

RESEARCH ARTICLE

History-dependent perturbation response in limb muscle

Thomas Libby¹, Chidinma Chukwueke² and Simon Sponberg^{3,*}

ABSTRACT

Muscle mediates movement but movement is typically unsteady and perturbed. Muscle is known to behave non-linearly and with history-dependent properties during steady locomotion, but the importance of history dependence in mediating muscle function during perturbations remains less clear. To explore the capacity of muscles to mitigate perturbations during locomotion, we constructed a series of perturbations that varied only in kinematic history, keeping instantaneous position, velocity and time from stimulation constant. We found that the response of muscle to a perturbation is profoundly history dependent, varying 4-fold as baseline frequency changes, and dissipating energy equivalent to ~6 times the kinetic energy of all the limbs in 5 ms (nearly 2400 W kg⁻¹). Muscle energy dissipation during a perturbation is predicted primarily by the force at the onset of the perturbation. This relationship holds across different frequencies and timings of stimulation. This history dependence behaves like a viscoelastic memory producing perturbation responses that vary with the frequency of the underlying movement.

KEY WORDS: Muscle, Work loop, Force, Running, Cockroach, Viscoelastic

INTRODUCTION

Muscle produces, dissipates, stores, returns and transmits mechanical energy to adopt diverse functions during locomotion (Dickinson et al., 2000). Even the same muscle can adopt different functions in unsteady or perturbed conditions (Biewener and Daley, 2007; Azizi and Roberts, 2010). A single muscle in the leg of a cockroach normally dissipates energy during steady-state running (Ahn et al., 2006; Full et al., 1998). Yet, when the animal is perturbed, neural feedback can categorically switch the muscle's function from one stride to the next (Sponberg et al., 2011b). Under unsteady conditions, the muscle can dissipate more than 10 times the energy that it does in steady state or convert its function to that of non-linear motor (Sponberg et al., 2011a). It remains challenging to predict function from the quasi-static length–tension and force–velocity relationships, especially under unsteady conditions (Ahn et al., 2006; Sponberg et al., 2011a; Daley and Biewener, 2011; Tytell et al., 2018). Nonetheless, such conditions likely pose greater performance demands than steady state (Biewener and Daley, 2007).

Strain history-dependent muscle properties are well known to affect muscle stress development especially over the course of a whole contraction cycle. These properties include force depression during shortening and force enhancement during lengthening. While the specific mechanisms for history dependence remain controversial and are likely multifaceted (Rassier, 2012), there are established consequences for steady, transition and impulsive behaviors (Josephson, 1999; Roberts and Azizi, 2011; Herzog et al., 2015; Nishikawa, 2016). These influences, which we will call long term because they manifest over tens to hundreds of milliseconds, are part of what makes muscle a versatile material. However, the short-term mechanical response and immediate function during perturbations to movement is much less explored at least under dynamic conditions. Short-range stiffening of muscle occurs when muscle is held at a constant length and then undergoes small strains, and consequently contributes to the postural perturbation response but is likely not involved in perturbations to running (Getz et al., 1998; Campbell and Moss, 2000). Perturbations to otherwise constant, typically tetanic, conditions are ubiquitous and simple material models like a viscoelastic Voigt body or a three-component Hill model can typically capture muscle behavior in these cases (e.g. Kirsch et al., 1994; Zajac, 1989; Cannon and Zahalak, 1982). Even in these cases, stiffness is activation dependent and damping may also vary (Nguyen et al., 2018).

However, perturbations around dynamic (time periodic or unsteady) conditions can create even more unexpected shifts in muscle performance (Robertson and Sawicki, 2015; Tytell et al., 2018). During running, muscle can experience large and rapid perturbations against a background strain trajectory where history has the potential to alter function (Daley and Biewener, 2006; Sponberg et al., 2011b). The response of muscle even to small perturbations during periodic strain can be non-linear (Tytell et al., 2018) and the coupling of muscle to robotic and simulated loads shows that environmental influences can change the classic velocity and strain dependencies in muscle (Robertson and Sawicki, 2015; Clemente and Richards, 2013). History could have profound effects on the muscle response to unsteady perturbations encountered during running, including slips or impacts with the substrate. Does history significantly modulate work output during rapid perturbations to periodic trajectories and can we reconcile any non-linearity with the simple material models that capture perturbations in static conditions?

To test these ideas, we constructed a systematic perturbation to a cockroach limb muscle to reveal the importance of history on transient behavior and identify simple predictors of function (Fig. 1B,C). To do this, we maintained the same Hill model (Hill, 1938; Zajac, 1989) contractile properties (stimulation, strain trajectory and velocity), while changing the strain history leading up to a perturbation. We modified this history by changing the frequency of background strain. To ensure comparable conditions, we also changed the phase of electrical stimulation of the muscle to occur at the same amount of absolute time before the perturbation. We hypothesize that history dependence modulates the mechanical

¹SRI International, Menlo Park, CA 94025, USA. ²Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA. ³School of Physics and School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA.

*Author for correspondence (sponberg@gatech.edu)

 T.L., 0000-0002-5850-4979; C.C., 0000-0002-1216-4251; S.S., 0000-0003-4942-4894

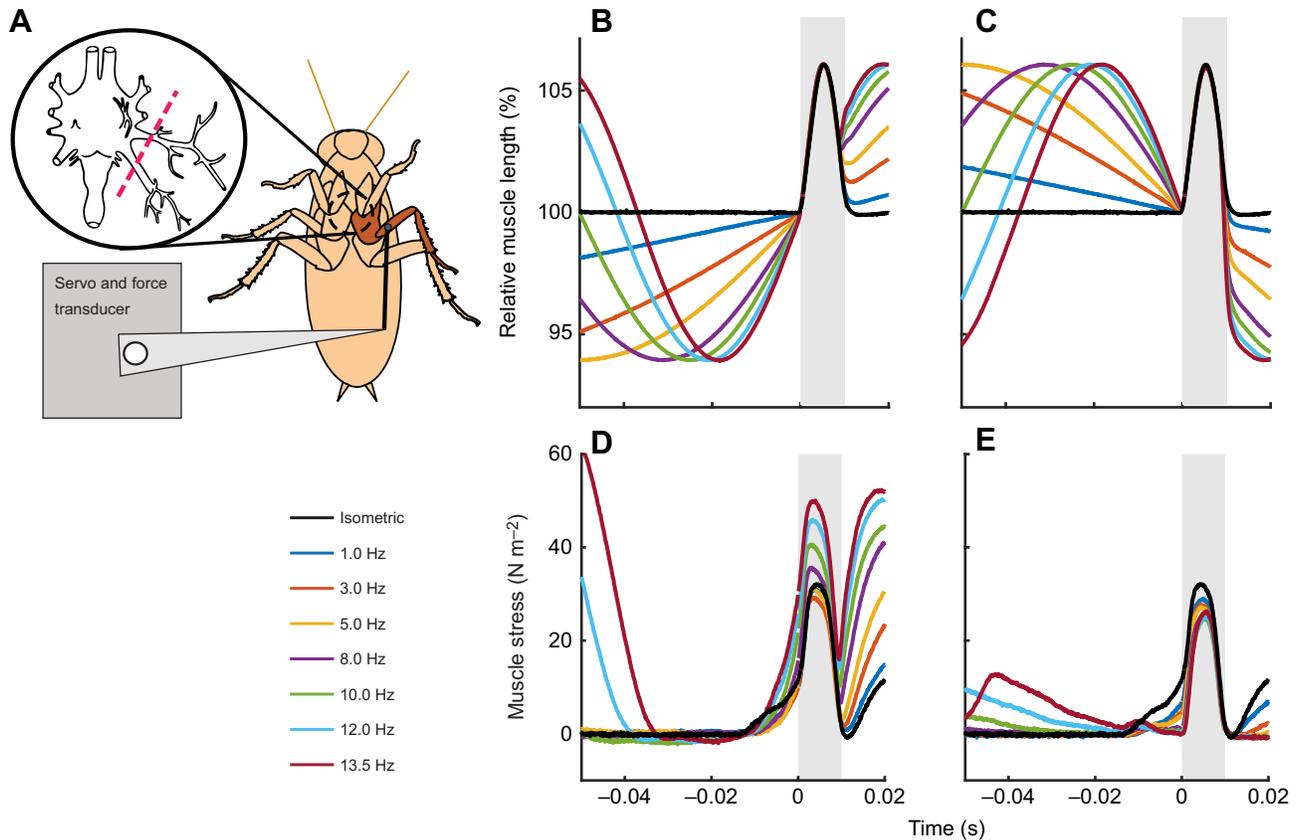


Fig. 1. Cockroach limb muscle preparation and perturbation response. (A) The intact joint work loop preparation extracellularly stimulates muscles in the denervated limb (inset shows ganglion nerves cut). Inset adapted from Pipa and Cook (1959). (B,C) During sinusoidal strain cycles of 1 to 13.5 Hz, identical eccentric perturbations (gray region) were applied mid-cycle during lengthening (B) and shortening (C). (D,E) Muscle stress was calculated by subtracting out the passive joint torque throughout the cycle (Sponberg et al., 2011a); stress varied during the perturbation for shortening (D) and lengthening (E), despite identical Hill determinants. Data are from $n=11$ muscle preparations from different cockroaches in all cases.

response of the muscle to rapid perturbations, but that the response will be predictable from the components of an active viscoelastic system. If history dependence has a functionally relevant consequence for rapid mechanical perturbations, then muscle work during the perturbation should vary systematically with history. If this history dependence matters for locomotion, then the modulation produced should be significant in light of the mechanical power required to alter limb movement.

MATERIALS AND METHODS

Intact joint work-loop preparation

Cockroaches (*Blaberus discoidalis* Audinet-Serville 1839) were housed on a 12 h:12 h light:dark cycle and fed dog chow *ad libitum*. Both males and females were used ($n=11$). We targeted the ventral femoral extensor of the middle leg (muscle 137), which is not the extensor that primarily powers limb extension, but rather a control muscle implicated in perturbation responses to locomotion on rough terrain (Full et al., 1998; Ahn and Full, 2002; Sponberg and Full, 2008). Instead of isolated muscle work loops (Josephson, 1985), we used intact joint work loops following previous methods (Fig. 1A; Sponberg et al., 2011a). In brief, all motor neurons innervating the middle leg were severed at the mesothoracic ganglion by surgical ablation of nerves 3, 4, 5 and 6. The limb was then mounted on a custom restraint stage and the coxa immobilized with epoxy. A muscle ergometer (Aurora Scientific 305C) was attached to the femur near the coxa femur joint via a two pin joint that allows for rotation. The femur and more distal segments were removed and the target

muscle was activated via implanted bipolar silver wire electrodes. The moment arm, pivot point and linear relationship between joint angle and muscle strain were taken from prior work on this muscle (Full and Ahn, 1995; Full et al., 1998). During steady-state work loops and imposed perturbations, the ergometer prescribed joint trajectories and simultaneously measured force. While this precludes the muscle from dynamically interacting with a load perturbation, it enabled us to use comparable conditions that vary only in history.

Besides the advantage of preserving the animal's nutrient and oxygen supply to the target muscle, the intact joint work loop allowed us to estimate the total passive work done on the joint. Here, muscle work loops are the active component of the work, calculated by measuring a passive work loop under identical strain conditions (including the perturbation) and subtracting the force measured in the passive trial from that of the active trial. The remaining force signal can be converted to muscle force (through the lever arm ratios) or used to calculate muscle work. Prior work (Sponberg et al., 2011a) validated this approach as reflective of the work output and muscle function reported in more traditional partially isolated, muscle work loops with direct neural stimulation (Full et al., 1998; Ahn et al., 2006).

Experimental conditions

Steady-state work-loop conditions reflected the strain trajectory and stimulation typical of *in vivo* 10 Hz running conditions. Three muscle potentials (spikes) of stimulation at 10 ms interspike intervals and 0.5 ms duration were applied at the onset of

shortening. Stimulus voltage for each preparation was tuned to the minimum voltage needed to elicit a plateaued twitch response plus 1 V. Extracellular stimulation does produce slightly faster twitches than neural stimulation but the overall work remains typical (Ahn and Full, 2002; Sponberg et al., 2011a).

Perturbations were imposed halfway through shortening (stance) phases. We constructed 10 ms (100 Hz) half-sine perturbations of amplitude equal to the stride amplitude. These strain perturbations were not summed with background periodic strain but rather pasted in place, so that perturbation kinematics were exactly identical across all history conditions (Fig. 1B,C). The initial and terminal 1 ms of the perturbation were smoothed into the underlying strain trajectory using a linear ramp filter to prevent discontinuities in velocity.

We modified kinematics and timing of the stimulus to test the effect of history on the perturbation response. To create different strain histories, we scaled the stride to different frequencies (1 to 13.5 Hz) without changing the overall amplitude or duty factor. To preserve Hill determinants during the perturbation, we varied the timing of the three spikes of stimulation so that the spike train always began 20 ms before the onset of perturbation. Thus, while the 10 Hz condition is reflective of *in vivo* conditions, the other frequencies do not specifically mimic the parameters of slower and faster movement. This is by design to isolate the effect due to changing strain history.

To examine the results of perturbations during both the shortening (stance) and lengthening (swing) phase, we repeated all conditions with perturbations at both mid-stance and mid-swing: 13.5 Hz was the fastest condition where we could maintain accurate perturbation conditions with our ergometer. Perturbations were always eccentric. We attempted concentric (shortening perturbations) of comparable magnitude, but the muscle would simply go slack under these conditions.

In a later set of experiments, we tested the effect of the amount of activation on the perturbation results. To do this, we repeated the perturbations but varied when the electrical stimulation occurred, so that it was 10, 20, 30 or 40 ms prior to the perturbation.

Analysis and statistics

Intact joint work loops produce significantly larger passive work than isolated muscles because of the presence of the entire limb. Following the approach of Sponberg et al. (2011a), we subtracted the passive work loop from the active to report the active muscle contribution, unless otherwise noted. While classic work loops combine both passive and active effects, the passive contribution is usually small. This has been verified for this particular muscle in prior studies, even at frequencies of 11 Hz (Sponberg et al., 2011a). Unless reported otherwise, all data are means \pm 95% confidence intervals (CIs) of the mean. Regression statistics report an *F*-test. To enable comparisons between individuals, we scaled perturbation work by a reference quantity approximately equal to the work done by a perturbation with isometric background (specifically, the average of the two 1 Hz perturbation conditions); we scaled total cycle work by the net cycle work at the 10 Hz condition. The average values of reference quantities are in Table 1. After scaling, measurements on different individuals were considered independent when fitting the models in Figs 2–7.

RESULTS

Muscle perturbation response is history dependent

Muscles absorbed energy during all perturbations. We characterized the muscle responses by the mechanical energy dissipated during

Table 1. Selected statistics and comparison measures from earlier work

Measure	Value
Average reference stress (isometric, 20 ms after first spike)	17.3 \pm 5.8 N cm ⁻²
Average reference perturbation work	-40.2 \pm 8.2 μ J
Perturbation work at 10 Hz (eccentric history)	-55.8 \pm 11.5 μ J
Passive joint perturbation work	-17.6 \pm 7.1 μ J
Average specific dissipation power at 10 Hz	-2360 \pm 798 W kg ⁻¹
Muscle 137 <i>in situ</i> negative work (Full et al., 1998)	-9 μ J
Muscle 137 <i>in situ</i> max. positive work, 6 spikes (Sponberg et al., 2011a)	25 μ J
Muscle 137 <i>in situ</i> max. negative work, 8 spikes (Sponberg et al., 2011a)	-45 μ J
Muscle 177c <i>in situ</i> positive work (Ahn and Full, 2002)	74 μ J
Peak kinetic energy of all limbs, 20 cm s ⁻¹ running (Kram et al., 1997)	8 μ J

Data are means \pm 95% confidence interval. Reference stress/work was used to normalize stress/work across conditions as in Figs 2–6. Reference work was the average across pre-lengthened and -shortened conditions at 1 Hz as in Fig. 2.

the stretch portion of the perturbation (the area under the force–length curve; Fig. 2A). Muscles absorbed 23.6 \pm 4.6 J kg⁻¹ during perturbations applied against a 10 Hz pre-lengthened background. A Hill-type contractile unit would respond with identical stress profiles to the perturbation regardless of kinematic history. By contrast, we found that active muscle stress and energy dissipation increased strongly as we increased frequency under pre-lengthened conditions, and decreased with frequency under pre-shortened conditions (Fig. 2B,D). Energy absorption varied almost 4-fold over the range of frequencies we tested. At the typical running stride frequency of 10 Hz, dissipation almost tripled if the muscle was perturbed in identical ways during shortening and lengthening.

Magnitude of net work is reduced in most perturbed strides

The effects of history dependence persisted after the perturbation. At the lowest frequencies (1 Hz), the strain rate on the muscle was sufficiently low that the situation was similar to isometric conditions (Figs 3, 4). To compare the effects of perturbations on the work loops across conditions, we first scaled each individual cockroach's work loop to the absolute magnitude of work done during the 10 Hz unperturbed, *in vivo* conditions typical of running (-64.4 \pm 6.7 μ J). We found that this was a better way to account for inter-animal variation than scaling by muscle mass, which can be imprecise. As a result, the value of work for all individuals at 10 Hz was -1. We set the normalization sign such that the sign of the scaled worked was still informative of whether the muscle was producing work (positive) or dissipating energy (negative).

The history dependence of muscle actually made the muscle produce less overall variation in work during perturbed strides than during unperturbed strides across frequency (Fig. 5). This is consistent with the rapid perturbation resetting crossbridge dynamics and a corresponding drop in muscle tension. When perturbations were imposed during the eccentric phase, the muscle was already doing negative work (Figs 3 and 5). Given the dissipative work of the muscle during the perturbation, we might assume that there would be overall more negative work across the entire cycle. However, the recovery significantly reduced the peak stress the muscle would otherwise experience. As a result, there was a significant decrease at all frequencies (compare blue and purple CIs of the mean in Fig. 5), except at 1 Hz, where the muscle was effectively isometric and the work during the 5 ms perturbation constituted about half of the total negative work in the stride (Fig. 3; 1 Hz condition).

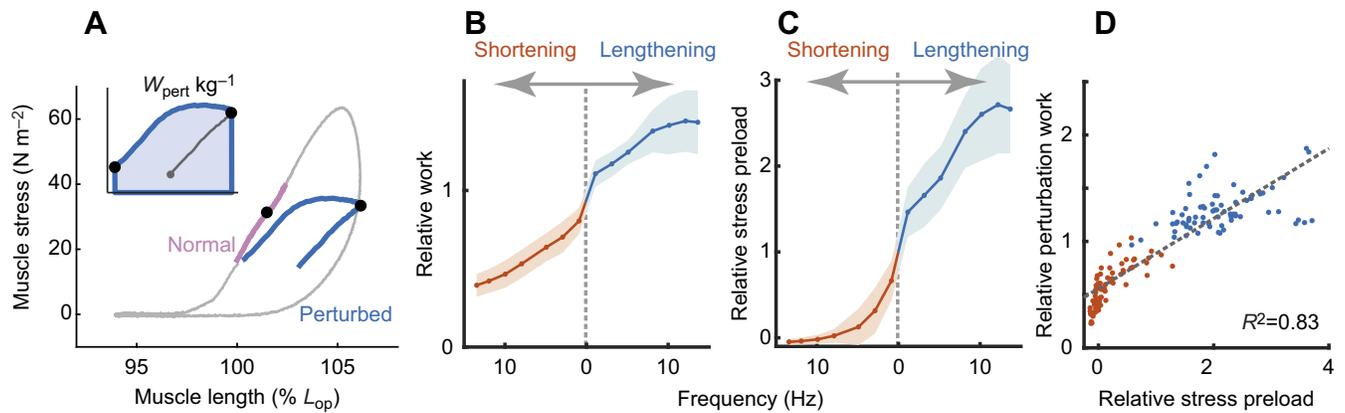


Fig. 2. Muscle perturbation response depends on preload. (A) Muscle stress was integrated over strain for the eccentric portion of the perturbation to calculate work, W_{pert} . L_{op} , muscle operating length. (B–D) Work during perturbation (B) and preload stress (C) both vary significantly and monotonically with frequency, resulting in a correlated response (D). Individual points in D correspond to individual trials across the range of frequencies from B and C. To normalize across animals, work was taken relative to the average of work done during just the perturbation in the two 1 Hz (shortening and lengthening) conditions. These most closely approximate the isometric work response. Relative stress was scaled to the peak isometric stress measured under *in vivo* stimulation conditions (3 spikes, 10 ms interspike interval). Preload refers to stress developed immediately prior to the onset of the perturbation.

When perturbations occurred during low-frequency (≤ 5 Hz) concentric perturbations, the normally net positive work loops became slightly negative because of the dissipation and reduced stress post-recovery (Fig. 4). Above a stride frequency of 5 Hz (left side of Fig. 5), this effect was enhanced and the muscle (now normally doing more negative work) produced almost no positive or negative work outside of the perturbation (Fig. 4, bottom row). This is likely due to both shortening inactivation and where the muscle operates on the force–velocity curve.

When perturbations were applied during the eccentric (stance) portion of the limb cycle, the muscle returned to its original work trajectory within about one half-cycle (Fig. 3). This is consistent with perturbations to individual cockroach legs, which damp out within the swing phase (Dudek and Full, 2006, 2007), although perturbations in those studies used deflections in a

different plane from the pure extension perturbations in the current experiment.

It is important to recognize that the conditions here were open loop, meaning that we set the stimulation conditions and enforced the recovery to prescribe the same Hill-type conditions during the perturbation. While this allowed us to isolate the effects of history on the short-term perturbation, it means that the overall work loop may be different from what is experienced during locomotion, where the precise recovery trajectory would involve a dynamic interaction of the perturbation force and the muscle work output. Even though the purpose of the experiment was not necessarily to match the long-term kinematics of strides operating with natural load perturbations, we can still assess the impacts of these prescribed perturbation trajectories in the context of the work-loop conditions we did perform.

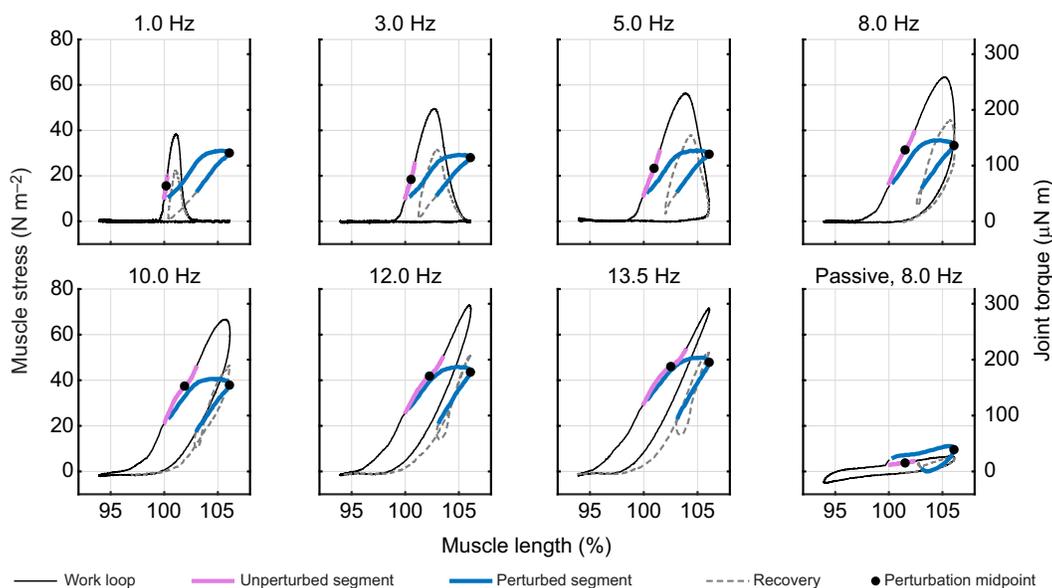


Fig. 3. Unperturbed workloops overlaid with perturbations during lengthening (blue), and stress trajectories after perturbation (dashed lines). Stress returned to the unperturbed trajectory (solid, black line) in less than half a stride. The purple line segment represents the unperturbed trajectory of the same time interval as the perturbation (midpoints are black dots).

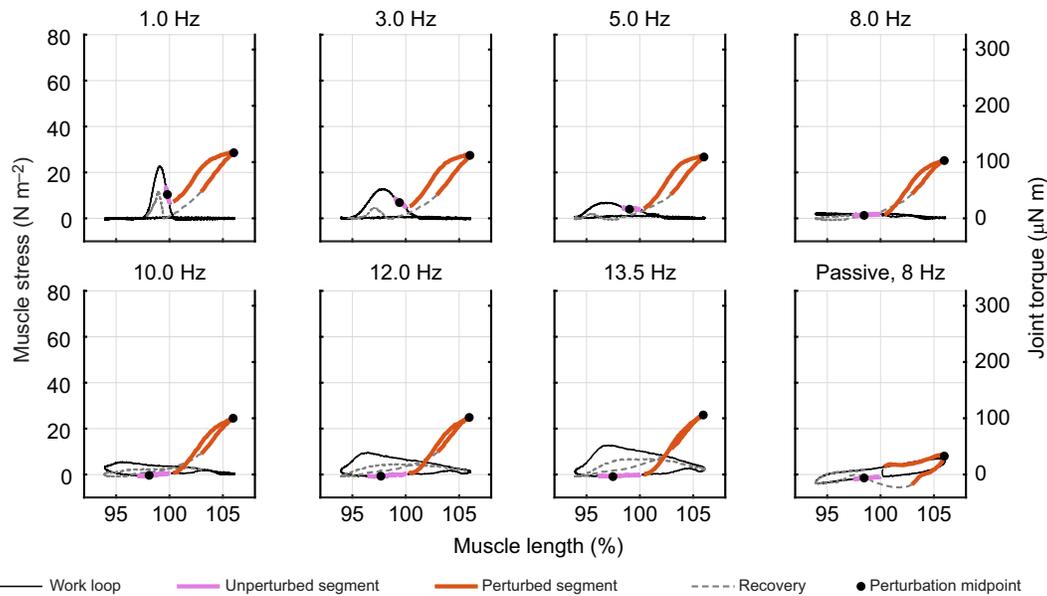


Fig. 4. Unperturbed work loops overlaid with perturbations during shortening (red). Other colors as in Fig. 3.

Force at onset of perturbation determines work

We next examined whether parameters of history prior to the perturbation could effectively predict the dissipative work during the perturbation. Muscle stress prior to perturbation onset (termed ‘preload’) showed a pattern of frequency dependence similar to work (Fig. 2B,C) and the two were highly correlated (Fig. 2D; $R^2=0.83$, $P<10^{-6}$). At the fastest shortening velocities (left side of Fig. 2D), work continued to fall off even after preload stress had reached zero, likely because of the need to take up slack from the transition of rapid shortening to lengthening.

Overall, the variable perturbation response and its dependence on preload show that a Hill-type contractile element model fails to predict the muscle perturbation response, even when time scales are quite rapid. Instead, we support the hypothesis that history dependence tunes the muscle mechanical response to perturbations. Muscle response to rapid stretches is known to have viscoelastic

properties (Kirsch et al., 1994; Zajac, 1989); here, we show that the context in which a perturbation occurs, meaning the muscle force history, modulates these properties and shapes muscle function even on short time scales. While Hill-like contractile elements fail to directly predict the functional modulation during the perturbation, they do play a role because the pre-perturbation forces follow a classic force–velocity curve (Fig. 2B; velocity is proportional to frequency). Despite history varying muscle mechanical work, the behavior of muscle is nonetheless predictable. This relationship holds regardless of whether the muscle is pre-shortened or pre-lengthened and across a range of frequencies spanning natural running (Fig. 2B,C).

Changing activation modulates work but not history dependence

To further test the robustness of this relationship, we repeated the frequency and phase experiments while varying the timing of muscle stimulation prior to perturbation. Doing so should alter the activation state of the muscle at the onset of the perturbation. Muscle stress prior to perturbation near static conditions increased almost 10-fold as timing advanced from 10 ms to 40 ms. In each case, the muscle showed an initial stiffening (Fig. 6B), but in the 40 ms condition, stress decreased rapidly at the end of the perturbation, likely because the muscle was beginning to relax. In all cases, recovery was complete with in half a cycle of the perturbation (compare dashed with solid black lines). Regardless of the activation time, the correlation between preload stress and work held across frequencies for all stimulation conditions (Fig. 6B; $R>0.85$, $P<10^{-6}$ for all timings). Activation did produce a shift in the overall dissipative work done as a function of stress, peaking during the perturbations occurring 30 ms post-activation. This corresponded to an additive dissipative force depending on activation magnitude. Regardless, the history-dependent properties of perturbed muscle persisted even when the level of activation varied.

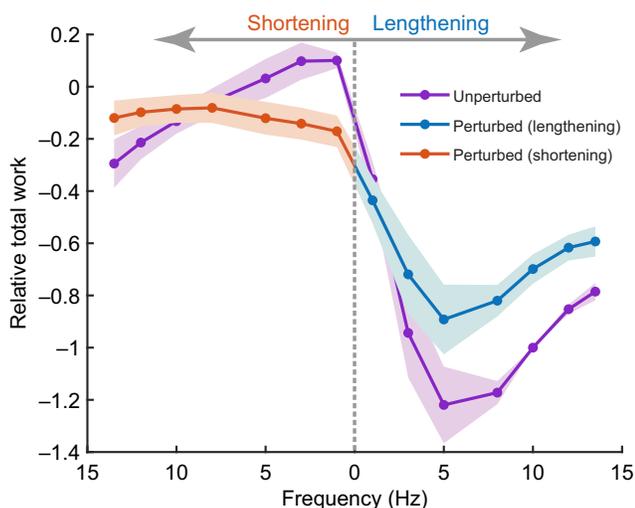


Fig. 5. Full cycle net work varies strongly with frequency in both perturbed and unperturbed conditions. Work is scaled relative to the magnitude of work done in the 10 Hz *in vivo* running conditions (-64.4 ± 6.7 μ J). Points are means $\pm 95\%$ confidence interval (CI) of the mean.

DISCUSSION

A viscoelastic model with memory captures muscle rapid perturbation response

As muscle force–length behavior during the perturbations appeared viscoelastic, we fitted a parallel spring–damper (Voigt) model with a

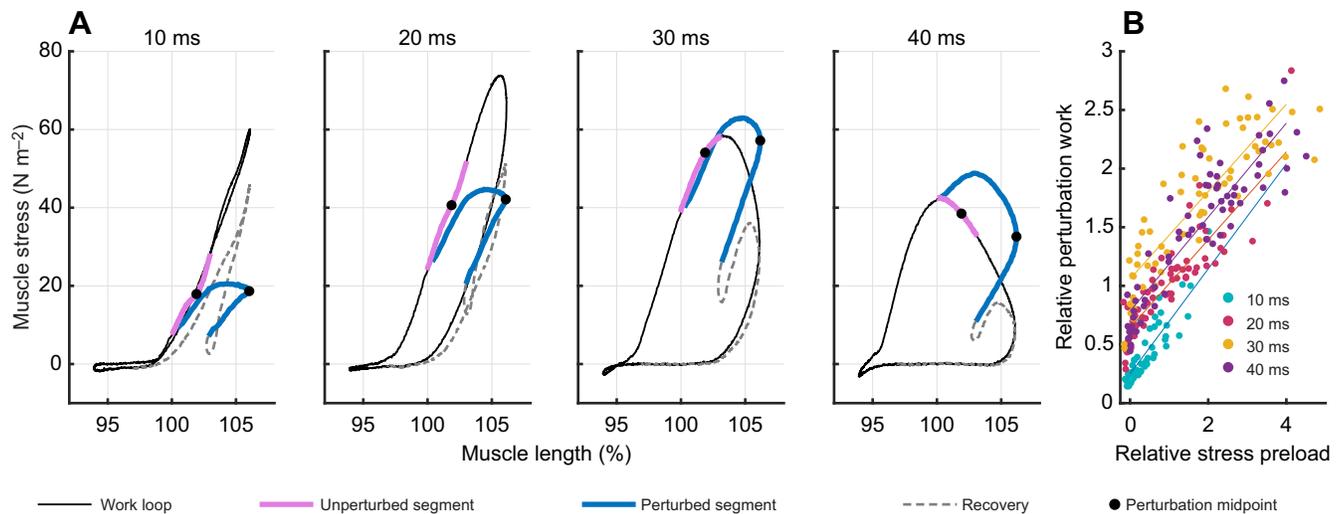


Fig. 6. The relationship between work and preload stress persists even if stimulation varies. Activation level during perturbation is modulated by changing how long before the perturbation the muscle is electrically stimulated. (A) Work during the perturbation and over the full cycle, with the typical work loop in black and the perturbation (blue) and recovery (dashed line) showing the overall work loop for the perturbed stride (10 Hz cycles shown). (B) Work was still predicted by preload stress for each timing condition; baseline changes in work shifted the relationship but did not change the slope. Points represent an individual trial at a specific frequency, with the different frequencies sweeping the relative stress preload. Work and stress preload are scaled as in Fig. 2D.

variable stress (or force) preload to the perturbation data (Fig. 7A). Changes in dissipative work against history could arise from (i) a change in stiffness, (ii) increased viscous damping or (iii) a change in the preload force. Changing the preload force is like changing the set point or equilibrium position of a spring because for a given length there is a larger or smaller force. The full model fitted these parameters separately for each condition (phase and frequency) and strongly predicted observed energy absorption (Fig. 7B; $R^2 > 0.99$). To examine which parameters were most predictive, we tested three models which each allowed only one parameter to vary (with the other two set to the average across all frequencies/phases). Doing so reduced the variance accounted for in all cases, but only when preload varied did the model retain any predictive ability (Fig. 7C–E).

The model property that best explained the functional variation was preload, rather than stiffness or damping. In prior studies with small sinusoid perturbation, muscle stiffness varied with frequency (Cannon and Zahalak, 1982; Kirsch et al., 1994). Here, variable stiffness did not account for the major differences in perturbation work, likely because total strain, velocity and prior stimulation were all kept the same. During perturbations to steady-state conditions,

work also varied with pre-perturbation force (Kirsch et al., 1994). We show that this adjustment can account for much of the difference in perturbation responses relevant to locomotion. A history-dependent preload or set point can also act as a viscoelastic memory effect, which typically arises from stored energy in elastic structures that cannot relax instantaneously or are themselves activation dependent.

Stiffness and damping likely do change under variable muscle conditions. First-order viscoelastic models have worked well to predict perturbations to cat soleus muscle at physiological, albeit constant conditions (Kirsch et al., 1994). However, stiffness does depend on the amplitude of the perturbations during small perturbations to static conditions (Weiss et al., 1988; Kirsch et al., 1994). Damping is also proposed to change, albeit non-linearly, to balance the needs of injury prevention and muscle contributions to stability (Nguyen et al., 2018). In our data, the full three free parameter model (Fig. 7B) did fit better than if just stress preload was allowed to vary (Fig. 7C), but only allowing damping (Fig. 7D) or stiffness (Fig. 7E) to vary produced a worse fit than a fixed model (sum of squared errors greater than total sum of squares). So, these parameters only have a secondary effect on predicting perturbation

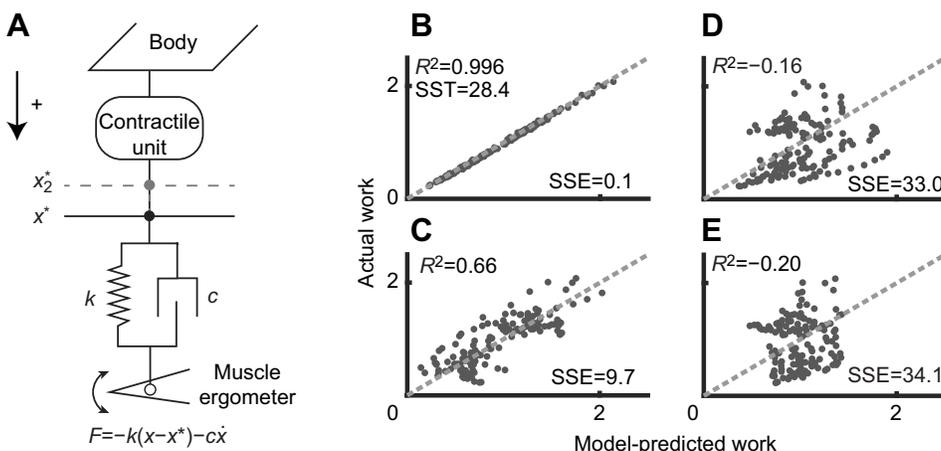


Fig. 7. A three-parameter viscoelastic model with memory. (A) The model predicts muscle work during the perturbation. The muscle force during a perturbation, F , can depend on a spring constant, k , a viscous damping coefficient, c , and the displacement of the muscle, x , away from its set-point, x^* , or equilibrium position. Muscle history offsets the equilibrium point, e.g. from x^* to x_2^* , proportional to the force it has at the perturbation onset, which is equivalent to a different preload force (or stress). (B–E) While all three parameters (x^* , k and c) contribute to the predictive power of the full model (B), preload (C) has a predictive effect on its own, unlike damping (D) and stiffness (E). A sum of squared errors (SSE) greater than the total sum of squares indicates a non-predictive model (D,E). SST, total sum of squares.

work after a variable preload is taken into account. Stiffness and damping may also have small non-linearities, but over the range of the perturbations here, the linear model works very well.

While variable stiffness and damping may be more important over longer time scales as the muscle further responds to a perturbation, we found that preloading accounted for the variation in the immediate material response of muscle to a rapid perturbation. Different histories could modulate the perturbation response in ways not predicted here. There are a large variety of trajectories possible during both steady-state and perturbed conditions. Many likely deviate from sinusoidal conditions common in isolated muscle experiments. Nonetheless, the preloading results were robust across frequencies (i.e. different strain rates) and activation levels. Most models of muscle material properties do not consider a memory-like preload. This term is usually held constant, but our results suggest that considering preload as a variable to incorporate history effects may improve muscle model performance under unsteady conditions.

The history-dependent perturbation effects may serve a role in locomotion analogous to the role of stiffening during postural perturbations, but through different mechanisms. Perturbations under static conditions lead to considerable short-range stiffness (SRS) when strains are short (1–3%) and there is prior activation (Getz et al., 1998). SRS is history dependent, depends on activation (Campbell and Moss, 2002) and is reduced or abolished under dynamic conditions (Campbell and Moss, 2000), likely because the short-range crossbridge-induced effects no longer apply (Campbell, 2014). These results suggest that SRS may be important for postural and static stabilization (De Groote et al., 2017), but not for perturbation responses during dynamic, locomotor conditions. In the case considered here, perturbation strains were larger and the muscle was moving. Moreover, it is not the stiffness that changes so much as the preload and hence dissipation.

Possible mechanisms of history dependence

What are the potential mechanisms for this history-dependent preload? Series elastic elements can modulate the state of the muscle fibers, contributing to energy storage and return, power amplification or dissipation (Roberts and Azizi, 2011). Yet, the role of series elastic elements in rapid perturbations is less explored. Our results might be explained by a contractile element with a series elastic component. However, the viscoelastic properties of insect apodeme and the exoskeletal attachment are typically an order of magnitude stiffer than vertebrate tendon (Bennet-Clark, 1975; Zajac, 1989). They are also short and in the case of the cockroach femoral extensors would require ~60% strain to account for the perturbation if the muscle remained isometric during the perturbation. It is likely that the muscle must be significantly involved in modulating the preloading and hence the work done during the perturbation. Nonetheless, we cannot reject the potential role of other contributors to series elasticity.

Non-uniformity in sarcomere strain and force production (Rassier and Herzog, 2004) is a likely contributor to history dependence, but it is unlikely to be the only explanation because history dependence typically manifests in single sarcomeres (Leonard et al., 2010; Rassier, 2012). There is also growing appreciation that components of the muscle lattice other than actin and myosin might contribute to history-dependent phenomena (Rassier, 2012; Herzog et al., 2015; Nishikawa, 2016). Titin, titin-like proteins (e.g. projectin and kettin; Bullard et al., 2006) and other large structural proteins have been implicated in history-dependent properties in muscle (Herzog et al., 2015). Recently, force spectroscopy between isolated actin and titin

has shown that calcium-dependent binding of the N2A domain effectively changes the stiffness and offset of the spring-like PEVK domain (Dutta et al., 2018). This feature alone might explain the phenomenon observed here. Titin also is suggested to have a further role in force generation via active winding of titin around the thin filaments (Nishikawa, 2016; Lindstedt and Nishikawa, 2017). Other components might play a role as well, especially regulatory elements that are strain dependent like tropomyosin (Tanner et al., 2012; Holt and Williams, 2018).

Muscle mechanical behavior during perturbations is significant for locomotion

Regardless of the mechanism, the history-dependent modulation of work during a perturbation can only have meaningful consequences for locomotion if the overall change is significant in the context of muscle, joint, limb and body. Surprisingly, this capacity is substantial at all scales even in a relatively small muscle (Table 1). The energy absorbed by the muscle during the perturbation we applied is at least comparable to the kinetic energy of all the limbs (Kram et al., 1997) and at most could absorb the center of mass kinetic energy of a 3 g animal running at 20 cm s⁻¹. Despite being about 1/10th the mass of the animal's largest femoral extensor, muscle 137 absorbed more energy during a single perturbation at 10 Hz than the larger muscle produces during running (Ahn and Full, 2002). Muscle 137 absorbed about 3-fold more energy than the entire joint did during the same perturbation. Even though this muscle is capable of very large dissipative power and can play a significant role in mitigating the perturbation, it does so without affecting its overall work production by more than a fraction of the normal stride (Fig. 5).

This single cockroach muscle therefore serves as an example of the versatile control role a muscle can adopt. At steady state during running, this muscle typically dissipates a small amount of energy during the swing period of each stride (Full et al., 1998). Its steady-state work is far below its capacity to either dissipate energy or do mechanical work when neural feedback modulates its function during locomotion (Sponberg et al., 2011b). Neural feedback can also turn this muscle into a motor assisting in obstacle traversal or turning (Sponberg et al., 2011b). Our results here indicate another control function: open-loop tuning of the limb response to disturbances. Cockroaches locomote with stride frequencies that vary over a wide band (at least 1–12 Hz); therefore, the time available to stabilize perturbations decreases dramatically as running speed increases (Sponberg and Full, 2008). Even with constant timing of stimulation, muscle 137's dissipative capacity during leg swing increases almost 50% over this frequency range.

Perturbations to steady periodic behavior are not like impulsive behaviors where muscle can do work over a relatively long time scale prior to release (Ilton et al., 2018). During perturbations both muscle's dissipative power and total work matter for an effective response. But with the capacity to dissipate 2400 W kg⁻¹, a muscle need not be large to absorb energy quickly. Despite the large dissipative capacity of muscle during strain perturbations, the net effect on the work output during the whole contraction cycle is minimal (Figs 3, 4), at least subject to the recovery trajectory that we imposed. The history-dependent properties that help muscles respond to impulsive perturbations may be especially important in the more distal limb muscle of vertebrates, which seem to have a greater role in maintaining stability and mitigating perturbations (Daley et al., 2007, 2009; Daley and Biewener, 2011).

Of course, stride history, intrinsic muscle properties and associated compliant tissues interact with neural feedback to

manage the response to perturbations during actual locomotion. However, rapid movements in animals at many scales challenge sensorimotor bandwidth (More and Donelan, 2018). Humans hopping with expected and unexpected changes in substrate stiffness, and hence different preparatory responses, can maintain their springy center of mass (COM) dynamics (Moritz and Farley, 2004). In contrast, rapidly running cockroaches with less time to react, show a COM response consistent with feedforward control of a clock torqued spring-loaded inverted pendulum (CT-SLIP; Seipel and Holmes, 2007) running model (Spence et al., 2010). However, guinea fowl running over relatively larger perturbations in surface height show a differential response. They are actually more capable of maintaining typical COM trajectories when the perturbation is visible, but also fail or stop more frequently (Daley et al., 2006). In unexpected drops, they more frequently use several modes of energy dissipation (Daley and Biewener, 2006; Daley et al., 2006) that involve both a slower reflex and faster intrinsic responses, at least in the gastrocnemius (Daley et al., 2009). Gordon et al. (2015) used an obstacle treadmill to control the advance notice that the animal had about an impending perturbation. A shorter reaction time produces a smaller shift in the timing of muscle activation in the distal leg, consistent with a greater reliance on the inherent properties of muscle, tendon and biomechanics to mediate control. During perturbations, reflexes may modulate muscles to differing degrees (Daley and Biewener, 2011), and some muscles, especially those associated with limited tendon architectures, may rely more on inherent material responses of the muscle. Context-dependent muscle behavior during perturbations can play an important role in stabilizing high-speed movements, especially if the animal can use anticipatory or feedforward control to tune the muscle perturbation response for greater dissipation at higher speeds, as seen here.

Natural perturbations are unlikely to be prescribed kinematic deviations, although rigid obstacles such as on rough terrain could produce these. Exploring unsteady muscle function with perturbed work loops coupled to impulsive forces or simulated loads could lead to a more complete picture of perturbation responsiveness in specific muscles (Robertson and Sawicki, 2015). However, our approach of prescribed perturbations imposed at different frequencies isolated the effect of history dependence and showed that increasing or decreasing the preload force has significant consequences for muscle function during perturbations.

Acknowledgements

The authors would like to thank Tom Daniel and C. Dave Williams for helpful comments regarding this manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: T.L., S.S.; Methodology: T.L., C.C., S.S.; Formal analysis: T.L., C.C.; Investigation: T.L., C.C., S.S.; Resources: S.S.; Data curation: T.L.; Writing - original draft: T.L., S.S.; Writing - review & editing: T.L., C.C., S.S.; Supervision: S.S.; Project administration: S.S.; Funding acquisition: S.S.

Funding

This work was supported by Army Research Office grant W911NF-14-1-0396 and National Science Foundation CAREER (PoLS) 1554790 to S.S. as well as the National Science Foundation PoLS Student Research Network 1205878. T.L. was supported by the University of Washington Institute for Neuroengineering and the Washington Research Foundation Funds for Innovation in Neuroengineering, and Air Force Office of Scientific Research grant no. FA9550-14-1-0398.

Data availability

Data used in this study are available from the Dryad digital repository (Sponberg et al., 2019): [dryad.f7m0cfxm](https://doi.org/10.22002/1.400000)

References

- Ahn, A. N. and Full, R. J. (2002). A motor and a brake: two leg extensor muscles acting at the same joint manage energy differently in a running insect. *J. Exp. Biol.* **205**, 379-389.
- Ahn, A. N., Meijer, K. and Full, R. J. (2006). In situ muscle power differs without varying in vitro mechanical properties in two insect leg muscles innervated by the same motor neuron. *J. Exp. Biol.* **209**, 3370-3382. doi:10.1242/jeb.02392
- Azizi, E. and Roberts, T. J. (2010). Muscle performance during frog jumping: influence of elasticity on muscle operating lengths. *Proc. R. Soc. B Biol. Sci.* **277**, 1523-1530. doi:10.1098/rspb.2009.2051
- Bennet-Clark, H. (1975). The energetics of the jump of the locust *Schistocerca gregaria*. *J. Exp. Biol.* **63**, 53-83.
- Biewener, A. A. and Daley, M. A. (2007). Unsteady locomotion: integrating muscle function with whole body dynamics and neuromuscular control. *J. Exp. Biol.* **210**, 2949-2960. doi:10.1242/jeb.005801
- Bullard, B., Garcia, T., Benes, V., Leake, M. C., Linke, W. A. and Oberhauser, A. F. (2006). The molecular elasticity of the insect flight muscle proteins projectin and kettin. *Proc. Natl. Acad. Sci. USA* **103**, 4451-4456. doi:10.1073/pnas.0509016103
- Campbell, K. S. (2014). Dynamic coupling of regulated binding sites and cycling myosin heads in striated muscle. *J. Gen. Physiol.* **143**, 387-399. doi:10.1085/jgp.201311078
- Campbell, K. S. and Moss, R. L. (2000). A thixotropic effect in contracting rabbit psoas muscle: Prior movement reduces the initial tension response to stretch. *J. Physiol.* **525**, 531-548. doi:10.1111/j.1469-7793.2000.00531.x
- Campbell, K. S. and Moss, R. L. (2002). History-dependent mechanical properties of permeabilized rat soleus muscle fibers. *Biophys. J.* **82**, 929-943. doi:10.1016/S0006-3495(02)75454-4
- Cannon, S. C. and Zahalak, G. I. (1982). The mechanical behavior of active human skeletal muscle in small oscillations. *J. Biomech.* **15**, 111-121. doi:10.1016/0021-9290(82)90043-4
- Clemente, C. J. and Richards, C. (2013). Muscle function and hydrodynamics limit power and speed in swimming frogs. *Nat. Commun.* **4**, 1-8. doi:10.1038/ncomms3737
- Daley, M. A. and Biewener, A. A. (2006). Running over rough terrain reveals limb control for intrinsic stability. *Proc. Natl. Acad. Sci. USA* **103**, 15681-15686. doi:10.1073/pnas.0601473103
- Daley, M. A. and Biewener, A. A. (2011). Leg muscles that mediate stability: mechanics and control of two distal extensor muscles during obstacle negotiation in the guinea fowl. *Philos. Trans. R. Soc. B Biol. Sci.* **366**, 1580-1591. doi:10.1098/rstb.2010.0338
- Daley, M. A., Usherwood, J. R., Felix, G. and Biewener, A. A. (2006). Running over rough terrain: guinea fowl maintain dynamic stability despite a large unexpected change in substrate height. *J. Exp. Biol.* **209**, 171-187. doi:10.1242/jeb.01986
- Daley, M. A., Felix, G. and Biewener, A. A. (2007). Running stability is enhanced by a proximo-distal gradient in joint neuromechanical control. *J. Exp. Biol.* **210**, 732. doi:10.1242/jeb.004226
- Daley, M. A., Voloshina, A. and Biewener, A. A. (2009). The role of intrinsic muscle mechanics in the neuromuscular control of stable running in the guinea fowl. *J. Physiol.* **587**, 2693-2707. doi:10.1113/jphysiol.2009.171017
- De Groot, F., Allen, J. L. and Ting, L. H. (2017). Contribution of muscle short-range stiffness to initial changes in joint kinetics and kinematics during perturbations to standing balance: a simulation study. *J. Biomech.* **55**, 71-77. doi:10.1016/j.jbiomech.2017.02.008
- Dickinson, M. H., Farley, C. T., Full, R. J., Koehl, M. A., Kram, R. and Lehman, S. (2000). How animals move: an integrative view. *Science* **288**, 100-106. doi:10.1126/science.288.5463.100
- Dudek, D. M. and Full, R. J. (2006). Passive mechanical properties of legs from running insects. *J. Exp. Biol.* **209**, 1502-1515. doi:10.1242/jeb.02146
- Dudek, D. M. and Full, R. J. (2007). An isolated insect leg's passive recovery from dorso-ventral perturbations. *J. Exp. Biol.* **210**, 3209-3217. doi:10.1242/jeb.008367
- Dutta, S., Tsiros, C., Sundar, S. L., Athar, H., Moore, J., Nelson, B., Gage, M. J. and Nishikawa, K. (2018). Calcium increases titin N2A binding to F-actin and regulated thin filaments. *Sci. Rep.* **8**, 14575. doi:10.1038/s41598-018-32952-8
- Full, R. J. and Ahn, A. N. (1995). Static forces and moments generated in the insect leg - comparison of a 3-dimensional musculoskeletal computer-model with experimental measurements. *J. Exp. Biol.* **198**, 1285-1298.
- Full, R. J., Stokes, D. R., Ahn, A. N. and Josephson, R. K. (1998). Energy absorption during running by leg muscles in a cockroach. *J. Exp. Biol.* **201**, 997-1012.
- Getz, E. B., Cooke, R. and Lehman, S. L. (1998). Phase transition in force during ramp stretches of skeletal muscle. *Biophys. J.* **75**, 2971-2983. doi:10.1016/S0006-3495(98)77738-0
- Gordon, J. C., Rankin, J. W. and Daley, M. A. (2015). How do treadmill speed and terrain visibility influence neuromuscular control of Guinea fowl locomotion? *J. Exp. Biol.* **218**, 3010-3022. doi:10.1242/jeb.104646
- Herzog, W., Powers, K., Johnston, K. and Duvall, M. (2015). A new paradigm for muscle contraction. *Front. Physiol.* **6**, 174. doi:10.3389/fphys.2015.00174

- Hill, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. Lond. B Biol. Sci.* **126**, 136-195. doi:10.1098/rspb.1938.0050
- Holt, N. C. and Williams, C. D. (2018). Can strain dependent inhibition of cross-bridge binding explain shifts in optimum muscle length? *Integr. Comp. Biol.* **58**, 174-185. doi:10.1093/icb/icy050
- Ilton, M., Bhamla, M. S., Ma, X., Cox, S. M., Fitchett, L. L., Kim, Y., Koh, J.-S., Krishnamurthy, D., Kuo, C.-Y., Temel, F. Z. et al. (2018). The principles of cascading power limits in small, fast biological and engineered systems. *Science* **360**. doi:10.1126/science.aao1082
- Josephson, R. K. (1985). Mechanical power output from striated-muscle during cyclic contraction. *J. Exp. Biol.* **114**, 493-512.
- Josephson, R. K. (1999). Dissecting muscle power output. *J. Exp. Biol.* **202**, 3369-3375.
- Kirsch, R. F., Boskov, D. and Rymer, W. Z. (1994). Muscle stiffness during transient and continuous movements of cat muscle: perturbation characteristics and physiological relevance. *IEEE Trans. Biomed. Eng.* **41**, 758-770. doi:10.1109/10.310091
- Kram, R., Wong, B. and Full, R. (1997). Three-dimensional kinematics and limb kinetic energy of running cockroaches. *J. Exp. Biol.* **200**, 1919-1929.
- Leonard, T. R., DuVall, M. and Herzog, W. (2010). Force enhancement following stretch in a single sarcomere. *Am. J. Physiol. Cell Physiol.* **299**, C1398-C1401. doi:10.1152/ajpcell.00222.2010
- Lindstedt, S. and Nishikawa, K. (2017). Huxleys' missing filament: form and function of titin in vertebrate striated muscle. *Annu. Rev. Physiol.* **79**, 145-166. doi:10.1146/annurev-physiol-022516-034152
- More, H. L. and Donelan, J. M. (2018). Scaling of sensorimotor delays in terrestrial mammals. *Proc. R. Soc. B Biol. Sci.* **285**, 20180613. doi:10.1098/rspb.2018.0613
- Moritz, C. T. and Farley, C. T. (2004). Passive dynamics change leg mechanics for an unexpected surface during human hopping. *J. Appl. Physiol.* **97**, 1313-1322. doi:10.1152/jappphysiol.00393.2004
- Nguyen, K. D., Sharma, N. and Venkadesan, M. (2018). Active viscoelasticity of sarcomeres. *Front. Robot. AI* **5**, 69. doi:10.3389/frobt.2018.00069
- Nishikawa, K. (2016). Eccentric contraction: unraveling mechanisms of force enhancement and energy conservation. *J. Exp. Biol.* **219**, 189-196. doi:10.1242/jeb.124057
- Pipa, R. L. and Cook, E. F. (1959). Studies on the hexapod nervous system. I. The peripheral distribution of the thoracic nerves of the adult cockroach, *Periplaneta americana*. *Ann. Entomol. Soc. Am.* **52**, 695-710. doi:10.1093/aesa/52.6.695
- Rassier, D. E. (2012). The mechanisms of the residual force enhancement after stretch of skeletal muscle: Non-uniformity in half-sarcomeres and stiffness of titin. *Proc. R. Soc. B Biol. Sci.* **279**, 2705-2713. doi:10.1098/rspb.2012.0467
- Rassier, D. E. and Herzog, W. (2004). Considerations on the history dependence of muscle contraction. *J. Appl. Physiol.* **96**, 419-427. doi:10.1152/jappphysiol.00653.2003
- Roberts, T. J. and Azizi, E. (2011). Flexible mechanisms: the diverse roles of biological springs in vertebrate movement. *J. Exp. Biol.* **214**, 353-361. doi:10.1242/jeb.038588
- Robertson, B. D. and Sawicki, G. S. (2015). Unconstrained muscle-tendon workloops indicate resonance tuning as a mechanism for elastic limb behavior during terrestrial locomotion. *Proc. Natl Acad. Sci. USA* **112**, E5891-E5898. doi:10.1073/pnas.1500702112
- Seipel, J. and Holmes, P. (2007). A simple model for clock-actuated legged locomotion. *Regul. Chaotic Dyn.* **12**, 502-520. doi:10.1134/S1560354707050048
- Spence, A. J., Revzen, S., Seipel, J., Mullens, C. and Full, R. J. (2010). Insects running on elastic surfaces. *J. Exp. Biol.* **213**, 1907-1920. doi:10.1242/jeb.042515
- Sponberg, S. and Full, R. J. (2008). Neuromechanical response of musculo-skeletal structures in cockroaches during rapid running on rough terrain. *J. Exp. Biol.* **211**, 433-446. doi:10.1242/jeb.012385
- Sponberg, S., Libby, T., Mullens, C. H. and Full, R. J. (2011a). Shifts in a single muscle's control potential of body dynamics are determined by mechanical feedback. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **366**, 1606-1620. doi:10.1098/rstb.2010.0368
- Sponberg, S., Spence, A. J., Mullens, C. H. and Full, R. J. (2011b). A single muscle's multifunctional control potential of body dynamics for postural control and running. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **366**, 1592-1605. doi:10.1098/rstb.2010.0367
- Sponberg, S., Chukwueke, C. and Libby, T. (2019). Data from: History-dependent perturbation response in limb muscle, v3. *Dryad Digital Repository*. <https://doi.org/10.5061/dryad.f7m0cfxrm>
- Tanner, B. C. W., Daniel, T. L. and Regnier, M. (2012). Filament compliance influences cooperative activation of thin filaments and the dynamics of force production in skeletal muscle. *PLoS Comput. Biol.* **8**, e1002506. doi:10.1371/journal.pcbi.1002506
- Tytell, E. D., Carr, J. A., Danos, N., Wagenbach, C., Sullivan, C. M., Kiemel, T., Cowan, N. J. and Ankarali, M. M. (2018). Body stiffness and damping depend sensitively on the timing of muscle activation in lampreys. *Integr. Comp. Biol.* **58**, 860-873. doi:10.1093/icb/icy042
- Weiss, P. L., Hunter, I. W. and Kearney, R. E. (1988). Human ankle joint stiffness over the full range of muscle activation levels. *J. Biomech.* **21**, 539-544. doi:10.1016/0021-9290(88)90217-5
- Zajac, F. E. (1989). Muscle and tendon: properties, models, scaling, and application to biomechanics and motor control. *Crit. Rev. Biomed. Eng.* **17**, 359-411.