Insect motor control: methodological advances, descending control and inter-leg coordination on the move

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Modern approaches, including high performance video, neurophysiology, and neurogenetics, allow to analyze invertebrate behavior on all levels of generation and performance in an unprecedented way. They allow observation and classification of behavior in controlled conditions, dissection of behavioral sequencing, identification of levels of processing and locations of associated sub-networks and, finally, identification of neuronal components and topologies contributing to specific aspects of behaviors. Recently conceptual and methodological progress has contributed to unraveling the neural structures underlying descending control of insect behavior as well as the mechanisms in charge of generating coordinated locomotor movements of the invertebrate extremities during walking. This brief review summarizes some of the most exciting new findings in these areas of research from the past years.

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Introduction

Insects show a huge repertoire of behaviors spanning from stereotypic ones, like courtship singing (Dickson [1]; Hedwig [2]), to highly complex behavioral sequences, like crossing obstacles and gaps during locomotion (e.g., [3–5]). Investigating insect motor behavior, such as postural control, crawling, walking, flying, jumping, intra-species communication and courtship, has a long tradition in the fields of motor control and neuroethology [6,7]. Insects are mostly considered simple compared to vertebrates. However, specifically with regard to their neural organization we are far from understanding how insects generate, adapt, and modify their behavior in a task-specific or ecologically

relevant manner. In recent years considerable progress has been made in understanding the organization and action of descending control the brain of an animal exerts within the framework of generating a particular motor behavior as well as with respect to the generation of a coordinated overall motor output of the multi-legged motor system of insects. The basis for these advances are developments in techniques for the analysis of behaviors ranging from automated tracking and annotation to neurogenetic tools. These approaches allow new insights into the sequential flow of behaviors as well as their neuronal realisation within the nervous system. This brief review highlights the most significant findings in this area of research. Due to space restrictions we can only refer to parts of the literature available, but will give reference to appropriate review articles for further reading.

Progress in the analysis of insect behavior

Recent progress in the analysis and understanding of insect behavior is mostly based on technological, and computational advances which have dramatically broadened the options for studying invertebrate behavior. Consequently, the current analysis of behavior in insects spans all possible levels: first, based on high-speed video recordings and powerful computer hardware and software, monitoring the behavioral portfolio in experimentally defined environments provides so called ethograms. This enables a quantitative view on behaviors together with detailed and often automated classification (Figure 1a). Impressive examples come from recent approaches to analyze *Drosophila* behavior for example [8–10]. These allow to analyze one specific behavior at a given time for large numbers of animals [9], the classification and quantification of behaviors of single flies as well as their social interaction [8] and, finally, the categorization and evaluation of behavior based on the analysis of leg movements in single animals [10]. Second, using these recent approaches, complemented by more traditional ones, researchers analyzed invertebrate behavior to the level of deciphering their sequential organization (Figure 1b). These behaviors comprise walking [11•,12,13••], locomotion under challenging environmental conditions, for example, the coordination of antennae and legs during obstacle negotiation [5,14] or the crossing of gaps [4], grooming behavior [15••], chemotactic orientation [9] and species-specific behaviors like courtship in fruit flies [16]. Particularly those approaches that are based on state-of-the-art video recording and analysis techniques have reached a level of unprecedented detail

Figure 1
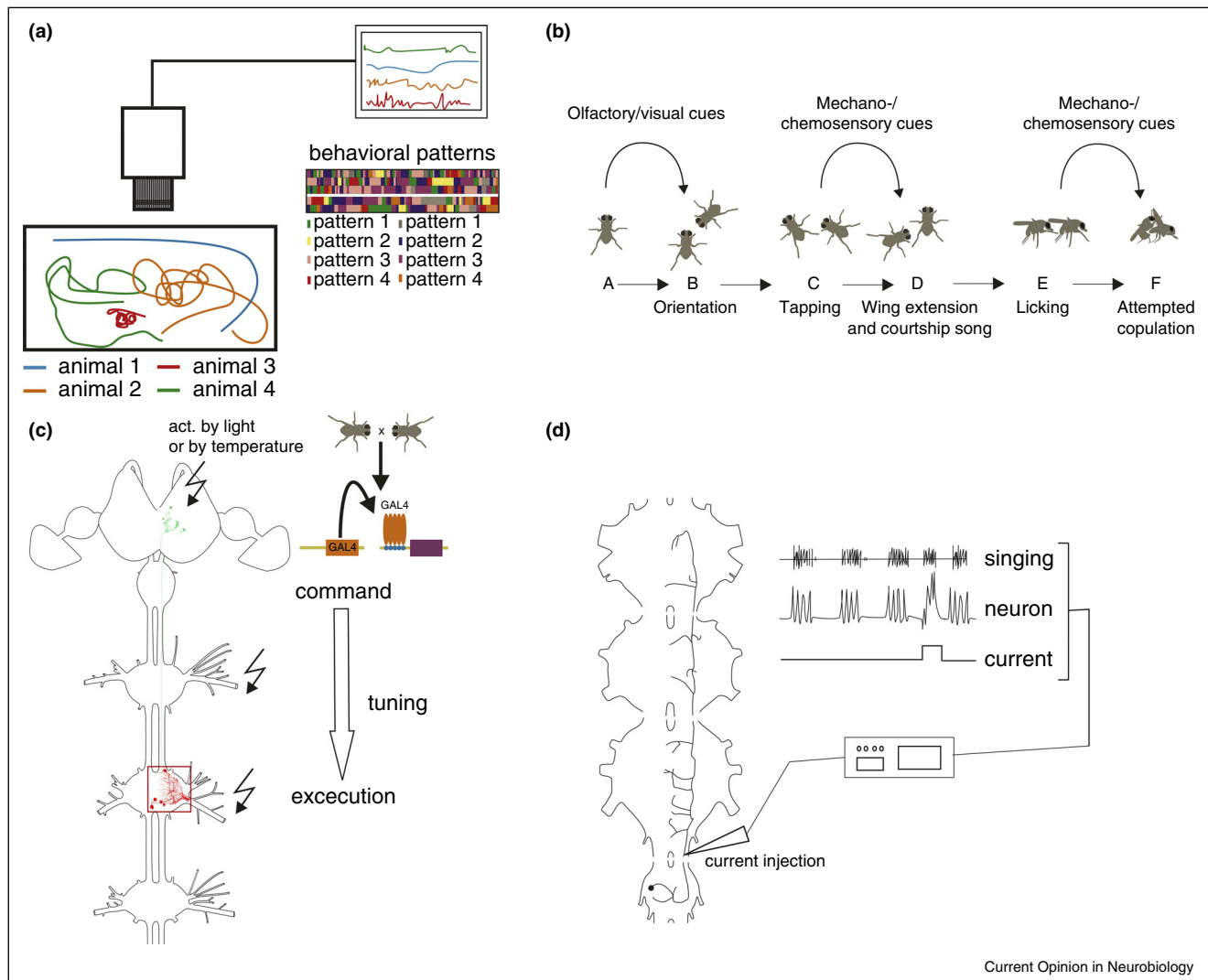
Contemporary approaches to the analysis of insect behavior: from neuroethology to network topology. **(a)** Automated tracking, classification and quantification of insect behavior: increasing computational power and elaborate video techniques allow the analysis of large amounts of behavioral data in comparably short time with an automated classification of the behaviors generated (lower panel, redrawn from Kain et al. [10]). Colors here indicate a certain behavior type. **(b)** Dissecting behavioral sequences: insect behavior can be broken down into different behavioral modules for example behavioral sequence of courtship steps performed by male *Drosophila* (redrawn from Billeter et al. [63]). **(c)** Identifying functional neural components and levels in the generation of behavior: neurogenetic approaches allow the generation and subsequent behavioral screening of hundreds of mutants to eventually identify neural modules that contribute to a certain behavior. Shown here: example of a command neuron (green) in the brain descending to a network of thoracic neurons (red) (left panel, redrawn and modified from Mugit and Feany [64]). **(d)** Neural modules and network topology: classical electrophysiological approaches as well as neurogenetic approaches ultimately aim to identify core neurons that interact to produce the motor output for a certain behavioral component. Here, intracellular current injection can elicit additional chirps in cricket singing (modified and redrawn from Schöneich and Hedwig [19])

and perfection regardless of the size of the studied animal. Analyzing the grooming behavior of fruit flies, Seeds and coauthors [15••] have shown that the sequence of grooming reflects the result of a competitive and inhibitory interaction of grooming-site specific neural modules. Exerting cleaning of one body part suppresses cleaning of the others. Third, current approaches, in particular neurogenetic tools, allow to dissect the generation of behaviors with respect to

localizing the components underlying specific aspects for example initiation, maintenance, tuning, execution, modulation of activity (Figure 1c). In the fruit fly different populations of interneurons were identified along the central nervous system that each serve particular functions in initiation, control, and motor pattern-generation of courtship song [17••]. Fourth, finally, also the kernel elements for generating behavior, that is, the neural networks

and their constituting neurons, have been investigated successfully (Figure 1d). For about 30 years the only neural network in insects, whose topology is known to a sufficient extent that can explain the generation of locomotor activity, has been the one generating the motor output for elevation and depression movements of fore and hind wing in locust flight review in [18]. In contrast, the available knowledge about neural network topologies underlying the generation of other locomotor behaviors is still rather incomplete (for summary see [19]). Recent results have broadened our knowledge with regard to the organization of the central neural circuits underlying insect behavior, that is, for courtship behavior in grasshoppers, crickets, and fruit flies [17••,19] by identifying intersegmental interneurons as kernel components. The analysis of neural network topologies responsible for insect behavior will need much more attention in the future in order to identify task-dependent modifications in neural processing for the generation of flexibility in behavior for example [20]. Behavioral, neurophysiological and genetic approaches allow to analyze all aspects of insect behavior from automated classification and quantification of behavior to network topology (Figure 1a–d).

Identifying the role and mechanisms of descending control

Two recent studies from von Philipsborn et al. [17••] and Bidaye et al. [21••] in fruit flies demonstrate how powerful the above mentioned approaches are to unravel the function of individual descending command neurons for the control of a specific behavior. The initiation and maintenance of most behaviors, with the exception of reflex and escape reactions, is controlled by higher order centers in the brain. This can be as complex as controlling goal-directed motor acts or as limited as the modification of state-dependent or phase-dependent local reflexes (comprehensive summary in [7]). One important question is still mostly unresolved: What kind of descending signals does the brain send downstream for the generation or modulation of a specific behavior? This question does not only address which behaviors rely on single command neurons for their execution, but also how detailed descending control needs to be in order to allow for the generation of the vast spectrum and flexibility of behaviors. In recent years, research in invertebrates has focused on this question, that is, the analysis of the role of descending control in the generation of behaviors in general and adaptivity in particular for example [22••,23].

Current findings for insects concern firstly the anatomical characterization of descending neurons from the supraoesophageal and gnathal ganglia forming the brain to the thoracic and abdominal ganglia that contain the neural networks for behavior for example [24–28]. Secondly, they give new insight into the specific influence of descending control on the local segmental networks

underlying the generation of motor activity for example [29–33] and, finally, the specific function of individual descending component neurons in controlling the execution of behaviors for example [17••,21••,34].

Two recent studies in the fruit fly have broadened our knowledge about the specificity of command neurons (see also [35]) for behaviors in the insect nervous system. von Philipsborn et al. [17••] provide evidence for a functional modularity of neural networks in the generation of courtship singing behavior in fruit flies. Courtship in fruit flies consists of a sequence of individual behaviors: firstly, orienting between the prospective mates; secondly, tapping of the male onto the abdomen of the female; thirdly, a species-specific fast movement of one wing of the courting male called 'singing', fourthly, the approach of the male to the abdomen of the female called 'licking' and, finally, fifthly, copulation between the two mates (review in [36]). von Philipsborn et al. [17••] have identified descending neurons in the fruit fly brain whose activity triggers and initiates the singing behavior during courtship (Figure 1C). Identified interneurons in the ventral nerve chord (VNC) time and shape the song. This organization of neuronal control is similar to the situation found in grasshopper and cricket singing (e.g., [2]). Recently, Bidaye et al. [21••] identified descending neurons that influence the walking direction in *Drosophila*: two pairs of brain neurons, the *moonwalker descending neurons* (MDNs), serve as command neurons in the control of backward walking (Figure 1c). After neurogenetically induced activation of MDNs, that is, by using the temperature sensitive cation channel TrpA1 (Hamada et al. [37]), backward walking with coordinated stepping sequences of all three pairs of legs was initiated. Activation of MDNs resulted both in a change of single-leg motor output as well as in intersegmental coordination. It is quite conceivable that MDNs target in the VNC the neural networks controlling the thoraco-coxal joints of the insect legs and the influence sensory feedback has on their activity. A critical difference between the forward and backward stepping motor output of an insect leg is the inverse activation of the thoraco-coxal muscles during both stepping directions [38], which is assisted by a task-dependent influence of load feedback signals from the legs [39].

The study by Bidaye et al. [21••] also addresses the aspect of maintenance of behavior with respect to descending control. MDNs exhibit the specific command neuron properties of necessity and sufficiency for the initiation of backward walking: when MDNs are activated experimentally backward stepping was elicited, while transient or permanent silencing of these neurons resulted in the loss of backward stepping. Activation of these neurons, however, does not induce continuous backward stepping, but only triggers recurrent short bouts of backward stepping that alternates with forward stepping periods. Only

when a pair of ascending VNC neurons, the *moonwalker ascending neurons* (MANs), was activated simultaneously continuous backward stepping behavior was induced. At present, it is not known whether MDNs and MANs interact with each other directly. It is important to note that simultaneous activation of MDN, as well as MDN together with MAN apparently initiate backward stepping by overriding the neural mechanisms initiating and maintaining forward stepping: in intermittent phases between activation of MDN and MAN fruit flies walk forward. Based on these results it is conceivable that MAN neurons stabilize backward stepping by inhibiting neural mechanisms for forward stepping. The results also indicate that additional descending neural channels may be needed that activate MANs which, in turn, maintain backward stepping.

Taken together, these current findings suggest that in the insect nervous system the production of behaviors relies on the activation of individual and specific descending command neurons in the brain. It should be noted, however, that the behaviors studied so far, except for backward walking, are all of the robust or inherent kind, specifically true for species specific singing behavior. Still, the question is open how transitional aspects in behaviors, for example changes in direction or speed of locomotion are controlled by descending signals and commands (e.g., [22••,29,30]).

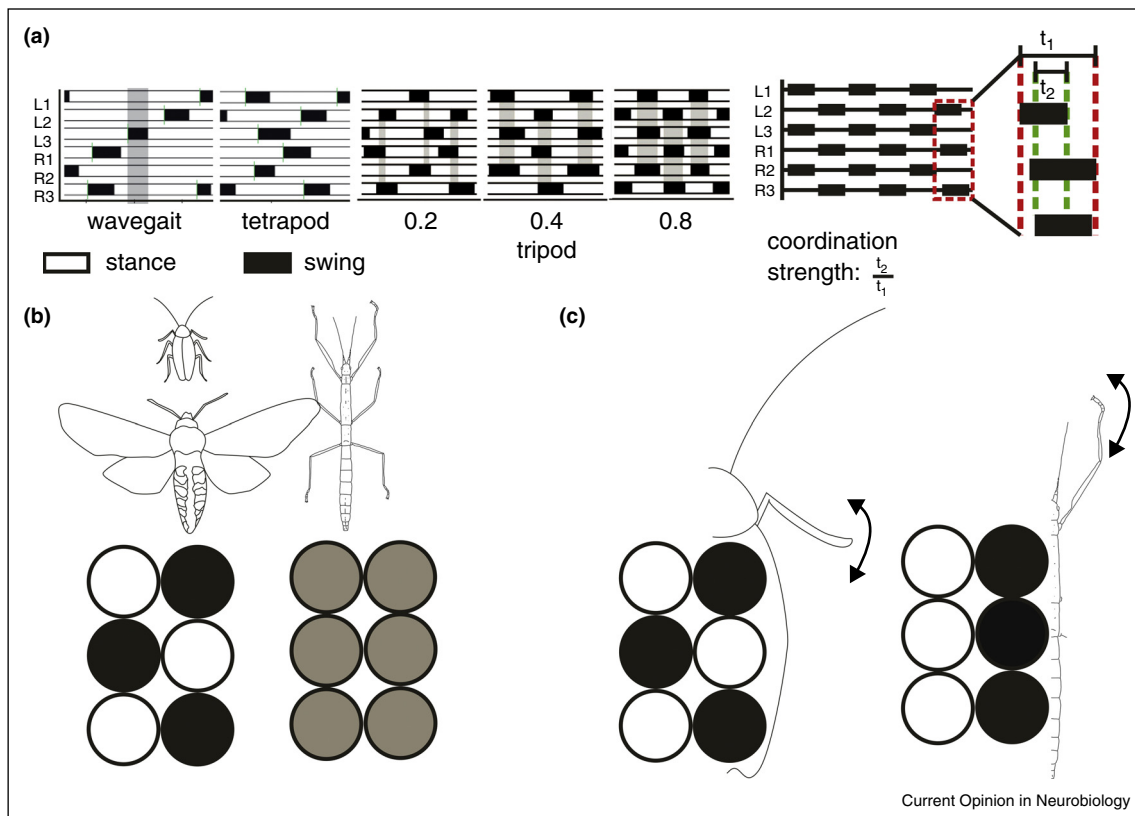## Inter-leg coordination in walking insects

As outlined above, descending signals from the brain activate specific local sub-networks residing in the thoracic ganglia and initiate the execution of a specific behavior. Especially for walking as a flexible and at the same time stereotyped behavior produced by the simultaneous movement of several limbs, the question arises how the nervous system generates the specific coordination between them. This is an issue which is particularly important during task-specific modifications, like changes in walking direction or under external perturbations, like uneven terrain.

Insects, like all other walking animals, use different forms of inter-leg coordination to optimize their motor output for specific locomotor tasks ([40]; Figure 2a): large, heavy, loaded, or very-slow walking insects coordinate their legs in a metachronal wave propagating from back to front on each body side with five legs on the ground at any given time (Figure 2; [41]). At higher walking speeds the number of legs that are on the ground simultaneously is reduced to four, called tetrapod coordination (Figure 2a; [42–44]). At high speeds, in lightweight, or unloaded insects the number is reduced to three, called tripod coordination (Figure 2a; [45,46]). As an interesting special case, even bipedal anti-phase coordination of cockroach hind legs has been reported during top speed running, while front and middle legs no longer touch the

ground [47]. Even though specific behavioral rules have been described for the influence of stepping legs on their neighbours (e.g., [48,49]), more detailed information with regard to neural network topologies contributing to inter-leg coordination is scarce. While the existence of intermediate forms of inter-leg coordination patterns has been reported (e.g., [50]), suggesting the existence of an adaptable neural control system covering a broad range of coordination patterns, it has long been common sense that we can distinguish between fast walking insect with a stereotyped tripod coordination pattern and slow walking insects with a more variable tetrapod coordination pattern. This resulted in studies on slow and fast walking insects, for example stick insects and cockroaches (e.g., [45,50,51]), respectively, elucidating either the role of central inter-leg coupling or the role of sensory feedback, thereby emphasizing the differences in inter-leg coordination. Recent studies on various insect species shed an interesting new light on this issue:

Compared to other insects, fruit flies show a high flexibility in locomotor behavior. They do not only use tripod coordination at medium to high walking speeds [52], but also tetrapod coordination for slower speeds and wave gait coordination for very slow speeds [13••]; see also [10,11•]. Interestingly, the strength of inter-leg coordination apparently depends on walking speed: at medium and higher speeds, that is, in the range from five to more than 15 body lengths per second the strength of tripod coordination changes about tenfold from as little as 10–90% (Figure 2a). Interestingly, tripod coordination is never perfect, a fact which has been attributed to a specific segmental phase lag between the three pairs of legs [50,52]. Such flexible performance in inter-leg coordination in the fruit fly challenges the hypothesis that coordination is solely based on coupling of central neural networks. This holds even for particular coordination patterns like tripod coordination. This is corroborated by the observation that inter-leg coordination shows characteristic modifications at slow walking speeds when sensory information is genetically reduced [11•]. For instance, Mendes et al. [11•] focused on type I ciliated sensory neurons and showed that silencing them in the distal leg segments leads to more variable inter-leg coordination patterns but does not abolish coordination. At the same time, however, walking speeds are strongly reduced. With respect to a potential interpretation of the role of sensory feedback for walking in the fruit fly it is important to note that there are also type II multipolar sensory neurons in each leg joint sensitive to angular changes. These may provide a redundant sensory pathway that contributes to the control of motor activity [53]. These findings on a velocity-dependence of inter-leg coordination in the fruit fly parallel observations in cockroaches, where [46] described a robust tripod coordination pattern only at high walking speed, but an ambling gait with variable coordination at slow speeds. In summary,

Figure 2

(a) Speed-dependent and task-dependent spectrum of inter-leg coordination patterns in insect walking. All insect species investigated so far show a preferred speed range and a corresponding inter-leg coordination pattern for walking from wave gait over tetrapod coordination to tripod coordination (see footfall patterns from left to right). Bottom numbers indicate tripod coordination strength calculated as time of overlap of swing phases (t_1, see right panel) divided by total time of swing of all three legs (t_2, see right panel). Nevertheless, recent studies show that all insects species are capable of using the whole spectrum of coordination patterns in a velocity and task dependent manner. Examples taken from recent *Drosophila* work by Wosnitza *et al*. [13••] (modified and redrawn). **(b)** Coupling architectures of locomotor networks in insects. Investigations on pharmacologically activated networks for motor pattern generation reveal preferred phases of coupling between motor outputs of different segments. Cockroaches, locusts and manduca show motor patterns as expected in tripod coordination (black circles, left panel), whereas stick insects only show recurrent intervals of in-phase activity but no strict cycle to cycle coupling (grey circles, right panel). **(c)** In cockroaches and stick insects single front leg stepping was found to stabilize the patterns generated in the otherwise deafferented thoracic nerve cord. In stick insects front leg stepping entrained ipsilateral rhythmic motor activity in phase. In cockroaches single front leg stepping strengthens a double tripod coordination pattern. Although all species display the entire behavioral spectrum the underlying neural networks appear to be particularly tuned towards a certain coordination pattern resembling closer to the one preferred in freely behaving insects.

recent results provide strong indications for a concept recognizing inter-leg coordination patterns in insects as part of a broad spectrum from which the most favorable coordination pattern is chosen in a speed-dependent and task-dependent manner.

Recent studies also highlight the influence of sensory feedback for establishing inter-leg coordination during walking. It has been known for some time that after pharmacological activation of the central pattern generating networks for walking in cockroach [54], locust [55], manduca [56] and stick insect [57] specific characteristics of intersegmental coupling become apparent (Figure 2b). In cockroach, locust, and the hawk moth intersegmental

coordination of motor activity is tuned towards antagonism between hemi-segments as well as between direct neighbouring segments, resembling tripod coordination (Figure 2b). In the stick insect recurrent intervals of in-phase activity between segmental homologue motoneuron pools were reported (Figure 2b). Interestingly, both in the stick insect [58] and cockroach [59•] the preferred species-specific intersegmental coupling between central pattern generating networks was stabilized when a front leg generated stepping movements (Figure 2c), indicating that local sensory feedback plays a remarkable role in the generation of intersegmental influences and contributes to inter-leg coordination. For the stick insect sensory signals not only stabilize central coupling but are important

to adjust the intersegmental coupling to a specific coordination pattern. Borgmann et al. [58,60•,61] show that the bias in the influence of local load signals versus intersegmental sensory signals varies for the pattern generating networks innervating MN pools of different joints. Thus, coordinated motor output appears to arise from a dynamic interplay of central coupling, intersegmental sensory signals from other legs, and sensory signals from local sense organs.

Quite recently, Zill et al. [62••] have added evidence for a highly significant sensory influence for the generation of inter-leg coordination in the cockroach. For the first time, they provide evidence for a mechanism, that is, load sensing, that acts between ipsilateral legs and establishes coordination during walking, thereby supporting one of the behavioral rules formulated by Schilling et al. [49]. Unloading of the hind leg at the transition between stance and swing phase increases load on the ipsilateral middle leg. Tibial campaniform sensilla of the middle leg [62] were shown to specifically sense this rapid increase in load when the hind leg lifts off. Such a mechanism is particularly noteworthy as it mediates an intersegmental sensory modality through interplay of body mechanics and sense organs local to the segment of effect. This mechanism reduces the time for information transfer as information is transferred mechanically and not neurally.

What can we deduce from recent approaches to understand the neural basis of intersegmental coordination? There appears to be a spectrum of different inter-leg coordination patterns in insects. Different coordination patterns are found in insect species with different probability: whereas adult stick insects walk mainly in a tetrapod coordination pattern, fruit flies and cockroaches mainly use tripod coordination patterns. However, these preferences are speed-dependent and task-dependent indicating that insects can execute the whole spectrum of inter-leg coordination (Figure 2a). The underlying coupling architectures of the locomotor networks (Figure 2b) appear to differ in a way that favors the species-specific preferred inter-leg coordination pattern (Figure 2c). The new results about locomotor behavior in different insect species clearly define the need to address the question with regard to the organization of the underlying neural networks of producing this broad spectrum of coordination patterns.

What is next? Based on the recent advances three by now unresolved issues appear in particular appealing and pressing. Firstly, which aspects of insect motor behavior, apart from initiation, maintenance and the gain of local reflexes are controlled by descending signals and what aspects are under exclusive local control? Secondly, what are the neural mechanisms mediating the interaction of descending pathways with local networks and which role does neuromodulation play? Are the neural network

topologies and mechanisms underlying intersegmental coordination in insects that preferentially use different coordination patterns different?

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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