EMG activation in the forelimb musculature of three-toed sloths (*Bradypus variegatus*)

by

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ABSTRACT

Suspensory behaviors require both strength and fatigue resistance of the limb flexors; however, the muscle mass of sloths is reduced compared to other arboreal mammals. Although suspensory locomotion demands that muscles are active to counteract the pull of gravity, it is possible that sloths minimize muscle activation and/or selectively recruit slow motor units, thus indicating neuromuscular specializations to conserve energy. Electromyography (EMG) was evaluated in a sample of three-toed sloths (*B. variegatus*: *N*=6) to test this hypothesis. EMG was recorded at 2000 Hz via fire-wire electrodes implanted into eight muscles of the left forelimb while sloths performed trails of suspensory hanging (SH), suspensory walking (SW), and vertical climbing (VC). All muscles were minimally active when performing SH. During SW and VC, sloths moved slowly (Duty Factor: 0.83–0.86) and activation patterns were consistent between behaviors; the flexors were activated early and for a large percentage of limb contact, while the extensors were activated later in the stride and showed biphasic (contact and swing) activity. EMG burst intensities were maximal for the elbow flexors and smallest for the carpal/digital flexors, and overall activation intensity was significantly greater for SW and VC compared with SH. Wavelet analysis indicated high frequency motor unit (MU) recruitment at low intensities for SH, whereas the opposite patterns of MU recruitment were similarly demonstrated for SW and VC, with the shoulder flexors and elbow flexor m. brachioradialis having the extremely low activation frequencies. Collectively, these findings support the hypothesis and suggest that sloths may selectively recruit smaller, fast-contracting MU for suspensory postures but have the ability to offset the cost of force production by recruitment of large, slow MU during locomotion.

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DEDICATION

I dedicate this Thesis to my Uncle Bob who I lost halfway through the completion of my MS degree. I am thankful for his unwavering support and guidance in many of my life decisions. He always encouraged me to keep pursuing my dreams no matter the obstacles I may endure, and he taught me to never let my happiness be determined by others.

November 21, 2016

Ms. Marissa Gorvet
Graduate Student Researcher
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Youngstown State University
One University Plaza
Youngstown, OH 44555

Dear Ms. Gorvet,

Thank you for your interest in performing an EMG study on muscle function in living tree sloths.

I am pleased to offer you access to collect data using fine-wire electromyography of N=5 *Bradypus variegatus* and N=5 *Choloepus hoffmanni* at the Sloth Sanctuary in Limón, Costa Rica. Our facility and sloths will be available for you to use in your study with 30-day advance notice, preferably in May and/or June of 2017. I understand that you will obtain the permits with Minae necessary to conduct research in Costa Rica, prior to your visit at our facility.

We are eager to help you and Youngstown State University with this exciting project to expand our knowledge on Costa Rican sloths.

Sincerely,

[redacted]

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INTRODUCTION

Skeletal muscle is contractile tissue that when recruited by the nervous system produces force and/or performs mechanical work. At the cellular level, muscle fibers are organized into motor units (MU) whereby the force per MU varies with the frequency of action potentials communicated by the motor nerve, as well as the number, size, and contractile velocity of the MU activated (Henneman, 1957; Henneman et al., 1965a, b). Thus, MU show a progressive level of recruitment by conventionally first recruiting smaller, slower MU followed by larger, faster MU (Henneman et al., 1974). This enables animals to match the functional demands of a locomotor or postural behavior, which is the primary means of regulating the force and power output of muscles (Roberts et al., 1997).

Electromyography (EMG) is a well-established technique used to record muscle activation (Gans, 1992), and further provides action potential intensity for wavelet analyses that are used to assess frequency dependent properties of slow and fast motor units recruited during locomotion (Wakeling, 2009, 2014). Some animals may perform locomotor behaviors in dynamic ways requiring greater power, while others move slowly and deliberately, emphasizing enhanced force. Regardless of the mode of locomotion employed, mammals have evolved numerous physiological strategies to conserve energy. In particular, the intensity of an EMG burst communicates the relative volume of muscle recruited, which reflects the metabolic energy cost of producing force for support (Roberts et al., 1997). Lower levels of muscle activation are possible when tensile loading of the tendinous and or elastic elements of muscle-tendon units (MTU) can act as an economical means to maintain limb posture, and this mechanism may be equally important for upright cursors (e.g., horses) as it is for mammals utilizing inverted behaviors (Nyakatura et al., 2010). However, while EMG activity has been sampled over a phylogenetic and functional range of taxa (Goslow et al., 1981; Wakeling et al., 2001; Licka et al., 2009; Lee et al., 2013; Fuchs et al., 2015), there are no available EMG data for species adapted for suspensory modes of locomotion and posture.

Tree sloths (superorder: Xenarthra) are an interesting lineage to study in this context because they are the only mammals for which suspensorial habits are almost obligatory (Hayssen, 2009, 2010). Sloths also have lower basal metabolism and mean body temperature compared with other xenarthan taxa, and placental mammals in general

(Gardner, 2008). Specifically, three-toed sloths (Genus: Bradypus) are high-canopy folivores (Montgomery, 1983; Cliffe et al., 2015) and have the lowest field metabolic rate observed among non-hibernating mammals (Pauli et al., 2016). The brown-throated three-toed sloth (*Bradypus variegatus*) is the most accessible (non-endangered) species of this genus and it is a specialized folivore that subsists on a very low food intake of exclusively leaves (Montgomery, 1983). Bradypus is diurnal but spends 15–18 hours/day sleeping (Sunquist and Montgomery, 1973; Janzen, 1983) and during this time, undergoes routine cycles of behavioral sleep (Barratt, 1965). The majority of the active, awake time in *B. variegatus* is filled by grooming or feeding while sitting in the recess of tree branches, thereby it does not often engage in locomotor behaviors (Urbani and Bosque, 2007). The collective sleep/wake patterns demonstrate that sloths are not particularly active mammals, thereby supporting the hypothesis that their physiology, as well as their behavior, reflects a system extremely modified to conserve metabolic energy (Cliffe et al., 2015; Spainhower et al., 2018a, 2018b).

Sloths exhibit arboreal locomotor behaviors that generally include suspensory (below branch) walking and vertical climbing, as well suspensory postures (hanging) by some combination of limbs for extended periods of time (Hayssen, 2009, 2010). Despite these abilities that necessitate both muscular strength and fatigue resistance, the muscle mass of sloths is greatly reduced (Grand, 1978), which further requires modifications to their distal limb skeletal morphology (Mendel, 1981b, 1985) and flexor muscle architectural properties (Olson et al., 2018), as well as the predominant expression slow myosin fibers (Spainhower et al., 2018a) with anaerobic metabolic characteristics for sustaining torque application (Spainhower et al., 2018b). In addition, their distinctively slow and controlled movement patterns are considered to be an adaptive strategy for energy conservation (Cliff et al., 2015; Cliff et al., 2018), and this may be understood as a means of enhancing muscle force while limiting contractile velocity (Olson et al., 2018), thus lowering the ATP cost of contraction (Taylor et al., 1982; Heglund and Taylor, 1988; Rome, 1990; Roberts et al., 1997). However, *in vivo* patterns of muscle activation are needed to verify muscle contractile properties in relation to locomotor performance in sloths.

This analysis aimed to evaluate EMG activation in the forelimb of *B. variegatus* to determine both the patterns and levels of muscle activation associated with suspensory

habits versus those of metabolically costly vertical climbing behavior. It is hypothesized that the neuromuscular properties of sloth muscles reflect a physiological system specialized to conserve metabolic energy via minimizing activation of flexor muscle mass for suspensory function. More specifically, it is expected that the shoulder, elbow, and digital flexors will be active for large portions of limb contact with the substrate. Additionally, it is predicted that maximum burst intensity will occur during vertical climbing, be intermediate during suspensory walking, and be minimal during suspensory posture. The results of this study improve the understanding of muscle recruitment patterns involved with suspensory habits and arboreal lifestyle, and help elucidate the influence of the neuromuscular system on motor unit properties in sloths. The outcomes also help clarify mechanical design requirements for limbs that evolved to experience primarily tensile versus compressive loading.

MATERIALS AND METHODS

Study animals

A total of N=6 thee-toed sloths (*B. variegatus*) were used for this study. No preference was given to the sex of the animals, although adult (or sub-adults) individuals were preferable to juveniles. Morphometric data for study animals are presented in Table 1. Data collection was conducted at The Sloth Sanctuary in Penshurt-Limon, Costa Rica in Summer 2017. Analyses were performed at Youngstown State University (YSU) during 2017–18. All experimental procedures complied with protocols approved by the Costa Rica Ministerio de Ambiente, Energia, y Telecomunicaciones (MINAE: R-008-2017 to M. Butcher) and adhered to the legal requirements of the United States of America.

Implantation of Electrodes

EMG was recorded with custom fine-wire electrodes. Briefly, the ends of Teflon® coated, stainless steel fine wire (0.002 bifilar: California Fine Wire, Grover Beach, CA) were bared and one end was routed through the eye of a 26g (0.45mm x 10mm) needle before the two wires were separated and fashioned into a hook-like projection. EMG electrodes were either 60 cm or 80 cm in length and all were sterilized (hydrogen peroxide plasma) prior to implantation.

Sloths were sedated with combination of Dexdomitor and Ketamine (0.1 ml/kg Dexdomitor + 0.1 ml/kg Ketamine) injected into the m. gluteus medius as done for routine nail clippings and teeth screenings of captive sloths. Because sloths do not respond to ketamine in a predictable manner (no anesthesia protocol currently available), supplemental doses of ketamine in increments of 0.1 ml were used as necessary to achieve the desired level of sedation. Body mass (Table 1) of each individual was recorded prior to sedation using a digital scale (Tree MRB 10000; LW Measurements Pte. Ltd., Singapore). While under sedation, electrodes were implanted into selected muscles of the shoulder, brachium, and antebrachium. Specifically, electrodes were implanted into either of two suites of four muscles in the left forelimb: Suite 1 (proximal musculature) – *m. pectoralis superficialis* (PS), *m. deltoideus* (DELT), *m. biceps brachii* (BB), and *m. triceps brachii-long head* (TBLO); Suite 2 (distal musculature) – *m. brachioradialis* (BCR), *m. palmaris longus* (PL), *m. flexor digitorum profundus* (FDP), and *m. extensor digitourm communis* (EDC). Pairs of 4 electrodes were implanted percutaneously into the center of each muscle belly within a suite (Wakeling et al., 2012; Lee et al., 2013) by using gentle force for the needle to penetrate the skin, then a one-half twist, and removal of the needle that left the hooked electrode end anchored in the muscle belly. Target muscles were identified by palpation of the limb while sloths were sedated; prior to experimentation, muscle locations were verified by gross dissection of three cadaver sloth forelimbs. The distance (in cm) from electrode placements in the shoulder, brachium and antebrachium regions of the forelimb to the spinal column was documented with a measurement tape.

The individual pairs of EMG electrode fine wires were loosely twisted and then taped together to form a 'cable' that was long enough to be looped for strain relief. Each electrode pair was marked with a channel number on the plastic portion of the needle housing. The bared ends of wires were fed into twist-pot connectors interfaced with a BioRadio™ (Great Lakes NeuroTechnologies, Cleveland OH, USA) that was harnessed to a Velcro® band fastened around the thorax of the animal. With the wireless EMG unit secure, sedation was reversed with injection of Antisedan (0.1 ml/kg) again into the m. gluteus medius.

EMG recordings

Upon recovery from sedation, the limbs of sloths were placed in position to grasp a horizontal beam (4.57m x 0.22m x 1.52m) constructed from bamboo. The beam was wrapped in veterinary adhesive (3M) to provide extra grip (Fig. 1A). Sloths were required to both hang in a static suspensory posture from the beam and walk across the beam. Several trials of suspensory hanging (SH) by forelimbs and hindlimbs were performed first in intervals of 1–3 min. Locomotor trials consisted of repeated short bouts of suspensory walking (SW), long enough to sample 2–3 footfalls of the instrumented forelimb. Sloths also performed repeated short bouts of vertical climbing (VC) where individuals were allowed to freely climb the outside of a fenced enclosure (2.00m x 3.97m x 3.12m) following walking trials (Fig. 1B). For each locomotor behavior, sloths were encouraged to move with the enticement of food (e.g., leaves or flowers) as needed, but were free to select their preferred speed.

All trials performed under the beam were videoed in the sagittal plane whereas VC trials were video captured in a coronal plane, both with a GoPro camera (HERO4: San Mateo, CA, USA). For each behavior, the camera was mounted to a tripod positioned 1.5m from the animal. This distance allowed nearly the entire length of the beam or height of the enclosure to be visualized. Video data (100 Hz) were synchronized with EMG recordings (2000 Hz) in real time by use of an LED light box placed in the field-of-view of the camera that was triggered with EMG data acquisition near the beginning of each trial. Trigger time appeared as a mark in the waveform records that sampled 4 channels of EMG signals (in millivolts) simultaneously via live broadband streaming to a laptop PC computer running the data acquisition software (BioCapture™: Great Lakes NeuroTechnologies). This method allowed for correlation of muscle activation with video recordings of footfalls during sloth locomotion, where a footfall (i.e., each grasp) was defined as the first frame when the claw of each digit was in full contact with the substrate and the limb showed evidence of weight-bearing support. EMG activity was recorded for one suite of four muscles (proximal vs. distal musculature) then the other on consecutive days for the same individual. Recording sessions lasted a total of ~2 h depending on the performance of an individual. Collectively, this sampling strategy resulted in a maximum number of trial recordings of EMG activation for eight forelimb

muscles across all behaviors and individuals. Our approach also limited the total time interval to which each animal was subjected to the rigors of the experimental process.

At the completion of EMG recordings, individuals were handled carefully and the fidelity of each implant was verified before the fine wire electrodes were removed. The animals were then placed back in their enclosure and observed for 1–2 h to ensure full recovery. In addition, veterinary personnel present at The Sloth Sanctuary monitored the health of the animals for several days post-experimental recordings for any signs of infection or unusual behavior due to sedation in accordance with their standard operating procedures and approved live animal protocols.

Data analysis

Video data were first analyzed for frame numbers that corresponded to the beginning and end of SH intervals, or to foot-on (grasp) and foot-off (release) events during locomotor trials. Frame number was then calibrated to time in seconds in the EMG waveform records using the frame marking the time that the LED light first illuminated. Next, EMG recordings were exported from BioCapture™ as a series of .CSV files in MS Excel (Microsoft Corp., Redmond, WA, USA) that were used as source data for quantitative analysis. EMG recordings for SW and VC, in particular, were analyzed as a series of strides consisting of consecutive pairs of limb contact and swing phases. LabScribe software (iWorx Systems Inc., Dover, NH) was used to measure and calculate all temporal variables (time in sec and % stride) associated with both the limb cycle and EMG recordings relative to the stride interval, where the sum of the contact and swing phases is 100% of the stride, and include duty factor, swing duration, EMG onset, EMG offset, and EMG burst duration. Velocity (ms^{-1}) for SW and VC was calculated by using the software Tracker (https://physlets.org/tracker/) to digitize several strides of each individual through a calibrated distance (15cm) in the frame of view. The BioRadio™ unit was used as the marker to digitize through one stride per trial analyzed. Instantaneous velocity was determined at 1 s intervals throughout a stride and averaged across data points where velocity was similar. A total of 117 trials, 227 strides, and 856 EMG bursts were analyzed (Table 1). All temporal measurements were averaged for each muscle and individual.

EMG intensity was analyzed two ways using custom code written for the software Mathematica© (Wolfram Research, Champaign, IL, USA). First, burst intensity was quantified by the Root Mean Square (RMS) method on rectified EMG data (Wakeling et al., 2001; Hodson-Tole and Wakeling, 2007; Wakeling, 2009). The maximum value (millivolts) was measured from each burst and stride sampled for final analysis, and averaged for each muscle and individual. Burst intensities for each muscle were normalized by calculating a series of intensity ratios ranging from 0 to 1 to compare EMG activation across behaviors, where a ratio of zero is no activation and one is maximum activation (Gillis and Biewener, 2001, 2002). Specifically, mean burst intensities for each muscle and behavior were divided by single maximum intensity measured (i.e., a proxy for maximum voluntary contraction) for each muscle. Second, a subset of 2–3 bursts per muscle and individual were chosen for wavelet analysis (von Tscharner, 2000; Wakeling et al., 2012), whereby mean normalized EMG intensity was evaluated across a frequency spectra for which contour plots were used to detect burst intensities, followed by extraction of EMG intensity at 17 selected characteristic frequencies (range: 6.9–902 Hz) sampled in 0.5–1 s packets (x5) of signal data. A representative wavelet analysis of EMG bursts from a sloth elbow flexor is shown in Figure 2. Mean and peak frequencies were then determined for each muscle and behavior across individuals in the format of binary data for low-end and high-end frequency characteristics present in the recorded EMG signals. Finally, the mean frequency properties of wavelets were output as a series of Principal Components Analysis (PCA). PCA is a multivariate analysis defined in terms of eigenvector-eigenvalue pairs. The eigenvector represents the PC weighting, while the eigenvalue represents the PC loading score and is responsible for describing the amount of each eigenvector in each measured spectrum (Wakeling, 2004; Hodson-Tole and Wakeling, 2007). In order to quantify high frequency versus low frequency components within the EMG signal, PCI (y-axis) loading scores were plotted against the PCII (x-axis) loading scores, resulting in an angle (θ). A larger θ has a negative contribution and specifies low-frequency content, while a smaller θ has a positive contribution and characterizes higher-frequency content. These PC loading plots were used to evaluate activation frequency grouping patterns for slow and fast motor units for each muscle across the three functional behaviors SH, SW, VC.

Statistics

Values are reported as means±s.d. (standard deviation) unless otherwise specified. Two-tailed *t*-tests were performed in MS Excel and used to measure differences for stride duration, duty factor, and velocity between SW and VC. Descriptive statistics for all temporal and EMG intensity measurements for each muscle were pooled and averaged across individuals to account for variance associated with the general patterns of activation timing and EMG maximum activation. For statistical analysis, Coefficients of Variation (CV) were calculated for each variable analyzed to justify use of mean data in multivariate testing. A MANOVA was performed in SPSS (v. 20: IBM, Inc., Armonk, NY, USA) to assess statistical significance in both EMG burst duration (%) relative to the stride cycle and normalized EMG burst intensity, specifically how these activation parameters varied among muscles (effect: muscle) and the three behavioral conditions (effect: behavior). In cases where the assumption of homoscedasticity was not met (e.g., relative EMG burst durations) a Kruskal-Wallis test was performed, and then significant results from either this test or those from a significant MANOVA were followed by a series of one-way ANOVAs and Dunnet's T3 (burst duration) or Tukey's (intensity) *post hoc* tests to determine all pair-wise significant outcomes. Significance for all statistical tests was accepted at $P \leq 0.05$.

Study Limitations

The exact placement of EMG electrode implantation could not be verified in the present study. We did not have access to ultrasound or MRI to verify muscle identity or the location of the electrode wire once implanted in a muscle mid-belly. Moreover, the implantation of fine-wire electrodes was approved as a recovery procedure with the stipulation that no animal would be sacrificed following experimentation. However, despite the potential lack of exact placement of our electrode implants in some muscles, EMG activation broadly sampled from flexor and extensor musculature (per limb region) is adequate to achieve our study objective and test our hypothesis and predictions. Also, we were not able to sample EMG from all muscles per behavior and individual due to electrode failure. This is typical of *in vivo* studies like ours and most often involved an individual scratching at the implantation site or implant failure following SW trials. We did not have the option (or time) to record SW and VC on separate days because one of

our goals was to minimize stress to the animals (see above). Perhaps it would have been advantageous to alternate the order of SW and VC trials, although suspensory walking is stereotypical in sloths and we wanted to ensure adequate sampling of this locomotor behavior *a priori*. The combination of all EMG recordings for each muscle and locomotor behavior are presented in Table 2.

Three other factors are suggested to be potential study limitations. First, all individuals were not capable of performing all behaviors equally well. This may be attributed to the time of day that sloths were required to perform our locomotion trials, and this time interval not coinciding with a typical active period for *Bradypus*, which is diurnal. Also, measurements from some muscles may vary due to fatigue and because muscle suites from the same individual were sampled on separate days. Second, it is also possible that sloths were not fully recovered from sedation at the outset of the recordings sessions. Sloths do not appear to metabolize drugs quickly, and despite the administration of the reversal agent, individuals often performed better after multiple SW trials. For this reason, we often made 10–15 records of each behavior to ensure that we were sampling optimum performance, which included consecutive strides of near steady-state walking and climbing. It is noted that velocity was not variable regulated in our experimental approach, although all trials analyzed show a low variation in duty factor indicating that preferred speeds were comparable across individuals. A third possibility could have been the large beam diameter. At 22 cm, this substrate would be considered very large by previous studies of two-toed sloths (Granatosky et al. 2018). However, *Bradypus* may prefer, or is more capable of grasping larger substrates (Mendel, 1985a), and none of our individuals appeared to have difficulty placing the entire foot on the beam, which should not have limiting factor in SW performance.

RESULTS

Stride Parameters

Three-toed sloths walked below the beam and climbed the enclosure primarily by employing diagonal sequence gaits. Walking strides were typified by placement of the left forelimb followed by sequential (nearly simultaneous) advancement of the right forelimb and left hindlimb pair, and completed by sequential advancement of the left forelimb and right hindlimb pair. Vertical climbing followed this limb placement pattern

for some individuals, whereas in others, climbing strides began with placement of the left forelimb followed by advancement of a right forelimb-hindlimb couplet, and completed by advancement of the left forelimb-hindlimb couplet. Stride duration averaged 21.3±28.3 s during SW, and was more variable than that during VC, which was 18.7±13.2 s (Table 3), but not significant (P=0.95). However, equally long stride durations resulted in low velocities for both SW and VC. On average, three-toed sloths (N=6) walked twice as fast (P=0.042) below the beam at 0.06±0.03 ms^{-1} than they climbed the enclosure at 0.03±0.01 ms^{-1}, but had similar (P=0.22) mean duty factors of 0.83±0.04 and 0.86±0.04 for SW and VC, respectively (Table 3).

Temporal EMG Activation Patterns

- *Suspensory hanging*

Exemplar raw EMG recordings for suspensory hanging (SH) are shown in Figure 3. EMG activations during SH were characteristically long in duration, ranging from 5–60 s, and each muscle generally showed intermittent activations over several minutes of recordings.

- *Suspensory walking*

Exemplar raw EMG recordings for suspensory walking (SW) are shown in Figure 4. Overall, absolute and relative EMG durations were remarkably similar between proximal and distal limb muscles, as well as flexor vs. extensor muscle activations. The proximal flexors PS, DELT, and BB exhibited long absolute burst durations that ranged from 7.25–10.5 s and were exceeded only by those of the distal flexors BCR, PL, and FDP (range: 10.3–11.6 s). The biarticular TBLO had the shortest mean burst duration at 3.08±3.13 s that was followed by mean of 3.89±4.81 s for the digital extensor EDC. Figure 5 shows the relative EMG burst durations (% stride cycle) for each muscle during SW. The distal flexors were activated for similarly long durations of the stride (56–60%), while PL had the longest relative EMG burst duration with a mean of 60.2±15.7% of the stride cycle (Table 4). TBLO and EDC had burst durations that were on average less than 20% of the stride during SW (Fig. 4). Moreover, all flexor muscles were mainly active during the contact phase (>88% of SW trials analyzed) compared to the TBLO and EDC, which showed activation during both the contact and swing phases. Most often the extensor muscles demonstrated activation late in the contact phase with continued activation into

early swing phase. Respectively, TBLO and EDC were activated 55% and 39% during the contact phase, 23% and 24% during swing phase, and showed biphasic activation in 22% and 37% of SW trials analyzed. A summary of the most typical EMG activation patterns of each muscle is presented in Table 5.

While all eight muscles displayed some amount of pre-activation (i.e., activation in late swing phase through the contact phase), the BB exhibited this pattern of activation most frequently by being observed in 20% of all trials sampled. On average, however, no muscle pre-activated before grasp (foot-on) of the left forelimb during SW (Fig. 5). Flexor muscles had earlier EMG onsets compared to the extensors and all were activated within the initial 30% of the stride during SW. The elbow flexor BCR activated the earliest of all muscles (mean EMG onset: 8.29±8.42%) followed by BB, FDP, and PL, which in general, all had early EMG onsets of muscle activation (Table 4). In contrast, TBLO and EDC both activated nearly midway into the stride cycle. A consistent pattern was observed for EMG offsets. Values for all flexors were generally similar and ranged from 61–73% of the stride (Fig. 5). EMG offset of the EDC was the latest of all muscles and on average activation ended during the swing phase (mean EMG offset: 86.9±27.7%) (Table 4). Last, although the forelimb flexors typically activated once per stride, in some trials these muscles occasionally displayed multiple bursts, which were accounted for by the measurement total EMG burst durations (Table 4).

- *Vertical climbing*

Exemplar raw EMG recordings for vertical climbing (VC) are shown in Figure 6. All flexor muscles had relatively long absolute burst durations that overlapped within the range of 5.7–8.1 s, whereas those of the EDC and the biarticular TBLO are comparable, both with absolute activations of ~4.0 s. Figure 7 shows the relative EMG burst durations (% stride) for each muscle during VC. The FDP and BCR showed the consecutively longest relative burst durations with means of 48.8±13.6% and 56.7±18.3% of the stride cycle, respectively (Table 4; Fig. 7). These two muscles also occasionally showed multiple bursts per stride and are accounted for by the metric total EMG activation in Table 4. With the exception of PL, which along with the EDC had average relative burst durations at 24% of the stride, the remaining forelimb flexors exhibited relative burst durations that on average approximated 42–46% of the stride. TBLO had the shortest

relative burst duration with a mean of 22.2±14.3% of the stride (Table 4; Fig. 7).

Similar to SW, the six forelimb flexors were active mainly during the contact phase (>83% of VC trials analyzed), with notably the elbow flexors BB and BCR, and digital flexor FDP activated almost exclusively (97–100%) during contact of VC trials analyzed (Table 5). The extensors TBLO and EDC displayed routine activation in both phases of the stride cycle. Throughout all trials sampled, respectively, TBLO and EDC were activated 68% and 30% during contact phase, 28% and 63% during swing phase, and showed biphasic activation in 4% and 7% of VC trials analyzed (Table 5). The DELT (11%), TBLO (4%), EDC (7%), and PL (17%) infrequently demonstrated activations during late contact into early swing phase.

Each muscle showed some pre-activation during VC, and it was observed most often in BB (40%) as well as in FDP (49%) for all trials sampled, although no muscle averaged EMG onsets that occurred prior to grasp (foot-on) (Fig. 7). Except for the DELT and PL, the flexors were activated within the first 8% of the stride, and the BCR again activated the earliest of all muscles (mean EMG onset: 3.64±8.60%); large error terms associated with these measurements represented pre-activations (i.e., negative %) of the muscles (Table 4). Conversely, TBLO and EDC both had EMG onsets of muscle activation beyond midway into the stride cycle (Fig. 7). EMG offsets also followed the patterns observed for SW, but with greater variability among the proximal flexors. Values for all flexors ranged from 49–72% of the stride with activation ending the earliest for BB and latest for DELT (Fig. 7). EMG offset of the biarticular TBLO was the latest of all muscles and on average activation ended during early swing phase (mean EMG offset: 87.1±17.0%) (Table 4; Fig. 7).

Relative EMG Burst Duration

The EMG onsets and offsets, as well as relative EMG durations observed were both consistent and similar between the functional behaviors SW and VC, but demonstrated differences among flexor vs. extensor muscle activations. For the effect of muscle, MANOVA found that differences in burst durations were significant ($P<0.001$). Relative EMG burst durations did not differ ($P=0.057–0.274$) among the flexors, whereas burst durations of both extensors were significantly lower than those of the BCR ($P=0.001$ vs. TBLO; $P<0.001$ vs. EDC), PL ($P=0.001$ vs. TBLO; $P=0.001$ vs. EDC), and FDP

(*P*=0.003 vs. TBLO; *P*=0.002 vs. EDC) (Table 4). Despite the lack of significance for the effect of behavior (*P*=0.546), the MANOVA results were significant (*P*=0.043) for the interaction of muscle and behavior effects, and *post hoc* tests revealed subtle differences in muscle activations between SW and VC. Relative burst duration for BB during VC was greater than that of TBLO (*P*=0.014) and EDC (*P*=0.001) during walking. Similarly, the relative burst duration of PS and BCR during VC was greater than that of TBLO during walking, albeit significance was marginal (*P*=0.05 vs. PS; *P*=0.048 vs. BCR).

Normalized EMG Maximum Intensity

Means of normalized EMG intensity for each individual and muscle are presented in Table 6, while pooled mean burst intensity ratios across all eight forelimb muscles and the three functional behaviors are presented in Table 7. Coefficients of variation for EMG burst intensities were not significant among muscles (*P*=0.827) or behaviors (*P*=0.513). For the effect of muscle, MANOVA found that normalized EMG intensity was significant (*P*=0.02). During each functional behavior, only the PL showed consistently low normalized burst intensity that was significantly different (*P*≤0.016–0.001) from all other muscles except the FDP (*P*=0.136). Although not significant, the flexors PS, BB, and BCR had the largest burst intensities with ratios ≥0.3 on average during suspensory hanging. Similarly, when three-toed sloths performed suspensory walking, activation of the elbow flexors BB and BCR remained the greatest with means of 0.71±0.19 and 0.72±0.16, respectively (Table 7). The patterns of maximum activation intensity during vertical climbing changed from those observed for both SH and SW, whereby the extensors TBLO and EDC showed the largest normalized EMG intensity.

Figure 8 shows normalized burst intensities for selected flexor muscles across the three behaviors. While normalized EMG intensities did not differ significantly among the PS, BB, BCR, and FDP (Fig. 8a) within any behavior, it was observed that activation of the digital flexor FDP was relatively low. However, MANOVA found that normalized EMG intensity for all muscles differed significantly (*P*<0.001) between SH, SW, and VC (effect: behavior). Compared to SH, the mean normalized burst intensities were nearly 2.0 times greater during SW and 1.3 times greater during VC, and *post hoc* tests revealed that these differences were significant: SW vs. SH (*P*<0.001), VC vs. SH (*P*<0.001).

Mean normalized burst intensity for all muscles during SW was also significantly greater (P=0.022) than that for VC (Fig. 8b).

Wavelet Intensity-Frequency EMG Properties

Wavelet analysis and PCA allowed generation of intensity-frequency spectra that were reconstructed from PC weightings and PC loading scores to reveal extreme low and high activation frequencies for each muscle. The first two principal components explained greater than 80.0% of the variance observed for each muscle sampled. Figure 9 shows mean normalized EMG intensity as a function of frequency for each muscle across all three functional behaviors. Low frequency spectra (i.e., slow motor units) for the BCR, PS, and DELT had minimal activation frequencies with values of 81.8 Hz, 88.9 Hz, and 95.3 Hz, respectively. The PL, BB, and TBLO demonstrated intermediate frequencies for activation of slow motor units that ranged from 134–173 Hz (Fig. 9). With mean values >200 Hz, the distal muscles EDC and FDP had the largest activation frequencies for slow motor units. The patterns for extreme high frequency spectra (i.e., fast motor units) generally followed those of the low level activations. The BCR and DELT had the lowest values of the high activation frequencies at 259 Hz and 269 Hz, respectively, whereas FDP (383 Hz) and EDC (472 Hz) exhibited the two overall highest activation frequencies of any muscle sampled (Fig. 9).

Differences in EMG activation frequencies during each functional behavior were also resolved and are presented in Table 8. The highest activation frequencies were generally observed for suspensory hanging. During SH, activation frequencies ranged from 231–355 Hz, where the elbow flexor BB had the lowest measured value among the eight muscles, and EDC and FDP had the overall highest frequency values of any behavior. Whereas activation frequencies for EDC were similar and highest across all three behaviors, those for FDP remained large but decreased from SH to SW (293 Hz) to VC (278 Hz). A pattern similar to that of the FDP was observed for the PS, although frequency values were lower for each functional behavior. The distal flexor PL, as well as the biarticular TBLO, also exhibited the high frequency activations of nearly 300 Hz across behaviors, with measured values for SH reaching 320 Hz or greater (Table 8). During SW, the range of activation frequencies was slightly lower than those for SH and showed minima and maxima between 209 and 347 Hz (Table 8). The DELT and BCR

demonstrated the lowest activation frequencies for SW, and in particular, these two flexors, along with the BB, TBLO, and PL, all exhibited their overall lowest activation frequencies during SW versus VC. This was the most typical pattern of activation frequencies among the three behaviors. However, activation frequencies determined during VC (range: 215–346 Hz) were similar between locomotor behaviors, and the overall lowest frequencies for both SW and VC were exhibited by DELT (Table 8). Despite the consistency in activation patterns across behaviors, BB was the only muscle where activation frequencies increased from SH to SW (262 Hz) to VC (285 Hz).

PCA resolved that EMG intensities occurred at variable frequencies across muscles for each functional behavior. The first two principal components of the EMG intensity spectra collectively explained 84.7% of the variance observed (PCI explained 72.1% and PCII explained 12.6%). Figure 10 shows a representative PCA eigenvector plot of EMG activation intensity-frequency properties of sloth forelimb muscles for each functional behavior. Here, PCI loading scores indicate the total EMG intensity, thereby providing an acceptable measurement of muscle activation, while PCII loading scores relative to those of PCI contribute a measurement of the relative activation frequency component within an EMG burst. SW had the highest intensity along the PCI (longest vector) that was similar in both direction and magnitude to VC, although the eigenvector for VC had a slightly larger value of θ, than that determined for SW. Combined, these data indicate lower frequency components resolved from all EMG bursts sampled and recruitment of more slow motor units. Conversely, SH had lowest intensity along the PCI (shortest vector), the smallest value of θ, and an eigenvector that was nearly 90º opposite in direction from that for both SW and VC (Fig. 10). The data for SH are indicative of higher frequency EMG components and fast motor unit recruitment.

DISCUSSION

Several major findings of this study verify the hypothesis that EMG activation of sloth forelimb muscles reflect a system specialized to conserve metabolic energy. First, the shoulder, elbow, and digital flexors of *B. variegatus* have typically long burst durations during the contact phase of locomotor behaviors, with notable early EMG onset of the well-developed, strong elbow and digital flexors. Second, relatively low levels of activation were detected during postural hanging compared to significantly greater

normalized EMG intensity for all muscles when individuals performed suspensory walking and vertical climbing. Third, the forelimb musculature collectively exhibited the highest activation (myoelectric) frequencies and recruitment of faster motor units (MU) for suspensory hanging, whereas the opposite pattern was similarly observed for walking and climbing. Forth, both shoulder flexors and the elbow flexor m. brachioradialis had the extremely low activation frequencies across locomotor behaviors. These data are both surprising and informative, and overall, they help to validate understanding of economical neuromuscular recruitment patterns involved with suspensory habits. Importantly, they develop our expectations for the mechanical design requirements of the forelimbs to endure tensile versus compressive loading, which will be explored in future studies with three-toed sloths. Moreover, a great deal of information on structure/function in sloths has been generated over recent decades (Mendel, 1981a, b, 1985; Montgomery, 1983; Urbani and Bosque, 2007; Nyakatura and Fischer, 2010, 2011; Nyakatura et al., 2010; Nyakatura, 2012; Cliff et al., 2015, 2018); however, no previous study has evaluated muscle function *in vivo* while sloths perform suspensory habits, or much less, been the focus for any mammalian taxa utilizing suspensory modes of locomotion. Moreover, the majority of locomotor studies involving sloths (Nyakatura et al., 2010; Nyakatura and Andrada, 2013; Granatosky and Schmitt, 2017; Granatosky et al., 2018) have focused on Linn's two-toed sloth (*Choloepus didactlyus*) and were limited to small sample sizes, whereas the present study observed *B. variegatus* (N=6) for which comparatively less data are available.

Three-toed sloths specifically occupy tree braches and lianas between the understory and emergent canopy levels of rainforest strata, whereas Choloepus typically will not exceed the understory (Montgomery and Sunquist, 1978). In addition to the slight differences in behavior and ecological preferences, members of both genera also appear to move at different locomotor speeds, albeit consistently slow velocities. During suspensory walking, *B. variegatus* averaged a velocity of 0.06 ms^{-1} that is up to ten times slower compared with speeds previously reported for *Ch. didactlyus* (Nyakatura et al., 2010). Broad dissimilarities in suspensory walking stride durations between studies can be explained by previous use of a motorized treadpole to influence speed dependent kinematics versus freedom to choose a preferred speed in a naturalistic setting for the

present study. And while two-toed sloths may move faster in the wild than Bradypus, a reported steady-state speed of 0.11 ms^{-1} (Granatosky and Schmitt, 2017) is still slow and duty factors for suspensory walking reliably range between 0.51 to 0.89 across studies (Nyakatura et al., 2010). The generally slow and deliberate locomotion of sloths is once more emphasized when compared with speeds of other mammals that employ facultative suspension, including a grey mouse lemur (0.39–0.89 ms^{-1}; Schmitt, 2008), squirrel monkey (0.39–1.00 ms^{-1}; Schmitt, 2008), and slow loris (0.73 ms^{-1}; Schmitt and Lemelin, 2004). Additionally, it is interesting that in the present study, a duty factor (0.86) for VC parallels that observed for SW. Thus, locomotor speed may be particularly constrained in Bradypus related to selection for stealth and cryptic movement patterns as its main form of predatory avoidance. We also suggest that slow and deliberate locomotion is strongly related to limb muscle fiber type characteristics (Spainhower et al., 2018a, b), which in turn are correlated with metabolic energy conservation in sloths.

Unlike upright quadrupeds, the suspensory habits of sloths require their musculoskeletal system to resist tensile loads instead of compressive forces. As predicted, the limb retractors/shoulder flexors (PS and DELT), elbow flexors (BB and BCR), and carpal/digital flexors (PL and FDP) are mainly active during the contact phase, whereas the elbow and digital extensors (TBLO and EDC, respectively) are active during both contact and swing (Table 5). These *in vivo* patterns of muscle activation match previous expectations (Nyakatura, 2012; Nyakatura and Andrada, 2013) that the functional demands placed on sloths limbs are the inverse of those of upright mammals; the pull of gravity causes extension of the limb joints that must be controlled by flexor muscles. Notably, the forelimb of *B. variegatus* contains strong flexors that are collectively capable of 2 to 5 times greater joint torque application than its extensor musculature (Olson et al., 2018). Hence, in contrast to pronograde arboreal mammals, sloths rely more on their forelimb flexor muscles rather than their extensors for below-branch stabilization (Miller, 1935; Mendel, 1985a) and propulsion (Granatosky and Schmitt, 2017; Granatosky et al., 2018) during suspensory locomotion.

One of the objectives of this study was to determine functional roles of limb muscles during suspensory locomotion. To be clear, formal studies of limb kinematics in Bradypus are still needed to confirm the following interpretations of muscle function.

During suspensory walking, the forelimb is in a protracted position at the beginning of a stride, and as the contact phase progresses, the humerus is retracted as shoulder joint is flexed. Early activation of the biarticular BB likely contributes to protraction of the forelimb during late swing and its continued large activation intensity could be related to its ability to stabilize both shoulder and elbow joints during contact. Muscle recruitment of the PS and DELT occurred nearly 20–30% into the stride cycle indicating their role in forelimb retraction. However, both their slightly delayed EMG onsets and intermediate activation intensities may be related to a limited degree of humeral retraction in Bradypus, which agrees with the limb kinematics observed for *Ch. didactylus* showing that the forelimb is most frequently in a neutral posture for suspensory walking in a naturalistic setting (Granatosky et al., 2018). Unlike Choloepus, the elbow joint of *B. variegatus* is flexed throughout the majority of the stride cycle. Lengthy (>50% stride) and large (>0.70) activations of elbow flexors BB and BCR can act to maintain flexion and mitigate tensile loading of the forelimb. Notably, the EMG activation parameters of the extremely well-developed BCR indicate the importance of its role as the main flexor of the elbow joint.

In contrast to the BCR, the biarticular TBLO is activated nearly halfway into the stride indicating that it does not play much of a role in elbow joint position control. We speculate that the medial and lateral heads of the m. triceps brachii perform this function in addition to active extension of the elbow joint during swing phase. Future EMG recordings from these muscles would be a test of these possibilities. Its activation occurs approximately when the ipsilateral hindlimb is being advanced and thus the TBLO may act to stabilize the shoulder joint late in forelimb contact through early swing phase. Some shortening of the TBLO may alternatively facilitate elbow joint extension near the offset of its activation and initiation of swing phase. Lastly, digital flexion generally occurs prior to complete purchase of the substrate by early activations of FDP. While carpal joint flexion and adduction (ulnar deviation) could not clearly be discerned from sagittal video recordings, the early, elongated, and moderately intense activations both the PL and FDP imply active maintenance of the carpus in this type of position. Moreover, *Ch. didactylus* maintained its carpus almost exclusively in ulnar deviation during suspensory walking (Granatosky et al., 2018), and our individuals of Bradypus

notably placed the majority of their palmar forefeet (hairy volar pads) on the substrate to accommodate the large beam diameter, which requires both strong carpal flexion and adduction. Confirmations of foot postures remain to be confirmed by comparable kinematic analyses in three-toed sloths. Nonetheless, active gripping of the substrate by the digits is prolonged until nearly the end of contact as would be expected. The EMG onset of EDC (45% stride) overlaps with activation of the carpal/flexors indicating that the digital extensors do counterbalance flexion of the digits where flexor activation intensity may be greatest. Similar to TBLO, deactivation of EDC near the initiation of the swing phase may demonstrate typical flexor vs. extensor EMG activation patterns in sloth forelimbs regardless of locomotor behavior.

Beyond the similarity in stride parameters, the consistency in EMG activation patterns observed between SW and VC was remarkable. The forelimb is protracted at the beginning of a climbing stride and is retracted (or adducted in a frontal plane) as the contact phase progresses primarily by the PS, which activates earlier (10% stride) compared to its onset during suspensory walking. It is expected that m. latissimus dorsi would also act as a major limb retractor during VC and may have a slightly delayed EMG onset in coordination with that of the DELT. However, reliable percutaneous implantation of fine wire electrodes in the m. latissimus dorsi of Bradypus was not possible, and the lack of complimentary data from this massive muscle could partially explain the relatively lower intensities of PS and DELT than those determined for these two limb retractor/shoulder flexors during SW. As with SW, the elbow joint remains flexed throughout the stride cycle except during swing phase when the animal reaches for new purchase of the substrate, thus accordingly the hypothesized roles of the BB, BCR, and TBLO likely remain unchanged for vertical climbing.

One difference between SW and VC, however, appears to be the position of the carpal joint. While early and prolonged activation of FDP is consistent to grasp the substrate, video recordings from a frontal view do not allow for estimation of how much carpal flexion occurs as sloths ascended the fenced enclosure. Delayed EMG onset and low burst intensities of the PL suggest the carpal joint may remain relatively extended during VC, although the findings for PL during VC are tenuous, especially those for burst intensity. It is also recognized that allowing individuals to climb a natural substrate would

have resulted in a forefoot position more similar to that during SW as was previously observed in Bradypus (Mendel, 1985a). Under these conditions, the PL should exhibit an early onset and prolonged activation as it does during SW. The EDC is recruited to extend the carpal joint as the elbow extends and the forefoot prepares to lift off the substrate as expected. TBLO and EDC also purportedly act to extend the elbow and carpus-digits, respectively, as the forelimb is initially protracted. Moreover, these two extensors were relatively more intensely activated compared to the six flexors and this was unexpected. It is speculated that because sloths are in an upright posture when climbing, their extensors play a larger role in resisting gravity than their flexors.

EMG Intensity and Energy Savings

The prediction that EMG burst intensity would be least for suspensory hanging was supported. However, because EMG intensity of the forelimb muscles peaked during suspensory walking, the prediction for vertical climbing could not be verified. Two aspects of sloth locomotor mechanics may explain these findings. First, greater levels of muscle activation during SW may be related to the application and maintenance of greater horizontal and medial substrate reaction forces (SRF) by the forelimbs compared to those of the hindlimbs, thus the forelimbs are primarily used for propulsion during suspensory locomotion while the hindlimbs of sloths are the braking appendages (Granatosky and Schmitt, 2017). Suspensory postures may require that muscles mainly counteract moments caused by vertical SRF. Second, the body weight has been shown to be equally distributed between fore- and hindlimb pairs during SW (Granatosky et al., 2108). Moving in a vertical orientation should place relatively greater demands on the hindlimbs versus the forelimbs for body weight support, and while comparable kinetics data are not yet available for vertical climbing behavior in sloths, it is suggested that muscle activation intensities were lower than those observed for SW because fore- and hindlimb couplets are involved in propulsion of the animal up the substrate. Similar studies involving EMG burst intensities of the hindlimb extensors would be a good test of this hypothesis. Moreover, it is of particular interest to study the functional roles of the hindlimb musculature of sloths during VC because climbing is an energetically expensive behavior in mammals (Cartmill, 1985) and EMG activation patterns of sloth muscles may be generally constrained, regardless of behavior, to offset metabolic cost. Such proposed

neuromuscular modification would match well with recent data indicating that sloth hindlimb muscles are notably slower contracting than their forelimb muscles (Spainhower et al., in prep).

Large activations of the two elbow flexors contributed to the significance in normalized EMG burst intensity during suspensory walking. Even without an EMG recording value of maximum voluntary contraction to normalize burst intensity, relative mean values greater than 0.70 for the BB and BCR further emphasize the importance of elbow joint for suspensory function in sloths. Sustained elbow flexion by strong activations of BB and BCR can mitigate tensile loading at the shoulder joint during weight-bearing as previously suggested (Nyakatura et al., 2010; Fujiwara et al., 2011; Granatosky et al., 2108). This could be particularly important for Bradypus, which has a highly mobile pectoral girdle (Nyakatura and Fischer, 2010a; Olson et al. 2018). Specifically, the m. brachioradialis has the ability to apply substantial flexor torque related to both its large physiological cross-sectional area (PCSA) and muscle moment arm at the elbow joint (Olson et al. 2018). However, large, sustained EMG bursts of elbow flexors may not be expected to contribute to energy conservation in sloths. This potential inconsistency may be reconciled with abundant expression of slow myosin heavy chain (MHC) isoform fibers in the proximal flexor muscles of *B. variegatus*. The BCR is specialized by a near homogenous distribution of large, slow-contracting MHC-1 fibers (Spainhower et al., 2018b).

Among the forelimb flexors, the lowest volumes of active muscle are recruited during suspensory hanging, but perhaps more informative, was the finding that distal flexors consistently showed the lowest levels of activation among all muscles and functional behaviors analyzed. These data potentially reveal that the structure-function of sloth digital flexors is somewhat similar to the suspensory apparatus of upright ungulates (Hildebrand, 1960; Butcher et al., 2007, 2010). For instance, the digital flexor complex is the most massive intrinsic muscle group in the forelimb of *B. variegatus* and each muscle belly has relatively short, pennate fascicles and appreciable PCSA (Olson et al., 2018). Additional evidence indicates that the m. flexor digitorum profundus may have appreciable tendinous fibers throughout its cross-section (Spainhower et al. 2018a), which suggests that active contractile muscle mass could be exchanged for enhanced

intramuscular tendon to maintain suspension by a strong grip on the substrate without increasing the cost of force production. By this mechanism, the FDP would produce enough force to counterbalance tensile loading of tendons to sustain flexion of the digits, all while requiring low volume of muscle activity. EMG recordings from the digital flexors and forelimbs extensors of horses support this hypothesis by showing minimal activation during standing (Jansen et al., 1992; Harrison et al., 2012).

However, unlike ungulates, which have long, thin suspensory tendons, the digital flexor tendons of sloths are robust (Olson et al., 2018), but the distal skeletal elements of the manus in both Bradypus (Mendel, 1985a) and Choloepus (Mendel, 1981a, b) are elongated and the digits have long, re-curved claws. Moreover, the combination of a hook-like confirmation to the manus and robust tendon (volar) tunnels have been argued to be convergent features for suspensory habits in sloths, whereby their body weight can be largely supported by passive tension on the flexor tendons (Mendel, 1985a). Support for this functional interpretation depends on the relative stiffness/compliance of the tendons, and while the material properties of the flexor tendons in sloths are unknown, it is speculated that the morphological modifications of the tendons may allow fascicles of the m. flexor digitorum profundus to undergo minimal length changes during tensile loading of the MTU. The muscle activation patterns observed for the FDP in *B. variegatus* match this expectation and conceivably lower metabolic energy expenditure.

Neuromuscular Recruitment Patterns

The activation parameters of the distal flexors further inform the potential recruitment patterns of slow and fast MU in sloth forelimbs. Wavelet analysis demonstrated that despite low levels of normalized EMG burst intensity, the collective low and high activation frequencies of the forelimb musculature sampled were overall the highest during SH compared to SW and VC. These results parallel the slow-to-fast gradient in myosin fiber type expression observed along the forelimbs of Bradypus, which are composed of only slow MHC-1 and fast MHC-2A fibers (Spainhower et al., 2018b). Specifically, high overall activation frequencies of FDP and PL are consistent with their large distributions (54–65%) of fast MHC-2A fibers, and although fiber type % was not quantified for EDC, it is expected to consist of a majority of fast MHC-2A fibers based on its activation frequency properties.

It is possible that selective activation of fast MHC-2A fibers, which are smaller than slow MHC-1 fibers in sloth muscles (Spainhower et al., 2018a), provides adequate levels of force to counterbalance tensile loading of the flexor tendons. Moreover, MU recruitment in sloth muscles may be modified in both size and number whereby smaller, faster-contracting fibers may be organized into smaller size MU (fewer fibers) that are initially recruited when lower levels of muscle force are needed to support the body weight during suspensory hanging. While this supposition may sound counterintuitive because MHC-1 fibers are most often reported to be capable of sustained force production, are fatigue resistant, and hydrolyze ATP more slowly to decrease the energy cost of force production compared to faster fiber types (Schiaffino and Reggiani, 2011), slow MHC-1 and fast MHC-2A fibers in sloth forelimb muscles equally rely on phosphagen energy systems to power muscle contraction (Spainhower et al., 2018b). This finding was interpreted as a means to save energy because cheap and rapid resynthesis of ATP is more than adequate to keep pace with the slow contractile demands of suspensory posture and locomotion. Thus, the expression of abundant MHC-2A and selective recruitment of smaller, faster MU in the distal flexors agrees with their consistently low EMG burst intensities across the three functional behaviors, and further supports the hypothesis that sloth forelimb muscles are specialized for metabolic energy conservation. Thus, low volumes of active muscle (i.e., active force) are needed to support the weight of the animal, placing greater emphasis on passive support properties of the distal muscle-tendon units as a whole.

The proposed pattern of MU recruitment in sloths also does well to explain the activation properties observed among all muscles and behaviors. Lower magnitudes of muscle force should be needed to stabilize the shoulder, elbow, and carpal/digital joints during SH. Overall, the high activation frequencies for the limb flexors indicate that adequate levels of force may be provided by recruitment of smaller MHC-2A fibers from both the proximal and distal musculature. The findings for activations of fast motor units then match the major functional role of the limb flexors in joint stabilization and support. However, the flexor muscles showed variable levels of activation and frequencies of recruitment of slow vs. fast MU during locomotor behaviors. For example, the BB showed modest but progressively increased activation frequencies from SH to SW to VC.

Activation intensity of BB was greatest during SW, which implies the need for larger force to facilitate flexion of the elbow during locomotion. Greater levels of force in BB again appear to be achieved by selective recruitment of smaller, faster contracting MU, which may offset any additional metabolic energy cost of recruiting greater numbers of larger, slow motor units to more strongly flex the elbow joint. Thus, the BB likely plays a progressively more important role in elbow joint stabilization during SW and VC, whereas the nearly homogeneous slow-contracting BCR (96% slow MHC-1; Spainhower et al. 2018b) is most capable of actively flexing via application of large flexor torque at the elbow for propulsion during contact phase of SW and VC. Not only is the BCR specialized by its slow MHC fiber type composition, but by having long, slow-contracting fibers with large CSA emphasizes contractile force while minimizing shortening velocity. These contractile features are generally reflective of sloth forelimbs and promote energy savings during locomotion.

Evaluation of activation properties of the BCR may also be particularly useful for resolving the lower and upper limits of for slow and fast MU recruitment frequencies. While the data presented in Table 8 reflect the influence of muscle activations for a specific functional behavior, it may be extrapolated that the upper bounds for slow MU recruitment in sloth muscles is approximately 200 Hz. In addition to BCR, recruitment of potentially larger, slow motor units for enhanced force production also reflects low average frequencies of activation in the proximal flexors PS and DELT. These muscles collectively showed the lowest average activation frequencies (82–95 Hz) for slow MU in *B. variegatus*. Since this is the first study of its kind to analyze EMG and neuromuscular recruitment properties in sloths, comparable data of other mammals capable of suspension or clinging behaviors are not available. However, several studies of terrestrial locomotion (Wakeling et al., 2001; Hodson-Tole and Wakeling, 2007; Lee et al., 2013) that have used wavelet analysis on EMG activations from limb muscles in mammals that co-express MHC-1 and 2A fibers indicate frequencies of activation for slow MU for limb extensors (e.g., m. gastrocnemius) that generally range from 120–200 Hz. The lower end frequencies of recruitment observed in BCR, PS, and DELT may confirm an unusually low maximum contractile velocity (V_{max}) suspected for slow MHC-1 fibers in sloth muscle tissue that does not closely follow body size scaling relationships for a reduction

in V_{max} (Pellegrino et al., 2003; Toniolo et al., 2007). Moreover, sacrificing contractile velocity for enhanced force output matches the most common pattern of the lowest frequencies of activation but significantly larger levels of EMG burst intensity during SW. Selectively recruiting larger, slow MU in the forelimb flexors appears to be necessary for propulsion during suspensory locomotion. Importantly, this feature of sloth locomotion still provides advantages for economical force production by the large volume of active muscle recruited (but at relatively low activation frequencies) for force production needed to pull the center of mass (CoM) forward by the forelimb flexors. The overall greater activation frequencies ranges for the forelimb muscles during VC also may be expected because of shared roles in propulsion by the hindlimbs and possibly less support by the forelimbs. This feature would allow less active muscle to be recruited and at higher activation frequencies because selective activation of fewer larger, slow MU.

Finally, the PCA loadings and eigenvector results (Fig. 10) were somewhat unexpected, and yet help to clarify use of selective or preferential recruitment of MU (Wakeling, 2004) in sloth muscles at its association with metabolic energy savings. The overall higher myoelectric frequencies and proportion of faster MHC-2A fibers recruited for suspensory hanging may be a fundamental example of how the neuromuscular system in sloths is tuned to economize force production by recruiting the most appropriate MU required for specific contractile tasks. By doing so, neuromuscular recruitment patterns in sloth forelimbs do not strictly follow the size principle (Henneman et al., 1974) and this helps sloths reduce the overall metabolic energy expenditure, similar to that suggested for the hindlimb muscles in other vertebrate taxa, including cats (Smith et al., 1980; Fowler et al., 1988) and humans (Wakeling, 2004) during terrestrial locomotion, as well as axial muscles in fish (Jayne and Lauder, 1994) during swimming. Therefore, despite higher myoelectric frequencies and faster fiber recruitment, the volume of active muscle recruited during SH is significantly lower than both SW or VC and this may critical to how Bradypus offsets the cost of flexor activation for limb joint stabilization during suspension. The species *B. variegetus* is well known to spend less of its active time in suspensory postures and suspensory locomotion than Choloepus (Urbani and Bosque, 2007; Granatosky et al. 2018), and the form of neuromuscular modification described may be even more specialized in two-toed sloths. Primates that perform facultative

suspensory behaviors may be expected to have evolved similar features in their limb muscles. Lastly, it was also informative to observe the similarity in motor unit recruitment patterns between SW and VC. When moving horizontal below a substrate or vertically up a substrate, sloth muscles appear to largely have similar muscle performance requirements. While velocity may be marginally related to the differences observed in EMG activation intensity between SW and VC, sloths seem to adaptively constrain their speed of movement related to both predator evasion and energy conservation. Nevertheless, selective MU recruitment during suspensory walking and vertical climbing in sloths is similar in conception, but may be achieved by slightly different muscle performance for the implied levels of force production needed for propulsion while in a suspended orientation.

New Insights into Locomotor Mechanics in Sloths

Previous models of suspensory locomotion predicted that sloths do not utilize pendular mechanics that dictate efficient exchanges between gravitational potential energy with horizontal kinetic energy (Nyakatura and Andrada, 2013). Pendulum-like oscillations of the CoM during suspensory walking would not be favorable for sloths because of the extra energy required to stabilize a moving substrate, as well as it would not allow them to remain unnoticed in the wild or react quickly enough if the substrate were to fracture. While upright quadrupeds may take advantage of some pendulum-like energy exchanges during walking, differences relating to suspensory locomotor dynamics dictate alterations in both the magnitude and timing of propulsive/braking impulses and the limb pairs that apply them to the substrate. Recent force pole data from *Ch. didactlyus* (Granatosky and Schmitt, 2017) verify the predictions of Nyakatura and Andrada (2013) and indicate that sloth forelimbs apply greater propulsive force to the substrate over the entire contact phase while the hindlimbs apply moderate braking force. In addition, two-toed sloths evenly distributed their body weight between fore- and hindlimb pairs during suspensory walking, yet the mechanism by which they maintain a constant horizontal position of their CoM is not well understood. Although, Choloepus and Bradypus demonstrate nuances in frequency of behaviors (Urbani and Bosque, 2007; Granatosky et al., 2018) and ecological preferences (Montgomery & Sunquist, 1978; Mendel, 1981a, 1985a; Hayssen 2010, 2011), broad evolutionary convergence (Nyakatura, 2012) for suspensory

habits evidenced by numerous shared morphological and physiological traits between the two genera should be reflected in similarity of locomotor mechanics. Thus, muscle activation data for *B. variegatus* may help to reconcile the previous findings.

Using preliminary data from this study (Gorvet et al., 2018), Granatosky et al. (2018) hypothesized that delayed EMG activation of the PS acting as a limb protractor during contact could be timed to co-contract with an ipsilateral hindlimb flexor to decelerate the CoM and prevent the substrate from oscillating. However, while it is reasonable to suggest how activation of protractor would act as an antagonist to propulsion and possibly control body position, we disagree that PS functions in protraction of the forelimb. Misinterpretation of the action of PS is likely related to its previous designation as m. pectoralis superficialis anterior, which is also grouped as a limb retractor (Olson et al. 2018). Moreover, it could not be verified that fine-wire electrodes were placed exactly in the anterior head of the m. pectoralis and EMG recordings are generally that of this shoulder flexor complex, but with no swing phase activation. Nonetheless, we agree that PS is a muscle most likely used to stabilize the shoulder joint as would be expected of a predominantly slow-contracting, fatigue resistance belly (Spainhower et al. 2018), and its slow MU recruitment properties discovered here support the hypothesis of Granatosky et al. (2018). Thus, the m. latissumus dorsi should be the main retractor of the forelimb during SW as it has a muscle moment arm nearly twice as long that for PS and is capable two times greater flexor joint torque application (Olson et al. 2018). The MHC fiber type compositions of the well-developed m. sartorious and m. adductor (all heads: Spainhower et al. unpublished data) are comparable to the those of the large PS (both heads), and we further speculate that co-activation of the slow MU from either or both these muscles in coordination with the PS may be the means by which sloths balance their body weight distribution during SW. Individuals of Bradypus in this study were often observed to the bring the body (abdomen) closer to the beam in advance of propulsion. The complimentary pairing of muscles with an ability to adduct the limbs may also counteract any abductor moments generated at the shoulder and hip joints.

The greatest overall torques (~1 Nm/kg) are applied at the thoraco-scapular articulation (i.e., articulation between scapula and thorax) and shoulder joint when the forelimb touches down during suspensory walking (Nyakatura and Andrada, 2013). This

keeps the CoM from being accelerated forward initially and allows the scapula and humerus to be retracted to propel the body forward later into contact. EMG activation patterns of PS (and DELT) match the timing of these events and its activity in part, would allow the body to progress in a slow, controlled, and highly uniform manner. For example, specifically co-activation of the slow MU of the m. sartorius may be recruited to brake forward motion of the body via lengthening contractions. This mechanism of activation of opposable forelimb and hindlimb flexor muscles may serve to make the body axis rigid, and also contribute to the slow, controlled movement patterns of sloths. Future EMG recordings from sloth hindlimb muscles aim to test the possibilities of co-contraction in fore- and hindlimb muscle pairs as a mechanical strategy for suspensory habits. Finally, Granatosky et al. (2018) observed that two-toed sloths exert appreciable and constant medial force when in contact with the substrate during SW. Large activations of the elbow flexors in *B. variegatus* may explain this previous finding. In particular, long activations of slow MU in the extremely well-developed and slow-contracting BCR may be critical to mitigating levels of forelimb loading and help to relieve stresses on the shoulder joint by maintaining medially-directed force. This mechanical outcome of muscle activation could be more important for the shoulder joint of Bradypus that is modified for mobility rather than stability due to the lack of muscular attachments to the cervical and cranial regions (Miller, 1935; Olson et al. 2018).

Conclusions

Sloth forelimbs demonstrate an interesting division of labor by having a combination of shoulder flexor muscles that appear to be consistently activated to either stabilize the shoulder joint and/or propel animals either below or up the substrate. These proximal muscles are paired with strong elbow flexors to stabilize the body mass in suspension and mitigate levels of limb loading, and other specialized distal limb muscles that may be constrained to be minimally activated to maintain grip on the substrate regardless of behavior. Despite the requirements of broad-scale flexor muscle activations during suspensory locomotion, sloths appear to have adapted several neuromuscular means to reduce the metabolic cost of active muscle, including minimal EMG activation intensity for suspensory hanging and several specialized slow-contracting muscles with low frequency slow MU recruitment properties. Moreover, *B. variegatus* may selectively

recruit smaller, fast motor units at lower activation levels to support their body weight during suspensory hanging, but uses recruitment of larger, slow motor units during suspensory walking and vertical climbing to offset the cost of force production during locomotion. These findings provide the clearest verification of the hypothesis that sloth forelimb muscles reflect a physiological system specialized to conserve metabolic energy. Thus, this study has clarified several muscular requirements for suspension and informed other limb mechanical features necessary to maintain even support of the body weight in sloths. Future studies will address EMG activations in the hindlimb muscles to correlate the motor unit recruitment patterns with their role in braking, as well as tests of tensile strength and stiffness of both limb long bones and digital flexor tendons in sloths to verify hypotheses regarding passive versus active force contributions for suspension.

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APPENDIX

Excitation/Contraction Coupling and Electromyography

Skeletal muscle is contractile tissue that when recruited by the nervous system, performs mechanical work. Unlike cardiac and smooth muscle, skeletal muscles contract only when stimulated by motor neurons. Muscle excitation and contraction occur through the process of **excitation-contraction coupling** (ECC). Excitation refers to the electrical depolarization which leads to the contraction of muscle fibers. Briefly, the hierarchy of muscle tissue is as follows: muscle bellies are comprised of fascicles; fascicles are comprised of bundles of muscle fibers; muscle fibers are comprised of myofibrils; myofibrils are comprised of myofilaments; and the assemblage of myofilaments compose *sarcomeres*. Sarcomeres are the functional unit of muscle contraction (Lieber, 2002; Matthews, 2002; Hill et al., 2004). Two types of myofilaments exist: 1. thick filaments composed of the motor protein **myosin** and 2. thin filament composed of actin, tropomyosin (TM), and troponin (TN). Myosin is composed of isoforms of both light and heavy amino acid chains know as myosin light chain (MLC) and myosin heavy chain (MHC), respectively. MHC form projections, or myosin heads, that in turn form actin-myosin *cross-bridges* by their physical interaction, with actin filaments overlapping the myosin filaments to allow for muscle contraction. Therefore, when a muscle contracts, thick and thin filaments 'slide' past one another rather than actin filaments physically shortening (Hill et al., 2004).

Cross-bridges cycle through periods of attachment and detachment with the thin filament during muscle contraction. However, **force** is produced only when cross-bridges are attached and pivot. Briefly, when calcium (Ca^{2+}) is present, the myosin head binds to a molecule of adenosine triphosphate (ATP). The ATP binding site on the myosin head is an ATPase enzyme that hydrolyzes the terminal inorganic phosphate from ATP and stores the energy so it can be used to power the cross-bridge interaction with actin. Next, the myosin head binds to actin to form cross-bridges. The myosin head then releases the inorganic phosphate to initiate a *power stroke*, the process which translates (pulls) the thin filament inward towards the center of the sarcomere (M-line), resulting in an overall shortening of the sarcomere (Lieber, 2002; Matthews, 2002; Hill et al., 2004). Additionally, the thin filament contains several isoforms of regulatory proteins, namely

tropomyosin (TM) and troponin (TN), that prevent myosin from binding to actin in the absence of Ca^{2+}. TM covers the myosin binding sites on each actin molecule in the resting state. For myosin to bind to actin to form an A-M cross-bridge, Ca^{2+} must initially bind to TN. This causes a conformational change to TN, thereby moving TM to expose active binding sites on the actin filament so myosin heads can bind. Therefore, the mineral Ca^{2+} is a key regulator of ECC (Hill et al., 2004). Furthermore, the *sarcolemma* (i.e., muscle cell membrane) of each muscle contains transverse tubules, which are narrow invaginations of the sarcolemma that interact with the sarcoplasmic reticulum (SR), the storage site of Ca^{2+}, in the internal milieu of the muscle fiber (Andersson-Cedergren, 1959; Lieber, 2002; Matthews, 2002; Hill et al., 2004). This interaction occurs through ryanodine receptors – voltage sensing proteins – embedded in the SR that detect the action potential (AP) triggered by a wave of depolarization traveling down the transverse tubules (Lieber, 2002). At rest, Ca^{2+} is mostly confined in the SR (Schneider and Chandler, 1973). However, during ECC, a chain of events causes the release of Ca^{2+} from the SR to initiate the transformation of chemical energy to mechanical work. The following is an ordered summary of the steps of ECC that result in muscle fiber contraction:

1) When the nerve action potential arrives in the axon terminal voltage-gated Ca^{2+} channels open and Ca^{2+} enters the axon terminal. Acetylcholine (ACh) is released from the presynaptic axonal terminal, crosses the synaptic cleft, and binds to ligand-gated channels on the motor end-plate of the skeletal muscle fiber. Opening of the ion channels associated with the ACh receptor then allows sodium (Na^{+}) to enter the muscle cell (Flucher and Franzini-Armstrong, 1996; Berchtold et al., 2000; Matthews, 2002).
2) The nervous impulse is transmitted across the neuromuscular junction (i.e., complex structure composed of the presynaptic motor neuron, postsynaptic muscle cell membrane, and axon terminal) to the muscle fiber (Lieber, 2002).
3) AP is conducted along the fiber surface and then deep into the fiber via the transverse tubule system (Lieber, 2002; Matthews, 2002).

4) Depolarization of the dihydropyridine receptors (i.e., voltage-gated receptors embedded in the membrane of the sarcolemma) is triggered by the action potential moving down the transverse tubule system.
5) Stimulation of the dihydropyridine receptors activates the ryanodine receptors, which are ion channels in the SR, to trigger the release of Ca^{2+} from the SR (Flucher and Franzini-Armstrong, 1996; Franzini-Armstrong and Protasi, 1997; Lamb, 2000).
6) TN, which is attached to TM, binds the released Ca^{2} to cause a conformational change to TM (Berchtold et al., 2000).
7) The steric blocking of TM, which normally inhibits myosin from binding to actin, is removed (Berchtold et al., 2000; Hill et al., 2004).
8) Globular heads of myosin molecules, which have been energized by splitting a high-energy phosphate bond of ATP, are then free to bind to actin (Berchtold et al., 2000; Lieber, 2002; Hill et al., 2004).
9) The stored energy of the of activated myosin is released to initiate a power stroke, propelling the thick and thin filaments past each other and the spent ADP is released from myosin (Lieber, 2002; Matthews, 2002; Hill et al., 2004).
10) A new ATP binds to myosin, releasing its attachment to the actin molecule and the new ATP is hydrolyzed to re-energize myosin and cause the contraction to occur again (Berchtold et al., 2000; Lieber, 2002; Matthews, 2002; Hill et al., 2004).
11) Contraction will be maintained as long as internal Ca^{2+} concentration is elevated. The calcium concentration falls as calcium ions are taken back into the SR via an ATP-dependent calcium pump (Lieber, 2002).
12) Ca^{2+} is pumped back into the sarcoplasmic reticulum when electrical impulses cease (Berchtold et al., 2000; Lieber, 2002; Hill et al., 2004).
13) Cross-bridges relax due to lack of Ca^{2+} filament activation (Lieber, 2002; Hill et al., 2004).

Skeletal muscles are innervated by motor neuron. A motor neuron and the set of muscle fibers that it innervates form a **motor unit** (Hill et al., 2004). Each fiber within a motor unit generates an AP that consequently produces gross muscle contraction (Gans, 1992).

When muscle fibers are activated to contract they produce **muscle tension**, which is the aggregate of the twitch force produced by each fiber comprising the motor unit. Moreover, since muscle activation/contraction is an all-or-none response, each fiber in the motor unit is activated at the same threshold of depolarization and produces the same magnitude of force.

The nervous system controls muscle tension by two different means: neuromuscular recruitment and temporal summation. An increase in the number and size of the motor units activated is known as **neuromuscular recruitment** (Henneman, 1957). Progressive recruitment of motor units is the primary means of controlling the amount of tension produced by skeletal muscles. That is, as more (or larger) motor units are recruited, the tension developed by the whole muscle increases. Differing the number of active motor units and the timing of their activation results in smooth and distinct movements (Hill et al., 2004). Notably, Henneman (1957) proposed the *size principle*, which is the foundation of our current understanding and application of motor-unit recruitment patterns (e.g., Henneman et al., 1965a, b, 1974; Hodson-Tole and Wakeling, 2008). Motor unit size varies by the numbers of muscle fibers innervated, where smaller motor units (e.g., 10-100 fibers) tend to contain slow-twitch fibers, while larger motor units (e.g., 500-1000 fibers) are often composed of fast-twitch fibers (McPhedran et al., 1965a, b). Contractile (twitch) velocity of muscle fibers are also matched with the AP conduction velocities of their motor neurons. For example, slow-twitch fibers are innervated by smaller, less myelinated motor neurons, which have slower conduction velocities. The converse is observed for fast-twitch muscle fibers and larger motor units (Bagust, 1974; Brooks and Faulkner, 1988). The *size principle* dictates that smaller, slower motor units will be recruited first, followed by the activation of progressively larger, faster motor units when the demand for mechanical work and power is higher (Henneman et al., 1965a, b). Henneman and Olson (1965) proposed that this order of recruitment ensures that the slowest, most fatigue resistant motor units are recruited first before employing the faster, more fatigable motor units. By this recruitment mechanism, animals save energy by exactly matching the mechanical power requirements for a given behavior (Rome et al., 1990). Lastly, muscle tension can be controlled through *temporal summation* of APs that can lead to maximal stimulation of muscle fibers known as

tetanus or tetanic force. Therefore, tension produced per motor unit varies with the frequency of APs communicated by the motor neuron (Hill et al., 2004).

Whole muscles produce force by either of three types of contractile activity: *lengthening, isometric,* or *shortening* contractions. The mechanics of these three types of contractions have been extensively studied (e.g., Burke et al., 1978; Brooks and Faulkner, 1994; Beltman et al., 2004) and will not be discussed. Instead the physiology of each contraction, namely the volume of muscle activated and energy cost per contraction, is germane to this review. Briefly, lengthening (eccentric) contractions produce the greatest amount of force, perform negative mechanical work (i.e., absorb power), and consume the least amount of ATP. Isometric (same length) contractions produce large force (F_{max}), perform no mechanical work, and consume moderate ATP per unit force. Shortening (concentric) contractions produce the least amount of force, perform mechanical work and power, but consume the greatest amount of ATP per unit of force production (Abbott et al., 1952; Beltman et al., 2004). For each type of contraction, the nervous system must recruit an appropriate number and size of motor units, realized as a volume of active muscle, to meet locomotor demands for force and power. Behaviors that demand higher mechanical work and power require a larger volume of active muscle to be recruited (Roberts et al., 1997). This is most easily understood by application of the **force-velocity relationship** of muscles during movement. For example, animals must always maintain enough force to support their body weight, and behaviors that demand higher power (e.g., vertical climbing) additionally require that muscles undergo strong shortening contractions (force × velocity = power). Greater shortening limits active muscle force production due to the trade-off between shortening velocity and force, therefore a greater volume of active muscle must be recruited to maintain force levels for support. Moreover, when limb muscles are generating peak power they are capable of producing only 0.3–0.5 of their maximum isometric force (Hill, 1938). In sum, contractions that perform work and power are energetically costly and for this reason, it is important that *muscle activation* be evaluated when relating the contributions of total muscle force to whole animal metabolic energy consumption.

Electromyography (EMG) is a well-established technique used to directly measure muscle activation (i.e., muscle AP) and assess recruitment of motor units in muscles

during locomotor behaviors (Gans, 1992; Wakeling, 2009). For example, EMG from live animals has historically been one of the least invasive and most fundamental ways to study patterns of muscle activity, which is a key to whole muscle function (Wakeling et al., 2001; Hodson-Tole and Wakeling, 2007; Wakeling et al., 2012; Lee et al., 2013; Fuchs et al., 2015). EMG can be achieved by percutaneous implantation of fire-wire electrodes into limb muscles of live animals. Fine-wire EMG is very useful for studying isometric contractions when there is a stable relationship between the electrode position and the motor units (DeLuca, 1997). It is also beneficial for studying muscle recruitment patterns during dynamic movements (Wakeling et al., 2012). This technique provides data about the relative timing and magnitude of motor unit recruitment patterns by onset-offset timing and intensity of muscle activation (Gans, 1992). In particular, the intensity (amplitude) of an EMG burst communicates the relative volume of active muscle recruited, which reflects the metabolic energy cost of producing force (Roberts et al., 1997). Therefore, EMG analysis can help identify several important features of the musculoskeletal system and how they affect the energetics (and mechanics) of animal locomotion. However, to date, no EMG data are available for any animals during suspensory modes of locomotion.

For the past century, the use of EMG has greatly increased to understand neuromuscular recruitment mechanisms, motor and postural control, and muscle physiology in the field of comparative biomechanics. EMG recordings are central to our knowledge of recruitment patterns of flexors/extensors during the gait cycle of quadrupeds. For example, Harrison et al. (2012) studied muscle activation in horses during different phases of locomotion. They discovered that during **stance phase** (i.e., when the limb is in contact with the ground), all distal forelimb muscles were active except the *m. extensor carpi radialis* (ECR). The onset of activation of these muscles occurred just prior to *toe-down* (footfall) or immediately thereafter. This latter event is known as **pre-activation**, and it is common in mammals during walking and running gaits to 'tense' muscles before the limb accepts the entire body weight of the animal. The same study also found that the common and lateral digital extensor muscles were intermittently active during **swing phase** (i.e., when the limb is off the ground and being

recycled forward), peaking in their activity near footfall, while the ECR was active during all of swing phase (Harrison et al., 2012).

Another study used EMG to study muscle recruitment during locomotion in rats (Cohen and Gans, 1975) and determined that during walking, the *mm. biceps brachii* and *brachialis* are activated at *toe-off* when the forelimb is lifted off the ground. In contrast, the long head of the *m. triceps brachii* is activated upon toe-down followed by activation of the medial head of the *m*. *triceps brachii*. This toe-down portion of the gait cycle immediately following footfall is known as **yield** and it is when the major limb extensors are active in resisting joint flexion as the limb is being loaded. The lateral head of the *m. triceps brachii* displays two types of activity patterns: it is pre-activated at low levels just before toe-down and a much larger volume of muscle is recruited after footfall.

Lastly, Anapol and Jungers (1987) studied activation in the quadriceps femoris in the brown lemur during walking, running, galloping, and leaping, and discovered that the *m. vastus intermedius* exhibits consistently high levels of EMG activity during all forms of locomotion, whereas the *m. vastus medialis* is recruited the least during walking and running, but it is highly active during galloping and leaping. The *mm. rectus femoris* and *vastus lateralis* are recruited at similarly high levels of EMG activity during all forms of locomotion (Anapol and Jungers, 1987). These studies, among many others, demonstrate that EMG has been used to study muscle activity in both a phylogenetic and function range of vertebrae taxa during different types and stages of locomotion.

Evolution and Speciation

Tree sloths belong to the superorder Xenarthra, which also includes armadillos and anteaters (Gardner, 2008). The two forms of sloths, commonly known as two-toed and three-toed sloths, belong to the order Pilosa and suborder Folivora (Patterson and Pascual, 1968; Gardner, 2005). The two families of sloths, Megalonychidae (two-toed sloths) and Bradypodidae (three-toed sloths), together contain six extant species (Höss, 1996; Greenwood et al., 2001; Gaudin, 2004). There are two species of two-toed sloths (Genus: Choloepus) named *Choloepus hoffmannii* and *Choloepus didactylus,* and four species of three-toed sloths (Genus: Bradypus) that include *Bradypus variegatus, Bradypus tridactylus, Bradypus torquatus,* and *Bradypus pygmaeus*. *Choloepus* is believed to be either the sister group of extant tree sloths or is related to the extinct

Mylodont ground sloths; however, *Bradypus* is more closely related to Megatheriid Mylodont lineage and diverged from ground sloths about 40 million years ago (Höss, 1996; Greenwood et al., 2001; Gaudin, 2004). Additionally, the last common ancestor of extant tree sloths most likely led a terrestrial or semi-arboreal lifestyle (White, 1993), and an arboreal lifestyle has evolved at least twice among sloths (Höss, 1996). Despite ecological, morphological, and behavioral similarities, recent genetic evidence suggests that *Choloepus* and *Bradypus* are distant relatives and independently evolved their suspensory behaviors, making these unusual mammals one of the most remarkable examples of convergent evolution (Nyakatura, 2012). The focus of this study will be on two species: Hoffmann's two-toed sloth, *Choloepus hoffmanni* (Peters, 1858), and the brown-throated three-toed sloth, *Bradypus variegatus* (Schinz, 1825).

Xenarthrans share a number of distinct **morphological traits**: 1. All species have xenarthrous or 'extra' zygapophyseal joints (i.e., joints between superior and inferior articular processes of vertebrae; includes both a dorsal and ventral articulation in xenarthrans) in their lumbar vertebrate (lumbar vertebrae reduced in extant sloths) in order to provide more rigid stability in the trunk; 2. The transverse processes of anterior caudal vertebrae are fused to the pelvis; 3. The scapula contains a well-developed secondary spine (also reduced in extant sloths); and 4. Palatine vacuities may be present in the roof of the mouth (Engelmann, 1985). Additional features that xenarthrans may share include robust skeletons (Vizcaíno et al., 2008; Vizcaíno, 2009) and rudimentary teeth with long crowns, short roots, and reduced enamel (Gaudin and McDonald, 2008; Charles et al., 2013; Gaudin and Croft, 2015), or the absence of teeth in some species. Extant sloths lack incisors and true canines, and their teeth are found only in the maxilla (Pujos et al., 2012). Lastly, xenarthrans are also well known to share distinct **physiological traits**, including a lower basal metabolism, slow digestion, and lower body temperatures compared to other eutherian (i.e., placental) mammals (see details below). These physiological characteristics are coupled with the slow movement and locomotion observed in sloths, which also may be viewed as evolutionarily adaptive (Engelmann, 1985; McDonald and De Iuliis, 2008).

Ecology, Physiology, and Locomotor Behavior

Sloths are found in the humid neotropical rainforests of Central and South America (Britton, 1941). More specifically, *Choloepus hoffmanni* occurs primarily in two disjunct areas. The northern population ranges from Honduras through Nicaragua, Costa Rica, Panama, and into the northern regions and Venezuela, Columbia, and Ecuador. Meanwhile, the southern population ranges north-central Peru through southwestern Brazil to central Boliva (Wetzel and De Avila-Pires, 1980; McCarthy et al., 1999). *Bradypus variegatus* is distributed in Nicaragua, Honduras, Ecuador, Colombia, Venezuela, and further south into Brazil and northern Argentina. *C. hoffmanni* has a larger average home range of 1.97 hectares compared to the 1.59 hectares for *B. variegatus* (Montgomery and Sunquist, 1975). Though the distributions of these two species differ, they do overlap, causing them to be sympatric primarily in the northern areas of their ranges. (Hayssen, 2010).

Sloths have very low metabolic rates. They are high-canopy folivores whose diets consist primarily of leaves, which are of low nutritional value (Montgomery, 1983). However, correlating with the native vegetation within their home ranges, there is diversity between the diets of both species. While *B. variegatus* is largely restricted to two types of leaves, mainly from Cecropia trees, *C. hoffmanni* feeds on buds, flowers, fruits, and twig tips (Montgomery, 1983; Meritt, 1985). Moreover, *C. hoffmanni* forages on 34 different species of trees compared to only 15 species for *B. variegatus* (Vaughan et al., 2007). In addition, sloths have a very low daily food intake of 17 g dry weight kg^{-1} day^{-1} on average, which is most likely linked to their remarkably low metabolic rates (Cliffe et al., 2015). For example, *B. variegatus* has the lowest recorded field metabolic rate among non-hibernating mammals of 162 $kJ/day*kg^{0.734}$ (Pauli et al., 2016). Correspondingly, sloths have low body temperatures, averaging only 32.7°C (*Bradypus*) and 34.5°C (*Choloepus*) (Pauli et al., 2016). *B. variegatus* is observed to employ **behavioral thermoregulation** by bathing in sunlight for warmth at the top of canopies during the mornings and descending into shade to 'sit' in the recesses of tree branches as temperatures increase during the day (Montgomery and Sunquist, 1978). Also, adult *B. variegatus* has a smaller body mass of 3.2-6.1 kg while *C. hoffmanni* is larger, averaging 7.0–9.0 kg (Genoways and Timm, 2003; Gillespie, 2003). Body size differences between

species are then correlated with variation in body temperature and thermoregulatory strategies.

Though sloths spend the majority of their time in the canopy or emergent strata of the rainforest, they descend to the forest floor to defecate, albeit only once every 8 days on average, due to their low rate of digestion (Montgomery and Sunquist, 1975). *C. hoffmanni* has a head first descent down trees, while *B. variegatus* has a backward (tail first) descent. *C. hoffmanni* is hypothesized to descend head first because it has more threatening defense mechanisms (e.g., hissing, baring teeth, slashing foreclaws) than *B. variegatus*. In contrast, the backwards descent for *B. variegatus* may be associated with its ability to rotate the head 180° (see section: Morphology and Myology) to scan the surrounding area for predators (Enders 1935; Hill and Tenney, 1974; Montgomery and Sunquist, 1978; Mendel, 1985a). Sloths are preyed on by a variety of carnivorous animals, with harpy eagles and jaguars being their primary predators (Garla, et al., 2001; Touchton et al., 2002). Ocelots, margays, and anacondas are also known to prey on *C. hoffmanni* (Goffart, 1971; Moreno et al., 2006).

In terms of daily activity, both species display different patterns of wakefulness. *C. hoffmanni* is primarily nocturnal, being active about 11 hours/day during the night and sleeping during the day on vines in the canopies of trees (Montgomery et al., 1973; Montgomery and Sunquist, 1975). On the other hand, *B. variegatus* is diurnal, being active both during the day and at night while spending 15-18 hours/day sleeping (Sunquist and Montgomery, 1973; Janzen, 1983); they are most likely to be active between 1200-1600 hours (Urbani and Bosque, 2007). Additionally, *Bradypus* is reported to have several modes of sleep/wake cycles including (a) “awake-exploring” where the animal is alert, moving the head, and blinking frequently, (b) “awake-alert” where the head is up and eyes open, blinking occasionally, (c) “awake-fixating” where the head is up and eyes open, but the animal shows a tonic immobility, (d) “behavioral sleep” where the animal sits or hangs with one limb or pairs of limbs around a tree, its head down, and its eyes closed (Barratt, 1965). During a 24-hour period, *B. variegatus* spends about 17.5% awake-exploring, 10.6% awake-alert, 2.5% awake-fixating, and 69.4% in behavioral sleep (De Moura Filho, 1983). The activity patterns of both genera demonstrate that sloths are not highly active animals, thereby supporting the supposition

that their behavior, as well as their physiology, is highly modified in ways to conserve metabolic energy (Cliffe et al., in review).

During their active awake cycles, sloths exhibit unusual arboreal behaviors that allow for suspension of their entire body weight beneath tree branches with one or more limbs for extended periods of time (Hayssen, 2009, 2010). Despite these abilities, which require great strength, skeletal muscle mass comprises only 23.6% of total body mass for *B. variegatus* and 26.2–27.4% for *C. hoffmanni* (Grand, 1978) in comparison to other arboreal mammals that average a muscle mass of 33% (Muchlinski et al., 2012). Their average velocity of under branch (i.e., anti-pronograde) walking along a tree branch or vine is merely 0.14 m s^{-1} (Britton and Atkinson, 1938) compared to human who have a preferred walking speed of 1.3 m/s (). Thus, their locomotion in trees is slow and deliberate. Both *C. hoffmanni* and *B. variegatus* exhibit a **diagonal-sequence gait** during under branch walking and upright vertical climbing (i.e., orthograde) and make use of diagonal-couplets (Mendel, 1985a). A diagonal-sequence gait is defined by consecutive footfalls (or grabs onto a substrate) of contralateral (opposite-side) fore- and hindlimb pairs. A diagonal couplet is slightly different by having opposite fore- and hindfeet grab the substrate simultaneously (Mendel, 1985a; Gillis, 2004; Strasser et al., 2013). Diagonal-sequence gaits are most often employed by mammals that are adapted to above branch (i.e., pronograde) locomotion for stability on arboreal substrates because it prevents rolling off of braches that would occur by bearing the majority of their weight on one side of the body as during lateral-sequence gaits. However, it is noted that *Choloepus* (*C. didactylus*) prefers a **lateral-sequence gait** and will freely choose this walking gait when performing inverted walking on a motorized treadpole during laboratory experimental trials (Nyakatura et al., 2010, Nyakatura and Fischer, 2010, 2011; Nyakatura and Andrada, 2013).

During inverted walking, the elbow joints are flexed and humeri are somewhat abducted and medially rotated at the shoulder joint, while their mani are held perpendicular to the substrate during contact, and their carpal joints are relatively extended (~180º) (Mendel, 1981a, 1985; Nyakatura and Andrada, 2013). Nyakatura et al. (2010) found that on average, the stance phase (manus or pes is gripping the substrate) lasts twice as long as swing phase (limb is transitioning between steps) when two-toed

sloths (*Choloepus didactylus)* performed inverted walking on a motorized treadpole. At touchdown, the scapula is protracted and lies dorsally while the glenoid fossa faces cranially. This allows the shoulder joint to be oriented more dorsal relative to the sternum. The clavicle is rotated cranially and points dorsally and the shoulder joint is extended thus causing the humerus to be protracted. However, humeral retraction begins momentarily before touch down due to application of a flexor torque by the limb retractors. Additionally, the antebrachium is held nearly parallel to the frontal plane, while the brachium is approximately vertical. During the first half of the contact phase, the scapula remains approximately in the same position as during touchdown (i.e., there is substantial movement in the shoulder joint), as the humerus undergoes retraction. Then, during the second half of the contact phase, the predominant motion is scapular retraction, whereas the shoulder joint remains flexed approximately 80–90°. In order for the limb to lift off the substrate, retraction of the scapula positions the shoulder joint at the level of the sternum or more ventral relative to the sternum. The glenoid cavity then faces laterally at lift off and the clavicle also points laterally, forming an angle of approximately 90° to the sagittal plane (Nyakatura and Fischer, 2010, 2011). Furthermore, steady-state locomotion across a horizontal branch, or arboreal support, is quasi-static, meaning mechanical loading is time-dependent, but slow enough that inertial effects are neglected. This mode of locomotion allows sloths to actively decrease dynamic changes in the forces they exert on the supports, thereby decreasing the chances of a support breaking. Also, moving as slowly as possible has the advantage of helping sloths avoid the attention of potential predators (Nyakatura and Andrada, 2013).

Although sloths spend the vast majority of their time in trees, they are also able to perform quadrupedal terrestrial locomotion. *C. hoffmanni* exhibits a lateral-sequence gait while walking over ground, while *B. variegatus* maintains a diagonal-sequence gait. For a lateral-sequence gait, a hindlimb footfall is followed by a footfall of the ipsilateral (same-side) forelimb, and the center of mass (CoM) is maintained within a large triangle of support for stability. This is because there are at least three feet in contact with the ground throughout most of the walking stride. Though sloths can perform both arboreal and terrestrial locomotion, they do not demonstrate a running gait during either mode of

transport (Mendel, 1981a, 1985). However, sloths are quite able swimmers and have some ability to dig (Enders, 1935; Carvalho, 1960).

Functional Morphology and Myology

C. hoffmanni and *B. variegatus* have distinguishable morphological characteristics. Notably, *C. hoffmanni* has 5–6 cervical vertebrae, which limits rotation of the neck but allows hyperextension, while *B. variegatus* has 8–9 cervical vertebrae enabling them to rotate their heads up to 180° and look forward when walking under a branch (Mendel, 1985a). Also, *B. variegatus* has a tail and their forelimbs are longer than their hindlimbs. In contrast, *C. hoffmanni* lacks a tail and their forelimbs and hindlimbs are of equal length (Britton, 1941). There is also evidence indicating that *C. hoffmanni* prefers arboreal supports that are parallel to the ground, and, on average, they spend longer in suspension than Bradypus (Miller, 1935). However, in order to engage in inverted walking and support their bodies while suspended, both genera have considerably specialized limbs with several synapomorphies (i.e., shared similar features).

All sloths have highly modified feet. *B. variegatus* has three functional digits on the manus – digits II, III, and IV – that are partially fused. Metacarpals I and V are vestigial and splint-like appendages. The distal phalanges are greatly curved and have long, narrow claws, and their volar pads are covered with fur. *C. hoffmanni* has two functional digits – digits II and III. Metacarpals I and IV of the manus are vestigial, splint-like metacarpals and digit V is absent. Their distal phalanges also have long, curved claws, however, their volar pads are hairless and covered by thick glabrous skin instead. The long, narrow claws of both species act as hook-like appendages, helping them to perform their unusual modes of **suspensory locomotion** (Mendel, 1981a, b; Mendel, 1985a). Also, regardless of the number of digits remaining on their forefeet, all six species of sloths retain three functional digits on their hindfeet.

In addition to their specialized forefeet, sloths have modified pectoral/pelvic girdles and shoulder/hip joints to support their limbs being loaded in a **tension** rather than in compression as required in upright mammals. Specifically, the scapulae of both species are comparatively small, and the thorax is considerably reduced cranially, and it has a circular cross-section. This modification enables the shoulder girdle (and shoulder joint) to efficiently rotate from a dorsal- to more lateral-position relative to the thorax.

Moreover, a ligament of irregular fibrous connective tissue connects the clavicle to the sternum thus permitting virtually all degrees of freedom at the sternoclavicular articulation (Nyakatura and Fischer, 2010). Nyakatura and Andrada (2013) also suggest that the greatest overall torques occur at the thoraco-scapular articulation (i.e., articulation between scapula and thorax) and shoulder joint and when the forelimb touches down during inverted walking; a torque of nearly 1 Nm/kg acts on these joints. This keeps the center of mass (CoM) from being accelerated and as a result, the body progresses in a very controlled and highly uniform manner (Nyakatura and Andrada, 2013). Similar to primates that employ forelimb suspension (e.g., gibbons and several species of monkeys), sloths are thought to have elongated forelimbs with limb muscles that support the weight of the body at the elbow and shoulder joints passively by tensile loading of the elastic elements, thereby reducing the need for muscular recruitment during postural suspension (Preuschoft and Demes, 1984).

Previous EMG studies on forelimb muscles in arboreal non-human primates provide a basis to evaluate muscle recruitment in other arboreal animals such as tree sloths. As an example, one study of forelimbs in Rhesus monkeys (Courtine et al., 2005), which employ a diagonal-sequence gait on arboreal substrates, verified similar patterns of EMG activation in the *mm. triceps brachii* and *biceps brachii* between pronograde arboreal locomotion and over ground locomotion. For the digital flexors, the *m. flexor digitorum profundus* was moderately active during limb contact and the *m. flexor digitorum superficialis* displayed greater EMG activity during the swing phase than during the stance phase. In contrast, *m. extensor digitorum communis* exhibited a modest, short burst of EMG activity near footfall of the forelimb (Courtine et al., 2005). A second study evaluated muscle activity in the forelimb during reaching movements in squirrel and owl monkeys (Santucci et al., 2005), and reported that when the limb is protracted to move the manus toward a food item, the *mm. triceps brachii* and *deltoideus* showed the greatest muscle activation. The bursts of EMG activity in these muscles were paired with recruitment of the *mm. biceps brachii* and *extensor digitorum communis* indicating **muscle co-activation**. However, the inverse activation pattern occurred when the manus was moved towards the mouth (Santucci et al., 2005). A third study examined the scapular rotators in chimpanzees during vertical climbing (Larson et al., 1991) and found

that the caudal portion of the *m. serratus anterior* become activated during the end of limb contact, and it also showed large EMG activity throughout limb swing that terminated just before footfall. For the cranial portion of the *m. trapezius*, there were consistent activation patterns beginning in the late stance phase and continued throughout the swing phase, whereas there was just a small burst of EMG activity just before the end of the swing phase in the caudal portion of the muscle (Larson et al., 1991). While these studies examine active muscle force production with arboreal habits, muscles can also act passively to provide supporting force through **strain** (deformation-elongation) of their series of elastic elements and tendons.

Tendons attach muscles to bone and transmit the force produced by the muscle fibers to cause movement at a joint, and this constitutes a mechanical lever system (Hill et al., 2004). A **muscle-tendon unit** (MTU) contains both internal and external tendon. Internal tendon is referred to as aponeurotic tendon. Tendinous structures consist of a large number of parallel collagen fibers (Goslow et al., 1981). At resting length, these collagen fibers are crimped, but unfold when tensile force is applied that causes the passive elements to undergo elongation. Collagen has high tensile strength and tendons are tough structures that are highly resistant to *tensile stress*. Because of these material properties, MTUs are capable of undergoing *tensile strain* and producing force passively. Therefore, during cyclic loading events such as those involved in locomotor behavior, MTUs help to save metabolic energy by the tendinous elements resisting strain (and not the muscle fiber changing length) and storing **elastic potential energy**.

Distal limb muscles that have long, thin tendons also tend to have short, pennate muscle fibers (see Section: Muscle Fiber Type and Fiber Architecture) and function like biological springs to store and recover elastic potential energy (Alexander et al., 1984). This functional morphology is most often observed in mammals specialized for cursorial (running) habits. However, these features of the distal limb are not exclusive to running animals. For example, during postural behaviors (e.g., standing, quiescent suspension, etc.), MTUs undergo length changes with alterations in joint angles and tensile limb loading, although there may be little-to-no muscle activation and force is largely the result of **passive tension** (Lieber, 2002; Hodson-Tole et al., 2016). Thus, passive tension plays a role in providing resistive force to maintain joint position control in the absence

of muscle activity, and this has important physiological and evolutionary implications; active contractile tissue may be replaced with intramuscular tendon (i.e., collagen fibers) without increasing the cost of total force production (passive force + active force = total muscle force). These MTUs are stiff and have a **length-tension relationship** favoring peak force at shorter sarcomere lengths; a narrow range of active contractile length change before passive tension significantly enhances force production. Enhanced passive tension and minimal recruitment of motor units decreases metabolic energy expenditure. Tree sloths may be model organisms to study these types of muscle modifications, as they are capable of hanging suspended underneath tree branches by one or more limbs for extended periods of time.

a. Forelimb Musculature

The musculature of the antebrachium and intrinsic manus acts to keep the radiocarpal joint firmly extended and the digits constantly flexed. The antebrachium and manus musculature of both *C. hoffmanni* and *B. variegatus* includes flexor/extensor muscles that act to flex/extend the manus at the radiocarpal joint and flex/extend the digits (Table 8; Olson et al., 2017). The cranial antebrachium contains the *m. extensor carpi radialis* and *m. extensor carpi ulnaris* that both act to extend the carpal joint, as well as abduction and adduction of the carpal joint, respectively. The major digital extensor is the *m. extensor digitorum communis*, and its function is synergistic with the *m. extensor digitorum lateralis*. Both of these muscles also act strongly to extend the carpal joint. On the caudal aspect of the antebrachium, the *m. flexor carpi radialis* is a muscle with a tendon that runs superficial to the flexor retinaculum giving it a greater moment arm for flexing the carpal joint. The *m. flexor carpi ulnaris* may have two bellies, with the superficial one being a weak carpal flexor and the deep head being both a flexor and adductor of the manus. Additionally, the *m. palmaris longus* is a very powerful flexor known not only for flexing the carpal joint, but also for flexing the metacarpal joints and proximal interphalangeal joints (Mendel, 1985b). It has often been observed that the forelimb of sloths does not have a separate belly for *m. flexor digitorum superficialis*. Any appearance of this muscle is inseparable from the *m. palmaris longus*. The *m. flexor digitorum profundus* is a large and complex muscle with 4 heads that inserts onto the distal phalanges of the forefeet allowing for strong flexion of the metacarpophalangeal

and interphalangeal joints to conform the manus into a hook-like appendage structure (Nyakatura and Andrada, 2013). It is also the only muscle capable of flexing the distal interphalangeal joints (Mendel, 1985b). Lastly, the intrinsic hand musculature is usually very difficult to distinguish and varies between individuals. These muscles produce virtually no adduction, abduction, or circumduction of the digits. Reduction in intrinsic hand musculature is primarily due to the reduction, loss, and partial fusion of the digits. However, the interossei are well developed and act to flex the metacarpophalangeal joints and proximal interphalangeal joints, while the thin lumbrical muscles extend the distal interphalangeal joints (Mendel, 1985b).

The muscles of the brachium are less complex (i.e., fewer muscles and muscle heads) than those of the antebrachium, but they must be able to resist extension of the elbow joint to support the body weight during postural hanging (Table 8). The *m. biceps brachii* primarily flexes the antebrachium at the elbow joint and unusually, it shares a common insertion on the antebrachial fascia with *m. pectoralis superficialis posterior* and the acromial head of the *m. deltoideus* in *Choloepus* to participate in protraction of the limb during early swing phase (Miller, 1935; Nyakatura and Fischer, 2011). Strong flexion at the elbow joint is also the action of *mm. brachialis* and *brachioradialis*. In regards to the latter, the *m. brachioradialis* takes its origin proximal to the supracondylar ridge of the humerus and thus has a large flexor muscle moment arm at the elbow joint. The *m. triceps brachii* (only 3 heads in sloths) acts to extend the elbow and on *Choloepus* only, it fuses with the scapular head of the *m. deltoideus* to participate in limb retraction (Nyakatura and Fischer, 2011). The standard mammalian condition is observed in *Bradypus* (Olson et al., 2017).

Additionally, muscles of the shoulder region protract and retract the humerus (and scapula) as well as act to stabilize the humerus at the shoulder joint (Table 8; Nyakatura and Andrada, 2013; Olson et al., 2017). The dorsal extrinsic muscles of this region (e.g., *mm. trapezius* and *rhomboideus*) have reduced, or absent, cervical and cranial attachments in *B. variegatus,* but *C. hoffmanni* maintains these attachments. The arrangement of modified origin/insertions allows for a greater range of motion for the shoulder girdle of *B. variegatus*, and this could be related to the motion of swinging upward from a suspended to sitting position that is common in this species (Miller, 1935).

In contrast, retaining the cervical and cranial attachments of the dorsal extrinsic muscles may better distribute weight bearing to the axial skeleton in *C. hoffmanni* since they spend more time suspended beneath arboreal supports (Miller, 1935). The intrinsic shoulder muscles are primarily shoulder and elbow joint flexors that counteract gravity-induced extension when hanging (Mendel, 1985a). Two extrinsic (limb retractor-shoulder flexor) muscles, the *m. pectoralis superficialis* and *m. tensor fasciae antebrachii* (TFA), support the weight of the thorax during the first part of the contact phase during inverted walking. In addition, the *m. latissimus dorsi* is the overall strongest limb retractor, but it has a less advantageous moment arm for application of flexor torque during the first part of the contact phase (Nyakatura and Fischer, 2011). The *m. teres major* and TFA act primarily to counteract extension, thereby stabilizing the shoulder joint (Nyakatura and Fischer, 2011; Olson et al., 2017). Both of these muscles are another feature that may be indicative of functional convergence between *Choloepus* and *Bradypus* because although both muscles flex the shoulder joint, the *m. teres major* is more prominent than the *m. tensor fasciae antebrachii* in *Bradypus*, but the opposite is true for *Choloepus* (Nyakatura, 2012).

b. Muscle Fiber Type and Fiber Architecture

While the size and conduction velocity of alpha motor neurons that innervate skeletal muscles directly influence their composition of slow and fast muscle fiber types, specialization for functional behaviors (i.e., locomotor habits) have the ability to modulate muscle contractile properties. Specifically, the expression of MHC isoforms in muscle fibers reflects the functional specialization of muscles because they are the main determinants of the intrinsic properties of muscle fibers (Schiaffino and Reggiani, 1996). Mammals can express four **myosin fiber types** in the skeletal muscles that are ordered as follows by increasing contractile velocity: MHC-1, 2A, 2X, and 2B. MHC-1 fibers are slow contracting, produce low force and power, and are oxidative in their metabolism (Kohn et al., 2007, 2011). These fibers also have small cross-sectional area (CSA), which is proportional to force production ability (Alexander et al., 1981; Bottinelli, 2001). MHC-2A fibers are fast contracting, produce higher force and power than slow MHC-1 fibers, and are highly oxidative despite having some glycolytic enzymatic properties (Schiaffino and Reggiani, 1996, 2011). Muscle fibers expressing the 2A isoform typically

have larger CSA than MHC-1 fibers (Kohn et al., 2007). MHC-2X fibers are fast contracting, produce higher force and power than MHC-2A fibers, and are moderately oxidative-glycolytic in their fiber type metabolism. These fibers have larger CSA and fatigue more easily than MHC-2A fibers. Lastly, MHC-2B are fast contracting, produce the highest power, and are more glycolytic than oxidative in their metabolism (Kohn et al., 2007). Muscle fibers expressing the 2B isoform are often smaller in CSA than MHC-2X fibers, and have lower fatigue resistance. MHC-2B fibers are most typically expressed in limbs of rodents and lagomorphs (Schiaffino and Reggiani, 1996) because their higher rate of ATP hydrolysis, and thus fast contractile velocity, play an important role in thermogenesis in small mammals.

Classical studies of myosin ATPase activity indicate that sloth muscles are slow contracting, as evidenced by their low rate of ATP cycling and protracted contraction times (Bárány, 1967; Bárány et al., 1967). Preliminary data on MHC isoforms in *B. variegatus* and *C. hoffmanni* support these findings by showing a primary expression of slow MHC-1 and fast MHC-2A fibers in their forelimb musculature (Spainhower et al., 2018a). Notably, the CSA of slow MHC-1 fibers is found to be larger than that of fast MHC-2A fibers, and an appreciable amount of connective tissue is observed in the muscle cross-sections (Spainhower et al., 2018a). Studies involving biochemical enzymatic assays to evaluate the overall metabolic (oxidative vs. glycolytic) profiles of sloth limb muscles are ongoing (Spainhower and Butcher; unpublished data).

Muscle fiber architecture also reflects the functional specialization of muscles. Muscle architecture is defined as the orientation of muscle fibers relative to the axis of force production (Lieber, 2002). Skeletal muscles demonstrate several types of fiber architecture ranging from parallel-fibered to pennate-fibered. While parallel muscles have long fascicles approximating the length of the muscle belly from origin-to-insertion, pennate muscle have fascicles oriented at varying angles to the long axis of the muscle or internal tendon(s). There are four types of pennation observed in mammal skeletal muscles: *unipennate*, *bipennate*, *multipennate*, and *circumpennate* (Lieber, 2002). The details of each muscle form are not critical to this review; however, it is important to understand that muscle fibers become progressively shorter and are arranged at steeper angles as the degree of pennation increases. This mechanical design is advantageous

because it allows muscles to be small while enhancing their ability to produce high force. The disadvantage is that pennate-fibered muscles are limited in their ability to perform work and power. The opposite is true for parallel-fibered muscles (Lieber, 2002).

The fundamental *architectural properties* of muscles include: muscle moment arm length (r_m), muscle mass, belly length, fascicle (fiber) length, pennation angle, and physiological cross-sectional area (PCSA). These properties are used to provide estimates of the functional parameters, maximum isometric force (F_{max}) and shortening velocity (V_{max}), peak joint torque and rotation velocity, and instantaneous power output (W) (Lieber, 2002). Collectively, these properties along with myosin fiber type, determine the amount of force and power a muscle can produce and sustain; performance requirements for force, joint torque, and power will vary with the constraints of the habitat that a species occupies. Architectural properties are available for the forelimb muscles of *B. variegatus* (Olson et al., 2017). To summarize, beyond gross observations of rope-like muscles with long, thin morphology, sloths demonstrate a general proximal-to-distal increase in pennation, and the flexors of the forelimb are stronger than the extensors. Notably, muscles with the largest PCSA and ability to apply the greatest amount of joint torque are those that act to retract the limb (shoulder flexors) and the carpal/digital flexors in the antebrachium. No muscles of *B. variegatus* are found to have the capacity for high power or large joint torques, although numerous muscles of the forelimb have the ability to rotate the joints rapidly by their relatively short muscle moment arms and long fascicles (Olson et al., 2017). Not only do these unpublished findings help to identify key muscles to target for *in vivo* study of function, but they point to the relationship between evolution and functional behavior, understood as a **structure-function relationship**. The most important implication of this relationship implies minimizing the metabolic cost associated with a functional-locomotor behavior (Zhong et al., 2001).

Objectives and Hypotheses: Significance

This study tests functional hypotheses about motor unit recruitment and active *versus* passive force development in the forelimbs of tree sloths. While muscle activity has been sampled over a phylogenetic and functional range of vertebrate taxa, no *in vivo* data are available for sloths, much less for the limb muscles of mammals during suspensory

behaviors. *The objective of this study is to evaluate EMG muscle activation in the limbs of sloths to determine the volume of muscle recruitment required to support body weight during both postural suspension and inverted walking*. This objective will be accomplished by studying two species of tree sloths: Hoffman's two-toed sloth (*Choloepus hoffmanni*) and the brown-throated three-toed sloth (*Bradypus variegatus*). **It is hypothesized that the neuromuscular function of sloth muscles will reflect a system specialized to conserve metabolic energy**. Specifically, sloth limbs are expected to be evolutionarily modified in ways that active contractile muscle mass has been exchanged for enhanced intramuscular tendon to maintain suspension without increasing the cost of force production. Verification of this hypothesis would indicate sloths may rely more on the passive tension (via tensile loading of tendinous elements: collagen fibers) of their limb muscles to maintain inverted position, thus matching physiological predictions of metabolic energy savings in sloths (Cliffe et al., in review). The ability to minimize the metabolic cost of muscle force is an important factor for survival and is related to adaptations observed among species.

The expected outcomes are that muscle EMG activity (by volume of active muscle) of forelimb flexors/extensors will be quiescent during postural hanging and moderately intense during inverted walking. The realization of these broad predictions would support the following interpretations: (i) passive elements of the musculoskeletal system loaded in tension are responsible for maintaining inverted posture in a way that is energetically beneficial for sloths, in much the same way that large terrestrial mammals (e.g., horses) support their body weight standing upright; (ii) limb design is such that relatively little muscular contractile activity (i.e., active force) is needed to support the body weight in favor of mechanisms for higher passive support via stiffer muscle-tendon units and limb joint modifications; and (iii) use of a walking gait for its favorable energy mechanics (i.e., low muscular effort) is comparable between inverted and upright mammals. With respect to the latter, the collected data will be critical to provide insight into the evolution of suspensory locomotion, and as it is evaluated in two species of sloths that are distantly related. Thus, the results may further validate our understanding of how muscle physiological properties may be convergent in sloths.

The findings of this study also aim to significantly improve our understanding of motor unit recruitment mechanisms involved with suspensory behaviors, the nervous system's influence on motor unit properties, and the inter-relationships between muscle architectural properties, fiber type, and energy metabolism. Importantly, the outcomes will also improve our knowledge of the mechanical design requirements for limbs that evolved to experience primarily tensile *versus* compressive loading. Finally, the outcomes of this investigation will provide critical functional data to explain the variation in muscle fiber physiology and structure observed in sloths by ongoing studies (Spainhower and Butcher, unpublished data). These data can only be provided by direct investigation of muscle activity in sloth skeletal muscles.

Table 1. Morphometric data for study animals and number of trials and strides analyzed.

Sloth	Sex	Limb	Body Mass (kg)	*N* Trials	*N* Strides	*N* Bursts
BV1	M	L	4.2	6_{SH} 8_{SW} 11_{VC}	31_{SW} 30_{VC}	183
BV2	M	L	4.5	2_{SH} 9_{SW} 1_{VC}	21_{SW} 1_{VC}	98
BV3	F	L	4.6	6_{SH} 7_{SW} 3_{VC}	26_{SW} 4_{VC}	116
BV4	F	L	3.2	10_{SH} 10_{SW} 1_{VC}	22_{SW} 1_{VC}	115
BV5	F	L	3.5	1_{SH} 13_{SW} 12_{VC}	32_{SW} 20_{VC}	217
BV6	F	L	3.2	2_{SH} 9_{SW} 6_{VC}	25_{SW} 14_{VC}	127
mean±s.d.; **totals**			**3.9±0.6**	**27_{SH}** **56_{SW}** **34_{VC}**	**157_{SW}** **70_{VC}**	**856**

N = number
SH, suspensory hanging; SW, suspensory walking; VC, vertical climbing

Table 2. Individuals, muscles, and functional behaviors for which EMG was recorded and analyzed.

Sloth	Behavior	PS	DELT	BB	TBLO	BCR	PL	FDP	EDC
	SH	X	X	X	X	X	X	X	X
BV1	SW	X	X	X	X	X	X	X	X
	VC	X	X	X	X	X	--	X	X
	SH	X	X	X	X	--	--	--	--
BV2	SW	--	X	X	X	X	X	X	X
	VC	--	--	X	X	--	--	--	--
	SH	X	X	X	X	X	X	X	X
BV3	SW	X	X	X	X	X	X	X	--
	VC	X	X	X	X	--	--	--	--
	SH	X	X	X	X	X	X	X	X
BV4	SW	X	X	X	X	X	X	X	X
	VC	--	--	--	--	--	X†	--	--
	SH	--	--	--	--	X	X	X	X
BV5	SW	X	X	X	X	X	X	X	X
	VC	X	X	X	X	X	X	X	X
	SH	X	X	X	X	--	--	--	--
BV6	SW	X	X	X	X	X	X	X	X
	VC	--	--	--	--	X	X	X	X

†No intensity or wavelet data available
SH, suspensory hanging; SW, suspensory walking; VC, vertical climbing
PS, pectoralis superficialis; DELT, deltoideus; BB, biceps brachii; TBLO, triceps brachii long head; BCR, brachioradialis; PL, palmaris longus; FDP, flexor digitorum profundus; EDC, extensor digitorum communis

Table 3. Stride parameters for suspensory walking and vertical climbing in *B. variegatus*.

Sloth	Stride Duration (s)	Duty factor (ratio)	Velocity ($m\ s^{-1}$)
BV1	$11.0\pm2.85_{sw}$ $15.5\pm6.71_{vc}$	$0.82\pm0.07_{sw}$ $0.83\pm0.05_{vc}$	$0.08\pm0.01_{sw}$ $0.05\pm0.01_{vc}$
BV2	$12.5\pm4.38_{sw}$ 15.6_{VC}	$0.76\pm0.08_{sw}$ 0.91_{vc}	$0.1\pm0.02_{sw}$ $0.04\pm0.01_{vc}$
BV3	$29.1\pm13.3_{sw}$ $26.5\pm8.95_{vc}$	$0.84\pm0.06_{sw}$ $0.83\pm0.05_{vc}$	$0.04\pm0.01_{sw}$ $0.02\pm0.01_{vc}$
BV4	$40.4\pm22.3_{sw}$ 46.60_{vc}	$0.83\pm0.11_{sw}$ 0.90_{vc}	$0.03\pm0.01_{sw}$ $0.01\pm0.02_{vc}$
BV5	$20.0\pm11.6_{sw}$ $15.0\pm3.78_{vc}$	$0.86\pm0.07_{sw}$ $0.83\pm0.04_{vc}$	$0.05\pm0.01_{sw}$ $0.02\pm0.004_{vc}$
BV6	$14.8\pm7.08_{sw}$ $11.5\pm3.52_{vc}$	$0.85\pm0.05_{sw}$ $0.83\pm0.06_{vc}$	$0.05\pm0.01_{sw}$ $0.03\pm0.01_{vc}$
mean±s.d.	**$21.3\pm28.3_{sw}$** **$18.7\pm13.2_{vc}$**	**$0.83\pm0.04_{sw}$** **$0.86\pm0.04_{vc}$**	**$0.06\pm0.03_{sw}$** **$0.03\pm0.01_{vc}$**

SW, suspensory walking; VC, vertical climbing

Table 4. Temporal variables of EMG activation of forelimb muscles in *B. variegatus*.

	PS	DELT	BB	TBLO	BCR	PL	FDP	EDC
Suspensory Walking								
EMG Onset (%)	28.8±20.3	17.6±20.3	8.36±19.4	47.4±36.6	8.29±8.42	13.7±15.1	11.5±13.4	45.5±37.2
EMG Offset (%)	63.2±23.5	61.1±24.0	61.7±19.5	81.5±30.9	68.0±13.2	73.2±9.17	69.9±14.9	86.9±27.7
EMG Duration (% Cycle)	38.3±20.2	42.5±19.1	50.3±20.9	17.6±16.5	57.9±15.8	60.2±15.7	56.2±18.4	18.5±16.5
Total EMG Activation (% Cycle)*	31.9±19.8	33.7±21.4	48.8±21.1		50.5±21.3	50.7±22.7	53.6±20.7	
Vertical Climbing								
EMG Onset (%)	3.68±21.1	23.6±27.3	7.30±18.8	61.8±25.5	3.64±8.60	32.8±28.6	5.41±14.5	56.7±39.9
EMG Offset (%)	52.3±16.4	72.1±18.5	48.8±16.5	87.1±17.0	52.4±12.8	56.6±35.9	61.2±17.8	86.4±25.2
EMG Duration (% Cycle)	45.3±14.7	41.5±27.2	45.6±14.1	22.2±14.3	48.8±13.6	23.8±17.7	56.7±18.3	23.7±18.3
Total EMG Activation (% Cycle)*	43.8±15.5	28.9±25.7	39.4±16.1		42.9±17.4		54.9±19.4	

*Total activation for flexor muscles that had multiple bursts per stride

Large error terms for EMG onset indicate pre-activations; averaging of negative % (those occurring before foot on-grasp)

Muscle abbreviations are the same as those in Table 2

Table 5. Summary of typical limb phase muscle activations during the stride cycle.

Muscle	Suspensory Walking	Vertical Climbing
PS	Contact	Contact
DELT	Contact	Contact
BB	Contact	Contact
TBLO	Contact/Swing	Contact/Swing
BCR	Contact	Contact
PL	Contact	Contact/Swing
FDP	Contact	Contact
EDC	Contact/Swing	Contact/Swing

SW, suspensory walking; VC, vertical climbing
Muscle abbreviations are the same as those in Table 2

Table 6. Means of normalized EMG intensity for each individual, muscle, and behavior.

Animal	Functional behavior	PS	DELT	BB	TBLO	BCR	PL	FDP	EDC
BV1	SH	0.24±0.12	0.06±0.04	0.54±0.18	0.06±0.05	0.31±0.06	0.15±0.12	0.14±0.05	0.11±0.02
	SH	0.61±0.15	0.53±0.67	0.69±0.17	0.38±0.26	0.56±0.06	0.93±0.11	0.33±0.24	0.47±0.11
	VC	0.45±0.14	0.08±0.01	0.58±0.11	0.32±0.21	0.44±0.16	--	0.24±0.05	0.60±0.19
BV2	SH	-*	0.69±0.19	0.36±0.10	0.02±0.0002	--	--	--	--
	SW	--	0.58±0.25	0.84±0.12	0.65±0.21	-*	-*	-*	-*
	VC	--	--	0.19±0.08	-*	--	--	--	--
BV3	SH	0.37±0.23	0.25±0.20	0.49±0.22	0.02±0.002	0.39±0.22	0.23±0.11	0.45±0.13	-*
	SW	0.54±0.17	0.57±0.11	0.69±0.13	0.51±0.27	0.72±0.15	0.59±0.24	0.77±0.17	--
	VC	0.51±0.13	0.67±0.25	0.57±0.02	0.65±0.11	--	--	--	--
BV4	SH	0.42±0.12	0.18±0.08	0.14±0.14	0.44±0.07	0.30±0.20	0.04±0.01	0.04±0.02	0.31±0.09
	SW	0.61±0.22	0.40±0.18	0.76±0.13	0.97±0.04	0.81±0.15	0.62±0.22	0.70±0.36	0.70±0.13
	VC	--	--	--	--	--	-*	--	--
BV5	SH	0.50±0.21	--	--	--	0.27±0.05	0.18±0.03	0.46±0.06	0.14±0.01
	SW	0.47±0.31	0.62±0.24	0.49±0.29	0.55±0.22	0.74±0.17	0.39±0.24	0.31±0.29	0.38±0.14
	VC	--	0.54±0.39	0.47±0.27	0.79±0.10	0.64±0.04	0.14±0.02	0.04±0.01	0.37±0.31
BV6	SH	0.24±0.06	0.22±0.03	0.38±0.04	0.19±0.14	--	--	--	--
	SW	0.41±0.24	0.63±0.18	0.87±0.19	0.54±0.24	0.75±0.15	0.33±0.21	0.68±0.13	0.75±0.02
	VC	--	--	--	--	0.37±0.16	-*	0.40±0.10	0.76±0.25

SH, suspensory hanging; SW, suspensory walking; VC, vertical climbing
*No data available because either only data for one behavior so normalization could not be done or only one trial available for analysis
Muscle abbreviations are the same as those in Table 2

Table 7. Pooled means of normalized EMG intensity for each muscle and behavior.

Functional behavior	PS	DELT	BB	TBLO	BCR	PL	FDP	EDC
SH	0.39±0.17	0.27±0.23	0.30±0.21	0.23±0.21	0.33±0.18	0.16±0.12	0.23±0.20	0.21±0.12
SW	0.54±0.22	0.55±0.22	0.71±0.19	0.55±0.26	0.72±0.16	0.48±0.27	0.50±0.31	0.52±0.19
VC	0.47±0.14	0.49±0.38	0.49±0.21	0.58±0.28	0.43±0.17	0.01±0.00*	0.25±0.15	0.57±0.24

SH, suspensory hanging; SW, suspensory walking; VC, vertical climbing
*Data analyzed for PL during vertical climb was limited due to usable EMG recordings from only two individuals
Muscle abbreviations are the same as those in Table 2

Table 8. Activation frequencies resolved by PCA across muscles for each behavior.

	Activation Frequency (Hz)		
Muscle	**SH**	**SW**	**VC**
PS	254	279	223
DELT	255	209	215
BB	231	262	285
TBLO	351	293	297
BCR	233	212	232
PL	320	296	317
FDP	354	293	278
EDC	355	347	346

Muscle abbreviations are the same as those in Table 2

Fig. 1 Photographs of the experimental setup. Shown is individual Bv4 on (**A**) a beam (4.57m x 0.22m x 1.52m) constructed from bamboo was used for trials of suspensory hanging (SH) and suspensory walking (SW), or (**B**) a fenced animal enclosure (2.00m x 3.97m x 3.12m) used for trials of vertical climbing (VC). The arrangement of the recording arenas includes a GoPro camera, LED light box, and ruler (scale: 15 cm) affixed to nylon shade screens or chain link fencing. Note: the BioRadio® attached to the back of the animal and the EMG electrode wire cables in both photos.

B

A

Fig. 2 Exemplar wavelet analysis of EMG bursts for a sloth flexor muscle. Shown in (**A**) is 5 s recording (2000 Hz) from the m. biceps brachii during the contact phase of an SW trial. The box marks (**B**) EMG burst data that were sub-sampled in 1 s data packets to generate (**C**) contour plots of central frequencies where EMG activation intensities were greatest. Mean normalized EMG intensities were sampled across a frequency spectrum that included 17 pre-determined activation frequencies (range: 6.9–902 Hz). Intensity-frequency datasets for all individuals, muscles, and functional behaviors were collated for principal component analysis (PCA) of intensity and activation frequency vector plot distributions.

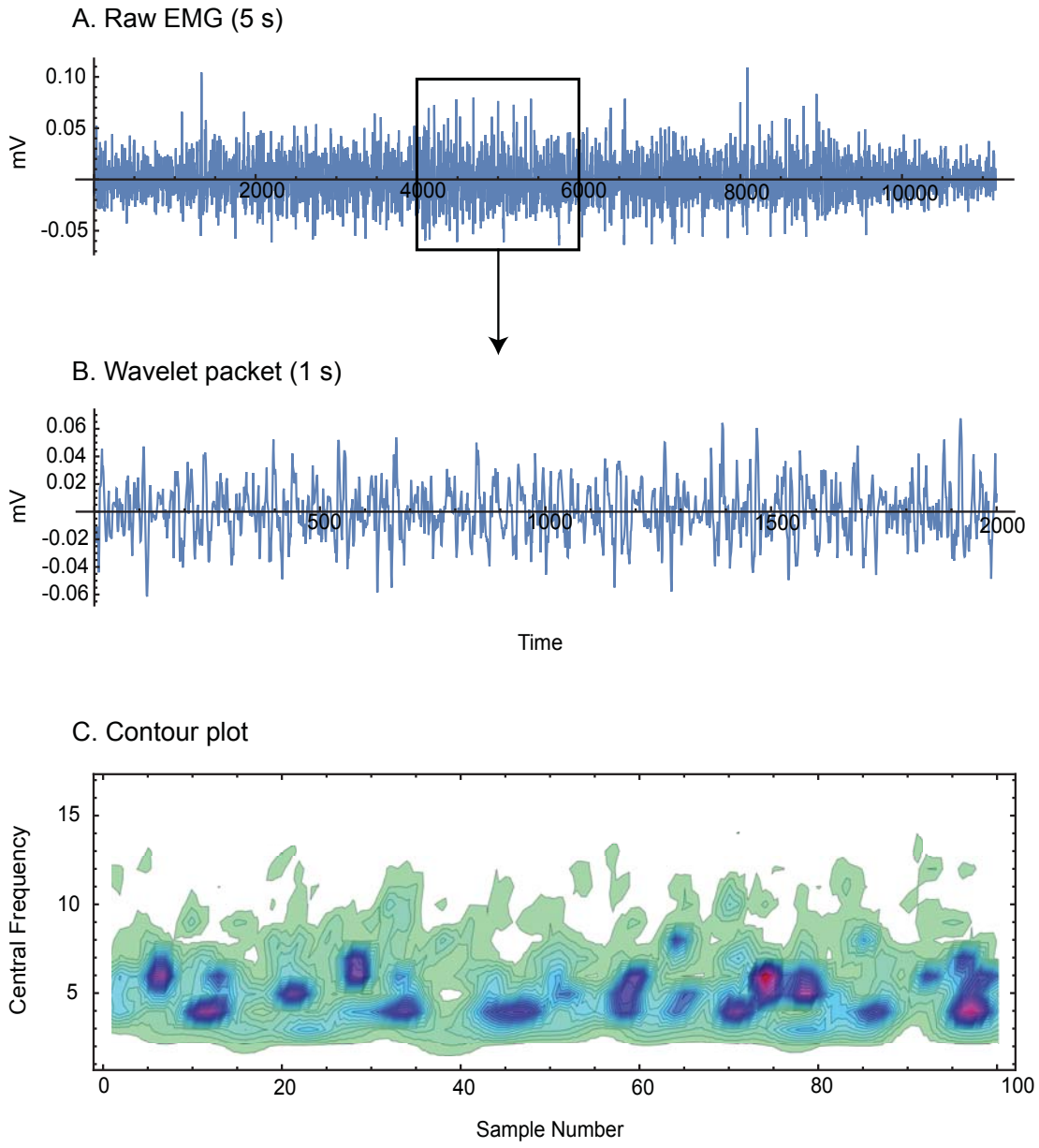

A. Raw EMG (5 s)
0.10
0.05
mV
-0.05
2000
4000
6000
8000
10000
B. Wavelet packet (1 s)
0.06
0.04
0.02
mV
-0.02
-0.04
-0.06
500
1000
1500
2000
Time
C. Contour plot
Central Frequency
15
10
5
0
20
40
60
80
100
Sample Number

Fig. 3 Raw EMG from 8 forelimb muscles during suspensory hanging (SH). EMG was recorded in 1–3 min intervals for trials with three-toed sloths hanging below the beam by their forelimbs and hindlimbs. Shown is a truncated portion of the EMG record with time in seconds (s). EMG recorded separately from four shoulder/brachium muscles and four antebrachium muscles are matched in time for illustrative purposes. PS, pectoralis superficialis; DELT, deltoideus; BB, biceps brachii; TBLO, triceps brachii long head; BCR, brachioradialis; PL, palmaris longus; FDP, flexor digitorum profundus; EDC, extensor digitorum communis.

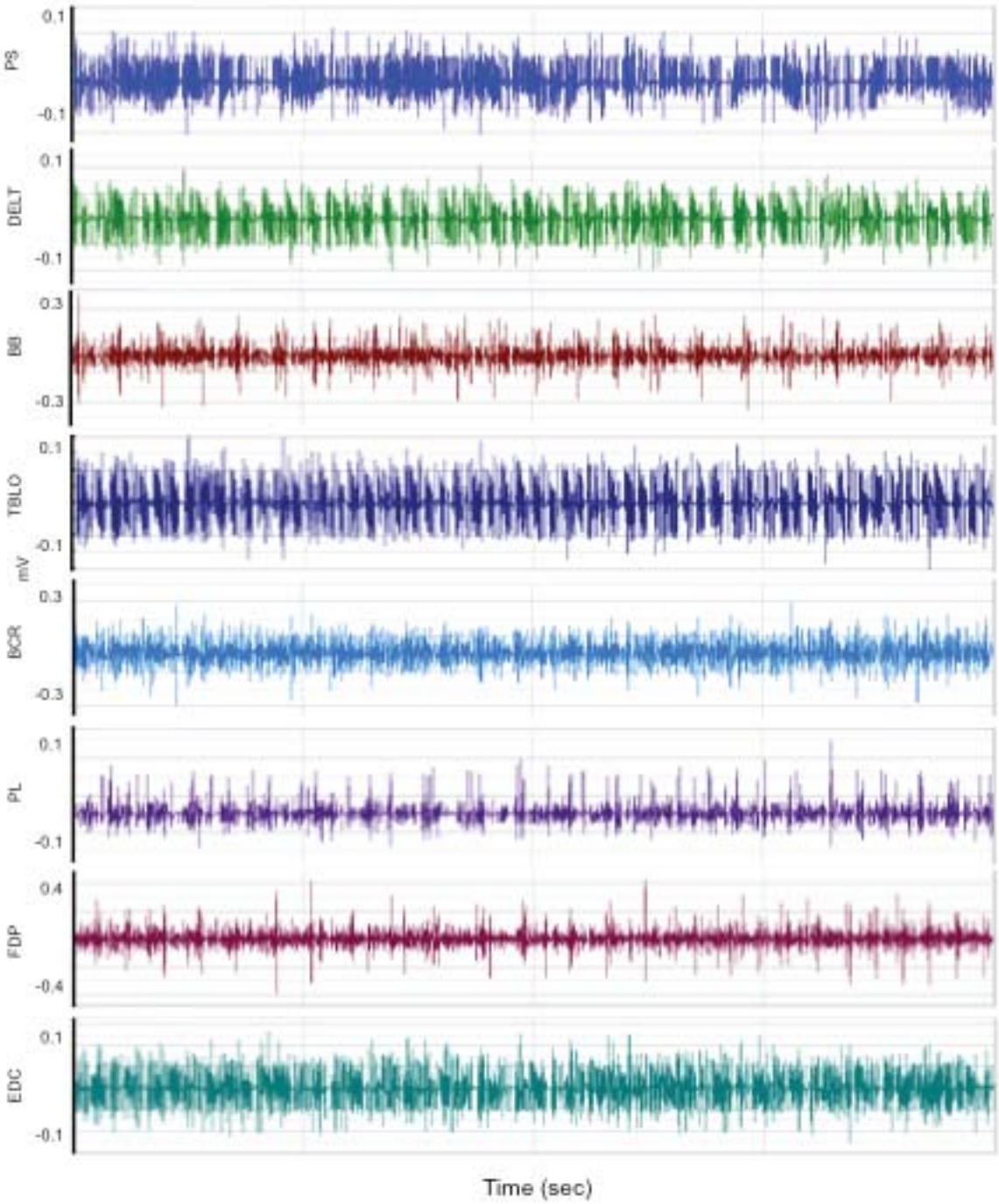

PS
0.1
-0.1
DELT
0.1
-0.1
BB
0.3
-0.3
TBLO
0.1
-0.1
mV
BCR
0.3
-0.3
PL
0.1
-0.1
FDP
0.4
-0.4
EDC
0.1
-0.1
Time (sec)

Fig. 4 Raw EMG from eight forelimb muscles during suspensory walking (SW). EMG recorded separately from four shoulder/brachium muscles and four antebrachium muscles are matched in time. Solid vertical line marks the beginning of one walking stride and corresponds to footfall (Foot On-grasp) of the left forelimb. Small dashed lines marks the events foot off (release) and subsequent footfall of the left forelimb for the proximal muscles. Large dashed lines mark the same events for the distal muscles. Animals were allowed to choose their preferred speed and all SW strides were not completed at the same velocity. Stride duration (0–100%) illustrates the percentage of the limb cycle (contact or swing) that the muscle is active. Muscle abbreviations are the same as those in Fig. 2.

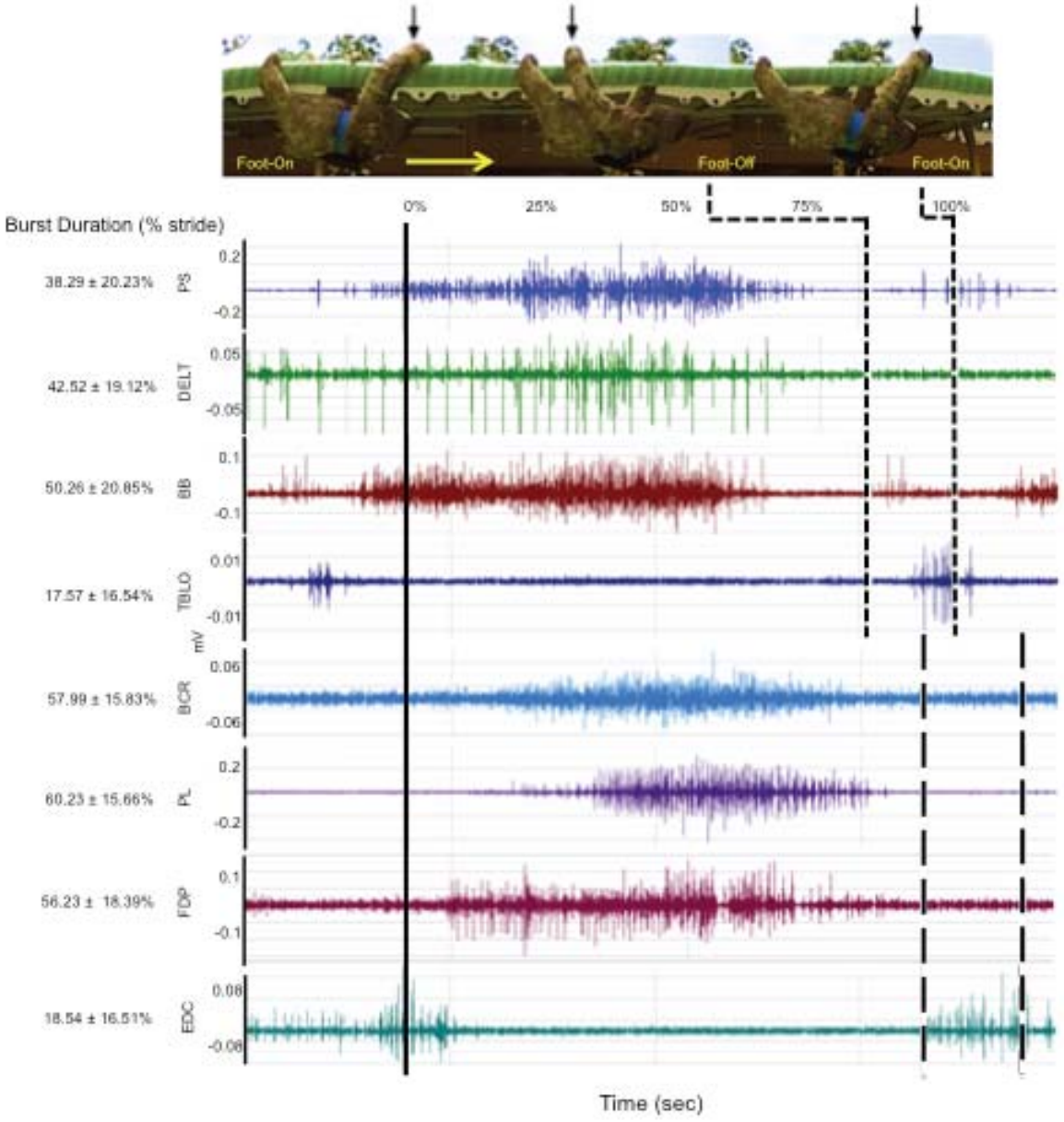
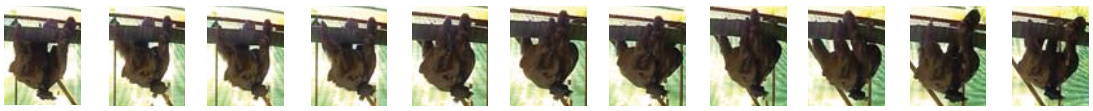

Foot-On
Foot-Off
Foot-On
0%
25%
50%
75%
100%
Burst Duration (% stride)
38.29 ± 20.23%
PS
42.52 ± 19.12%
DELT
50.26 ± 20.85%
BB
17.57 ± 16.54%
TBLO
57.99 ± 15.83%
BCR
60.23 ± 15.66%
PL
56.23 ± 18.39%
FDP
18.54 ± 16.51%
EDC
mV
Time (sec)

Fig. 5 Mean EMG duration (% stride) for sloth forelimb muscles when walking. Burst duration is expressed as a percentage of the stride (0–100%), with horizontal bars representing means for each muscle during a typical walking stride. Error bars are s.e. (standard error). Burst duration was averaged across individuals (N=4–6) to show the most typical patterns of EMG activations. Solid vertical line marks the end of limb contact (Foot Off-release) at an average duty factor of 0.83 for suspensory walking in sloths. Video frames (above) depict representative positions of the left forelimb at 10% intervals throughout a stride. Shown is individual Bv5. Muscle abbreviations are the same as those in Fig. 2.

Suspensory Walk 0.06 ms^{-1}



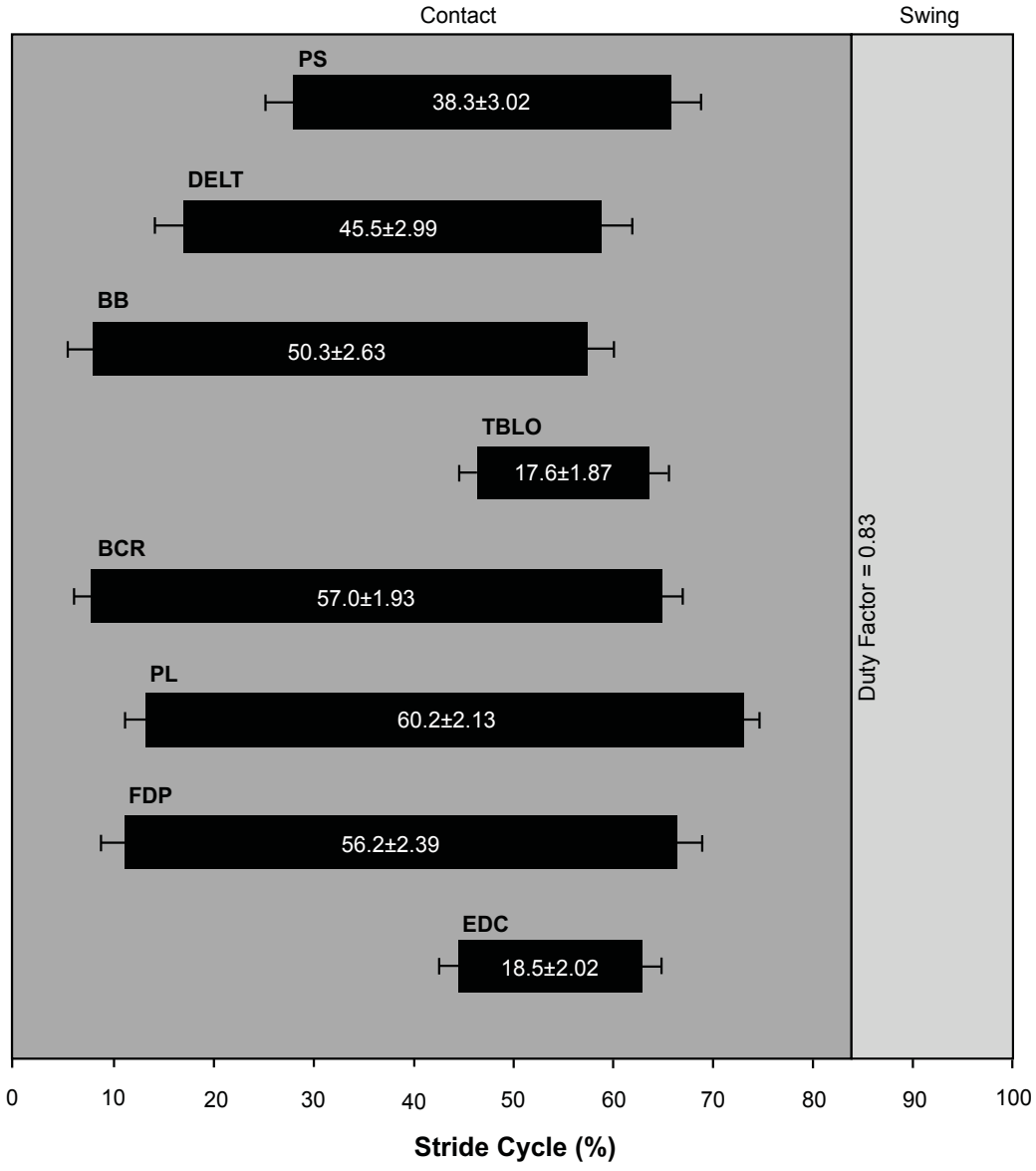


Fig. 6 Raw EMG from eight forelimb muscles during vertical climbing (VC). EMG recorded separately from four shoulder/brachium muscles and four antebrachium muscles are matched in time. Solid vertical line marks the beginning of one vertical climbing stride and corresponds to footfall (Foot On-grasp) of the left forelimb. Small dashed lines marks the events foot off (release) and subsequent footfall of the left forelimb for the proximal muscles. Large dashed lines mark the same events for the distal muscles. Animals were allowed to choose their preferred speed and all VC strides were not completed at the same velocity. Stride duration (0–100%) illustrates the percentage of the limb cycle (contact or swing) that the muscle is active. Muscle abbreviations are the same as those in Fig. 2.

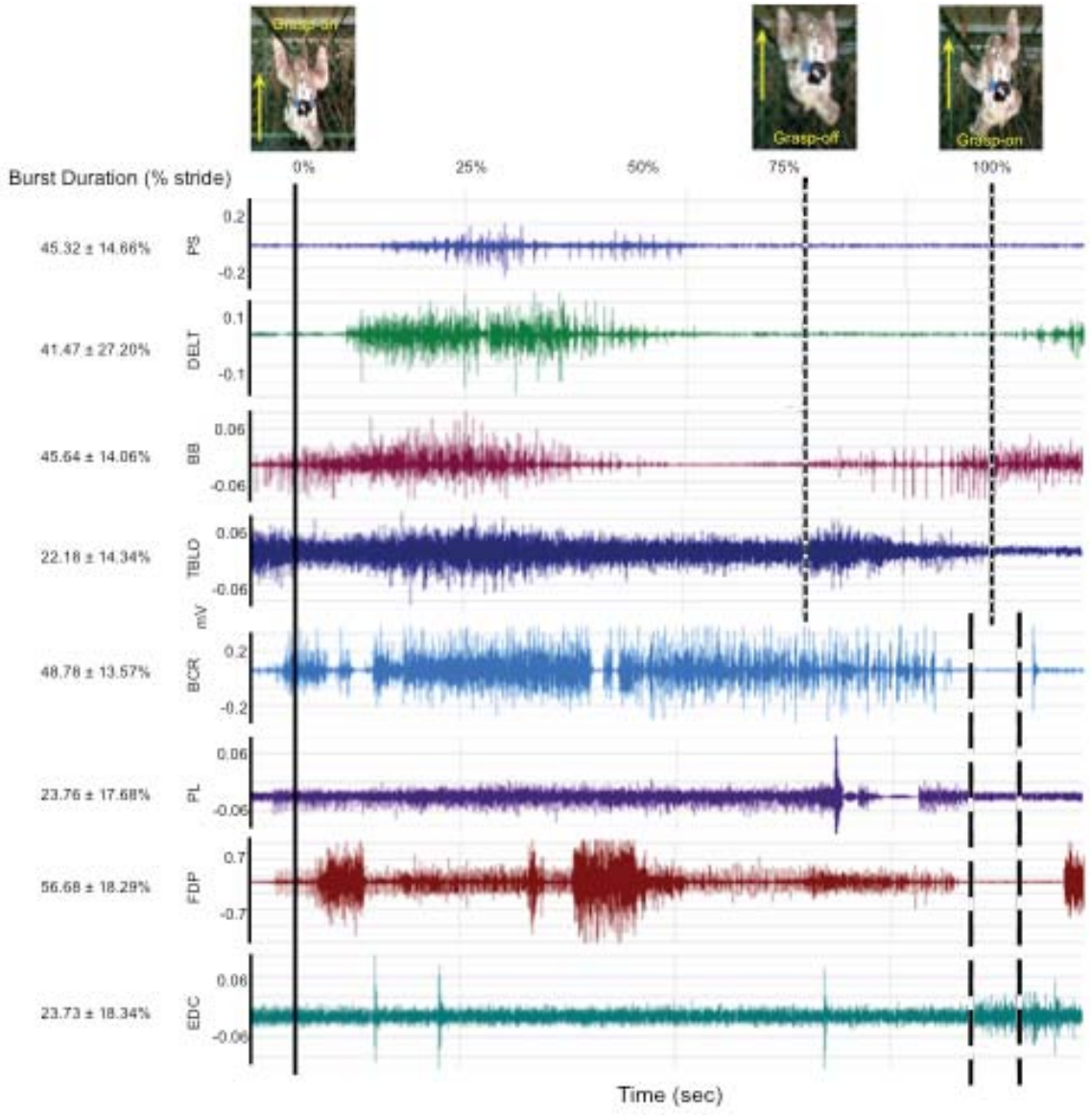
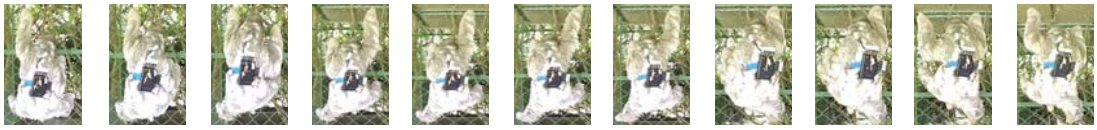

Grasp-on
Grasp-off
Grasp-on
Burst Duration (% stride)
0%
25%
50%
75%
100%
45.32 ± 14.66%
PS
0.2
-0.2
41.47 ± 27.20%
DELT
0.1
-0.1
45.64 ± 14.06%
BB
0.06
-0.06
22.18 ± 14.34%
TBLO
0.06
-0.06
mV
48.78 ± 13.57%
BCR
0.2
-0.2
23.76 ± 17.68%
PL
0.06
-0.06
56.68 ± 18.29%
FDP
0.7
-0.7
23.73 ± 18.34%
EDC
0.06
-0.06
Time (sec)

Fig. 7 Mean EMG duration (% stride) for sloth forelimb muscles when climbing. Burst duration is expressed as a percentage of the stride (0–100%), with horizontal bars representing means for each muscle during a typical climbing stride. Error bars are s.e. (standard error). Burst duration was averaged across individuals (*N*=3–6) to show the general patterns of EMG activation. Solid vertical line marks the end of limb contact (Foot Off-release) at an average duty factor of 0.86 for vertical climbing in sloths. Video frames (above) depict representative positions of the left forelimb at 10% intervals throughout a stride. Shown is individual Bv5. Muscle abbreviations are the same as those in Fig. 2.

Vertical Climb 0.03 ms^{-1}



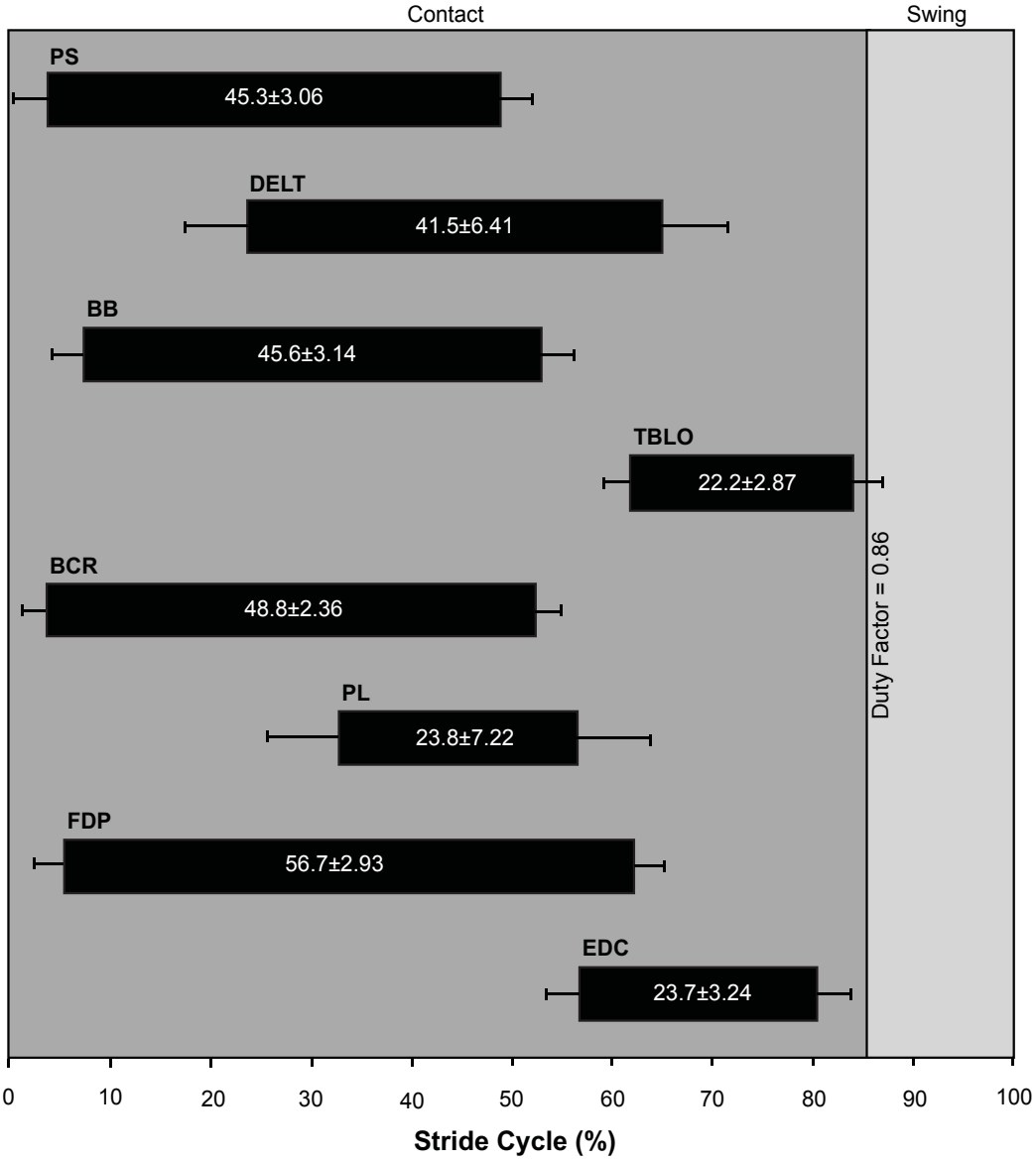


Fig. 8 Mean normalized EMG intensity for sloth forelimb muscles. Burst intensities (**A**) four major flexor muscles and (**B**) all forelimb muscles combined. Burst intensity was normalized to the maximum EMG burst recorded for each muscle during either the behaviors suspensory hanging (SH), suspensory walking (SW), or vertical climbing (VC). Normalized burst intensity is expressed as a ratio (0–1.0), with bars representing means for each muscle across all individuals (*N*=6). Error bars are s.d. (standard deviation). Ratios near or equal to 1.0 indicate maximum contraction recorded, which was most commonly observed during SW trials. *significantly greater than SH; **significantly greater than SH and VC.

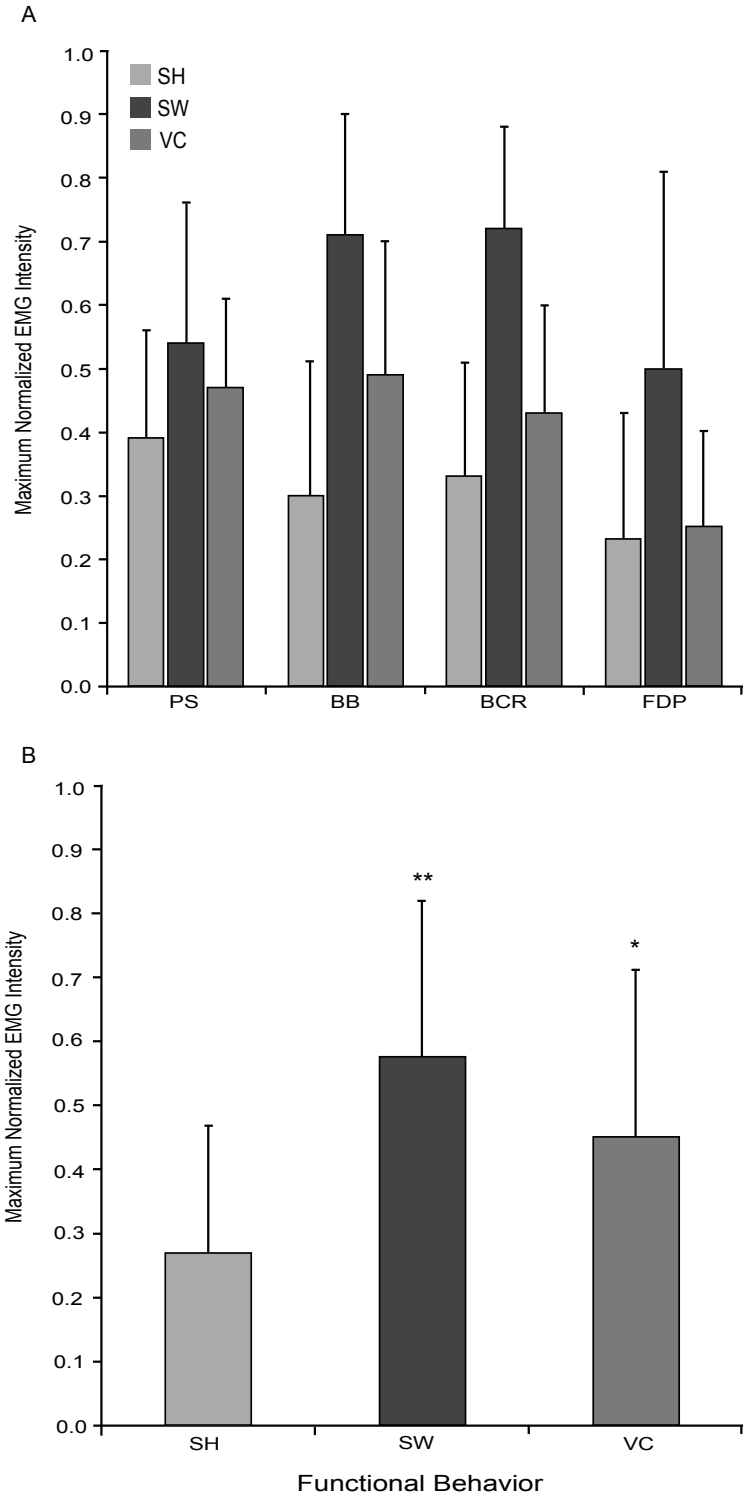

A
SH
SW
VC
Maximum Normalized EMG Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
PS
BB
BCR
FDP
B
Maximum Normalized EMG Intensity
**
*
SH
SW
VC
Functional Behavior

Fig. 9 Mean activation frequencies of slow and fast motor units from sloth forelimb muscles across three functional behaviors. Plots of mean normalized EMG intensity vs. activation frequencies for the muscles (**A**) PS, (**B**) DELT, (**C**) BB, (**D**) TBLO, (**E**) BCR, (**F**) PL, (**G**) FDP, and (**H**) EDC. Data shown are collated for SH, SW, and VC and are representative of forelimb motor unit (MU) recruitment patterns in *B. variegatus*. Blue curves (left) are low frequencies corresponding to slow MU activations whereas red curves (right) are high frequencies corresponding to fast MU activations. Color-coded values are pairs of low and high mean frequencies for each muscle.

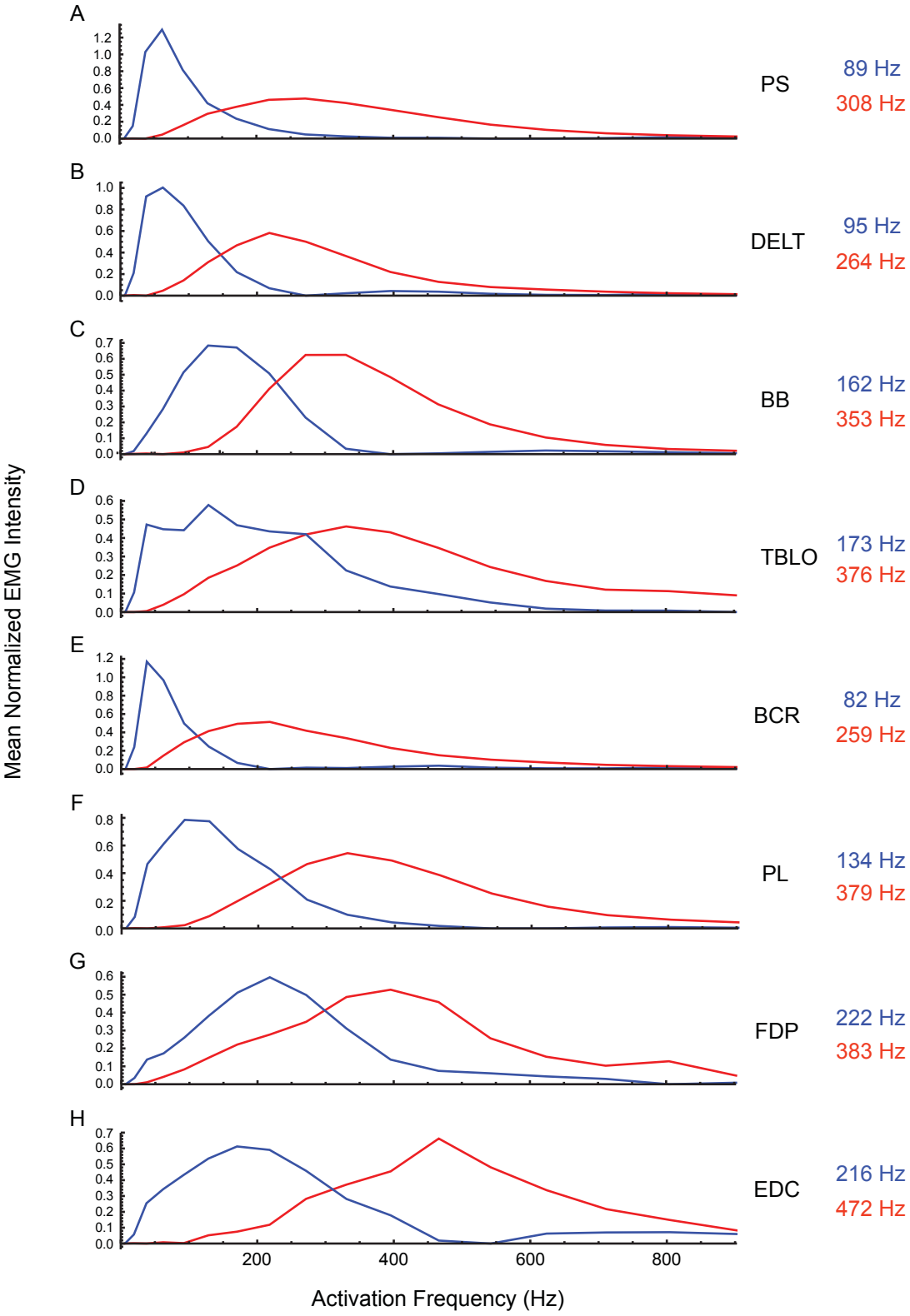
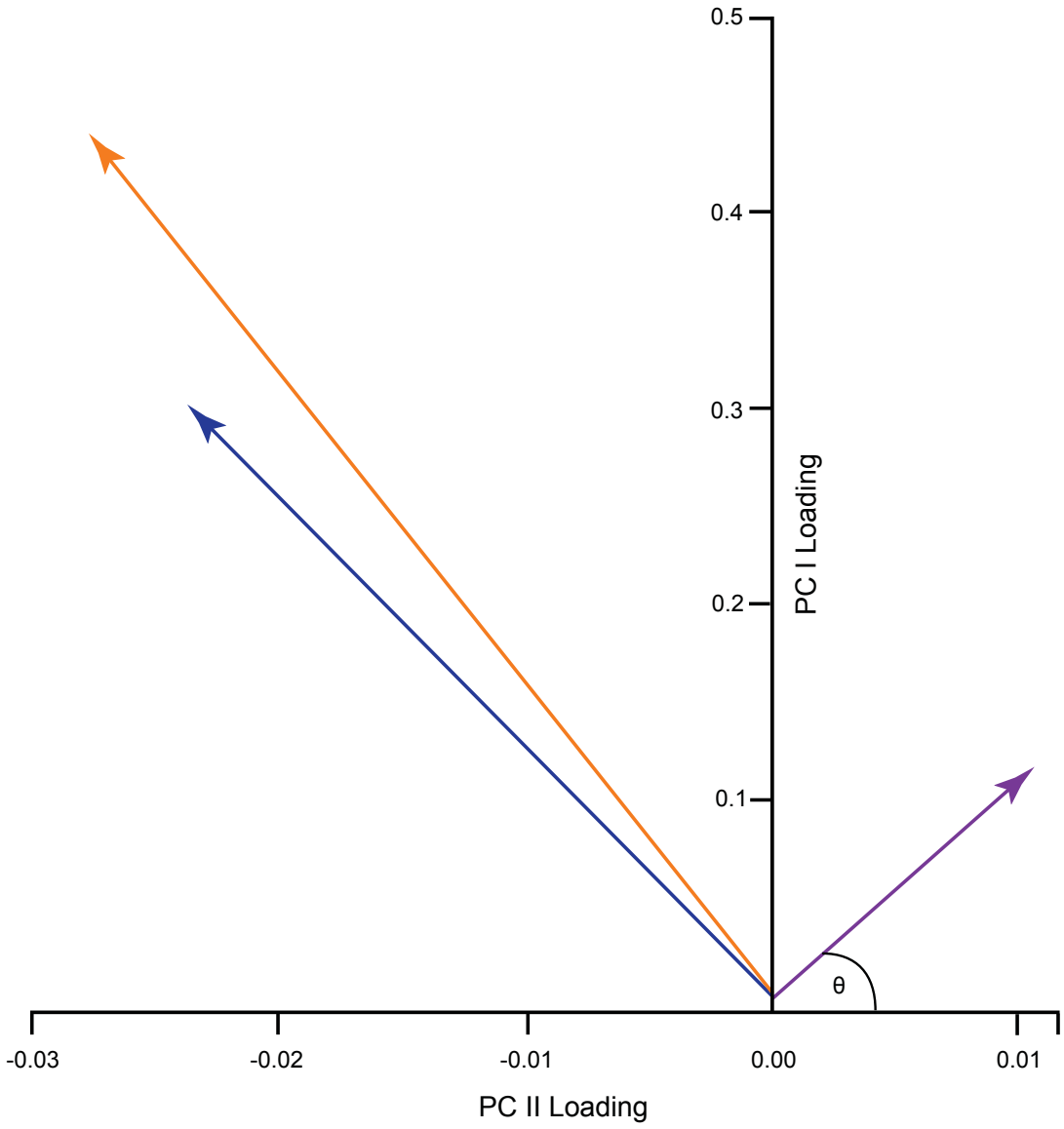

A
B
C
D
E
F
G
H
PS
89 Hz
308 Hz
DELT
95 Hz
264 Hz
BB
162 Hz
353 Hz
TBLO
173 Hz
376 Hz
BCR
82 Hz
259 Hz
PL
134 Hz
379 Hz
FDP
222 Hz
383 Hz
EDC
216 Hz
472 Hz
Mean Normalized EMG Intensity
200
400
600
800
Activation Frequency (Hz)

Fig. 10 PCA vector plot of muscle activation intensity and frequency distributions for each functional behavior in three-toed sloths. Principal component loading scores are plotted 2D on vertical (PCI) and horizontal (PCII) axes. Vectors from the origin are color-coded for the functional behaviors SH (purple), SW (orange), and VC (blue). Loadings along PCII represent recruitment frequencies with positive scores indicating higher frequency activations and negative loadings indicating lower frequency activations. Loadings along PCI represent EMG intensities of activation where shorter vector length indicates smaller burst intensity and longer vector length indicates larger burst intensity. Data shown are collated for all muscles and functional behaviors sampled and represent overall neuromuscular recruitment patterns in *B. variegatus*.


0.5
0.4
0.3
0.2
0.1
PC I Loading
θ
-0.03
-0.02
-0.01
0.00
0.01
PC II Loading