# RESEARCH ARTICLE

# Proprioceptive feedback reinforces centrally generated stepping patterns in the cockroach

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## SUMMARY

**The relative importance of sensory input for the production of centrally generated motor patterns is crucial to our understanding of how animals coordinate their body segments to locomote. In legged locomotion, where terrain heterogeneity may require stride-by-stride changes in leg placement, evidence suggests that sensory information is essential for the timing of leg movement. In a previous study we showed that in cockroaches, renowned for rapid and stable running, a coordinated pattern can be elicited from the motor centres driving the different legs in the absence of sensory feedback. In the present paper, we assess the role of movement-related sensory inputs in modifying this central pattern. We studied the effect of spontaneous steps as well as imposed transient and periodic movements of a single intact leg, and demonstrate that, depending on the movement properties, the resulting proprioceptive feedback can significantly modify phase relationships among segmental oscillators of other legs. Our analysis suggests that feedback from front legs is weaker but more phasically precise than from hind legs, selectively transferring movement-related information in a manner that strengthens the inherent rhythmic pattern and modulates local perturbations.**

Key words: central pattern generator, frequency entrainment, intersegmental coordination, locomotion, phase relationship, proprioceptive feedback.

## INTRODUCTION

The ability of animals to produce both stereotyped and adaptive locomotive behaviours is largely dependent on the interplay among centrally generated motor patterns and the sensory inputs that shape them. Previous studies of various preparations cumulatively suggest that the weight accorded to sensory feedback in constructing motor commands depends on the complexity of the environment through which the animals move, and the frequency with which they sample it. For example, animals that travel fast through water exhibit a tightly coordinated wavelike motor program that is produced centrally in the absence of sensory feedback [see Hill et al. (Hill et al., 2003) and references therein]. In contrast, in legged locomotion, where properties of the substrate with which the legs interact can drastically change, local information about current leg states plays an essential role in coordinating the limbs (Cattaert and Le Ray, 2001; Pearson, 2004; Ritzmann and Büschges, 2007; Borgmann et al., 2007; Borgmann et al., 2009). An important context-specific role for sensory feedback was also suggested by Johnston and Levine (Johnston and Levine, 2002), following their detailed analysis of differences between patterned motor activities evoked from isolated larvae or adult central nervous systems in *Manduca*, and activity patterns observed in the intact animals.

In this respect, cockroaches, which are renowned for their rapid and stable gaits, present a unique opportunity for examining legged locomotion. Their body and limb mechanics provide stability during rapid running over rough terrain and in the face of impulsive perturbations, without requiring precise changes in motor patterns (Jindrich and Full, 2002; Sponberg and Full, 2008). In the presence of larger perturbations, however, neural feedback modifies the centrally produced patterns to adjust limb movement (Sponberg and Full, 2008; Sponberg et al., 2011). This suggests a combined feedforward–feedback control strategy, in which feedforward pathways from centrally connected central pattern generators (CPGs), in conjunction with nonlinear properties of muscles, suffice to produce basic locomotion (Jindrich and Full, 2002; Holmes et al., 2006; Kukillaya and Holmes, 2007; Kukillaya et al., 2009), whereas proprioception enhances flexibility in response to unexpected perturbations (Sponberg and Full, 2008; Proctor and Holmes, 2010; Proctor et al., 2010).

In a previous study, we verified that a coordinated motor pattern can be elicited in cockroaches in the absence of sensory feedback (Fuchs et al., 2011). This pharmacologically induced pattern comprises alternating activity in antagonistic coxal depressor and levator motor neuron (MN) groups with intersegmental phase relationships that share similarities with functional gaits of cockroaches (Delcomyn, 1971; Pearson and Iles, 1973; Delcomyn, 1987). Estimates of coupling strengths made by fitting a stochastic oscillator model (Kiemel and Cohen, 1998) and experiments with a single intact front leg both indicated that central intersegmental couplings are contralaterally symmetric but ipsilaterally asymmetric, being stronger in the descending (rostro-caudal) than the ascending direction. They are also relatively weak and produce variable intersegmental phase relationships, which become tighter during spontaneous leg stepping. Such reinforcement of the central pattern could either result from movement-related inputs or from central

modulation causing both the generation of steps and stabilization of the central pattern, or from a mixture of both.

The nature of the coupling between hemisegmental oscillators in the absence of sensory feedback and the effect of phasic input from a major limb proprioceptor have been previously addressed by Sillar et al. (Sillar et al., 1987) in the crayfish. In the present study, we further investigate the role of movement-related feedback on the cockroach CPG. We analyze the intersegmental effects of spontaneous steps (free walking), imposed single steps and imposed periodic stepping of intact hind and front legs. In addition, we quantify the responsiveness of the segmental CPGs to impulsive leg displacements. The latter is important in explaining how sensory feedback can adjust CPG outputs during locomotion, without allowing perturbations of one leg to disrupt coordination at the others. Our results suggest that proprioceptive feedback can modify and entrain phase relationships between neighbouring hemisegments of CPGs. Our analysis also shows that feedback from hind and front legs differs in strength and phasic precision, supporting earlier evidence for rostro-caudal asymmetry in the sensory-motor network (Fuchs et al., 2011).

## MATERIALS AND METHODS

Experiments were conducted on adult female cockroaches [*Periplaneta americana* (Linnaeus 1758)] obtained from our colony at Tel Aviv University. The neurophysiological procedure for electrode implantation followed (Fuchs et al., 2011). After a short anesthesia, animals were fixed dorsal side up. We opened their head capsule and cut the circumesophageal connectives between the brain and subesophageal ganglion (SEG) to minimize descending influences from the brain while maintaining the SEG intact to allow normal leg coordination during walking (Ridgel and Ritzmann, 2005). Animals were then subjected to a second anesthesia in $CO_2$ and fixed to a tethering plate that supported the pronotum and thorax. All legs, except a single intact front or hind leg, were amputated at mid-coxa and rotated to expose the dorsal surface for electrode implantation (see Pearson and Iles, 1970). Electrodes were fixed with a drop of polyepoxide adhesive. The soft cuticle connecting the dorsal coxal rim to the abdomen was removed, and fat tissue and trachea were carefully cleared to expose nerves 6Br4 and 5r1, containing depressor and levator coxal motor neurons (Pearson and Iles, 1970), in order to fix 0.003 mm silver-wire hook electrodes to the overlying cuticle. To ensure that segmental ganglia were completely deafferented, all nerves to be recorded from were crushed distal to the recording sites, and all other nerves leaving the ganglia were cut.

After electrode fixation and implantation, animals were carefully rotated dorsal side up and left undisturbed for 1 h to recover, before being placed above a lightweight low-friction treadmill that allows stepping with the single intact leg. To generate fictive activity at the deafferented ganglia, 20 μl of the muscarinic ACh agonist pilocarpine ($10^{-4}$ mol l$^{-1}$; Sigma-Aldrich, St Louis, MO, USA) were injected to the thoracic cavity. Pilocarpine provides non-specific activation to the motor centers and, for all experiments, we used it at the minimal concentration that induced bursting activity in the deafferented segments (for details, see Fuchs et al., 2011). We monitored the activity in leg MNs of the deafferented segments, while recording sequences of stepping movements with the intact leg using a DC motor attached to the treadmill that served as a tachometer to monitor the leg's angular velocity.

We carried out three sets of experiments. In the first, we investigated the effect of spontaneous steps on the pharmacologically induced central pattern. This was carried out by allowing a single intact front or hind leg to step spontaneously on the treadmill while

recording the bursting activity from depressor and levator MNs at other, deafferented segments. During each such step, the treadmill was accelerated through the stance phase and velocity returned to zero during the swing phase.

In the second set of experiments, we measured the responsiveness of CPGs in neighbouring segments to impulsive leg movements. These were induced by sending a short pulse of current to the treadmill motor that briefly accelerated the treadmill and created leg displacement. The magnitude and duration of the pulse were determined to be sufficient to cause a single leg movement in every trial.

In the third set of experiments, we studied whether periodic motion of a single intact leg could entrain bursting frequencies in neighbouring segments. To generate controlled periodic movements that resemble steps, the tarsus of the intact leg was tethered to the treadmill and periodic negative and positive current stimuli from a master 8 pulse generator were sent to the DC motor, moving the treadmill backward and forward. Pulse durations were programmed to reproduce typical stance and swing-like leg movements in durations similar to natural steps at different speeds, based on data from Pearson (Pearson, 1972). Each preparation was tested at frequencies ranging from 0.5 to 5 Hz, with 20 periodically imposed steps in each trial.

Because we were interested in the properties of the motor patterns in the deafferented segments, we included in our analysis only those preparations that showed persistent bursting in the levator MNs of their deafferented segments, following the application of pilocarpine. Such rhythmic activity was seen in approximately 40% of the preparations overall. Ten rhythmically active preparations were included from the spontaneous steps experiments (five with intact front legs and five with intact hind legs); five preparations were included from the second set of experiments to measure the responsiveness of the CPGs to single impulses; and nine preparations were included from the final set of periodically imposed movement studies (five with intact front legs and four with intact hind legs). We note that the smaller number of preparations included in our analysis of CPG responsiveness to single perturbations resulted from the fact that the calculation required uniform pre-stimulus rhythmicity, which only 30% of the preparations exhibited.

Analogue voltages transmitted from the extracellular recordings and the DC motor were sampled, played back in real time and recorded at 10 kHz using a four-channel differential amplifier (model 1700, A-M Systems, Carlsborg, WA, USA), an A-D board (Digidata 1200, Molecular Devices, Sunnyvale, CA, USA) and Axoscope software (Molecular Devices).

## RESULTS

In a previous study (Fuchs et al., 2011), we showed that in the absence of sensory feedback from legs, the deafferented motor centres can produce fictive rhythmic activity, which is characterized by intersegmental phase differences that share similarities with a double-tripod gait (pairs of neighbouring contralateral and ipsilateral hemiganglia show out-of-phase activity), albeit one with a high degree of variability. Here we build on previous work (Fuchs et al., 2011) to study the effects of input from moving legs on the centrally generated rhythmic pattern. Throughout the study, recordings were started and data were collected for analysis only after consistent pilocarpine-induced rhythmic bursts of activity were observed in the deafferented segments.

### Effects of spontaneous steps of an intact leg
We started by reconfirming and extending our previous results, including spontaneous steps of a single intact leg [see fig. 4 in Fuchs et al. (Fuchs et al., 2011)]. Fig. 1A shows an example of extracellular
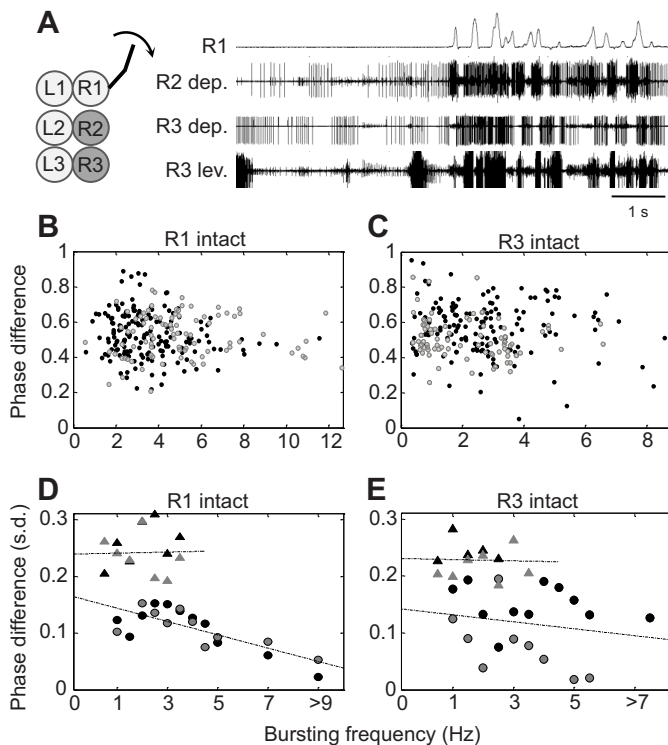
Fig. 1. Motor patterns during spontaneous steps of a single intact leg (black line extending from R1) in the cockroach *Periplaneta americana*. (A) Simultaneous extracellular recordings from depressor (dep.) motor neurons (Mns) of R2 and R3 and from levator (lev.) MNs of R3 before and during front leg (R1) stepping; treadmill velocity is plotted in the top row. The arrow in the cockroach limb schematic (left) indicates the direction of movement during stance part of step. (B,C) Phase differences between bursts in the levator MNs of neighbouring contralateral and ipsilateral hemiganglia (black and grey circles, respectively) during front leg (B; N=5 preparations) and hind leg (C; N=5) stepping sequences. (D,E) Circular standard deviations of phase differences as a function of burst frequency. Black and grey circles are circular standard deviations of the phases plotted in B and C, calculated separately for each burst frequency. Black and grey triangles represent circular standard deviations of contralateral and ipsilateral phase differences of the same preparations during periods when the leg was still. For panels B–E, we analysed phases for the contralateral pair R2–L2, and analysed the ipsilateral pair R2–R3 when R1 was intact (B,D) and R1–R2 when R3 was intact (C,E).

recordings from the deafferented middle-right (R2) and hind-right (R3) leg MNs, while the intact front-right (R1) leg was freely moving on the low-friction treadmill. Relatively irregular bursting activity can be seen in the deafferented segments during time intervals in which the intact leg did not move. This activity intensified (both in burst frequency and number of spikes within each burst) in conjunction with initiation of spontaneous stepping in the intact leg.

We analyzed sequences containing 50 steps per preparation to calculate phase differences among bursts at the deafferented segments. The mean (±s.d.) phase difference between a step in an intact leg R1 and a burst in the adjacent R2 levator MNs was 0.195±0.02, and between a step in intact leg R3 and a burst in R2 levators it was 0.224±0.09. Here the phase of a spontaneous step is defined as onset of treadmill motion, i.e. when velocity rises above zero. Phase differences between neighbouring contralateral and ipsilateral pairs of levator MNs during stepping sequences in R1 or in R3 are plotted in Fig. 1B and 1C, respectively. As in the completely deafferented preparations analyzed by Fuchs et al. (Fuchs et al., 2011), phase

differences between neighbouring contralateral and ipsilateral pairs of MNs are scattered around an average value of 0.5, corresponding to anti-phase relationships between each neighbouring pair

We found that variability in phase differences (estimated from the circular standard deviation of phase differences; Fig. 1D,E) was reduced during spontaneous stepping of both front legs and hindlegs, compared with quiescent periods in which the leg was still. Because both stepping and burst frequencies changed during the recording sessions, we calculated phase variability separately for each range of burst frequencies, using bins of 0.5 Hz width (except above 5 Hz, where fewer data were available and bin widths were 2 Hz).

The phase variability analysis further revealed that although the intact legs remained motionless, the level of phase variability was independent of burst frequency (see upper left of panels D and E in Fig. 1). However, phase variability of both contralateral (R2–L2) and ipsilateral (R2–R3) pairs was frequency dependent when R1 was stepping (Pearson correlation, P=0.013 and 0.02), whereas the effect of steps in R3 on the contralateral pair R2–L2 was not significantly frequency dependent (P=0.248). This was supported by a Bartlett's multiple group variance test, performed using the circular variance values of phase differences at each frequency with contralateral and ipsilateral phases grouped together. The test indicated that phase variability significantly differed at the different bursting frequencies during R1 steps (P=0.009), but not during R3 steps (P=0.089). Because burst frequencies at the deafferented segments were highly correlated with stepping speed of the intact legs, and phase variability decreased as frequency increased (especially for front leg steps; Fig. 1B,D), we suggest that more movement-related input arriving per unit time could result in tighter intersegmental coupling.

**Brief perturbations and phase response curves**

The second set of experiments was carried out to assess the responsiveness of the CPG to movement-related input from adjacent legs. Leg movements were induced by sending a short pulse of current to the treadmill motor, resulting in an unexpected movement of the intact leg resting on it, while recording the pharmacologically induced burst pattern in neighbouring segments. Pulses were applied at different phases throughout the burst cycle in order to construct a phase response curve (PRC) that characterizes the phase-dependent nature of the impulsive stimulus effects on an ongoing rhythm. In theory, the PRC describes the transient phase change of an oscillator resulting from a perturbation of an impulsive delta function that is infinite in amplitude and infinitesimal in duration (for details, see Holmes et al., 2006; Ermentrout and Terman, 2010). Here we approximated such an impulse by means of a short rectangular current pulse, with the minimum duration sufficient to create leg movement (Fig. 2A, third row).

We defined the cycle period, $T_{mean}$, of the pilocarpine-induced rhythm as the average period over five cycles preceding the one in which the stimulus was applied. The phase of the stimulus, θ, was determined by the interval, $s$, from the beginning of the levator burst to the beginning of the induced movement (see Fig. 2A), and the dimensionless phase θ and PRC $Z(θ)$ were defined as follows:

$$\theta = \frac{s}{T_{mean}}, \qquad (1)$$

$$Z(\theta) = \frac{T_{mean} - T_s}{T_{mean}}, \qquad (2)$$

where $T_s$ is the interval between the last burst prior to stimulus onset and the first burst after it.
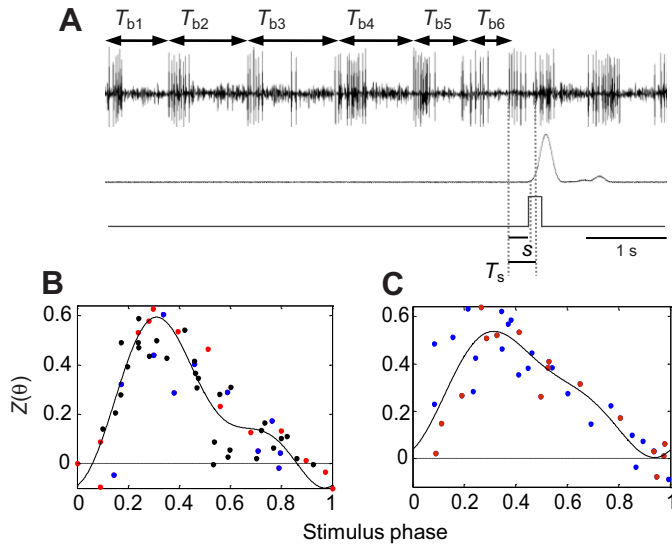
Fig. 2. (A) Effect of an induced movement of an intact R1 leg during pilocarpine-activated rhythms in levator MNs in the deafferented hemi-segment R2 (top trace) in the cockroach *P. americana*. $T_{b1}$–$T_{b6}$ show the durations of bursts prior to the stimulus from which $T_{mean}$ was calculated. Treadmill velocity and current pulse delivered to the motor are shown in the second and third traces, respectively; backwards leg movement was followed by swing to its original location. (B,C) Relative changes in cycle period of bursts in R2 levator MNs are plotted against the phase of stimulation onset in R1 (B) and R3 (C), and fitted to periodic functions using second-order Fourier series (solid curves). Different colours represent different preparations (three with intact R1 and two with intact R3). Each preparation was tested as long as its rhythmic bursting persisted, hence the difference in the number of data points in each case. Burst frequencies varied between 0.5 and 2.5 Hz, with a mean value of 1.24±0.72 Hz.

The PRCs shown in Fig. 2 display changes in burst phase recorded from levator MNs at the right side of the mesothoracic ganglion (R2) against the phase of induced leg movement in the intact leg; PRCs for steps induced in R1 and R3 are shown in Fig. 2B and C, respectively. Values of both PRCs are positive for almost all stimulus phases, indicating that leg movements typically advance the next levator burst of the neighbouring leg.

The PRC also quantifies the sensitivity of the CPG oscillators to steps in neighbouring legs and indicates differences in the intersegmental transfer of input arriving from the front leg *versus* that from the hind leg. In both cases, maximal responsiveness occurs near θ=1/3, shortly before the next stance would begin in a natural double-tripod gait. The narrower peak of the PRC for inputs from R1 compared with R3 (widths of the graphs at half their heights differ by 38%) implies a more selective effect of input from the front *versus* the hind legs. We quantified the level of this selectivity, $S$, as the ratio between the maximal height and the area under the two responsiveness curves as follows:

$$S = \frac{L_{norm}^{max}}{L_{norm}^{1}} , \qquad (3)$$

where $L_{norm}^{max}=\max|Z(\theta)|$ and $L_{norm}^{1}=\int_{\theta=0}^{1}|Z(\theta)|d\theta$. This gave values of $S_F$=2.4961 and $S_H$=1.9041 for selectivity to inputs arriving from the front and the hind leg, respectively – a difference of 24%.

### Periodic perturbations and intersegmental frequency entrainment

In the third set of experiments, to determine whether repetitive sensory inputs from an intact stepping leg can entrain the central
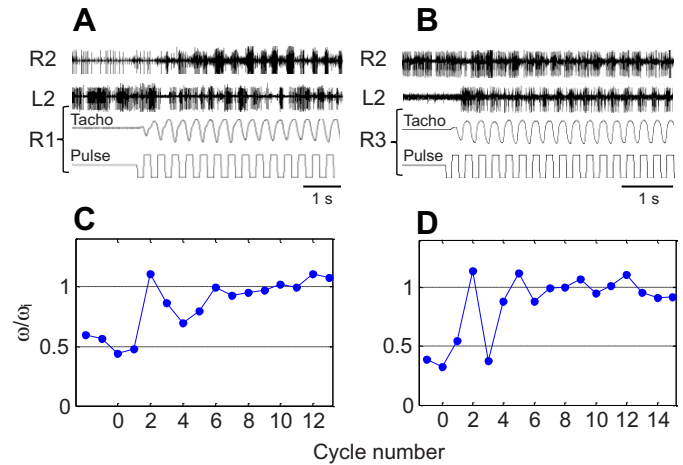


Fig. 3. Examples of recorded burst sequences in R2 and L2 levator MNs during periodically imposed front leg (A,C) and hind leg (B,D) stepping in the cockroach *P. americana*. Treadmill movements imposing R1 and R3 steps (tachometer) and pulse currents that generate these movements are plotted in A and B in the two rows below the burst sequences. The two examples show entrainments to a frequency $\omega_i$ that is higher than the initial bursting frequency $\omega_0$. (C,D) Ratios between the evolving burst frequency $\omega$ and the imposed stepping frequency $\omega_i$ in A and B, respectively, as functions of cycle number following onset of imposed stepping at cycle 0.

motor pattern seen in other legs, we first generated a stable ongoing motor pattern by application of pilocarpine and then forced a single front or hind leg to move periodically back and forth, at different frequencies, while recording the activity in the neighbouring ganglion. The relative duration of the backward and forward movements of each imposed step (corresponding to the simulated steps' stance and swing phases) was programmed to have properties similar to those of natural steps at different speeds.

Fig. 3 shows two examples of pilocarpine-induced bursting in the meso-thoracic ganglion R2–L2 during periodic movement of R1 (Fig. 3A) and R3 (Fig. 3B), at a frequency that was faster than the mean burst frequency ($\omega_0$) calculated before periodic perturbations were imposed (averaged over five preceding cycles, as above). The bottom trace in each example shows the evolution of the ratio between the burst frequency in R2 ($\omega$) and the frequency of the imposed stepping ($\omega_i$) in the forced leg. As can be seen in both examples, after several cycles the frequency of the burst sequences recorded from MNs in R2 and L2 converged to the frequency of imposed leg movements, indicating that periodic movement of a single leg can entrain the central pattern. Each rhythmically active preparation was tested at imposed frequencies ranging from 0.5 to 4 Hz; this frequency range was determined based on it generating consistent results in preliminary tests over a wider range of imposed frequencies. Examples of bursting sequences in R2 during periodic forcing of R3 at different frequencies are shown in Fig. 4. We note that in two preparations, two bursts within a single stepping cycle were occasionally seen in one of the deafferented segments. This could be related to the double bursts previously reported in amputee preparations (Delcomyn, 1991a; Delcomyn, 1991b). For simplicity, and because double bursts were very rare, we included in our analysis only cases in which one burst per cycle (1:1 frequency locking) was observed.

The propensity of stimuli at the different frequencies to generate entrainment was assessed by counting the number of cycles until entrainment was achieved (the entrainment criterion being that the
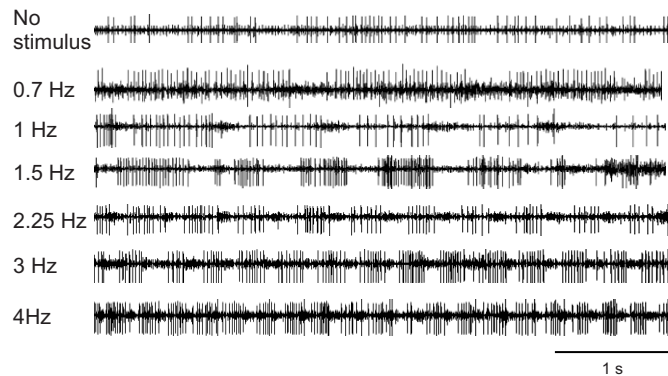
Fig. 4. Typical extracellular recordings from R2 levator MNs in the cockroach *P. americana* during imposed periodic forcing of a hind leg at various frequencies. Traces show segments of the recording prior to (top trace) and during forced stepping at increasing frequencies (listed on left). Periods are plotted after entrainment had been reached.



Fig. 5. Numbers of cycles (±1 s.d.) in R2 until the bursting frequency reached that of the imposed steps in R1 (A; *N*=5 preparations) and R3 (B; *N*=4) in the cockroach *P. americana*. Each animal was tested over a range of imposed stepping frequencies between 0.5 and 4 Hz, and the values of the imposed frequency divided by the pre-stimulus bursting frequency, $\omega_i/\omega_0$, were calculated *post hoc* after determining the initial frequency $\omega_0$ as described in the text. Each point represents the mean number of cycles from the onset of imposed leg motions until entrainment as a function of $\omega_i/\omega_0$ in bins of 0.2 Hz. Shaded areas represent statistical significance when comparing the number of cycles needed to entrain the central pattern generator using front leg *versus* hind leg stepping (light grey, $P<0.05$; dark grey, $P<0.01$). Note that, in common with entrainment studies in nonlinear oscillator theory and unlike in Fig. 3 above, the frequency ratio $\omega_i/\omega_0$ is used here.

ratio between the imposed and the bursting frequencies should satisfy $0.9<\omega_i/\omega<1.1$ for at least three successive cycles). Fig. 5 shows the entrainment results for both intact front and hind legs. The number of cycles before reaching entrainment serves as a proxy for coupling strength in the entrainment domains typically computed for models of periodically forced oscillators in theoretical studies (Guckenheimer and Holmes, 1983). In both front and hind legs, above and below the resonance condition ($\omega_i/\omega_0$=1, at which the pre-stimulus and imposed frequencies match), there is a consistent increase in the number of cycles needed to reach entrainment as the imposed frequency diverges from the initial frequency (Fig. 5). Comparison of Fig. 5A and 5B reveals that, over most of the frequency range tested, periodic stimulation of a hind leg produced significantly faster entrainment than stimulation of a front leg, suggesting stronger proprioceptive feedback from hind legs than front legs.

Closer examination of the coordination pattern elicited by periodic entrainment reveals that the resulting fictive gait has characteristics similar to those observed during normal walking. Specifically, as in free walking (Delcomyn, 1971), neighbouring pairs of hemisegmental oscillators burst out of phase, with a mean phase difference of 0.514 for R2–L2 (data not shown). Furthermore, phase delays between the beginning of stance in the mechanically driven leg and the onset of levator burst at the neighbouring leg oscillator are similar to those expected in free walking (see Fig. 6A,B). The latter delay was extrapolated by taking the average values of retraction/protraction ratios of steps at each frequency observed in free walking (Hughes, 1952; Spirito and Mushrush, 1979), and calculating expected delays between stance onset in R1 or R3 and the following swing onset in R2.

In addition, similar to the preparations with a spontaneously walking intact leg, in our repetitive stimulus experiments the motor pattern variability decreased as the frequency of the imposed leg movement increased (Fig. 6C,D). Finally, and again consistent with our observations with a free-stepping leg (Fig. 1), driving the front leg resulted in less variable phase delays and clearer speed dependency compared with driving the hind leg. These findings together suggest that our mechanically imposed motions successfully mimic natural steps and that step-related input strengthens the central coordination pattern in a speed-dependent manner.

## DISCUSSION

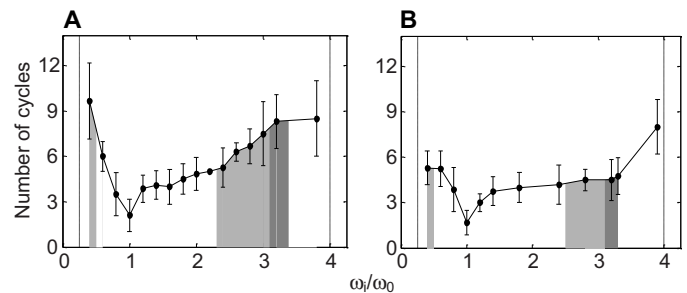The present study demonstrates that movement-related input from stepping legs reinforces centrally generated patterns to create a coordinated walking gait. Inter-segmental input from front and hind legs is combined with internal coupling among segmental oscillators in a manner that strengthens the inherent rhythmic pattern and reduces cycle-to-cycle variability.

### Proprioceptive feedback reinforces the centrally generated motor pattern

In a recent study, we observed tighter coupling between motor outputs of segmental CPGs following steps of a single intact front leg (Fuchs et al., 2011). This could result from leg movement-related feedback and/or from central modulation that causes both phasic excitation of steps and strengthening of phase coupling. Distinguishing between these two scenarios to assess the role of movement-related sensory feedback in modifying the motor pattern is not straightforward. It requires opening the closed loop between motor control and sensory processing *via* careful experimental manipulation. The present study approaches this by inducing mechanical movements in a single intact leg and showing that they result in reduction of intersegmental phase variability in a frequency-dependent manner, reminiscent of the effect of natural steps.

Similarities between the effects of spontaneous and imposed steps of a single leg on the motor pattern at other hemiganglia suggest that both free and forced steps activate the same pathways, and indicate that movement-generated proprioceptive inputs can significantly strengthen the CPG's internal coordination. However, not all leg movements necessarily influence the motor pattern of the other ganglia. Our results suggest that single steps, when coordinated with the inherent rhythm and rhythmic stimuli at steady rates, suffice to influence the activity of other segments, whereas single, irregularly timed movements may not (PRCs in Fig. 2B,C show low susceptibility to inputs arriving near the beginning and end of the burst cycle).

Reduction in phase variability upon increase in availability of sensory inputs is consistent with previous observations on the effects of addition and removal of sensory information from legs in free-walking preparations (Tang and Macmillan, 1986; Delcomyn,
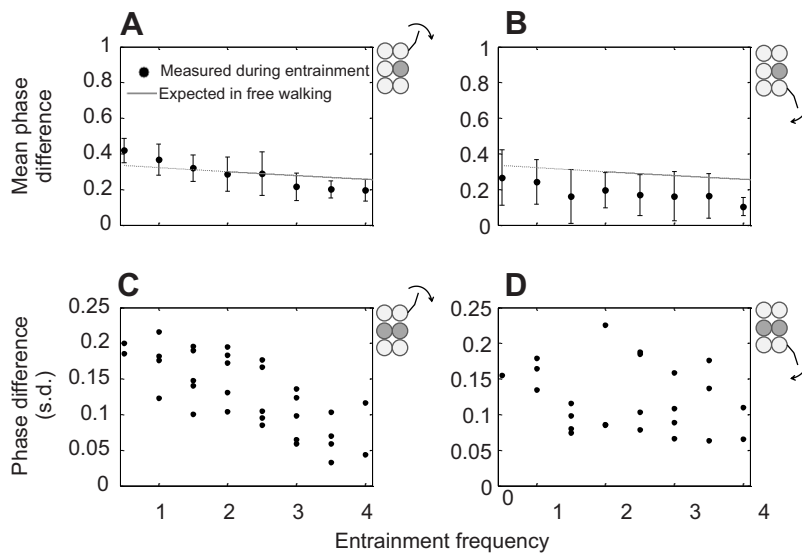
Fig. 6. Phase analysis of bursts entrained by periodic steps in front (left column) and hind legs (right column) of the cockroach *P. americana*. (A,B) Mean ±1 s.d. phase differences between stance onset of the periodically forced leg (R1 or R3) and burst onset in the levator MN of R2, calculated separately for each entrainment frequency. Grey lines show the average values between stance onset in one leg and swing onset of the ipsilateral leg derived from previous behavioural data (Hughes, 1952; Spirito and Mushrush, 1979). (C,D) Circular standard deviations of contralateral phase relationships (R2–L2) for each preparation against the entrainment frequency $\omega_e$. In all panels, for each entrainment trial, we averaged over the first eight bursts after entrainment had been reached. Schematics of cockroach limbs and hemisegments are as described in Fig. 1A.

1991a; Delcomyn, 1991b). Specifically, using muscle potential recordings from intact and amputated leg stumps, Delcomyn (Delcomyn, 1991a; Delcomyn, 1991b) showed that leg amputation, and the concomitant reduction of sensory input, resulted in an increase in motor pattern variability in all leg pairs.

Our current results, obtained by means of a complementary approach of starting with a reduced preparation and studying the effect of gradual addition of phasic input from a single leg, strengthen these observations. Our approach also allows better isolation of proprioceptive feedback with minimal intrusion of the complex mechanical interactions that are exhibited during walking. Within the frequency ranges obtained for our single-legged preparations (0.5–4 Hz), it appears that as the frequency of movement-related sensory information increases, the motor pattern tightens (Figs 1, 6). Related experiments in the lamprey indicated that mechanical movement has significant impact both on the frequency of the motor output and on the strength of intersegmental coupling (Guan et al., 2001). Intersegmental coupling, in turn, affects phase differences among CPG outputs. We emphasize that, during intervals in which the leg was motionless and phasic input was absent, intersegmental phase variability did not appear to depend on frequency (Fig. 1). This suggests that changes observed in the degree of intersegmental coordination during leg movements are not only a direct consequence of changes in bursting frequency, but also depend on the phasic inputs.

The question of whether inputs are integrated similarly at both high and low speeds is a central challenge in research of the motor control of locomotion (Koditschek et al., 2004; Holmes et al., 2006; Sponberg et al., 2011). When speed increases, not only does the type of sensory information available during each stride change (Duysens et al., 2000; Zill et al., 2009), but the translation of neural commands into mechanical actions is also affected (Sponberg et al., 2011). The present study and other recent papers indicate that the mechanics of a given task can shape the neural signals delivered to control movement (Dickinson et al., 2000; Holmes et al., 2006; Pearson et al., 2006; Cowan and Fortune, 2007; Pfeifer et al., 2007; Chiel et al., 2009; Proctor et al., 2010; Tytell et al., 2011). Collectively, this work emphasizes the need for an integrated neuro-mechanical approach.

### Leg-specific contributions of proprioceptive inputs

A comparison between the influence of front and hind leg movements on the motor patterns in our study suggests rostral-to-

caudal asymmetries. Specifically, hind-leg movements resulted in faster entrainment but less accurate phase relationships than front-leg movements (Figs 5, 6). We also found that feedback from hind legs was less frequency dependent than that from front legs (Fig. 1). These findings are in accordance with previous observations in the stick insect (Borgmann et al., 2007) and the cockroach (Delcomyn, 1991a; Delcomyn, 1991b), which indicate that activity in the mesothoracic segments is more strongly phase coupled to front-leg steps than hind-leg steps.

PRCs quantify the responsiveness of a limit cycle oscillator to inputs arriving at different phases in its cycle, and thus may be used to assess functional relationships between sensory signals from one leg and motor patterns of another. Comparing the PRCs shown in Fig. 2 further illustrates the differences noted above. The narrower peak of the PRC shown in Fig. 2B indicates a more selective transfer of phasic information in signals arriving caudally from the front leg than the broader PRC shown in Fig. 2C. Coupled with the entrainment rates shown in Fig. 5, these PRCs support our observations of phase variability during stepping (Figs 1, 6), suggesting weak but more precise phasic feedback from front legs, and stronger, albeit more diffuse, phasic feedback from hind legs.

These asymmetries could result from segmental differences on multiple levels. Estimates of central intersegmental coupling strengths show stronger descending than ascending coupling between deafferented mesothoracic and metathoracic ganglia in the cockroach [see fig. 6 in Fuchs et al. (Fuchs et al., 2011)]. Further sources of asymmetry could derive from differences in the composition of sensors on different legs, differences in sensory pathways for mechanical responses, or differences in intersegmental transfers of movement-generated inputs. Indeed, front and hind legs are specialized to allow the performance of different functions. In crustacean walking, for example, different legs contribute differently to locomotion: the hind legs push the body whereas the more anterior leg pairs 4, 3 and 2 pull the body forward (Jamon and Clarac, 1995). In insects, the smaller front legs are used under direct visual control to guide behaviours such as grasping of food and exploration of surfaces during walking, whereas the more massive hind legs allow generation of large forces and movements in the horizontal plane for escape running (Quimby et al., 2006). Despite these differences, studies of sensory encoding of load in cockroaches indicate that motor responses to different load signals are similar in each of the serially homologous legs (Quimby et al., 2006).

In addition to tonic signals encoding graded measures of load during stance (Zill and Moran, 1981; Zill et al., 1999), phasic signals encode state information, such as position, in the timing of individual spikes (Wong and Pearson, 1976). Because most of the complexity of front-leg usage occurs during the swing phase, differences between homologous legs could result from differences in the encoding of phasic sensory information to allow the finer precision needed for front leg control. Overall, a combination of load, position and velocity feedback modulates stepping patterns in insects (Büschges et al., 2008; Proctor and Holmes, 2010), and different weighting of phasic and tonic sensory information could explain the more accurate, speed-dependent feedback from front legs in comparison to hind legs.

Is the need for greater precision in the control of rostral segments a general principle in forward locomotion? In anguilliform (eel-like) swimming, phase lags between the onset of muscle activation and mechanical body curvature increase along the body (Williams et al., 1989; McMillen et al., 2008), and differences in translations between neural commands and mechanical movement are seen (Tytell and Cohen, 2008). Some commonalities in differing requirements of rostral *versus* caudal segments that are observed in anguilliform swimming are also supported by our current findings in cockroach walking. These include requirement of high force production in caudal segments compared with relatively low force production by the rostral segments, the latter being responsible for initiation of turns and accelerations (Fagerstedt and Ullén, 2001; Tytell and Cohen, 2008). However, because viscoelastic interactions between the body and the surrounding water, as well as muscle mechanics and body geometry, play significant roles in determining phase lags during swimming (McMillen et al., 2008; Tytell et al., 2010), distinct mechanisms are also likely to modulate differential force production and phasic contributions in legged locomotion.

In summary, our finding that feedback from front legs is relatively weak but phasically precise, whereas feedback from hind legs is stronger but phasically diffuse, is consistent with the rostro-caudal asymmetries noted in previous studies; namely, high force production in caudal segments and phasic accuracy in rostral ones. Further studies to investigate the combined effects of different sensory and central pathways using experiments and modelling will assist our understanding of how these asymmetries interact to produce symmetric gaits and stable locomotion.

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