Post-activation muscle potentiation and its relevance to cyclical behaviours

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Abstract

Muscle can experience post-activation potentiation (PAP), a temporary increase in force and rate of force development, when contractions are closely timed; therefore, cyclical behaviors are likely affected by PAP, as succeeding contraction cycles can lead to potentiation over several subsequent cycles. Here, we examined PAP during *in situ* cyclical contractions of the mallard lateral gastrocnemius (LG). Surface swimming, a cyclical behavior, was mimicked with work-loops utilizing *in vivo* LG length change and stimulation parameters. Tests were performed at mallards' preferred cycle frequency as well as at lower and higher frequencies. Like muscles from mammals, anurans, and arthropods, the mallard LG exhibited PAP with increases in peak force, average force rate, and net work. Staircase potentiation occurred over two or more work-loop cycles, resulting in gradual increases in PAP. The number of cycles needed to reach maximum work varied with cycle frequency, requiring more cycles at higher cycle frequencies. PAP occurred under *in vivo*-like stimulation parameters, suggesting a potentially important role of PAP in animal locomotion, especially in cyclical behaviors.

Introduction

The contraction history of a muscle can influence its performance. Post-activation potentiation (PAP) is one such history-dependent phenomenon where muscle force is higher for a given submaximal stimulation, if the muscle has been recently stimulated. The potentiated state may be the predominate condition for muscles *in vivo* [1]. PAP is caused by phosphorylation of the myosin regulatory light chain (MRLC), which increases the rate of cross-bridge formation [2], thereby increasing both the magnitude of force and rate of force development for submaximal activation [3,4]. PAP has been studied for more than a century (for review [5]). Much of the foundational work on PAP focused on isolated muscle fibers [4] or whole muscles [1,2,3,6] *in vitro.* PAP was also measured in humans as an increase in twitch force following a maximal voluntary contraction [7,8], demonstrating that voluntary activation could elicit PAP. More recently, increases in human performance during high-power behaviors following a tetanic contraction have been studied [5,9,10]; however, differences in timing of MRLC phosphorylation relative to improved exercise performance led to concerns that these studies measured a different phenomenon [5]. Thus,

a gap in understanding remains between measurements of PAP and naturally-occurring contractile behaviors.

Cyclical behaviors are likely affected by PAP since conditioning by one cycle can lead to potentiation in subsequent cycles. MRLC phosphorylation levels remain high for up to a minute following a conditioning stimulus [2,11], and PAP can affect muscle forces for several minutes [1,7,8] or longer under non-fatiguing conditions [6]. Observations of PAP during low-frequency isometric contractions uncovered a phenomenon known as staircase potentiation – a gradual rise in muscle force over several contractions [3,6,12,13]. Staircase potentiation has been observed during twitch [3,6,13] and incompletely-fused tetanic contractions [12]. We, therefore, hypothesize that staircase potentiation may also affect force and work production during dynamic contractions.

Relatively few studies have examined PAP during dynamic contractions [2,11,14]. The work-loop technique is a powerful tool for mimicking dynamic muscle contractions and is particularly useful for studying cyclical behaviors [15]. Work is the area inside of a force versus length loop, so both increased force and force rate should together increase work output. An experiment examining a single work-loop in the potentiated state suggests PAP increases work output [2], and work has been observed to increase over several work-loops [15, 16, 17]. Although the term post-activation potentiation has been restricted to certain conditioning stimuli and responses [5,13], we define PAP as any increases in muscle force, rate of force development, or work following prior activation.

Here, we used the work-loop technique *in situ* to examine how PAP affects work output over repeated cycles in mallard lateral gastrocnemius (LG). The LG is an important ankle extensor during walking and surface swimming, and *in vivo* LG function during these behaviors has been described [18]. The current study differs from previous studies of PAP by measuring PAP while mimicking the *in vivo* LG length change and activation patterns observed during surface swimming, a cyclical behavior. Mallards use a cycle frequency of 2.6 Hz during surface swimming [18,19], but use a range of cycle frequencies during terrestrial locomotion [20]. To explore the effect of cycle frequency, we measured work across successive cycles at 1.0, 2.6, and 4.0 Hz. We tested three hypotheses: (H1) PAP increases muscle work output under *in vivo*-like conditions; (H2) because of the staircase

effect [13], PAP builds over cycles, resulting in increased work with each subsequent cycle until it reaches some maximum; and (H3) cycle frequency affects the degree of potentiation.

Methods

All animal procedures were approved by Harvard University's Institutional Animal Care and Use Committee (protocol 20-09). Mallard ducks (*Anas platyrhynchos*, n=5) were anesthetized with 1-2% isoflurane:oxygen gas mixture using mask induction. To measure fascicle length, sonomicrometry crystals (2 mm, Sonometrics Corporation) were implanted at each end of a mid-belly LG fascicle (Fig. S1) and secured (5-0 silk suture, Ethicon). The muscle's origin remained intact, but all other connective tissue attachments were removed to allow free movement of the LG. To measure muscle force and impose length changes, kevlar thread was tied proximal to the tendon's fibrocartilage pad and connected to a muscle ergometer lever arm (10 N, Aurora Scientific Inc.) modified by drilling a hole at half its length to increase its upper force limit (18.3 N). The animal was mounted on a moveable stage by clamping the femur mid-shaft and securing the foot to a flat plate (Fig. S1); this setup ensured that the limb and muscle origin remained stationary and the muscle line of action was aligned with the ergometer. The muscle was stimulated (pulse duration: 0.2 ms, Grass S48, Grass Instruments, Warrick, RI) intramuscularly through medial and lateral pairs of 24 gauge, stainless steel wire electrodes separated proximally and distally as far possible (~3 cm) and sutured in place (Fig. S1).

The voltage that induced maximal twitch force was determined, with voltage reduced (~2-3 V) to elicit submaximal force (35 ± 5% maximum), resulting in dynamic forces likely similar to forces produced during slow swimming [18]. Using stimulation frequencies of 125 (n=3) or 250 Hz (n=2), we determined the submaximal isometric L_o (length at which submaximal tetanic force is produced). We then used the work-loop technique [15] to measure muscle work while mimicking strain and activation patterns observed during surface swimming *in vivo* [18]. Specifically, ramp changes in muscle length were applied over 5-15 cycles with stimulation onset at -1.6% phase, stimulation duration of 26%, and lever arm excursion of 9 mm (Fig. 1), which corresponded to fascicle length changes of 5.4 ± 0.9 mm and strains (maximum: 0.03 ± 0.04, minimum: -0.21 ± 0.04) consistent with *in vivo* strains observed during surface swimming [18, KTB personal

observations]. Keeping the length change, activation phase, and duty factor constant, we tested three cycle frequencies (1.0, 2.6, and 4.0 Hz) in a random order with a 5 min rest between series, consistent with previous PAP work-loop experiments [2]. Because of in-series compliance, minimum strains decreased over cycles with increased force production, but these differences were small (change in strain from first to last cycle ~0.02 for 2.6 & 4 Hz and ~0.001 at 1 Hz). Isometric force production at L_o was monitored throughout the experiment to ensure that force decreases were <10%.

Post-mortem dissections confirmed sonomicrometry crystal alignment. Resting LG fascicle and inter-crystal lengths were measured and the ratio of these was used to calculate time-varying fascicle lengths (time-varying segment lengths * resting fascicle length/inter-crystal distance). Force and length data were filtered (low-pass, 1st order, dual pass Butterworth, 20 Hz cutoff; Fig. 1C,D) before plotting length versus force to calculate resulting loop areas (i.e., net work, Fig. 1E). Peak force and the average rate of force development (F_{rate}=change in force / duration from stimulation onset to maximum force) were measured. 2nd order polynomials were fitted to net work, peak force, and F_{rate} versus cycle number for each frequency to calculate (1) the maximum net work, peak force, or F_{rate} and (2) the cycle when the maximum occurred. Percent change in net work, peak force, and F_{rate} were calculated as: ((maximum – cycle 1 value)/ cycle 1 value)*100. Percent change data are available on Dryad (doi:10.5061/dryad.69p8cz8z4).

Statistical tests were performed in R (v. 3.6.0), with mixed-effects linear models used to predict the percent change in net work, peak force, and F_{rate} and the maximum work cycle number. Individual was a random effect and cycle frequency, stimulation frequency, and strain at peak force (taken from cycle 2) were added iteratively as fixed effects. Models were compared using ANOVA with α=0.05. Stimulation frequency and strain at peak force did not significantly improve the models ($p>0.05$), so were not included in further analysis. Frequency significantly improved the models ($p<0.05$) for all variables. Diagnostic plots for the selected model (frequency as a fixed effect and individual as a random effect) revealed assumptions of homoscedasticity and normal distribution of residuals were reasonable. We performed pairwise comparisons using the emmeans package and Tukey's adjustment. All reported values are mean ± s.e.m.

Results

We performed work-loop experiments to determine the degree of work, force, and F_{rate} PAP over 5-15 work-loop cycles at 1.0, 2.6, and 4.0 Hz. The number of work-loop cycles required to reach maximum work varied by frequency ($p<0.001$, Fig. 2A,B, Table 1). The maximum work cycle number significantly differed between 1 Hz and 2.6 Hz and 1 Hz and 4 Hz, but not between 2.6 and 4 Hz (Table 2).

Cycle frequency explained a significant amount of the variation in percent change (i.e., PAP) for net work (Fig. 2A,C; $p=0.001$), peak force (Fig. D; $p<0.001$), and F_{rate} (Fig. E; $p=0.001$). The percent change in net work was significantly different from zero at 4 Hz, but not 2.6 and 1 Hz (Table 1). Peak force and F_{rate} both significantly increased over work-loop cycles at 2.6 and 4 Hz, but not at 1 Hz (Table 1). Pairwise comparisons showed PAP in net work, peak force, and F_{rate} was significantly different between 2.6 and 4 Hz and 1 and 4 Hz, but not between 1 and 2.6 Hz (Fig. 2; Table 2).

Discussion

We examined the effect of PAP on mallard LG cyclical force and work performance, by imposing *in vivo* length change and activation data to mimic the contractile behavior of this muscle during surface swimming [18]. Under these conditions, PAP resulted in a 37% increase in peak force, a 64% increase in F_{rate}, and a 52% increase (n.s.) in net work (Fig. 2, Table 1), suggesting PAP is a relevant and substantial contractile phenomenon at the mallard's preferred cycle frequency.

Past work provided evidence of PAP in mammals [1,2,3,4,7,8,17], anurans [6,21], fish [16], and insects [15]; that birds also experience PAP is not surprising. The mallard LG is a mixed fibered muscle, with mostly type II fibers (74-100%, depending on the sample location, [22]). Previous work has shown that muscles with large populations of type II fast fibers demonstrate larger increases in force and F_{rate} than type I slow-fibered muscles [8].

We observed increases in both force and F_{rate} (Fig. 2, Table 1), consistent with previous findings [2,3,4]. Unlike isometric contractions where force and F_{rate} can be considered separately, force and F_{rate} are interrelated during dynamic contractions. Force drops when muscles shorten to lengths below L_o, so a greater F_{rate} will permit more force

development prior to substantial shortening. Thus, we cannot draw independent conclusions about these factors here.

During a dynamic contraction, changes in force and F_{rate} should also correspond to a change in work (H1). We observed a significant increase in peak force and F_{rate} for both 2.6 and 4 Hz, but a significant increase in net work only at 4 Hz (although there was a tendency toward significance at 2.6 Hz). Work potentiation has previously been observed during a single work-loop of a mouse fast-fibered muscle (extensor digitorum longus) following a conditioning stimulus [2]. The current study builds on this finding by examining work potentiation over several cycles of the mallard LG. Our observation of gradual increases in net work, peak force, and F_{rate} over 4-10 cycles (Figs. 1E,2A) indicates that PAP is cumulative during dynamic contractions (H2). Our findings are consistent with (1) staircase potentiation, where force increases over several isometric stimulations [3,6,12,13], and (2) observations of work increasing over several cycles [15,16,17]. PAP can build and possibly be maintained over a large number of cycles for sustained locomotion.

At higher cycle frequencies, (1) net work, peak force, and F_{rate} potentiation was larger and (2) a larger number of cycles were needed to achieve this potentiation, supporting our hypothesis that cycle frequency would affect PAP (H3). Krarup [3] similarly observed that higher contraction frequencies elicited larger twitch force potentiation but, in contrast to our findings, found that peak potentiation was reached with fewer stimuli at higher contraction frequencies. The lack of a significant effect of PAP on LG work at lower frequencies could be due to longer inter-cycle times, allowing the effects of PAP to diminish before the next cycle commences. Alternatively, the effects of fatigue due to longer contraction durations at lower frequencies could counteract the effects of PAP, causing work potentiation to be lower and to peak with fewer contractions. Because we maintained a constant duty factor of activation across frequencies, the total stimulation duration and number of spikes was higher at lower frequencies. PAP is thought to offset the effects of fatigue [7,23], possibly helping to maintain force production over a series of cycles. Thus, PAP may very well affect work output by mitigating the effects of fatigue and reducing the drop in force that might otherwise be observed.

The present study demonstrates that PAP occurs under conditions that mimic a cyclical swimming behavior and could elicit meaningful increases in muscle work, force,

and F_{rate} under certain cycle frequencies. Further exploration is needed of how changes in force and work production resulting from PAP relate to locomotor performance in naturally-behaving animals.

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Tables & Figure Captions

Figure 1. Work-loop experiments. (A) Ramp length change pattern applied via the lever arm and stimulation timing. Stimulation offset (1.6%) and duration (26%) are expressed as % of cycle and scaled to cycle frequency. Example (B) lever position and stimulation, (C) fascicle length change from sonomicrometry, and (D) force from the ergometer during a work-loop series at 2.6 Hz. (E) Force versus length work (area inside the loop) loops at 2.6 Hz. Length is expressed as an absolute measure (mm) and as strain (relative to $L_{o,\,submax}$). All loops are counterclockwise (positive work). Loops progress from light to dark with each subsequent loop. Thicker lines indicate the period of activation.

Figure 2. Post-activation potentiation occurred during work-loops in the mallard LG. (A) Percent change in net work versus cycle number at three frequencies: 1 Hz (gray, filled circles), 2.6 Hz (black, filled circles; mallards' preferred surface swimming cycle frequency), and 4 Hz (open circles). Solid lines are 2^{nd} order polynomial fits. (B) Maximum work cycle number was significantly higher at 2.6 Hz and 4 Hz than at 1 Hz, but not between 2.6 and 4 Hz (Table 2). Percent change in (C) net work, (D) peak force, and (E) F_{rate} were significantly greater at 4 Hz than 1 Hz and 2.6 Hz, but not between 1 Hz and 2.6 Hz (Table 2). All points are averages ± s.e.m. Lower case letters (a,b) indicate groups for Tukey's pairwise

comparison, with different letters assigned to groups that were significantly different ($p<0.05$). Sample size is indicated by the number on each point.

Table 1. Maximum work cycle number and mean percent change for net work, peak force, and F_{rate} by frequency. P-values<0.05 (bolded) indicate percent change is significantly different from zero.

Frequency		Max Work Cycle	Work (%)	Force (%)	F_{rate} (%)
1 Hz	mean ± s.e.m.	4 ± 1	16 ± 9%	10 ± 4%	29 ± 8%
	p-value		0.577	0.535	0.570
2.6 Hz	mean ± s.e.m.	8 ± 1	52 ± 14%	37 ± 11%	64 ± 20%
	p-value		0.051	**0.016**	**0.026**
4 Hz	mean ± s.e.m.	10 ± 1	115 ± 35%	69 ± 17%	117 ± 27%
	p-value		**<0.001**	**<0.001**	**0.002**

Table 2. P-values for a Tukey-adjusted pairwise-comparison for maximum work cycle number and percent change in net work, peak force, and F_{rate} by frequency. Bolded p-values indicate a significant difference (<0.05).

Comparison	Max Work Cycle	Work	Force	F_{rate}
1 Hz vs. 2.6 Hz	**0.011**	0.234	0.065	0.072
2.6 Hz vs. 4 Hz	0.288	**0.045**	**0.048**	**0.046**
1 Hz vs. 4 Hz	**0.001**	**0.004**	**0.001**	**0.002**

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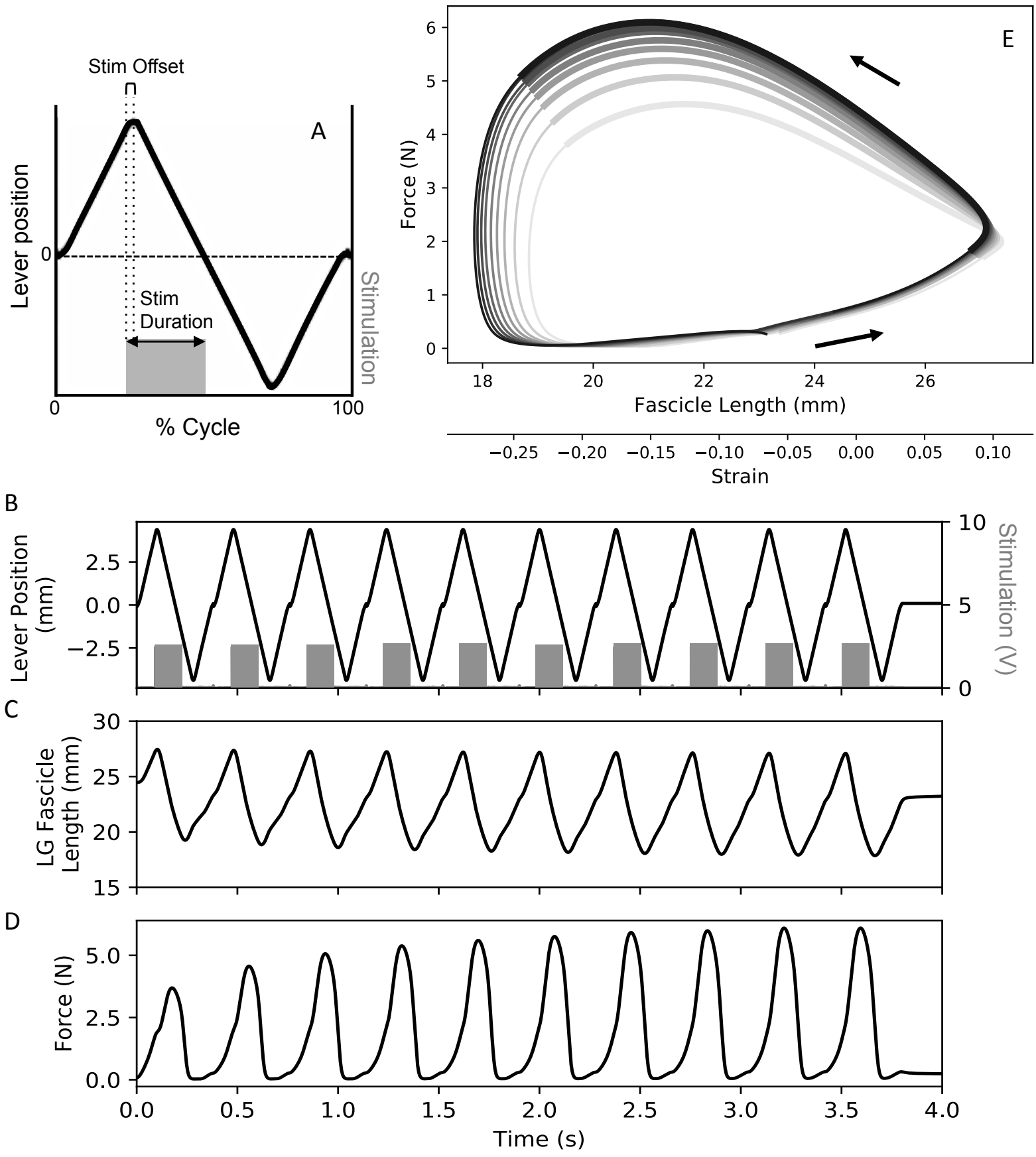
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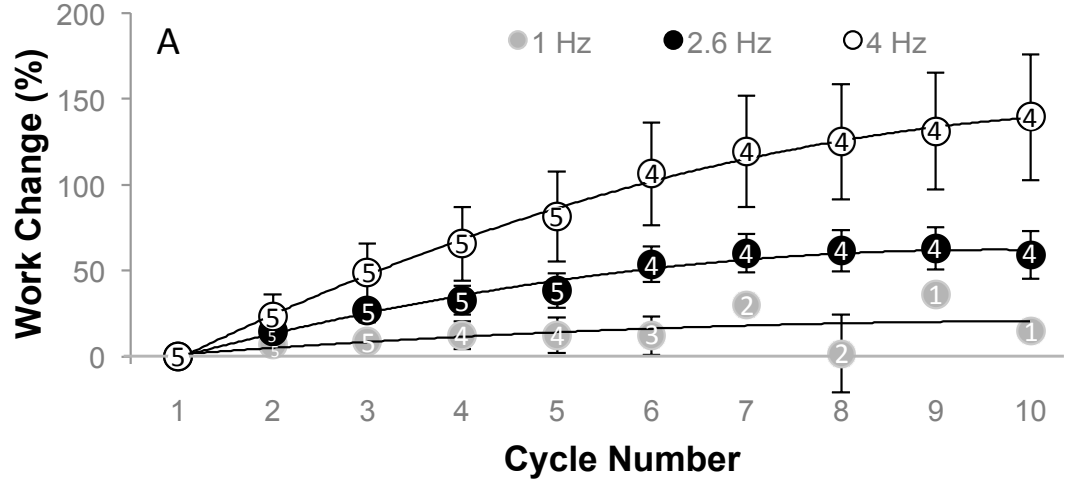
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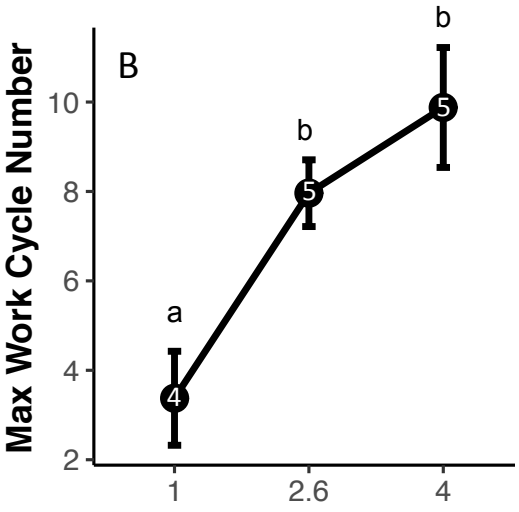
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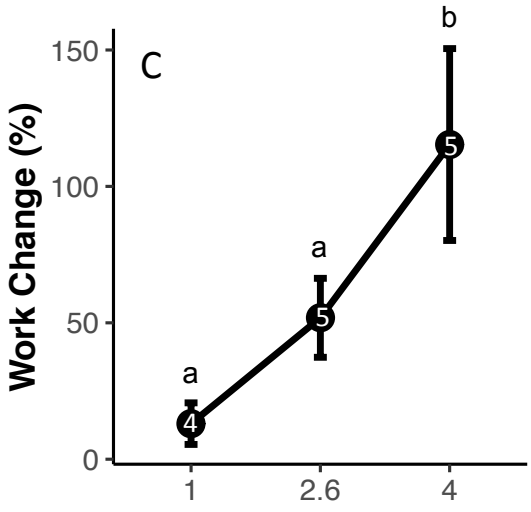
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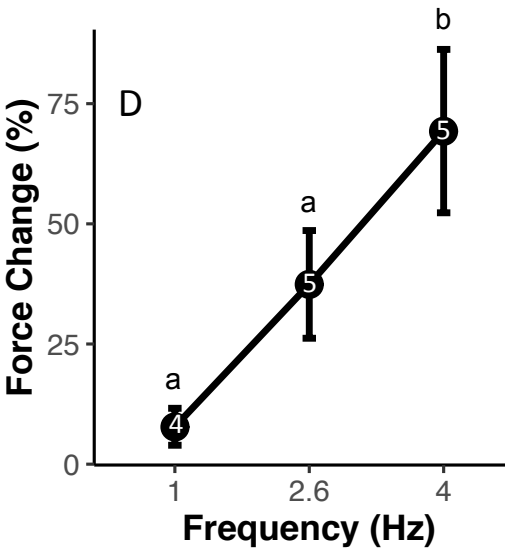
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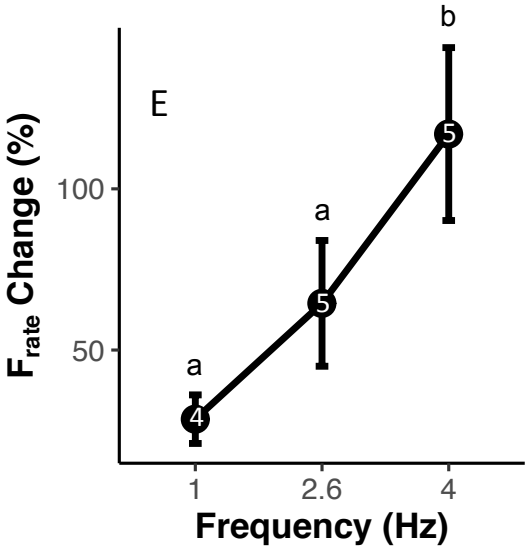
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F rate Change (%)
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Supplemental Information for:

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Biology Letters

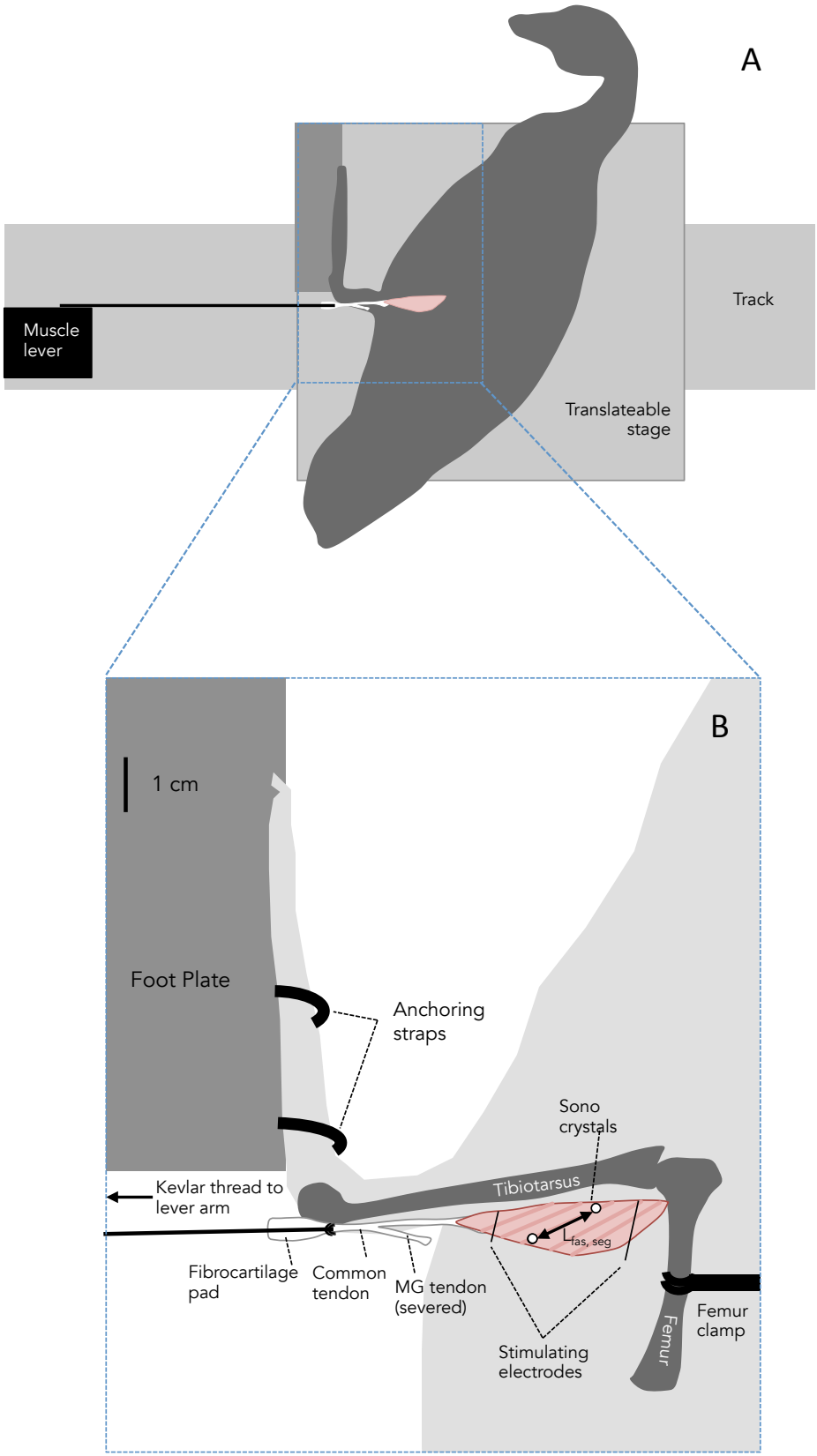

Figure S1. In situ experimental setup to measure force and length in the mallard lateral gastrocnemius (LG). (A) Schematic showing the *in situ* rig. The animal was placed on a stage that could be translated along a track to change the distance between the animal and muscle lever. (B) Larger view of a portion of the *in situ* rig (blue box). The femur was clamped mid-shaft and the foot was anchored to a plate to hold the leg and muscle origin stationary. The LG tendon was severed below the fibrocartilage pad, and a Kevlar thread was secured above the pad and connected to a muscle ergometer lever arm. The medial gastrocnemius (MG) tendon was severed and the muscle was freed from connective tissue attachments to surrounding tissues except for the point of origin on the femur. A pair of sonomicrometry crystals was inserted along the fascicle to measure a segment of fascicle length ($L_{fas, seg}$). An effort was made to measure as much of the fascicle as possible. We assumed the shortening in the segment was representative of shortening along the full length of the fascicle. Post-mortem 'resting' measurements of the segment ($L_{fas, seg, rest}$) and the full fascicle length ($L_{fas, rest}$) permitted us to correct sonomicrometry measurements of segment length ($L_{fas, seg}$) to fascicle lengths ($L_{fas, calculated} = L_{fas, seg} * L_{fas, resting} / L_{fas, seg, rest}$). Two pairs of stainless steel stimulating electrodes were inserted through the muscle belly with one polarity as far proximal and the other as far distal as possible. Although only one pair is shown, two pairs were inserted in medial and lateral sagittal planes.