Keep Calm and Hang On: EMG Activation in the Forelimb Musculature of Three-toed Sloths (*Bradypus variegatus*)

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ABSTRACT

Sloths exhibit below branch locomotion whereby their limbs are loaded in tension to support the body weight. Suspensory behaviors require both strength and fatigue resistance from the limb flexors; however, skeletal 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 to maintain support, 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 fine-wire electrodes implanted into two suites of four muscles in the left forelimb while sloths performed suspensory hanging (SH), suspensory walking (SW), and vertical climbing (VC). All muscles were minimally active for SH. During SW and VC, sloths moved slowly (Duty Factor: 0.83) and activation patterns were consistent between behaviors; the flexors were activated early and for a large percentage of limb contact, whereas the extensors were activated for shorter burst durations on average and showed biphasic (contact and swing) activity. Muscle activities were maximal for the elbow flexors and lowest for the carpal/digital flexors, and overall activity was significantly greater for SW and VC compared with SH. Wavelet analysis indicated high mean EMG frequencies from the myoelectric intensity spectra coupled with low burst intensities for SH, although the opposite pattern occurred for SW and VC, with the shoulder flexors and elbow flexor, m. brachioradialis, having extremely low mean EMG frequencies that are consistent with recruitment of slow fibers. Collectively, these findings support the hypothesis and suggest that sloths may selectively recruit smaller, fast motor units for suspensory postures but have the ability to offset the cost of force production by recruitment of large, slow motor units during locomotion.

Keywords: flexor, motor units, muscle, suspension

Summary: Three-toed sloths minimize activation of their limb muscles while hanging and appear offset the cost of force production when walking and climbing by preferentially recruiting large, slow-contracting muscle fibers.

INTRODUCTION

Tree sloths (Xenarthra, Pilosa) are one of only a few mammals for which suspensorial habits are almost obligatory (Hayssen, 2009, 2010). Sloths also have lower basal metabolism and mean body temperature compared with other xenarthran 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 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 reclined in the recesses of tree branches, thereby it does not engage in suspensory (below branch) behaviors as often as two-toed sloths (Urbani and Bosque, 2007). The collective sleep/wake patterns demonstrate that sloths are not particularly active mammals supporting the hypothesis that their physiology, as well as their behavior, reflects a system extremely modified to conserve metabolic energy (Cliffe et al., 2015, 2018; Spainhower et al., 2018).

Sloths exhibit arboreal locomotor behaviors that include suspensory walking and vertical climbing, as well as suspensory postures (i.e., 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 necessitates modifications to their distal skeletal elements (Mendel, 1981b, 1985) and flexor muscle architectural properties (Olson et al., 2018), as well as the predominant expression slow myosin heavy chain (MHC) fibers that atypically have anaerobic metabolic characteristics (Spainhower et al., 2018) to power contraction. Our previous work specifically reported that the extrinsic muscles of the forelimb in *Bradypus* have parallel fiber architecture and several have long moment arms, and with the exception of mainly the distal carpal/digital flexors and extensors, the majority of the intrinsic musculature is slow-contracting with relatively long, parallel or unipennate fibers (Olson et al., 2018). For example, the shoulder flexors mm. pectoralis superficialis and deltoideus are ~70% slow in their MHC fiber type distribution as are the elbow flexors/extensors, although large m. brachioradialis is the strongest

elbow flexor and has a 95% expression of the slow MHC-1 isoform in *B. variegatus*. Conversely, the carpal/digital flexors have a larger percentage of fast MHC-2A fibers (Spainhower et al., 2018). In addition to generally strong, slow-contracting muscles, the distinctively slow and controlled movement patterns of sloths are considered to be an adaptive strategy for energy conservation (McDonald and De Iuliis, 2008; Cliff et al., 2018), and this may be understood as a means of enhancing muscle force while limiting contractile velocity, thus lowering the ATP cost of contraction (Taylor et al., 1982; Heglund and Taylor, 1988; Roberts et al., 1997). However, *in vivo* patterns of muscle activation are needed to verify the muscle fiber type properties that we found previously in relation to locomotor performance in sloths.

The expectations for muscle function in a tensile limb system are formed by foundational fiber typing (Jouffroy and Medina, 2004) and telemetered electromyography (EMG: Jouffroy and Stern, 1990; Jungers and Stern, 1980, 1981) studies in suspensorial primates. A near homogeneous expression of large, slow MHC-1 fibers is typical in a single belly (or head) of each major extensor functional group in primate limbs. These muscles show a modest level of EMG activation during arboreal postural behaviors, which correlates with an anti-gravity role in joint stabilization (Jouffroy et al., 1999). Sickles and Pinkstaff (1981a, b) notably observed a near uniform distribution of slow-contracting fiber types in both flexor and extensor muscles of the slow loris (*Nycticebus*) corresponding with EMG activation patterns of selective flexors vs. extensors during suspension (Jouffory and Stern, 1990), thus suggesting the possibility of shared body weight support between fore- and hindlimb muscles. The absence of fast isoform MHC-2X and -2B fibers in *Nycticebus* and sloths (Spainhower et al., 2018) further suggests specialization of their limb muscles for joint stabilization during their slow, deliberate suspensory habits, but this possibility has yet to be reported by companion EMG analyses from sloth limb muscles.

In this study, we aim to evaluate EMG from the forelimb musculature of *B. variegatus* to determine the patterns and levels of muscle activation, in addition to the EMG spectral properties associated with suspensory habits versus those of metabolically costly vertical climbing (Hanna et al., 2008). Muscle activation patterns determined using EMG record electrical signals from action potentials as the motor units (MU) within muscles are recruited. Action potentials elicited from different MU that comprise slow and fast fiber types leave characteristic signatures in the EMG frequency spectrum that can be detected using wavelet decomposition of EMG (Hodson-Tole and Wakeling, 2007; Blake and Wakeling, 2014). By use of wavelet analysis, the

relationship between MHC fiber type expression and muscle activation in sloth limbs can be more critically assessed, and may offer insight into suspensory muscle function beyond that typical of numerous previous reports in primates.

We hypothesize 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 slow fibers of the shoulder, elbow, and digital flexors will be active for large portions of limb contact with the substrate. It is also predicted that maximum EMG intensity will occur during vertical climbing (via greater recruitment of fast fibers), be intermediate during suspensory walking, and be minimal during suspensory posture. It is not known if the biomechanics of sloths resemble the forelimb-biased propulsion during vertical climbing in slow-moving lorisids (Hanna et al., 2017), but it is possible that sloth forelimbs have a even greater role in propulsion while moving vertically than they do when walking below-branch (Granatosky and Schmitt, 2017). The results of this study improve the understanding of muscle fiber 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

Six three-toed sloths (*B. variegatus*, 3.9±0.6 kg) were used for this study at The Sloth Sanctuary in Penshurst-Limon, Costa Rica. No preference was given to the sex of the animals, although adult (or sub-adult) individuals were preferable to juveniles. Morphometric data for individuals are presented in Supplemental Table 1. 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

Sloths were sedated (0.1 ml/kg Dexdomitor + 0.1 ml/kg Ketamine, injected into the m. gluteus medius) prior to electrode implantation. Supplemental doses of ketamine in increments of 0.1 ml were used as necessary to achieve the desired level of sedation. While under sedation, custom fine-wire, bipolar EMG electrodes (0.002 bifilar: California Fine Wire, Grover Beach, CA, USA)

were implanted into selected muscles of the shoulder, brachium, and antebrachium. Specifically, electrodes (sterilized by hydrogen peroxide plasma) 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 digitorum communis (EDC). The electrodes for the PS were placed in the head approximating the m. pectoralis superficialis anterior (PSA) that originates from the manubrium/sternum and inserts on the pectoral ridge (line) of the humerus. The three heads of the DELT are inseparable in three-toed sloths and we placed the electrodes about the dorsolateral aspect of the shoulder joint approximating the merged bellies of the m. deltoideus pars scapularis/acromialis. See Olson et al. (2018) for a review of forelimb myology in *B. variegatus*.

Pairs of 4 electrodes were all implanted percutaneously into the center of each muscle belly within a suite by using gentle force with a 26g 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. EMG wires were cabled, secured, and connected to a wireless EMG unit (BioRadio™, Great Lakes NeuroTechnologies, Cleveland, OH, USA) that was harnessed to the thorax of the sloth. Sedation was reversed with injection of Antisedan (0.1 ml/kg) 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 flexible veterinary bandaging tape (3M) to provide extra grip. Sloths were required to both hang in a static suspensory posture and walk suspended below 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 (Fig. 1A). Sloths also performed 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 were videoed with a GoPro camera (HERO4: San Mateo, CA, USA) positioned 1.5 m from the animal; in the sagittal plane for the suspensory trials and in a coronal plane for the VC trials. 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 (2000 Hz) with an LED light in the field-of-view of the camera. EMG was recorded via broadband streaming using BioCapture™ software (Great Lakes NeuroTechnologies) for one suite of four muscles (proximal vs. distal musculature) then the other on consecutive days for the same individual. At the completion of EMG recordings (~2 h) 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.

Data analysis

Video data were first analyzed for frame numbers that corresponded to time (in s) in the EMG records marking the beginning and end of SH intervals, or to foot-on (grasp) and foot-off (release) events during locomotor trials. Footfall (i.e., each grasp) was defined as the first frame that we could determine when the claw of each digit was in full contact with the substrate and the limb showed evidence of weight-bearing support. EMG for SW and VC were analyzed as a series of strides consisting of consecutive pairs of limb contact and swing phases. Velocity (ms⁻¹) for SW and VC was calculated across a calibrated distance (15 cm) in the frame of view (Tracker software; https://physlets.org/tracker/): instantaneous velocity was determined at 1s intervals throughout a stride and averaged across data points where velocity was similar. Except for velocity, temporal variables were calculated (LabScribe software; iWorx Systems Inc., Dover, NH, USA) for both the limb cycle (in s) and relative stride interval (% stride), where the sum of the contact and swing phases is 100% of the stride, and include duty factor, EMG onset-offset, and EMG burst duration. All temporal measurements were averaged for each muscle and individual.

Because both the magnitude of the EMG and the spectral properties that occurred in each EMG burst are the parameters of interest, this requires a time-frequency analysis that is not possible from more traditional approaches such as the root-mean-square (RMS) EMG (Jouffroy and Stern, 1990; Jouffroy et al., 1999). We used a wavelet technique that has been specifically

adapted for EMG analysis (von Tscharner, 2000), and extensively tested across different species (e.g., Wakeling and Syme, 2002; Wakeling et al., 2002; Wakeling et al., 2012). The wavelet analysis determined an EMG intensity that was a close approximation of the power in the EMG signal. *A priori*, only bursts that were at least 2 times greater than baseline EMG voltage were considered for analysis. EMG intensities via wavelets were determined for a subset of 2–3 bursts per muscle for each individual across numerous strides (for locomotor trials) using custom software in Mathematica (v. 11.3; Wolfram Research, Champaign, IL, USA), with this intensity being localized across both time and frequency. Briefly, the normalized EMG was decomposed by a bank of 17 wavelets (range of central frequencies: 6.9–902 Hz), and EMG intensity spectra were generated for 0.5–1 s windows within each burst of activity (about 5 spectra per burst).

The EMG intensity was normalized to the mean of the peak EMG intensities across all individual, trials and behaviors. The EMG intensity spectra for all muscles and functional behaviors across individuals were classified by Principal Components Analysis (PCA) (Wakeling, 2004; Hodson-Tole and Wakeling, 2007): the EMG intensity was represented by the PC1 loading score for each window while the PC2 loading score represented activation frequency, and the shape of the EMG intensity spectra were given by the angle theta (θ) of the PC1-PC2 loading score eigenvector. A large θ characterized EMG intensity spectra that with lower frequency components than spectra with small θ.

Statistics

Values are reported as means±s.d. (standard deviation) unless otherwise specified. MANOVA performed in SPSS (v. 20: IBM, Inc., Armonk, NY, USA) was used to test for mean differences in stride duration, stride frequency (inverse of 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. Coefficients of Variation (CV) were calculated for each EMG variable analyzed to justify use of mean data in additional multivariate testing. MANOVA was also employed to assess statistical significance in both EMG burst duration (% stride) and normalized EMG intensity, specifically how these parameters varied among flexor muscles (effect: muscle) and SH, SW, and VC (effect: behavior). In cases where the assumption of homoscedasticity was not met (e.g., EMG burst durations) a Kruskal-Wallis test was performed, and then significant results from either this test or those from

MANOVA were followed by a series of one-way ANOVAs and Dunnet's T3 (burst duration) or Tukey's (burst activation) *post hoc* tests to determine all pair-wise significant outcomes. Significance for all statistical tests was accepted at $P\leq0.05$.

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, implantation of fine-wire electrodes was approved as a recovery procedure where no animal could be sacrificed. However, despite the potential lack of exact placement of our electrode implants in some muscles, EMG activation broadly sampled from flexor/extensor musculature (per limb region) is adequate to achieve the objective and test our hypothesis. 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 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 to minimize stress on the animals. 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 individual, muscle, and behavior are presented in Supplemental Table 2.

Three other factors are offered as 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 locomotor trials, and this time not coinciding with a typical active period for *Bradypus*. Additionally, measurements from some muscles could vary due to fatigue and/or 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 EMG recordings. Sloths do not appear to metabolize drugs quickly, and despite administration of a reversal agent, individuals often performed better after multiple bouts of SW. 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 locomotor velocity was not controlled for in our experimental approach, although all trials analyzed show little variation in duty factor indicating that preferred speeds were comparable across individuals. Moreover, sloths do not employ steady-state locomotion favoring

instead short bouts of intermittent movement. A third possibility could have been the large beam diameter. At 22 cm, the substrate was considered to be very large in previous studies of two-toed sloths (Granatosky et al., 2018). However, *Bradypus* may prefer, or is more capable of grasping larger substrates (Mendel, 1985), and none of our individuals appeared to have difficulty placing their entire foot on the beam, and thus should not have a factor limiting SW performance.

RESULTS

Gait and Stride Parameters

Three-toed sloths performed SW using a lateral sequence diagonal couplet (LSDC) gait and VC using more diagonal sequences of footfalls (Fig. 2A, B). The LSDC gait observed during SW corresponded with original the formulations of Cartmill et al. (2002), as well as the predictions of Usherwood and Self Davies (2017) for slow gaits with large duty factors. Diagonality varied slightly among individuals, duty factors, and behaviors, overall with VC correspondingly showing greater diagonality than SW in *B. variegatus* (Fig. 2C). Stride parameters for each individual are presented in Supplemental Table 3. Stride frequencies were low and similar (P=0.62) between SW and VC, while stride durations were long but variable, and also did not differ significantly (P=0.95) resulting in slow velocities for both locomotor behaviors. Nevertheless, on average, three-toed sloths (N=6) walked nearly twice as fast (P=0.02) than they climbed (0.07 m s^{-1} vs. 0.04 m s^{-1}), but had the same (P=0.22) mean duty factors of 0.83±0.07 and 0.83±0.05 for SW and VC, respectively. Taken all together, strides between SW and VC are not different (P=0.14).

EMG Activation Patterns

A total of 117 trials, 224 strides, and 856 EMG bursts were analyzed. Raw EMG traces are included as Supplemental Figs. 1–3. EMG activations during SH were long in duration (5–60 s), and each muscle generally showed intermittent activations over several minutes of recordings. For the locomotor behaviors, relative EMG durations were broadly similar between proximal and distal limb muscles, as well as flexor vs. extensor muscle activations for both SW and VC. Means for all temporal variables during SW and VC are presented in Table 1.

All flexor muscles showed some pre-activation (i.e., activation in late swing phase into contact phase), and it was observed most frequently in BB, as well as in FDP (for VC), although no muscle averaged EMG onsets that occurred prior to grasp (negative %). In general, the timing

of flexor muscle activity within stride cycles was similar between SW and VC (Fig. 3). Flexor muscles had earlier mean EMG onsets compared the extensors and all were activated within the initial 30% of the stride during SW, or except for DELT and PL, within the first 8% of the stride during VC (Table 1). The elbow flexor BCR activated earliest of all muscles, whereas TBLO showed the latest activation, typically at mid-contact phase during SW, and its activation was further delayed during VC (Fig. 3). Specifically, the PSA was activated latest among the flexors during SW, although it was active in early contact phase of VC. Due to multiple activations, the extensors averaged EMG onsets that approximated 50–60% of the stride, yet the initial activation for EDC was earlier, overlapping with EMG onsets of most flexors during SW and VC (Fig. 3). Consistent patterns were also observed for mean EMG offsets, but with additional variability among the proximal flexors between SW and VC (49–73% stride: Table 1). Flexor muscle activations ended earliest for BB and latest for FDP and DELT. Mean EMG offsets of EDC and TBLO were the latest of all muscles (Table 1). However, except for TBLO during VC, the initial activation of both extensors ended within the range of the flexor EMG offsets, while the second activation ended in late swing phase (Fig. 3).

Overall, relative EMG burst durations were similar (P=0.057–0.274) among the forelimb flexors for both SW and VC (P=0.546; MANOVA, effect: Behavior). In particular, the distal flexors (BCR, PL, FDP) were activated for consistently long durations of the stride (~50–60%), while the extensors TBLO and EDC had EMG burst durations that were on average 14–22% of the stride (Table 1), and these differences were significant (P<0.001; MANOVA, effect: Muscle). All flexor muscles were mainly active during the contact phase of SW (>88% of trials analyzed) and VC (>83% of trials analyzed), with notably BB, BCR, and FDP activated almost the entirety (97–100%) of limb contact during VC. The TBLO and EDC, which were most typically activated during both the contact and swing phases of the stride cycle, are the only muscles that showed biphasic activity, hence the generally shorter average EMG burst durations for both SW and VC (Fig. 3). The protracted EMG burst duration shown for EDC during the contact phase of VC resulted from fewer measurements for this muscle and behavior.

Mean EMG intensities across all muscles and functional behaviors are presented in Table 2. EMG intensity did not differ significantly among the main flexors PSA, BB, BCR, and FDP across behaviors, although activity of FDP was consistently the lowest on average for each functional behavior (Fig. 4A). EMG intensity for the elbow flexors BB and BCR were the largest

across all muscles and behaviors during SW with means of 0.71±0.19 and 0.72±0.16, respectively. The extensors TBLO and EDC showed the largest EMG intensity during VC (Table 2). In general, EMG intensity for all muscles was greatest during SW, moderate during VC, and least during SH (Fig. 4B), and these differences were significant ($P<0.001$; MANOVA, effect: Behavior). Compared to SH, the mean EMG intensities were nearly 2.0 times greater during SW (pairwise data: $P<0.001$) and 1.3 times greater during VC (pairwise data: $P<0.001$). Mean EMG intensity for all muscles during SW was also greater (pairwise data: $P=0.022$) than that for VC.

EMG Spectral Properties

PCA for the EMG intensity spectra revealed that PC1 and PC2 weights explained 75.8±5.2 % and 12.2±4.7 % of the spectral signal, respectively. Combinations of these PCs allowed extreme low- and high-frequency components to be identified from the spectra for each muscle across all functional behaviors: the low frequency component had a mean of 147±55.7 Hz (range: 82–222 Hz) and the high frequency component had a mean of 350±69.7 Hz (range: 259–472 Hz) (Fig. 5). Notably, the low frequency components from PS, DELT, and BCR (Fig. 5A, B, E) had low mean frequencies that averaged 88.9 Hz, 95.3 Hz, and 81.8 Hz, respectively, while BB, TBLO, and PL demonstrated intermediate low frequency components (Fig. 5C, D, F) and those for EDC and FDP had higher mean frequencies >200 Hz. The high frequency components from the EMG intensity spectra generally followed the same pattern for each muscle. On average, the FDP (383 Hz) and EDC (472 Hz) exhibited the two largest mean frequencies for their high frequency components (Fig. 5G, H).

The PC loading scores allowed the major properties of the EMG intensity spectra to be distinguished among the three functional behaviors and showed a lower angle theta (θ) for SH than that for either SW or VC, which were equally large (Fig. 6). SW had the highest intensity (shown by its PC1 loading score), while VC had the lowest frequency properties. Conversely, SH had lowest intensity and highest frequency properties of the three functional behaviors.

DISCUSSION

Interest in structure-function of sloths has increased over recent decades (Mendel, 1981a, b, 1985; Montgomery, 1983; Urbani and Bosque, 2007; Nyakatura and Fischer, 2010, 2011; Nyakatura et al., 2010; Nyakatura, 2012; Cliffe et al., 2015, 2018), although no previous study has evaluated sloth muscle function *in vivo* during suspensory locomotion and posture. 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 only 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 less data are available.

Several of our major findings support the hypothesis that activation of sloth forelimb muscles reflects a neuromuscular system specialized to conserve metabolic energy. To summarize, first the shoulder, elbow, and digital flexors of *B. variegatus* typically have long burst durations during the contact phase of locomotor behaviors, with notable early EMG onsets of the well-developed, strong elbow and digital flexors. Second, relatively low levels of muscle activation were detected during SH compared to significantly greater levels for all muscles when individuals performed SW and VC. Third, the forelimb musculature collectively had the signature of high frequencies in their EMG intensity spectra for SH, whereas the opposite pattern was similarly observed for SW and VC. Forth, the shoulder (PSA and DELT) and elbow (BCR) flexors had the characteristically low frequencies in their EMG intensity spectra across locomotor behaviors. These data are both surprising and informative, and overall, they help to validate understanding of neuromuscular recruitment patterns involved with suspensory habits. Importantly, our results refine our expectations for the mechanical properties of limb bones to endure tensile versus compressive loading, which we are currently testing in tree sloths.

The suspensory habits of sloths require their musculoskeletal system to resist mainly tensile loads. As predicted, the forelimb flexors are active during the majority of the contact phase of SW, whereas the extensors are activated in the contact and swing phases, and these *in vivo* patterns of muscle activation match mechanical predictions for sloths (Nyakatura, 2012; Nyakatura and Andrada, 2013) and previous EMG findings for *Nycticebus* (Jouffroy and Stern, 1990). The forelimb of *B. variegatus* notably contains strong flexors that are collectively capable of 2–5 times greater joint torque application than its extensor musculature (Olson et al., 2018). Hence, unlike pronograde arboreal locomotion, sloths rely on their forelimb flexors for below-branch stabilization (Miller, 1935; Mendel, 1985) and propulsion (Granatosky and Schmitt, 2017) during suspensory locomotion. However, during SW, the forelimb flexors of the slow loris appear to function mainly in support/stabilization, whereas activation of its hindlimb extensors late in the contact phase indicate a greater role in propulsion (Jouffroy and Stern, 1990). Comparable EMG data are not yet available for sloth hindlimbs.

One of the goals of this study was to interpret the functional roles of specific limb muscles during suspensory locomotion. Although, we acknowledge that formal studies of limb kinematics and kinetics in *Bradypus* are needed to confirm the our interpretations of muscle function from EMG. From the video recordings, the forelimb is in a protracted position at the beginning of an SW stride, and as the contact phase progresses, the limb is retracted. Early activation of the biarticular BB may contribute to protraction of the forelimb during late swing and its sustained large activation intensity could be related to its ability to stabilize both the shoulder and elbow joints during contact. It is also possible that the lengthy (>50% stride) and large activations of BB (and BCR) provide some propulsion over the first half of limb contact. EMG onsets of the PSA and DELT occurred nearly 20–30% into the stride cycle also indicating a role in shoulder joint stabilization and controlled forward progress. Moreover, their intermediate activation intensities may be related to a limited degree of forelimb retraction in *Bradypus*, which agrees with kinematics observed for *C. didactylus* showing that its forelimb is most frequently in a neutral posture for SW (Granatosky et al., 2018). Unlike *Choloepus*, however, the elbow joint of *B. variegatus* is flexed throughout the majority of the stride cycle. In particular, the EMG activation patterns of the strong BCR most likely indicate the importance of its role as the main flexor of the elbow joint for body weight support.

The function of BCR in *Bradypus* during SW mimics the anti-gravity role of the m. brachialis in *Nycticebus* (Jouffroy and Stern, 1990). Both muscles are activated early in and maintain elbow flexion over the contact phase to control the distance between the body and support. Individuals in our study were observed to the bring the body closer to the support early in the stride. Dissimilar to 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 either the medial or lateral head of the m. triceps brachii performs this function in addition to active extension of the elbow joint during swing phase. However, the late, bi-phasic activation pattern of TBLO matches that of the m. triceps brachii lateral head in *Nycticebus* (Jouffroy and Stern, 1990), suggesting the lack of early co-activation of major forelimb flexors and extensors during SW. The initial activation of TBLO occurs approximately when the ipsilateral hindlimb is being advanced and thus its activity could provide stabilization to the shoulder joint late in contact. Alternatively, activity of TBLO during its first burst likely provides some propulsion

following mid-contact as indicated for the slow loris walking below-branch (Jouffroy and Stern, 1990), while activity of its second burst facilitates elbow joint extension in swing phase.

Digital flexion typically occurs just prior to complete purchase of the substrate by early activations of FDP during SW. While carpal joint flexion and abduction (ulnar deviation) could not clearly be discerned from only sagittal video recordings, the early, elongated, and moderately intense activations of both PL and FDP imply active maintenance of the carpus in this position. Kinematic evaluations of *Choloepus* show that it maintains the carpus almost exclusively in abduction during SW (Granatosky et al., 2018), and our individuals of *Bradypus* notably placed the majority of their forefeet on the beam to accommodate its large diameter, which requires both strong carpal flexion and abduction. Exact foot posture remains to be confirmed by comparable kinematic analyses in three-toed sloths. Nonetheless, active gripping of the substrate is prolonged until late into contact as would be expected. The initial activation of EDC overlaps with activity of the distal flexors suggesting that the digital extensors counterbalance flexion of the carpus and digits where PL and FDP activation intensity is largest. Similar to TBLO, deactivation of EDC in contact and reactivation in swing phase (to extend the carpus/digits in preparation of a new grasp) of SW may demonstrate typical flexor vs. extensor EMG activations in sloth limbs.

Beyond the similarity in stride parameters, the consistency in EMG patterns between SW and VC was notable as was previously observed in climbing *Nycticebus* (Jouffroy and Stern, 1990). The forelimb is protracted at the beginning of a climbing stride and is retracted as the contact phase progresses, possibly by the action of PSA, which activates earlier (10% stride) compared to its delayed onset during SW. We speculate that m. latissimus dorsi would act as a major limb retractor during both SW and VC. Unfortunately, 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 muscle could partially explain the relatively lower intensities of PSA and DELT during VC compared to those determined for SW. Nonetheless, as with walking, the elbow joint remains relatively flexed throughout the stride cycle except during extension in late contact and swing phase when the animal reaches for new purchase of the substrate. Thusly, the hypothesized roles of BB, BCR, and TBLO remain largely unchanged, albeit TBLO likely provides greater propulsion by pushing throughout late contact during VC.

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, our video

recordings of climbing in a frontal view do not allow for estimation of how much carpal flexion occurs as sloths ascended a fenced enclosure. Delayed EMG onset and low burst intensities of the PL suggest the carpal joint may remain relatively extended during VC on a fence, although our findings for PL during VC are tenuous, especially those for burst intensity. We recognize that allowing individuals to climb a natural substrate would have resulted in a forefoot position more similar to that previously observed in *Bradypus* (Mendel, 1985). Under these conditions, PL would be expected exhibit an early EMG onset and prolonged activation as it does during SW. The EDC, however, shows the opposite pattern of longer, more intense activation in the contact phase of VC to maintain a relatively extended carpal joint. The TBLO and EDC are activated again to purportedly extend the elbow and carpus/digits, respectively, as the forelimb is protracted when sloths ascend the substrate. Moreover, both extensors were more intensely activated relative to the flexors during VC, indicating that beyond mid-contact, the extensors play a larger role in resisting gravity than do flexors when climbing (Jouffroy and Stern, 1990).

Integrated Suspensory Limb Function

Function of the forelimb flexors appears to play a role that minimizes overall muscle activity in postural suspension consistent with our predictions. Perhaps most revealing was that the distal flexors consistently showed the lowest levels of activation among all flexor muscles across the three functional behaviors analyzed. This finding suggests that structure-function in sloth digital flexors may be analogous to a suspensory apparatus found in upright ungulates (Hildebrand, 1960; Butcher et al., 2007, 2010). For example, EMG recordings from the digital flexors and forelimb extensors of horses support this hypothesis by showing minimal activation during standing (Jansen et al., 1992; Harrison et al., 2012). The muscle activation patterns for the FDP in *Bradypus* are in accord with this previous observation and thus sloth distal flexor muscles may function to lower metabolic energy expenditure. By this mechanism, the FDP would produce enough force to counteract tensile loading of its tendons to sustain flexion of the digits/claws for grip on the substrate, all while requiring low-level muscle activity.

In contrast, EMG intensity of the forelimb muscles peaked during SW, which was not originally anticipated. Two aspects of sloth locomotor mechanics may explain this result: 1) greater levels of muscle activation during SW may be related to the application and maintenance of larger horizontal and medial substrate reaction forces (SRF) by the forelimbs compared to those of the hindlimbs (Granatosky and Schmitt, 2017); and 2) body weight has been shown to

be equally distributed between fore- and hindlimb pairs during SW (Granatosky et al., 2018). Climbing should place relatively greater demands on the hindlimbs for body weight support, and while comparable kinetics data are not yet available for VC in sloths, EMG intensities were likely lower than those observed for SW because of shared roles in propulsion by fore- and hindlimbs to move up the substrate as found in primates (Hanna et al., 2017). Studies of muscle activities in the hindlimb extensors similar to those for lorisids (Jouffroy and Stern, 1990) would be a good test of this hypothesis. Moreover, it is of particular interest to assess the functional roles of the hindlimb musculature because climbing is an energetically expensive behavior in mammals (Hanna et al., 2008) and EMG patterns from sloth hindlimbs also could be modulated, regardless of behavior, to offset metabolic cost. Such a proposed neuromuscular modification would match well with novel data (Spainhower et al., in revision) showing that sloth hindlimb muscles are considerably slower contracting than their forelimb musculature.

Large activations of the two elbow flexors contributed most to the significance in EMG intensity during suspensory walking. Even without an EMG recording of maximum voluntary contraction to normalize the EMG, relative mean ratios of EMG intensity greater than 0.70 for BB and BCR further emphasize the importance of elbow joint for suspensory function (Fujiwara et al., 2011). Sustained, strong activations by the elbow flexors can mitigate tensile limb loading to relieve stresses acting at the shoulder joint during weight-bearing as previously suggested (Nyakatura et al., 2010; Granatosky et al., 2018). This could be chiefly important for *Bradypus*, which has a highly mobile pectoral girdle (Nyakatura and Fischer, 2010) due to the lack of muscular attachments to the cervical and cranial regions of the neck and head, respectively, unlike the condition in *Choloepus* (Miller, 1935). Outside of these differences the intrinsic forelimb myology of *B. variegatus* is more conservative compared with *Choloepus*. In particular, two-toed sloths enhance elbow flexion for anti-gravity support via a muscle chain among mm. biceps brachii long head, deltoideus, and pectoralis superficialis posterior (Nyakatura and Fischer, 2011) that is somewhat similar to that in gibbon forelimbs (Jungers and Stern, 1980). In three-toed sloths, however, the strong BCR has the ability to apply substantial flexor torque due to its large area and long moment arm at the elbow joint (Olson et al., 2018). Large activations of the elbow flexors in *B. variegatus* may explain the previous finding by Granatosky et al. (2018) that sloths exert appreciable and constant medial force on the substrate during SW.

In addition to the mechanics at the elbow joint, EMG activation patterns for the shoulder musculature offer new insights into locomotion in sloths. Suspensory locomotion in sloths does not involve pendulum-based energy exchanges (Nyakatura and Andrada, 2013), yet the mechanism by which they maintain a constant horizontal position of their center-of-mass (CoM) throughout a stride (Granatosky et al., 2018) is not fully understood. Muscle activations for *B. variegatus* help to reconcile this set of previous findings by the observed delayed activation of PSA, which may coincide with a hypothesized (Granatosky et al., 2018) co-activation of an ipsilateral hindlimb flexor to decelerate the CoM and prevent the substrate from oscillating. Although the two heads of m. pectoralis superficialis are slightly distinct in *B. variegatus* (Olson et al., 2018), we agree that the slow-contracting PSA (Spainhower et al., 2018) is most likely a main stabilizer of the shoulder joint during SW. Notably, slow MHC fiber type % of m. sartorius and m. adductor (Spainhower et al., in revision) is comparable to that of PS (both heads), and we further hypothesize that co-activation of these two muscles with PSA may be the mechanism by which sloths balance their body weight between the fore- and hindlimbs during SW. The complimentary pairing of opposing muscles with an ability to adduct the limbs at the shoulder and hip joints may also serve to make the body axis rigid and prevent swinging.

A suspensory system entirely driven by muscle activation requires modifications beyond passive tendon support (Mendel, 1981b) to minimize the metabolic cost of contraction. Two additional features of sloth limbs are possible strategies to economize locomotion. First, despite differences in behavioral and ecological preferences between *Bradypus* and *Choloepus* (Mendel, 1981a, 1985; Hayssen, 2010, 2011), members of both genera move at low speeds (Britton, 1941; Granatosky and Schmitt, 2017) and have large duty factors (Nyakatura et al., 2010). Their slow movements are emphasized by the LSDC gait of *B. variegatus* during SW. This pattern of footfall phasing may not only be more stable (Granatosky et al., 2019), but it is also expected to minimize mechanical work (Usherwood and Self Davies, 2017). It is interesting that diagonality and duty factors for VC parallel those observed for SW, thus velocity may be constrained in *Bradypus* as related to selection for stealth and cryptic movements, its main form of predatory avoidance (Carillo et al., 2009). Second, we suggest that sloth locomotor patterns are strongly related to selective recruitment of slow versus fast MU and limb muscle fiber type characteristics (Spainhower et al., 2018), which in turn are correlated with extremely low basal metabolism (Pauli et al., 2016; Cliffe et al., 2018), therefore reflecting their critical need to conserve energy.

Neuromuscular Recruitment Patterns in Sloths

The activation parameters of flexor muscles inform the potential recruitment patterns of slow and fast MU in sloth forelimbs consistent with our main objective. Wavelet analysis demonstrated that despite low EMG intensity, the EMG mean frequencies (from the EMG frequency spectra) of the forelimb musculature were overall the highest during SH. Higher mean EMG frequencies have previously been associated with faster muscle 'fiber types' when recorded using fine-wire EMG in animal studies (e.g., Anapol and Jungers, 1987; Hodson-Tole and Wakeling, 2007; Lee et al., 2013), and these results match the slow-to-fast gradient in MHC expression observed distally along the forelimbs of *Bradypus*. Specifically, high mean EMG frequencies of FDP and PL are consistent with their large distributions (54–65%) of fast MHC-2A fibers (Spainhower et al., 2018), and although fiber type % was not quantified for EDC, it is expected to consist of a majority of fast MHC-2A fibers based on the spectral properties of its EMG.

Wavelet results further imply that it is possible that MU in sloth muscles are modified in both size and number, whereby smaller, faster-contracting MHC-2A fibers may be organized into smaller-sized MU (fewer fibers) that are initially recruited when less muscle force is needed to balance the body weight. Lower magnitudes of muscle force should be needed to stabilize the shoulder, elbow, and carpal/digital joints during SH, consequently placing greater emphasis on the strength/stiffness properties of the distal flexor tendons for postural support, which is supported by recent preliminary data (Mossor et al., 2019). Overall, the high mean EMG frequencies for the forelimb flexors during SH infer recruitment of small, fast MHC-2A fibers (i.e., activations of fast MU) from both the proximal and distal musculature, matching a major functional role of the limb flexors in joint stabilization and support.

Recruitment of potentially larger, slow MU for enhanced force production is best reflected by the low mean EMG frequencies in the proximal flexors PSA and DELT, as well as the distal BCR. These muscles collectively showed the lowest mean EMG frequencies (82–95 Hz) in *B. variegatus*. While comparable wavelet data from other mammals capable of suspensory or clinging behaviors are not available, several studies of terrestrial locomotion (Wakeling et al., 2001; Hodson-Tole and Wakeling, 2007; Lee et al., 2013) in mammals with limb muscles that co-express MHC-1 and -2A fibers only, report mean EMG frequencies for slow MU of limb extensors (e.g., m. gastrocnemius) that generally range from 120–200 Hz. The lower mean EMG frequencies observed in PSA, DELT, and BCR of *Bradypus* suggest a very low maximum

contractile velocity (V_{max}) for their slow MHC-1 fibers that does not closely follow body size scaling relationships for a reduction in V_{max} with increasing body size (Toniolo et al., 2007).

The mean EMG frequencies for these three flexor muscles in sloths are also lower than those that have been estimated from the slowest motor units in fish and cats (Wakeling et al., 2002), rats (Wakeling and Syme, 2002), and goats (Lee et al., 2013), thus they may represent some of the slowest contractile properties of twitch muscle ever reported. Moreover, trading off contractile velocity for enhanced force output would match the most common pattern observed for sloth muscles having the lowest mean EMG frequencies, but significantly larger EMG intensity during SW (Fig. 4). Selectively recruiting potentially larger, slow MU in the forelimb flexors appears to be necessary for propulsion during suspensory locomotion. Notably, this purported neuromuscular function involved sloth locomotor habits could provide advantages for enhanced force production despite a larger volume of active muscle recruited, but activation of large, slow fibers at relatively low EMG frequencies. The extremely slow-contracting BCR in *B. variegatus* (Spainhower et al., 2018) is perhaps the best example of a muscle that emphasizes force while minimizing shortening velocity (Olson et al., 2018), and these contractile features are generally reflective of sloth forelimbs.

Finally, the collective modulations in muscle activations between postural and locomotor behaviors are shown by the PCA loading scores (see Fig. 6), and these key findings help to clarify use of selective or preferential recruitment of MU (Wakeling, 2004) in sloth muscles. The overall higher mean EMG frequencies observed for SH associated with recruitment of fast MHC-2A fibers, 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, MU recruitment patterns in sloth forelimbs do not strictly follow the size principle (Henneman et al., 1974). However, this potentially unique feature in sloth limbs helps them reduce their overall metabolic energy expenditure, which is similar to that suggested for numerous other axial (Jayne and Lauder, 1994) and limb muscles (Smith et al., 1980; Wakeling, 2004) in diverse vertebrate taxa. Such a pattern of muscle recruitment may be critical to how *B. variegatus* offsets the cost of flexor activation for postural suspension, and the degree of neuromuscular modification that we propose may be even more specialized in *Choloepus*. We also hypothesize that suspensory primates have evolved similar neuromuscular control features in their forelimb muscles. For example, lorisids are ecologically and

physiologically convergent with sloths. Last, it was essential to observe the similarity in muscle activation patterns between SW and VC. When moving either horizontally below or vertically up the substrate, sloth forelimb muscles appear to have largely similar performance requirements, which could be representative of energy savings by coupling the adaptive strategies of slow/intermittent movements with selective recruitment of large, slow MHC-1 fibers.

Conclusions

Despite the requirement of lengthy and large flexor muscle activations during suspensory locomotion, sloths appear to have neuromuscular modifications that can offset the cost of muscle contraction, including reduced EMG intensity for postural support and several specialized slow-contracting muscles with low mean EMG frequencies. In particular, *B. variegatus* may selectively recruit smaller, fast motor units at lower activation levels during suspensory hanging, but uses recruitment of larger, slow motor units during suspensory walking and vertical climbing when greater force production is needed. Three-toed sloths therefore demonstrate a division of labor in their forelimb musculature and this major finding provides the clearest support for the hypothesis that sloth forelimb muscles reflect a critical part of a physiological system specialized to conserve metabolic energy. We further clarified several muscular functional requirements for suspension and informed other limb mechanical features necessary to maintain even suspensory support of the body weight in sloths.

Competing interests

The authors declare no competing financial interests.

Author contributions

M.A.G. developed the concepts and experimental approach, collected and analyzed data, and drafted and edited the manuscript; J.M.W. performed data analysis, prepared figures, and edited the manuscript; D.M.M. performed data analysis, prepared figures, and edited the manuscript; D.H-S. performed data collection and edited the manuscript; J. A-A. developed the concepts and experimental approach, performed data collection, and edited the manuscript; M.T.B. developed the concepts and approach, performed data collection, and drafted and revised the manuscript.

List of symbols and abbreviations

DF – duty factor

EMG – electromyography

EMG_{dur} – EMG burst duration

EMG_{on} – onset time of muscle activation

EMG_{off} – offset time of muscle activation

EMG_{int} – EMG burst intensity

FF – footfall (grasp)

FO – foot off (release)

PCA – principle component analysis

SH – suspensory hanging

ST – stride time

SW – suspensory walking

VC – vertical climbing

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Figures
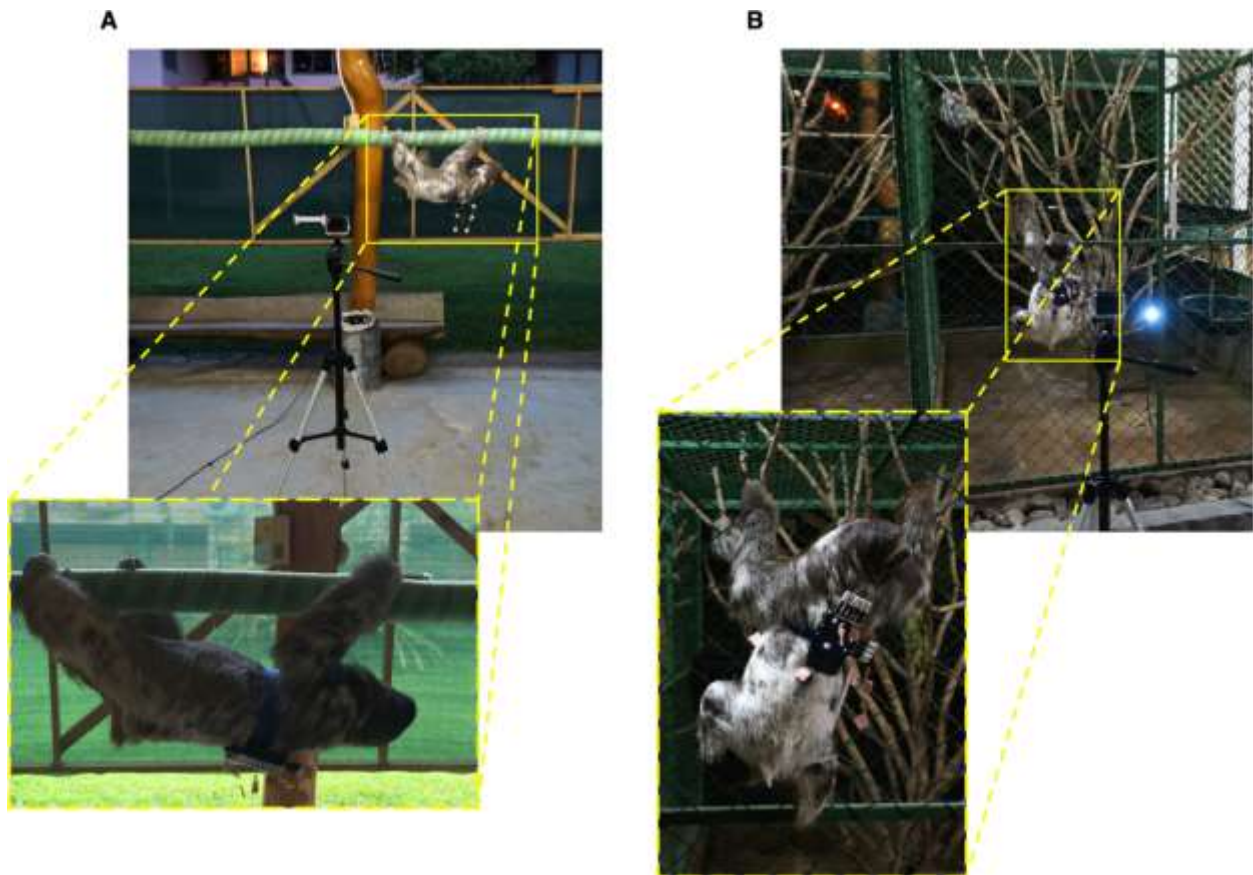
Fig. 1 Experimental set-up. Shown is individual Bv4 on (**A**) a beam (4.57m x 0.22m x 1.52m) constructed from bamboo was used for trials of suspensory hanging (SH) and suspensory walking (SW), or (**B**) a fenced animal enclosure (2.00m x 3.97m x 3.12m) used for trials of vertical climbing (VC). The 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.
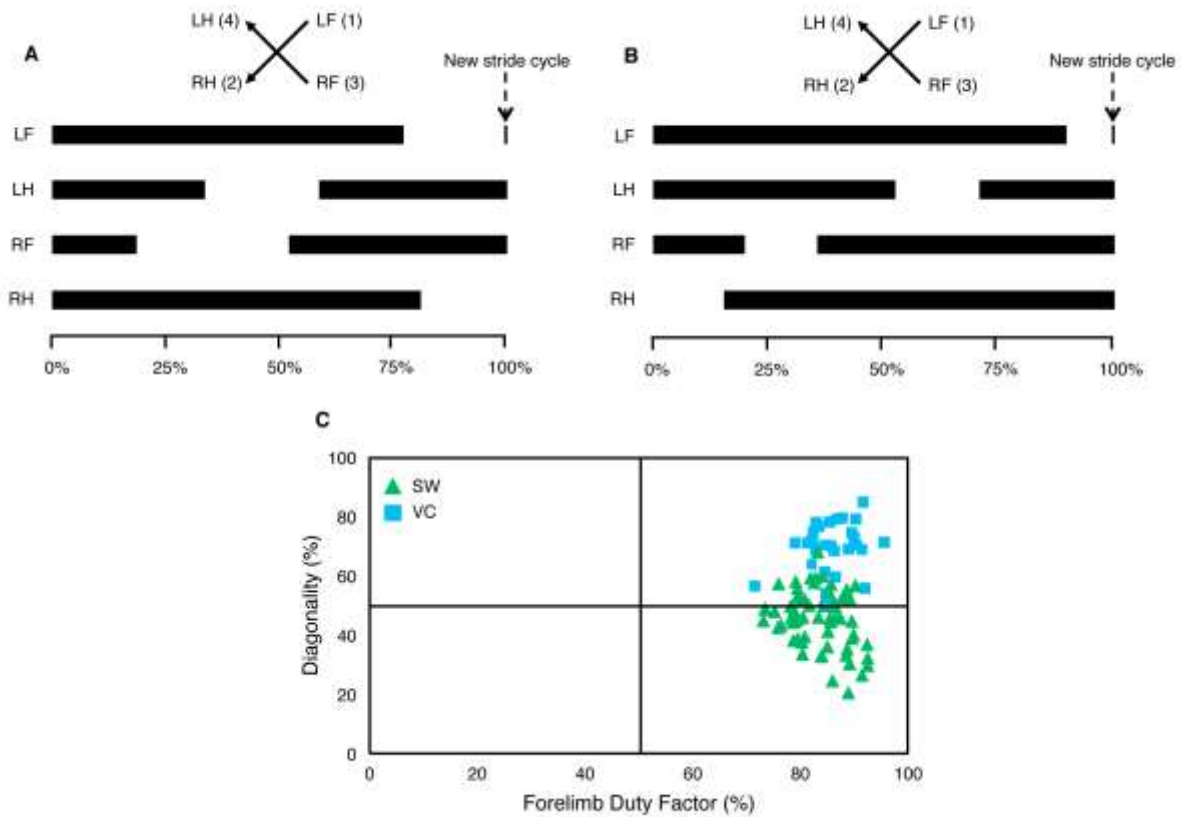
Fig. 2 Gait patterns in three-toed sloths. Strides for (**A**) suspensory walking (SW) and (**B**) vertical climbing (VC) were typified by placement of the left forelimb (reference limb) followed by sequential contact (near couplet) of the right hindlimb and completed by sequential advancement of the right forelimb and left hindlimb pair. Limb phase metrics of (**C**) diagonality and forelimb duty factor indicate that *Bradypus* is largely restricted to use of the lateral sequence diagonal couplet (LSDC) gait. Diagonality was calculated as the percentage of the stride cycle by which the hindlimb footfall precedes the forelimb footfall on the same side (Lemelin et al., 2003).
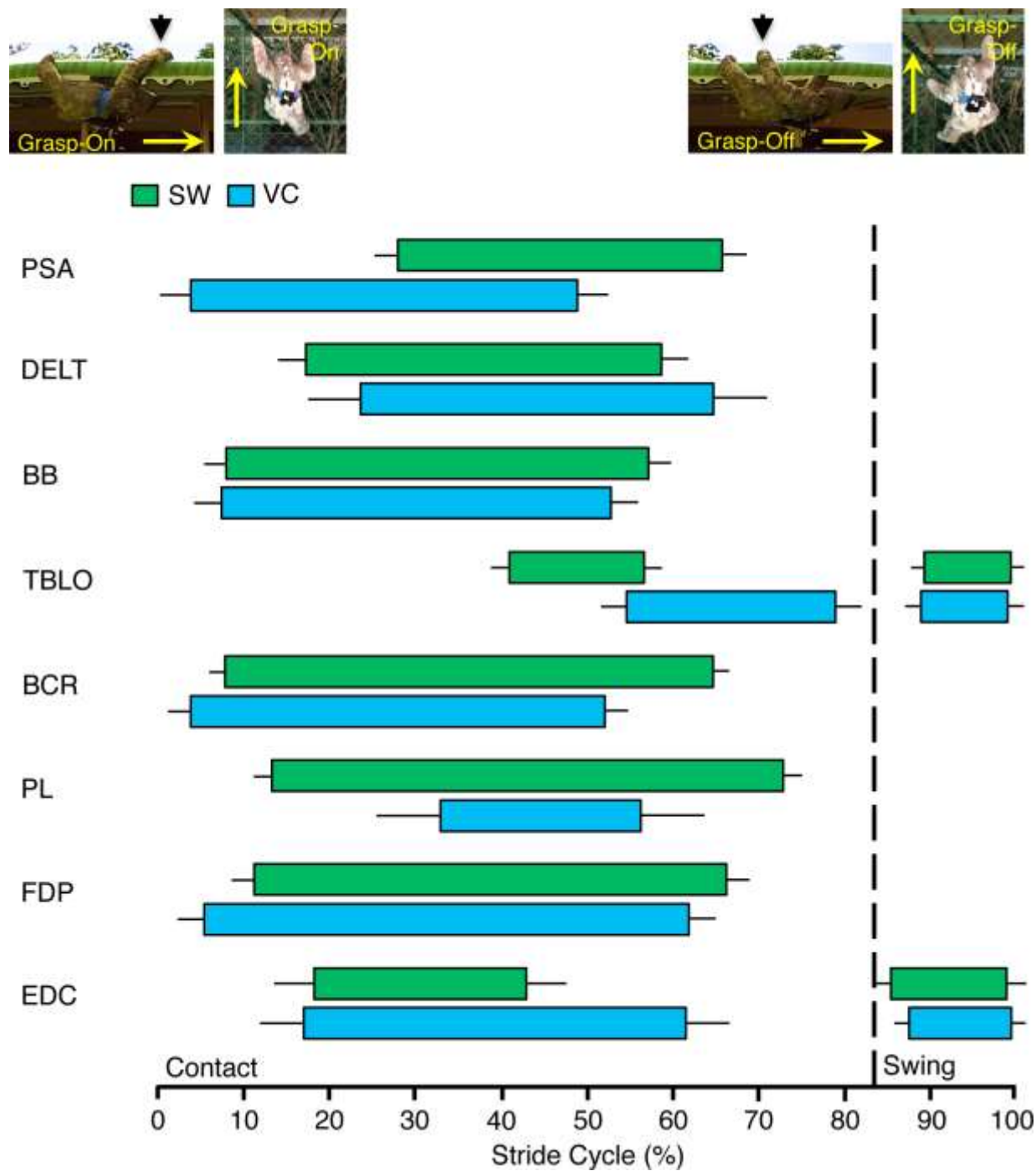
Fig. 3 Mean EMG burst duration (%stride) for sloth forelimb muscles when suspensory walking (SW) and vertical climbing (VC). Burst duration is expressed as a percentage of the stride (0–100%), with horizontal bars representing averages for each muscle during a stride.

Values are means±s.e. (standard error) across individuals (N=3–6 individuals per muscle) and represent a data subset of the only the most typical pattern of EMG activations (bi-phasic activity for TBLO and EDC). The solid vertical line marks the end of limb contact (Grasp Off-release) at a average duty factor of 0.83 for both SW and VC in three-toed sloths. Video frames (above) depict representative positions of the left forelimb at 10% intervals throughout a stride. Shown are individuals Bv1 and Bv5 for SW and VC, respectively. Muscle abbreviations: PSA, pectoralis superficialis anterior; DELT, deltoideus; BB, biceps brachii; TBLO, triceps brachii long head; BCR, brachioradialis; PL, palmaris longus; FDP, flexor digitorum profundus; EDC, extensor digitorum communis.
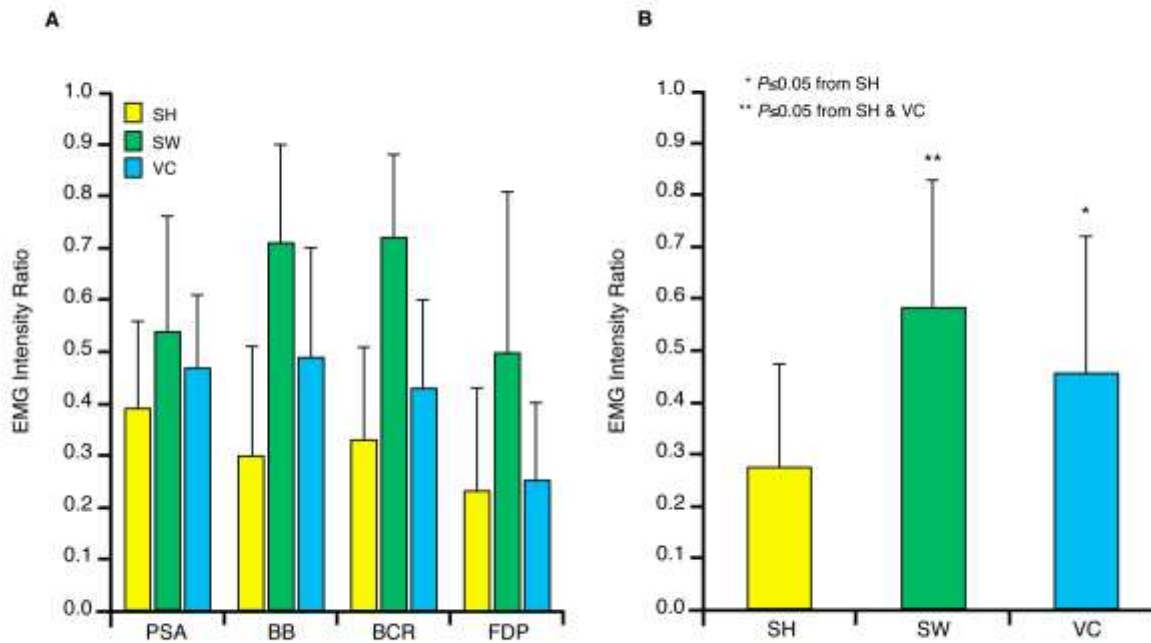
Fig. 4 Mean EMG intensity for sloth forelimb muscles. Burst activity from (**A**) four major flexor muscles and (**B**) all forelimb muscles combined. Mean EMG intensity was normalized to the maximum EMG intensity recorded for each muscle and functional behavior: suspensory hanging (SH-yellow), suspensory walking (SW-green), and vertical climbing (VC-turquoise). EMG intensity is expressed here as ratios (0–1.0: Gillis and Biewener, 2001), with columns representing averages for each muscle during contact of the left forelimb. Values are means±s.d. (standard deviation) across all individuals (*N*=6). 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.
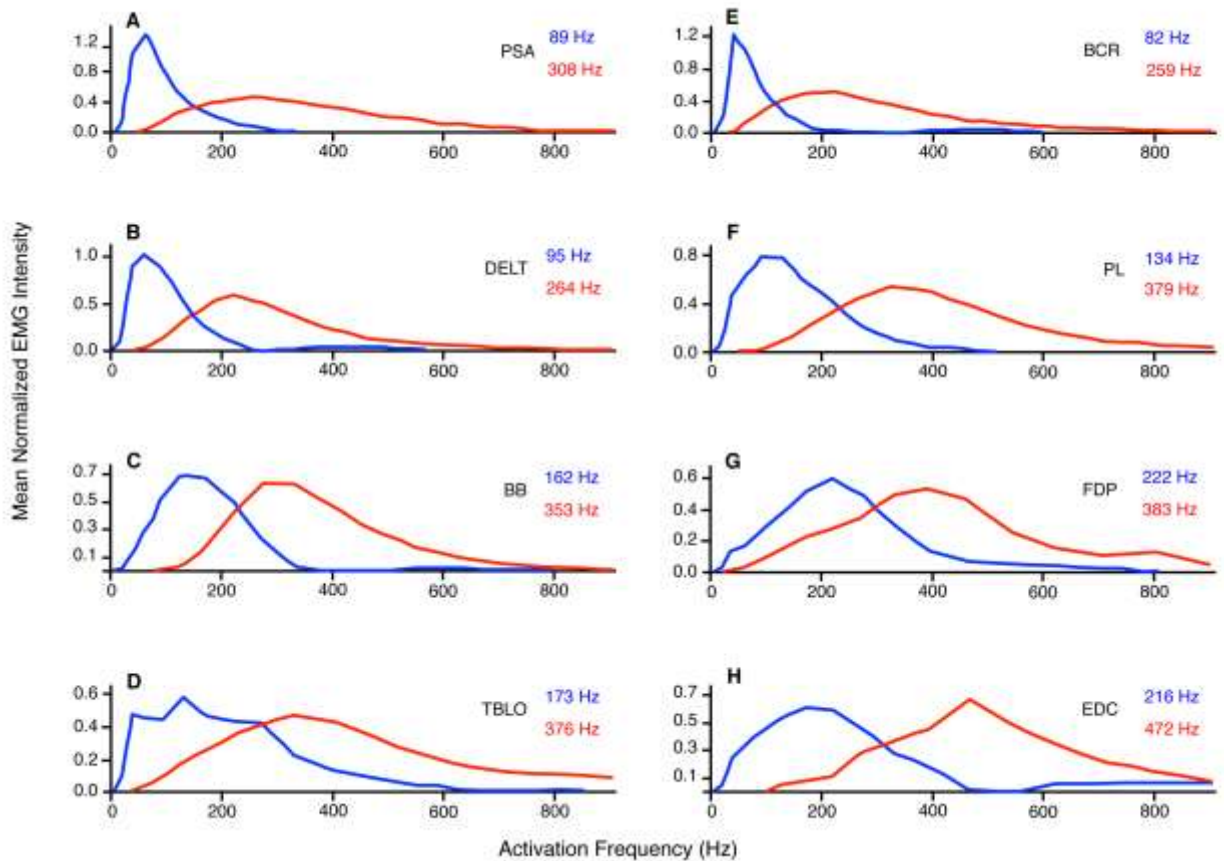
Fig. 5 Mean EMG frequencies from sloth forelimb muscles across three functional behaviors. Plots of mean normalized EMG intensity vs. frequency for the muscles (**A**) PSA, (**B**) DELT, (**C**) BB, (**D**) TBLO, (**E**) BCR, (**F**) PL, (**G**) FDP, and (**H**) EDC. Data shown are collated for functional behaviors SH, SW, and VC in *B. variegatus*. Blue curves (left) are low-frequency components whereas red curves (right) are high-frequency components. Color-coded values are pairs of low and high mean frequencies for each muscle.
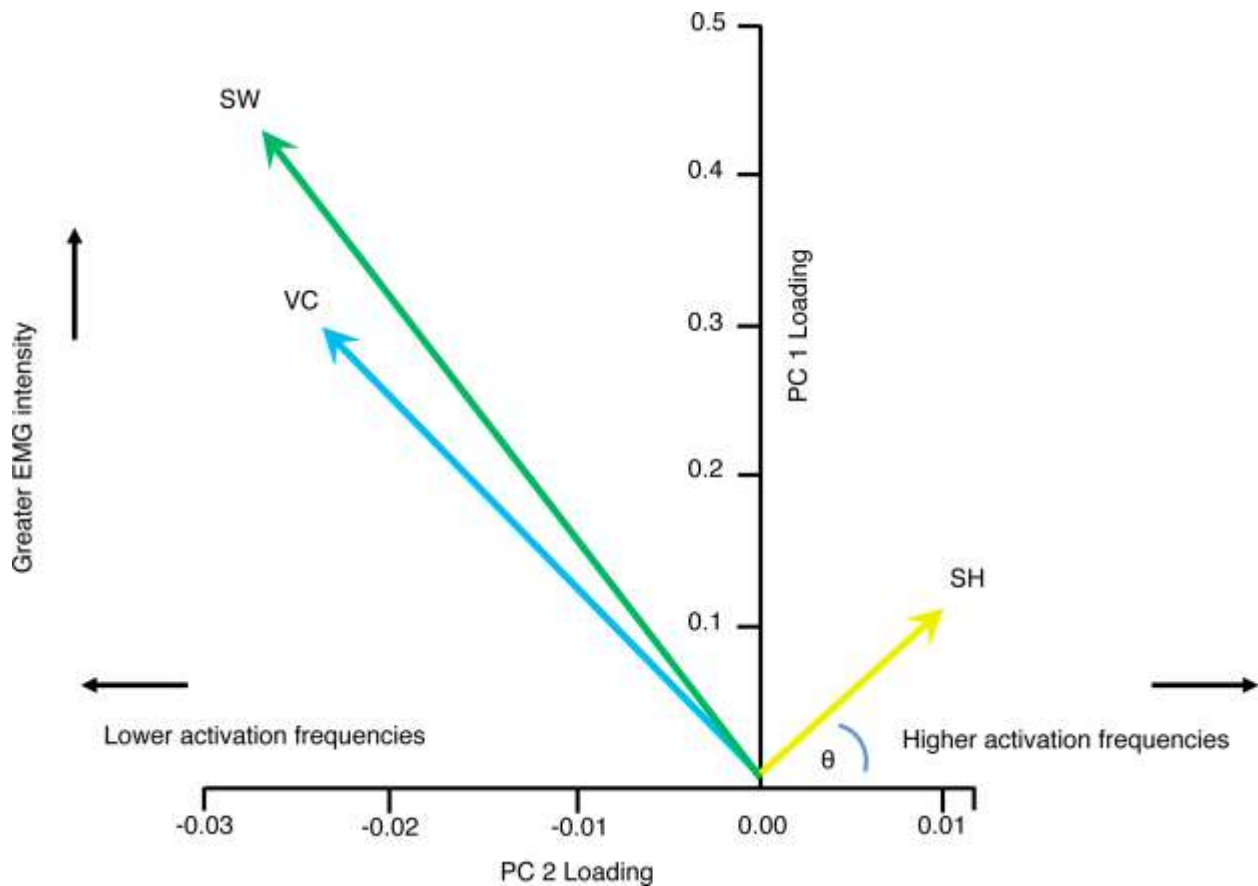
Fig. 6 PCA plot of muscle intensity-frequency spectral distributions for each functional behavior in three-toed sloths. Principal component loadings are plotted 2D on vertical (PC1) and horizontal (PC2) axes. Eigenvectors have an angle theta (θ) from the origin and are color-coded for the functional behaviors SH (yellow), SW (green), and VC (turquoise). Loadings along PC1 represent EMG intensities of activation where a shorter vector length indicates smaller burst intensity and a longer vector length indicates larger burst intensity. Loadings along PC2 represent recruitment frequencies with positive scores (smaller θ) indicating higher frequency activations and negative scores (larger θ) indicating lower frequency activations. Data shown are collated for all muscles and functional behaviors sampled in *B. variegatus*.

Table 1. Temporal variables of EMG activation of forelimb muscles in *B. variegatus*.

	PSA	DELT	BB	TBLO	BCR	PL	FDP	EDC
Suspensory Walking								
EMG Onset (%)	28.8±20.3	17.6±20.3	8.36±19.4	56.4±30.9	8.29±8.42	13.7±15.1	11.5±13.4	50.0±37.2
EMG Offset (%)	63.2±23.5	61.1±24.0	61.7±19.5	77.6±30.4	68.0±13.2	73.2±9.17	69.9±14.9	86.8±33.9
EMG Burst Duration (% Cycle)	38.3±20.2	42.5±19.1	50.3±20.9	13.8±11.3	57.9±15.8	60.2±15.7	56.2±18.4	19.3±16.7
Total EMG Activation (% Cycle)*	31.9±19.8	33.7±21.4	48.8±21.1	18.1±16.8	50.5±21.3	50.7±22.7	53.6±20.7	19.5±17.1
Vertical Climbing								
EMG Onset (%)	3.68±21.1	23.6±27.3	7.30±18.8	61.1±20.8	3.64±8.60	32.8±28.6	5.41±14.5	57.0±39.3
EMG Offset (%)	52.3±16.4	72.1±18.5	48.8±16.5	86.0±12.3	52.4±12.8	56.6±35.9	61.2±17.8	93.1±21.6
EMG Burst Duration (% Cycle)	45.3±14.7	41.5±27.2	45.6±14.1	20.6±13.7	48.8±13.6	23.8±17.7	56.7±18.3	21.6±17.7
Total EMG Activation (% Cycle)*	43.8±15.5	28.9±25.7	39.4±16.1	21.2±13.7	42.9±17.4	--	54.9±19.4	23.6±17.6

Values are pooled means±s.d. of all data per EMG burst (= large deviations)

*Total activation duration across each burst when muscles showed multiple bursts per stride (most typical for TBLO and EDC)

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

Muscle abbreviations: PSA, pectoralis superficialis anterior; DELT, deltoideus; BB, biceps brachii; TBLO, triceps brachii long head; BCR, brachioradialis; PL, palmaris longus; FDP, flexor digitorum profundus; EDC, extensor digitorum communis

Table 2. Pooled means of EMG intensity ratios for each muscle and behavior.

Functional behavior	PSA	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

In bold are the largest mean±s.d. ratios calculated
*Data analyzed for PL during vertical climb was limited due to usable EMG recordings from only two individuals
SH, suspensory hanging; SW, suspensory walking; VC, vertical climbing
Muscle abbreviations are the same as those in Table 1

Suppl. Fig. 1 – Raw EMG Hang (SH)

Fig. S1 Raw EMG from eight forelimb muscles during suspensory hanging (SH) in sloths. 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. PSA, pectoralis superficialis anterior; DELT, deltoideus; BB, biceps brachii; TBLO, triceps brachii long head; BCR, brachioradialis; PL, palmaris longus; FDP, flexor digitorum profundus; EDC, extensor digitorum communis.

Suppl. Fig. 2 – Raw EMG Walk (SW)

Fig. S2 Raw EMG from eight forelimb muscles during suspensory walking (SW) in sloths. 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 mark 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. S1.
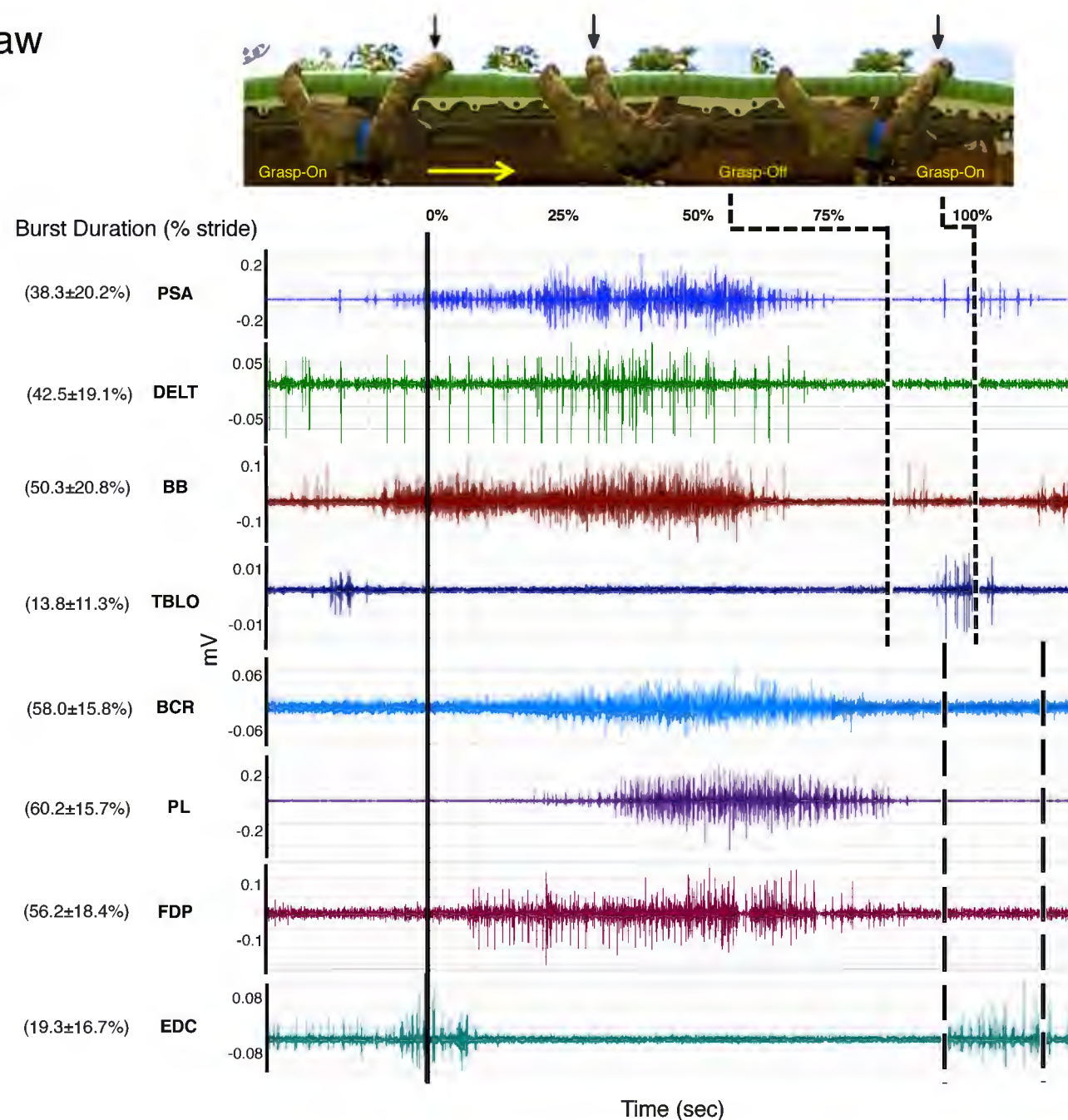
Suppl. Fig. 3 – Raw EMG Climb (VC)

Fig. S3 Raw EMG from eight forelimb muscles during vertical climbing (VC) in sloths. 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 mark 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. S1.
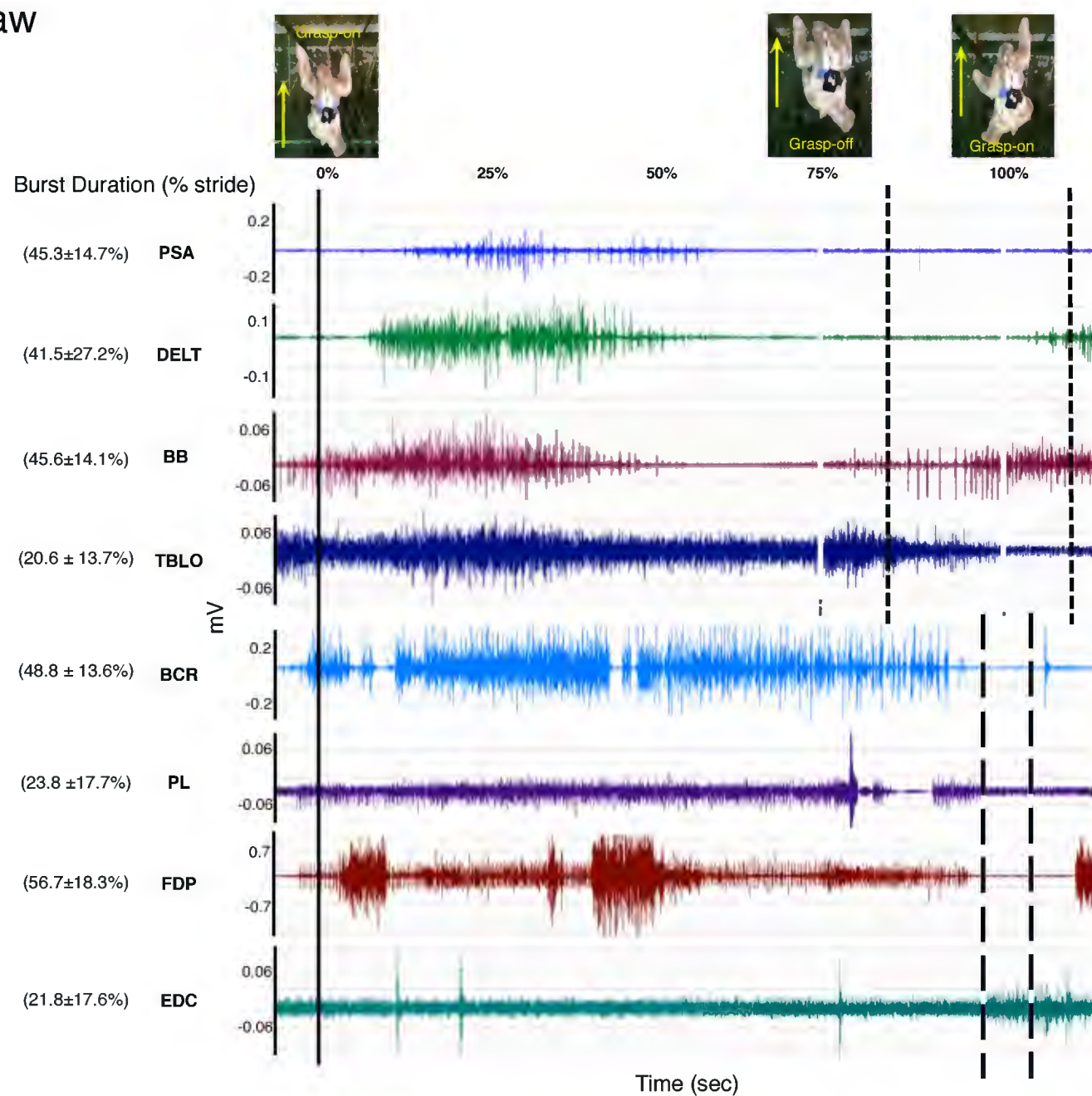
Table S1. Morphometric data for study animals and number of trials/strides/ bursts analyzed.

Sloth	Sex	Limb	Body Mass (kg)	*N* Trials	*N* Strides	*N* Bursts
BV1	M	L	4.2	6_{SH} 8_{SW} 11_{VC}	34_{SW} 29_{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}	21_{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**	$\mathbf{27_{SH}}$ $\mathbf{56_{SW}}$ $\mathbf{34_{VC}}$	$\mathbf{155_{SW}}$ $\mathbf{69_{VC}}$	**856**

N = number
In bold are either pooled mean±s.d. or total counts
SH, suspensory hanging; SW, suspensory walking; VC, vertical climbing

Table S2. Individuals, muscles, and functional behaviors for which EMG was recorded and analyzed.

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

†No EMG intensity or wavelet data available

SH, suspensory hanging; SW, suspensory walking; VC, vertical climbing

PSA, pectoralis superficialis anterior; DELT, deltoideus; BB, biceps brachii; TBLO, triceps brachii long head; BCR, brachioradialis; PL, palmaris longus; FDP, flexor digitorum profundus; EDC, extensor digitorum communis

Table S3. Stride parameters for suspensory walking and vertical climbing in *B. variegatus*.

Sloth	Stride Duration (s)	Stride Frequency (Hz)	Duty factor (ratio)	Velocity (m s^{-1})
BV1	11.0±2.85$_{sw}$	0.10±0.02$_{sw}$	0.82±0.07$_{sw}$	0.13±0.07$_{sw}$
	15.5±6.71$_{vc}$	0.08±0.03$_{vc}$	0.83±0.05$_{vc}$	0.04±0.02$_{vc}$
BV2	12.5±4.38$_{sw}$	0.09±0.03$_{sw}$	0.76±0.08$_{sw}$	0.11±0.06$_{sw}$
	15.6$_{vc}$	0.06$_{vc}$	0.91$_{vc}$	0.04$_{vc}$
BV3	29.1±13.3$_{sw}$	0.04±0.02$_{sw}$	0.84±0.06$_{sw}$	0.07±0.06$_{sw}$
	26.5±8.95$_{vc}$	0.04±0.02$_{vc}$	0.83±0.05$_{vc}$	0.03±0.02$_{vc}$
BV4	40.4±22.3$_{sw}$	0.04±0.04$_{sw}$	0.83±0.11$_{sw}$	0.05±0.02$_{sw}$
	46.60$_{vc}$	0.02$_{vc}$	0.90$_{vc}$	0.01$_{vc}$
BV5	20.0±11.6$_{sw}$	0.07±0.05$_{sw}$	0.86±0.07$_{sw}$	0.05±0.01$_{sw}$
	15.0±3.78$_{vc}$	0.07±0.02$_{vc}$	0.83±0.04$_{vc}$	0.04±0.02$_{vc}$
BV6	14.8±7.08$_{sw}$	0.08±0.03$_{sw}$	0.85±0.05$_{sw}$	0.06±0.02$_{sw}$
	11.5±3.52$_{vc}$	0.10±0.04$_{vc}$	0.83±0.06$_{vc}$	0.03±0.01$_{vc}$
mean±s.d.	**20.3±15.2**$_{sw}$	**0.07±0.04**$_{sw}$	**0.83±0.07**$_{sw}$	**0.07±0.05**$_{sw}$
	15.6±7.33$_{vc}$	**0.08±0.03**$_{vc}$	**0.83±0.05**$_{vc}$	**0.04±0.02**$_{vc}$

In bold are pooled means±s.d.
Stride frequency is the inverse of stride duration
SW, suspensory walking; VC, vertical climbing

Suppl. Fig. 1 – Raw EMG Hang (SH)

Fig. S1 Raw EMG from eight forelimb muscles during suspensory hanging (SH) in sloths. 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. PSA, pectoralis superficialis anterior; DELT, deltoideus; BB, biceps brachii; TBLO, triceps brachii long head; BCR, brachioradialis; PL, palmaris longus; FDP, flexor digitorum profundus; EDC, extensor digitorum communis.

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Fig. S2 Raw EMG from eight forelimb muscles during suspensory walking (SW) in sloths. 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 mark 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. S1.
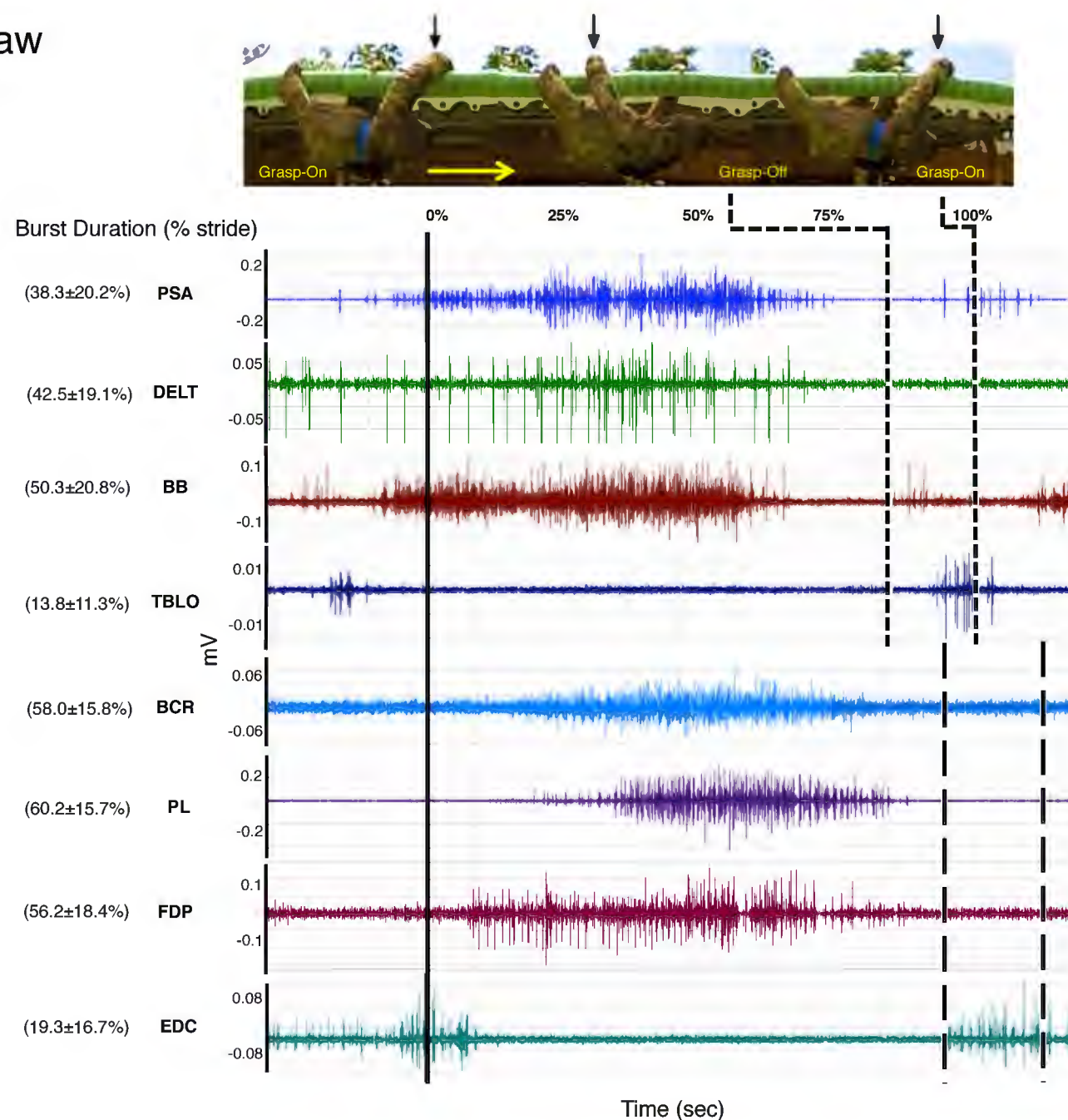
Suppl. Fig. 3 – Raw EMG Climb (VC)

Fig. S3 Raw EMG from eight forelimb muscles during vertical climbing (VC) in sloths. 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 mark 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. S1.
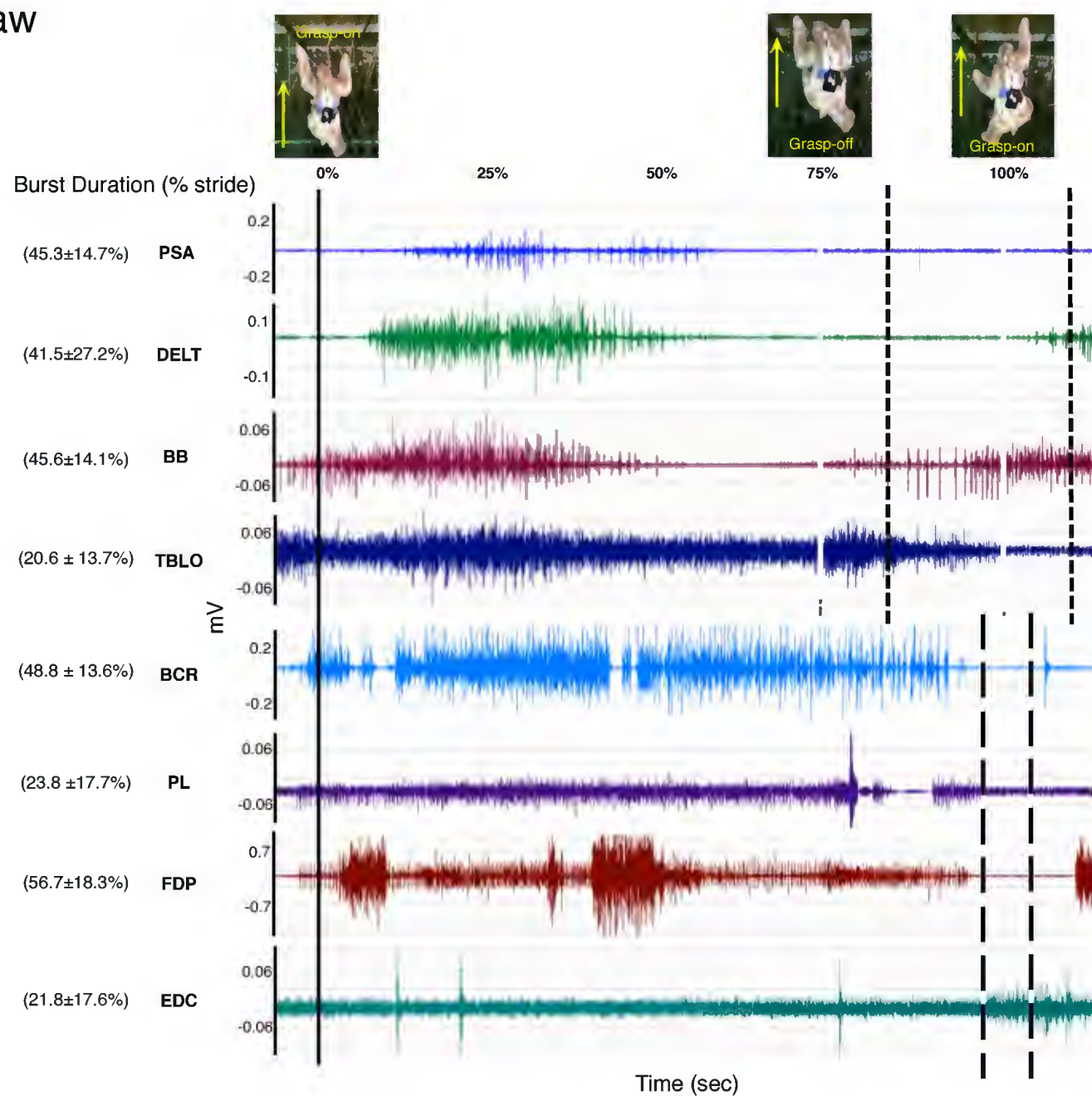
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BV5	F	L	3.5	1$_{SH}$ 13$_{SW}$ 12$_{VC}$	32$_{SW}$ 20$_{VC}$	217
BV6	F	L	3.2	2$_{SH}$ 9$_{SW}$ 6$_{VC}$	25$_{SW}$ 14$_{VC}$	127
mean±s.d.; totals			**3.9±0.6**	**27$_{SH}$ 56$_{SW}$ 34$_{VC}$**	**155$_{SW}$ 69$_{VC}$**	**856**

N = number

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SH, suspensory hanging; SW, suspensory walking; VC, vertical climbing

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BV1	SH	X	X	X	X	X	X	X	X
	SW	X	X	X	X	X	X	X	X
	VC	X	X	X	X	X	--	X	X
BV2	SH	X	X	X	X	--	--	--	--
	SW	--	X	X	X	X	X	X	X
	VC	--	--	X	X	--	--	--	--
BV3	SH	X	X	X	X	X	X	X	X
	SW	X	X	X	X	X	X	X	--
	VC	X	X	X	X	--	--	--	--
BV4	SH	X	X	X	X	X	X	X	X
	SW	X	X	X	X	X	X	X	X
	VC	--	--	--	--	--	X†	--	--
BV5	SH	X	--	--	--	X	X	X	X
	SW	X	X	X	X	X	X	X	X
	VC	--	X	X	X	X	X	X	X
BV6	SH	X	X	X	X	--	--	--	--
	SW	X	X	X	X	X	X	X	X
	VC	--	--	--	--	X	X	X	X

†No EMG intensity or wavelet data available

SH, suspensory hanging; SW, suspensory walking; VC, vertical climbing

PSA, pectoralis superficialis anterior; DELT, deltoideus; BB, biceps brachii; TBLO, triceps brachii long head; BCR, brachioradialis; PL, palmaris longus; FDP, flexor digitorum profundus; EDC, extensor digitorum communis

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BV2	12.5±4.38$_{sw}$ 15.6$_{vc}$	0.09±0.03$_{sw}$ 0.06$_{vc}$	0.76±0.08$_{sw}$ 0.91$_{vc}$	0.11±0.06$_{sw}$ 0.04$_{vc}$
BV3	29.1±13.3$_{sw}$ 26.5±8.95$_{vc}$	0.04±0.02$_{sw}$ 0.04±0.02$_{vc}$	0.84±0.06$_{sw}$ 0.83±0.05$_{vc}$	0.07±0.06$_{sw}$ 0.03±0.02$_{vc}$
BV4	40.4±22.3$_{sw}$ 46.60$_{vc}$	0.04±0.04$_{sw}$ 0.02$_{vc}$	0.83±0.11$_{sw}$ 0.90$_{vc}$	0.05±0.02$_{sw}$ 0.01$_{vc}$
BV5	20.0±11.6$_{sw}$ 15.0±3.78$_{vc}$	0.07±0.05$_{sw}$ 0.07±0.02$_{vc}$	0.86±0.07$_{sw}$ 0.83±0.04$_{vc}$	0.05±0.01$_{sw}$ 0.04±0.02$_{vc}$
BV6	14.8±7.08$_{sw}$ 11.5±3.52$_{vc}$	0.08±0.03$_{sw}$ 0.10±0.04$_{vc}$	0.85±0.05$_{sw}$ 0.83±0.06$_{vc}$	0.06±0.02$_{sw}$ 0.03±0.01$_{vc}$
mean±s.d.	**20.3±15.2**$_{sw}$ **15.6±7.33**$_{vc}$	**0.07±0.04**$_{sw}$ **0.08±0.03**$_{vc}$	**0.83±0.07**$_{sw}$ **0.83±0.05**$_{vc}$	**0.07±0.05**$_{sw}$ **0.04±0.02**$_{vc}$

In bold are pooled means±s.d.
Stride frequency is the inverse of stride duration
SW, suspensory walking; VC, vertical climbing