

REVIEW | *Central Pattern Generators*

Six-legged walking in insects: how CPGs, peripheral feedback, and descending signals generate coordinated and adaptive motor rhythms

Salil S. Bidaye,^{1*}  Till Bockemühl,^{2*} and Ansgar Büschges²

¹Department of Molecular and Cell Biology and Helen Wills Neuroscience Institute, University of California, Berkeley, Berkeley, California; and ²Department of Animal Physiology, Zoological Institute, University of Cologne, Cologne, Germany

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Bidaye SS, Bockemühl T, Büschges A. Six-legged walking in insects: how CPGs, peripheral feedback, and descending signals generate coordinated and adaptive motor rhythms. *J Neurophysiol* 119: 459–475, 2018. First published October 25, 2017; doi:10.1152/jn.00658.2017.—Walking is a rhythmic locomotor behavior of legged animals, and its underlying mechanisms have been the subject of neurobiological research for more than 100 years. In this article, we review relevant historical aspects and contemporary studies in this field of research with a particular focus on the role of central pattern generating networks (CPGs) and their contribution to the generation of six-legged walking in insects. Aspects of importance are the generation of single-leg stepping, the generation of interleg coordination, and how descending signals influence walking. We first review how CPGs interact with sensory signals from the leg in the generation of leg stepping. Next, we summarize how these interactions are modified in the generation of motor flexibility for forward and backward walking, curve walking, and speed changes. We then review the present state of knowledge with regard to the role of CPGs in intersegmental coordination and how CPGs might be involved in mediating descending influences from the brain for the initiation, maintenance, modification, and cessation of the motor output for walking. Throughout, we aim to specifically address gaps in knowledge, and we describe potential future avenues and approaches, conceptual and methodological, with the latter emphasizing in particular options arising from the advent of neurogenetic approaches to this field of research and its combination with traditional approaches.

descending control; intersegmental coordination; motor control; motor flexibility; pattern generation

INTRODUCTION

Motility is often cited as one of the defining characteristics of animals. Most animals are motile during some time in their life cycle. Some movements are acute responses to a particular stimulus, whereas other longer timescale motor behaviors (also termed “motor rhythms”) are made up of repeating patterns of movements and encompass the basic behaviors essential for the survival of the animal, such as feeding, breathing, locomotion, and courtship. These motor rhythms are brought about by rhythmic contractions of the effector muscles, and the site for generation of these rhythms lies in the animal’s nervous system. How nervous systems generate such rhythms has been an active topic of investigation in neuroscience for several decades. Some motor rhythms, such as respiration or heartbeat,

consist of relatively few muscles contracting at fairly regular intervals throughout the life of the animal, and the underlying neural mechanisms producing such rhythms are also relatively easy to conceptualize. Motor rhythms such as walking, on the other hand, require coordinated contractions of a large number of muscles and are much more dynamic. Still, the rhythmic motor activities for all the motor behaviors mentioned above has collectively been shown to be generated by the contribution of specialized networks in the central nervous system (CNS) that can intrinsically generate a rhythmic motor output upon continuous activation by external inputs, either from the brain or from other regions of the nervous system, so-called “central pattern generators” (CPGs) (for reviews see, e.g., Katz and Hooper 2007; Marder and Rehm 2005; Orlovsky et al. 1999; Smith et al. 2013).

In contrast to most of the other rhythmic motor patterns mentioned above, the stepping pattern of an animal walking on a variable substrate needs to be constantly updated based on the unevenness of the ground as well as other environmental and

* S. S. Bidaye and T. Bockemühl contributed equally to this work.

Address for reprint requests and other correspondence: A. Büschges, Dept. of Animal Physiology, Zoological Institute, Zùlpicher Str. 47b, 50964 Cologne, Germany (e-mail: ansgar.bueschges@uni-koeln.de).

internal stimuli. This necessitates the integration of complex and state-dependent sensory information and CPG-derived output to produce the proper motor output for walking. As a consequence, the mechanistic details of the underlying neural circuits producing coordinated and adaptable rhythms are not yet clear and are targets of active investigation. Specifically, understanding neural circuits producing coordinated walking has been a focus of investigation across several animal species, including humans (Hooper and Büschges 2017), in part for the following reasons. First, walking is an essential constituent of the behavioral repertoire of most terrestrial animals. Furthermore, these studies offer an opportunity to investigate complex neural circuit functioning, where the output of the circuit is easily quantifiable (e.g., Orlovsky et al. 1999). Finally, walking forms one of the best modes of reliable terrestrial locomotion, and principles derived from the studies could be extrapolated to robotics and other engineering applications (e.g., Buschmann et al. 2015; Hooper and Büschges 2017).

With regard to the number of neurons, invertebrates in general, and insects in particular, possess a relatively simple nervous system and yet show complex walking behavior. This makes them ideal models for finding out the neural circuit principles that generate such a complex rhythmic behavior. In fact, studies spanning several decades of research have generated a great amount of literature in this field; in combination with the recent advances in neurophysiology and neurogenetics techniques, it seems an opportune time for unravelling key conceptual insights in this field, particularly with respect to the question, what role CPGs in the CNS play in the generation of a coordinated and adaptable locomotor output. In this article, we aim to review the current knowledge in this field with a special focus on experimental evidence for functioning of CPGs underlying insect walking across different insect species. Although for this special issue we focus our overview on CPGs in the context of walking, it is important to note that CPGs alone are not sufficient to produce and explain walking behavior in insects. We will therefore specifically report on observations illuminating how CPGs interact with sensory information and descending brain inputs to orchestrate coordinated movement of the six legs of a walking insect. We hope to synthesize an overall conceptual overview of where the field stands as a whole and lay out perspectives for promising future investigations aimed at addressing the gaps in our current understanding.

EVIDENCE FOR CPGS AS PART OF THE LEG MUSCLE CONTROL SYSTEM FOR WALKING IN INSECTS

Starting in the 1960s, investigations on the role and organization of rhythmically active neural networks in the CNS of animals underlying the generation of a rhythmic motor output for locomotion experienced a real boost (e.g., Clarac and Pearlstein 2007). Since then, central neural networks intrinsically capable of generating rhythmic motor outputs have been identified in a variety of systems. In 1960, Hughes and Wiersma showed that the completely isolated abdominal nerve cord of crustaceans was capable of generating a rhythmic motor output for beating of the abdominal swimmerets, small leglike appendages used for swimming (Hughes and Wiersma 1960). A year later, Wilson (1961) published a highly influential study, in which he showed that the completely deafferented

thoracic nerve cord of the locust only attached to the head would generate alternating activity in wing elevator and depressor muscles, when a continuous wind flow was directed at the animal's head. Such networks were termed central pattern generators (CPG), because they are capable of generating rhythmic motor output in the absence of phasic or rhythmic input. In the following years, the origin of rhythmic motor output in a variety of animals could be traced back to the activity of CPGs, e.g., chewing movements in crustaceans (Maynard 1966), respiration in dragonflies (Mill and Hughes 1966), cardiac activity in crustaceans (Hagiwara and Bullock 1957), locomotor rhythmicity in leeches (Kristan and Calabrese 1976), swimming in mollusks (Arshavsky et al. 1985), and feeding in snails (Kater and Rowell 1973). Together with research on vertebrate motor systems (for reviews see, e.g., Clarac 2008; Orlovsky et al. 1999), the aforementioned studies extended the insight into how the nervous systems can generate rhythmic motor output centrally. They built on work at the beginning of the 20th century by George Brown (1911, 1914), who showed in his classical experiments the existence of central rhythmogenic mechanisms in the cat spinal cord.

Interestingly, early studies that focused on insect walking as a form of rhythmic behavior emphasized the importance of sensory signals and reflexes and their putative sufficiency for establishing a proper motor output in this context (i.e., Wendler 1964; Wilson 1965). It was not until some time later that the first evidence for a role of CPGs for walking was provided in a study by Pearson (1972). This study showed evidence for the activity of a central neural network underlying the generation of alternating activity between the antagonistic coxal leg muscles and the motor neuron pools that innervate them. Subsequent studies on insects verified the existence of central neural networks underlying the generation of alternating motor activity. Bässler and Wegner (1983) showed that alternating activity in antagonistic leg motor neuron pools can be induced in the deafferented thoracic nervous system by tactile stimulation of the animal's head or abdomen (see also Büschges et al. 2004). Compelling evidence for the existence of CPGs driving alternating and rhythmic activity in leg motor neurons for locomotion in the segmental thoracic ganglia became available by the findings of Ryckebusch and Laurent (1991) in the locust, following up on previous findings in crustaceans (Chrachri and Clarac 1987). After pharmacological activation of muscarinic cholinergic receptors in the locust's isolated thoracic nerve cord, the authors reported the induction of long-lasting alternating and rhythmic activity in antagonistic leg motor neurons driving single leg joints. Since then, similar findings have been reported for the stick insect (Büschges et al. 1995), the hawkmoth (Johnston and Levine 2002), and the cockroach (Fuchs et al. 2011).

In locust and stick insect, data are also available on the phase relation in activity of motor neuron pools driving the three main leg joints of the insect leg, i.e., the thorax-coxa joint, generating horizontal protraction and retraction movements, the coxa-trochanter joint, generating vertical levation and depression, and the femur-tibia joint, generating extension and flexion of the tibia. In both species, the muscarinic agonist pilocarpine induced long-lasting alternating rhythmic activity in antagonistic leg motor neuron pools (Fig. 1A). Interestingly, in the locust (Ryckebusch and Laurent 1991) compared with the stick insect (Büschges et al. 1995), the authors reported

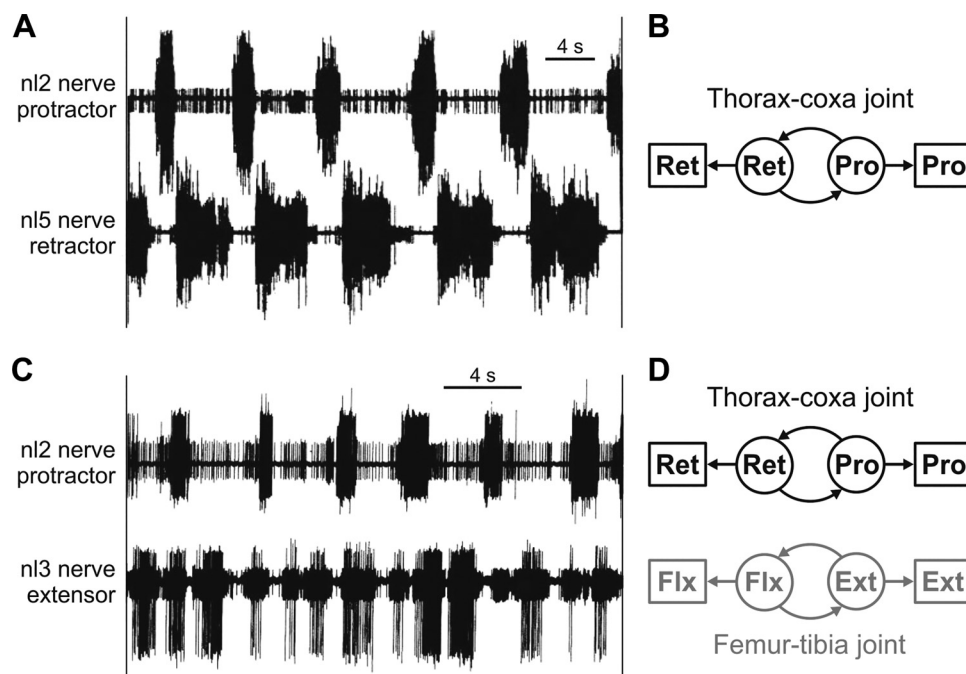


Fig. 1. Motor activity in leg nerves after application of pilocarpine in the deafferented thoracic nervous system (A and C) and schematics of the associated CPGs (B and D) (Büschges et al. 1995). A: alternating burst activity in the protractor/retractor motor system. Protractor muscles are innervated by the efferents in the nl2 nerve; retractor muscles are innervated by the efferents in the nl5 nerve. B: schematic of the half-center oscillator forming the protractor/retractor CPG driving the thorax-coxa joint. Circles denote mutually inhibiting interneuronal structures, which, in turn, activate retractor and protractor motor neuron pools and muscles (rectangles). C: protractor nerve activity (nl2) and simultaneous extensor nerve activity (nl3). In contrast to the protractor/retractor system, there is no clear coupling between the activities in these 2 nerves. D: schematics of the 2 half-center oscillators involved in the activity shown in C. Both of them can generate rhythmic motor output, but direct connections between the 2 CPGs are absent. Pro, protractor; Ret, retractor.

some coordination between motor neuron pools responsible for the three main leg joints: motor neuron pools that are typically active at the same time during the stance movement showed a preference for in-phase activity in this deafferented preparation. Because of this similarity, the authors concluded that the locust thoracic ganglia are capable of generating a “fictive stepping motor pattern,” albeit with higher variability. In contrast, data from similar stick insect preparations did not show a preferred phase coupling for consecutive cycles of motor activity (Fig. 1, C and D). Instead, a general independence of rhythmic activity in the antagonistic motor neuron pools of the three leg joints was observed; only rare and transient coordination between these motor neuron pools was reported. In summary, the stick insect data suggest that there exist at least three individual CPGs governing the three main leg joints, i.e., the thorax-coxa joint, the coxa-trochanter joint, and the femur-tibia joint (Büschges et al. 1995). It may be worthwhile to mention that in the locust, only those periods of the recordings were evaluated which showed obvious coordinated activity (Ryckebusch and Laurent 1991), whereas in the stick insect, all recordings were evaluated (Büschges et al. 1995). Further lines of evidence support the contribution of CPGs in insect walking: Borgmann et al. (2009) showed that a stepping front leg can activate and entrain alternating and rhythmic motor activity in the deafferented caudal segments. In turn, this activity can then be modified and entrained by stimulation of local sensory feedback pathways (Borgmann et al. 2009, 2011). Taken together, the results on the stick insect provide evidence for the existence of multiple CPGs in the thoracic ganglia of a stick insect. Each of those is capable of generating alternating activity in the antagonistic motor neuron pools innervated by them. These findings constituted the first verification of the unit-burst generator (UBG) concept formulated hypothetically by Grillner and Zangger (1979) for the organization of the neuronal networks controlling the cat hindlimb. This concept was formulated on the basis of notion that the various hindlimb motor patterns that can be produced by the cat’s spinal cord

could not result from just one CPG network. Recently, this organization of CPG networks was also found to exist in the mouse lumbar spinal cord controlling the hindlimb movements (Büschges and Borgmann 2013; Hägglund et al. 2013).

On the basis of these results, it is clear that CPGs play an important role in generating the motor output for stepping in insects. This gives rise to the question of how these CPGs are structured. Currently, there is no conclusive identification of the topology of the CPGs that contribute to this motor output in any insect. One persistent hurdle is the challenge of gaining targeted and reproducible electrophysiological access to central neurons in the insect nerve cord. A further complication in characterizing interneurons that constitute CPGs is differentiating neurons that process or relay external signals, intersegmental or phasic sensory signals, for instance, that occur naturally during stepping, from neurons that are actually part of the CPG network producing the rhythmic motor output.

Nevertheless, with these caveats in mind, there exist morphologically and electrophysiologically characterized interneurons, some across different insect species, that have been implicated in the generation of rhythmic motor output in the context of walking. It was first in the cockroach that Pearson and Fourtner (1975) identified a local nonspiking interneuron (interneuron I) in the metathoracic ganglion, which they proposed as a candidate CPG neuron. This interneuron showed membrane potential oscillations in synchrony with leg movements. Furthermore, small depolarizing or hyperpolarizing current injections in this interneuron caused changes in firing rates of several motor neuron pools, notably the levator motor neurons. Most significantly, small depolarizing current injections into the interneuron during particular phases of the motor neuron firing cycle could reset the rhythm. This indicated that this interneuron is an essential component of the pattern generation network. Similarly, in the stick insect, three local premotor nonspiking interneurons were identified as elements of the CPGs controlling the activity of motor neurons driving the coxa-trochanter and the femur-tibia joint in the mesotho-

racic leg, i.e., two interneurons of type E4 (Bässler and Büschges 1998; Büschges 1990; Büschges et al. 1994, 1995; Büschges and Wolf 1995; Sauer et al. 1996) and one interneuron of type I4 (Büschges 1995; Büschges and Wolf 1995; Sauer et al. 1996). In the pharmacologically activated isolated mesothoracic ganglion as well as during semi-intact leg stepping, current injection into interneuron E4 resulted in the reset of ongoing motor neuron activity and even stepping (Bässler and Büschges 1998; Büschges et al. 1994; Büschges and Wolf 1995). Currently, interneuron I4 is only known to exert this role in a pharmacologically activated preparation of the isolated mesothoracic ganglion (Büschges 1995). Interestingly, these interneurons identified in the stick insect show morphological similarities with interneuron I, described by Pearson and Fourtner (1975). Further studies cracking the topology of segmental CPG networks in the thoracic ganglia of insects are still needed to unravel the organization and operation of the CPGs that contribute to the generation of a coordinated locomotor activity during walking.

As outlined above, there is clear evidence that CPGs are important for walking in insects. However, as we will show repeatedly throughout this review, CPGs alone are not sufficient for establishing the proper motor output required for walking in insects. Instead, their basic rhythmicity is strongly shaped and influenced by sensory information; only combining these two elements results in *in vivo*-like walking behavior.

COORDINATING MULTIPLE JOINTS FOR SINGLE-LEG STEPPING

Depending on the species, an insect leg consists of at least five segments that can be moved individually. These are the coxa, trochanter, femur, tibia, and at least four tarsal segments. The segments are driven by more than a dozen muscles (Bässler 1983; Burrows 1996; Goldammer et al. 2012). The main leg joints between thorax-coxa, coxa-trochanter, and femur-tibia are controlled by pairs of antagonistic muscles; the coxa, for instance, is controlled by the protractor and retractor coxae muscles (Fig. 1A). The tarsal segments are under unified control, in particular by a single tripartite muscle, the retractor unguis. This muscle mediates bending of the tarsal segments. To generate leg movements, e.g., steps for walking, a specific temporal sequence of muscle activations has to be generated (e.g., Duch and Pflüger 1995; Fischer et al. 2001; Graham 1985; Graham and Epstein 1985; Graham and Wendler 1981; Rosenbaum et al. 2010).

Currently, the most comprehensive knowledge with regard to the neural mechanisms responsible for the generation of coordinated motor output for stepping exists for the middle legs of the stick insect (Büschges et al. 2008). The middle legs in the stick insect lend themselves well to the study of walking because they are functionally the least specialized compared with front and hind legs (Dallmann et al. 2016). The front legs, in addition to the fact that they are often used tactually for finding support in an often unpredictable environment (Cruse 1976; Grabowska et al. 2012; Graham 1985), are situated on the prothoracic segment. In phasmids, the small size and the specific internal structure of the prothoracic segment make it difficult to access, particularly when it comes to electrophysiological recordings in semi-intact preparations. The hind legs, on the other hand, are specialized for dealing with the physical

challenge that the center of mass of the animal is situated right between both hind leg coxae.

In the middle leg the role of signals from sensory organs on the leg has been studied with regard to their role in the generation of motor output for stepping movements. Particular focus was directed toward the questions of which of these signals modify motor output via reflex-like pathways and which act directly on CPGs. The signals that have been studied in greatest detail originate in two classes of sensory structures: the femoral chordotonal organ (fCO) (e.g., Bässler 1983; Hess and Büschges 1999), a movement sensor, and the campaniform sensilla (CS) (e.g., Akay et al. 2001, 2004, 2007; Borgmann et al. 2011), responsible for the detection of force and load. The specific influences of phasic and tonic signals of these sense organs on the activity of the CPGs driving the three main leg joints have been found to substantially contribute to the generation of the proper temporal sequence of motor activity during stepping (Fig. 2). During forward walking, for example, load signals from CS on the trochanter and femur initiate and maintain retractor and terminate protractor coxae activity (Akay et al. 2007). Furthermore, they initiate and maintain depressor and terminate levator trochanteris activity (Borgmann et al. 2011), and, finally, initiate flexor tibiae activity (Akay et al. 2001). In doing so, they assist the initiation and maintenance of the motor output required for stance movements. In contrast, CS signals mediating unloading of the leg support the transition to swing movement. Flexion signals from the fCO initiate levator trochanteris activity and terminate depressor trochanteris activity, whereas extension signals induce the opposite effect (Hess and Büschges 1999). This contributes to the transition between stance and swing movement and vice versa, respectively. Individual aspects of this sensory control have also been shown for other insects, e.g., for the role of movement feedback in the cockroach (Mu and Ritzmann 2008a, 2008b) and the locust (Knop et al. 2001; Zill 1985). Detailed accounts on the nature of the known sensory-to-central influences and their role in establishing the motor output for leg stepping can be found in previous reviews (Büschges 2005; Büschges et al. 2008, 2011; Daun-Gruhn and Büschges 2011; Ritzmann and Büschges 2007). Importantly, the sufficiency of the identified mechanisms for the generation of a single-leg stepping pattern was shown in three-dimensional dynamic simulations that integrated morphological features, muscle properties, and a neural control architecture based on neurobiological results (Ekeberg et al. 2004; von Twickel et al. 2011).

It is important to note that there are still major gaps in our understanding of the neural mechanisms underlying the insect leg stepping cycle:

1) Most of our current knowledge concerns the generation of straight forward stepping in a single middle leg. This movement pattern occurs only in a very specific condition, i.e., when the animal walks on a level surface. In addition, it only represents one of the many and flexibly intermingled movements an insect leg can generate. This becomes apparent when we consider the changes in leg kinematics necessary for climbing, walking vertically and upside down, negotiating obstacles, turning, or changes in walking direction. Leg movements during all these behaviors differ markedly in their kinematics compared with straight walking (Dürr and Ebeling 2005; Gruhn et al. 2006, 2009; Mu and Ritzmann 2008a). On the

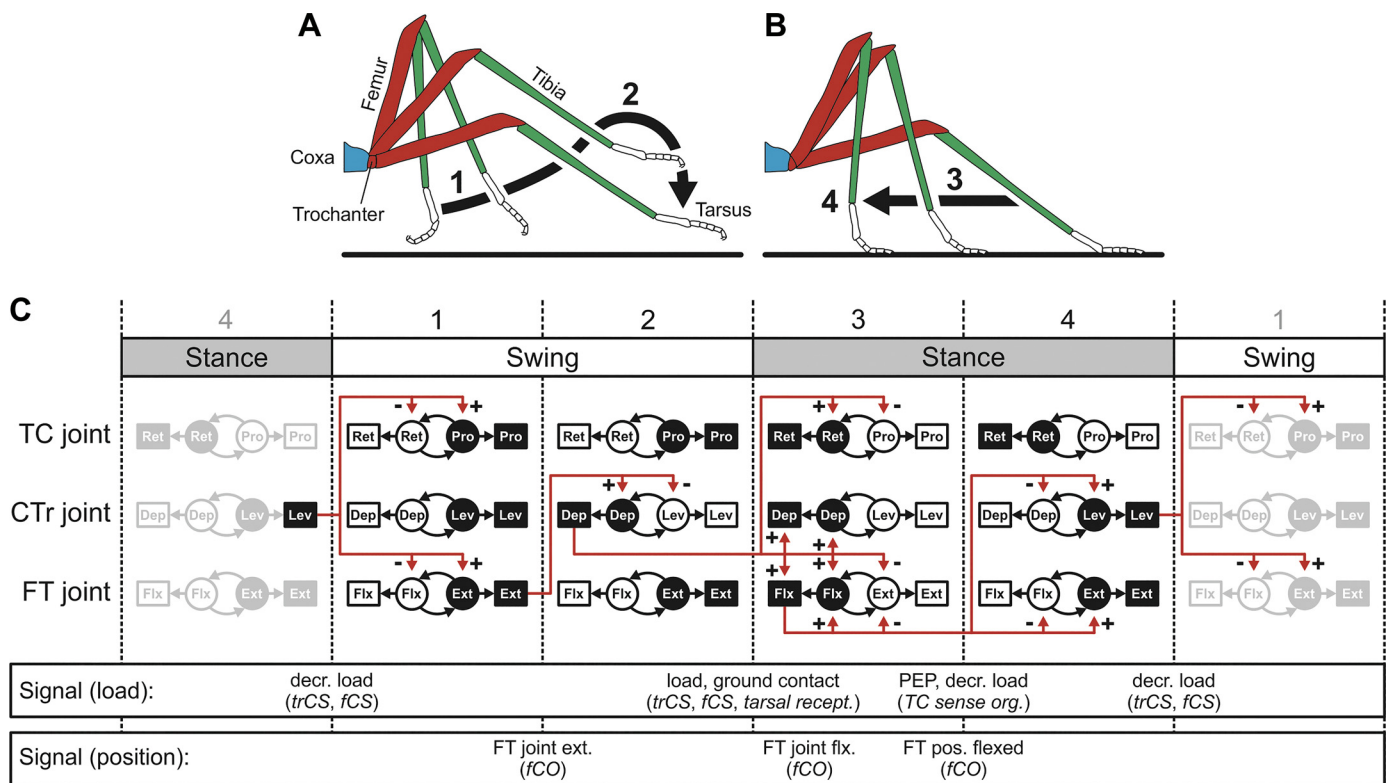


Fig. 2. Single-leg stepping in the stick insect. *A*: swing movement. *B*: stance movement. *C*: schematic overview of 4 distinguishable phases in one stepping cycle and the associated transitions in the activities of the 3 main leg joints, their muscles, and the CPGs driving them (TC, thorax-coxa; CTr, coxa-trochanter; FT, femur-tibia). *Phases 1 and 2* refer to swing movement, and *phases 3 and 4* refer to stance movement; due to the cyclic nature of stepping, *phase 4* is followed by *phase 1*. Each joint is driven by its own CPG (circles), which, in turn, drives motor neurons and leg muscles (rectangles). Ret, retractor; Pro, protractor; Dep, depressor; Lev, levator; Flx, flexor; Ext, extensor. Sensory signals [load, mediated by campaniform sensilla (CS), or position, mediated by the femoral chordotonal organ (fCO)] facilitate state transitions between the 4 phases, thus coupling the otherwise independent CPGs into a coordinated motor output.

basis of results presented here, it is quite conceivable that they may depend on different influences of sensory signals from other leg sensory organs. An insect leg is equipped with a fascinating setup of proprioceptors, the role of many of which for walking is still unclear. To name just a few, there are hair plates, internal stretch receptors, and muscle receptor organs (e.g., Bässler 1983; Bräunig et al. 1981; Burrows 1996; Tuthill and Wilson 2016).

2) Does the generation of leg stepping in insects other than the slow-walking stick insect depend on sensory feedback signals in a similar way? This question arises from the fact that online sensory feedback has a certain latency. In fast walking insect, with stepping frequencies of 20 Hz or higher, the time available for the execution of swing or stance movements drops below 20–30 ms. Under these conditions it becomes questionable whether transitions from swing to stance, or vice versa, can be generated exclusively by sensory feedback when delays in signal transmission by sensory activation, neural conduction times, and muscle fiber activation (Guschlbauer et al. 2007) are taken into consideration. Mendes et al. (2013) provided data on this issue for the fruit fly, which shows a broad range of stepping frequencies ranging from less than 1 Hz to more than 18 Hz (Wosnitza et al. 2013a). Mendes et al. (2013) used genetic silencing of sensory organs in the leg by expressing tetanus toxin light chain in most of the leg sensory neurons in adult flies. This led to the suppression of many synaptic outputs from leg sensory projections. Flies lacking this sensory feedback were still capable of producing stepping

activity in individual legs; the variability of the motor patterns was increased, however. These results suggest that, during fast walking, segmental coordination of single-leg stepping does not exclusively depend on sensory feedback. Additional central mechanisms might play a role for the coordination of muscle activity.

MOTOR FLEXIBILITY IN A STEPPING INSECT LEG: FORWARD AND BACKWARD WALKING, CURVE WALKING, AND SPEED CHANGES

How are the changes in stepping movements of an insect leg generated when the animal needs to bring about a change in direction or walking speed? These are key questions in understanding the mechanisms of how an animal generates goal-directed locomotor behavior. Some progress in this regard has been achieved by the characterization of changes in the influence and processing of sensory feedback in the generation of an appropriate motor output mediating the intended behavioral outcome. Although these experiments do not monitor the activity taking place in the central circuits, the timing and nature of activity changes at the motor neuron level provide important insights into the underlying mechanisms.

Forward vs. Backward Walking

We will first consider the relatively simple task of switching from forward to backward walking. A stick insect can be reliably induced to walk forward by touching its abdomen with

a brush. Backward walking can be elicited by touching or pulling its antennae. This stimulus-evoked and direction-specific walking is maintained even in tethered and partially reduced preparations (Akay et al. 2007; Graham and Epstein 1985). The main difference in motor output for a middle leg between the two directions is found in the activation of the motor neurons that innervate the muscles that move the leg forward and backward, i.e., the protractor coxae (ProCx) and retractor coxae (RetCx) motor neurons. Compared with forward walking, these muscles reverse their antagonistic activity pattern in such a way that during backward stepping, the ProCx is active during stance, whereas the RetCx is active during swing. The activation patterns of the motor neurons innervating the muscles of the more distal joints do not change in this direction-dependent manner (Rosenbaum et al. 2010). During forward stepping, RetCx motor neurons are active during stance; this is assisted by load signals mediated via CS on the trochanter. When the insect switches to backward walking, the same load signals induce activity in the antagonistic ProCx motor neurons. Importantly, the influence of CS signals has been shown to act on the CPG controlling the RetCx and ProCx motor neurons (Akay et al. 2007, see also Fig. 5). In a recent study, Rosenbaum et al. (2015) investigated the synaptic drive that motor neurons receive during the generation of motor activity in forward and backward stepping. Although no difference was detected in the tonic excitatory synaptic drive received by motor neurons supplying all leg joints after activation of the locomotor system (Büschges et al. 2004; Schmidt et al. 2001), the authors presented evidence for an exclusive reversion of the alternating phasic excitatory and inhibitory synaptic drive to coxal motor neurons, i.e., RetCx and ProCx motor neurons. This switch results in the reversal of their activity and, as a result, in the change in stance phase direction. Apart from load signals, also movement signals from the leg have been shown to contribute to a change in the motor output that is necessary for backward stepping for all legs. Hellekes et al. (2012) showed that flexion signals from the fCO exert walking direction-dependent influences on the activity of the motor neurons and muscles that control the femur-tibia joint of front and hind leg.

Curve Walking

When an animal switches from forward to backward walking the motor output of all legs has to be changed in a similar fashion; as outlined above, only the exact phase of activity in stance motor neurons has to be shifted. The situation is more complex when we consider curve walking. In insects, curve walking is brought about by body side-specific changes in leg kinematics. Legs on the inside of the curve pull the animal into the direction of movement, while the legs on the outside push the animal into the new direction of heading (Dürr and Ebeling 2005; Jander 1985). Tethered stick insects, like most other animals, can be induced to turn by using visual optomotor stimuli presented in their visual field (Gruhn et al. 2009). To investigate the central mechanisms involved in curve walking, the legs can be mechanically decoupled from each other by having the tethered insect walk on a slippery surface. Under these conditions, legs on the inside of a turn show strong tibial flexion during stance, whereas the legs on the outside show little, if any, flexion during stance (Gruhn et al. 2009). This

suggests that, again, task-dependent processing of sensory signals from both movement and load sensors plays an important role in generating the appropriate leg movements and kinematics for curve walking in a body side-dependent way. Hellekes et al. (2012) presented evidence for the contribution of flexion signals from the femoral CO to the body side-specific adaptation of the motor output: although flexion signals assisted ongoing flexor motor neuron activity in legs on the inside of a curve, the frequency of such an influence was reduced for legs on the outside of the curve. Gruhn et al. (2016) showed that load feedback signals from a middle leg have body side-dependent influences: on the outside of a curve, load signals initiated and maintained RetCx activity, whereas on the inside of a curve, no systematic influence of load feedback signals was detectable. Again, these differing influences are based on sensory feedback influencing the activity of, or even inducing a phase switch in, the CPGs contributing to the generation of the motor output for leg stepping.

Speed Changes

Changes in walking speed are generated by changes in the kinematics of single legs, mainly in their stepping frequency, as well as by changes in interleg coordination. We will first consider the knowledge on those mechanisms that influence the stepping frequency. Presently, two lines of evidence suggest that changes in the stepping frequency in insect legs result from modifications in the gain of sensory feedback; pathways that directly influence the CPGs might not be involved. Berendes et al. (2016) recently have shown that after single-leg amputation in the fruit fly, the remaining stump oscillates at a constant and comparatively high frequency. Crucially, this was independent of walking speed and the stepping frequency of the remaining intact legs. These findings suggest that descending signals from the brain have no or only little control over segmental CPGs active during walking. Second, Gabriel and Büschges (2007) have shown that in semi-intact stepping legs of a stick insect, it is the amount of phasic depolarizing synaptic inputs to stance motor neurons only that affects changes in stepping speed. Importantly, alterations in synaptic drive occurred during ongoing stance activity, and no changes were detected for synaptic inputs during swing phase in stance motor neurons. Also, no changes occurred in swing motor neurons with respect to peak and trough potentials. These findings are supported by analyses of the activity of the associated premotor interneurons (Rosenbaum et al. 2015; von Uckermann and Büschges 2009). Taken together, these findings suggest that sensory feedback pathways may be the target of descending influences that control walking speed in insects.

INTERSEGMENTAL COORDINATION

As described previously, walking in insects entails the proper spatial and temporal coordination among all six legs that participate in this behavior. The high-level description of this as well as the underlying mechanisms can be summarized with the term “intersegmental” or “interleg coordination.” During walking, each leg executes the cyclic stepping behavior of swing and stance movements; the step cycles of individual legs have to be coordinated with respect to each other to produce an overall activity pattern that can reliably propel the animal through its environment. It is self-evident that some

interleg coordination patterns are better suited for this than others: synchronously executing a swing movement with all six legs, for instance, would result in the animal tumbling. Beyond such basic stability-centric considerations, interleg coordination must bring about and accommodate changes in walking speed and heading, switches from forward to backward walking, and changes to different substrates, and must even take into account the accidental or intentional loss of one or more legs (Maginnis 2006). In general, two mechanisms might contribute to the coordination of rhythmic activity in several legs: direct coupling between the involved CPGs and sensory signals transmitting information about the state a leg is currently in.

Below, we will first briefly summarize the current knowledge about interleg coordination on the behavioral level. We will then discuss the contribution of central coupling to coordination. Finally, we will outline the sensory-dependent nature of the rules for interleg coordination and their putative neural basis.

Behavioral Aspects of Interleg Coordination

Basic (forward) walking behavior in insects consists of the successive protraction of three ipsilateral legs on each side of the body. This metachronal wave of activity is always directed anteriorly: after the protraction of a hind leg, the ipsilateral middle leg protracts, followed, in turn, by the protraction of a front leg, after which the cycle is repeated. This can be observed on both body sides and, apart from special situations, seems to be a relatively strict invariant in insect walking; there is, however, a phase difference between the two contralateral metachronal waves. Taken together, these two metachronal waves of activity constitute the normal walking behavior.

Several coordination patterns (often also termed gaits) have been described in the literature on insect walking. At low walking speeds, insects exhibit an interleg coordination pattern that most clearly illustrates the metachronal nature of ipsilateral leg protraction. Sometimes called “wave gait,” this coordination pattern is characterized by very long stance durations (Hughes 1952). As a result, there is at most one leg that executes its swing movement at a given time. At intermediate walking speeds, insects use a coordination pattern in which, maximally, two legs are lifted off at the same time (Graham 1972). This coordination pattern is called “tetrapod coordination,” which refers to the fact that at least four legs have ground contact at all times. The anteriorly directed metachronal wave of protractions of ipsilateral legs is maintained in this coordination pattern. However, the stance durations of individual legs are shorter; as a result, the swing movements of two legs on contralateral body sides can overlap. The phase shift between the two body sides ensures that two contralateral legs of the same segment never execute their swing phase at the same time. Finally, at high walking speeds, insects use a coordination pattern called “tripod coordination” (Graham 1972; Strauss and Heisenberg 1990; Wahl et al. 2015; Wosnitza et al. 2013a). As the term implies, at least three legs are on the ground at any given time. Strikingly, two groups of three legs each (a front leg, an ipsilateral hind leg, and a contralateral middle leg) execute their swing movements synchronously, and the two groups alternate with a phase difference of 180°. During tripod coordination, the duration of stance movements

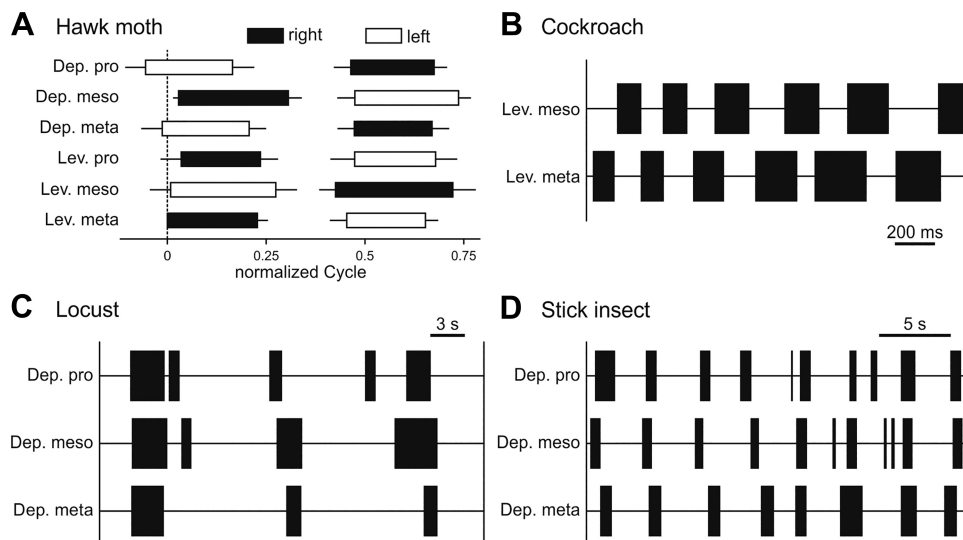
becomes even shorter, to the point where it is similar to the duration of swing movements or even shorter in very fast running insects (Wahl et al. 2015). As a consequence, the metachronal wave of protractions is not clearly visible anymore, because the stepping frequency of a single leg is higher than the frequency of the metachronal wave. Tripod coordination is often regarded as the typical coordination pattern insects use in general. Although it may be true that many insects predominantly use tripod coordination, one reason for a biased observation of tripod coordination might be based on the fact that many of the studies that investigated interleg coordination during walking have been carried out on fast walking insects such as the cockroach. But even for the cockroach, typically a fast runner, and the desert ant, among the fastest insects, there is strong evidence that they use other interleg coordination patterns when they walk at slower speeds (Bender et al. 2011; Wahl et al. 2015).

Central Aspects of Interleg Coordination

Several studies on different insect species have provided evidence for direct connections between CPGs involved in walking. As described in EVIDENCE FOR CPGs AS PART OF THE LEG MUSCLE CONTROL SYSTEM FOR WALKING IN INSECTS, these studies take advantage of the fact that the ventral nervous cord of insects can readily be deafferented, thus removing all potential sensory influences and allowing for the investigation of potential direct connections between CPGs in the different ganglia. Furthermore, it has been found that rhythmic motor output, alternating activity in the depressor/levator motor system, for instance, can be elicited in these preparations by application of pilocarpine, a muscarinic cholinergic agonist (Ryckebusch and Laurent 1991). The resulting motor output is often called “fictive locomotion.” In general, these studies invariably find that direct connections between rhythmogenic networks exist in insects; however, the extent to which these couplings establish functional locomotor patterns with regard to interleg coordination varies.

Johnston and Levine (2002) studied fictive locomotor patterns in the deafferented ventral nervous system of the hawkmoth (*Manduca sexta*) after the application of pilocarpine (Fig. 3A). They recorded from the nerves that innervate the levator and depressor muscles in all ipsilateral ganglia. These muscles are antagonists and are active in alternating fashion. In intact animals, the switch in activity between levator and depressor occurs during the late swing movement, when the leg is lowered onto the ground, or during the late stance movement, when the leg starts to lift off (Fig. 2). Not only did this study find that the motor neuron pools driving antagonistic muscles in a single leg produce alternating motor output, but it also provided evidence that the output of all three ipsilateral hemiganglia is coordinated: the levator motor output of the prothoracic ganglion occurred simultaneously with that of the metathoracic ganglion; at the same time, the depressor motor output of the mesothoracic ganglion was active. The same type of coordination was observed for the pro- and metathoracic depressors and the mesothoracic levator. In general, this fictive coordination pattern suggests a direct central connection between the rhythmogenic networks in the individual ganglia; furthermore, it resembles what would be expected during tripod coordination in the intact animal. In the cockroach

Fig. 3. Fictive locomotion patterns in 4 different insect species. For clarity, original recordings were schematized in *B–D*. In all preparations shown, rhythmic activity was elicited by application of pilocarpine, and all were deafferented. *A*: relative phase of the burst activity in depressor and levator nerves in all ipsilateral hemiganglia of the hawkmoth (Johnston and Levine 2002). Activity in *A* was normalized to the onset of the metathoracic levator burst. *B*: levator nerve recordings in the ipsilateral mesothoracic and metathoracic hemiganglia of the cockroach (Ayali et al. 2015). *C*: depressor nerve recordings in all ipsilateral hemiganglia of the locust (Knebel et al. 2017). *D*: depressor nerve recordings in all ipsilateral hemiganglia of the stick insect (unpublished data, but see Mantziaris et al. 2017). Dep, depressor; Lev, levator; meso, mesothoracic; meta, metathoracic.



(*Periplaneta americana*), the situation seems to be comparable (Fig. 3*B*). Deafferented and pilocarpine-activated cockroach preparations have the propensity to produce fictive locomotor patterns that resemble tripod coordination found during actual walking (Ayali et al. 2015; Fuchs et al. 2011).

Studies on the locust and the stick insect support the idea that direct connections between walking-related CPGs exist. Contrary to the findings in the hawkmoth and the cockroach, however, the putative connections in the locust and the stick insect do not produce motor output that resembles interleg coordination in intact animals. Knebel et al. (2017) simultaneously recorded pilocarpine-induced activity from the efferents of the depressor motor neurons in the locust (*Schistocerca gregaria*), either in all ipsilateral hemiganglia or in contralateral hemiganglia. Interestingly, they found that the ipsilateral depressor motor neurons of all the three hemiganglia tend to fire simultaneously, or in phase (Fig. 3*C*). The same general tendency was found for the intrasegmental activity in contralateral hemiganglia (left-right coordination) in the pro- and mesothoracic ganglia. Only the metathoracic hemiganglia had a tendency to burst in an alternating fashion. A similar study (Mantziaris et al. 2017) in the stick insect (*Carausius morosus*) found essentially the same: ipsilateral depressor motor neuron pools had a tendency for in-phase activity (Fig. 3*D*). The same was true for contralateral depressor motor neurons of the same segment. In the locust as well as in the stick insect, the centrally generated fictive motor patterns observed in the presence of pilocarpine do not constitute a functional interleg coordination pattern observed in intact animals during walking.

In conclusion, these studies generally suggest that there are direct connections between at least some of the CPGs involved in walking, mainly the depressor/levator motor system of different legs. A further general finding in these studies is that there is a higher variability in coordination compared with intact animals. In the hawkmoth and cockroach, the motor patterns that result from these couplings even have similarities to coordination patterns (mainly tripod) observed in intact animals. The deafferented nervous systems of the locust and the stick insect, on the other hand, do not produce activity patterns resembling coordinated walking. In addition, even in the hawkmoth and the cockroach, fictive motor output seems to be a fixed and preferred pattern; in contrast, intact insects,

especially the cockroach, are known to be able to use other coordination patterns (Bender et al. 2011). These considerations raise the question of how behaviorally relevant functional interleg coordination is achieved at all (in the locust and stick insect) or how it can be adapted flexibly (in the hawkmoth and cockroach).

Interleg Coordination Rules

A considerable number of studies, mainly in the stick insect, have investigated the behavioral mechanisms that might give rise to interleg coordination in insects. Although there is evidence that the basic motor output for individual joints is strongly shaped by CPG-like neural structures and individual CPGs might be coupled weakly by direct connections, these studies suggest that coordination among legs strongly relies on sensory signals. Sensory signals directly relevant for interleg coordination mainly originate in sensory structures in the legs, such as hair fields and rows, chordotonal organs (Field and Matheson 1998), and campaniform sensilla (Zill et al. 2004). Furthermore, these signals seem to be mainly active between directly neighboring legs, i.e., adjacent ipsilateral legs or contralateral legs of the same segment. Based on these studies, a small set of comparatively simple behavioral rules have proved to be sufficient for the coordination of six-legged walking (Fig. 4). Sometimes these rules are subsumed under the term “Cru(i)se control,” in allusion to Holk Cruse, who coauthored many of the relevant studies (for further reviews see, e.g., Cruse 1990; Cruse et al. 2007; Dürr et al. 2004). Most of these rules ensure the proper temporal coordination between legs, whereas one rule facilitates spatial coordination. In the following, we will briefly characterize five of the six behavioral rules important for interleg coordination (*rule 6* will not be discussed because it is relatively specialized).

Rule 1. This rule is a temporal rule and is active in anterior direction between ipsilateral legs, i.e., between a hind leg and a middle leg, and between a middle leg and a front leg (Cruse and Epstein 1982; Graham 1978). *Rule 1* states that when a leg is lifted off, i.e., during its swing movement, liftoff in the adjacent anterior target leg is suppressed and the stance movement of this target leg is prolonged.

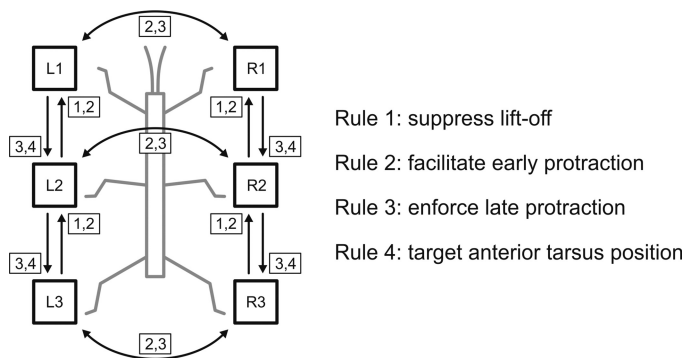


Fig. 4. Schematic overview of the coordination rules sensu Cruse (Cruse 1990; Dürr et al. 2004). Four coordination rules are sufficient for the temporal and spatial coordination observed in walking insects (for clarity, rules 5 and 6 are not discussed here). Individual legs are indicated by numbers (1, 2, and 3 for front, middle, and hind leg, respectively) and letters (L and R for left and right, respectively). Coordination rules act locally; i.e., they are active only between directly neighboring legs (ipsilateral and contralateral); there is no transsegmental information transfer. Rules 1–3 contribute to temporal coordination; rule 4 contributes to spatial coordination.

Rule 2. This temporal rule is active in anterior direction between ipsilateral legs and between contralateral legs of the same segment (Cruse and Knauth 1989; Cruse and Schwarze 1988). Rule 2 states that when a leg touches down, i.e., at the end of its protraction, it facilitates the onset of protraction in the adjacent target leg.

Rule 3. This temporal rule is active in posterior direction between ipsilateral legs and between contralateral legs of the same segment (Cruse and Knauth 1989; Cruse and Schwarze 1988). During an ongoing stance movement, there is an influence on the adjacent target leg to start its swing movement if it has not yet done so. The strength of this influence increases the closer the anterior leg is to its liftoff position.

Rule 4. This rule is responsible for spatial coordination and is active in posterior direction between ipsilateral legs (Cruse 1979; Dean and Wendler 1983; Wosnitza et al. 2013b). A leg's current tarsal touchdown position influences the prospective touchdown position of a directly posterior leg; this rule acts as a targeting mechanism between a middle leg and a hind leg, and a front leg and a middle leg.

Rule 5. This rule does not act specifically between individual leg pairs but is more of a global coordination rule: when load is increased in one leg, the stance duration of all other legs is prolonged, thereby adjusting force to keep the animal's body lifted up (Cruse 1985; Schmitz 1993). This effect can occur due to normal liftoff of legs during walking and the subsequent temporary reduction in the number of supporting legs, or to a general increase in load after the complete loss of a leg, for instance. For clarity, this rule has been omitted from the schematic drawing in Fig. 4.

It is important to note that in their original formulation, all of these rules are local in nature; i.e., their influence is active between adjacent (ipsilateral or contralateral) leg pairs. In that notion, the overall coordination of all six legs during walking is an emergent property of the coordination between leg pairs. Furthermore, these coordination rules are thought to be based on acute sensory information that is continuously transmitted between legs. Thus they are able to take into account external perturbations due to substrate variability, for instance, or general motor noise degrading the accuracy of motor output. In

combination with the basic motor output provided by CPG activity, these sensory influences are able to shape the rhythmic stepping of individual legs into a coordinated whole.

Sensory Mechanisms for Interleg Coordination

As mentioned in the previous paragraph, the neural signals mediating interleg coordination are thought to be mainly sensory in nature and originate from several sensory organs in the legs of walking insects. Among those are chordotonal organs (Field and Matheson 1998), hair fields and rows, and campaniform sensilla (Zill et al. 2004). The first two types of sensory structures (chordotonal organs and hair fields/rows) measure kinematic aspects of leg movements, i.e., position, velocity, and acceleration, whereas campaniform sensilla respond to cuticular strain that occurs when the leg is loaded. All of these structures are putative sources for the neural signals facilitating interleg coordination and, in fact, have been shown to have, at least in principle, intersegmental influences. We briefly review some of the studies that have shown the relevance of these sensory structures for interleg coordination. It has to be noted, though, that explicit information on neural pathways and the specific contribution of sensory signals for interleg coordination is still sparse.

Stein et al. (2006) stimulated the femoral chordotonal organ (fCO) in the middle legs of the stick insect while monitoring the activity of the motor neurons responsible for tibia extension and flexion in several other legs. Their results show that there is a clear influence of this sensory stimulation on the recorded motor neurons in the contralateral middle leg whose spiking activity followed the applied stimulus. Furthermore, application of picrotoxin to decrease general inhibition of the CNS revealed that this stimulus-locked response was present in all legs, indicating that acute sensory information originating in the middle leg is transmitted to all other legs. It is not directly clear, however, how these results relate to walking, since the preparation they used was largely deafferented, thus abolishing sensory signals in the affected legs.

To characterize the neuronal basis of interleg targeting (rule 4; see above), Brunn and Dean (1994) recorded neuronal activity from interneurons in the metathoracic ganglion while moving the ipsilateral mesothoracic leg; the aim of this study was to find the neural correlates for targeting information in the ganglion associated with the targeting leg. Previous studies (Cruse et al. 1984; Dean and Wendler 1983) had already implicated hair fields and rows, as well as the femoral chordotonal organ, as the source of this sensory information for targeting. Indeed, this study found several interneurons projecting to the metathoracic ganglion, as well as one local interneuron that responded systematically to changes in the current posture of the middle leg. Interestingly, the responses of these neurons can be explained as a neural representation of the middle leg's joint angles, rather than a representation of its tarsus position in external coordinates.

Ludwar et al. (2005) investigated the influence of the front leg fCO on the output of mesothoracic protraction/retraction motor neurons as well as extensor/levator motor neurons in the stick insect. In this study, the animal was completely deafferented, apart from sensory structures in a fixed front leg stump. During short bouts of alternating activity in mesothoracic motor neurons, the front leg fCO was elongated, mimicking

flexion of this leg. This fCO stimulation promoted flexor and retractor activity in the ipsilateral mesothoracic motor neurons, indicating an intersegmental influence of sensory signals originating in the fCO. Crucially, in the same study, a completely deafferented preparation showed no intersegmental coupling between the same motor neurons during pilocarpine induced activity.

The results shown by Ludwar et al. (2005) are supported by recent findings in the cockroach. In their study, Fuchs et al. (2012) used movements that were imposed externally on the front and hind leg and investigated the effects of these stimulations on fictive locomotor rhythms evoked by pilocarpine in mesothoracic efferents. Similarly to the study by Ludwar et al. (2005), they found front or hind leg movement-related influences on the activity in the mesothoracic levator efferents that were entrained by the imposed rhythm. Furthermore, the entrainment was frequency dependent, i.e., movement frequency in the intact legs determined the frequency of the fictive locomotor rhythm.

The studies outlined so far in this section investigated sensory signals related to positional information, i.e., sensors like hair plates and the fCO. Campaniform sensilla (CS) might be involved in interleg coordination, as well, but in a more indirect manner. Zill et al. (2009) showed in the freely walking cockroach that the unloading of a middle leg elicits strong activity in tibial CS in this leg. Surprisingly, the observed unloading was not induced by the onset of the swing movement in the middle leg, as one would expect, but even earlier by the touchdown and onset of the stance movement in the hind leg. The touchdown of the hind leg takes over some of the animal's body weight, thus reducing it in the middle leg, in turn inducing unloading. Activity in the middle leg's tibial CS might then promote liftoff. This elegant mechanism partly relies on mechanical interactions between legs that are picked up locally by sensory structures.

In concluding this section, it has to be noted that a lot is known about interleg coordination in walking insects on the behavioral level. This has led to the formulation of a small set of decentral coordination rules that have been shown in simulations such as the WalkNet (e.g., Cruse et al. 1998; Schilling et al. 2013) and in walking robots (e.g., Schmitz et al. 2008) to be sufficient for proper and insect-like interleg coordination. The picture is much less clear with regard to the neural mechanisms and structures underlying these rules. There are several pieces of information regarding the interplay between central and sensory processes and how sensory signals shape the basic rhythmic output. However, a coherent, comprehensive, and integrated view on the neural level of interleg coordination during walking is still elusive. All current findings with regard to neural processes are based on reduced preparations in which only isolated aspects have been investigated. Many of the findings discussed in this review have been investigated in experiments in which animals have been activated pharmacologically or in which only few legs, or even one leg, actually showed behavior that resembled walking. To arrive at a more complete picture of how, for instance, the Cruse rules are implemented neuronally, intact walking preparations are necessary that, on the other hand, allow for the simultaneous recording and stimulation of CPG neurons, sensory neurons, and interneurons. This will let researchers answer questions that address the individual contributions of

direct central coupling between rhythmogenic networks and more indirect coupling of legs via sensory signals mediated, for instance, by mechanical coupling.

DESCENDING CONTROL OF MOTOR OUTPUT IN INSECT WALKING

Although most of the neural machinery to generate a coordinated hexapod walk resides in the insect nerve cord, descending inputs from the brain are instrumental for initiating and manipulating these neurons so as to achieve goal directed locomotion. The principles of descending control appear to bear similarities to vertebrates (Orlovsky et al. 1999). In walking insects, however, knowledge on the specifics of descending control of locomotion and the contributing descending neuronal pathways, i.e., initiation, maintenance, directional control, and stopping, is still very limited (e.g., Bidaye et al. 2014; Böhm and Schildberger 1992; Martin et al. 2015).

Importance of descending neurons in walking control. One of the most striking “experiments” that shows the importance of descending influence on insect walking has been performed with very high reproducibility for millions of years by parasitoid jewel wasps (*Ampulex compressa*) on cockroach subjects. These wasps are parasites that hatch and feed as larvae on live cockroaches. The mother wasp has evolved an exceptional host control mechanism to provide the perfect source of nutrition for its progeny (reviewed in Libersat and Gal 2014; Libersat and Gal 2013). When the parasitic wasp encounters its host cockroach, it performs an intricate “surgery” on the cockroach involving a quick thoracic sting to temporarily inhibit host leg movements, followed by a slower and highly precise mechanoreceptor-guided injection of a neurotoxin into a specific brain region of the cockroach. The wasp toxin specifically disables the ability of the cockroach to self-initiate walking. Radioisotope tracing of the wasp venom in the cockroach brain indicates that the venom is mostly concentrated in the gnathal ganglion (previously called subesophageal ganglion, but see Ito et al. 2014) and a few higher brain areas such as the central complex neuropil (Haspel et al. 2003). Following this successful host control surgery, the wasp leads the cockroach to its nest by pulling on its antenna. Only higher level control signals are affected by the wasp venom, so the local control circuits situated in the ventral nerve cord, including CPGs responsible for walking, are potentially functioning in response to the wasp pull. The wasp is thus able to guide the walking cockroach all the way to its nest. Once there, the wasp lays an egg on the host and seals the nest so that its progeny will hatch in a protected and nutritive environment. If an experimenter interferes with this process and removes the wasp egg before hatching, the cockroach is able to regain complete control of its walking after a few days. This shows that the wasp toxin specifically and transiently inactivates certain brain or gnathal ganglion neurons responsible for the initiation of walking. In fact, researchers have now reproduced the effects by focal injection of wasp venom to different brain regions of the cockroach and have shown that injection of the venom into the region of the gnathal ganglion or the region of the central complex alone is sufficient to induce hypokinesia in the cockroach. The wasp probably injects venom in multiple regions to ensure prolonged hypokinesia in its host. These observations of the wasp-cockroach interaction clearly demonstrate the impor-

tance of descending signals from both the gnathal ganglion and regions in the cerebral ganglia in controlling the initiation of walking.

In addition to the initiation of walking, descending signals also play an important role for the cessation of walking and in controlling speed and direction. Furthermore, they control sensory-motor influences that contribute to the generation of leg stepping. The latter function has recently been shown for the first time by Martin et al. (2015), who provided evidence that descending signals from the central complex in the insect brain can modify the sign of local reflexes in the cockroach walking system. All these functions of the descending brain commands are manifested due to the precise manipulation of the downstream CPGs and local circuits that were described in earlier sections, in response to specific environmental and internal stimuli. To fully appreciate the flexible and adaptable nature of insect walking, it is imperative to first understand how sensory information is represented in the activity of descending interneurons and to then understand how these activity patterns transform the output of downstream thoracic neural circuits consisting of CPGs and local and intersegmental neurons involved in walking. To this end, researchers have either recorded activity of descending interneurons in response to sensory stimuli or experimentally manipulated the activity of descending interneurons and observed its effect on behavior or motor activity during walking. In the following paragraphs, we review key experimental observations that provide insights in this direction.

Similar to the observations in the wasp-cockroach interaction, early studies on decapitated and decerebrate (cervical connective lesioned) insects showed that information from descending inputs is critical for self-initiation of walking. This is a consistent observation via different types of manipulation (cutting, crushing, or decapitation) across several insect species, including fruit flies (Yellman et al. 1997), cockroaches (Ridgel and Ritzmann 2005), stick insects (Graham 1979), or praying mantises (Roeder 1937). This shows that also in insects, as in vertebrates (Orlovsky et al. 1999), descending inputs from the brain are essential to switch on the CPG activity required for walking. In contrast, crushing or lesioning of circumesophageal connective (connection between the higher brain regions and the gnathal ganglion) does not lead to a cessation of walking. In fact, these insects are still able to demonstrate a coordinated tripod interleg coordination. The only impairment in their walking behavior seems to be the lack of proper speed and direction control. These observations indicate that interneurons situated in the gnathal ganglion are sufficient for initiation of normal forward walking, whereas descending interneurons in higher brain regions are potentially important for the control of walking speed and direction (Gal and Libersat 2006; Graham 1979; Ridgel and Ritzmann 2005).

Electrophysiological characterization of descending interneurons. To precisely pinpoint descending interneurons involved in walking, researchers have recorded from several descending interneurons across different insect species. Böhm and Schildberger (1992) identified an ipsilateral descending interneuron (IDIN) in crickets whose activity was highly correlated with walking initiation. Moreover, on current injection, this neuron caused initiation of walking, whereas on hyperpolarization, it led to cessation of walking. However, the authors note that on several occasions the animal continued to walk while IDIN

stopped firing. This indicates that it is not required for the maintenance of a walking state. Nevertheless, IDIN is indeed a very interesting electrophysiologically characterized descending interneuron that might be instrumental to switching on the downstream nerve cord walking circuits. Another more recent study (Zorović and Hedwig 2013) has identified three other descending interneurons in the cricket that respond to auditory stimuli and can induce walking on current injection. Interestingly, in addition to inducing walking, these neurons also induced a rotational component. B-DI1(1) is an ipsilateral descending interneuron that induced ipsilateral turns, B-DI1(2) is an ipsilateral descending interneuron that induced contralateral turns, and B-DC(5) is a contralateral descending interneuron that induced contralateral turns. However, only B-DC(5) led to cessation of ongoing walking when it was hyperpolarized. Also, the authors note that the latency for the onset of walking after current injection by all three neurons was much longer (150 ms) compared with latency of spontaneous spiking-induced walking (30 to 50 ms). This suggests that normally these neurons might be working in cohort and are not individually responsible for walking initiation. Also, their response to auditory stimuli indicates that these neurons might be essential for steering the cricket toward chirping stimuli produced by conspecifics. These neurons must therefore bring about both the activation and modulation of downstream local circuits. Along similar lines, neurons responsive to antennal touch and implicated in steering control have been characterized in stick insects (Ache et al. 2015) and cockroaches (Burdoan and Comer 1996). However, their precise role in walking control has not been identified.

These electrophysiological characterizations of descending interneurons provide important information regarding how sensory information received via head sensory organs is encoded in the activity of single descending interneurons. This will greatly aid in understanding how a particular stimulus drives a specific change in the walking pattern of the insect. However, as seen from many of the recording experiments, often multiple descending interneurons respond to similar stimuli, and to fully understand how naturalistic goal-directed walking is achieved, it may be necessary to record from multiple interneurons at the same time. Moreover, to understand how activity of descending interneurons is translated into changes in local CPG-generated stepping patterns and intersegmental coordination patterns, it is necessary to record activity of these downstream neurons while manipulating activity of the descending interneurons. Such experiments will require reproducible access to the same descending interneuron. Although electrophysiological access to these neurons provides a powerful method to record and manipulate the neuronal activity, this technique does not always allow reproducible experiments on the same identified neurons. Physiologists use anatomical landmarks to guide their electrode to specific sites in the insect brain and then dye-fill the neurons after recording to capture their full morphology. In certain cases, the unique shape, position, and electrical properties of the cell aid in reproducible access to the same cell; however, in case of neurons in the insect CNS, this is rarely true. This makes it difficult to have multiple recordings in a given study, and even more difficult for researchers in other laboratories or future studies to reproduce and build upon existing results. *Genetic*

targeting of descending neurons highlights genetics-based strategies to achieve this in a reliable way.

Genetic targeting of descending neurons. Genetic access to a neuron not only overcomes the hurdle of physically targeting the central neurons with electrodes, but it also provides a means for reproducible targeting, thereby allowing future studies across several laboratories to successfully reproduce the original results and even characterize the neuron further. However, the surge in sophistication of genetic targeting techniques and development of neurogenetic reagents for neuronal manipulation and activity imaging is fairly recent compared with electrophysiological techniques. As of now, most of the genetic targeting efforts are focused on the highly explored genetic model insect, *Drosophila melanogaster*. The predominant method for gaining genetic access to central neurons in *Drosophila* is via the bipartite GAL4/UAS system (Brand and Perrimon 1993). This system (reviewed in Hampel and Seeds 2017) relies on a genomic enhancer controlled expression of the yeast transcriptional activator *GAL4* in specific neuronal subsets. When *GAL4* expression is coupled with the presence of another transgene, the effector, under the control of a *GAL4*-specific upstream activator sequence (UAS), the *GAL4*-targeted neurons will also express the effector protein. At present,

there is only one case of a specific genetically targeted descending interneuron implicated in insect walking.

Bidaye et al. (2014) used a neural activation *GAL4* screen to search for potentially interesting genetically targeted neurons in the context of walking. The screen relied on use of a newly created *GAL4* library (the Vienna Tiles *GAL4* collection, VT-*GAL4*) that targeted specific and sparse neuronal populations in the entire CNS of the fly (Bidaye et al. 2014; von Philipsborn et al. 2011; Dickson BJ, unpublished observations). They crossed the *GAL4* driver lines of this collection with a recently discovered thermogenetic activation reagent, UAS-TrpA1 (Hamada et al. 2008). This approach leads to expression of TrpA1 (a heat-sensitive cation channel) in targeted neuronal populations, which could then be depolarized by raising the temperature of the assay chamber. In this way, the authors screened over 3,400 different *GAL4* lines and found one particularly interesting line, dubbed “moonwalker.” On thermogenetic activation, flies of this line performed continuous long backward walking bouts. Further intersectional genetic approaches involving the split-*GAL4* system (Luan et al. 2006; Pfeiffer et al. 2010) led the authors to identify a specific descending interneuron, called “moonwalker descending neuron” (MDN). This neuron was sufficient to initiate backward walking on activation (Fig. 5C). Moreover, silencing

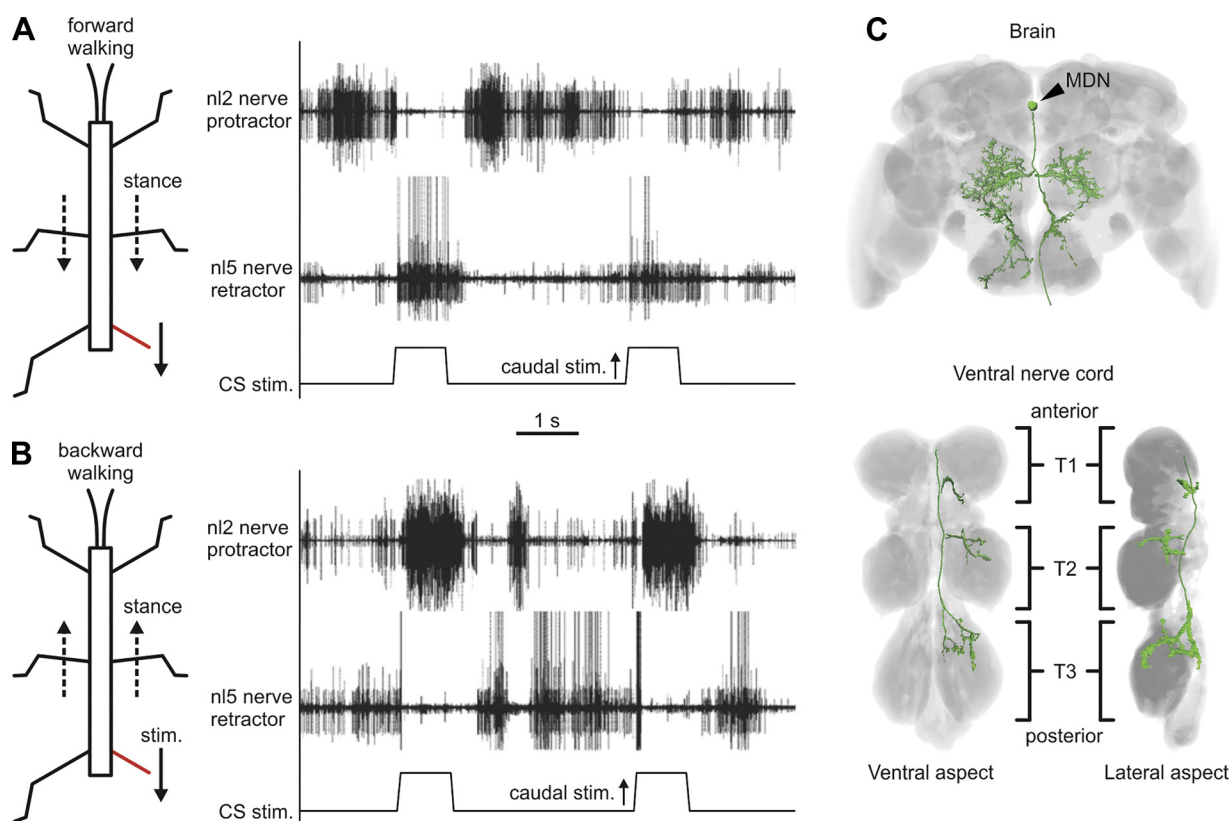


Fig. 5. Differential processing of sensory information during forward and backward walking in the stick insect (A and B; Akay et al. 2007) and descending control of walking direction in the fruit fly (C; Bidaye et al. 2014). A: recordings of the protractor (nl2) and retractor (nl5) nerves in a fixed hind leg (red) of the stick insect during a walking episode of the remaining 5 legs. During forward walking, bending the hind leg caudally, thus stimulating campaniform sensilla (CS), terminates protractor activity and initiates retractor activity. This can be interpreted as a fictive stance-swing transition. B: the same recordings during backward walking. In this situation, bending the hind leg caudally induces the opposite reaction, i.e., termination of retractor activity and initiation of protractor activity. This can be interpreted as a fictive swing-stance transition. C: descending interneuron in the fruit fly's brain that is sufficient and necessary for the initiation of backward walking (soma and arborizations labeled green, soma indicated by arrow). Called moonwalker descending neuron (MDN), this neuron projects to all 3 ganglia in the ventral nerve cord (T1, T2, and T3, homologous to the prothoracic, mesothoracic, and metathoracic ganglia, respectively). After activation, it induces backward walking, whereas silencing it results in the inability of the fly to walk backward.

this neuron by genetically expressing tetanus toxin light chain (TNT) made the flies lose the ability to walk backward in an assay where control flies preferred to walk backward in mechanically constrained environment. This indicated that MDN is both necessary and sufficient for initiation of backward walking. Moreover, this implies that targeted investigation of MDN outputs will provide more detailed information of how backward walking is initiated. MDN-induced backward walking causes every leg to switch from forward to backward stepping. In *MOTOR FLEXIBILITY IN A STEPPING INSECT LEG: FORWARD AND BACKWARD WALKING, CURVE WALKING, AND SPEED CHANGES*, we discussed that antenna pull-induced backward stepping in stick insects involves a switch in the phasic inputs on the ProCx and RetCx motor neurons (Fig. 5A and 5B), compared with forward walking. It is very likely that MDN activation induces a similar switch in the local circuits of every leg in the fly. Interestingly, quantification of the stepping pattern of “moonwalking” flies showed that in addition to switching every leg movement from forward to backward stepping, MDN also switches the interleg coordination sequence. The backward gait is usually at slow speed and typically has a reversed metachronal wave (ipsilateral front-mid-hind sequence). This shows that activity of the same descending interneuron is able to achieve two different functions: switch in single-leg stepping direction and a change in interleg coordination. Whether the interleg coordination switch is a byproduct of sensory feedback alterations induced by a backward stepping leg or whether it is a direct manipulation of central intersegmental coordinating neurons is still unknown, but genetic access to MDN indeed provides a handle in planning such hypothesis-driven future studies. In fact, in a follow-up study using the genetic tools for targeting MDN neurons, Sen et al. (2017) successfully showed that these neurons receive inputs from looming-sensitive visual projection neurons. Similarly, studies investigating how MDN output affects the ventral nerve cord CPG circuits are now under way and will provide further mechanistic insight into how activity of this neuron is translated into a change in the walking direction. In addition to MDN, the authors also identified an ascending neuron in the original moonwalker line, the “moonwalker ascending neuron” (MAN). This neuron aided in maintenance of a prolonged backward walking state, typical of the moonwalking phenotype. By careful quantification of neural activation and inactivation data of genetic reagents targeting MDN alone, MAN alone, and both MDN and MAN, the authors concluded that MAN is not necessary for backward initiation but inhibits a competing forward walking drive, thereby prolonging the backward walking state when coactivated with MDN. Genetic access to MAN thus provides a potentially very useful tool to look for interneurons involved in forward walking.

SOME NEXT STEPS IN UNDERSTANDING THE CONTROL OF INSECT WALKING

On the basis of what we have outlined in this review, we can now summarize a general schematic of how insects generate a coordinated motor output for six-legged walking. The rhythmic activity of each leg generating stance and swing movements during stepping arises from the rhythmic activity of central neural networks, so-called CPGs. Modular CPGs, each driving

the antagonistic muscles of one leg joint, cooperatively generate the motor output required for single-leg stepping. The magnitude and timing of this motor activity is modified and coordinated by specific feedback signals originating in sensory structures in the leg. These encompass information about movement, force or load, and ground contact. In some fast walking insects, such as cockroaches, central coupling between modular CPGs might also contribute to coordinated motor output with strongly reduced sensory feedback. Descending inputs from the brain relayed via descending interneurons are important for both the initiation of CPG activity in the thoracic ganglia and for modulating the motor output, to generate different leg kinematics that are adequate for goal-directed walking. Current evidence suggests that the influence of descending inputs is joint specific and modifies how sensory feedback influences CPG output specifically at that joint.

A variety of modeling approaches have shown that this general idea of an interplay between CPGs, sensory signals from the leg, and coordinating influences between legs is able to sufficiently capture the basic characteristics of stepping and walking in insects (Daun-Gruhn 2011; Ekeberg et al. 2004; Szczecinski et al. 2014, 2015; Toth and Daun-Gruhn 2011; von Twickel et al. 2011). The circuit designs used in these simulations explain, to a certain extent, many of the available behavioral and motor recording data and provide conceptual insights and specific hypotheses for circuit mechanisms. Tóth et al. (2012), for instance, created a neuromechanical model of a single middle leg for simulating the switch from forward to backward stepping. To account for the behaviorally observed fast switches between forward and backward stepping, they introduced two layers of interneurons between the CPG and the motor neurons in their model. With this configuration, the descending inputs responsible for the switch to backward stepping act at a point downstream of the CPGs, thereby inducing an almost instantaneous switch in stepping direction, similar to what is often observed in behaving insects. This provides a testable hypothesis regarding the mechanics of direction selection for future research. Currently, these simulations have some shortcomings that necessitate further experimental data as well as more detailed modeling. First, the realization of sensory feedback signals is often much simpler and not as complete as what is known from biological systems, for example, when movement and force signals are combined to a single sensory-state variable (e.g., Toth and Daun-Gruhn 2011; Tóth et al. 2012). Furthermore, the simulations are forced to make major assumptions about all of the central neural elements (CPGs, premotor interneurons, intersegmental neurons, and descending interneurons) in the walking circuit. Most central neurons are unknown, and to truly understand the central transformations, it is imperative to identify and characterize the central neurons in this circuit. Finally, as outlined above, very little is currently known about how and by which network topology intersegmental and descending signals influence segmental networks and CPGs during the generation of coordinated walking behavior in insects (for reviews see Ayali et al. 2015; Borgmann and Büschges 2015). Resolving these issues could make the simulations studies even more insightful and, in fact, drive formulation of new hypotheses to unravel central principles of the circuit functioning.

Electrophysiological approaches on large insects can be helpful in that task, because they allow for a preparatory

reduction, and therefore simplification, of the walking system. Furthermore, these approaches make it possible to precisely target information processing in the ventral nerve cord with in vivo-like stimulation of leg sense organs while performing single-cell recordings from identified neurons (e.g., Zill et al. 2015, 2017). Similarly, they allow for the reduction of the six-legged animal to a single- or two-legged preparations, thereby excluding specific information from neighboring segments or legs during the execution of stepping behavior (Berg et al. 2015; von Uckermann and Büschges 2009). However, identification of network elements may profit from methodological advances in motor control: although some of the central neurons involved in the control of walking have been characterized, these are most often isolated cases across different insect species. This makes it difficult to put it all together in a single circuit diagram. Moreover, since most of the characterized central neurons do not have transgenic driver lines, it is difficult to reproducibly access these neurons for further investigations. The ability to genetically target specific central neurons indeed opens up avenues for a large array of future investigations as exemplified by the discovery of the moonwalker descending neurons (outlined above; Bidaye et al. 2014). In addition to reproducible access, genetic targeting also makes these neurons amenable to ever more sophisticated tools in optogenetics, thermogenetics, and calcium/voltage imaging techniques. It is therefore not surprising that several efforts are underway to create genetic driver lines (especially split-GAL4 lines) that target specific neurons in most of the CNS of *D. melanogaster*. In fact, a large resource targeting one-third of all descending interneurons in *Drosophila* has been created at the Janelia Research Campus (unpublished observations; Namiki S and Card G, personal communication; <https://www.janelia.org/project-team/fly-descending-interneuron>). Similar resources are also being created for the ventral nerve cord neurons. These tools will greatly aid in identifying central neurons in the walking circuit of *Drosophila*. However, identifying neurons is only the first step; these then need to be put into an anatomically and functionally annotated circuit diagram. Electron microscopy (EM) reconstruction of the entire nervous system of *Drosophila* is under way; a recent publication shows a completed electron micrograph of the brain (Zheng et al. 2017), and similar efforts with regard to EM are under way for the ventral nerve cord. This electron microscopy data will be invaluable for reconstructing a complete wiring diagram of the walking circuit. Although these efforts are currently limited to *D. melanogaster*, techniques optimized in this process will aid similar analysis in other insect species as well. Also, with the use of newly developed CRISPR-based genetics strategies (Fineran and Charpentier 2012; Wiedenheft et al. 2012), it has now become possible to create transgenic resources in many insect species, such as mosquitos (Kistler et al. 2015), ants (Trible et al. 2017), silk moths (Wang et al. 2013), locusts (Li et al. 2016), and crickets (Awata et al. 2015). This is particularly important, because some studies, such as simultaneous recordings in behaving animals, are in fact better implemented in larger insects such as the stick insect and the cockroach. The behavioral repertoire of a given insect species is also limited, and it will therefore always be beneficial if techniques advance simultaneously in several model insects. That may then also allow researchers to address how specific behavioral specializations come about. For instance, the walking speed ranges for

different insects are dramatically different (stick insects walk much slower on average than cockroaches), and the neural circuit properties of different species might have some interesting specializations. Thus, eventually, it will indeed be valuable to have comparative as well as specific data sets across several insect species.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

S.S.B., T.B., and A.B. prepared figures; S.S.B., T.B., and A.B. drafted manuscript; S.S.B., T.B., and A.B. edited and revised manuscript; S.S.B., T.B., and A.B. approved final version of manuscript.

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