Neuromechanical response of musculo-skeletal structures in cockroaches during rapid running on rough terrain

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SUMMARY

A musculo-skeletal structure can stabilize rapid locomotion using neural and/or mechanical feedback. Neural feedback results in an altered feedforward activation pattern, whereas mechanical feedback using visco-elastic structures does not require a change in the neural motor code. We selected musculo-skeletal structures in the cockroach (*Blaberus discoidalis*) because their single motor neuron innervation allows the simplest possible characterization of activation. We ran cockroaches over a track with randomized blocks of heights up to three times the animal's 'hip' (1.5 cm), while recording muscle action potentials (MAPs) from a set of putative control musculo-skeletal structures (femoral extensors 178 and 179). Animals experienced significant perturbations in body pitch, roll and yaw, but reduced speed by less than 20%. Surprisingly, we discovered no significant difference in the distribution of the number of MAPs, the interspike interval, burst phase or interburst period between flat and rough terrain trials. During a few very large perturbations or when a single leg failed to make contact throughout stance, neural feedback was detectable as a phase shift of the central rhythm and alteration of MAP number. System level responses of appendages were consistent with a dominant role of mechanical feedback. Duty factors and gait phases did not change for cockroaches running on flat *versus* rough terrain. Cockroaches did not use a follow-the-leader gait requiring compensatory corrections on a step-by-step basis. Arthropods appear to simplify control on rough terrain by rapid running that uses kinetic energy to bridge gaps between footholds and distributed mechanical feedback to stabilize the body.

Key words: locomotion, motor control, muscle.

INTRODUCTION

Rapid locomotion in natural environments can drive neuromechanical control systems to their functional limits. Despite this fact, far more progress has been made on the neural control of terrestrial locomotion for slower, quasi-static movement (Buschges, 2005; Cruse et al., 2007; Pearson, 2004). In a dynamic, unpredictable environment, a muscle and its tendon or apodeme often must do more than generate the force and work required for steady-state locomotion (Dickinson et al., 2000). These musculo-skeletal structures (Delp and Loan, 1995; Zajac, 1989) must stabilize locomotion by managing any energetic deviations from steady state produced by perturbations from the environment (Holmes et al., 2006; Koditschek et al., 2004). A stable solution may demand that a structure produce, absorb, store and return and/or transfer energy (Biewener and Daley, 2007; Biewener and Gillis, 1999; Full et al., 1998). A given structure can respond with no change in its neural activation from steady state or by adjusting the neural signals to the muscle. A structure that is passive with no activation or one with unaltered rhythmic feedforward activation from a central pattern generator (CPG) can provide stabilization through mechanical feedback. These responses are sometimes referred to as preflexes (Brown and Loeb, 2000). A structure that reacts to a perturbation through sensory modulation of the efferent motor code or neural feedback can provide stabilization using reflexes. In the present study, we focus on rapid running that may limit the bandwidth of neural feedback (Koditschek et al., 2004), as well as the time a musculo-skeletal structure has to generate force and relax for the next cycle (Marsh and Bennett, 1985; Swoap

et al., 1993). In addition, while there is a growing understanding of the control strategies that animals employ in response to single perturbations (Biewener and Daley, 2007; Daley et al., 2006; Jindrich and Full, 2002; Kohlsdorf and Biewener, 2006; Revzen et al., 2005; Watson et al., 2002a), locomotion is typically characterized by negotiation of irregular terrain that repeatedly perturbs the steady state locomotor limit-cycle (Spagna et al., 2007). Our primary objective was to measure the activation of musculo-skeletal structures during rapid running on rough, irregular *versus* flat, regular terrain to determine the type of feedback a musculo-skeletal structure employs for stabilization.

We selected musculo-skeletal structures of an insect that are exclusively innervated by a single motor neuron, because their identifiable muscle action potentials (MAPs) provide the simplest possible characterization of muscle activation. The cockroach *Blaberus discoidalis* possesses a pair of dorsal/ventral femoral extensors[1] (178 and 179) (Carbonell, 1947) that are putative control muscles and innervated only by a single fast motor neuron (Df) (Pearson and Iles, 1971; Pipa and Cook, 1959). A single action potential in Df produces one, relatively large MAP in its target muscles, resulting in nearly identical patterns of activation in both extensors muscles (Ahn et al., 2006; Full et al., 1998; Watson and Ritzmann, 1995). When first recruited during running, these

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[1]These muscles have also been termed trochanteral extensors or coxal depressors, although we adopt the femoral extensor usage as it is the closest match to anatomical function. These specific muscles have also been referred to as the anterior/posterior pair of femoral extensors in literature because of their orientation when the leg is fully extended.

femoral extensors have been hypothesized to shorten the transition from flexion to extension and possibly increase joint angular velocity at higher speeds (Levi and Camhi, 1996; Watson and Ritzmann, 1998b). The relatively rapid force development in response to Df action potentials (10 ms latency and 30 ms to peak force) (Full et al., 1998; Watson and Ritzmann, 1995), compared with the much longer time (225 ms) to first movement in 'slow' Ds motor neuron activation (Watson and Ritzmann, 1995), could enable effective neural feedback control in these extensors at higher running speeds. Quantifying energy management in these muscles has shown that they are capable of producing, storing and returning and/or absorbing energy depending on activation level (i.e. the number of MAPs), phase of activation and strain (Ahn et al., 2006; Full et al., 1998). The low twitch to tetanus ratio (0.2) found in the ventral femoral extensor (179) permits a fine gradation of force, suggesting considerable control potential *via* neural feedback. Here, we directly compare the activation pattern of these musculo-skeletal structures as cockroaches run at their preferred speed over flat *versus* rough terrain containing randomized block-like obstacles up to three times 'hip' height (1.5 cm). We hypothesize a change in MAP number, inter-stimulus interval, burst phase and/or interburst period reflecting the use of neural feedback to stabilize rough terrain locomotion. Alternatively, no change from the feedforward motor activation pattern would support the hypothesis that these structures contribute to stability by mechanical feedback.

Connecting feedback provided by individual musculo-skeletal structures to higher level, task-relevant variables remains a challenge (Biewener and Daley, 2007; Cappellini et al., 2006; Flash and Hochner, 2005; Todorov et al., 2005). Although approaches correlating muscle activation patterns with kinematic variables have been successful at reducing dimensions (Ivanenko et al., 2005; Wang et al., 2006), we must ultimately find the mechanistic link between musculo-skeletal structures' responses to a perturbation and the recovery dynamics of the body or center of mass (Biewener and Daley, 2007; Holmes et al., 2006; Ting, 2007). By comparing a musculo-skeletal structure's perturbation response to that of the whole animal, we can begin to understand how the mechanical and neural feedback of individual structures couple to result in task-level stability. Therefore, our secondary objective was to determine if a gait change during perturbed and unperturbed locomotion is consistent with the mechanical and/or neural feedback response measured at the level of the individual musculo-skeletal structures.

To characterize the task-level feedback response of the animal, we measured speed, pitch, roll and yaw of the body, static stability margin, duty factor, stance initiation phase and stance termination phase for cockroaches running on flat *versus* rough terrain. If cockroaches are perturbed on the rough terrain, then pitch, yaw and roll and static stability margin should show greater variation and perhaps a different pattern than on flat terrain. If animals can negotiate this rough terrain by completing the course without large decrements in speed, then they must be recovering from perturbations. Significant alterations in gait could point to an important role of neural feedback to musculo-skeletal structures for recovery (Cruse, 1976; Pearson and Franklin, 1984). This would be particularly true if animals showed a shift away from the typical steady state alternating tripod gait or demonstrated compensatory corrections on a step-by-step basis. We tested the latter hypothesis by determining whether cockroaches use a follow-the-leader (FTL) gait on rough terrain where each posterior foot is placed on the secure foothold used by a more anterior leg

on the same side (Song and Choi, 1989). Alternatively, animals running over rough terrain that rely on collections of mechanically controlled musculo-skeletal structures might show little change in the feedforward alternating tripod gait generated during unperturbed locomotion and demonstrate no evidence of precise stepping (Spagna et al., 2007).

Characterizing the mechanical and neural feedback contributions from a musculo-skeletal structure in the context of the body's response to perturbations is a necessary step to understand how responses at lower levels interact to produce control at higher levels of the hierarchy. Yet, we view this approach as one of a suite of approaches. Complementing perturbation studies with characterization of musculo-skeletal structures' capabilities in isolation, direct manipulation of their motor and sensory neural code during locomotion, and modeling using a dynamical systems approach, will enable a far more complete understanding of the control of locomotion.

MATERIALS AND METHODS
Animals
We used male cockroaches *Blaberus discoidalis* L. (Carolina Biological Supply, Burlington, NC, USA), as females were often gravid and therefore under different load-bearing conditions. The six males included in this study had an average mass of 1.98±0.29 g (mean ± s.d.). Prior to experimentation, we kept the cockroaches in communal plastic containers (initially 10–20 adults) at room temperature (22°C) on a 12 h:12 h light dark cycle and provided water and food (fruit and dog chow) *ad libitum*.

Rough and level terrain track
To simulate cockroaches running over natural, rough terrain, we constructed an artificial wooden terrain with a random distribution of surface heights to ensure that no regularity in the substrate would contribute to stabilization. The rough terrain surface was constructed using 1 cm×1 cm variable height blocks of wood formed into a track 22 cm long by 10 cm wide (Fig. 1A). The height of each block was randomly assigned to a value selected from a Gaussian distribution with a mean of zero and a standard deviation of 0.5 cm (i.e. near cockroach 'hip' or coxa-body joint height), so that perturbations in the running surface reached up to three times the cockroach's hip height (Fig. 1).

We attached 30 cm-long flat balsa wood tracks to the beginning and end of the rough terrain to allow the cockroach to encounter and leave the blocks without stopping. Balsa wood walls along the approach and exit trackways and mirrors surrounding the rough terrain restricted the cockroach to running along the track. The terrain was raised by approximately 2 cm with respect to the approach and exit trackways to ensure that the cockroach always encountered a large initial and final step perturbation (2–4 cm) during running. The rough terrain was replaced with a third segment of level balsa wood for the flat (unperturbed) terrain runs.

Kinematics
We video recorded the cockroaches running over rough and flat terrains with two cameras (Ektapro Model 2000 cameras, Eastman Kodak, Rochester, NY, USA) recording at 500 frames s^{-1} with a resolution of 512×384. Large mirrors were placed at a 15–30° incline along the terrain to reflect the image. The position of each camera above the trackway was oriented 5–10° off-center to provide both a view of the track and a reflected view of the cockroach in one of the mirrors. This arrangement provided four views distributed in a 60° arc above the terrain.
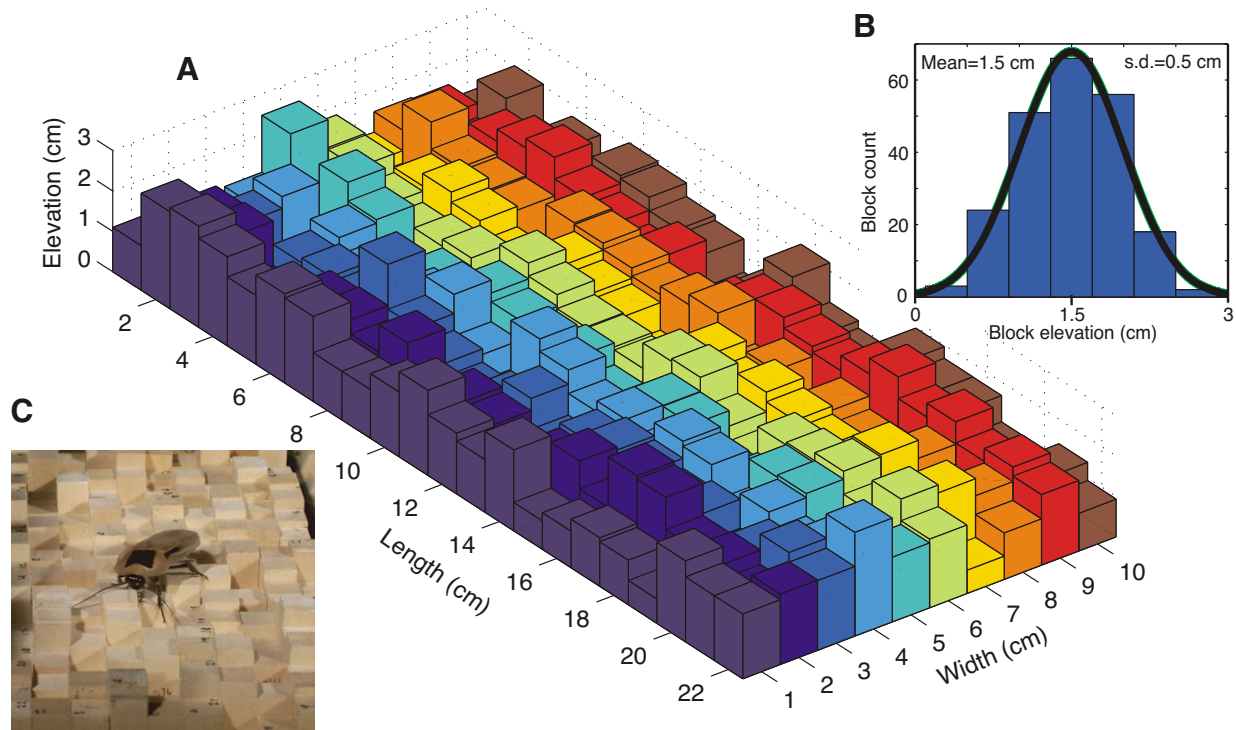
Fig. 1. Cockroaches (*B. discoidalis*) were run over a rough terrain (A) with a Gaussian distribution of surface heights (B) up to three times the hip height of the animal (C). The cockroach experienced repeated random perturbation while negotiating the terrain. Offsetting the terrain from the entry and exit tracks resulted in very large (4–6 times hip height) perturbations to the first and last steps on the terrain. The level surface of all blocks was 1 cm×1 cm.

We used a flood lamp (Lowel, Brooklyn, NY, USA) located adjacent to the cameras to illuminate the surface and two double goose-neck lights located on either end of the arena to reduce shadows and provide more even lighting particularly on the rough terrain. Images were buffered through the camera memory until post-triggering, after which a 1024 frame (2048 ms) clip from each camera was reviewed and cropped to the relevant time segment (usually 1000–1600 ms). Frames were then downloaded as a series of images (.tiff) and converted to movie files (.avi) for digitization. All video capture, downloading, and conversion were done with a software program (MotionCentral v2.7.5; Redlake MASD, Tuscon, AZ, USA).

Muscle action potentials (MAPs)

We recorded the activation patterns of the muscle 179 (Carbonell, 1947) that parallels the proximal-distal axis of the coxa on the medio-ventral side. Its dorsal counterpart, muscle 178, receives identical activation from the same motor neuron, Df (Ahn et al., 2006). Muscle action potential recordings follow previously published methods (Ahn and Full, 2002; Watson and Ritzmann, 1998a; Watson and Ritzmann, 1998b). Briefly, we created two small holes in the cuticle using size 0 insect pins. Two 50 μm silver wires (California Fine Wire Company, Grover Beach, CA, USA), whose tips were stripped of insulation and formed into small balls, acted as a bipolar electrode directly under the exoskeleton along the proximal–distal axis of the coxa. The silver wires were kept in place by making the balls slightly larger than the holes and covering the insertion points with a small quantity of dental wax. Care was taken to prevent wax from contacting joints. A third wire was placed in the third or fourth most posterior abdominal segment to serve as the reference for the bipolar recording. Finally, we epoxied the three wires to the dorsal

abdominal surface to prevent entanglement with the legs during running.

MAP signals coming from the running cockroach were collected differentially using an AC pre-amplifier (Grass-Telefactor, West Warwick, RI, USA) and amplified 2000× with 30 Hz low pass, 1 kHz high pass and 60 Hz line-in filters. We acquired the data through an acquisition board (National Instruments, BNC 2090, Austin, TX, USA) and PCI card using custom programs (Matlab, MathWorks, Natick, MA, USA). The electrophysiological recording synchronized with the video *via* a custom external trigger box. We imported the resulting raw data into a program (Spike2 v5.07, Cambridge Electronic Design, Cambridge, England) for analysis and applied a 60 Hz notch filter (alpha=1). Thresholding and peak finding searches discriminated spikes from the recording. The resulting spike times were exported and synchronized with the kinematic data.

Experimental protocol

In preparation for experimentation, cockroaches were cold-anesthetized, although direct contact with ice was avoided by placing the cockroach in a submerged plastic well. After motion ceased in about 30 min, we removed the specimen from the bath. The distal regions of both pairs of wings were removed to expose the dorsal side of the abdomen. Kinematic markers were composed of small dots of white liquid paper and one was placed on the tarsus of each leg. Markers were placed on the distal and proximal extremes of each tibia to provide additional reference points to determine leg movement during tracking. Finally, markers were added on the dorsal side of the cockroach body. One marker was added on the center of the pronotum (referred to as the head point), one on the second thoracic segment, and three along the abdomen. To measure pitch, roll and yaw during traversal, we affixed three

cockroaches with a balsa wood cross above the COM with a fifth arm rising above the cockroach. Matching a three-dimensional model of the cross to the recovered cross markers in the resulting videos enabled calculation of body rotations about all three axes.

If the cockroach was not motionless after marking, it was returned to the ice bath for 15–30 min. We then secured the specimen to a rubber platform using staple-shaped pins to hold the body and coxa in proper orientation for MAP wire insertion. At no time was the cockroach's cuticle pierced except to create the holes required for the recording wires. A small amount of ice was placed around the cockroach during operation to keep the air cool and maintain sedation. Cockroaches were allowed at least 1.5 h to recover at room temperature prior to running trials.

After recovery, we released the cockroach onto the approach track and elicited rapid running by gently probing the posterior abdominal segments and cerci with a small rod. Cockroaches quickly ran down the track and traversed either the rough or flat terrain before entering a shaded region at the end of the exit ramp. If the cockroach stopped or attempted to climb the wall of the track, we repositioned it manually on the approach track. After a successful trial, the cockroach had at least 5 min to recover as the video was downloaded from the camera buffer. The track was illuminated only during recording to encourage the cockroaches to remain stationary in between trials and to prevent a change in temperature.

We randomly chose whether each animal would first run on flat or rough terrain. We continued recording until six trials were obtained that met our operational definition and then switched to the other terrain type. There was at least a 30 min transition time between the two terrain types. Occasionally, the animal would snare and break the long trailing recording wire during the experiment, resulting in fewer than six trials on the second terrain. We analyzed only individuals with at least one flat and one rough terrain run.

We recorded a trial when a cockroach made one complete traversal of the rough or flat terrain. Each trial was divided into constituent strides, which are considered individually. We defined a stride as starting when the hind left leg, from which we recorded MAPs, first initiated stance. Since we wished to test cockroaches' stability in the face of repeated perturbations to high-speed running, some strides were not included in our analysis. Specifically, we rejected strides under four conditions. We rejected strides when the cockroach's body contacted one of the mirrored side walls. In these trials, cockroaches could experience a lateral perturbation due to contact and often the tarsi were obscured throughout the analysis. Secondly, under normal and perturbed running the cockroach would naturally yaw, but cockroaches would occasionally exhibit substantial turns, often in response to contacting and tracking the wall (Camhi and Johnson, 1999). We therefore removed strides in which the cockroach exhibited turns of greater than 15°. Thirdly, we excluded strides in which the cockroach started or ended with a velocity of zero (i.e. when the animals stopped). Finally, strides occasionally occurred in which one leg failed to make contact with the ground throughout the entire stride (duty factor=0) due to the leg being placed in a large trough formed by the rough blocks. We separately consider the few strides containing these mis-steps (N=6).

Kinematic analysis

After the experiments, we imported uncompressed videos (.avi) of each run into a commercial motion analysis software package (Peak Motus v8.5, Peak Performance Technologies division of Vicon,

Centennial, CO, USA) for digitization. We analyzed the actual image (non-reflected) from whichever video provided the least obscured view of the animal. Other views were used to confirm placement of legs and visually corroborate our analysis.

We used a 9.6×8.0 cm calibration object (Lego blocks, Lego Systems, INC., Enfield, CT, USA) with 24 digitization points. In all images at least 16 calibration points were visible. Distances between each pair of points provided references to calibrate the video images. The calibration object was large enough to fill approximately half of the camera screen to ensure distortion in the image did not significantly affect the calibration.

All markers on the cockroach's body and legs were digitized in each frame. The resulting data were exported to a spreadsheet editor (Excel, Microsoft Corp., Redmond, WA, USA) and synchronized with the MAP data. We used custom scripts (Matlab) to represent the trajectory of each leg and stance onset was operationally defined as the time when leg movement relative to the surface went to zero. We constructed gait diagrams from the leg timing data. Steps in which the stance timing of the hind left leg could not be determined due to occlusion were not used in the analysis. Absolute footfall position and the block where foot placement occurred were recorded for analysis of FTL stepping.

To calculate running speed on a stride-to-stride basis, we took the linear distance between the thoracic body marker at the time of stance initiation in the hind left leg in two subsequent steps and divided by the stride time between them. We also calculated the yaw angle of the cockroach in each camera frame as the angle between the line segment formed by the head and thorax markers on the cockroach and a line segment oriented along the long axis of the terrain that was constant for each run. The difference in this angle between subsequent stance initiation times of the hind left leg determined the stride-to-stride heading adjustment of the animal.

MAP analysis

Each stride had a corresponding set of MAPs defined by their peak spike times associated with the hind left leg. Since both number of spikes and their timing affect muscle activation, it was important to characterize several variables to determine if the neural activation patterns were changing from flat to rough terrain running (Fig. 2A). Therefore, we analyzed the number of spikes occurring in each step, the time between spikes (interspike interval, ISI), the relative phase of the burst of spikes with respect to the initiation of stance in that leg, and the timing between the initiations of bursts (interburst interval, IBI; Fig. 2B). Since the number of spikes per stride is categorical and ordinal, we performed χ^2-tests for statistical differences. Interspike interval, burst phase and interburst interval are all continuous variables for which we compared means and variances across the two terrain types. Since stepping speed impacted the timing between bursts and spikes and each animal shifted its activation phase slightly, we used partial t-tests from multiple regressions of the timing variables with respect to terrain, speed, and/or individual. We implemented all statistics in a data analysis program (JMP v5.1, SAS Institute, Inc., Cary, NC, USA) except for multiple regressions, which were accomplished with another program (STATA v8.1, Stata Corp., College Station, TX, USA).

RESULTS

Overall, we analyzed 20 flat terrain and 19 rough terrain trials from six cockroaches comprising 144 and 74 steps, respectively. Cockroaches ran with a preferred speed of 27.1±5.9 cm s^{-1} (mean
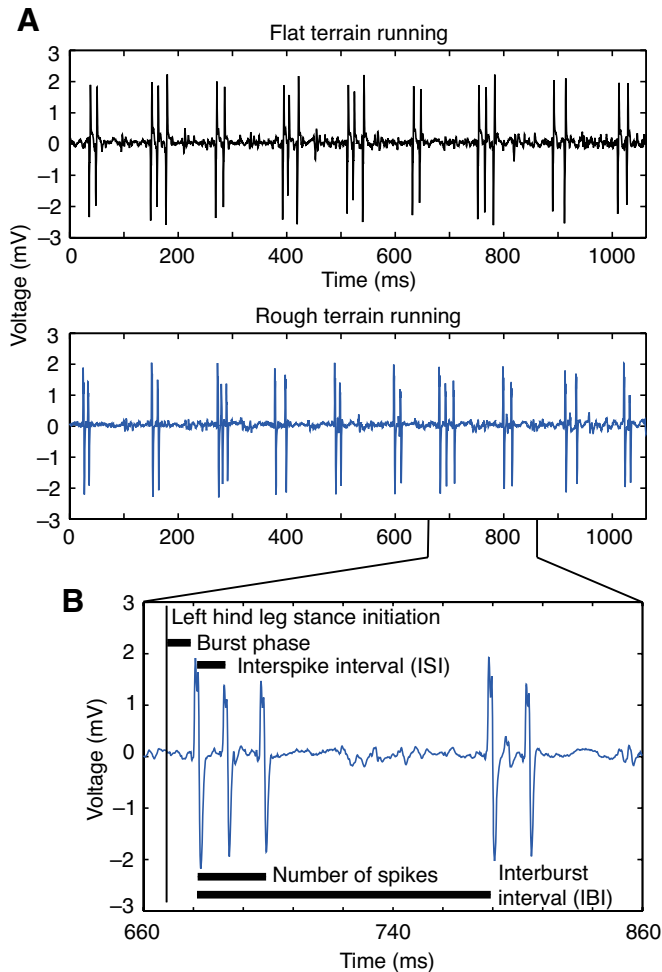
Fig. 2. Muscle action potentials during running over flat *versus* rough terrain. (A) Recordings of the muscle action potentials (MAPs) activating the ventral femoral extensor (179) in the hind left leg of a cockroach running on flat (black trace) and rough terrain (blue trace). Each cluster of spikes occurs during a single stance phase. Changes in neural stimulation would necessitate neural feedback in the control of locomotion over the rough terrain. (B) Four measures of the activation burst describe the degree of stimulation (number of spikes) and the relevant timing parameters (interspike interval, interburst interval and burst phase).

± s.d.) while traversing the flat terrain. There was an approximate 18% decrease in speed while traversing the rough terrain (22.0 ± 6.6 cm s^{-1}, t-test, $P<0.001$), which remained significant when we corrected for effects of individual animals (F test, $P<0.001$). It is important to note that these measurements did not take into account the increased vertical distance the cockroach was forced to traverse during rough terrain navigation. This difference in speed did not affect our kinematics or electrophysiological analyses, except when considering interspike interval and burst period, where we discuss its impacts.

System level perturbations

We intended the rough terrain treatment to significantly perturb steady-state running behavior. The repeated surface height perturbations increased body pitch (Fig. 3A), roll (Fig. 3B), and yaw (Fig. 3C) when cockroaches ran on the rough terrain. Comparing across all flat and rough terrain trials from the three animals with the tracking cross, pitch, roll and yaw all showed

statistically significant greater variation while on the rough terrain (F tests for equivariance, $P<0.0001$).

Rough terrain running also strongly disrupted the static stability of the cockroach. Static stability occurs when the center of mass (COM) rests within the support area defined by the legs in contact with the substrate (Ting et al., 1994). Both the degree of static stability, defined as the actual distance between the COM and the nearest edge of the support tripod, and the ideal margin of stability, the maximum possible degree of static stability, were significantly lower while traversing the rough terrain (t-tests, $P<0.0001$, Kruskal–Wallis tests, $P<0.0001$). Cockroaches running on both rough and flat terrains showed significant periods of static instability where the COM fell outside of the tripod of support, but the rough terrain treatment significantly increased the time spent statically unstable (t-test, $P<0.0001$, Kruskal–Wallis test, $P<0.0001$). This necessitates that inertial effects preserve posture through dynamic stability (Ting et al., 1994) or external forces (e.g. adhesion/friction) counteract the destabilizing gravitational effects. Taken together the pitch, roll and yaw as well as the static stability data confirm the visual observation that cockroaches running over the randomly rough terrain experienced significant system level perturbations.

Musculo-skeletal structure activation response to perturbations
MAP spikes per burst

We could not show any significant difference in the distribution of the number of MAPs between flat and rough terrain trials for the hindleg femoral extensors (Fig. 4A). During flat terrain running, MAP recordings of muscle 179 demonstrated a stereotyped pattern of 2–3 spikes during each stance period. Occasionally steps occurred with 1 or 4 spikes. Despite repeated perturbations to body height and orientation, a contingency analysis of spike counts found that the distribution of spike number did not significantly vary between flat and perturbed running (Pearson χ^2-test, $P=0.25$; Likelihood ratio test, $P=0.26$). We note that a contingency analysis is sensitive to differences in both the mean number of spikes and, more importantly, the variance of spike occurrences. Therefore, if a significant number of rough terrain steps demonstrated both increases and decreases in activation, as might be expected from the random nature of the perturbation, then these tests would show significant differences ($P<0.05$) in the distribution of spike activity.

To test the effect of inter-animal variability obscuring results in the spike count distributions, we performed a Cockran–Mantel–Haenszel (CMH) test, which controls for a grouping variable (animal) in comparing the distribution of one variable (spike count) across a second grouping variable (flat, unperturbed and rough, perturbed running conditions). Controlling for individuals did not change the results. The difference in motor activation pattern distributions remained insignificant (CMH test, $P=0.18$).

When we further challenged the dynamic requirements of the running cockroach with larger initial (Fig. 4B) and final steps (Fig. 4C), neural activation did change. Prior to moving across the rough terrain, we required the cockroaches to first ascend a large step. The cockroaches had to descend an equally large downwards step when leaving the rough terrain track. The initial and terminal step varied from 2 to 4 cm in height, depending on the section of the track the cockroach encountered. Motor activation patterns elicited by ascending (Fig. 4B) and descending (Fig. 4C) steps showed significant changes in per step spike count from flat terrain running. Ascending steps demonstrated a significant increase in spikes per burst (χ^2-test, $P<0.0001$) with up to seven spikes being
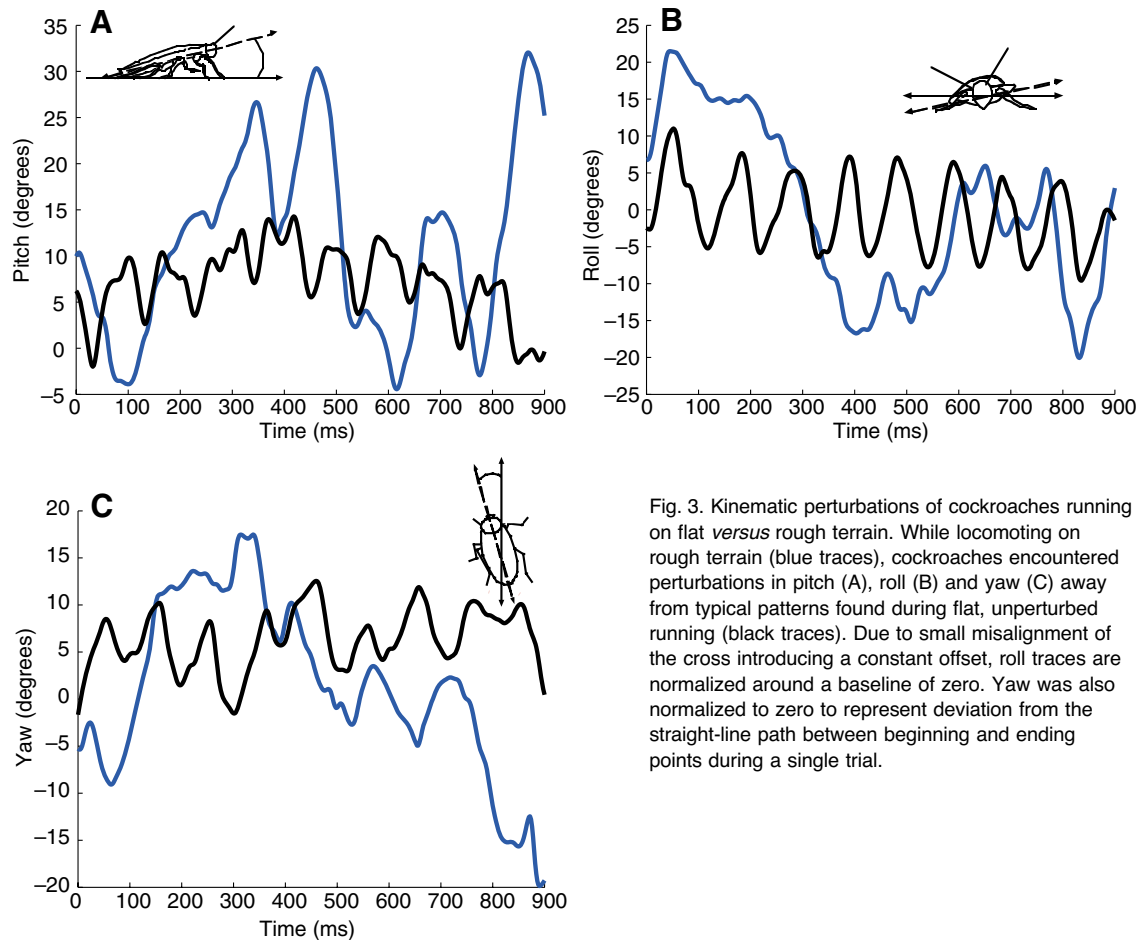
Fig. 3. Kinematic perturbations of cockroaches running on flat *versus* rough terrain. While locomoting on rough terrain (blue traces), cockroaches encountered perturbations in pitch (A), roll (B) and yaw (C) away from typical patterns found during flat, unperturbed running (black traces). Due to small misalignment of the cross introducing a constant offset, roll traces are normalized around a baseline of zero. Yaw was also normalized to zero to represent deviation from the straight-line path between beginning and ending points during a single trial.

recorded, whereas the plurality of descending steps required only a single Df spike and the overall motor activation pattern was significantly decreased (χ^2-test, $P<0.0001$).

Interspike interval

The number of MAPs per burst does not solely determine muscle force and power output, which can also depend on the amount of time between spikes or interspike interval (ISI). ISI decreased as speed increased bringing the spikes closer together in time as stance period decreased (regression F test, $P<0.0001$, $r^2=0.40$). However, mean ISI did not vary significantly between flat and rough terrain running independently of speed differences (Fig. 5, partial t-test, $P=0.46$). To control for non-normal distributions of ISIs, we also tested for statistical differences under Poisson and logarithmic transformations as well as the non-parametric Kruskal–Wallis test. No alternative method affected the statistical outcome. ISI means remained statistically indistinguishable for flat and rough terrain (Poisson transform: t-test, $P=0.33$; log transform; t-test, $P=0.33$; Kruskal–Wallis test, $P=0.26$). Additionally, no differences were detectable when we considered all ISIs as coming from the same sample or separated them into groups depending on which spikes in the burst were being analyzed (e.g. ISI between spike 1 and 2, ISI between spike 2 and 3 etc.).

Since perturbations elicited by the rough terrain can be positive, negative, or mixed changes in surface height, it is possible that while the mean interspike interval values would remain unchanged, the variance might increase. Using an F test for comparing standard deviations, we found that the variance was not significantly

different between perturbed and unperturbed locomotion (Fig. 5, F test, $P=0.12$). These results were also robust to Poisson or logarithmic transformations to the ISI distributions (Poisson transform F test, $P=0.31$; log transform F test, $P=0.18$).

Burst-to-burst period and phase

Since the burst of MAPs occurs during stance, the period of bursting may depend strongly on speed, which was not constrained from step to step. We therefore normalized the period from the first spike of one burst to the first spike of the subsequent burst with respect to speed. No significant difference in the corrected interburst interval (IBI) mean or variance was observable between flat and rough terrain running (Fig. 6A, partial t-test, $P=0.58$; non-parametric Kruskal–Wallis test, $P=0.64$, F test for equivariance, $P=0.27$).

In addition to changing the bursting period, cockroaches could adjust the phase of the femoral extensor burst during stance in response to perturbations. When controlling for individual animal differences, we could detect no change in onset of activity in muscle 179 compared to the stride period. Across all runs, the average burst phase (mean ± s.d.) for flat and rough terrain running were 0.116±0.033 and 0.102±0.056, respectively (Fig. 6B). Prior to correcting for individual, these values were not significantly different given appropriate Bonferroni correction (t-test, $P=0.044$, Bonferroni corrected significance threshold=0.0125), but flat terrain running appeared to strongly trend towards longer phase delays. However, this potential difference results from different animals having different preferred phases (F test, $P=0.037$). When
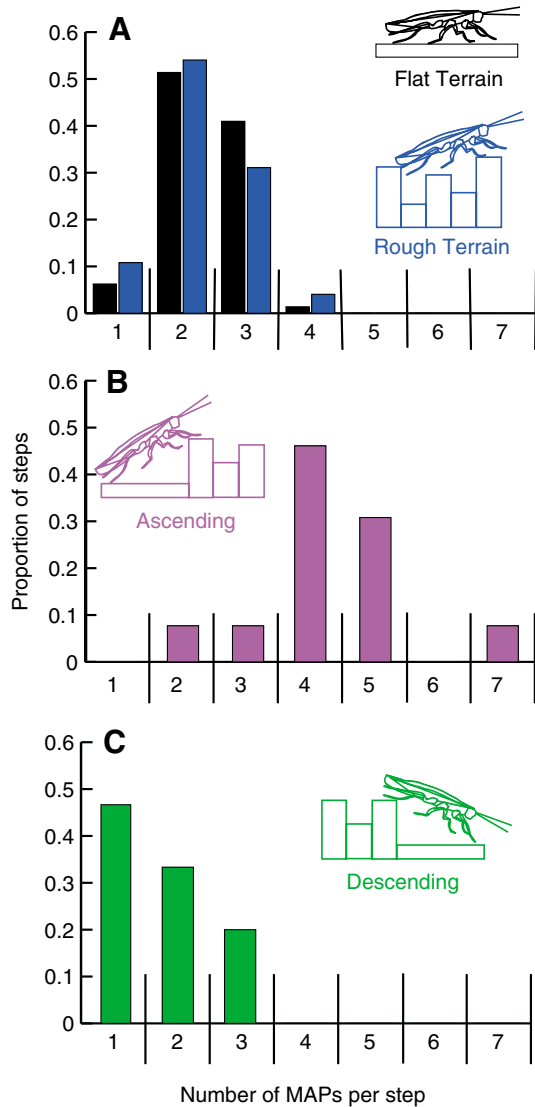
Fig. 4. Histograms of the proportion of steps that contained one through seven action potentials. (A) The distributions are statistically indistinguishable between flat (black) and rough (blue) terrain running, indicating that this parameter (spike number) was not modified by typical rough terrain perturbations. Neural modulation of spike number was evident during the large ascending perturbation at the start of the rough terrain (B) and during the final large descending step (C).

these differences were taken into account the trend disappeared (*t*-test, *P*=0.404) indicating no significant shifts in MAP phase during perturbed running.

Mis-steps

During six steps of the 150 analyzed for rough terrain running, cockroaches experienced a particularly large elevation difference between blocks. The resulting orientation of the cockroach's body and the roughness of the surfaces caused one of the hindlegs to swing through its stride without making contact with the surface at any time. We analyzed these events as particularly extreme perturbations to steady-state running. In the instances where the hind left leg missed its step completely, we observed a normal feedforward pattern of Df activation, despite the duty factor of the leg being reduced to zero (Fig. 7A,B). To test if there was feedback
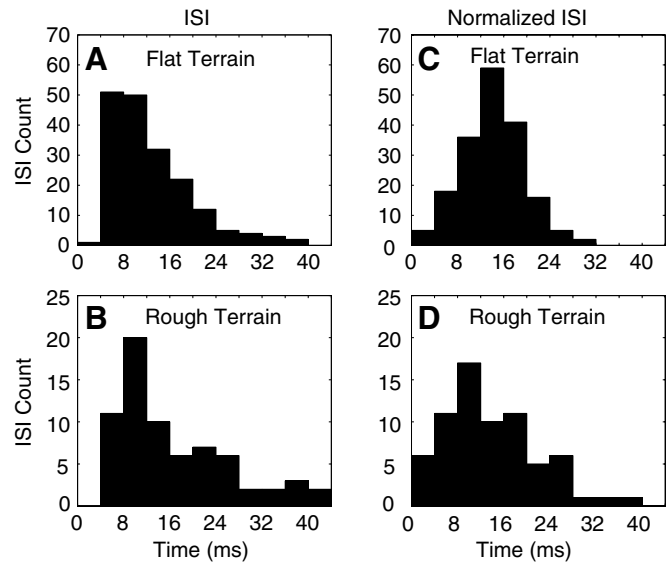

Fig. 5. Distribution of interspike intervals (ISIs) between all spikes within a burst compared between flat (A) and rough (B) terrain running. Neither the individual animal nor the order of the spiking event in the step (time between first and second spike *vs* time between second and third spike) significantly affected the distribution. Running speed did correlate with ISI and was corrected for in the normalized ISI values (C,D). No significant differences between terrain type were present in normalized ISI distributions.

occurring during or following the missed steps, we compared the stride period two strides prior to the perturbation, following the perturbation, and two strides subsequent to the perturbation (Fig. 7C). Compared to the normal period (0.119±0.022 s, mean ± s.d.), the period significantly increased by 30.9% in the stride immediately following the perturbation (0.158±0.047 s, mean ± s.d; *t*-test, *P*<0.004). After recovery, the period returned to a level indistinguishable from its original value (0.130±0.013 s, mean ± s.d.; *t*-test, *P*=0.57). This transient increase in stride period requires neural feedback, as the timing of the cyclical neural activation of the muscles must change (Revzen et al., 2005).

System level response to perturbations
Gait and leg phasing

We measured the duty factors and relative phasing of the legs with a phase of zero set at the moment of stance initiation in the hind left leg. Gait analysis (duty factors and statistics in Table 1; gait phases in Table 2) demonstrated no detectable change in duty factor, stance initiation phase or stance termination phase for any leg between flat and rough terrain running. Throughout flat and rough terrain trials, the cockroach maintained an alternating tripod gait.

Follow-the-leader gait and interleg coordination

A follow-the-leader (FTL) gait occurs when the organism targets a posterior leg to land on the successful foothold that the ipsilateral leg anterior to it used in a previous step (Song and Choi, 1989; Spagna et al., 2007). Functionally it constitutes feeding back information of stable foot placement to guide the following leg movements. On the rough terrain, a simple FTL gait would predict that cockroach foot placements should minimally occupy the same 1 cm×1 cm equiplanar block. Using the definition that posterior tarsi gain purchase on the same block as their anterior ipsilateral
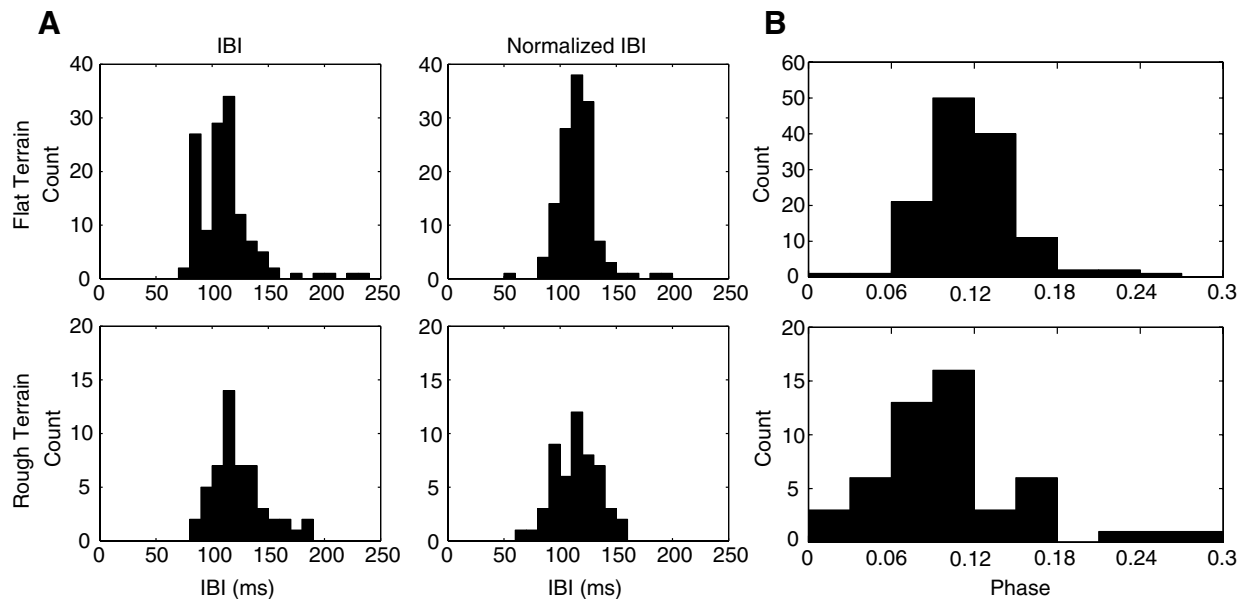
A

B

Fig. 6. Distributions of the interburst interval (IBI) of the MAP burst (A) and the phase of the MAP burst (B) compared between flat and rough terrain running. As with ISI values, IBI values correlated with running speed and were speed normalized (A, right panels). Phase is defined relative to stance initiation events of the hind left leg. No significant differences between terrain type were present in normalized IBI or phase distributions.

partner, we found that only 23.3% and 25.5% of the hind left and hind right legs, respectively, followed in the footsteps of the preceding middle leg (Fig. 8) on the rough terrain. The percentage for middle legs following front legs was higher (29.5 and 30.1% for left and right, respectively), but the vast majority of steps still resulted in footholds on completely different blocks (Fig. 8).

Despite not targeting the same foothold block, cockroaches could attempt to use FTL stepping, but often fail given the challenging substrate. This strategy would still target posterior foot placements to land near the successful anterior placement. Determination of how close placement has to be to constitute FTL stepping requires definition. Since the posterior leg cannot coincide exactly with anterior placement without the cockroach stepping on itself, a strict criterion for a posterior leg targeting a successful foothold would be placement within one-foot-length of the anterior leg, as is observed in biological FTL gaits (Song and Choi, 1989). In this case, the strict criterion would be the long dimension of the cockroach's tarsus, which is ~3 mm. A reasonable minimal criterion is the half-width of a rough terrain block and, therefore the target area of constant surface height for the posterior foot, assuming ideal anterior foot placement in the center of the block.

For the rough terrain condition, this minimal criterion is 5 mm, which is ~15% of the body length and ~25% of the stride length of the cockroach.

We calculated the absolute magnitude of the deviation between the placement of each anterior and posterior pair of tarsi (Fig. 9B). In 95.1% of rough terrain steps, the deviation from precise FTL stepping exceeded the longest dimension of the tarsus (~3 mm), whereas 85.1% exceeded the 5 mm minimal FTL criterion. Flat terrain running exceeded these criteria in 91.4% and 76.4% of steps, respectively. FTL deviation did not differ in terms of means (rough 8.40±3.75 mm, mean ± s.d.; flat 8.80±5.83 mm; t-test, P=0.13). Additionally, foot placement variance was larger during rough terrain running (F test for equivariance, P<0.0001), indicating less precise stepping during perturbed conditions, counter to the FTL hypothesis of decreasing variation in challenging environments.

To separate the deviation axes we further plotted the footfall position of each middle and hindleg with respect to the footfall location of its anterior partner (Fig. 9A). Precise FTL gaits would predict narrow distributions within the minimal or strict FTL criterion for all four footfall position plots. However, deviations for each pair of legs differed significantly from a distribution centered on the origin (t-test of each dimension to hypothesized mean of zero, P<0.0001 for at least one dimension in each case). Since this FTL metric did not depend on *a priori* blocks, it was amenable to quantification for flat terrain running as well. Stepping patterns were identical during flat running with significant divergent stepping.

Interestingly, the direction of systematic deviation in foot placement depended on which pair of legs was compared (Fig. 9A). On the left side of the body middle legs tended to fall to the left of front leg foothold with hindlegs stepping progressively further left. A mirror image pattern was observed for

Table 1. Duty factors and gait statistics for flat and rough terrain running

Leg*	Duty factor		P-values for Flat vs Rough terrain (t-tests)		
	Flat terrain	Rough terrain	Duty factor	Stance initiation phase	Swing initiation phase
FL	0.51±0.01	0.54±0.02	0.454	0.409	0.079
FR	0.53±0.01	0.54±0.02	0.974	0.112	0.318
ML	0.60±0.01	0.61±0.02	0.602	0.456	0.346
MR	0.58±0.01	0.58±0.02	0.904	0.487	0.998
HL	0.50±0.01	0.52±0.03	0.130	N/A†	0.130
HR	0.50±0.01	0.50±0.03	0.437	0.348	0.056

Values are means ± s.e.m.
*FL, front left; FR, front right; ML, middle left; MR, middle right; HL, hind left; HR, hind right.
†Since hind left leg stance initiation defines a phase of zero there can be no comparison.
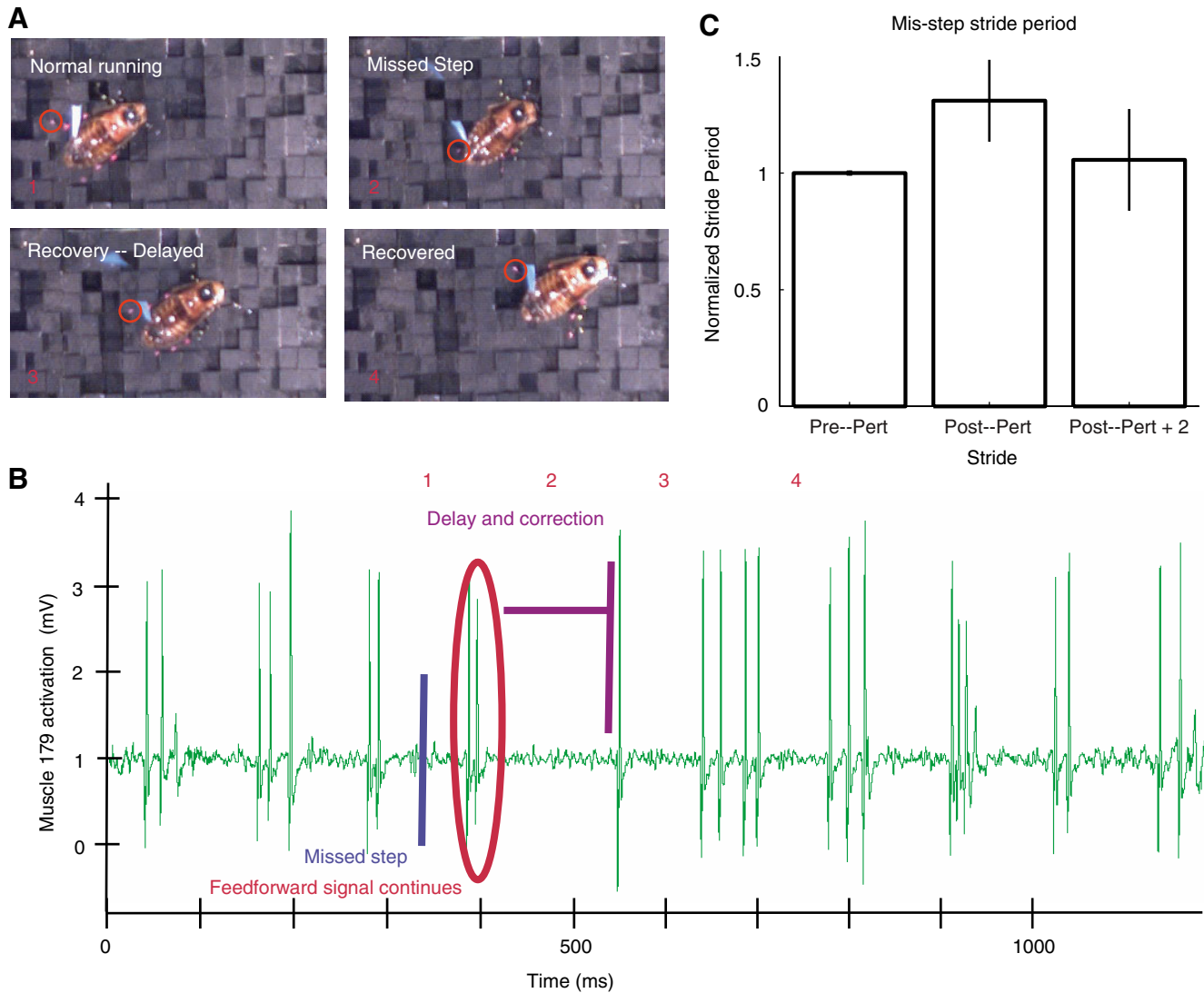
Fig. 7. Large perturbation mis-steps. A small number of rough terrain steps included a leg that failed to contact the substrate during stance. Here, the hind left leg (three pink points in image 1) swings through a trough formed by several particularly high obstacles (A). Normal activation of muscle 179 occurs even though contact is absent (B); however, there is a subsequent increase in stride period in the following step (B,C), indicating a stride-to-stride change in the clock-like activation of 179. This change requires neural feedback. Stride period returns to the original time within two strides of the mis-step following kinematic recovery from the perturbation. Histograms in C are normalized with respect to the baseline stride period taken 2 steps before the perturbation.

the right legs. These results indicate that more posterior legs assume more sprawled footholds. This constant offset suggested that leg placement may be coordinated as in walking stick insects (Cruse, 1979; Dean and Wendler, 1982; Dean and Wendler, 1983). Indeed, the variation in body-centered posterior foot placement is correlated with anterior foot placement for all pairs of legs (regression F tests, $P<0.05$), although the strength of this coordination is weak (r^2=between 0.05 and 0.55).

The cockroach could possibly accomplish this coordination through neural feedback, as in the stick insect (Buschges, 2005; Cruse et al., 2007), but mechanical coupling between the legs could also produce significant correlation. In fact, simple geometry of the running

animal suggests that as the body yaw angles away from a leg pair or the body pitches up, both foot placements in an anterior/posterior pair should move forward and lateral in world coordinates centered

Table 2. Individual leg gait phase for flat and rough terrain running

	Flat terrain		Rough terrain	
Leg	Stance onset phase	Swing onset phase*	Stance onset phase	Swing onset phase*
FL	−0.09±0.01	0.40±0.01	−0.07±0.02	0.48±0.04
FR	0.40±0.01	0.90±0.01	0.45±0.03	0.94±0.04
ML	0.42±0.01	1.02±0.01	0.45±0.02	1.04±0.02
MR	−0.07±0.01	0.51±0.01	−0.06±0.02	0.51±0.02
HL	0[†]	0.51±0.01	0[†]	0.54±0.02
HR	0.49±0.01	1.02±0.01	0.51±0.02	1.06±0.02

Values are means ± s.e.m.; FL, front left; FR, front right; ML, middle left; MR, middle right; HL, hind left; HR, hind right.
*Swing onset phase=stance termination phase.
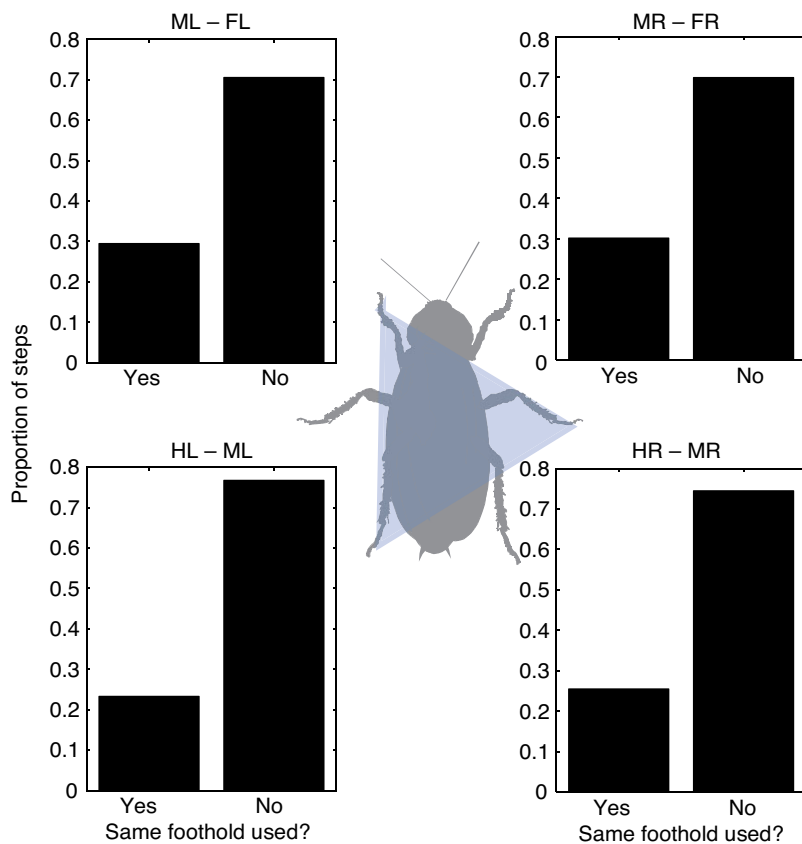[†]Hind left leg stance onset was defined as the reference phase of 0.

Fig. 8. Proportion of steps demonstrating follow-the-leader gait. Histograms report the proportion of steps during rough terrain running in which pairs of ipsilateral legs (FL—ML, ML—HL, etc.) use the same 1 cm×1 cm block on two sequential steps. This indicator of follow-the-leader (FTL) stepping occurs in less than a third of steps despite the relatively large target area. Middle/front leg pairs use the same blocks more often than do hind/middle leg pairs. FL, front left; FR, front right; ML, middle left; MR, middle right; HL, hind left; HR, hind right.

about the COM. We can partially test for a mechanical basis for the coordination observed by including yaw in the coordination regression. In nearly all coordination comparisons, yaw accounts for a significant portion of foot placement correlation (14 of 16 cases, partial correlation and restriction F tests, $P<0.05$) and in 8 of the 16 cases is sufficient to explain all of the coordination (partial correlation of the posterior foot position to anterior position is no longer significant, $P>0.05$). Further, coordination was generally weaker and more completely explained by yaw mechanics during rough terrain running than on flat terrain, rejecting more careful neural coordination operating during perturbed running. Overall, while coordination itself does not necessarily indicate that the system is adopting a neural or mechanical feedback strategy, our results are consistent with simple mechanical models.

DISCUSSION
System level perturbations
Rough terrain resulted in significant perturbations to the steady-state running behavior of cockroaches (Fig. 3). Repeated, unpredictable steps, varying in height by as much as three times the animal's hip height, produced highly visible disruptions to pitch, yaw, roll and body elevation. Despite these perturbations to system level variables, cockroaches recovered from randomized perturbations to complete the obstacle track with less than a 20% decrement in speed. Animals became less statically stable running over the rough terrain, thereby requiring more dynamic corrections. Particular combinations of forces and energy exchanges from the legs maintained dynamic stability. The resulting control of system-level state variables likely occurs along particular modes that differ in their rate of recovery (Full et al., 2002). A mode is a direction of recovery that can be as simple as a dynamic response in a single

degree of freedom, such as rolling to the left after a rightward perturbation. They can also be more complex, involving coupled degrees of freedom and energy production, absorption or exchange, such as a pitching moment and vertical acceleration mode driven by increased stress in a muscle in response to sudden loading. Modes that decay rapidly are more likely influenced by mechanical feedback, whereas those that are slower or recover with a significant gait change may require neural feedback (Full et al., 2002). Musculo-skeletal structures acting alone or in concert to enable these recovery modes can be considered control modalities. Control modalities can include a neural feedback strategy if muscle activation is altered in response to a perturbation, or adopt a primarily mechanical feedback strategy if there is no alteration in the rhythmic feedforward activation from a CPG.

Musculo-skeletal structure activation response to perturbations
A hypothesized mechanical control modality over rough terrain
The hindleg musculo-skeletal structures (femoral extensors, 178 and 179) of cockroaches appear to be operating as a mechanical control modality during rapid running over rough terrain with large unpredictable perturbations in surface height. Data showed no significant difference in the distribution of the number of MAPs, the interspike interval, burst phase or interburst period between flat and rough terrain trials (Figs 4–6).

Locomotion over the rough terrain likely caused considerable variation in the ground reaction force during the stance phase. Yet, the summed forces were sufficient to produce stable running over the rough terrain with only a small decrease in speed. While some steps resulted in secure footholds by the tarsi similar to those used on flat terrain, many steps showed contact with the blocks
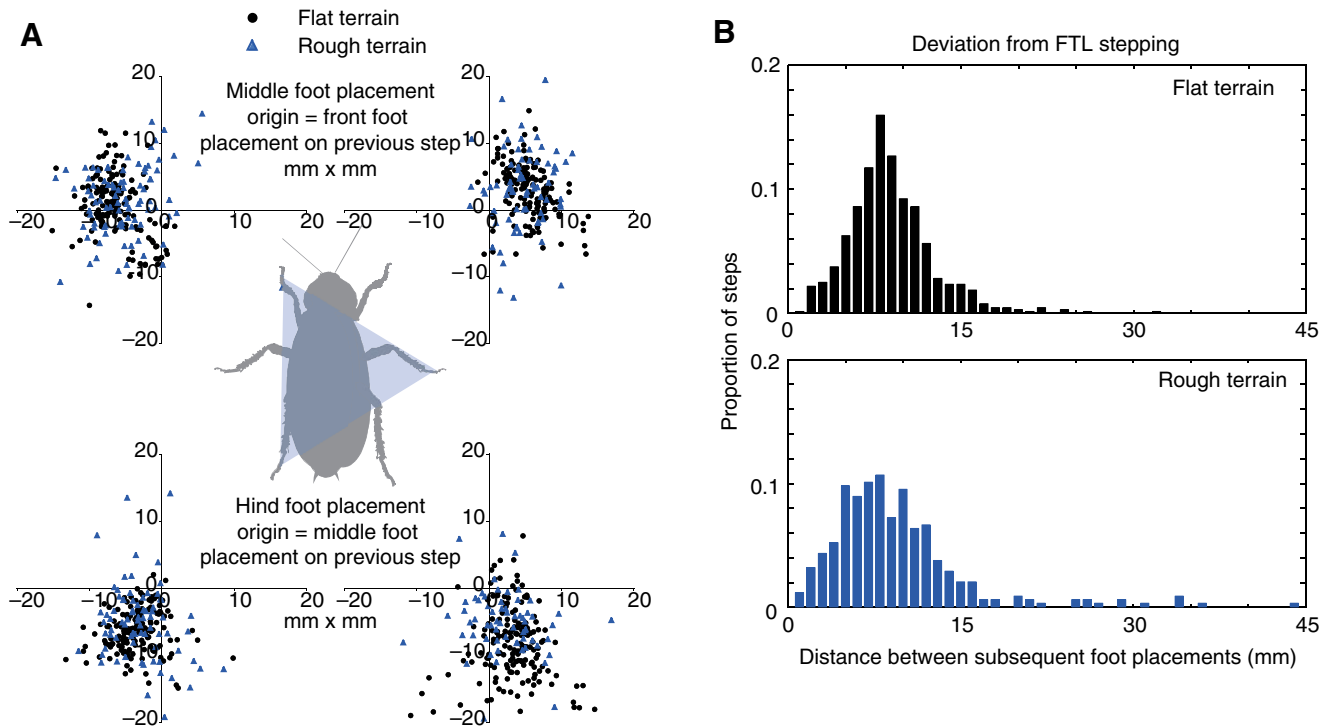
Fig. 9. Follow-the-leader (FTL) foot placements. FTL deviation plotted for each of the four pairs of legs (A). In each plot the origin was defined as the point of anterior leg placement with positive axes pointed anteriorly and to the right side of the animal. Points in quadrant I therefore represent a stance position in front of and to the right of where the anterior leg had foot placement. Flat terrain steps are in black, rough terrain in blue. In both cases, footfalls were significantly skewed away from the origin, indicating lack of FTL stepping. The distance of the deviations are represented as histograms for flat (top) and rough (bottom) terrain running (B). Deviation distance is the Euclidean distance to the origin for each point from the four plots in A, pooled across all pairs of legs. The peaks of the distributions are shifted from zero (perfect FTL stepping) and the means are above both the strict (3 mm) and minimal (5 mm) FTL deviation criteria.

distributed along the leg. The resulting shift in contact point and moment arms will alter the force output of a given activation pattern. Contacting the block nearer the joint can increase force output resulting in destabilizing pitch, yaw and roll of the body. This effect has been shown in a physical model, a hexapedal robot (RHex) attempting to run over a pile of bricks (Weingarten et al., 2004). Rapid neural feedback to the dorsal/ventral pair of femoral extensors altering their activation patterns has the potential to modulate forces that enhance stability, but we found no changes in the neural code during typical rough terrain traversal.

Generating stabilizing neural feedback in dorsal/ventral femoral extensors during a step may be challenging at high speeds due to bandwidth limitations and muscle properties. For speeds greater than 20 cm s^{-1}, stance and swing duration decrease to less than 40 ms (Fig. 10). Sensory response latencies measured in American cockroaches *Periplaneta americana* range from 6 to 15 ms (Ridgel et al., 2001; Wilson, 1966). Adding to this reflex delay is the 10 ms required for dorsal/ventral femoral extensors to begin to generate force following stimulation (Ahn et al., 2006). Therefore, the fastest reflex response following a leg perturbation is not likely to occur before 16–20 ms have lapsed, nearly one-half the stance duration. This prediction is supported by Schaefer et al. (Schaefer et al., 1994), who report the latency between tactile leg stimulus and first leg movement in the American cockroach at 17 ms. *In situ* measurements of these muscles show that their twitch kinetics might further limit their effective changes of force development (Ahn et al., 2006; Full et al., 1998). The time to peak isometric force for muscles 178 and 179 stimulated with 2–3 MAPs is 47 ms.

The time to 50% relaxation in force after the peak is 62–66 ms. Even though shortening deactivation will decrease the duration of twitches (Josephson and Stokes, 1989; Rome and Swank, 1992), femoral extensor kinetics do not allow peak force development and recovery to occur within the duration of a half-cycle. As a result, when muscle 178 is cycled to mimic rapid running, stimulation at the beginning of stance results in peak force being attained at the end of stance (Ahn et al., 2006). Work and power are generated during extension in the stance phase, but an equal amount of energy is absorbed during the swing phase as force declines.

Mechanical feedback using the visco-elastic properties of the feedforward activated dorsal and ventral femoral extensors could assist in dissipating destabilizing energy changes. *In situ* measurements of these muscles mimicking rapid running do not support the view that these muscles necessarily function only as their anatomical designation suggests (Ahn et al., 2006; Full et al., 1998). Muscle 178 does develop force and produce energy during extension in the stance phase, but absorbs an equal amount of energy during the swing phase (Ahn et al., 2006). Despite being activated by the same motor neuron at the beginning of stance, muscle 179 generates no power during stance and only absorbs energy during the swing phase (Full et al., 1998). Perhaps, the dorsal/ventral pair of femoral extensors responds to perturbations in both stance and swing by absorbing, storing and returning, and transferring energy.

For example, legs frequently struck blocks on the rough terrain during the swing phase. Perturbation studies of the isolated legs of *B. discoidalis* give us insight into how these femoral extensors may

be contributing to mechanical feedback. Dudek and Full (Dudek and Full, 2007) imposed large, dorsal-ventrally directed impulsive perturbations to isolated hindlegs. These sagittal plane perturbations were out of the plane of coxa–femur joint rotation, so any response resulted from the passive properties of the exoskeleton alone. Leg position attained its peak amplitude within 4–6 ms following an impulse. Position was recovered completely within 16–47 ms, depending on leg configuration. Feedforward activation of muscles 178 and 179 could set an effective stiffness that allows rapid rejection of perturbations in the plane of joint rotation through mechanical feedback.

Our conclusion that hindleg musculo-skeletal structures 178 and 179 are functioning as a mechanical control modality for rough terrain running is not a result of an inability to detect changes in the neural motor code. Detectable changes in neural activation are produced when the cockroach encountered the even larger step perturbations at the beginning and end of the rough terrain track. MAP number increased when cockroaches ascended steps above 1.5 cm (Fig. 4B) and decreased when they descended from similar height blocks (Fig. 4C).

A hypothesized neural control modality for the largest perturbations – mis-steps

Mis-steps during rough terrain running occurred when one leg of the cockroach failed to make contact with the surface throughout its stride, completely eliminating the stance phase. This is equivalent to a duty factor of zero. Stepping through a hole where no footfall occurs can offer further insight into neuromechanical control of musculo-skeletal structures. Earlier experiments on stick insects and cockroaches (Blaesing and Cruse, 2004a; Blaesing and Cruse, 2004b; Duerr, 2001; Tryba and Ritzmann, 2000a; Tryba and Ritzmann, 2000b) moving more slowly demonstrate a neural feedback strategy for negotiating mis-steps that involves a foothold searching behavior, where the leg is repeatedly retracted and protracted until surface contact is established. By contrast, cockroaches running at rapid speeds did not exhibit this behavior, but continued to swing the leg throughout retraction before resuming a normal swing protraction (Fig. 7A). The failure to make contact during its normal gait cycle resulted in the largest perturbation. Rhythmic activation of Df persisted for one step, despite the lack of stance initiation, suggesting a continuation of the feedforward, clock-like signal (Fig. 7B). However, in the next step, neural feedback acted to delay stance initiation (Fig. 7C). During these very large perturbations, the dorsal/ventral femoral extensors operated as a neural control modality that used sensory information, not to adjust within a step, but to shift the phase of the CPG's clock-like signal in the subsequent stride.

Running speed and perturbation size

The response of the dorsal/ventral femoral extensors likely depends on both running speed and the magnitude of the perturbation. At the slowest 1/6 of the cockroach's speed range (<10 cm s^{-1}), the fast motor neuron (Df) stimulating muscles 178 and 179 is not active (Fig. 10). At these slow speeds, particularly during exploratory walking behaviors, neural control modalities seem to dominate locomotor control primarily through the 'slow' motor neuron (Ds) that innervates two other femoral extensors, muscles 177d and 177e (Pearson and Iles, 1970; Pearson and Iles, 1971; Pipa and Cook, 1959; Watson and Ritzmann, 1998a; Watson and Ritzmann, 1998b; Watson et al., 2002a). Load sensing (Noah et al., 2004; Ridgel et al., 2001; Zill et al., 2004), proprioceptive hair plates (Pearson et al., 1976) and antennal/visual detection of
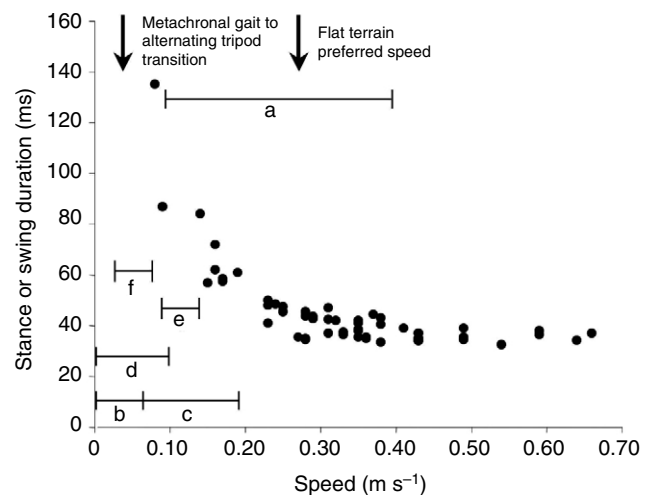

Fig. 10. Stance or swing duration as a function of speed. Stance and swing duration decrease as a function of speed until mid-speed (Full and Tu, 1990). No change in duration is observed at high speeds. Animals ran at the mid-speed range in the present study. Most previous studies of cockroach locomotor control, other than escape response elicitation, have been conducted at speeds less than 1/6 the maximal speed. [a]Present study; [b](Watson and Ritzmann, 1998a); [c](Watson and Ritzmann, 1998b); [d](Ridgel and Ritzmann, 2005); [e](Tryba and Ritzmann, 2000a; Tryba and Ritzmann, 2000b); [f](Watson et al., 2002a; Watson et al., 2002b).

obstacles (Watson et al., 2002a) can all alter these femoral extensors' activity patterns. Ds activity is correlated with a graded increase in joint velocity when activated in isolation during slow locomotion (Watson and Ritzmann, 1998a; Watson and Ritzmann, 1998b). Cutting the descending inputs to the thoracic ganglia of cockroaches adversely affects walking including Ds firing (Ridgel and Ritzmann, 2005). However, thoracic circuits are still capable of generating locomotion.

As running speeds reach 1/3 maximal speed (10–20 cm s^{-1}) and dynamics become increasingly more important in locomotor control, single Df spikes activate the dorsal/ventral femoral extensors. Df also innervates femoral extensor muscles 177d and 177e, where it acts on top of persisting Ds activity (Levi and Camhi, 1996; Pearson and Iles, 1970; Pipa and Cook, 1959; Watson and Ritzmann, 1998b). At these speeds, Df activation in these femoral extensors is correlated with the shortening of the transition from flexion to extension (Watson and Ritzmann, 1998b).

At one-half maximal speed (0.30 cm s^{-1}; the speed measured in the present study), stance and swing duration approach their minimum values (Fig. 10). *In situ* measurements of the dorsal/ventral femoral extensors mimicking running show that one muscle generates power in the stance phase, but both muscles show significant energy absorption during the swing phase (Ahn et al., 2006; Full et al., 1998). At this speed, the musculo-skeletal structures may assist in rejecting relatively large perturbations resulting from changes in ground reaction forces or forces imposed in the plane of joint motion when the leg collides with an obstacle. Effective within step, rapid stabilization appears to result from a visco-elastic, mechanical feedback response set by the feedforward activation of these structures, since no changes in the activation pattern occurred (Figs 4–6). When perturbed outside this already surprisingly large region of mechanical stability, neural feedback can alter the activation levels or the timing of the feedforward motor pattern in the next step (Fig. 7). This nested approach to neuromechanical

control relies on stride-to-stride neural feedback to set the general activation 'state' of the control modalities, while rejecting moderate within-stride perturbations with rapid mechanical feedback in these modalites that might include damping (Dudek and Full, 2007), energy exchange (Ahn et al., 2006; Full et al., 2002), energy storage and return (Dudek and Full, 2006), distributed contact (Spagna et al., 2007) and/or momentum trading (Holmes et al., 2006; Kubow and Full, 1999; Seipel and Holmes, 2006).

From moderate speed to the cockroaches' maximal speed, the function of the femoral extensors, or any muscle, is unknown. At these speeds, cycle period no longer decreases (Fig. 10). Speed instead increases by taking longer strides (Full and Tu, 1990). This transition may arise from functional constraints, but our hexapedal model (Seipel et al., 2004) supports a different view that relates to stability. The dynamic model shows that an increase in stride frequency with speed provides stability at slower speeds, but the resulting gaits become unstable or graze the stability boundary near mid-speed where cockroaches attain their maximum stride frequency. When foot touchdown positions are modified to approximate the increased stride lengths measured at speeds greater than mid-speed, then the mechanical feedback stability boundary moves further out and a reasonable dynamic stability margin is obtained throughout the speed range. Increased Df activation to the femoral extensors could enable this mechanical stability by increasing stride length. Indeed, Df activity during escape response has been correlated with increased coxa-trochanter-femur joint excursion in the American cockroach (Levi and Camhi, 1996). Still, unraveling the causal control potential of Df activation on task-level dynamics, particularly in this speed range, will likely require direct manipulation of the motor code.

System level response to perturbations

The system level response to rapid running over rough terrain perturbations was consistent with the mechanical feedback response of the musculo-skeletal structures. Despite large perturbations to the body (Fig. 3), variations in ground reaction forces and legs striking obstacles during swing, animals successfully negotiated unpredictable terrain with less than a one-fifth reduction in speed. Cockroaches retained the use of the alternating tripod gait on the rough terrain with no significant changes in duty factor (Table 1) or leg phase (Table 2).

We found no evidence that cockroaches used a feedback dependent follow-the-leader gait (Figs 8 and 9) that enforces precise stepping through perception of limb placement to aid in negotiation of the rough terrain (Song and Choi, 1989). Even under the broadest definition of a FTL gait, where some minimal amount of information concerning effective footholds in the surrounding environment is passed to posterior legs, only a small percentage of steps satisfied the condition. This does indicate that such a gait is not physically constrained by leg geometry at high speeds, but simply that it occurred infrequently (Fig. 9).

Anterior-posterior pairs of legs did demonstrate coordinated variation in tarsus placement, but much or all of this variation could be accounted for by the animal's variation in yaw. While it is likely that the cockroach's body pose can provide mechanical coupling to coordinate the legs, the neural or mechanical basis of coordination cannot be fully resolved without separate experiments similar to those revealing the intricate neural coordination of walking sticks and other organisms (reviewed in Buschges, 2005; Cruse et al., 2007; Pearson, 2004). Rather, we can reject any of the FTL hypotheses that the animal is providing useful coordination to reference effective foot placements by preceding legs.

A similar absence of FTL stepping and gait change was discovered when cockroaches and spiders run over surfaces possessing a very low probability of contact (Spagna et al., 2007). Animals attained high running speeds on a simulated terrain made of wire mesh with 90% of the surface contact area removed. These arthropods appear to simplify control on low contact surfaces by rapid running that uses kinetic energy to bridge gaps between footholds. Using dynamics for stability was possible because these many-legged arthropods can take advantage of distributed mechanical feedback, resulting from passive contacts along legs positioned by CPG pre-programmed trajectories favorable to their attachment mechanisms. Distributed mechanical feedback appeared to play the same role for traversing rough terrain. Recovery from system level perturbations during rapid running on challenging terrain is consistent with the use of mechanical feedback for self-stabilization during controlled lateral perturbations in cockroaches (Jindrich and Full, 2002) and dynamic models (Kubow and Full, 1999; Schmitt et al., 2002; Schmitt and Holmes, 2000a; Schmitt and Holmes, 2000b; Seipel and Holmes, 2006; Seipel et al., 2004).

We envision several important next steps if we are to understand how musculo-skeletal structures contribute to system level locomotor stability at high speeds. Characterization of the body's recovery using dynamical systems approaches can provide directions along which recovery of system level variables occurs (Full et al., 2002). Groups of musculo-skeletal structures that act together to stabilize the body along these particular directions (or modes) can be thought of as a control modality. We contend that control modalities can be composed of musculo-skeletal structures that provide mechanical and/or neural feedback. As we identify control modalities, it will be advantageous if we can determine the feedback strategy employed by the musculo-skeletal structures that contribute to stability. Typically, only muscles that respond by neural feedback are considered part of the controller, whereas passive or feedforward responses are relegated to body mechanics (i.e. the plant). Yet mechanical feedback acting to these musculo-skeletal structures can be integral to stability. To gain a deeper understanding of both of the performance and morphology enabling controlled behavior, the concept of control must include musculo-skeletal structures that reject perturbations by mechanical feedback.

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