A., Kittmann, R., and Ramirez, J. M. (1993). Octopamine effects mimick state-dependent changes in a proprioceptive feedback system. *J Neurobiol*, 24(5):598–610. [19, 59, 128]

Büschges, A., Ludwar, B. C., Bucher, D., Schmidt, J., and DiCaprio, R. A. (2004). Synaptic drive contributing to rhythmic activation of motoneurons in the deafferented stick insect walking system. *Eur J Neurosci*, 19(7):1856–1862. [16, 22, 23, 37, 147]

Büschges, A., Schmitz, J., and Bässler, U. (1995). Rhythmic patterns in the thoracic nerve cord of the stick insect induced by pilocarpine. *J Exp Biol*, 198:435–456. [18, 22, 56, 124]

Butt, S. J. B. and Pitman, R. M. (2002). Modulation by 5-hydroxytryptamine of nicotinic acetylcholine responses recorded from an identified cockroach (*Periplaneta americana*) motoneuron. *Eur J Neurosci*, 15(3):429–438. [132]

Butt, S. J. B. and Pitman, R. M. (2005). Indirect phosphorylation-dependent modulation of postsynaptic nicotinic acetylcholine responses by 5-hydroxytryptamine. *Eur J Neurosci*, 21(5):1181–1188. [18]

Calabrese, R. L., Nadim, F., and Olsen, O. H. (1995). Heartbeat control in the medicinal leech: a model system for understanding the origin, coordination, and modulation of rhythmic motor patterns. *J Neurobiol*, 27(3):390–402. [13, 16]

Cayre, M., Buckingham, S. D., Yagodin, S., and Sattelle, D. B. (1999). Cultured insect mushroom body neurons express functional receptors for acetylcholine, GABA, glutamate, octopamine, and dopamine. *J Neurophysiol*, 81(1):1–14. [18]

Claassen, D. E. and Kammer, A. E. (1986). Effects of octopamine, dopamine, and serotonin on production of flight motor output by thoracic ganglia of *Manduca sexta*. *J Neurobiol*, 17(1):1–14. [19, 20]

Clemens, S. and Katz, P. S. (2001). Identified serotonergic neurons in the *Tritonia* swim CPG activate both ionotropic and metabotropic receptors. *J Neurophysiol*, 85(1):476–479. [20]

Cornford, A., Kristan, W. B., Malnove, S., Kristan, W. B., and French, K. A. (2006). Functions of the subesophageal ganglion in the medicinal leech revealed by ablation of neuromeres in embryos. *J Exp Biol*, 209:493–503. [98]

Crisp, K. M. and Mesce, K. A. (2003). To swim or not to swim: regional effects of serotonin, octopamine and amine mixtures in the medicinal leech. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*, 189(6):461–470. [20]

Cruse, H. (1976). The function of the legs in the free walking stick insect, *Carausius morosus*. *J Comp Physiol A*, 112:235–262. [36]

Davenport, A. P. and Evans, P. D. (1984). Stress-induced changes in the octopamine levels of insect haemolymph. *Insect Biochem*, 14(3):135–143. [19]

David, J. and Sattelle, D. (1984). Actions of cholinergic pharmacological agents on the cell body membrane of the fast coxal depressor motoneurone of the cockroach *Periplaneta americana*. *J Exp Biol*, 108:119–136. [18, 125]

David, J. A. and Pitman, R. M. (1996). Cyclic-AMP regulation of calcium-dependent K^+ channels in an insect central neurone. *Neurosci Lett*, 203(3):151–154. [18]

Deliagina, T. G., Zelenin, P. V., and Orlovsky, G. N. (2002). Encoding and decoding of reticulospinal commands. *Brain Res Rev*, 40(1-3):166–177. [14]

Di Prisco, G., Pearlstein, E., and Le Ray, D. (2000). A cellular mechanism for the transformation of a sensory input into a motor command. *J Neurosci*, 20(1):8169–8176. [134]

Dierkes, P. W., Wende, V., Hochstrate, P., and Schlue, W.-R. (2004). L-type Ca^{2+} channel antagonists block voltage-dependent Ca^{2+} channels in identified leech neurons. *Brain Res*, 1013(2):159–167. [175]

DiFrancesco, D. (1993). Pacemaker mechanisms in cardiac tissue. *Annu Rev Physiol*, 55:455–472. [122]

Dörr, H., Heß, D., and Gramoll, S. (1996). Interstitial voltage and potassium concentration in the mesothoracic ganglion of a stick insect at rest and during neuronal activation. *J Insect Physiol*, 42:967–974. [27]

Driesang, R. B. and Büschges, A. (1996). Physiological changes in central neuronal pathways contributing to the generation of a reflex reversal. *J comp Physiol [A]*, 179:45–57. [145]

Dringenberg, H. C. (2000). Serotonergic receptor antagonists alter responses to general anaesthetics in rats. *Br J Anaesth*, 85(6):904–906. [129]

Edwards, D. H., Yeh, S.-R., Musolf, B. E., Antonsen, B. L., and Krasne, F. B. (2002). Metamodulation of the crayfish escape circuit. *Brain Behav Evol*, 60(6):360–369. [131]

el Manira, A., Tegnér, J., and Grillner, S. (1994). Calcium-dependent potassium channels play a critical role for burst termination in the locomotor network in lamprey. *J Neurophysiol*, 72(4):1852–1861. [17, 175]

Eldefrawi, A. T. and O'Brien, R. D. (1970). Binding of muscarone by extracts of housefly brain: relationship to receptors for acetylcholine. *J Neurochem*, 17(8):1287–1293. [18]

Elson, R. C. and Selverston, A. I. (1997). Evidence for a persistent Na^+ conductance in neurons of the gastric mill rhythm generator of spiny lobsters. *J Exp Biol*, 200:1795–1807. [16]

Erspamer, V. and Boretti, G. (1951). Identification and characterization, by paper chromatography, of enteramine, octopamine, tyramine, histamine and allied substances in extracts of posterior salivary glands of octopoda and in other tissue extracts of vertebrates and invertebrates. *Arch Int Pharmacodyn Ther*, 88(3):296–332. [19]

Evans, P. D. (1981). Multiple receptor types for octopamine in the locust. *J Physiol*, 318:99–122. [70, 129]

Evans, P. D. and O'Shea, M. (1977). An octopaminergic neurone modulates neuromuscular transmission in the locust. *Nature*, 270(5634):257–259. [19, 127]

Felder, C. C. (1995). Muscarinic acetylcholine receptors: signal transduction through multiple effectors. *FASEB J*, 9(8):619–625. [17]

Fischer, H., Schmidt, J., Haas, R., and Büschges, A. (2001). Pattern generation for walking and searching movements of a stick insect leg. I. Coordination of motor activity. *J Neurophysiol*, 85(1):341–353. [22, 124]

Flamm, R. E., Fickbohm, D., and Harris-Warrick, R. M. (1987). cAMP elevation modulates physiological activity of pyloric neurons in the lobster stomatogastric ganglion. *J Neurophysiol*, 58(6):1370–1386. [80]

Fleidervish, I. A., Friedman, A., and Gutnick, M. J. (1996). Slow inactivation of Na^+ current and slow cumulative spike adaptation in mouse and guinea-pig neocortical neurones in slices. *J Physiol*, 493:83–97. [17]

Foth, E. and Bässler, U. (1985a). Leg movements of stick insects walking with five legs on a treadwheel and with one leg on a motor-driven belt. I. General results and 1:1-coordination. *Biol Cybern*, 51:313–318. [141]

Foth, E. and Bässler, U. (1985b). Leg movements of stick insects walking with five legs on a treadwheel and with one leg on a motor-driven belt. II. Leg coordination when step-frequencies differ from leg to leg. *Biol Cybern*, 51(5):319–324. [141]

Fouad, K., Fischer, H., and Büschges, A. (2003). *Comprehensive Handbook of Psychology, Biological Psychology, Volume 3*, chapter Comparative locomotor systems, pages 109–137. Wiley, New Jersey. [13]

Frank, T. M. and Fein, A. (1991). The role of the inositol phosphate cascade in visual excitation of invertebrate microvillar photoreceptors. *J Gen Physiol*, 97(4):697–723. [134]

Frost, W. and Katz, P. (1996). Single neuron control over a complex motor program. *Proc Natl Acad Sci USA*, 93:422–426. [14]

Gabriel, J. P. (2005). *Activity of leg motoneurons during single leg walking of the stick insect: From synaptic inputs to motor performance*. PhD thesis, University of Cologne. [22, 120, 124, 131, 139, 142, 144, 147, 149]

Gabriel, J. P. and Büschges, A. (2007). Control of stepping velocity in a single insect leg during walking. *Philos Transact A Math Phys Eng Sci*, 365(1850):251–271. [142, 149]

Gabriel, J. P., Scharstein, H., Schmidt, J., and Büschges, A. (2003). Control of flexor motoneuron activity during single leg walking of the stick insect on an electronically controlled treadwheel. *J Neurobiol*, 56(3):237–251. [30, 31, 121]

Gal, R. and Libersat, F. (2006). New vistas on the initiation and maintenance of insect motor behaviors revealed by specific lesions of the head ganglia. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*, 192(9):1003–1020. [15, 143]

Gatellier, L., Nagao, T., and Kanzaki, R. (2004). Serotonin modifies the sensitivity of the male silkmoth to pheromone. *J Exp Biol*, 207:2487–2496. [79, 130]

Goldberg, F., Grünewald, B., Rosenboom, H., and Menzel, R. (1999). Nicotinic acetylcholine currents of cultured Kenyon cells from the mushroom bodies of the honey bee *Apis mellifera*. *J Physiol*, 514:759–768. [18]

Graham, D. (1972). A behavioural analysis of the temporal organisation of walking movements in the 1st instar and adult stick insect (*Carausius morosus*). *J comp Physiol*, 81:23–52. [149]

Graham, D. (1979a). The effects of circum-oesophageal lesions on the behavior of the stick insect *Carausius morosus*. I. Cyclic behaviour patterns. *Biol Cybern*, 32:139–145. [15, 29, 98, 104, 144]

Graham, D. (1979b). The effects of circum-oesophageal lesions on the behavior of the stick insect *Carausius morosus*. II. Changes in walking coordination. *Biol Cybern*, 32:147–152. [15, 98, 144]

Graham, D. (1985). Pattern and control of walking in insects. *Adv Insect Physiol*, 18:31–40. [15, 22, 27]

Grillner, S. (2003). The motor infrastructure: from ion channels to neuronal networks. *Nat Rev Neurosci*, 4(7):573–586. [13]

Grillner, S. (2006). Biological pattern generation: the cellular and computational logic of networks in motion. *Neuron*, 52(5):751–766. [13]

Grillner, S., Ekeberg, Ö., El Manira, A., Lansner, A., Parker, D., Tegner, J., and Wallen, P. (1998). Intrinsic function of a neuronal network - a vertebrate central pattern generator. *Brain Res Rev*, 26:185–197. [14]

Grillner, S. and Wallen, P. (2002). Cellular basis of a vertebrate locomotor system - steering, intersegmental and segmental co-ordination and sensory control. *Brain Res Rev*, 40:92–106. [13, 14]

Grolleau, F., Lapied, B., Buckingham, S. D., Mason, W. T., and Sattelle, D. B. (1996). Nicotine increases $[Ca^{2+}]_i$ and regulates electrical activity in insect neurosecretory cells (DUM neurons) via an acetylcholine receptor with 'mixed' nicotinic-muscarinic pharmacology. *Neurosci Lett*, 220(2):142–146. [18, 127]

Gronenberg, W. and Strausfeld, N. J. (1990). Descending neurons supplying the neck and flight motor of diptera: physiological and anatomical characteristics. *J Comp Neurol*, 302(4):973–991. [144, 145]

Gundelfinger, E. and Schulz, R. (2000). *Insect nicotinic acetylcholine receptors: genes, structure, physiological and pharmacological properties. in: Handbook of Experimental Pharmacology.*, chapter Neuronal nicotinic Receptors, pages 497–521. Springer, Berlin. [17]

Han, K. A., Millar, N. S., and Davis, R. L. (1998). A novel octopamine receptor with preferential expression in *Drosophila* mushroom bodies. *J Neurosci*, 18(10):3650–3658. [79]

Hancox, J. and Pitman, R. (1991). Plateau potentials drive axonal impulse bursts in insect motoneurons. *Proc R Soc Lond B*, 244:33–38. [16]

Harris, J. W. and Woodring, J. (1992). Effects of stress, age, season, and source colony on levels of octopamine, dopamine and serotonin in the honey bee (*Apis mellifera L.*) brain. *J Insect Physiol*, 38(5):29–35. [19]

Harris-Warrick, R. (1985). Amine modulation of extension command element evoked motor activity in the lobster abdomen. *J Comp Physiol [A]*, 154:875–884. [133]

Harris-Warrick, R. M., Coniglio, L. M., Barazangi, N., Guckenheimer, J., and Gueron, S. (1995). Dopamine modulation of transient potassium current evokes phase shifts in a central pattern generator network. *J Neurosci*, 15:342–358. [17]

Harris-Warrick, R. M. and Kravitz, E. A. (1984). Cellular mechanisms for modulation of posture by octopamine and serotonin in the lobster. *J Neurosci*, 4(8):1976–1993. [133]

Heckmann, C. J., Gorassini, M. A., and Bennett, D. J. (2005). Persistent inward currents in motoneuron dendrites: implications for motor output. *Muscle Nerve*, 31(2):135–156. [16]

Hedwig, B. (2000). Control of cricket stridulation by a command neuron: Efficacy depends on the behavioral state. *J Neurophysiol*, 83:712–722. [15]

Heidel, E. and Pflüger, H.-J. (2006). Ion currents and spiking properties of identified subtypes of locust octopaminergic dorsal unpaired median neurons. *Eur J Neurosci*, 23(5):1189–1206. [17]

Heinrich, R. (2002). Impact of descending brain neurons on the control of stridulation, walking, and flight in orthoptera. *Microsc Res Tech*, 56(4):292–301. [143, 148]

Heinrich, R., Wenzel, B., and Elsner, N. (2001). A role for muscarinic excitation: control of specific singing behavior by activation of the adenylate cyclase pathway in the brain of grasshoppers. *Proc Natl Acad Sci U S A*, 98(17):9919–9923. [80, 84, 135]

Heitler, W. and Burrows, M. (1977). The locust jump II. Neural circuits of the motor programme. *J Exp Biol*, 66:221–241. [145]

Hirashimai, A. and Eto, M. (1993). Effect of stress on levels of octopamine, dopamine and serotonin in the american cockroach (*Periplaneta americana L.*). *Comp Biochem Physiol C*, 105(3):279–284. [19]

Homberg, U., Vitzthum, H., Müller, M., and Binkle, U. (1999). Immunocytochemistry of GABA in the central complex of the locust *Schistocerca gregaria*: identification of immunoreactive neurons and colocalization with neuropeptides. *J Comp Neurol*, 409(3):495–507. [132]

Hooper, S. L. and DiCaprio, R. A. (2004). Crustacean motor pattern generator networks. *Neurosignals*, 13(1-2):50–69. [16]

Hounsgaard, J., Hultborn, H., Jespersen, B., and Kiehn, O. (1984). Intrinsic membrane properties causing a bistable behaviour of alpha-motoneurones. *Exp Brain Res*, 55(2):391–394. [16]

Hounsgaard, J., Hultborn, H., Jespersen, B., and Kiehn, O. (1988). Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *J Physiol*, 405:345–367. [16]

Hounsgaard, J. and Kiehn, O. (1989). Serotonin-induced bistability of turtle motoneurones caused by a nifedipine-sensitive calcium plateau potential. *J Physiol*, 414:265–282. [16]

Howell, K. and Evans, P. (1998). The characterization of presynaptic octopamine receptors modulating octopamine release from an identified neurone in the locust. *J Exp Biol*, 201:2053–2060. [129]

Hoyer, D., Clarke, D. E., Fozard, J. R., Hartig, P. R., Martin, G. R., Mylecharane, E. J., Saxena, P. R., and Humphrey, P. P. (1994). International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev*, 46(2):157–203. [20]

Hoyle, G. and Barker, D. L. (1975). Synthesis of octopamine by insect dorsal median unpaired neurons. *J Exp Zool*, 193(3):433–439. [19]

Hughes, G. M. and Wiersma, C. A. (1960). The coordination of swimmeret movements in the crayfish, *Procambarus clarkii (Girard)*. *J Exp Biol*, 57:657–670. [14]

Hultborn, H., Brownstone, R. B., Toth, T. I., and Gossard, J.-P. (2004). Key mechanisms for setting the input-output gain across the motoneuron pool. *Prog Brain Res*, 143:77–95. [17]

Hyngstrom, A. S., Johnson, M. D., Miller, J. F., and Heckman, C. J. (2007). Intrinsic electrical properties of spinal motoneurons vary with joint angle. *Nat Neurosci*, 10(3):363–369. [16]

Jan, C.-R., Lu, Y.-C., Jiann, B.-P., Chang, H.-T., and Huang, J.-K. (2002). Effect of riluzole on cytosolic Ca^{2+} increase in human osteosarcoma cells. *Pharmacology*, 66(3):120–127. [171]

Johnston, R. and Levine, R. (1996). Locomotory behavior in the hawkmoth *Manduca sexta*: kinematic and electromyographic analyses of the thoracic legs in larvae and adults. *J exp Biol*, 199:759–774. [18]

Jordan, L. (1998). Initiation of locomotion in mammals. In Kiehn, O., Harris-Warrick, R., Jordan, L., Hultborn, H., and Kudo, N., editors, *Annals of the New York Academy of Sciences*, pages 83–93. The New York Academy of Sciences, New York. [14]

Kandel, E., Schwartz, J., and Jessell, T. (1996). *Neurowissenschaften.* Spektrum Akademischer Verlag. [21]

Kandel, E. R. (2001). The molecular biology of memory storage: a dialog between genes and synapses. *Biosci Rep*, 21(5):565–611. [20]

Kanzaki, R. and Shibuya, T. (1986). Descending protocerebral neurons related to the mating dance of the male silkworm moth. *Brain Res*, 377(2):378–382. [145]

Katz, P. S. and Frost, W. N. (1997). Removal of spike frequency adaptation via neuromodulation intrinsic to the *Tritonia* escape swim central pattern generator. *J Neurosci*, 17(20):7703–7713. [17]

Kien, J. (1983). The initiation and maintenance of walking in the locust: an alternative to the command concept. *Proc R Soc Lond B*, 219:137–174. [15, 98, 143, 144]

Kien, J. (1990). Neuronal activity during spontaneous walking–I. starting and stopping. *Comp Biochem Physiol A*, 95(4):607–621. [98, 138]

Knipper, M. and Breer, H. (1988). Subtypes of muscarinic receptors in insect nervous system. *Comp Biochem Physiol C Comp Pharmacol*, 90:275–280. [18, 54]

Knipper, M. and Breer, H. (1989). Muscarinic receptors modulating acetylcholine release from insect synaptosomes. *Comp Biochem Physiol C*, 93(2):287–292. [79]

Krans, J. L. and Chapple, W. D. (2005). The action of spike frequency adaptation in the postural motoneurons of hermit crab abdomen during the first phase of reflex activation. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*, 191(2):157–174. [17]

Kristan, W. B. and Weeks, J. C. (1983). Neurons controlling the initiation, generation and modulation of leech swimming. *Symp Soc Exp Biol*, 37:243–260. [14]

Kupfermann, I. and Weiss, K. (1978). The command neuron concept. *Behav Brain Sci*, 1(5):3–10. [14]

Lange, A. B. (2002). A review of the involvement of proctolin as a cotransmitter and local neurohormone in the oviduct of the locust, *Locusta migratoria*. *Peptides*, 23(11):2063–2070. [59, 132]

Lapied, B., le Corronc, H., and Hue, B. (1990). Sensitive nicotinic and mixed nicotinic-muscarinic receptors in insect neurosecretory cells. *Brain Res*, 533(1):132–136. [18]

Laughlin, S. B., de Ruyter van Steveninck, R. R., and Anderson, J. C. (1998). The metabolic cost of neural information. *Nat Neurosci*, 1(1):36–41. [137]

Laurent, G. and Burrows, M. (1989a). Distribution of intersegmental inputs to nonspiking local interneurons and motor neurons in the locust. *J Neurosci*, 9:3019–3029. [136, 137]

Laurent, G. and Burrows, M. (1989b). Intersegmental interneurons can control the gain of reflexes in adjacent segments of the locust by their action on nonspiking local interneurons. *J Neurosci*, 9(9):3030–3039. [136, 137]

Le Bon-Jego, M., Cattaert, D., and Pearlstein, E. (2004). Serotonin enhances the resistance reflex of the locomotor network of the crayfish through multiple modulatory effects that act cooperatively. *J Neurosci*, 24(2):398–411. [131]

le Corronc, H. and Hue, B. (1993). Pharmacological and electrophysiological characterization of a postsynaptic muscarinic receptor in the central nervous system of the cockroach. *J exp Biol*, 181:257–278. [56]

Leslie, R. A. (1973). A comparison of the fine structure of thoracic and abdominal interganglionic connectives in the newly hatched and adult stick insect, *Carausius morosus* Br. *Z Zellforsch Mikrosk Anat*, 145(3):299–309. [148]

Levi, R. and Selverston, A. I. (2006). Mechanisms underlying type I mGluR-induced activation of lobster gastric mill neurons. *J Neurophysiol*, 96(6):3378–3388. [21]

Lin, C.-H., Liu, M.-C., Lin, M.-S., Lin, P.-L., Chen, Y.-H., Chen, C.-T., Chen, I.-M., and Tsai, M.-C. (2005). Effects of a new isoquinolinone derivative on induction of action potential bursts in central snail neuron. *Pharmacology*, 75(2):98–110. [136]

Lu, J., Gramoll, S., Schmidt, J., and Calabrese, R. L. (1999). Motor pattern switching in the heartbeat pattern generator of the medicinal leech: membrane properties and lack of synaptic interaction in switch interneurons. *J Comp Physiol [A]*, 184(3):311–324. [16]

Ludwar, B. C. (2003). *Mechanisms for Intersegmental Leg Coordination in Walking Stick Insects*. PhD thesis, University of Cologne. [22, 23, 41, 42, 131, 141, 142, 149]

Ludwar, B. C., Göritz, M. L., and Schmidt, J. (2005a). Intersegmental coordination of walking movements in stick insects. *J Neurophysiol*, 93(3):1255–1265. [41, 140, 141]

Ludwar, B. C., Westmark, S., Büschges, A., and Schmidt, J. (2005b). Modulation of membrane potential in mesothoracic moto- and interneurons during stick insect front-leg walking. *J Neurophysiol*, 94(4):2772–2784. [16, 22, 41, 42, 50, 74, 93, 120, 121, 123, 131, 137, 138, 139, 140, 144, 147, 149]

Lundberg, J. M. (1996). Pharmacology of cotransmission in the autonomic nervous system: integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. *Pharmacol Rev*, 48(1):113–178. [132]

Lundell, M. J. and Hirsh, J. (1998). Eagle is required for the specification of serotonin neurons and other neuroblast 7-3 progeny in the *Drosophila CNS*. *Development*, 125(3):463–472. [20]

Lundquist, C. and Nässel, D. (1997). Peptidergic activation of locust dorsal unpaired median neurons: depolarisation inducted by locustatachykinins may be mediated by cyclic AMP. *Inc J Neurobiol*, 33:297–315. [80]

Marder, E. (1991). Plateaus in time. *Curr Biol*, 1(5):326–327. [15]

Marder, E. (1999). Neural signalling: Does colocalization imply cotransmission? *Curr Biol*, 9(21):R809–R811. [132]

Marder, E., Bucher, D., Schulz, D. J., and Taylor, A. L. (2005). Invertebrate central pattern generation moves along. *Curr Biol*, 15(17):685–699. [13]

Marder, E., Christie, A. E., and Kilman, V. L. (1995). Functional organization of cotransmission systems: lessons from small nervous systems. *Invert Neurosci*, 1(2):105–112. [132]

Marder, E. and Hooper, S. (1985). *Model Neural Networks and Behavior*, chapter Neurotransmitter modulation of the stomatogastric ganglion of decapod crustaceans, pages 319–337. New York: Plenum. [18]

Marquardt, F. (1940). Beiträge zur Anatomie der Muskulatur und der peripheren Nerven von *Carausius (Dixippus) morosus* Br. *Zoologische Jahrbücher Abt Anat*, 66:63–128. [27]

Matsumoto, Y. and Sakai, M. (2000). Brain control of mating behavior in the male cricket *Gryllus bimaculatus DeGeer*: brain neurons responsible for inhibition of copulation actions. *J Insect Physiol*, 46:539–552. [15]

Matsushima, T., Tegnér, J., Hill, R. H., and Grillner, S. (1993). $GABA_B$ receptor activation causes a depression of low- and high-voltage-activated Ca^{2+} currents, postinhibitory rebound, and postspike afterhyperpolarization in lamprey neurons. *J Neurophysiol*, 70(6):2606–2619. [16]

McCormick, D. A. (1992). Neurotransmitter actions in the thalamus and cerebral cortex. *J Clin Neurophysiol*, 9(2):212–223. [125]

Melnick, I. V., Santos, S. F. A., and Safronov, B. V. (2004). Mechanism of spike frequency adaptation in substantia gelatinosa neurones of rat. *J Physiol*, 559(2):383–395. [17]

Mentel, T., Duch, C., Stypa, H., Wegener, G., Müller, U., and Pflüger, H.-J. (2003). Central modulatory neurons control fuel selection in flight muscle of migratory locust. *J Neurosci*, 23(4):1109–1113. [19]

Menzel, R., Wittstock, S., and Sugawa, M. (1990). *The Biology of Memory*, chapter Chemical codes of learning and memory in honey bees, pages 335–358. Schattauer Verlag, Stuttgart. [19]

Mercer, A. R., Kloppenburg, P., and Hildebrand, J. G. (2005). Plateau potentials in developing antennal-lobe neurons of the moth, *Manduca sexta*. *J Neurophysiol*, 93(4):1949–1958. [16, 17]

Meyer, M. R. and Reddy, G. R. (1985). Muscarinic and nicotinic cholinergic binding sites in the terminal abdominal ganglion of the cricket *(Acheta domesticus)*. *J Neurochem*, 45(4):1101–1112. [18]

Millar, N. S., Baylis, H. A., Reaper, C., Bunting, R., Mason, W. T., and Sattelle, D. B. (1995). Functional expression of a cloned *Drosophila* muscarinic acetylcholine receptor in a stable *Drosophila* cell line. *J Exp Biol*, 198(Pt 9):1843–1850. [75]

Mills, J. D. and Pitman, R. M. (1997). Electrical properties of a cockroach motor neuron soma depend on different characteristics of individual Ca components. *J Neurophysiol*, 78(5):2455–2466. [16, 75]

Möhl, B. and Bacon, J. (1983). The tritocerebral comissure giant (TCG) wind-sensitive interneurone in the locust. II. Directional sensitivity and role in flight stabilization. *J Comp Physiol [A]*, 150:453–465. [145]

Mori, S., Matsui, T., Kuze, B., Asonome, M., Nakajima, K., and Matsuyama, K. (1998). Cerebellar-induced locomotion: Reticulospinal control of spinal rhythm generating mechanism in cats. In Kiehn, O., Harris-Warrick, R., Jordan, L., Hultborn, H., and Kudo, N., editors, *Annals of the New York Academy of Sciences*, pages 94–105. The New York Academy of Sciences, New York. [14]

Morton, D. and Evans, P. (1984). Octopamine release from an identified neurone in the locust. *J exp Biol*, 113:269–287. [19]

Nagayama, T. (2002). Serotonergic modulation of nonspiking local interneurones in the terminal abdominal ganglion of the crayfish. *J Exp Biol*, 205(19):3067–3076. [138]

Nässel, D. R. (1988). Serotonin and serotonin-immunoreactive neurons in the nervous system of insects. *Prog Neurobiol*, 30(1):1–85. [65]

Nation, P. and Roth, S. (1988). The effects of neomycin on membrane properties and discharge activity of an isolated sensory neuron. *Can J Physiol Pharmacol*, 66:27–31. [89]

Negro, C. A. D., Morgado-Valle, C., and Feldman, J. L. (2002). Respiratory rhythm: an emergent network property? *Neuron*, 34(5):821–830. [171]

Nickisch-Rosenegk von, E., Krieger, J., Kubick, S., Laage, R., Strobel, J., Strotmann, J., and Breer, H. (1996). Cloning of biogenic amine receptors from moths (*Bombyx mori* and *Heliothis virescens*). *Insect Biochem Mol Biol*, 26(8-9):817–827. [70]

Nusbaum, M. P., Blitz, D. M., Swensen, A. M., Wood, D., and Marder, E. (2001). The roles of co-transmission in neural network modulation. *Trends Neurosci*, 24(3):146–154. [132]

Orchard, I. (1982). Octopamine in insects: neurotransmitter, neurohormone, and neuromodulator. *Can J Zool*, 60:659–669. [19, 59, 127]

Orlovsky, G., Deliagina, T., and Grillner, S. (1999). *Neuronal Control of Locomotion*. Oxford University Press, New York. [14, 149]

Orr, G. L., Orr, N., and Hollingworth, R. M. (1991). Distribution and pharmacological characterization of muscarinic-cholinergic receptors in the cockroach brain. *Arch Insect Biochem Physiol*, 16(2):107–122. [18]

Osborne, R. H. (1996). Insect neurotransmission: neurotransmitters and their receptors. *Pharmacol Ther*, 69(2):117–142. [18, 20, 54, 125, 127]

O'shea, M. and Rowell, C. H. (1976). The neuronal basis of a sensory analyser, the acridid movement detector system. II. response decrement, convergence, and the nature of the excitatory afferents to the fan-like dendrites of the LGMD. *J Exp Biol*, 65(2):289–308. [145]

Parker, D. (1995). Serotonergic modulation of locust motor neurons. *J Neurophysiol*, 73(3):923–932. [20, 65, 79, 135]

Parker, D. (1996). Octopaminergic modulation of locust motor neurons. *J Comp Physiol A*, 178:243–252. [127, 129]

Parker, D. and Newland, P. (1995). Cholinergic synaptic transmission between proprioceptive afferents and a hind leg motor neuron in the locust. *J Neurophysiol*, 73:586–594. [18]

Pearson, K. (1993). Common principles of motor control in vertebrates and invertebrates. *Annu Rev Neurosci*, 16:265–297. [13]

Pearson, K. (2004). Generating the walking gait: role of sensory feedback. *Prog Brain Res*, 143:123–129. [13]

Peck, J. H., Gaier, E., Stevens, E., Repicky, S., and Harris-Warrick, R. M. (2006). Amine modulation of I_h in a small neural network. *J Neurophysiol*, 96(6):2931–2940. [122]

Pichon, Y. and Treherne, J. (1972). The insect blood-brain barrier. *Adv Insect Physiol*, 9:257–313. [54, 126]

Qazi, S. and Trimmer, B. A. (1999). The role of nitric oxide in motoneuron spike activity and muscarinic-evoked changes in cGMP in the CNS of larval *Manduca sexta*. *J Comp Physiol [A]*, 185(6):539–550. [22]

Ramirez, J.-M., Büschges, A., and Kittmann, R. (1993). Octopaminergic modulation of the femoral chordotonal organ in the stick insect. *J Comp Physiol [A]*, 173:209–219. [19, 59, 128]

Ramirez, J.-M. and Pearson, K. (1991a). Octopamine induces bursting and plateau potentials in insect neurones. *Brain Res*, 549:332–337. [16, 19, 128]

Ramirez, J.-M. and Pearson, K. (1991b). Octopaminergic modulation of interneurons in the flight system of the locust. *J Neurophysiol*, 66(5):1522–1537. [19, 128]

Ridgel, A., Frazier, S., DiCaprio, R., and Zill, S. (2000). Encoding of forces by cockroach tibial campaniform sensilla: implications in dynamic control of posture and locomotion. *J Comp Physiol [A]*, 186:359–374. [140]

Ridgel, A. L. and Ritzmann, R. E. (2005). Effects of neck and circumoesophageal connective lesions on posture and locomotion in the cockroach. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*, 191(6):559–573. [15, 144, 148]

Roberts, A. and Tunstall, M. J. (1990). Mutual re-excitation with post-inhibitory rebound: A simulation study on the mechanisms for locomotor rhythm generation in the spinal cord of *xenopus* embryos. *Eur J Neurosci*, 2(1):11–23. [16]

Robinson, R. B. and Siegelbaum, S. A. (2003). Hyperpolarization-activated cation currents: from molecules to physiological function. *Annu Rev Physiol*, 65:453–480. [122]

Roeder, K. (1937). The control of tonus and locomotor activity in the praying mantis *(Mantis religiosa L.)*. *J exp Zool*, 76:353–374. [143, 144]

Roeder, T. (1991). A new octopamine receptor class in locust nervous tissue, the octopamine 3 (OA3) receptor. *Life Sciences*, 50:21–28. [129]

Roeder, T. (1994). Biogenic amines and their receptors in insects. *Comp Biochem Physiol C*, 107:1–12. [65, 129]

Roeder, T. (1999). Octopamine in invertebrates. *Prog Neurobiol*, 59(5):533–561. [19, 75, 127, 128]

Roeder, T. (2002). Biochemistry and molecular biology of receptors for biogenic amines in locusts. *Microsc Res Tech*, 56(3):237–247. [60]

Ronacher, B., Wolf, H., and Reichert, H. (1988). Locust flight behavior after hemisection of individual thoracic ganglia: evidence for hemiganglionic premotor centers. *J Comp Physiol [A]*, 163:749–759. [146]

Ryckebusch, S. and Laurent, G. (1993). Rhythmic patterns evoked in locust leg motor neurons by the muscarinic agonist pilocarpine. *J Neurophysiol*, 69:1583–1595. [18]

Sachs, L. (1974). *Statistische Auswertungsmethoden*, chapter Abhängigkeitsmasse: Korrelation und Regression, pages 328–330. Springer (Berlin). [38]

Sattelle, D. (1980). Acetylcholine receptors of insects. *Adv Insect Physiol*, 15:215–315. [17, 54]

Sawczuk, A., Powers, R. K., and Binder, M. D. (1995). Spike frequency adaptation studied in hypoglossal motoneurons of the rat. *J Neurophysiol*, 73(5):1799–1810. [17]

Schmidt, B. J. and Jordan, L. M. (2000). The role of serotonin in reflex modulation and locomotor rhythm production in the mammalian spinal cord. *Brain Res Bull*, 53(5):689–710. [129]

Schmidt, J., Fischer, H., and Büschges, A. (2001). Pattern generation for walking and searching movements of a stick insect leg II. Control of motoneuronal activity. *J Neurophysiol*, 85:354–361. [17, 22, 124, 139, 142, 175]

Schmitz, J., Büschges, A., and Delcomyn, F. (1988). An improved electrode design for *en passant* recording from small nerves. *Comp Biochem Physiol A*, 91(4):769–772. [32]

Schmitz, J., Delcomyn, F., and Büschges, A. (1991). Oil and hook electrodes for *en passant* recording from small nerves. *Methods in Neurosciences*, 4(4):266–278. [32]

Schofield, P. K. (1979). Ionic permeability of the blood-brain barrier system of an insect *Carausius morosus*. *J Exp Biol*, 82:385–388. [27]

Sekirnjak, C. and du Lac, S. (2002). Intrinsic firing dynamics of vestibular nucleus neurons. *J Neurosci*, 22(6):2083–2095. [16]

Selverston, A., Elson, R., Rabinovich, M., Huerta, R., and Abarbanel, H. (1998). Basic principles for generating motor output in the stomatogastric ganglion. In Kiehn, O., Harris-Warrick, R., Jordan, L., Hultborn, H., and Kudo, N., editors, *Annals of the New York Academy of Sciences*, pages 35–50. The New York Academy of Sciences, New York. [136]

Selverston, A. I. (2005). A neural infrastructure for rhythmic motor patterns. *Cell Mol Neurobiol*, 25(2):223–244. [13]

Shik, M. L., Severin, F. V., and Orlovsky, G. N. (1966). Control of walking and running by means of electric stimulation of the midbrain. *Biophysics 11*, 11(4):756–765. [149]

Sombati, S. and Hoyle, G. (1984). Generation of specific behaviors in a locust by local release into neuropil of the natural neuromodulator octopamine. *J Neurobiol*, 15(6):481–506. [19, 60, 127]

Staudacher, E. and Schildberger, K. (1998). Gating of sensory responses of descending brain neurons during walking in cricket. *J Exp Biol*, 201:559–572. [145, 148]

Steeves, J. and Pearson, K. (1982). Proprioceptive gating of inhibitory pathways to hindleg flexor motoneurons in the locust. *J comp Physiol*, 146:507–515. [145]

Steeves, J. D., Sholomenko, G. N., and Webster, D. M. (1987). Stimulation of the pontomedullary reticular formation initiates locomotion in decerebrate birds. *Brain Res*, 401(2):205–212. [14]

Stein, R. B. and Parmiggiani, F. (1979). Optimal motor patterns for activating mammalian muscle. *Brain Res*, 175(2):372–376. [175]

Stein, W., Büschges, A., and Bässler, U. (2006). Intersegmental transfer of sensory signals in the stick insect leg muscle control system. *J Neurobiol*, 66(11):1253–1269. [137, 140, 141, 145]

Stevenson, P. (1989). *Neural mechanisms of behaviour*, chapter Central effects of octopamine in the locust, page 31. Thieme, New York. [130]

Stevenson, P. and Kutsch, W. (1986). Basis circuitry of an adult specific motor program completed with embryogenesis. *Naturwissenschaften*, 73:741–743. [19]

Stevenson, P. A., Dyakonova, V., Rillich, J., and Schildberger, K. (2005). Octopamine and experience-dependent modulation of aggression in crickets. *J Neurosci*, 25(6):1431–1441. [19]

Straub, V. A. and Benjamin, P. R. (2001). Extrinsic modulation and motor pattern generation in a feeding network: a cellular study. *J Neurosci*, 21(5):1767–1778. [16]

Suarez-Kurtz, G. (1974). Inhibition of membrane calcium activation by neomycin and streptomycin in crab muscle fibers. *Pflügers Arch*, 349(4):337–349. [89]

Swensen, A. M., Golowasch, J., Christie, A. E., Coleman, M. J., Nusbaum, M. P., and Marder, E. (2000). GABA and responses to GABA in the stomatogastric ganglion of the crab *Cancer borealis*. *J Exp Biol*, 203(14):2075–2092. [138]

Taylor, A. L., Cottrell, G. W., Kleinfeld, D., and Kristan, W. B. (2003). Imaging reveals synaptic targets of a swim-terminating neuron in the leech CNS. *J Neurosci*, 23(36):11402–11410. [14]

Teshiba, T., Shamsian, A., Yashar, B., Yeh, S. R., Edwards, D. H., and Krasne, F. B. (2001). Dual and opposing modulatory effects of serotonin on crayfish lateral giant escape command neurons. *J Neurosci*, 21(12):4523–4529. [79, 130]

Thoby-Brisson, M., Telgkamp, P., and Ramirez, J. M. (2000). The role of the hyperpolarization-activated current in modulating rhythmic activity in the isolated respiratory network of mice. *J Neurosci*, 20(8):2994–3005. [122]

Tierney, A. J. (2001). Structure and function of invertebrate 5-HT receptors: a review. *Comp Biochem Physiol A Mol Integr Physiol*, 128(4):791–804. [129]

Trimmer, B. (1994). Characterization of a muscarinic current that regulates excitability of an identified insect motoneuron. *J Neurophysiol*, 72:1862–1873. [18, 123, 125]

Trimmer, B. (1995). Current excitement from insect muscarinic receptors. *Trends in Neurosciences*, 18:104–111. [18, 124, 125]

Trimmer, B. and Berridge, M. (1985). Inositol phosphates in the insect nervous system. *Insect Biochem*, 15:811–815. [85]

Trimmer, B. and Weeks, J. (1989). Effects of nicotinic and muscarinic agents on an identified motoneurone and its direct afferent inputs in larval *Manduca sexta*. *J exp Biol*, 144:303–337. [18, 56]

Trimmer, B. A. and Weeks, J. C. (1993). Muscarinic acetylcholine receptors modulate the excitability of an identified insect motoneuron. *J Neurophysiol*, 69(6):1821–1836. [18, 54, 123, 125]

Tyrer, N., Turner, J., and Altman, J. (1984). Identifiable neurons in the locust central nervous system that react with antibodies to serotonin. *J Comp Neurol*, 227(3):313–330. [65]

Urbani, A. and Belluzzi, O. (2000). Riluzole inhibits the persistent sodium current in mammalian CNS neurons. *Eur J Neurosci*, 12(10):3567–3574. [171]

Venkatesh, K., Siddhartha, G., Joshi, R., Patel, S., and Hasan, G. (2001). Interactions between the inositol 1,4,5-trisphosphate and cyclic AMP signaling pathways regulate larval molting in *Drosophila*. *Genetics*, 158(1):309–318. [85]

Waldrop, B. and Hildebrand, J. G. (1989). Physiology and pharmacology of acetylcholinergic responses of interneurons in the antennal lobes of the moth *Manduca sexta*. *J Comp Physiol [A]*, 164(4):433–441. [18]

Walther, C. and Zittlau, K. E. (1998). Resting membrane properties of locust muscle and their modulation II. Actions of the biogenic amine octopamine. *J Neurophysiol*, 80(2):785–797. [79, 84, 135]

Watson, A. H. (1984). The dorsal unpaired median neurons of the locust metathoracic ganglion: neuronal structure and diversity, and synapse distribution. *J Neurocytol*, 13(2):303–327. [128]

Weidler, D. and Diecke, F. (1969). The role of cations in conduction in the central nervous system of the herbivorous insect *Carausius morosus*. *Z vergl Physiologie*, 64:372–399. [27, 32]

Weiger, W. A. (1997). Serotonergic modulation of behaviour: a phylogenetic overview. *Biol Rev Camb Philos Soc*, 72(1):61–95. [20]

Wendler, G. (1964). Laufen und Stehen der Stabheuschrecke *Carausius morosus*: Sinnesborstenfelder in den Beingelenken als Glieder von Regelkreisen. *Z vergl Physiologie*, 48:198–250. [149]

Wenzel, B., Elsner, N., and Heinrich, R. (2002). mAChRs in the grasshopper brain mediate excitation by activation of the AC/PKA and the PLC second-messenger pathways. *J Neurophysiol*, 87(2):876–888. [18, 21, 79, 84, 89, 93, 135, 136]

Widmer, A., Höger, U., Meisner, S., French, A. S., and Torkkeli, P. H. (2005). Spider peripheral mechanosensory neurons are directly innervated and modulated by octopaminergic efferents. *J Neurosci*, 25(6):1588–1598. [135]

Wiersma, C. and Ikeda, K. (1964). Interneurons commanding swimmeret movements in the crayfish, *Procambarus clarkia* (Girard). *Comp Biochem and Physiol*, 12:509–525. [14]

Wildman, M., Ott, S. R., and Burrows, M. (2002). GABA-like immunoreactivity in non-spiking interneurons of the locust metathoracic ganglion. *J Exp Biol*, 205(23):3651–3659. [137]

Wilson, D. (1961). The central nervous control of flight in a locust. *J Exp Biol*, 38:471–490. [98]

Würden, S. and Homberg, U. (1995). Immunocytochemical mapping of serotonin and neuropeptides in the accessory medulla of the locust, *Schistocerca gregaria*. *J Comp Neurol*, 362(3):305–319. [132]

Wüstenberg, D. G. and Grünewald, B. (2004). Pharmacology of the neuronal nicotinic acetylcholine receptor of cultured Kenyon cells of the honeybee, *Apis mellifera*. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*, 190(10):807–821. [126]

Wu, L.-J., Li, Y., and Xu, T.-L. (2002). Co-release and interaction of two inhibitory co-transmitters in rat sacral dorsal commissural neurons. *Neuroreport*, 13(7):977–981. [59]

Yeh, S. R., Fricke, R. A., and Edwards, D. H. (1996). The effect of social experience on serotonergic modulation of the escape circuit of crayfish. *Science*, 271(5247):366–369. [130]

Yellman, C., Tao, H., He, B., and Hirsh, J. (1997). Conserved and sexually dimorphic behavioral responses to biogenic amines in decapitated *Drosophila*. *Proc Natl Acad Sci U S A*, 94(8):4131–4136. [20, 133]

Zhang, B. and Harris-Warrick, R. (1995). Calcium-dependent plateau potentials in a crab stomatogastric ganglion motor neuron. I. Calcium current and its modulation by serotonin. *J Neurophysiol*, 74:1929–1937. [16]

Zill, S., Schmitz, J., and Büschges, A. (2004). Load sensing and control of posture and locomotion. *Arthropod Structure and Development*, 33:273–286. [13]

Zorovic, M. and Hedwig, B. (2007). Towards a better understanding of the complex auditory behavior in crickets: Combining a sensitive trackball system with single-cell recording from brain neurons. In *Proceedings of the 31th Göttingen Neurobiology Conference and the 7th Meeting of the German Neuroscience Society 2007. Abstract and Poster, T18-4B*. [98, 145, 148]

Appendix

A Effect of Riluzole on the tonic depolarization

Riluzole is known to have a variety of effects in vertebrate systems, for example, it was shown to cause a rapid and sustained plateau increase in calcium (Ca^{2+}) in human osteosarcoma cells (Jan et al. 2002). In the mentioned study it was suggested, that the increase in intracellular Ca^{2+} was caused by stimulation of extracellular Ca^{2+}-influx and intracellular Ca^{2+}-release from the endoplasmatic reticulum. At low concentrations (0.5 to 20 µM), riluzole seems to selectively block the persistent sodium current (Negro et al. 2002; Urbani and Belluzzi 2000).

Using riluzole, it was investigated if a persistent sodium current might contribute to the tonic depolarization. The effect of riluzole on the tonic depolarization in mesothoracic flexor MNs was tested in four animals. The concentrations of riluzole ranged from 25 to 150 µM. Riluzole induced in three of four experiments an increase in the amplitude of the tonic depolarization. Figure I shows a representative experiment. Under control condition in saline the tonic depolarization in an ipsilateral flexor MN was 2 mV (7 steps, Fig. I(a)). Superfusion of 100 µM riluzole increased the tonic depolarization amplitude to 2.5 mV during a sequence of 5 steps (Fig. I(b)).

The time course for another experiment is shown in figure II(a). The amplitude of the tonic depolarization is plotted against time. In saline (control, ○), the tonic depolarization

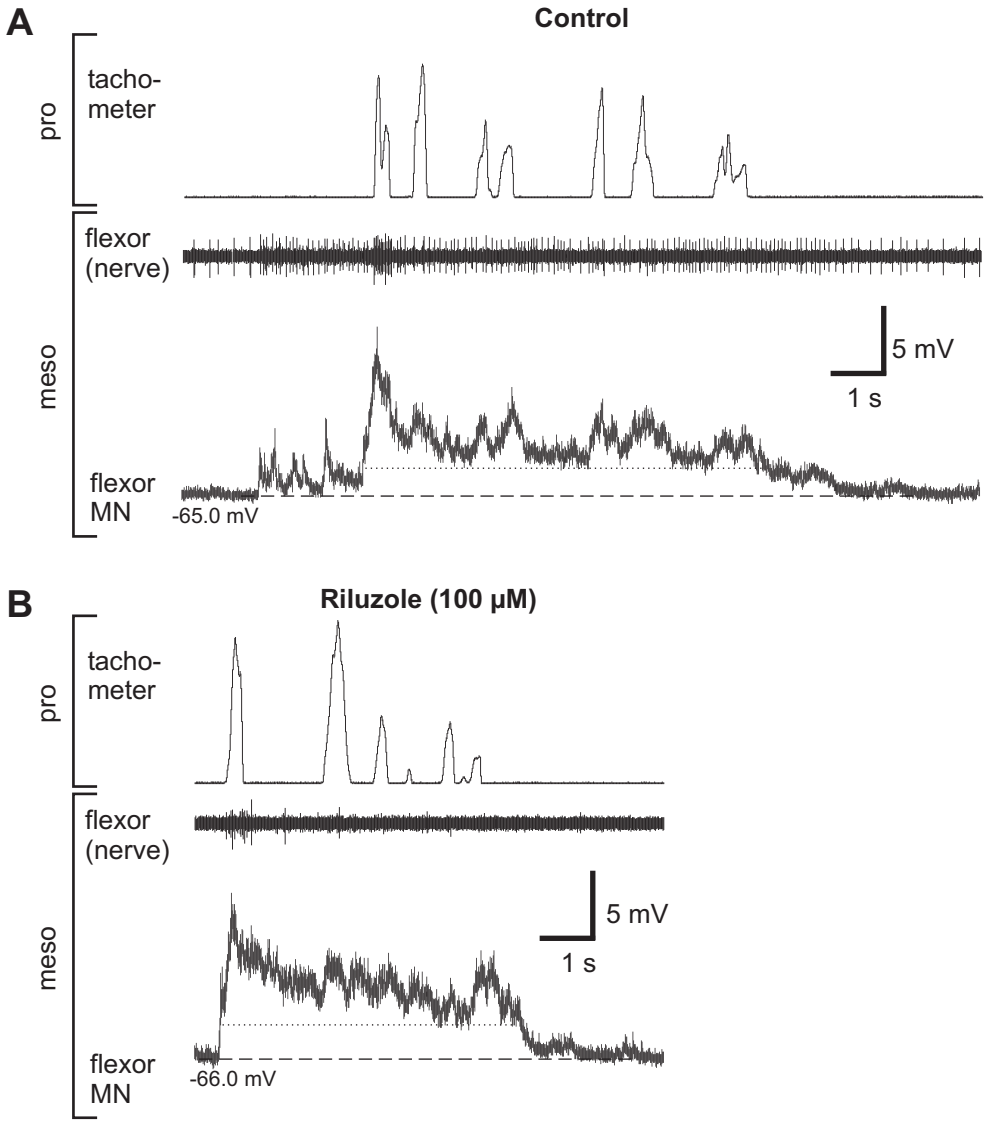


Figure I: *Riluzole increases the tonic depolarization in ipsilateral mesothoracic flexor MNs during front leg stepping.* ***A:*** *The tonic depolarization in saline was 2.0 mV during a stepping sequence of the front leg (7 steps).* ***B:*** *During superfusion of 100 µM riluzole the amplitude of the tonic depolarization during a sequence of 5 steps increased (6 min, 2.5 mV).*

amplitude was 2.8 ±0.1 mV and increased during superfusion of 150 µM riluzole (●) to 3.8 mV (6 min). The coefficient of correlation for the linear fit in this experiment was significant at the 5 % level. The analysis for all experiments is shown in figure II(b)). Different concentrations of riluzole were used: 25 µM (N=1), 100 µM (N=1) and 150 µM (N=2). In three experiments riluzole increased the tonic depolarization amplitude by 25 % (range 7 to 43 %), in one experiment a decrease by 75 % during superfusion of 150 µM riluzole was observed (data calculated from linear fits). One flexor MN was successfully recorded during wash and showed a partial recovery of the tonic depolarization amplitude.

In summary, riluzole had an opposing effect on the tonic depolarization in flexor MNs. In three of four experiments, riluzole increased the tonic depolarization amplitude by up to 43 %, in the fourth experiment a decrease by 75 % was observed.

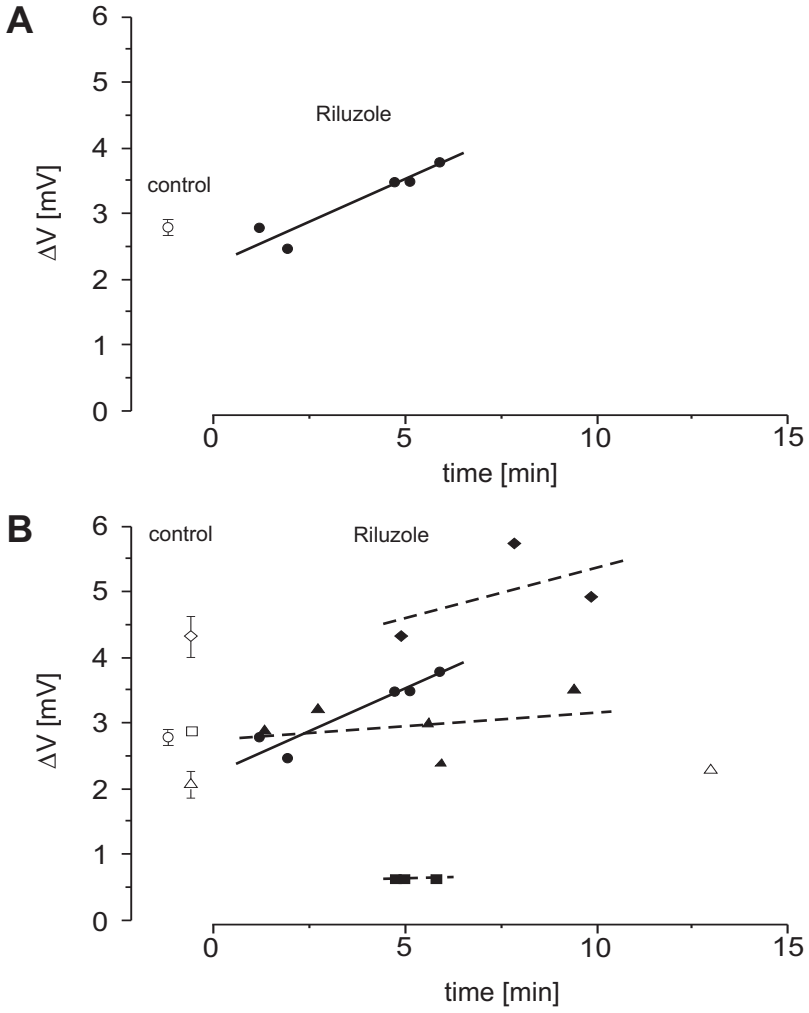


Figure II: *Tonic depolarization amplitude over time during riluzole superfusion.* ***A:*** *Detailed analysis for one experiment. Control in saline: 2.8 ±0.1 mV (n=2). The amplitude of the tonic depolarization increased to 3.8 mV after 6 min superfusion of 150 µM riluzole. Data points are fitted by linear regression (n=5), p < 0.5.* ***B:*** *Time course for all experiments. Riluzole increased the tonic depolarization in three of four animals. In the fourth experiment riluzole had the opposite effect (■). The concentrations are labeled as follows: 25 µM ♦; 100 µM ▲; and 150 µM ●, ■. Only linear fits are shown for ΔV during riluzole superfusion. Same symbol is one experiment. Solid line: level of significance 5 %, dashed lines: no significance.*

B Spike frequency adaptation

A fundamental property of many classes of neurons, e.g. MNs, is the exhibition of a time-dependent decrease in action potential (AP) discharge rate, which is termed spike frequency adaptation (SFA). A function of SFA in MNs might be to increase the speed of force generation in muscle fibres which can be sustained with lower frequencies (Stein and Parmiggiani 1979).

The mechanism that generates SFA could be slow activation of outward currents, a reduction in the availability of fast inactivating sodium channels or a summation of the slow afterhyperpolarization. A calcium-dependent potassium current causes SFA in e.g. MNs in the lamprey locomotor network, whereas SFA is thought to play a critical role in burst termination (el Manira et al. 1994). Stick insect MNs also show SFA (Schmidt et al. 2001). How SFA is generated in stick insect neurons is not known. In preliminary experiments the effect of verapamil on SFA in flexor MNs was analyzed. The phenylalkylamine verapamil is known to inhibit K^+-induced increase of intracellular calcium, for example in the leech (Dierkes et al. 2004), and Benquet et al. (1999) showed that verapamil acts as a non-selective blocker of voltage activated calcium channel currents in cockroach brain neurones. Thus, if SFA in stick insect bases on an increased calcium entry during repetitive firing, that causes greater activation of Ca^{2+}-dependent K^+-channels which in turn leads to greater interspike intervals, verapamil might reduce SFA.

The effect of verapamil on SFA was analyzed in three experiments, one of which is shown in figure III. In saline, a flexor MN showed SFA during injection of a depolarizing current (2 s, Fig. III(a)), which was decreased during superfusion of verapamil (Fig. III(b)). After several minutes wash in saline, SFA increased slightly (Fig. III(c)).

The adaptation of the instantaneous spike frequency over time for one flexor MN is shown in figure IV. The initial frequency in this flexor MN was on average approximately 40 Hz on onset of a depolarizing current pulse (2 nA, Fig. IV(a),(b)). The spike frequency reached a steady state about 500 ms after stimulus onset, whereas the instantaneous spike frequency was reduced by 58 % when reaching the steady state (17 Hz). During superfusion of 200 µM verapamil the initial frequency was increased to approximately 55 Hz on onset of a depolarizing current pulse (2 nA, Fig. IV(c),(d)). The steady

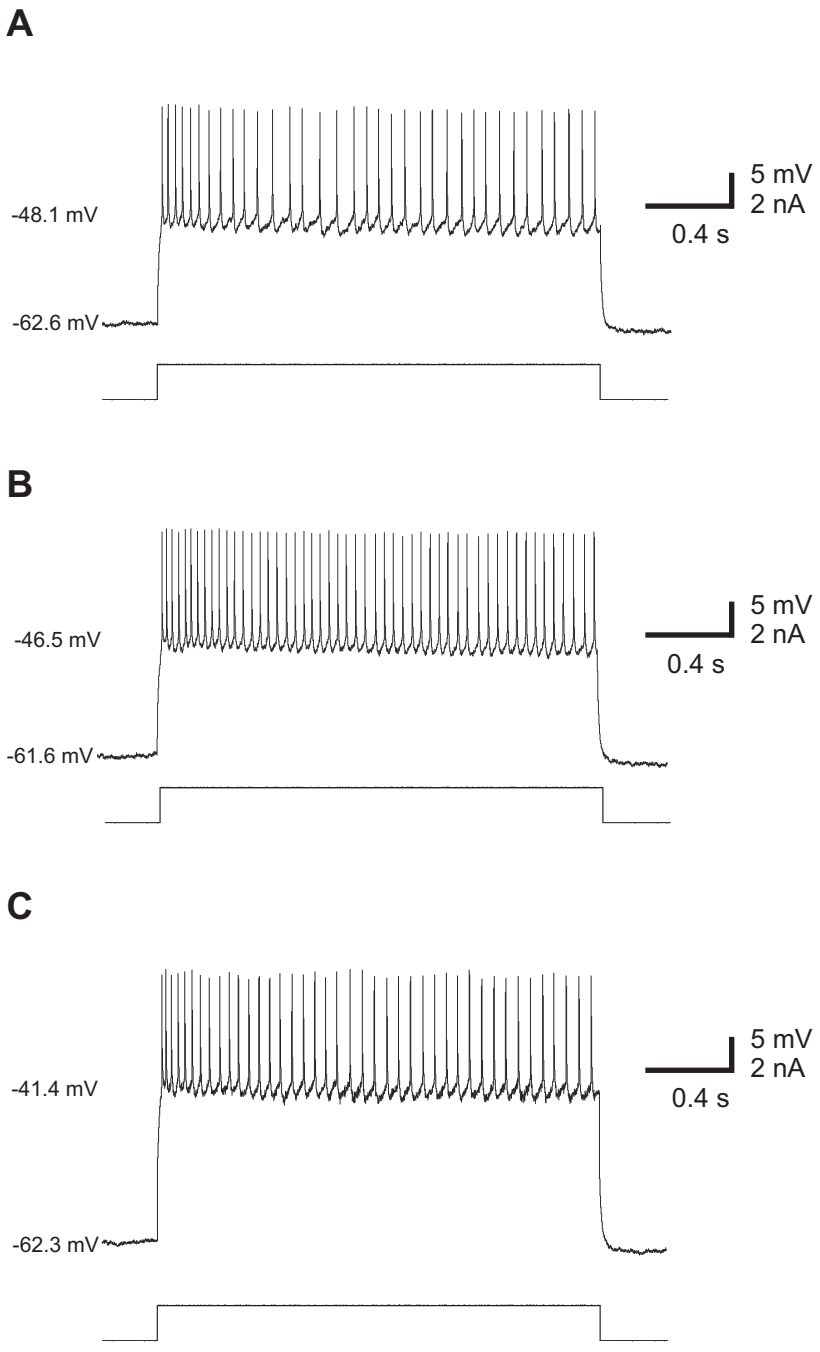


Figure III: *Spike frequency adaptation in a flexor MN. DCC recordings of repetitive firing elicited by current injection (2 nA, 2 s duration) in* ***A:*** *control,* ***B:*** *during superfusion of 200 µM verapamil and* ***C:*** *during wash. Spike frequency was increased after 3 min superfusion of verapamil (****B****).*

state was reached about 600 ms after stimulus onset. The frequency during the steady state was reduced to approximately 22 Hz (60 %). Compared to control values in saline, the initial frequency in this flexor MN was increased by 27 % in the presence of verapamil. Furthermore, also the steady state was increased by 29 % compared to control in saline.

In summary, the initial spike frequency in flexor MNs was increased by 27 % to 146 % during superfusion of verapamil (N=3). The steady state of the instantaneous spike frequency was increased up to 60 % (38 ±19 %) compared to control in saline. A delay in reaching a steady state was observed in the presence of verapamil in all three experiments (range 13 % to 89 %). Thus verapamil seems to decrease SFA, indicating that SFA in flexor MNs might include the activation of Ca^{2+}-dependent K^{+}-channels.

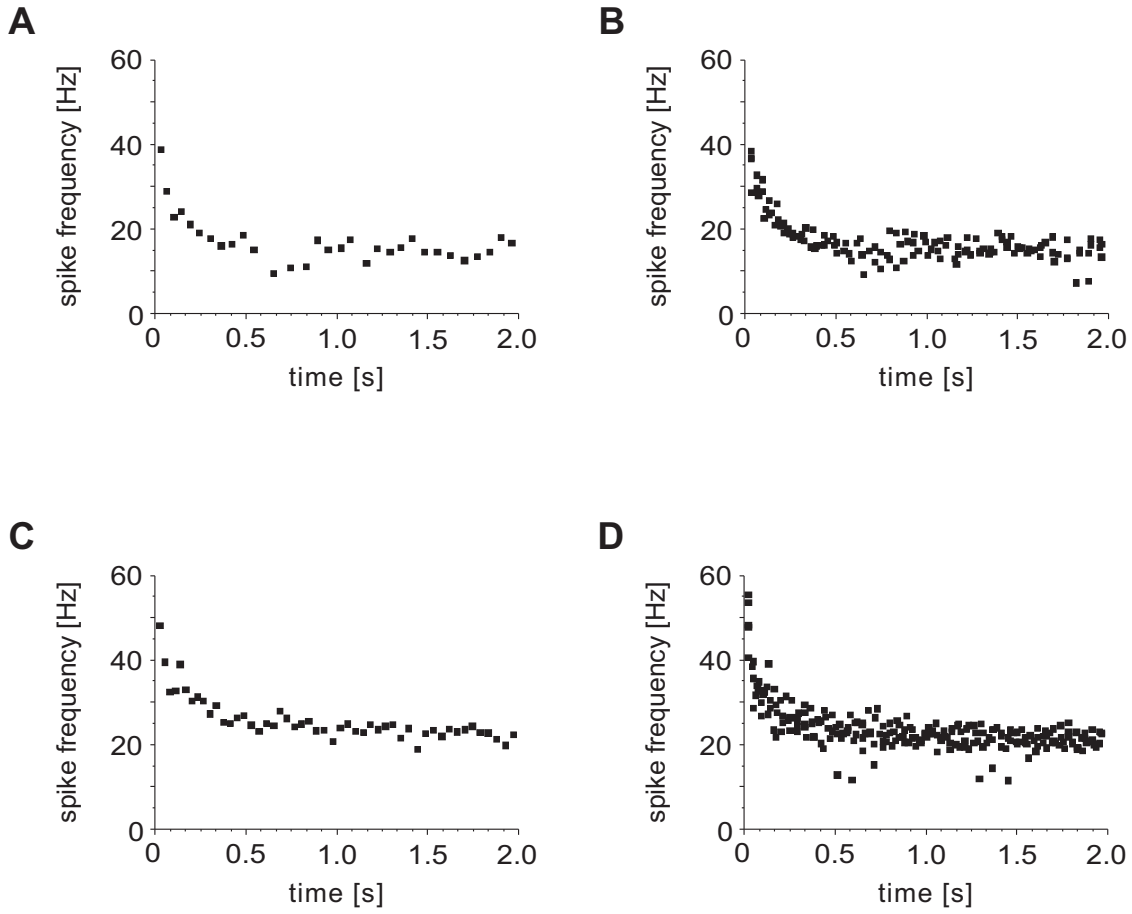


Figure IV: *Plots of spike frequency over time in a flexor MN.* ***A,B:*** *Control in saline.* ***A:*** *SFA during one current pulse (2 nA).* ***B:*** *Overlay of frequencies during five current pulses. A flexor MN showed an initial instantaneous spike frequency of approximately 40 Hz during a depolarizing current pulse. The instantaneous spike frequency was reduced by ~58 % when reaching the steady state after 500 ms (~17 Hz).* ***C,D:****: SFA during superfusion of 200 µM verapamil.* ***C:*** *SFA during one current pulse (2 nA) and* ***D:*** *Overlay of frequencies during five current pulses. The initial instantaneous spike frequency increased on average to approximately 55 Hz during a depolarizing current pulse. A steady state of the instantaneous spike frequency was reached after ~600 ms (22 Hz).*

Abbreviations

AC	Adenylate cyclase
ACh	Acetylcholine
AHP	Afterhyperpolarization
AP	Action potential
BAPTA	1,2-bis-(o-aminophenoxy)ethane-N,N,N′,N′-tetra-acetic acid
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CPG	Central pattern generator
CS	Campaniform sensilla
DCC	Discontinuous current clamp
DCMD	Descending contralateral movement detector
DIN	Descending interneuron
DUM	Dorsal unpaired median
EMG	Electromyogram

fCO	Femoral chordotonal organ
GABA	γ-amino butyric acid
IP_3	Inositol trisphosphate
KAc	Potassium acetate
KCl	Potassium chloride
mAChR	Muscarinic acetylcholine receptor
MLR	Mesencephalic locomotor region
MN	Motoneuron
nAChR	Nicotinic acetylcholine receptor
ncr	Nervus cruris
OAR	Octopamine receptor
oxo-M	Oxotremorine-M
PIC	Persistent inward current
PIR	Post-inhibitory rebound
PKA	Protein kinase A
PLC	Phospholipase C
RS	Reticulospinal system
SEG	Subesophageal ganglion
SFA	Spike frequency adaptation
STG	Stomatogastric ganglion
TCG	Tritocerebral commisure giant
VUM	Ventral unpaired median

List of Figures

List of Tables

Teilpublikationen

Article

Ludwar B.Ch., **Westmark S**., Büschges A., Schmidt J. (2005)
Modulation of Membrane Potential in Mesothoracic Moto- and Interneurons during Stick Insect Front-Leg Walking. *J Neurophysiol, (94)4, 2772-84*.

Poster and Abstracts

Westmark S., Schmidt J. (2007)
Evidence for a role of 2nd messengers in the control of motoneuron activity in the walking stick insect. *Proceedings of the 31th Göttingen Neurobiology Conference and the 7th Meeting of the German Neuroscience Society 2007*.

Westmark S., Büschges A., Schmidt J. (2006)
Motoneuron activity during walking in stick insects involves 2nd messenger pathways. *Neurovisionen: Perspektiven in NRW. Düsseldorf, Germany 2006*.

Westmark S., Borgmann A., Büschges A., Schmidt J. (2006)
Tonic input to leg motoneurons during stick insect walking: A pharmacological analysis

Neuroscience 2006 SfN 36th Annual Meeting.

Westmark S., Borgmann A., Büschges A., Schmidt J. (2006)
Pharmacological analysis of tonic input to leg motoneurons during walking in stick insects *99th Annual Meeting of the Deutsche Zoologische Gesellschaft (Münster) 2006. Supplement V, 67.*

Westmark S., Ludwar B. Ch., Schmidt J. (2005)
Intersegmental Influence of Tonic and Phasic Modulation on Leg Motoneurons in Walking Stick Insects. *Proceedings of the 30th Göttingen Neurobiology Conference and the 6th Meeting of the German Neuroscience Society 2005, Program S.177 Nr.83B*.

Westmark S., Ludwar B. Ch., Gabriel J., Schmidt J. (2004)
Tonic input to leg motoneurons during walking in stick insects. *Neuroscience 2004 SfN 34th Annual Meeting*.

Acknowledgements

An dieser Stelle möchte ich mich besonders bedanken bei:

- PD Dr. Joachim Schmidt, für die sehr gute Betreuung, seine tollen Anleitungen und jegliche Unterstützung, die Überlassung des Themas und vor allem für seine Begeisterung dafür, seine guten Ideen und für sein Lachen.
- Prof. Dr. Ansgar Büschges, für seine Unterstützung und Förderung, die Bereitstellung des Arbeitsplatzes, seine hilfreichen Anregungen, seine Begeisterung und Motivation.
- Prof. Dr. Peter Kloppenburg, für die Bereitschaft zur Erstellung des Zweitgutachtens.
- Anke Borgmann, Géraldine v. Uckermann, Christoph Guschlbauer und Sharon Meyen-Southard für gute Unterhaltung(en), viel Spaß, Zusammenhalt, seelisch-moralische Unterstützung, Korrekturlesen und Hilfestellung jeder Art.
- Dr. Hans Scharstein für seine Hilfestellungen und Ideen bei der Auswertung. Lydia Berlingen, Markus Blümel, Hans-Peter Bollhagen, Michael Dübbert, Volker Dürr, Jens Peter Gabriel, Matthias Gruhn, Scott Hooper, Tim Mentel, Eugenio Oliveira, Christian Porres, Sherylane Seeliger, Michael Schöngen für zahlreiche Unterstützungen und die gute Atmosphäre.
- Meinen Eltern Ursula und Henrik Westmark, sowie meiner Tante Sigrid van Hoorn, dafür, dass sie immer für mich da waren, an mich geglaubt haben, und mich in jeglicher Hinsicht unterstützt haben.
- Meinen Schwestern Silvia Weihrauch und Daniela Westmark für all die Dinge, wofür Schwestern da sind.
- Martina Schürmann für die lange währende, tolle Freundschaft.
- Thomas von Braunschweig für seine Rücksichtnahme und Liebe und seine Fähigkeit, mich immer wieder aufzubauen.

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 PD Dr. Joachim Schmidt betreut worden.

Köln, den 15.05.2007

Curriculum vitae

Sandra Westmark
Bilker Allee 211
40215 Düsseldorf
S.Westmark@online.de
*Sep 15, 1976 in Emden

Education

- 2006 Jun-Aug Neural Systems and Behavior Course at the Marine Biological Laboratory, Woods Hole, USA.
- 2003–present Department of Animal Physiology, University of Cologne; PhD program. Advisor: Prof. Dr. A. Büschges
- 2002–2003 Diploma thesis at the Institute of Neurobiology, Heinrich-Heine-University Düsseldorf; thesis title: *Properties and Pharmacology of Caffeine-sensitive Cation Channels in Identified Neurons in the Leech Central Nervous System*. Thesis advisor: Prof. Dr. W.-R. Schlue.
- 1996–2002 Studies in biology at the Heinrich-Heine-University Düsseldorf.

- 1996 Abitur at the Bettina-von-Arnim Gymnasium in Dormagen

Professional experience

- 2003-2006 Assistant teacher, undergraduate and graduate courses in animal physiology/neurobiology at the University of Cologne
- 2003 July–Sept Research assistant in the lab of Prof. Dr. W.-R. Schlue (Institute of Neurobiology, Düsseldorf).
- 2003 Assistant teacher, neurobiology (graduate course) at the Heinrich-Heine-University Düsseldorf
- 2002 Assistant teacher, neurophysiology (undergraduate course) at the Heinrich-Heine-University Düsseldorf
- 2000–2003 Technical assistant in the oncological immunological lab (University Hospital Düsseldorf, Gynecology).

Honors

- 2006 Boehringer Ingelheim Travel Allowance for participation in the course Neural Systems and Behavior at the Marine Biological Laboratory (Woods Hole, USA).