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# Pattern Generation for Walking and Searching Movements of a Stick Insect Leg. II. Control of Motoneuronal Activity

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**Schmidt, Joachim, Hanno Fischer, and Ansgar Büschges.** Pattern generation for walking and searching movements of a stick insect leg. II. Control of motoneuronal activity. *J Neurophysiol* 85: 354–361, 2001. In the stick insect, *Cuniculina impigra,* intracellular recordings from mesothoracic motoneurons that control flexion and extension of the tibia and depression and levation of the trochantero-femur were made while the leg performed walking-like movements on a treadband or stereotyped rhythmic searching movements. We were interested in how synaptic input and intrinsic properties contribute to form the activity pattern of motoneurons during rhythmic leg movements without sensory feedback from other legs. During searching and walking, motoneurons expressed a rhythmic bursting pattern that was formed by a depolarizing input followed by a hyperpolarizing input in the inter-burst interval. This basic pattern was similar in all fast, semi-fast, and slow motoneurons that were recorded. Hyperpolarizations were in synchrony with activity in the antagonistic motoneurons. De- and hyperpolarizations were associated with a decrease in input resistance. All motoneurons showed spike frequency adaptation when depolarized by current injection to a membrane potential similar to that observed during walking. In the hyperpolarizing phase of fast flexor motoneurons, the initial maximum hyperpolarization was followed by a sag in potential toward more depolarized values. Consistent with this observation, only fast flexor motoneurons developed a depolarizing sag potential when hyperpolarized by injection of constant negative current.

## INTRODUCTION

In a walking animal, the coordinated activity of muscles that control movements of different leg joints depends on the precise timing of the activity of motoneurons that innervate these muscles (recent reviews: Orlovsky et al. 1999; Pearson 1993). The timing and magnitude of motor activity during walking is known to result from interaction of sensory signals and the output of central rhythm generating networks (recent reviews: Büschges and El Manira 1998; Clarac 1991; Duysens et al. 2000; Pearson 1995). The complete structure of central walking rhythm generating networks is not known in any animal (Orlovsky et al. 1999), although in insects some interneurons have been identified that are part of premotor networks involved in postural control and locomotion (see Bässler and Büschges 1998; Burrows 1996). However, not only is the information about central premotor elements fragmentary, but there is little information about the membrane potential changes in motoneurons during walking, which would show the pattern of synaptic inputs (cockroach: Pearson and Fourtner 1975; Tryba and Ritzmann 2000; stick insect: Godden and Graham 1984: locust: Wolf 1990, 1992). In addition, there is only sparse data on the intrinsic properties of insect motoneurons that contribute to forming their activity pattern (David and Pitman 1996; Hancox and Pitman 1991, 1993; Mills and Pitman 1999; Ramirez and Pearson 1991).

The membrane potential of leg motoneurons has been studied in preparations of the cat, the rat, and the locust in which walking-like motor activity has been induced by electrical stimulation of higher locomotor regions (Shefchyk and Jordan 1985) or by application of drugs (Cazalets et al. 1996; Ryckebusch and Laurent 1993). Under these conditions, rhythmic activity of motoneurons results from alternating excitatory and inhibitory synaptic input. These data are consistent with the observation in the cockroach that phasic inhibition and phasic depolarization contribute to activity of a coxal motoneuron and an extensor tibiae motoneuron during walking (Tryba and Ritzman 2000) Application of the cholinergic agonist pilocarpine onto stick insect ganglia activates central neuronal networks that generate alternating rhythmic activity in antagonistic leg motoneurons (Büschges et al. 1995). However, under these conditions, rhythmic activity in mesothoracic flexor and extensor motoneurons appears to be based on tonic excitation and cyclic hyperpolarizing synaptic input (Büschges 1998). It is the objective here to study the intracellular activity pattern in, and the role of synaptic inputs to, stick insect motoneurons in a semi-intact walking preparation that is suitable for long-term intracellular recordings (see companion paper, Fischer et al. 2001).

Along with synaptic inputs, the activity pattern of motoneurons is also sculpted by their intrinsic membrane properties. Spike frequency adaptation (SFA) and depolarizing sag potentials and plateau potentials affect the activity pattern of motoneurons in different systems (SFA: lamprey: El Manira et al. 1994; phrenic motoneurons of cat: Jodkowski et al. 1988; hypoglossal motoneurons of rat: Sawczuk et al. 1995; depolarizing sag potentials: stomatogatric nervous system: Kiehn and Harris-Warrick 1992; hypoglossal and facial motoneurons of rat: Bayliss et al. 1994; Magariños-Ascone et al. 1999; plateau potentials: for review, see Hultborn 1999; Kiehn 1991). Plateau potentials in insect motoneurons have been shown in cockroach leg motoneurons (Hancox and Pitman 1991, 1993)

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and locust flight motoneurons (Ramirez and Pearson 1991), but it is not known whether insect leg motoneurons exhibit SFA and depolarizing sag potentials. We have looked for both properties in stick insect motoneurons because they might affect the motoneuronal activity pattern during walking. SFA would decrease spike frequency during depolarizations and depolarizing sag potentials could counteract membrane hyperpolarizations.

In the companion paper (Fischer et al. 2001), we have introduced a preparation in which after amputation of all legs but one middle leg, the remaining leg performs walking movements on a treadband or stereotyped rhythmic searching movements when ground contact is absent. We made intracellular recordings from mesothoracic motoneurons of the coxa-trochanteral joint and the femur-tibial joint and investigated the overall synaptic drive underlying patterning of motoneuronal activity in the locomotor cycle. We found that in all motoneurons recorded rhythmic activity during walking and searching movements was due to alternating inhibitory and excitatory synaptic inputs. When studying cellular properties we found that slow, semi-fast, and fast motoneurons exhibited marked spike frequency adaptation. In addition, fast flexor tibiae motoneurons were found to develop a depolarizing sag potential when hyperpolarized by injection of constant current.

## METHODS

All experiments were carried out on adult female stick insects, *Cuniculina impigra* Redthenbacher (syn. *Baculum impigrum* Brunner) that were raised at the Zoological Institut, University of Cologne. The experiments were performed at room temperature of 20–22°C under dimmed light conditions.

The experimental setup and the methods of preparation are described in detail in the accompanying paper (Fischer et al. 2001). In short, the animals were mounted on a platform using dental wax. All legs but one middle leg were amputated between trochanter and coxa. The stumps were glued to the platform to prevent movements and the coxa of the remaining leg was glued to the body wall to prevent pro- and retraction movements. After opening the thoracic cavity, the mesothoracic ganglion was placed on a platform, and all nerve roots on the side of the leg stump and the nerves nl2 and nl5 were crushed.

The activity of motoneurons was recorded intracellularly from their neuropilar arborizations in the ipsilateral hemi-ganglion with microelectrodes (1 mm OD, 0.78 mm, Science Products, Hofheim, Germany) that had resistances of 15–25 MΩ and were filled with a solution of 3 MKAc/0.05 M KCl. An NPI-10 L amplifier (npi electronic GmbH, Tamm, Germany) was used in bridge or discontinuous current-clamp mode. Monopolar hook electrodes were used for extracellular recording from nerve roots. Electromyographic recordings were obtained by inserting two 50-$\mu$m copper wires into the respective muscles of the leg segments. Motoneurons were identified either by a one-to-one relationship of their action potentials with muscle potentials in EMG recordings and/or by induction of leg movements on injection of depolarizing current. Each spike in a fast motoneuron evoked a distinct fast movement. Individual spikes in semi-fast motoneurons evoked movements that were much smaller and hardly detectable by eye. However, a spike train in semi-fast motoneurons evoked a smooth movement that was clearly faster than movements evoked by spike trains in slow motoneurons. A single spike in a slow motoneuron was never able to evoke a movement of a joint.

For data processing, see Fischer et al. (2001). For creating *x, y* data plots, we used PlotIT for Windows (Scientific Programming Enterprises, Haslett, MI). PlotIT was used for smoothing the *y* values in *x, y* data using a smoothing factor of 0.6. Measurements are given as means ± SE. We used a modified *t*-test (Dixon and Massey 1969) to compare data sets and samples were regarded significantly different for $P < 0.05$.

## RESULTS

### *Activity pattern of motoneurons during walking and searching movements*

Electromyographic recordings (EMG) from leg muscles have shown that the motoneurons of the coxa-trochanteral joint and the femur-tibial joint generate bursts of action potentials when the middle leg performs walking-like movements on a

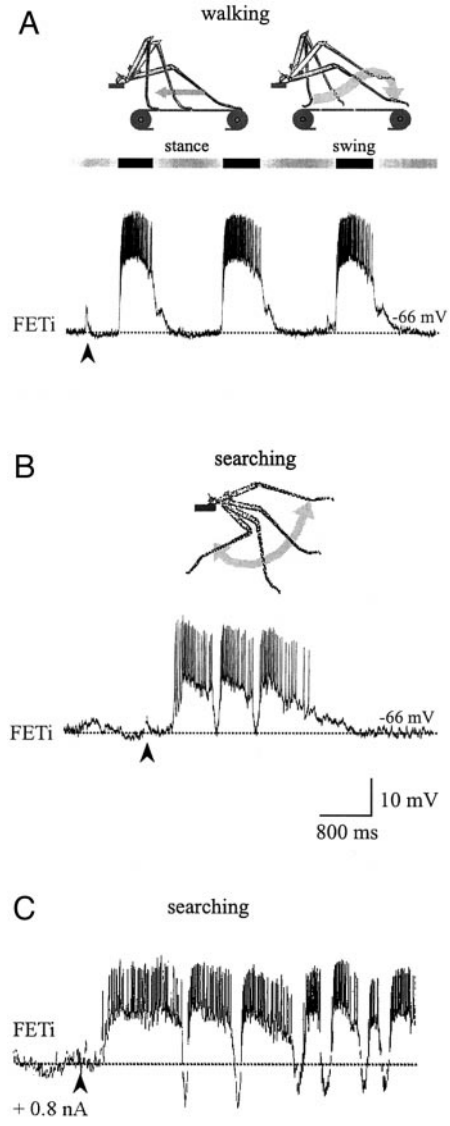


FIG. 1. Rhythmic activity of a fast extensor tibiae motoneuron during walking and searching movements of the leg. *A*: the middle leg of a stick insect walks on a treadband in the transverse plane, i.e., no pro- and retraction is possible. Swing phase is defined as fast extensor tibiae motoneuron (FETi) activity (see Fischer et al. 2001). The intracellular recording of FETi shows burst activity during 3 consecutive steps. In this and subsequent figures, arrowheads indicate time of tactile stimulation that elicited the sequence of leg movements. *B*: during searching movements, FETi activity is less vigorous but also organized in bursts. *C*: when depolarized by intracellular current injection, the membrane potential of FETi in the interburst interval was more negative than the new resting potential.

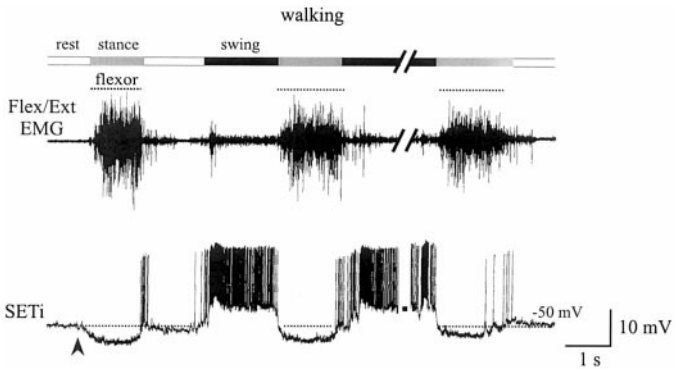


FIG. 2. Alternation of inhibition and excitation in slow motoneurons during walking. The membrane potential of a slow extensor motoneuron (SETi) is hyperpolarized below the resting potential during flexor activity (Flex/Ext EMG), indicating the stance phase of leg movement.

treadband or performs searching-like movements. For a discussion of some differences between these walking- and searching-like movements of the single middle leg and walking and searching leg movements in intact animals that result from the constrains of the single leg preparation, see companion paper by Fischer et al. (2001). For the sake of readability, we will refer to walking- and searching-like movements further on as walking and searching.

The intracellular recording of the fast extensor tibiae motoneuron (FETi) gives an example of the basic features of the typical rhythmic motoneuronal pattern. During walking, FETi ($n = 5$) generated bursts of action potentials (Fig. 1*A*). Between bursts, FETi repolarized and the membrane potential settled at −67 mV, 1 mV more negative than its potential at rest. Each burst in FETi indicated the swing phase of a walking cycle (Fischer et al. 2001). A similar rhythmic activity pattern was generated in FETi during searching movements of the leg (Fig. 1*B*). Spike frequency was generally lower and cycle period was faster during searching than during walking. During searching, FETi did not hyperpolarize below its potential at rest between bursts. However, when FETi was held at a more depolarized membrane potential (−40 mV) by current injection during searching, the membrane hyperpolarized below the new resting potential (Fig. 1*C*), indicating that FETi not only repolarized due to decreasing depolarizing input but also received inhibitory synaptic input in the inter-burst interval.

During walking, extensor motoneurons also appear to receive inhibitory input. Bursting of SETi activity during walking was formed by depolarizations and hyperpolarizations around the potential at rest ($n = 5$; Fig. 2). It was a general pattern for all motoneurons that the hyperpolarization was in synchrony with burst activity in the antagonistic motoneurons. Starting from a potential at rest of −50 mV, SETi hyperpolarized by about 4 mV during flexor activity (stance phase) and generated bursts of action potentials during swing phase. The walking sequence shown started with a hyperpolarization of SETi because the walking cycle started with a stance phase.

Flexor motoneurons expressed the same basic activity pattern during walking and searching as extensor motoneurons as is shown in Fig. 3. The membrane of a slow flexor motoneuron

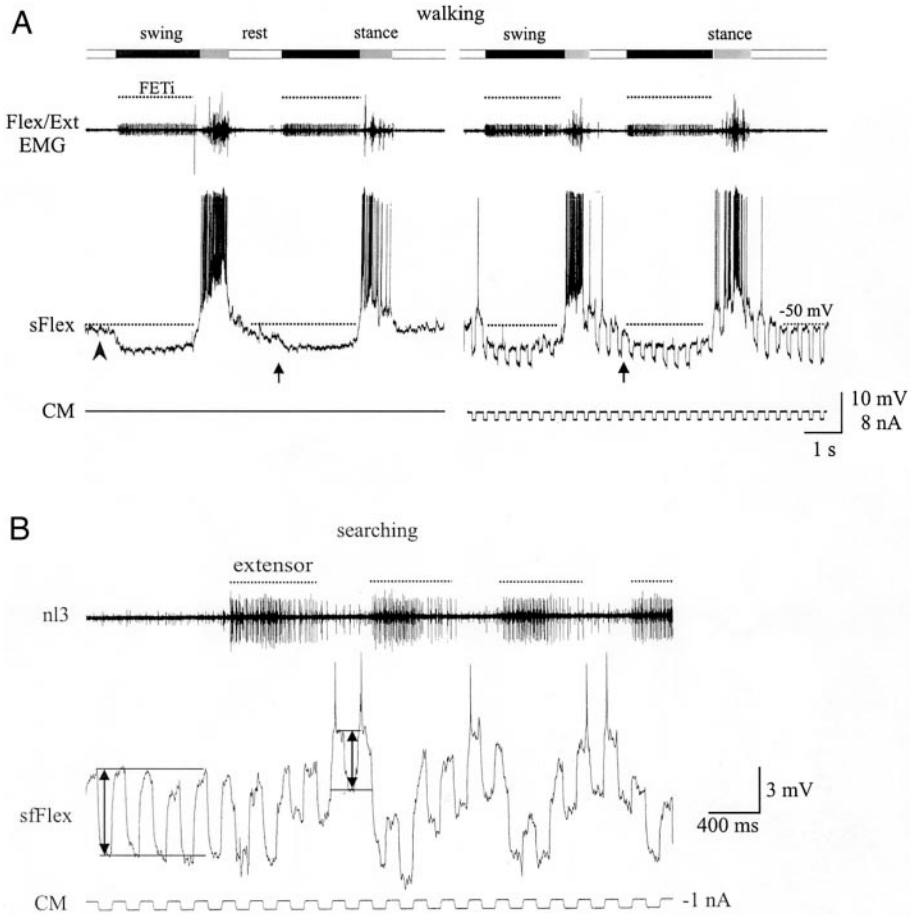


FIG. 3. Rhythmic activity of flexor motoneurons during walking and searching. *A*: during walking, the membrane potential of a slow flexor motoneuron (sFlex) is hyperpolarized below resting potential during extensor activity (Flex/Ext EMG), indicating swing phase of the walking cycle. Burst activity in the flexor motoneuron indicates the stance phase of the leg movement. Injection of negative current pulses (CM, current monitor) into the slow flexor motoneuron reveals that the input resistance is decreased during swing phase (extensor activity) as compared with rest. ↑, onset of hyperpolarizing input. *B*: during spontaneous searching, a semi-fast flexor motoneuron (sfFlex) was rhythmically depolarized and hyperpolarized. ↕, potential change during injection of negative current pulses. The input resistance was reduced during the depolarizing phase.

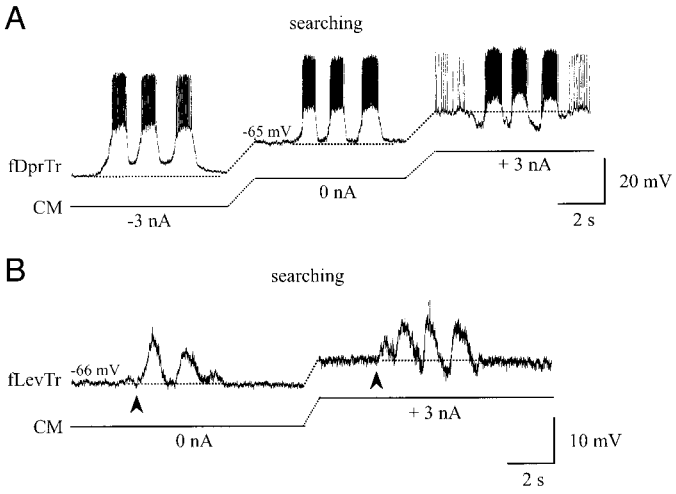


FIG. 4. Alternation of excitation and inhibition in fast depressor and levator trochanteris motoneurons during searching. *A*: the intracellular recording of a fast depressor trochanteris motoneuron (fDprTr) shows bursts of action potentials during spontaneous searching movements of the leg. When the cell is held hyperpolarized by constant injection of negative current, the membrane potential does not reach baseline in the interburst interval. When the cell is depolarized by constant injection of positive current, the membrane potential is hyperpolarized below baseline. *B*: a fast levator trochanteris motoneuron (fLevTr) mainly exhibits subthreshold depolarizations during searching movements of the leg. When the cell is depolarized by constant injection of positive current, the membrane potential is hyperpolarized below rest between depolarizations. Two spikes were clipped.

(sFlex, Fig. 3*A*) hyperpolarized some 4 mV below the resting membrane potential of −50 mV in the interburst interval in synchrony with activity in extensor motoneurons. The walking sequence shown started with a hyperpolarization of sFlex because the sequence started with a swing phase. In this preparation, the rhythm paused for about a second after each swing-stance cycle. The pause was characterized by a repolarization of the membrane to resting potential and an increase in input resistance. A sudden decrease in input resistance and further hyperpolarization indicated the onset of inhibitory synaptic input (see ↑).

During walking and searching (Fig. 3*B*), the hyperpolarizing phase in all motoneurons tested was associated with a decrease in input resistance. The mean input resistance of 6.5 ± 0.91 MΩ [$n$ = 7, 5 slow and 2 fast motoneurons (MNs)] at rest was reduced to 3.1 ± 0.61 MΩ in the inhibitory phase of a walking or searching cycle. The depolarizing phase during walking and searching is also associated with a decrease in input resistance (Fig. 3*B*). During searching, a semi-fast flexor motoneuron was rhythmically depolarized by 2–3 mV associated with a decrease in input resistance of about 40%.

Depolarizations and hyperpolarizations around the resting potential also formed the activity pattern of depressor and levator trochanteris motoneurons during searching (Fig. 4). A fast depressor trochanteris motoneuron (fDprTr) generated bursts of action potentials during the down stroke of the leg and repolarized back to its resting potential between bursts. When depolarized by current injection, fDprTr hyperpolarized below the new resting potential between bursts (Fig. 4*A*). These hyperpolarizations were not due to K currents activated by a preceding depolarization because the sequence started with a hyperpolarization of fDprTr. When the membrane potential was held more negative than the resting potential of −65 mV, the membrane did not reach the new resting potential between bursts indicating a reversal potential of −65 mV for the interburst hyperpolarization (Fig. 4*A*). The fLevTr motoneuron in Fig. 4*B* usually did not reach spike threshold when depolarized and did not hyperpolarize below its resting potential of −66 mV during leg depression. Again, when depolarized by current injection the membrane hyperpolarized below the new resting potential between depolarizations, indicating hyperpolarizing synaptic input during depression of the leg.

Only 8% of the fast motoneurons ($n$ = 14) that were recorded while the animal performed searching movements hyperpolarized below the resting potential between bursts. When depolarized by current injection, however, all neurons hyperpolarized below the new resting potential. A far greater percentage of slow motoneurons, 42%, hyperpolarized below rest during searching movements ($n$ = 26). Of those slow motoneurons that were held at a more positive membrane potential by current injection ($n$ = 7), all but one hyperpolarized below the new resting potential. A similar picture emerged for semi-fast motoneurons. The mean resting potential of the neurons that hyperpolarized below rest was −49 ± 2.0 mV ($n$ = 17), whereas the resting potential of neurons that did not hyperpolarize below the resting potential during searching was −58 ± 1.2 mV ($n$ = 45) and thus significantly more negative. These data suggest that a hyperpolarization below rest was more often observed in slow and semi-fast motoneurons because the mean resting potential of those was significantly more positive, −51 ± 1.0 mV ($n$ = 47) and −52 ± 1.6 mV ($n$ = 27), respectively, than that of fast motoneurons, which was −62 ± 1.2 mV ($n$ = 35; see also Table 1).

Our data suggest that in all fast, semi-fast, and slow extensor, flexor, depressor trochanteris, and levator trochanteris motoneurons that were recorded, the activity pattern during searching and walking appeared to be formed by depolarizing and hyperpolarizing synaptic input. Rhythmic activity in motoneurons during walking and searching appears to be based on the same mechanisms; the only difference was that spike

TABLE 1. *Intrinsic properties of motoneurons*

| | $V_m$ | SFA | Depol. Sag Potential |
|---|---|---|---|
| FETi | −63 ± 1.1 (8) | yes (4) | no (4) |
| SETi | −52 ± 1.1 (15) | yes (10) | no (8) |
| fFlex | −61 ± 2.3 (14) | yes (6) | **yes (6)** |
| sfFlex | −51 ± 2.1 (18) | yes (11) | no (9) |
| sFlex | −47 ± 2.4 (10) | yes (3) | no (6) |
| fDprTr | −65 ± 1.2 (4) | yes (1) | no (1) |
| sDprTr | −53 ± 1.6 (18) | yes (10) | no (9) |
| fLevTr | −63 ± 2.6 (9) | yes (4) | no (4) |
| sfLevTr | −54 ± 2.6 (9) | yes (3) | no (2) |
| sLevTr | −50 ± 6.6 (4) | yes (1) | no (1) |

Resting membrane potential ($V_m$) of motoneurons that control movements of the femoro-tibial and the coxa-trochanteral joint. Number in parentheses gives total number of respective cells record. SFA: spike frequency adaptation. Numbers in parentheses give number of cells tested. All cells tested exhibited SFA. Depol. sag potential: depolarizing sag potential. Numbers in parentheses give numbers of cells tested. Only fFlex neurons developed depolarizing sag potentials. Abbreviations for motoneurons: FETi, fast extensor tibiae; SETi, slow extensor tibiae; fFlex, fast flexor; sfFlex, semi-fast flexor; sFlex, slow flexor; fDprTr, fast depressor trochanteris; sDprTr, slow depressor trochanteris; fLevTr, fast levator trochanteris; sfLev Tr, semi-fast levator trochanteris; sLevTr, slow levator trochanteris.

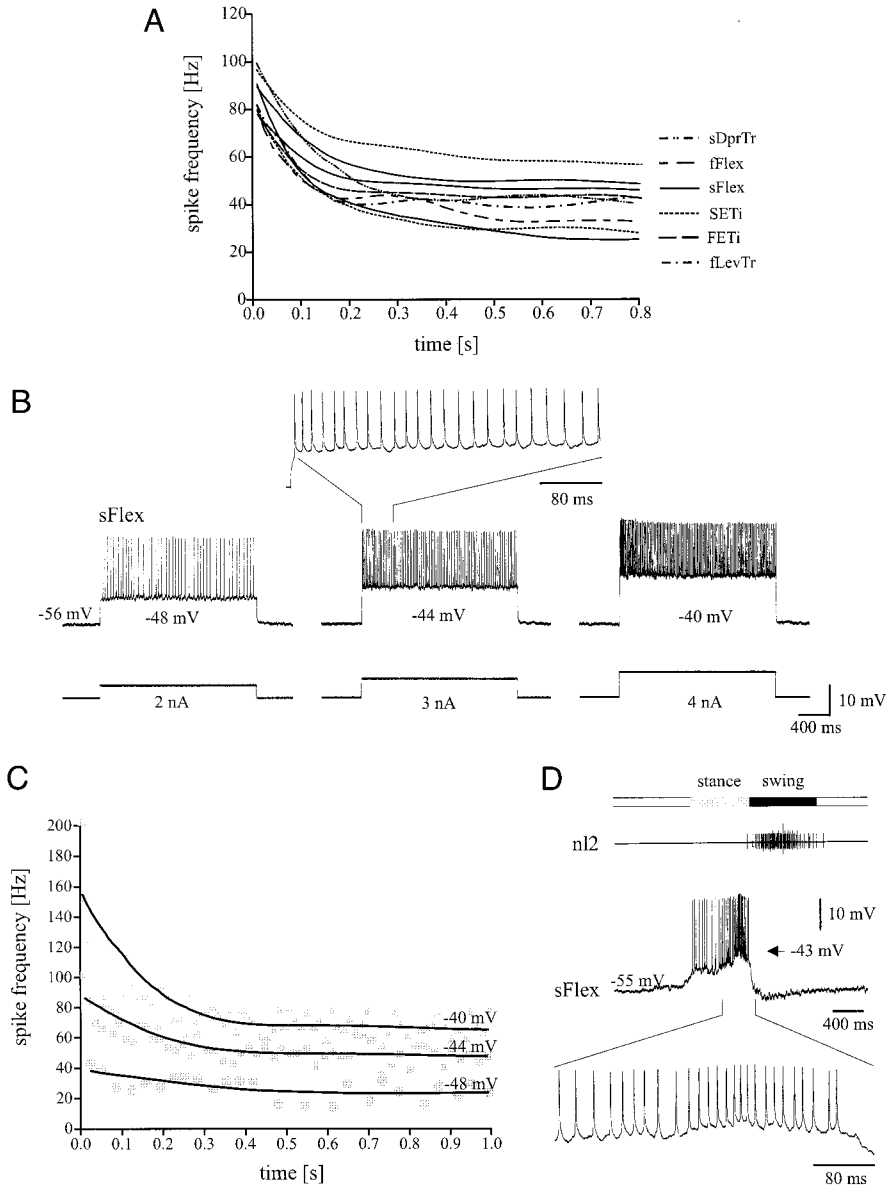


FIG. 5. Leg motoneurons exhibit spike frequency adaptation. *A*: smoothed curve fits of spike frequency over time, based on data from 3 slow flexor motoneurons (sFlex MN), 1 fast flexor (fFlex MN), 2 slow extensor tibiae (SETi), 1 fast extensor MN (FETi), 1 slow depressor trochanteris MN (sDprTr), and 1 fast levator trochanteris MN (fLevTr). The motoneurons were depolarized to give an initial frequency of 100 ± 15 Hz. *B*: a slow flexor motoneuron was depolarized by current pulses of 2 s duration. The spike frequency over time is plotted in *C*. The data points are overlaid by smoothed curve fits (same smoothing factor as in *A*). *D*: maximum spike frequency and membrane potential in the same slow flexor motoneuron shown in *B* during the stance phase of a step is similar to spike frequency and membrane potential during injection of 3-nA current.

activity during searching was usually less strong and cycle period was faster than during walking (see also Fischer et al. 2001).

### *Spike frequency adaptation*

The activity pattern of neurons is not only determined by their synaptic input but also by intrinsic cellular mechanisms, one of which is spike frequency adaptation (SFA). All motoneurons that were tested exhibited SFA (Table 1). Figure 5*A* shows the adaptation of the instantaneous spike frequency in nine different motoneurons that assumed an initial frequency of 100 ± 15 Hz on onset of a depolarizing current pulse (2–5 nA). The spike frequency reached a steady state about 500 ms after stimulus onset. Differences in SFA between different types of motoneurons were not apparent, and the steady-state spiking rate within one type was quite large. For example, in two different slow flexor tibiae motoneurons that were depolarized by current injection of 3 nA to a holding potential of −44 and −41 mV, respectively, the initial instantaneous spike frequency was 102 Hz in both cells and adapted to a steady-state frequency of 47 ± 1.4 and 26 ± 1.2 Hz, respectively (Fig. 5*A*).

The magnitude of adaptation depended on the membrane potential, as shown for a slow flexor motoneuron in Fig. 5, *B* and *C*. A current pulse of 2 nA for 2 s depolarized the membrane from −56 to −49 mV and initially evoked an instantaneous spike frequency of 43 Hz that was reduced by 42% when reaching a steady state of 25 Hz after about 500 ms. When the membrane was depolarized to a potential of −44 mV, the instantaneous frequency was reduced by 51% when reaching a steady state, and a reduction by 65% was observed when the membrane was depolarized to a membrane potential of −39 mV.

During the stance phase of a step cycle (Fig. 5*D*), the same neuron depolarized to a membrane potential of −44 mV and generated spikes with a slightly higher maximum instantaneous frequency, which was 125 Hz. Similar examples could be shown for other motoneurons, indicating that SFA is likely to

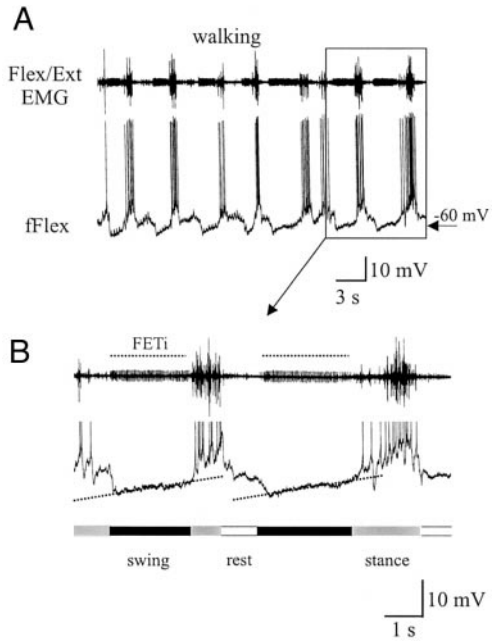


FIG. 6. Depolarizing sag potential in a fast flexor motoneuron during walking. *A*: a fFlex generated bursts of action potentials during stance phase and was hyperpolarized during swing phase of the leg. *B*: enlargement from *A*. fFlex was hyperpolarized throughout extensor (FETi) activity. Spikes were clipped. The hyperpolarization declined steadily until spike activity started.

contribute to determining spike frequency in the excitatory phase during walking activity.

### *Restorative depolarizing sag*

Figure 6 shows rhythmic activity in a fast flexor motoneuron (fFlex) during walking. fFlex received hyperpolarizing input during extensor activity (Fig. 6, *A* and *B*). The membrane was initially hyperpolarized by 4–5 mV at the onset of extensor activity and slowly repolarized by 3–4 mV until extensor activity stopped (Fig. 6*B*). A similar decline of hyperpolarization during swing phase was observed in two other fFlex motoneurons but not in any other motoneuron. In a variety of systems, there are neurons that when hyperpolarized by constant current injection reveal a restorative sag in membrane potential that depolarizes the cells (Pape 1996). We tested the leg motoneurons for such a sag potential by injecting negative current pulses (−1 to −4 nA) of 1–2 s. Only the fast flexor motoneurons ($n = 6$) of all the motoneurons that were recorded exhibited a depolarizing sag potential when hyperpolarized (Fig. 7, Table 1). The depolarizing sag in the inhibitory phase of fast flexor motoneurons during walking is consistent with this observation.

## DISCUSSION

Walking movements of a single middle leg on the treadband consisted of stance and swing phases in the transversal plane with successive steps sometimes separated by short pauses. The coordination pattern of leg movements in the step cycle was somewhat different from those observed during straight walking of intact animals (for detailed discussion, see companion paper, Fischer et al. 2001) but similar to those used for forward walking movements of front legs in the intact stick insect (Bässler 1986), and it resembled the coordination pattern in the middle leg during curve walking (Jander 1982; Rixe and Dean 1995).

### *Alternating excitatory and inhibitory synaptic drive sculpts rhythmic locomotor activity of leg motoneurons*

During walking and searching movements of a leg, rhythmic activity patterns in motoneurons innervating muscles of the coxa-trochanteral joint and the femoro-tibial joint control the movements of these joints. We found that the rhythmic bursting pattern in these motoneurons is based on alternating depolarizing and hyperpolarizing synaptic inputs to the motoneurons associated with an increase in input resistance. This finding was true for all fast, semi-fast, and slow motoneurons that were recorded (Table 1). During walking, leg motoneurons always received hyperpolarizing synaptic input in synchrony with activity in the antagonistic motoneuron pool. Our findings substantiate previous data from Godden and Graham (1984), who observed termination of bursts by strong hyperpolarization in coxal motoneurons recorded in a semi-intact six-legged preparation of the stick insect that walked on a tread wheel. The depolarizing input that we observed during walking was not described probably because Godden and Graham (1984) could not obtain a stable threshold for spike initiation in their recordings. Similar to the stick insect, extensor and depressor activity in the cockroach during walking appears to be patterned by inhibitory and excitatory synaptic input (Tryba and Ritzmann 2000). Therefore in both, stick insect and cockroach, the active and the inactive phase of leg motoneurons during walking is under control of premotor neurons.

We do not know how the alternation of inhibitory and excitatory input to motoneurons and switching between antagonists is controlled during walking. It is quite conceivable that signals from proprioceptors of the leg play an important role, such as the femoral chordotonal organ (fCO), which measures parameters of movement of the femoro-tibial joint or force sensors in the cuticle, like campaniform sensilla in the trochanter and femur. For example, when the stick insect locomotor system is active, flexion signals from the fCO inhibit extensor motoneurons and activate flexor motoneurons (Bässler 1988).

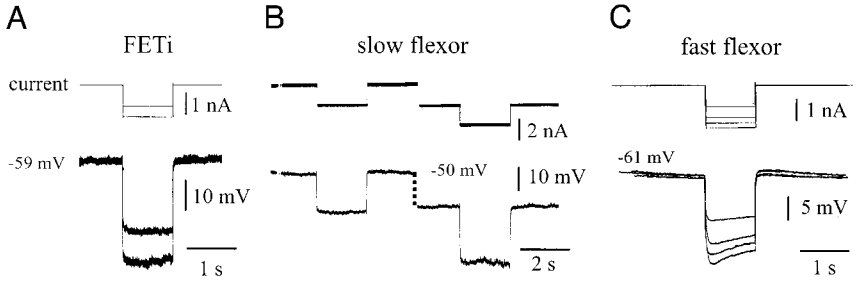


FIG. 7. Hyperpolarizing pulses of 1- to 2-s duration were injected into a fast extensor tibiae motoneuron (*A*), a slow flexor motoneuron (*B*), and a fast flexor motoneuron (*C*). Only the fast flexor motoneuron exhibited a restorative depolarizing sag potential during hyperpolarization.

At a rather flexed position of the femoro-tibial joint, the influence of fCO signals on tibial motoneuron activity is reversed as extensor motoneurons are excited and flexor motoneurons are inhibited (Bässler 1988). The switch from inhibition to excitation in extensor motoneurons and from excitation to inhibition in flexor motoneurons is very similar to the activity switch in these motoneurons at the transition from stance to swing. Thus signals from the fCO may contribute to the control of synaptic input to flexor and extensor motoneurons during walking.

In a variety of isolated nerve systems of different species, it is possible to pharmacologically evoke rhythmic activity patterns in motoneurons that resemble activity patterns during locomotion. Some of these preparations show motor activity that is also based on alternating excitatory and inhibitory synaptic input to motoneurons, for example, swimming in lamprey (Russel and Wallén 1983) and tadpole (Soffe and Roberts 1982) and fictive walking in rat (Cazalets et al. 1996) and locust (Ryckebusch and Laurent 1993). In the stick insect, application of the muscarinic agonist pilocarpine evokes alternating activity of antagonistic motoneurons (Büschges et al. 1995). In such a preparation, rhythmic activity in extensor and flexor motoneurons appeared to be based on tonic depolarization and cyclic hyperpolarizing synaptic input to the motoneurons (Büschges 1998). The apparent lack of rhythmic depolarizing input in this preparation is in contrast to the motoneuronal activity patterns observed during real walking or searching movements of the leg.

### *Membrane properties of motoneurons*

The resting membrane potential of fast motoneurons was on average −62 mV and thus about 10 mV more negative than that of semi-fast and slow motoneurons. In the light of numerous functional and physiological differences between fast and slow motoneurons (Burrows 1996), the difference in resting membrane potential is likely to contribute to differences in excitability, for example during sensory input. Elongation of the femoral chordotonal organ excites slow and fast extensor tibiae motoneurons in nonactive stick insects (Bässler 1983). Suprathreshold activation of the fast extensor tibiae motoneuron occurs at high stimulus velocities, whereas the slow extensor tibiae motoneuron responds over a wider range of stimulus velocities. Similar observations have been made in the locust (Field and Burrows 1982). Burrows (1996) suggested that sensory neurons coding different velocities make different connections with members of a motor pool, e.g., with the fast motor neurons receiving greater inputs from neurons coding higher velocities. In addition, the more negative resting membrane potential of fast motoneurons might contribute to the different responses of slow and fast motoneurons to input from the femoral chordotonal organ. Depolarizing input from the same source will most probably evoke more spikes in slow motoneurons than in fast motoneurons due to their lower spike threshold.

All motoneurons tested exhibited SFA when depolarized by current injection. Differences among slow, fast, or semi-fast motoneurons were not apparent. SFA was found to be effective at membrane potentials and spike frequencies that occur during walking and is therefore likely to affect the activity pattern of motoneurons during their phase of activity in the locomotor cycle. SFA has also been found in a variety of vertebrate motoneurons (Del Negro et al. 1999; El Manira et al. 1994; Magariños-Ascone et al. 1999; Sawczuk et al. 1995), although the functional implication of SFA in stick insect motoneurons is not yet apparent. As a general function of SFA, the prevention of excessive discharge is discussed by Sawczuk et al. (1995). In the lamprey locomotor network, a calcium-dependent potassium current that causes SFA plays a critical role in burst termination (El Manira et al. 1994). In any case, SFA is likely to shape the final output of the motoneurons during burst activity within the locomotor cycle.

During walking, fast flexor motoneurons were hyperpolarized in the swing phase of the leg movement. After the maximum initial hyperpolarization, the membrane potential declined steadily until burst activity started. A similar behavior was never observed in any of the other neurons. Consistent with this observation is that fast flexor motoneurons expressed a depolarizing sag potential when hyperpolarized by intracellular current injection. However, we cannot exclude decreasing hyperpolarizing input to fFlex that contributes to the depolarizing sag. The depolarizing sag potential that develops during hyperpolarization in swing phase would support a fast transition to depolarization in the subsequent stance phase.

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