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HINDLIMB MUSCLE FUNCTION IN RELATION TO SPEED AND GAIT: *IN VIVO* PATTERNS OF STRAIN AND ACTIVATION IN A HIP AND KNEE EXTENSOR OF THE RAT (*RATTUS NORVEGICUS*)

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Summary

Understanding how animals actually use their muscles during locomotion is an important goal in the fields of locomotor physiology and biomechanics. Active muscles *in vivo* can shorten, lengthen or remain isometric, and their mechanical performance depends on the relative magnitude and timing of these patterns of fascicle strain and activation. It has recently been suggested that terrestrial animals may conserve metabolic energy during locomotion by minimizing limb extensor muscle strain during stance, when the muscle is active, facilitating more economical force generation and elastic energy recovery from limb muscle–tendon units. However, whereas the ankle extensors of running turkeys and hopping wallabies have been shown to generate force with little length change (<6 % strain), similar muscles in cats appear to change length more substantially while active. Because previous work has tended to focus on the mechanical behavior of ankle extensors during animal movements, the actions of more proximal limb muscles are less well understood. To explore further the hypothesis of force economy and isometric behavior of limb muscles during terrestrial locomotion, we measured patterns of electromyographic (EMG) activity and fascicle strain (using sonomicrometry) in two of the largest muscles of the rat hindlimb, the biceps femoris (a hip extensor) and vastus lateralis (a knee extensor) during walking, trotting and galloping. Our results show that the biceps and vastus exhibit largely overlapping bursts of electrical activity during the stance phase of each step cycle in all gaits. During walking and trotting, this activity typically commences shortly before the hindlimb touches the ground, but during galloping the onset of activity depends on whether the limb is trailing (first limb down) or leading (second limb down), particularly in the vastus. In the trailing limb, the timing of the onset of vastus activity is slightly earlier than that observed during walking and trotting, but in the leading limb, this activity begins much later, well after the foot makes ground contact (mean 7 % of the step cycle). In both muscles, EMG activity typically ceases approximately two-thirds of the way through the stance phase. While electrically active during stance, biceps fascicles shorten, although the extent of shortening differs significantly among gaits (P<0.01). Total average fascicle shortening strain in the biceps is greater during walking (23±3 %) and trotting (27±5 %) than during galloping (12±5 % and 19±6 % in the trailing and leading limbs, respectively). In contrast, vastus fascicles typically lengthen (by 8–16 %, depending on gait) over the first half of stance, when the muscle is electrically active, before shortening slightly or remaining nearly isometric over much of the second half of stance. Interestingly, in the leading limb during galloping, vastus fascicles lengthen prior to muscle activation and exhibit substantial shortening (10±2 %) during the period when EMG activity is recorded. Thus, patterns of muscle activation and/or muscle strain differ among gaits, between muscles and even within the same muscle of contralateral hindlimbs (as during galloping). In contrast to the minimal strain predicted by the force economy hypothesis, our results suggest that proximal limb muscles in rats operate over substantial length ranges during stance over various speeds and gaits and exhibit complex and changing activation and strain regimes, exemplifying the variable mechanical roles that muscles can play, even during level, steady-speed locomotion.

Key words: terrestrial, locomotion, gait, muscle, function, electromyography, sonomicrometry, rat, *Rattus norvegicus*, biceps femoris, vastus lateralis.

Introduction

Muscles provide the forces and mechanical work necessary for moving animals through their environments over a range of speeds. Muscles of animals that interact with fluids to generate propulsion (such as fish and birds) have been shown to undergo relatively high levels of active strain and to generate substantial amounts of work and power during locomotion

(Altringham and Ellerby, 1999; Biewener et al., 1998a). In contrast, work by Taylor and colleagues (summarized in Taylor, 1994) suggests that, during terrestrial locomotion, the limb muscles of cursorial animals probably contract with small length excursions, doing little mechanical work but generating high levels of force. Recent direct analyses of muscle force and strain during locomotion in hopping wallabies and running turkeys have corroborated Taylor's ideas (Biewener et al., 1998b; Roberts et al., 1997). Specifically, the ankle extensors of wallabies and turkeys undergo very low levels of muscle strain while generating high levels of force during the stance phase of steady, level locomotion.

By minimizing fascicle strain and, hence, the mechanical work generated by muscles within the limb, muscular forces can remain high while the energetic cost of producing these forces can be reduced, increasing the performance (Weyand et al., 2000) and economy (Roberts et al., 1997; Taylor, 1994) of the locomotor system. In addition, elastic energy stored in and released from tendons can offset much of the mechanical work that might otherwise be required by limb muscles during level, constant-speed running (Alexander, 1988; Cavagna et al., 1977). Therefore, if the mechanics of terrestrial locomotion allows limb muscles to operate generally with low strains when generating force during stance, animals may conserve metabolic energy expenditure. However, because turkeys and wallabies constitute such a small subset of terrestrial vertebrates, and ankle extensors are distal muscles, and thus provide a limited sampling of the muscle–tendon systems within the whole limb, it remains important to investigate how widespread *in vivo* isometric contractile behavior actually is in the limb muscles of terrestrial animals.

Are wallabies and turkeys in some ways unique or do the distal hindlimb muscles of a wide variety of bipeds and quadrupeds also operate under largely isometric conditions during locomotion? Work by Walmsley et al. (Walmsley et al., 1978), Whiting et al. (Whiting et al., 1984), Gregor et al. (Gregor et al., 1988) and Prilutsky et al. (Prilutsky et al., 1996) measuring *in vivo* muscle force production in relation to limb kinematics suggests that, unlike in running turkeys or hopping wallabies, the fibers of various ankle extensors in cats moving across a range of speeds can undergo substantial length changes during stance (up to 16 % reported by Prilutsky et al., 1996). Furthermore, in some of these studies, it was reported that muscle length excursions increased with locomotor speed, suggesting that speed (or gait) may also influence patterns of limb muscle strain during locomotion. In contrast to the relatively large body of work that exists concerning *in vitro* and *in vivo* functional characteristics of various ankle extensors in a variety of animals, much less is known about functional aspects of more proximal muscles acting at the knee and hip. Architecturally, muscles acting at the hip and knee tend to have longer fibers, lower pennation angles and higher muscle-to-tendon length ratios. Do these proximal-to-distal differences in muscle architecture underlie differences in strain patterns, such that limb muscles acting more proximally tend to undergo greater length changes than those acting more distally? Finally, animal size influences various structural aspects of an animal's limbs, including their configuration (Biewener, 1989) and compliance (Farley et al., 1993). Given the crouched posture and increased limb compliance of small animals, might their limb muscles be expected to undergo greater length excursions than homologous muscles in large animals with more upright postures and stiff limbs?

The use of sonomicrometry for transducing muscle length changes *in vivo* during locomotion has now been applied successfully in a wide variety of animals (Biewener et al., 1998a; Biewener et al., 1998b; Carrier et al., 1998; Coughlin et al., 1996; Griffiths, 1991; Olson and Marsh, 1998; Roberts et al., 1997) and provides an excellent opportunity to address some of these basic questions regarding strain patterns in individual limb muscles during animal movement. In the present study, we use electromyography and sonomicrometry to measure directly patterns of activity and length change in two thigh muscles of the rat (*Rattus norvegicus*) over a broad range of speeds. We have undertaken this study with several goals in mind. First, we seek to test whether more proximally located muscles operate with higher strain levels during the stance phase than those previously measured in the more distal ankle extensors of wallabies and turkeys. Second, we want to test whether patterns of muscle strain during stance differ among speeds and gaits. Specifically, do muscles exhibit greater degrees of strain as speed increases and as animals change gaits? Third, we seek to compare recent *in vivo* measurements of activity and strain in the hip and knee extensors of horses (de la Paz et al., 1999; D. F. Hoyt, personal communication) and dogs (Carrier et al., 1998; Gregersen et al., 1998) with data obtained from rats to determine whether differences in body size and associated shifts in limb posture and compliance might influence the mechanical function of homologous limb muscles during locomotion.

Materials and methods

Animals and training

Six female Sprague Dawley rats (*Rattus norvegicus*) weighing 225–260 g (mean 242 g) were obtained from Charles River Laboratories. All experimental procedures and animal housing and training received prior IACUC approval. Animals were housed in pairs in cages and maintained on a diet of rat chow (ProLab Isopro 3000). Temperature within the holding room was kept at 22 °C, with a photoperiod of 12 h:12 h light:dark. For at least 3 weeks prior to experiments, individuals were trained to locomote on a small motorized treadmill with a Plexiglas cover to prevent escape. The treadmill was fitted with an NVT-256 endless belt (Motion Industries, Somerville, MA, USA), providing a running surface 60 cm long and 20 cm wide. The belt was covered with small dimples to facilitate traction. Initial training sessions consisted of slow-speed walking to familiarize the animals with the equipment and routine. In further training sessions, the animals underwent independent 1- to 3-min intervals at speeds that would elicit each of three gaits: walking, trotting and galloping.

Once animals could perform this routine regularly, they were considered ready for study.

Surgical procedures

Prior to experimental recordings, sonomicrometry crystals and bipolar electrodes were implanted unilaterally under sterile anesthetic conditions into the vastus lateralis and biceps femoris muscles of the rat thigh to transduce their length changes and electromyographic (EMG) activity patterns *in vivo* during locomotion. The vastus lateralis is a parallel-fibered muscle that originates proximally on the femur and inserts onto the proximal tibia *via* the large patellar tendon, where it converges with the other muscles of the quadriceps complex. It is the largest muscle of the quadriceps complex (Armstrong and Phelps, 1984) and thus is a major extensor of the knee. The biceps femoris is a broad, parallel-fibered, sheet-like muscle with multiple origins from the distal sacral and proximal caudal (tail) vertebrae. It has an extensive insertion onto the proximal two-thirds of the tibia, and its most anterior fibers insert onto the distal aspect of the femur. It is the largest muscle in the hindlimb (Armstrong and Phelps, 1984) and, because of its broad insertion, has multiple actions including thigh abduction, hip extension (more anterior fibers) and knee flexion (more posterior fibers).

Rats were initially anesthetized with an intraperitoneal injection of sodium pentobarbital (35 mg kg^{-1} body mass). Supplemental doses of 0.01–0.02 ml were used over the course of the surgical procedure to maintain an appropriate plane of anesthesia. After the initial anesthetization, the fur covering the experimental hindlimb and skull was shaved using small-animal clippers, and the bared skin was scrubbed and disinfected with a Povidone-Iodine solution (E-Z Prep; Becton Dickinson). To expose limb muscles for implantation, a 3 cm incision was made through the skin over the femur using a scalpel. A second 2 cm incision was made through the skin covering the dorsum of the skull along its sagittal midline. Using a long pair of curved blunt scissors, the skin was carefully spread away from the underlying fascia between the hindlimb and the skull until an unobstructed subcutaneous passage between the two incisions was formed.

At the site of the head incision just lateral to the sagittal suture, the muscle, connective tissue and periosteum were scraped off the skull surface in a small rectangular area approximately 15 mm×10 mm in size. The area was swabbed with ethanol, and a small hole was drilled into its center through the skull using a dental drill. A small epoxy block was screwed onto the skull surface using a stainless-steel machine screw (MX-080-4; Small Parts Inc.). Wet dental cement (HY-Bond) was spread onto the bottom of the epoxy block prior to its being screwed down and was subsequently applied at the interface between the block and skull surface to further bond them together. Prior to surgery, all lead wires from EMG electrodes and sonomicrometry crystals were soldered into female connectors (GF-6; Microtech) which, in turn, were glued with cyanoacrylate to the sides of the epoxy block anchored to the skull. After attaching the epoxy block to the skull, all wires were then pulled subcutaneously to the incision in the hindlimb.

Pairs of 1.0 mm sonomicrometry crystals (Sonometrics Corp.) were implanted into each muscle 8.1–12.7 mm apart (mean 10.3 mm for biceps and 9.6 mm for vastus). See Biewener et al. (Biewener et al., 1998a) and Olson and Marsh (Olson and Marsh, 1998) for thorough reviews of using sonomicrometry to transduce muscle fascicle length changes *in vivo* during animal locomotion. Small pockets 2–3 mm deep were created in the muscles parallel to the fiber axes by puncturing the muscle surface with the tips of small stainless-steel watchmaker's forceps. Crystals were planted into these pockets using forceps and aligned with one another until their signal-to-noise ratio was maximized (as determined by their output to an oscilloscope). The pockets were then sutured shut, and the wires from the crystals were sutured to the surface of the muscle using 6-0 silk to help maintain alignment and prevent any dislocation during experiments. Crystals were placed in the central region of the vastus and in the more cranial portions of the biceps. Fibers in this location of the biceps insert onto the most proximal aspect of the tibia and thus largely act in extension and abduction at the hip, rather than flexion at the knee.

Electrodes (offset twist hook; Loeb and Gans, 1986) made of fine insulated silver wire (0.1 mm diameter; California Fine Wire Co.) with their tips bared of insulation (0.5 mm) were then implanted into muscles using a 21 gauge needle. EMG electrode tips were placed approximately equidistant from each crystal of a pair and 2–3 mm adjacent to a line that would connect crystal pairs. Electrode wires were sutured onto the muscle surface at the site of implantation using 6-0 silk to prevent their being dislodged. After the implantations had been completed, skin incisions were sutured shut using 4-0 silk, and a small layer of silicone adhesive was spread between the skin on the scalp and the epoxy block to protect any exposed wires. Finally, a fine-tipped permanent black marker was used to designate the anterior border of the ilium, the hip joint, the knee joint and the ankle joint. These marks could be seen on the video recordings and were used to determine the basic patterns of joint angle excursions at the hip and knee during locomotion.

Data recording and collection

After surgery, rats were allowed 24–48 h to recover before starting experiments. Before beginning locomotor trials, the female connectors glued to the epoxy block on the skull were connected to the recording equipment *via* lightweight shielded cables and matching male connectors (GM-6, Microtech). EMG signals were amplified 1000× and filtered (60 Hz notch filter and 100–3000 Hz bandpass) through Grass P511 preamplifiers. Wires conveying signals from the sonomicrometry crystals were connected to a sonomicrometer unit (model 120-1001, Triton Technology Inc.), the output of which was monitored by an oscilloscope (Tektronix 2235A 100 MHz). The voltage outputs from the Grass amplifiers and Triton sonomicrometer were digitized at 5000 Hz *via* a 12-bit

A/D converter (Digidata 1200B system; Axon Instruments Inc.) and recorded onto a personal computer.

Because of a phase delay introduced by filters in the sonomicrometer unit, all length change data were offset 5 ms in time relative to the EMG and imaging recordings. In addition, the Triton sonomicrometer system calculates distances between crystals assuming a speed of sound of 1500 m s^{-1} (i.e. the speed of sound in water). Because sound travels slightly faster through muscle tissue (1540 m s^{-1}; Goldman and Hueter, 1956) than through water, all distances were adjusted by 2.7 % to account for this. Voltage outputs from the sonomicrometer were converted to distances (mm) based on this calibration and offset. To verify this relationship, we attached several pairs of sonomicrometry crystals to the tips of digital calipers placed in a water bath, and varied the distance between crystals across the range 7–20 mm for comparison with the output of the Triton sonomicrometer. In all cases, the distance obtained by the sonomicrometer slightly overestimated that measured by the calipers (mean 0.6 mm), so all length data were adjusted by 0.6 mm to account for this.

Locomotor trials were performed using the same treadmill on which the rats were trained. During trials, the treadmill was illuminated using two 800 W lights. Once the rats had been placed onto the treadmill, they were exercised through a series of 8–11 trials, typically spanning a speed range of 25–100 cm s^{-1} to elicit walking, trotting and galloping gaits and to compare low *versus* high speeds within each gait. Locomotor sequences saved for analysis typically consisted of 4–15 step cycles, during which the animal held position on the treadmill belt. At the highest speeds, it was difficult for the animal to maintain as steady a position for more than approximately 4–5 step cycles.

A lateral view of each locomotor sequence was recorded to computer at 125 frames s^{-1} using a digital video system (Redlake Motionscope PCI 500). Electromyographic and length-change data were synchronized with the video files using a voltage pulse that acted as the trigger to stop video recording and was recorded on its own channel along with the EMG and sonomicrometry signals. Experiments performed prior to locomotor trials that videotaped a light-emitting diode, also triggered by the voltage pulse, revealed a 4 ms delay between the video system and A/D board, which was later accounted for during data analysis. After all trials had been completed, the rats were killed with an overdose of sodium pentobarbitol, and dissections were performed to locate and confirm the positions of the EMG electrode tips and sonomicrometry crystals. Because some implantations were pulled out or were unreliable, we do not have simultaneous strain and activity recordings for both muscles in all six rats. However, strain and/or activity were recorded from each muscle from a total of four rats (Table 1).

Table 1. *Individual rats from which muscle activity and strain were recorded for the biceps femoris and vastus lateralis*

	Biceps femoris		Vastus lateralis	
Individual	EMG	Strain	EMG	Strain
Rat 1	X	X	X	X
Rat 2	X	–	X	X
Rat 3	X	–	–	–
Rat 4	X	X	X	X
Rat 5	–	X	—	–
Rat 6	–	X	X	X

Note that X reflects successful data collection and use in analysis whereas – reflects a lack of successful data collection.

Data analysis

Data from 3–5 step cycles were analyzed for each locomotor trial. For each step cycle, the timing of two kinematic events was determined *via* the video files: (i) the onset of the stance phase, defined as the video field in which the foot of the implanted hindlimb first touched the ground, and (ii) the onset of the swing phase, defined as the first video field in which the same foot left the ground completely. The durations of the stance and swing phases from each step cycle were calculated from these data. In addition, the positions of the anterior margin of the ilium, hip joint, knee joint and ankle joint were digitized (Didge software; courtesy of Alistair Cullum, Creighton University) for three step cycles during each of the gaits in four individuals: walking, trotting and galloping. During galloping trials, most rats shifted which hindlimb was used as the leading limb (the hindlimb that leaves the ground last during a step cycle) *versus* the trailing limb (the hindlimb that first makes ground contact) among sequences. As a result, joint movements were digitized for each of these conditions. Coordinate data were smoothed using a binomial algorithm in IGOR Pro (Wavemetrics Inc.) to minimize gross digitizing errors. Coordinate data were then converted into joint angle data, and average joint angle profiles were constructed for the hip and knee by scaling all strides to the same duration and determining the mean joint angles at 15 equivalent times in a stride for each gait.

Numerous variables quantifying the timing and magnitude of each burst of muscle activity were also determined for each step cycle. The onset and offset of EMG activity in both muscles were measured relative to the start of the stance phase during each step. The magnitude of each EMG burst was calculated by averaging the spike amplitude (intensity) of the rectified EMG signal. For each muscle, values of EMG intensity were converted to relative values within each individual by dividing each intensity by the largest respective value recorded in that muscle for that individual. Therefore, the largest intensity for a muscle in an individual was given a value of 1, and all other bursts in that muscle were assigned a value between 0 and 1.

Fascicle strain was measured as a fractional length change relative to resting length (defined as the distance between crystals measured directly after surgery when the rat was still anesthetized). Changes in muscle fascicle length were estimated on the basis of the changes in distance between pairs of sonomicrometry crystals in each muscle of interest. Any change in distance between crystals was assumed to represent a proportional change in length over the entire muscle fascicle. Small errors in distance measurements between crystals

potentially introduced by changes in muscle stiffness during contraction were not accounted for but were likely to be quite small (<2–3 %; Hatta et al., 1988). Because patterns of fascicle strain were complex in both muscles, particularly in the vastus, the strain cycles from each muscle were divided into five discrete intervals, and the total strain occurring in each interval was determined. In both muscles, the stance phase was divided into four intervals of equal duration for each step cycle analyzed, and the strain during each interval was determined. In the biceps, strain typically shifted from shortening to lengthening during the fourth interval, so both shortening and lengthening strains were determined for this interval. The fifth and final interval encompassed the entire swing phase and, in both muscles, also consisted of both shortening and lengthening strains. Average strain rates were also determined over certain intervals in each muscle. In the biceps, average strain rates were calculated over the entire shortening period. In the vastus, average strain rates were calculated over the initial period of lengthening associated with knee 'yield' or flexion during stance.

Statistical analyses

For each muscle, the average values for each of the variables quantifying patterns of muscle activity were determined for each locomotor sequence: onset and offset of EMG activity, EMG duration (as a percentage of the locomotor cycle and as a percentage of the stance phase only) and EMG intensity. Mean levels of fascicle strain, temporally partitioned as described above, were also calculated for each locomotor sequence. To test for significant differences among gaits, two-way mixed-model analyses of variance (ANOVAs) were performed with gait and individual as the fixed and random factors respectively. ANOVAs were performed on multiple variables for each muscle: EMG onset and offset time, EMG duration (percentage of cycle and percentage of stance), relative EMG intensity, total shortening strain (for the biceps) and yield phase lengthening (for the vastus). The mean values of the variables for each sequence from every individual were used in these ANOVAs. During galloping, sequences could be divided into those in which the experimental limb was used as the trailing limb (i.e. the first hindlimb to make ground contact) and those in which the experimental limb was the leading limb (the second hindlimb to make ground contact). To assess potential differences between the leading and trailing limb, paired *t*-tests were used on the mean values of each of the variables from all the galloping sequences (divided into leading- *versus* trailing-limb sequences). No manipulations were performed to account for the use of multiple statistical tests; however, *F*-statistics, degrees of freedom and *P*-values are reported so that the relative degree of significance can be assessed in all cases.

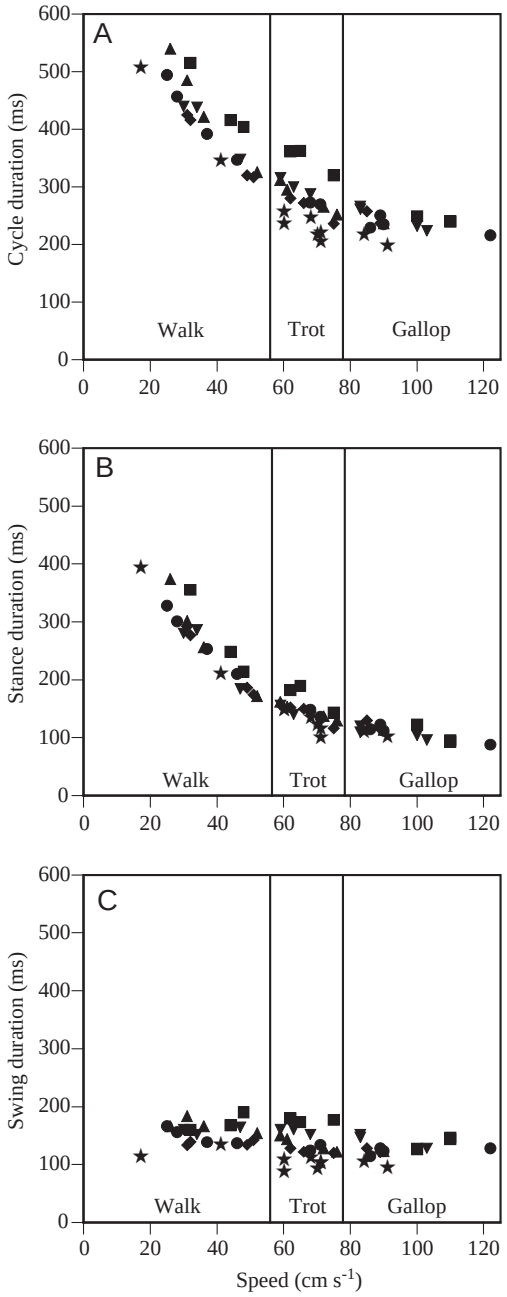

Fig. 1. Effects of speed on cycle (A), stance (B) and swing (C) duration during rat locomotion. Cycle duration decreases with increases in locomotor speed (A). This decrease mirrors the decrease in stance phase duration with increasing speed (B). In contrast, swing phase duration remains relatively constant over a large speed range (C). Vertical lines generally separate data into walking, trotting and galloping trials. However, several slow-speed galloping trials are found between 60 and 75 cm s^{-1}. Each symbol represents the average values from locomotor sequences at various speeds and gaits. Different symbols represent different individuals.

Results

Locomotor kinematics

Rats use different gaits depending on their speed of locomotion. At relatively slow speeds (17–48 cm s^{-1}), rats utilize a lateral sequence walk, at intermediate speeds (59–71 cm s^{-1}) they typically use a trot, and at high and occasionally at intermediate speeds (60–122 cm s^{-1}) they use a transverse gallop. Among, and occasionally within, galloping sequences, rats alternate the leading *versus* trailing hindlimb.

Although the speeds at which the animals chose to walk *versus* trot were distinct, this was not always true of trotting and galloping, in which one individual exhibited both trotting and galloping at speeds between 60 and 70 cm s^{-1}. Stride durations decrease with locomotor speed, and gait transitions did not affect this relationship (Fig. 1A). The parabolic decrease observed reflects a decrease in the stance phase duration that occurs as speed increases (Fig. 1B) rather than a change in the swing phase duration, which remains relatively constant across speeds (Fig. 1C).

Rats undergo cyclic angular excursions at all the major limb joints during any gait. As this paper focuses on muscles acting at the hip and knee, only the excursions of these two joints are addressed here. The hip exhibits a consistent pattern of extension, followed by flexion, during each step cycle regardless of gait (Fig. 2A–D). The hip extends throughout most of the stance phase and flexes during much of the swing phase. In all gaits, the knee undergoes cycles of flexion and extension during the stance as well as during the swing phase, although the absolute angular excursions during these cycles vary among gaits (Fig. 2E–H). As the foot initially contacts the ground at the onset of the stance phase, the knee begins to flex, and continues flexing through approximately the first half of stance. Over the second half of the stance phase, the knee extends, although to a lesser extent than its original flexion, particularly during walking and trotting (Fig. 2E,F). During the first third of the swing phase, the knee again flexes as the foot is lifted off the ground. Finally, over the latter two-thirds of the swing phase, the knee again extends (this time more substantially) as the foot swings forward to re-establish ground contact for the next step cycle (Fig. 2E–H).

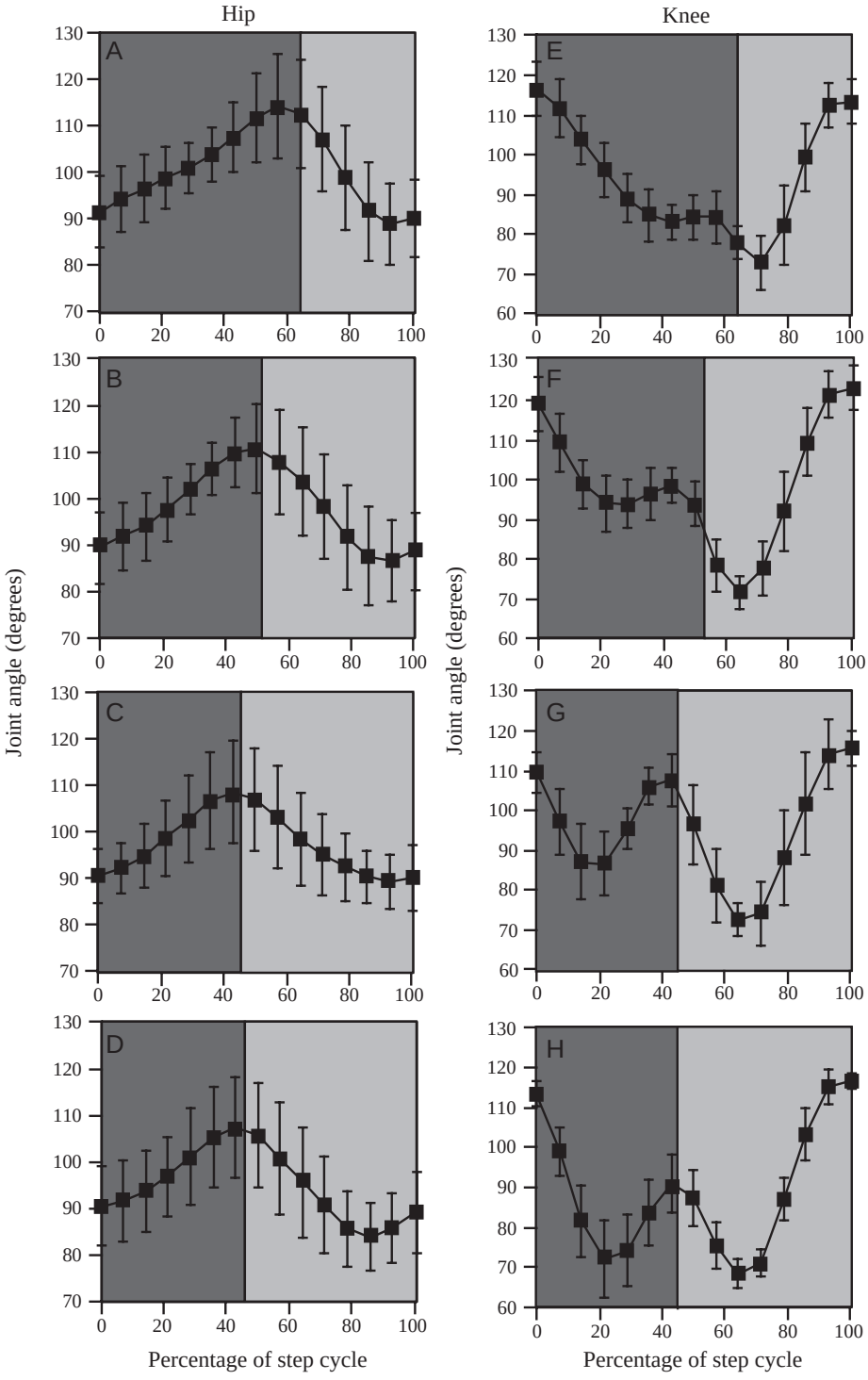

Fig. 2. Two-dimensional angular excursions of the hip and knee joints during walking (A,E), trotting (B,F) and galloping (leading limb, C,G; trailing limb, D,H). These plots reveal the general kinematic patterns observed at the hip and knee during rat locomotion and some of the variation related to changes in gait. Data are from four individuals, three strides per individual. Dark background shading represents the stance phase, and light background shading represents the swing phase. Values are means ± S.D. In all gaits, the hip mainly extends during stance and flexes during swing. The knee typically exhibits flexion followed by extension during both the stance and swing phases. The timing and extent of these angular excursions differ among gaits.

Patterns of muscle activity and strain

EMG activity

The onset of EMG activity in the biceps typically occurs slightly before the start of the stance phase (Fig. 3, Fig. 4A), and this timing does not vary significantly among gaits. There

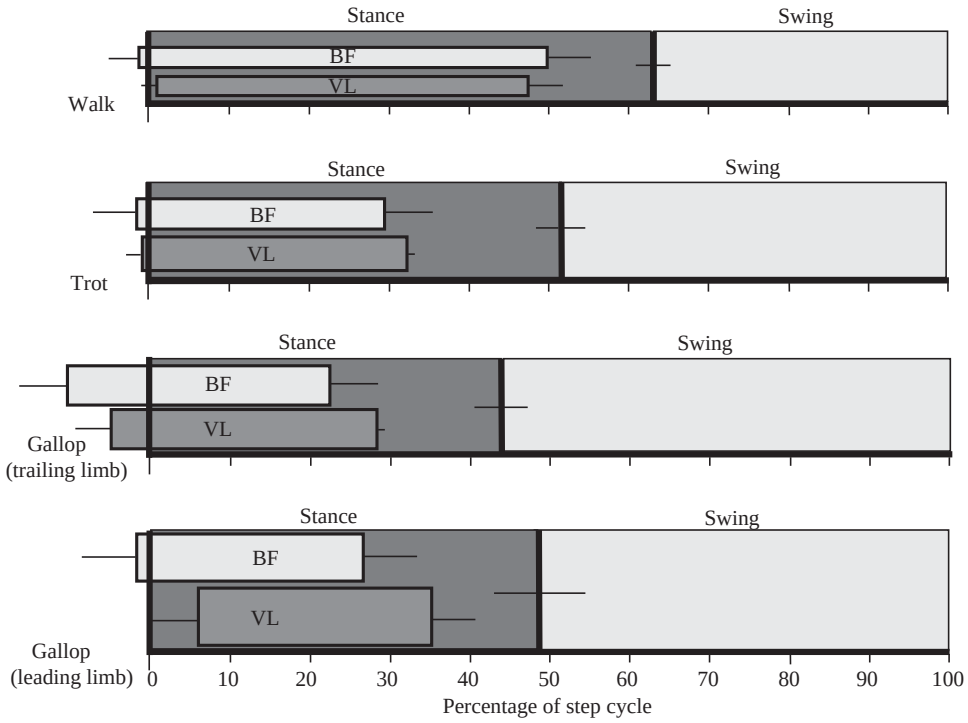

Fig. 3. Average patterns of electromyographic activity in the biceps femoris (BF) and vastus lateralis (VL) during walking (mean speed 36 cm s^{-1}), trotting (mean speed 64 cm s^{-1}), galloping trailing limb (mean speed 96 cm s^{-1}) and galloping leading limb (mean speed 83 cm s^{-1}). Horizontal bars represent average temporal periods of EMG activity. For each muscle, bar thickness denotes the average relative intensity of activity. Black vertical lines represent the onset of the stance phase (foot down) and the onset of the swing phase (foot up). Values are means ± S.D. (N=4 individuals per muscle).

is a tendency during galloping for average onset times to be earlier in the trailing limb than in the leading limb (Fig. 3, Fig. 4A), but this difference is not significant (P=0.32; paired t-test). Biceps activity ceases during the last half of the stance phase in all gaits (Fig. 3, Fig. 4A), lasting an average of approximately 70 % of the stance phase regardless of gait (Table 2; Fig. 4C). However, because stance phase durations decrease with speed (Fig. 1B), absolute EMG burst durations also decrease with speed, being greatest during walking and least during galloping (Fig. 4B). In contrast, the relative intensity of biceps EMG activity increases with speed (Table 2; Fig. 4D), being greatest during galloping and least during walking. However, biceps EMG intensity does not differ significantly between the leading and trailing hindlimb during a gallop (P=0.35; paired t-test).

The vastus lateralis exhibits largely overlapping periods of EMG activity with the biceps during the stance phase of all gaits (Fig. 3) and often also exhibits a short, low-intensity burst late in the swing phase (Fig. 5). The main burst of EMG activity in the vastus begins near the start of the stance phase (Fig. 3, Fig. 4E), and this onset time does not vary significantly among gaits (Table 2). However, the precise EMG onset time

Table 2. *Results of a two-way ANOVA examining the effects of gait and individual on muscle activation and strain parameters*

Muscle	Variable	Gait	Individual	Gait × Individual
Biceps femoris		d.f.=2, 6	d.f.=3, 23–25	d.f.=6, 23–25
	EMG onset	4.14	15.31***	0.68
	EMG offset	14.33**	8.91***	1.83
	EMG duration (% of cycle)	9.68*	1.81	1.51
	EMG duration (% of stance)	1.37	3.57*	1.76
	EMG intensity (relative)	20.21**	0.74	1.20
	Shortening strain during stance	17.76**	16.03***	1.52
Vastus lateralis		d.f.=2, 5	d.f.=3, 23	d.f.=5, 23
	EMG onset	1.29	4.42*	2.56
	EMG offset	16.82**	1.84	0.99
	EMG duration (% of cycle)	7.88*	9.29***	3.29*
	EMG duration (% of stance)	1.66	5.95**	3.03*
	EMG intensity (relative)	34.83**	3.80*	0.39
	Yield-phase lengthening strain during stance	7.14*	31.53***	1.51

Table entries are F-values.
*Significant at P<0.05; **significant at P<0.01; ***significant at P<0.001.

in the vastus during a gallop varies significantly between the leading and trailing limb (P=0.001; paired t-test). Specifically, EMG activation in the vastus of the leading limb during a gallop typically begins relatively later in the step cycle (well after foot contact) than in the trailing limb (prior to foot contact). Vastus EMG activity ends in the last half of the stance phase and, as in the biceps, absolute EMG burst durations are significantly longer during walking than during galloping (Fig. 4F), but scale proportionally to stance phase durations (Table 2; Fig. 4G). Also similar to the biceps, vastus EMG intensity increases with locomotor speed, so that during galloping limb muscles exhibit their most intense EMG bursts (Fig. 3, Fig. 4D,H). Vastus burst intensity is also greater, on average, in the leading limb than in the trailing limb during a gallop, (Fig. 3, Fig. 4H), but this difference is not significant (P=0.09; paired t-test).

Fascicle strain

During each stride, the muscle fascicles of the biceps and vastus undergo consistent patterns of length change. In general, the biceps typically shortens over much of the stance phase and lengthens over much of the swing phase (Fig. 6A). However, the transition from fascicle shortening to lengthening does not occur precisely as the foot leaves the ground, but instead occurs slightly before this (Fig. 6A). Similarly, the transition from fascicle lengthening to shortening does not occur precisely as the foot makes ground contact, but consistently occurs slightly before this (Fig. 6A). The total amount of fascicle shortening in the biceps differs significantly among gaits (Table 2; Fig. 6A), with shortening strains being greater during walking (23±3 %) and trotting (27±5 %) than during galloping (19±6 % in the leading limb and 12±5 % in trailing limb). In fact, if the stance phase is divided into four intervals, the amount of shortening that occurs in each of the first three intervals tends to be greater during walking and trotting than during galloping (Fig. 7A,B). Average fascicle shortening strain rates also vary substantially across speeds and gaits (Fig. 8A). During walking and trotting, fascicle strain rates are relatively constant over the stance phase and are lowest during slow walking, increasing with speed until they reach their maxima during fast trotting (Fig. 8A). During galloping, shortening strain rates vary greatly over the course of the stance phase (Fig. 6A), precluding a meaningful assessment of the muscle's average strain rate.

Patterns of fascicle strain in the vastus are more complex than in the biceps. In general, vastus fascicles tend to lengthen

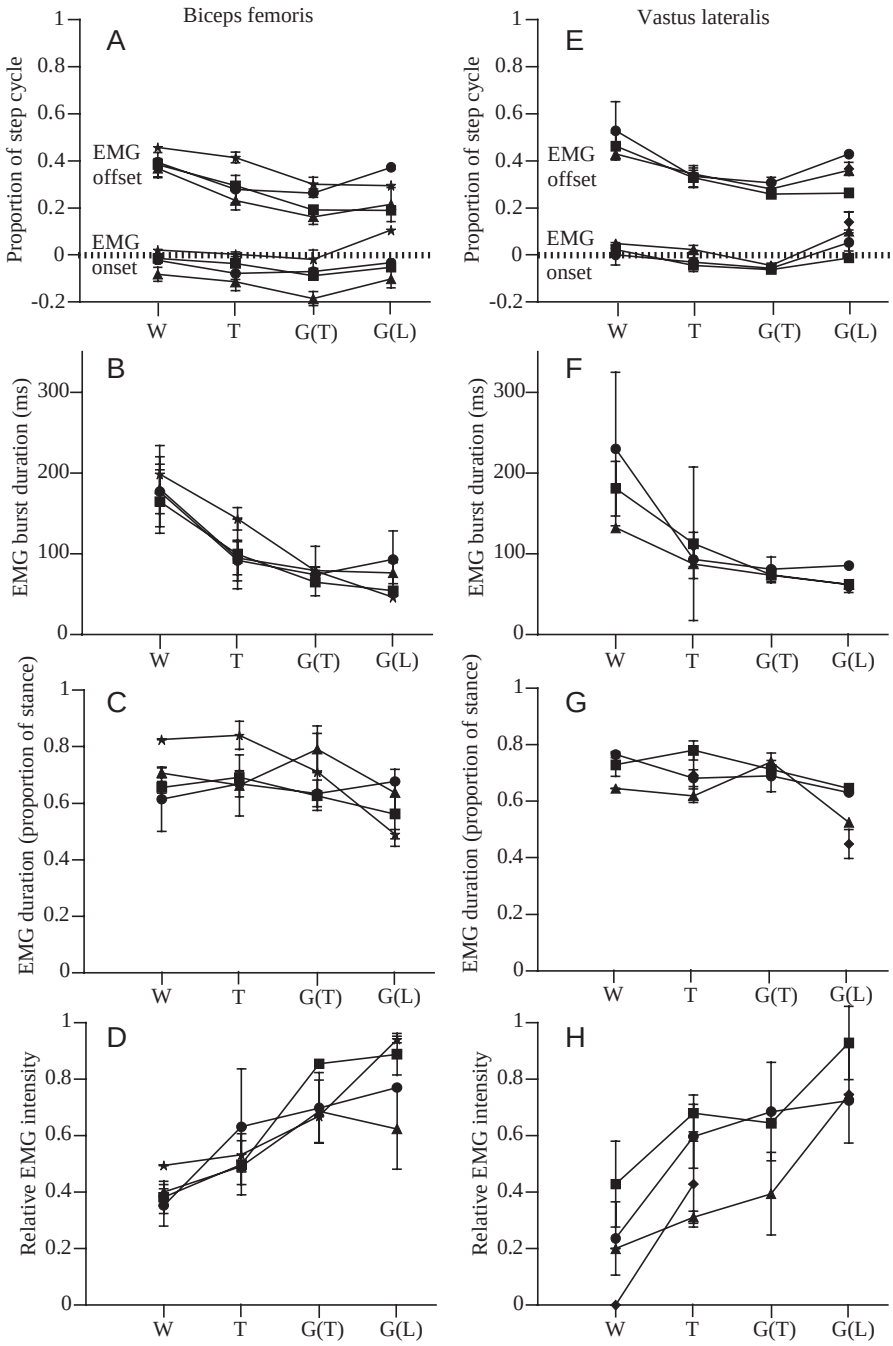

Fig. 4. The relative timing of EMG onset and offset (A,E), absolute EMG burst duration (B,F), relative burst duration (C,G) and relative EMG intensity (D,H) for the biceps femoris (left column) and vastus lateralis (right column). Data are shown for walking (W), trotting (T) and galloping [trailing limb G(T), leading limb G(L)]. Mean locomotor speeds for each gait are the same as for Fig. 3. The dashed horizontal lines in A and E represent the onset of the stance phase (time of foot down). Each symbol represents the average values of all analyzed trials from a particular individual for that particular gait. Different symbols stand for different individuals (N=4 for each muscle). Values are means ± S.D.

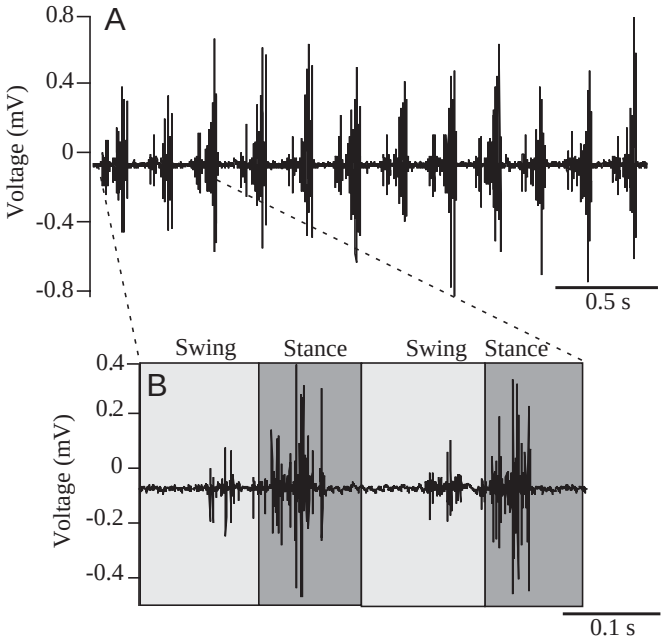

Fig. 5. (A) Electromyographic trace from the vastus lateralis of the trailing limb from a rat during level galloping. (B) An expanded view of two cycles of EMG activity showing an initial small burst of vastus activity late in the swing phase followed by a larger burst of activity commencing near the onset of the stance phase (demarcated by the vertical line separating the light and dark shading). The initial bursts of activity late in the swing phase were always of much lower intensity than the stance-related bursts and have been discussed in some detail previously (de Leon et al., 1994).

over the initial half of the stance phase in all gaits (i.e. the yield phase), as the knee flexes or 'yields' to the weight of the animal (Fig. 6B, Fig. 7C,D). The amount of this yield phase lengthening, however, varies significantly among gaits (Table 2) and is greatest in the trailing limb during galloping (15±6 %), mirroring the large degree of knee flexion that occurs over the first half of stance in the trailing limb (Fig. 2H). Lengthening velocities during this period also vary substantially across gaits (0.6–3.7 $L\,s^{-1}$, where L is resting fascicle length) but, as with the magnitudes of lengthening strain, are typically highest during galloping (Fig. 8B). In the second half of stance, vastus fascicles remain nearly isometric during walking and trotting (Fig. 7C,D), consistent with the nearly constant knee angle during this time (Fig. 2E,F), and they shorten to a variable extent during galloping (Fig. 7C,D) when the knee exhibits more notable extension (Fig. 2G,H). In the leading limb during galloping, this fascicle shortening (10±2 %) is twice as great, on average, as in the trailing limb (5±3 %) and occurs while the muscle is electrically active. During approximately the initial third of the swing phase, the fascicles re-lengthen as the knee flexes and the foot is lifted off the ground. The fascicles then shorten substantially and rapidly over the remainder of the swing phase as the knee is extended and the foot is brought into position to begin the next stance phase (Fig. 6B, Fig. 7C,D).

Discussion

Our major goals in this study were to address how patterns of activation and strain in two proximal hindlimb muscles of the rat change across gaits and how they compare with the function of limb muscles studied previously in other taxa. Our findings show that, unlike the nearly isometric contractions predicted for the limb muscles of hopping and running animals (Taylor, 1994) and observed in the ankle extensors of hopping wallabies and running turkeys (Biewener et al., 1998b; Roberts et al., 1997), both the biceps femoris and vastus lateralis of rats typically contract with substantial length changes (10–25 %) during the stance phase of different gaits. Although we could not directly measure the time course of force production in these muscles, the temporal patterns of EMG activity in relation to strain are generally suggestive of active shortening in the biceps and active lengthening in the vastus (except in the leading limb during galloping, during which EMG activity is coincident with shortening). In both muscles, levels of activation and strain and strain rate are all modulated with speed and gait, and overall patterns of strain differ among gaits. In addition, even within the same gait (galloping), the timing of muscle activation, with respect to strain, can differ between the leading and trailing limbs (as exemplified by the vastus). Finally, the lengthening strains observed during stance in the vastus of the rat (during trotting) differ from the more isometric or shortening strains observed in the vastus of dogs (Carrier et al., 1998) and horses (D. F. Hoyt, personal communication) during the same gait. However, the substantial shortening strains in the biceps over the stance phase in the rat are similar to what occurs in the semimembranosus (another hamstring muscle) in dogs (Gregersen et al., 1998). Such results suggest that differences in animal size, and corresponding differences in limb configuration and compliance, may influence the function of some homologous limb muscles, but not others, during locomotion.

Hindlimb muscle function during different gaits

A characteristic feature of limb-based locomotion among many tetrapods is the use of discrete gaits at different speeds (Gambaryan, 1974; Hildebrand, 1976; Hildebrand, 1980). Among the various gaits that have been described and categorized, the walk, trot and gallop represent three distinguishable patterns of limb movements that rats and many other quadrupedal mammals use to move at slow, intermediate and fast locomotor speeds. Similar to other quadrupeds that have been studied, rats exhibit a smooth and continuous decrease in step cycle duration as speed increases, despite changes in gait. Decreases in step cycle duration occur almost completely at the expense of the stance phase rather than the swing phase and, hence, the duration of the swing phase remains largely unchanged between walking, trotting and galloping gaits, as has been observed in other quadrupeds (Grillner, 1975). Therefore, shifts in speed or gait during locomotion are based primarily on changes in the recruitment and mechanics of limb muscles that are active during the stance phase. The biceps and vastus represent two of the largest

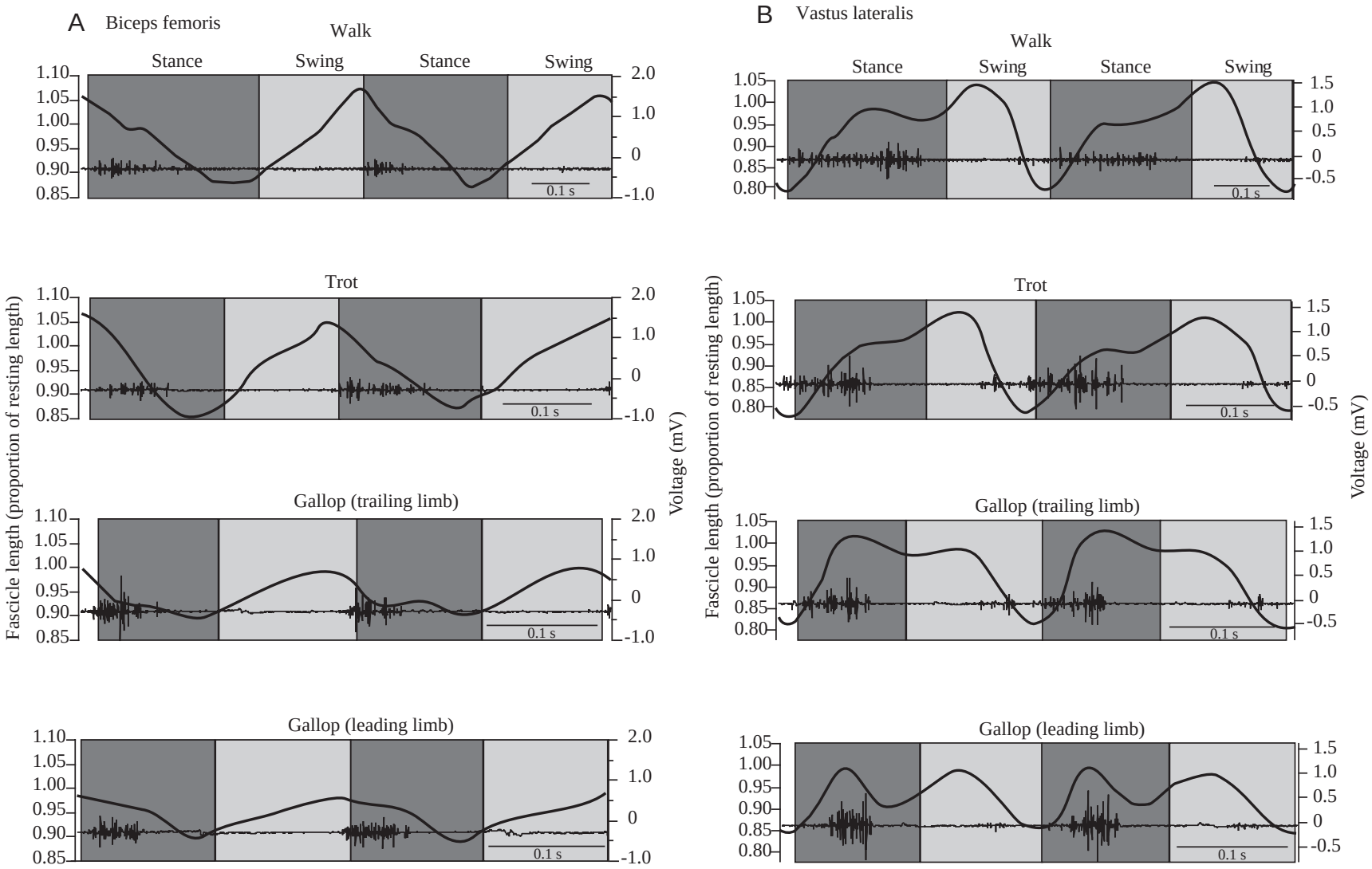

Fig. 6. *In vivo* patterns of strain and activation in the biceps femoris (A) and vastus lateralis (B) during two strides of walking, trotting and galloping (trailing limb and leading limb). Dark shading represents the stance phase, and light shading represents the swing phase. Slightly different scales are used between muscles for both strain and voltage. Note that total shortening strain levels in the biceps femoris are reduced during galloping relative to other gaits. In addition, the vastus lateralis during most gaits is active (as prescribed by EMG activity) during lengthening, but exhibits very little shortening during this time. However, in the leading limb during galloping, vastus fascicles exhibit more shortening when active.

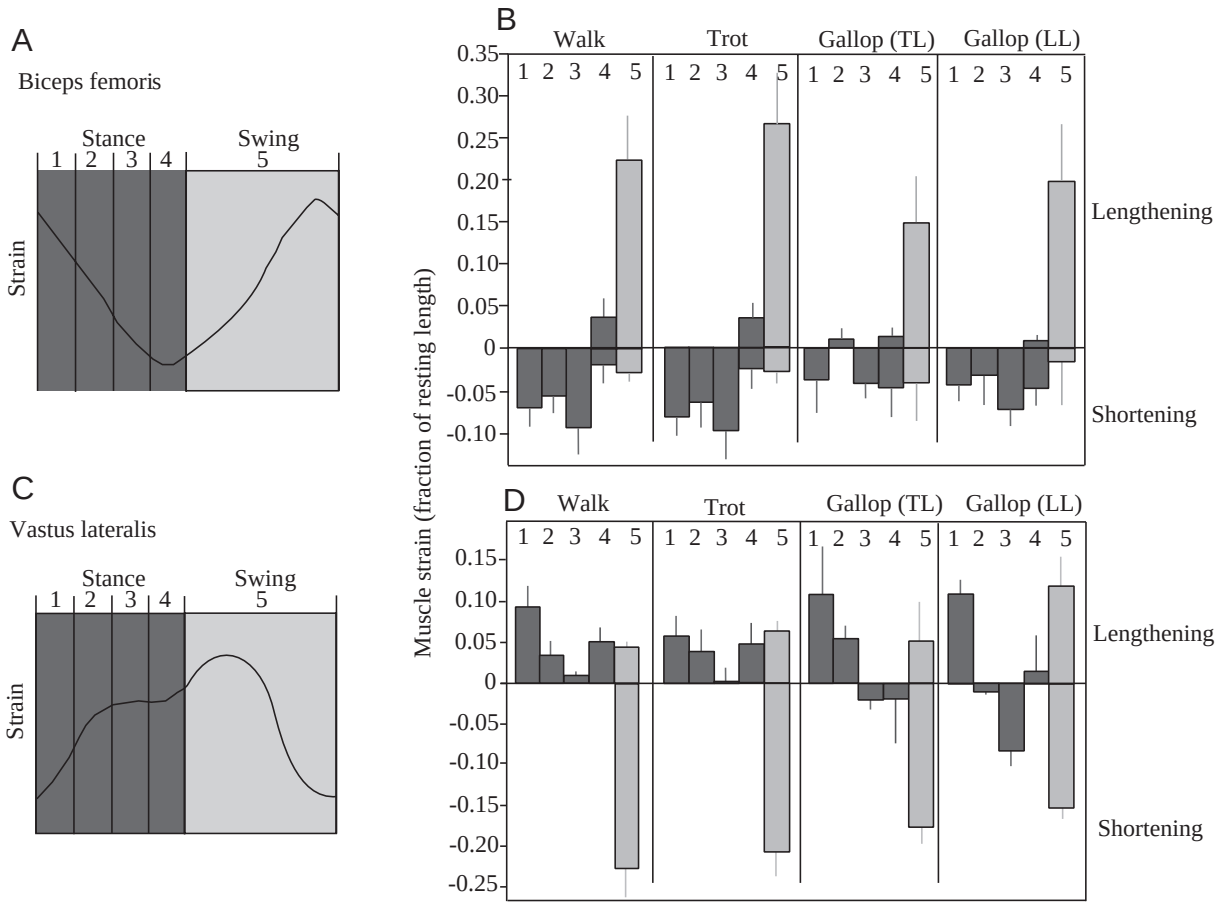

Fig. 7. Strain levels in the biceps femoris and vastus lateralis during different portions of the stance and swing phases. Generalized fascicle strain patterns (based on average strain levels during trotting) for the biceps and vastus are shown in A and C, respectively, as are the five subdivisions of the step cycle for the two muscles (four stance and one swing). Average strain levels for walking, trotting, galloping trailing limb (TL) and galloping leading limb (LL) are shown for each of the phases in the biceps (B) and vastus (D) (*N*=4 individuals per muscle). Methods used for obtaining these fascicle strain levels are described under 'Data analysis' in the Materials and methods section. Negative values for muscle strain indicate fascicle shortening, whereas positive values reflect fascicle lengthening. If both shortening and lengthening occur during one of the intervals, both positive and negative columns are present for that interval. Error bars are standard deviations. Dark gray shading represents strain occurring during the stance phase, and light gray shading represents strain occurring during the swing phase.

hindlimb muscles that are active during stance in these gaits and, consequently, their actions are probably important to whole-limb mechanics and to the accompanying energetic cost of locomotion.

Our recordings show that the cranial regions of the biceps femoris shorten substantially during the stance phase in walking, trotting and galloping gaits. EMG activity, typically present over the first 50–70 % of stance, increases in intensity with speed as limb muscles must generate force over shorter periods (as the stance phase duration decreases). Shortening strains and strain rates also increase with speed, as has been found in various ankle extensors of cats (Whiting et al., 1984; Gregor et al., 1988; Prilutsky et al., 1996). However, shortening strains reach their maxima during fast trotting and decrease at speeds above the trot–gallop transition. The magnitude of shortening remains relatively low even as galloping speed and EMG intensities continue to increase. In addition, fascicle length-change trajectories become less consistent during galloping, with periods of rapid shortening followed and/or preceded by periods of nearly isometric behavior. This combination of increasing EMG intensity and decreasing muscle shortening implies that higher levels of muscle force generation underlie the trot–gallop transition in rats. However, whether this transition also occurs at the expense of significant mechanical work production (e.g. Taylor, 1994; Roberts et al., 1997) remains unclear. As muscles must shorten while producing force to generate mechanical work, our inability to assess the timing and magnitude of force production by the biceps during galloping limits interpretations of its work performance.

Unlike the biceps, the vastus exhibits EMG activity when its fascicles are lengthening rather than shortening (except in the leading limb during galloping, which we discuss below). EMG intensity as well as the magnitude and rate of vastus lengthening are greater during galloping than during trotting and walking. Some degree of active lengthening and work

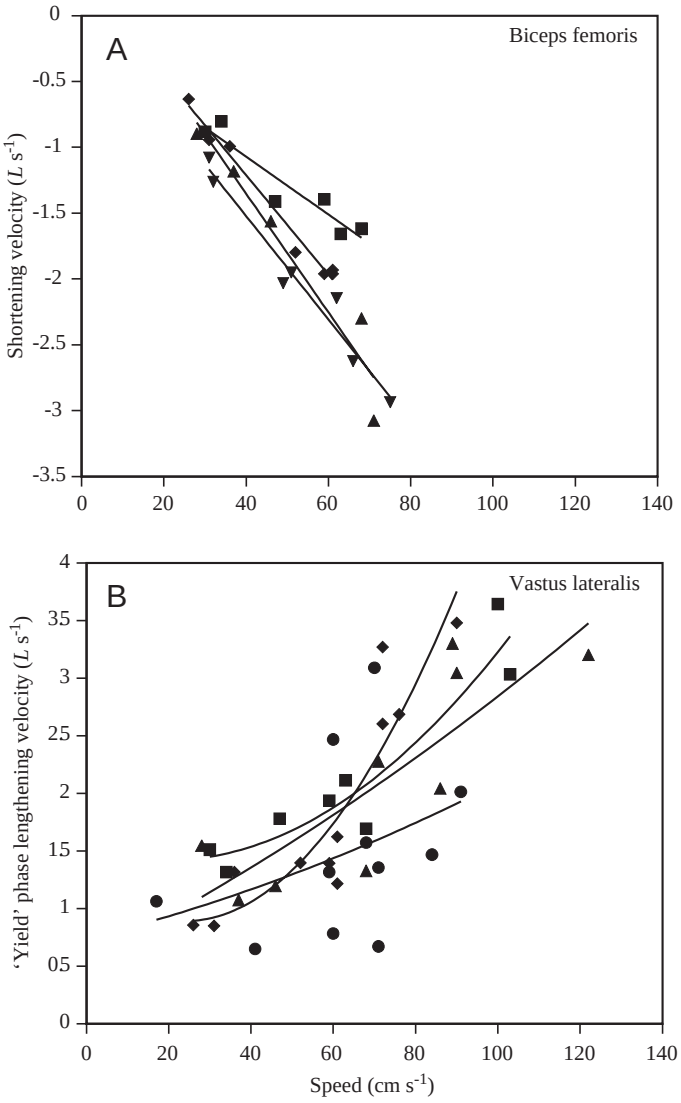

Fig. 8. Average biceps shortening velocity (A) and vastus lengthening velocity (B) as a function of locomotor speed. Biceps shortening velocities are calculated by dividing the total distance shortened by the biceps by the duration of shortening. Only shortening velocities during walking and trotting are shown because, during these gaits, shortening velocity remained relatively constant over most of the shortening period (unlike during galloping, for which shortening velocities varied substantially). Negative values are used to indicate shortening velocities. Shortening velocities increase with speed and are highest during fast trotting. Average vastus lengthening velocities are calculated by dividing the total distance lengthened during the first half of the stance phase (the 'yield' phase) by the duration of this lengthening phase. Positive values are used to indicate lengthening velocities. Lengthening velocities increase with speed and are, therefore, greatest during fast galloping. Different symbols represent the average values from locomotor sequences at various speeds and gaits. Different symbol types represent different individuals. *L*, resting fascicle length.

absorption is also observed in the ankle extensors of cats in the initial portions of the stance phase (Whiting et al., 1984; Gregor et al., 1988; Prilutsky et al., 1996). However, in contrast to cat ankle extensors, any fascicle shortening that does occur in the rat's vastus later in the stance phase is small compared with the amount of lengthening and occurs mostly after EMG activity in the muscle has ceased (except in the leading limb during galloping). Thus, it seems likely that net work absorption characterizes the rat's vastus during walking and trotting and in the trailing limb during galloping. In addition to absorbing work, active lengthening of the muscle may also facilitate more rapid and greater force development, particularly when EMG burst intensities are high, which may be critical for joint stabilization as the knee yields to, but continues to support, the loads placed on the limb during each stride.

During all gaits, the largest length changes in the vastus occur late in the swing phase, as the fascicles shorten during subsequent knee extension prior to foot–ground contact. However, all this shortening occurs well after the main burst of vastus EMG activity has stopped. As demonstrated previously (Gruner and Altman, 1980; de Leon et al., 1994), small bursts of vastus lateralis activity are also often present late in the swing phase as the knee extends to place the foot on the ground for the next step. Such activity, despite its relatively low intensity and short duration, suggests that the vastus may contribute to knee extension late in the swing phase. Similar small bursts of activity have been recorded during the swing phase in the vastus lateralis of various other mammals (lemurs, *Lemur fulvus*, Anapol and Jungers, 1987; dogs, Carrier et al., 1998; cats, Engberg and Lundberg, 1969) as well as in uni-articular knee extensors of various other phylogenetically diverse tetrapods (for a brief review, see Earhart and Stein, 2000). As suggested by Earhart and Stein (Earhart and Stein, 2000), it appears that double bursting patterns in uni-articular knee (and elbow) extensors are generally common to limbed vertebrates and, thus, may represent a relatively primitive feature of the motor pattern underlying tetrapod locomotion.

Because galloping is an asymmetrical gait (Hildebrand, 1980) and each hindlimb experiences a different amount (or pattern) of loading during stance (Biewener et al., 1988), muscles in the leading and trailing hindlimb of a gallop may be expected to function somewhat differently. Such a functional difference is evident in the vastus lateralis, in which the timing of activation depends on whether the hindlimb is the first (i.e. trailing limb) or second (i.e. leading limb) to touch down during a stride. EMG activity begins in the vastus of the trailing limb prior to when the foot touches the ground but does not commence in the leading hindlimb until well after foot–ground contact. In addition, there are also differences in vastus strain patterns between the leading and trailing hindlimbs. During the first half of stance, vastus fascicles in the trailing limb are stretched more, on average, than in the leading limb, and this increased stretching occurs almost entirely while the muscle is active. During the second half of stance, vastus fascicles in the leading limb shorten to a greater degree than in the trailing limb, and this increased shortening also occurs while the muscle exhibits EMG activity. As a result, the same muscle apparently serves rather different

functional roles between the leading and trailing limbs at a gallop. In the trailing limb, the vastus probably absorbs more energy than it generates during stance (as in walking and trotting). In the leading limb, the vastus probably generates more energy than it absorbs. Because all vasti exhibit similar temporal patterns of activation in rats running at high speeds (see fig. 3A in Nicolopoulos-Stournaras and Iles, 1984) and insert together *via* the patellar tendon over the knee, they probably function in a manner that is similar to our observations for the vastus lateralis.

Comparative aspects of muscle function during locomotion

Over the last several decades, work performed by Dick Taylor and his students and colleagues has established an intriguing framework for understanding the energetics and mechanics of terrestrial locomotion across a broad range of animal species, sizes and speeds (summarized in Taylor, 1994). Briefly, Taylor (Taylor, 1994) suggested that, unlike swimmers and fliers, cursorial animals need not generate large amounts of mechanical work to move at a constant speed across a level, solid surface (see Taylor, 1994, and references therein for a more thorough explanation of this argument). Rather, it is the generation of muscular forces to support body weight and the costs of generating these forces at different rates that underlie the energetics of terrestrial locomotion (Kram and Taylor, 1990). With regard to limb muscle function during cursorial locomotion, Taylor reached the novel conclusion that 'it seems likely that muscles of running animals remain nearly isometric over the entire range of body size and running speeds' (Taylor, 1994, p. 201).

Work directly measuring the *in vivo* patterns of force, strain and activation in the distal ankle extensors of running turkeys (Roberts et al., 1997) and hopping wallabies (Biewener et al., 1998b) has shown that these muscles are indeed active and generating force nearly isometrically (strain levels $\leqslant 6\,\%$) during the stance phase of fast level locomotion. However, kinematic estimates of muscle strain in various ankle extensors of cats moving on the level at a variety of speeds suggest that these muscles do change length during stance and can absorb and/or generate mechanical work during each stride (Gregor et al., 1988; Prilutsky et al., 1996; Whiting et al., 1984). In fact, in their summary, Prilutsky et al. (Prilutsky et al., 1996; p. 801) conclude that, in the cat, 'ankle extensor muscles play a significant role in the generation of mechanical energy for locomotion'. Furthermore, direct strain measurements in hip and knee extensors of dogs (Carrier et al., 1998; Gregersen et al., 1998) as well as those performed in the present study on the more proximal hip and knee extensors of rats, also show that muscles can both actively shorten or actively lengthen to different degrees and at different rates during the stance phase over a range of walking and running speeds. Thus, while Taylor's (Taylor, 1994) framework for understanding the mechanics and energetics of animal locomotion works well at the level of the whole limb or animal, individual muscles within the limb need not perform exclusively isometric contractions during running.

So why then do energetic patterns across a broad range of animals match so well the hypothesis set forth by Taylor (Taylor, 1994) that limb muscles contribute minimal mechanical work during running, and that it is the cost of muscle force generation that underlies locomotor energetics? It seems likely that the net amount of mechanical work performed by a cursorial animal against its environment during each stride during level running is relatively low. Levels of air resistance that must be overcome are generally small, and some of the energy required for swinging the limbs and re-accelerating the body during each stride can be obtained through the recovery of strain energy stored in elastic elements of the musculoskeletal system (Alexander, 1977). However, although the net work output of the limbs' muscles may be relatively low in each stride, the net work output of any individual muscle within the limb need not be low. Rather, given the architectural diversity of limb muscles, it seems probable that, during any stride, some limb muscles might act to generate net work, others to absorb net work and others may generate or absorb almost no net work (through nearly isometric contractions or equal amounts of energy absorption and generation). Furthermore, as reported by Roberts et al. (Roberts et al., 1998), there are probably other factors, in addition to the rate of force generation, that influence the energetic cost of animal locomotion. One possibility they suggest (Roberts et al., 1998) is a change in the relative shortening velocities of muscles across speeds. As shown in the present study for rats, as well as in studies of cats (Whiting et al., 1984; Gregor et al., 1988; Prilutsky et al., 1996), the length excursions and strain rates of various limb muscles do change substantially with speed, and these differential levels of muscle work output or absorption may contribute to any speed-related changes in locomotor energetics that remain unexplained by the costs of generating muscular forces during stance.

Given that certain individual muscles (and tendons) may perform net positive or net negative work, it becomes important to understand how the mechanical actions of individual muscle–tendon units throughout the limb are coordinated to yield the spring-like behavior of the whole limb during running (Farley and Morgenroth, 1999). Studies modeling animal limbs as a spring–mass system can generally predict basic features of running mechanics (Blickhan, 1989; McMahon and Cheng, 1990). To some extent, the spring-like properties of certain limb muscle–tendon units must underlie these predictive qualities of the whole limb's function. However, it is extremely unlikely that all limb muscle–tendon units contribute equally or at all to a limb's spring-like behavior. Ankle extensors and their long tendons provide an obvious site for the storage and release of mechanical energy and have been shown to operate in this fashion during both hopping and running (Biewener et al., 1998b; Roberts et al., 1997). In contrast, muscles such as the biceps in the rat or semimembranosus in the dog (Gregersen et al., 1998), which possess less tendon at their insertion and shorten over substantial distances during the entire stance phase of trotting,

are unlikely to contribute significantly to the spring-like behavior of the whole limb during this gait. Future studies of the *in vivo* patterns of activation and strain (and force production when possible) of muscles throughout the limb will help us to understand better how the actions of individual muscles within the limb contribute to patterns of whole-limb stiffness (e.g. Ferris et al., 1998).

In addition to trying to understand the relationships between the mechanical behavior of individual limb muscles, on the one hand, and the mechanics of the whole limb, on the other, the question of how differences in animal size affect limb muscle function during terrestrial locomotion is also of considerable importance. Because of scale differences in locomotor mechanics (Biewener, 1989), rats move with more 'crouched' postures than much larger animals such as horses. This crouched posture probably increases the relative compliance of a rat's hindlimb (Gatesy and Biewener, 1991) which, in turn may be expected to lead to different patterns of strain in certain limb muscles of large and small animals. Recent recordings of *in vivo* strain and activity in the vastus lateralis during the stance phase of trotting horses have shown that fascicles remain nearly isometric during this period, with some shortening (approximately 10 %) occurring late in the stance phase (D. F. Hoyt, personal communication). Similar recordings from the vastus lateralis of trotting dogs show that fascicles can remain nearly isometric or actively shorten during stance (Carrier et al., 1998). Nevertheless, these patterns differ considerably from what we observed in the vastus of trotting rats, in which the muscle initially lengthens substantially over the first half of stance (coincident with EMG activity) and remains nearly isometric over the remainder of support. These observations suggest that homologous muscles in different-sized animals can function quite differently with regard to fascicle length change and, presumably, muscle work production or absorption.

The extent to which muscles of different-sized animals perform different amounts of work remains unclear, but is important in regard to interpreting the size-independence of the cost coefficient (defined by Kram and Taylor, 1990). If the muscles of smaller animals perform greater mass-specific work than the muscles of larger animals (at equivalent speeds or gaits), one would expect their cost coefficient for supporting body-weight-related forces to be greater. However, the results of Kram and Taylor (Kram and Taylor, 1990) and Roberts et al. (Roberts et al., 1998) suggest that there is no size-dependence to the cost coefficient in mammalian quadrupeds or avian bipeds. Consistent with these observations, Heglund et al. (Heglund et al., 1982) observed that different-sized terrestrial mammals and birds perform the same mass-specific whole-body work when compared across speed and size. As a result, Heglund et al. (Heglund et al., 1982) concluded that differences in mass-specific work could not explain differences in mass-specific energy expenditure and were the first to suggest that differences in the rate and magnitude of muscle force generation probably explain differences in metabolic cost as a function of animal size. A key consideration is the extent to which the rate and magnitude of force generation *per se versus* net muscle work affect locomotor energy cost. Even if muscles in smaller animals perform more mass-specific work, the increased work may not be sufficiently large to affect significantly the energy cost that appears to be tied to muscle activation and force generation, independent of shortening work. Nevertheless, it will be important to examine this issue further, and *in vivo* studies of muscle mechanics in other muscle groups and in different-sized species will help to distinguish the extent to which size affects how much work the limb muscles of terrestrial mammals and birds perform during locomotion.

In summary, shifts in gait exhibited by rats over a range of speeds are accompanied not only by alterations in levels of muscle activation (as previously shown by Roy et al., 1991; de Leon et al., 1994, and others) but also by shifts in the patterns and degree of muscle fascicle strain. Subtle shifts in the timing, degree and pattern of muscle strain and activation can play a substantial role in regulating the mechanical performance of the musculature underlying diverse locomotor systems (Josephson, 1999). It is also becoming increasingly apparent that muscles do not simply shorten while active, generating positive work and power. Some muscle–tendon complexes might serve as springs, storing and releasing mechanical energy, while others might serve as brakes, actively lengthening to absorb mechanical energy in the system (e.g. Dickinson et al., 2000; Marsh, 1999). In the present study, we have shown that even the same muscle can perform quite different mechanical functions during the same gait (i.e. the vastus lateralis in the leading *versus* trailing hindlimb of a gallop). Such results not only exemplify the variable mechanical roles different muscles can play but also indicate the functional plasticity of individual muscles, even during locomotion across a flat, uniformly solid surface. As we continue to explore muscle function within the limbs of terrestrial animals over more broadly varying locomotor behaviors and environments, the mechanisms by which muscles are integrated to provide animals with the dynamic behaviors they exhibit in nature will become better understood.

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References

Alexander, R. McN. (1977). Terrestrial locomotion. In *Mechanics and Energetics of Animal Locomotion* (ed. R. McN. Alexander and G. Goldspink), pp. 168–203. London: Chapman & Hall.

Alexander, R. McN. (1988). *Elastic Mechanisms in Animal Movement*. Cambridge: Cambridge University Press.

Altringham, J. D. and Ellerby, D. J. (1999). Fish swimming: patterns in muscle function. *J. Exp. Biol.* **202**, 3397–3403.

Anapol, F. C. and Jungers, W. L. (1987). Telemetered electromyography of the fast and slow extensors of the leg of the brown lemur (*Lemur fulvus*). *J. Exp. Biol.* **130**, 341–358.

Armstrong, R. B. and Phelps, R. O. (1984). Muscle fiber type composition of the rat hindlimb. *Am. J. Anat.* **171**, 259–272.

Biewener, A. A. (1989). Scaling body support in mammals: limb posture and muscle mechanics. *Science* **245**, 45–48.

Biewener, A. A., Corning, W. R. and Tobalske, B. W. (1998a). *In vivo* pectoralis muscle force–length behavior during level flight in pigeons (*Columba livia*). *J. Exp. Biol.* **201**, 3293–3307.

Biewener, A. A., Konieczynski, D. D. and Baudinette, R. V. (1998b). *In vivo* muscle force–length behavior during steady-speed hopping in tammar wallabies. *J. Exp. Biol.* **201**, 1681–1694.

Biewener, A. A., Thomason, J. J. and Lanyon, L. E. (1988). Mechanics of locomotion and jumping in the horse (*Equus*): *in vivo* stress in the tibia and metatarsus. *J. Zool., Lond.* **214**, 547–565.

Blickhan, R. (1989). The spring-mass model for running and hopping. *J. Biomech.* **22**, 1217–1227.

Carrier, D. R., Gregersen, C. S. and Silverton, N. A. (1998). Dynamic gearing in running dogs. *J. Exp. Biol.* **201**, 3185–3195.

Cavagna, G. A., Heglund, N. C. and Taylor, C. R. (1977). Mechanical work in terrestrial locomotion: two basic mechanisms for minimizing energy expenditure. *Am. J. Physiol.* **233**, R243–R261.

Coughlin, D. J., Valdes, L. and Rome, L. C. (1996). Muscle length changes during swimming in scup: sonomicrometry verifies the anatomical high-speed cine technique. *J. Exp. Biol.* **199**, 459–463.

de la Paz, K. L., Hoyt, D. F., Cogger, E. A. and Wickler, S. J. (1999). The effects of speed and incline on function of the gluteus medius during trotting in the horse. *Am. Zool.* **39**, 64A.

de Leon, R., Hodgson, J. A., Roy, R. R. and Edgerton, V. R. (1994). Extensor- and flexor-like modulation within motor pools of the rat hindlimb during treadmill locomotion and swimming. *Brain Res.* **654**, 241–250.

Dickinson, M. H., Farley, C. T., Full, R. J., Koehl, M. A. R., Kram, R. and Lehman, S. (2000). How animals move: an integrative view. *Science* **288**, 100–106.

Earhart, G. M. and Stein, P. S. G. (2000). Step, swim and scratch motor patterns in the turtle. *J. Neurophysiol.* **84**, 2181–2190.

Engberg, I. and Lundberg, A. (1969). An electromyographic analysis of muscular activity in the hindlimb of the cat during unrestrained locomotion. *Acta Physiol. Scand.* **75**, 614–630.

Farley, C. T., Glasheen, J. and McMahon, T. A. (1993). Running springs: speed and animal size. *J. Exp. Biol.* **185**, 71–86.

Farley, C. T. and Morgenroth, D. C. (1999). Leg stiffness primarily depends on ankle stiffness during human hopping. *J. Biomech.* **32**, 267–273.

Ferris, D. P., Louie, M. and Farley, C. T. (1998). Running in the real world: adjusting leg stiffness for different surfaces. *Proc. R. Soc. Lond. B* **265**, 989–994.

Gambaryan, P. P. (1974). *How Mammals Run: Anatomical Adaptations*. New York: John Wiley and Sons.

Gatesy, S. M. and Biewener, A. A. (1991). Bipedal locomotion: effects of speed, size and limb posture in birds and humans. *J. Zool., Lond.* **224**, 127–147.

Goldman, D. E. and Hueter, T. F. (1956). Tabular data of the velocity and absorption of high frequency sound in mammalian tissues. *J. Acoust. Soc. Am.* **28**, 35–53.

Gregersen, C. S., Silverton, N. A. and Carrier, D. R. (1998). External work and potential for elastic storage at the limb joints of running dogs. *J. Exp. Biol.* **201**, 3197–3210.

Gregor, R. J., Roy, R. R., Whiting, W. C., Lovely, R. G., Hodgson, J. A. and Edgerton, V. R. (1988). Mechanical output of the cat soleus during treadmill locomotion: *in vivo* vs. *in situ* characteristics. *J. Biomech.* **21**, 721–732.

Griffiths, R. I. (1991). Shortening of muscle fibres during stretch of the active cat medial gastrocnemius muscle: the role of tendon compliance. *J. Physiol., Lond.* **436**, 219–236.

Grillner, S. (1975). Locomotion in vertebrates: central mechanisms and reflex interaction. *Physiol. Rev.* **55**, 247–303.

Gruner, J. A. and Altman, J. (1980). Swimming in the rat: analysis of locomotor performance in comparison to stepping. *Exp. Brain Res.* **40**, 374–382.

Hatta, I., Sugi, H. and Tamura, Y. (1988). Stiffness changes in frog skeletal muscle during contraction recorded using ultrasonic waves. *J. Physiol., Lond.* **403**, 193–209.

Heglund, N. C., Fedak, M. A., Taylor, C. R. and Cavagna, G. A. (1982). Energetics and mechanics of terrestrial locomotion. IV. Total mechanical energy changes as a function of speed and body size in birds and mammals. *J. Exp. Biol.* **97**, 57–66.

Hildebrand, M. (1976). Analysis of tetrapod gaits: general considerations and symmetrical gaits. In *Neural Control of Locomotion* (ed. R. M. Herman, S. Grillner, P. S. G. Stein and D. G. Stuart), pp. 203–206. New York: Plenum Press.

Hildebrand, M. (1980). The adaptive significance of tetrapod gait selection. *Am. Zool.* **20**, 255–267.

Josephson, R. K. (1999). Dissecting muscle power output. *J. Exp. Biol.* **202**, 3369–3375.

Kram, R. and Taylor, C. R. (1990). Energetics of running: a new perspective. *Nature* **346**, 265–267.

Loeb, G. E. and Gans, C. (1986). *Electromyography for Experimentalists*. Chicago: University of Chicago Press.

Marsh, R. L. (1999). How muscles deal with real-world loads: the influence of length trajectory on muscle performance. *J. Exp. Biol.* **202**, 3377–3385.

McMahon, T. A. and Cheng, G. C. (1990). The mechanics of running: how does stiffness couple with speed? *J. Biomech.* **23S**, 65–78.

Nicolopoulos-Stournaras, S. and Iles, J. F. (1984). Hindlimb muscle activity during locomotion in the rat (*Rattus norvegicus*) (Rodentia: Muridae). *J. Zool. Lond.* **203**, 427–440.

Olson, J. M. and Marsh, R. L. (1998). Activation patterns and length changes in hindlimb muscles of the bullfrog *Rana catesbeiana* during jumping. *J. Exp. Biol.* **201**, 2763–2777.

Prilutsky, B. I., Herzog, W. and Allinger, T. L. (1996). Mechanical power and work of cat soleus, gastrocnemius, and plantaris muscles during locomotion: possible functional significance of muscle design and force patterns. *J. Exp. Biol.* **199**, 801–814.

Roberts, T. J., Kram, R., Weyand, P. G. and Taylor, C. R. (1998). Energetics of bipedal running. I. Metabolic cost of generating force. *J. Exp. Biol.* **201**, 2745–2751.

Roberts, T. J., Marsh, R. L., Weyand, P. G. and Taylor, C. R. (1997). Muscular force in running turkeys: the economy of minimizing work. *Science* **275**, 1113–1115.

Roy, R. R., Hutchison, D. L., Pierotti, D. J., Hodgson, J. A. and Edgerton, V. R. (1991). EMG patterns of rat ankle extensors and flexors during treadmill locomotion and swimming. *J. Appl. Physiol.* **70**, 2522–2529.

Taylor, C. R. (1994). Relating mechanics and energetics during exercise. In *Comparative Vertebrate Exercise Physiology* (ed. J. H. Jones), pp. 181–215. San Diego: Academic Press.

Walmsley, B., Hodgson, J. A. and Burke, R. E. (1978). Forces produced by medial gastrocnemius and soleus muscles during locomotion in freely moving cats. *J. Neurophysiol.* **41**, 1203–1216.

Weyand, P. G., Sternlight, D. B., Bellizzi, M. J. and Wright, S. (2000). Faster top running speeds are achieved with greater ground forces not more rapid leg movements. *J. Appl. Physiol.* **89**, 1991–1999.

Whiting, W. C., Gregor, R. J., Roy, R. R. and Edgerton, V. R. (1984). A technique for estimating mechanical work of individual muscles in the cat during treadmill locomotion. *J. Biomech.* **17**, 685–694.