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Minimal shortening in a high-frequency muscle

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Summary

Reducing the cost of high-frequency muscle contractions can be accomplished by minimizing cross-bridge cycling or by recycling elastic strain energy. Energy saving by contractile minimization has very different implications for muscle strain and activation patterns than by elastic recoil. Minimal cross-bridge cycling will be reflected in minimal contractile strains and highly reduced force, work and power output, whereas elastic energy storage requires a period of active lengthening that increases mechanical output. In this study, we used sonomicrometry and electromyography to test the relative contributions of energy reduction and energy recycling strategies in the tailshaker muscles of western diamondback rattlesnakes (*Crotalus atrox*). We found that tailshaker muscle contractions produce a mean strain of 3%, which is among the lowest strains ever recorded in vertebrate muscle during movement. The relative shortening velocities (V/V_{max}) of 0.2–0.3 were in the optimal range for maximum power generation, indicating that the low power output reported previously for tailshaker muscle is due mainly to contractile minimization rather than to suboptimal V/V_{max}. In addition, the brief contractions (8–18 ms) had only limited periods of active lengthening (0.2–0.5 ms and 0.002–0.035%), indicating little potential for elastic energy storage and recoil. These features indicate that high-frequency muscles primarily reduce metabolic energy input rather than recycle mechanical energy output.

Key words: tailshaker muscle, rattlesnake, *Crotalus atrox*, muscle contraction, elastic recoil, cross-bridge, biomechanics.

Introduction

Sustaining high-frequency muscle contractions involves substantially reducing the metabolic energy cost per contraction. This can be accomplished by forming only a limited number of cross-bridges per contraction (for example, see Conley and Lindstedt, 1996; Marsh, 1990; Rome et al., 1999), or using energy-saving mechanisms such as elastic recoil (for example, see Biewener, 1998; Huxley and Simmons, 1971), or both mechanisms. Although these energy-saving strategies are not mutually exclusive, contractile minimization has very different implications for muscle strain and activation patterns than does elastic recoil. For example, minimal cross-bridge cycling will be reflected in minimal contractile strains and highly reduced force, work and power output (Conley and Lindstedt, 1996, 2002; Rome et al., 1999), whereas elastic energy storage requires a period of isometric contraction or active lengthening that dramatically increases mechanical output (Cavagna et al., 1994; Lindstedt et al., 2001). The relative contributions of these strategies for reducing the cost of high-frequency contractions can be tested with direct measurements of *in vivo* muscle strain and activation.

Minimal cross-bridge cycling has been determined in insect muscles using energetic measurements, work loop experiments and strain recordings (Casey and Ellington, 1989; Chan and Dickinson, 1996; Gilmour and Ellington, 1993; Josephson, 1973, 1985). Although muscle strain has not yet been measured *in vivo* in vertebrate muscles contracting at very high frequencies, several lines of evidence suggest that these contractions involve minimal strains produced by only one or two cross-bridge cycles per contraction. For example, muscle fibres can generate work *in vitro* at very low strains of 0.5% and very high contraction frequencies (90 Hz in rattlesnake tailshaker muscle and 200 Hz in toadfish swimbladder muscle; Rome et al., 1996). Tailshaker muscle and swimbladder muscle use very little energy and generate very low forces, work and power (Conley and Lindstedt, 1996; Moon et al., 2002; Rome et al., 1999). Modelling of the key sources and sinks for ATP in active muscle indicates that the major factor keeping the cost of contraction low is a small number of cross-bridge cycles per contraction (Conley and Lindstedt, 2002). These results argue strongly for minimal cross-bridge cycling during high-frequency contractions. However, direct measurements of muscle shortening are needed to determine whether high-frequency contractions in vertebrate muscle involve truly

minimal strains produced by only 1–2 cross-bridge cycles per contraction.

One major consequence of minimal strains and cross-bridge cycling may be that the resulting low forces and low work output limit power output. However, because power is proportional to contraction frequency, very high frequencies may compensate for the limited work per contraction and still generate high power. At moderate to high contraction frequencies, muscle power is typically optimized whenever the relative shortening velocity (V/V_{max}, the ratio of actual to maximal shortening velocity) is between 0.2 and 0.3 (Askew and Marsh, 1998; Hill, 1938; Rome and Lindstedt, 1997). Therefore, one indicator of contractile minimization would be low power output, but despite high contraction frequencies and optimal V/V_{max}. We have previously reported high frequencies of contraction and low power output in rattlesnake tailshaker muscle (Conley and Lindstedt, 1996; Moon et al., 2002), although we were unable to relate the low power to suboptimal V/V_{max} or to contractile minimization because *in vivo* muscle strains and shortening velocities were not yet known. Direct measurements of muscle shortening can be used to test whether low power output derives mainly from suboptimal V/V_{max} or from truly minimal strains.

Another major consequence of minimal strains and minimal cross-bridge cycling in high-frequency muscle contractions may be limited potential for elastic energy storage and recoil. For example, the low work and mechanical efficiency of rattlesnake tailshaker muscle contractions suggest limited energy savings by elastic recoil (Moon et al., 2002). A simple model of contractile energetics also indicates that energy recycling is not necessary to account for the low cost of contraction (Conley and Lindstedt, 2002). Elastic strain energy can only be stored in muscle or connective tissue during periods of isometric or eccentric contraction (Biewener, 1998; Cavagna et al., 1994; Lindstedt et al., 2001). Therefore, the duration of isometric or eccentric contraction is an indicator of the potential for energy savings by elastic energy storage and recoil. Limited isometric or eccentric contraction would indicate little potential for elastic energy storage and recoil.

In the present work, we used sonomicrometry and electromyography to record tailshaker muscle strains and activation patterns *in vivo* during rattling in western diamondback rattlesnakes *Crotalus atrox*. Our specific goals were to test the hypotheses that (1) the high-frequency contractions of tailshaker muscle involve minimal strains, (2) suboptimal V/V_{max} does not explain the low power output, and (3) the high-frequency contractions involve little isometric or eccentric contraction and therefore have little potential for strain energy storage and recoil.

Materials and methods

Animals and handling

We used 10 western diamondback rattlesnakes *Crotalus atrox* Baird and Girard from southern Arizona. To handle a snake, we first drew the snake up into a clear acrylic tube and then transferred it into a Rubbermaid® container. The tail and rattle passed out horizontally through a hole in the side of the container and were able to move freely. The tail was secured in place by Velcro® tape wrapped around the body just anterior to the cloaca, and the tape around the body bonded to complementary tape lining the hole in the container. This prevented the tail from being pulled into the container during the experiments.

Rattling frequency is temperature-dependent (Chadwick and Rahn, 1954; Martin and Bagby, 1972). We used a thermocouple placed in the cloaca to measure each animal's body temperature as it was controlled over a range of 10–30°C by circulating temperature-controlled water through tightly coiled copper tubing in the bottom of the container. We varied the starting temperature and direction of temperature change (heating or cooling by approximately 5°C per hour) for each animal, and collected data at 10, 20 and 30°C.

Muscle mass and anatomy

Three major tailshaker muscles insert directly onto the bony shaker element in the base of the rattle without any measurable tendons (Clark and Schultz, 1980; Czermak, 1857; Martin and Bagby, 1973; Zimmerman and Pope, 1948). We used external measurements to determine the volume of the tail around the tailshaker muscles, and then subtracted 15% to account for non-muscle components such as vertebrae, blood vessels and scent glands; this adjustment for non-muscle components was based on measurement from magnetic resonance images of *in vivo* tail anatomy (Moon et al., 2002). This method gives a more accurate measure of tailshaker muscle mass than by estimating it as a function of body mass, as in Schaeffer et al. (1996), because tailshaker muscle mass appears to be conserved even when body mass changes. We then converted the volume into mass by assuming a muscle density of 1.06 g ml^{-1}.

Muscle strain and shortening velocity

We measured muscle strain using sonomicrometry, in which a pair of small piezoelectric crystals is implanted in each muscle. The crystals use the time delay between the transmission and reception of ultrasound pulses to measure muscle length as it changed during contraction. We implanted pairs of 0.75 mm sonomicrometer crystals in alignment with the muscle fibres by first making a 1–2 mm incision in the skin, then puncturing the epimysium with the tip of a 16-gauge needle, and then inserting the crystals into the muscles where they cross the joint between the last caudal vertebrae and the shaker element in the base of the rattle. After crystal implantation, the incisions were sealed with surgical glue. Crystals were implanted in the lateral muscle in every snake, and also in the dorsolateral muscle and the inferior part of the ventrolateral muscle in some snakes.

For data collection, the sound velocity in muscle was set to 1540 m s^{-1}, which is most accurate at 20°C (based on data from Goss et al., 1978, 1980; Sonometrics Corporation, 2001). The velocity of sound in muscle changes with temperature,

however, with a Q_{10} of 1.025 (based on Goss et al., 1978, 1980). Therefore, to correct for differences in sound velocity in muscle at different temperatures, lengths measured at 10°C were reduced by 2.5%, and lengths measured at 30°C were increased by 2.5%. We were unable to measure resting muscle lengths and temperature-induced changes in the velocity of sound in tailshaker muscle directly because the snakes typically rattled whenever we moved to start recording data.

For analysis, the signals were digitized at 1050 Hz with a Sonometrics TRX Series 4 sonomicrometer (Sonometrics Corp., London, ON, Canada) with a distance resolution of 0.024 mm. The muscle length signals were digitally smoothed using a 3-point moving average. A ninth order polynomial curve-fitting algorithm was used on some signals from which sequences of a few data points had been dropped during signal acquisition.

From the digitized signals we measured the times and muscle lengths at the points of activation, maximum length and minimum length. We then calculated contraction frequency, muscle strain (shortening distance divided by the resting length; presented here as a percentage), average shortening velocity from peak to trough (in muscle lengths per second, $L\ s^{-1}$) and activation phase (as a percentage of contraction cycle). At each temperature, we used our shortening velocity data along with V_{max} and Q_{10} values from Rome et al. (1996) to compute the relative shortening velocity, V/V_{max}, which is the ratio of actual shortening velocity (V) to maximum shortening velocity (V_{max}).

Muscle activation

We recorded electromyograms (EMGs) simultaneously with the muscle length changes. For the EMG recordings, we used bipolar hook electrodes made from 0.08 mm diameter insulated stainless steel wire (California Fine Wire; Grover Beach, CA, USA). The electrodes had 1–2 mm bipole spacings and 1–2 mm bare tips, and were inserted into the muscle with 23-gauge hypodermic needles. The EMG signals were amplified with an A-M Systems differential AC amplifier Model 1700 (A-M Systems, Carlsborg, WA, USA). The amplifier gain was 1000 with a bandwidth of 10–500 Hz and a 60 Hz notch filter. Although some of the EMG spikes occurred in the range of 60 Hz, the waveform of each spike was much higher than 60 Hz and was not severely attenuated by the notch filter.

Statistical analyses

For every individual snake and tailshaker muscle, we selected three consecutive contractions from each of three different rattling bouts at each temperature, and then computed mean values for strain, shortening velocity and V/V_{max}. Therefore, each data point analyzed here represents a mean value for nine contractions by a single muscle at a single temperature. Prior to the statistical analyses, we inspected plots of the strain data to be sure that the slopes of strain against contraction frequency for each individual had the same polarity and similar magnitude as the overall slope for the entire sample. To determine whether the three tailshaker muscles could be analyzed together, we first used analysis of variance (ANOVA) to test for differences in strain among the three muscles. In these analyses, the dependent variables were strain, shortening velocity, and V/V_{max}; the independent variable was muscle identity (dorsolateral, lateral, ventrolateral), and the covariates were contraction frequency and muscle mass. We chose to use frequency rather than temperature as an independent variable because it varies as a direct function of temperature and it better accounts for variation in muscle function.

The ANOVA results showed that the dorsolateral muscle shortened less and more slowly than the other two muscles, which did not differ from each other in these variables. Consequently, we pooled lateral and ventrolateral muscle data and then used multiple regressions to test for the effects of twitch frequency on muscle strain. We did not compute regressions for the small number of data points from the dorsolateral muscle. Because the ANOVA results also showed that shortening variables were size-dependent, we included muscle mass as an independent variable along with contraction frequency. The dependent variables in the regression analyses were muscle strain, shortening velocity and V/V_{max}. Values are given as means ± S.D.

Results

The 10 snakes used in this study averaged 924 mm (range = 730–1160 mm) in snout to vent length, 420 g (range =173–911 g) in body mass, and 6.5 g (range = 2.7–11.6 g) in tailshaker muscle mass. Three major tailshaker muscles insert directly onto the bony shaker element in the base of the rattle without any measurable tendons. Rattling frequency was 27–64 Hz over a temperature range of 10–30°C, which indicates that contraction periods were 16–37 ms.

Muscle strain

Tailshaker muscle strain was approximately sinusoidal, with contralateral muscles shortening out of phase with each other (Fig. 1). Strains were very small and averaged 3.1±0.95% (mean ± S.D.) for the lateral and ventrolateral muscles over all temperatures. The average strain of 1.5% in the dorsolateral muscle was significantly lower than that of the other two muscles (F=8.2, d.f.=2, P=0.001). Muscle shortening increased slightly with temperature and twitch frequency (Fig. 2; Tables 1, 2).

Shortening velocity

Shortening velocity averaged 2.8±1.5 $L\ s^{-1}$ for the lateral and ventrolateral muscles over all temperatures, and increased substantially with temperature and twitch frequency (Tables 1, 2, Fig. 3). In the dorsolateral muscle, the mean shortening velocity of 1.4 $L\ s^{-1}$ was significantly lower than for the other two muscles (F=8.3, d.f.=2, P=0.001).

In the lateral and ventrolateral muscles, mean relative shortening velocity (V/V_{max}) was 0.26±0.07 over all

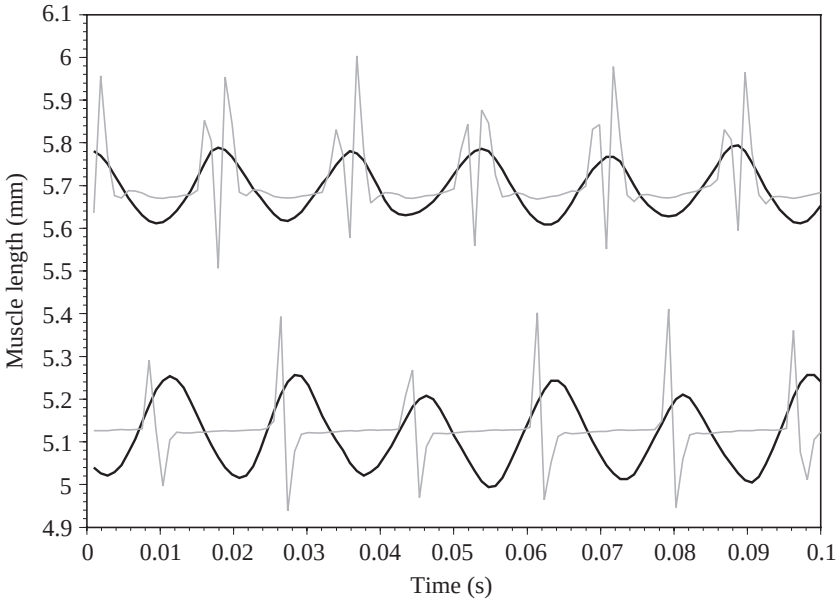

Fig. 1. Sinusoidal strain (thick black lines) and activation patterns (thin grey lines) in the lateral tailshaker muscle of a western diamondback rattlesnake *Crotalus atrox* rattling at 30°C and 58 Hz. Data for the right side (top) and left side (bottom) muscles show different lengths because the distance between sonomicrometry crystals varied slightly, not because the muscles have different resting lengths or strains.

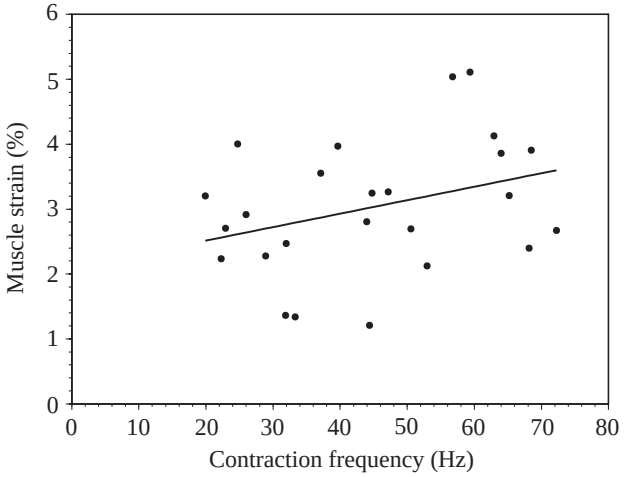

Fig. 2. *In vivo* strain of the lateral and ventrolateral tailshaker muscles during rattling in western diamondback rattlesnakes *Crotalus atrox*. The multivariate regression results are given in Table 2.

temperatures, increasing to 0.31±0.06 at 30°C. In the dorsolateral muscle, the average V/V_{max} of 0.19 was significantly lower than for the other two muscles (F=3.8, d.f.=2, P=0.03).

Muscle activation

Tailshaker muscles were activated by a single, or occasionally a double, EMG spike (Fig. 1). Muscles were activated 4.6 ms (at 10°C) to 2.4 ms (at 30°C) before reaching maximal stretch. Therefore, the contraction on one side of the tail appeared to involve a maximum of 0.5% (at 10°C) and 1.2% (at 30°C) active stretching by the contralateral muscles. This active lengthening, called eccentric contraction, is necessary for the storage of elastic strain energy, and it may dramatically increase muscle force exertion per unit energy consumed. However, because there is a delay between electrical excitation (as indicated by the EMG spike) and actual contraction (cross-bridge formation), some of the time between excitation and the onset of shortening does not involve actual

Table 1. *Mean strain patterns of tailshaker muscles during rattling in 10 western diamondback rattlesnakes* Crotalus atrox

Variable	Temperature (°C)		Increase (ratio of 30°C to 10°C) values
	10	30	
Twitch frequency (Hz)	27±4.6	64±5.3	2.4
Muscle strain (%)	2.5±0.8	3.7±0.9	1.5
Shortening velocity ($L\,s^{-1}$)	1.3±0.3	4.5±0.9	3.5
V/V_{max}	0.22±0.05	0.31±0.06	1.4

Values are mean±S.D.

$L\,s^{-1}$=muscle lengths per second.

V/V_{max}=ratio of actual to maximum shortening velocity; V_{max} taken from Rome et al. (1996).

Muscle strain and shortening velocity results are from the lateral and ventrolateral muscles only, whereas twitch frequency is from all three muscles combined.

Table 2. *Regression results for changes in lateral and ventrolateral tailshaker muscle strain with increasing twitch frequency in western diamondback rattlesnakes* Crotalus atrox

	Muscle strain (%)	Shortening velocity ($L\,s^{-1}$)	V/V_{max}
Frequency slope	0.03**	0.08**	0.002**
Muscle mass slope	0.21*	0.18**	0.01**
Intercept	0.52	–2.1**	0.09*
Adjusted r^2	0.38	0.77	0.38
Overall F	9.9**	50.6**	9.9**

*Significant at $P<0.05$.

**Significant at $P<0.01$.

Slope values for independent variables are unstandardised partial regression coefficients.

$L\,s^{-1}$=muscle lengths per second.

V/V_{max}=ratio of actual to maximum shortening velocity; V_{max} taken from Rome et al. (1996).

N=30.

Each slope indicates the bivariate relationship between the dependent variable and the particular independent variable when the other independent variable is held constant at the mean. For example, when muscle mass is held constant, contraction frequency significantly affects muscle strain with a slope of 0.03.

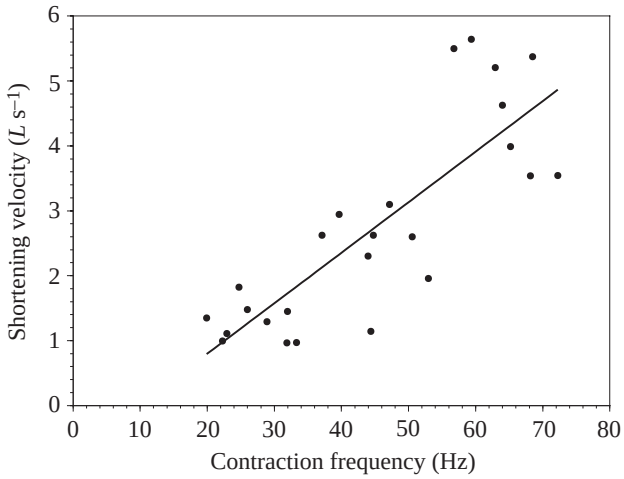

Fig. 3. *In vivo* shortening velocities of the lateral and ventrolateral tailshaker muscles during rattling in western diamondback rattlesnakes *Crotalus atrox*. The multivariate regression results are given in Table 2.

contraction. Assuming excitation–contraction delays of 4.4 ms at 10°C and 1.9 ms at 30°C (based on data for an analogous high-frequency muscle; Josephson, 1973), tailshaker muscle contractions actually involved only 0.2 ms and 0.002% active lengthening at 10°C, and only 0.5 ms and 0.035% active lengthening at 30°C.

Discussion

Rattle motion

Contractile strains were very small in all three tailshaker muscles, but the strains and shortening velocities varied among the three muscles. The differences in strain and shortening velocity among the muscles may explain the dramatic rattle twisting that we reported previously (Moon et al., 2002). Twisting appears to be produced by the lateral and ventrolateral muscles shortening more and faster than the dorsolateral muscle, thus pulling the ventral half of the rattle ahead of the dorsal half.

Tailshaker muscle strain increases slightly with contraction frequency, but rattle displacement decreases (Moon et al., 2002). Rattle motion must therefore be ballistic: muscle contraction accelerates the rattle but does not limit how far it moves. Instead of being limited by muscle strain, rattle displacement is limited by the timing of contralateral muscle contraction and perhaps by tissue stiffness. Ballistic motion is also indicated by the relationship between contraction frequency and shortening velocity. If rattle motion were limited by muscle strain, then an increase in shortening velocity should produce a corresponding one to one decrease in contraction period, and hence a one to one increase in rattling frequency. However, shortening velocity increases more (3.5 times) from 10° to 30°C than does twitch frequency (2.4 times), which accelerates the rattle faster but does not appear to limit its maximal displacement.

Are muscle strains minimal?

The 2–4% strains of tailshaker muscle contractions are among the lowest ever recorded in vertebrate muscle during movement (Conley and Lindstedt, 1996, 2002; Rome and Lindstedt, 1998; Rome et al., 1996). These low strains explain the low work and power of rattling reported by Moon et al. (2002). Although tailshaker muscle contractions are nearly isometric, they are quite different from isometric contractions in typical skeletal muscles, in which substantial cross-bridge cycling produces large forces. In contrast to these typical isometric contractions, tailshaker muscle contractions generate very low forces (Moon et al., 2002), which suggests that cross-bridge cycling is very limited.

The extremely low costs of contraction in sound-producing muscles, including tailshaker muscles, indicate that approximately 10% of available cross-bridges form and undergo only one cycle per contraction (Conley and Lindstedt, 2002). One cross-bridge cycle comprises a truly minimal contraction. Do the small strains in tailshaker muscle contractions reflect minimal cross-bridge cycling? It is possible to estimate the number of cross-bridge cycles required to produce the observed muscle strains if the sarcomere length and compliance are known. For example, if the sarcomere length in tailshaker muscle is approximately 2.4 μm (Clark and Schultz, 1980), then muscle strains of 0.025–0.037 correspond to half-sarcomere strains of 30–44 nm. If a cross-bridge stroke is 20 nm (Ishijima et al., 1996) and stiffness is moderately high (which appears to be the case; Martin and Bagby, 1973), then

the observed muscle strains would be truly minimal, with only 1–2 cross-bridge cycles per contraction. The minimal cross-bridge cycling explains the low force and cost of tailshaker muscle contractions.

Is low muscle power best explained by contractile minimization or by suboptimal V/V_{max}?

Power is the rate at which work is done by muscle, and it is proportional to the frequency of contraction. Muscle power is optimized when the relative shortening velocity (V/V_{max}) is between 0.2 and 0.3 (Askew and Marsh, 1998; Hill, 1938; Rome and Lindstedt, 1997). Therefore, a major indicator of contractile minimization would be low power output, but despite high contraction frequencies and optimal V/V_{max}.

Although the contraction frequencies of tailshaker are very high, power output is very low (mean of 3.0 W kg^{-1} muscle at 30°C; based on data from Moon et al., 2002). Is the low power due to contractile minimization or to suboptimal V/V_{max}? In our previous study (Moon et al., 2002), we were unable to relate the low power to suboptimal V/V_{max} or to contractile minimization because *in vivo* muscle strains and shortening velocities were not yet known. In this study, we measured V and used V_{max} and Q_{10} values from Rome et al. (1996) to determine that V/V_{max} varies between 0.2 (at 10°C) and 0.3 (at 30°C) in tailshaker muscle. These values conform to the optimal range for maximum power generation (Askew and Marsh, 1998; Hill, 1938; Rome and Lindstedt, 1997), which indicates that suboptimal V/V_{max} is not the primary cause of low power output by tailshaker muscle. Instead, the minimal strains involving only one or two cross-bridge cycles per contraction limit muscle force, work and power, despite high contraction frequencies and optimal V/V_{max}. These results support the hypothesis of contractile minimization in tailshaker muscles.

Do high-frequency contractions involve elastic recoil?

The lack of measurable tendons in the tailshaker muscle segments that we measured indicates limited structural potential for elastic strain energy storage outside the muscle compared to muscles that have long tendons. However, elasticity may occur in other connective tissues or in cytoskeletal elements such as titin molecules and the cross-bridges themselves (Huxley and Simmons, 1971; Lindstedt et al., 2002; Reich et al., 2000).

There are three lines of evidence that there is little potential for storing and recycling elastic strain energy in tailshaker muscle. First, if energy recycling were important in tailshaker muscle contractions, then it should produce high apparent efficiencies. However, the mechanical efficiency of tailshaker muscle is very low, 0.3–11%, which indicates that energy recycling is limited (Moon et al., 2002).

Second, eccentric contraction (active stretching), or isometric contraction together with stretching in a tendon, is required for storing elastic strain energy in muscle (Biewener, 1998; Cavagna et al., 1994; Lindstedt et al., 2001). However, there are no measurable tendons and eccentric contraction is limited in the tailshaker muscles that we measured. At low contraction frequencies, nearly all of the apparent eccentric contraction can be accounted for by the excitation–contraction coupling (ECC) delay. In contrast to tailshaker muscle activation 0.7–3% of the cycle before the onset of shortening, some mammal and bird muscles that recycle substantial amounts of strain energy are activated 14–29% of the cycle in advance of shortening (Biewener, 1998). Although the moderate passive stiffness (see fig. 6 of Martin and Bagby, 1973) and the limited eccentric contraction (0.002–0.035%) may enhance force output and allow some strain energy to be stored and recycled, the low mechanical efficiency (≤11%; Moon et al., 2002) suggests that energy recycling is considerably less than 11%. Limited strain energy recycling (approximately 10%) also occurs in the high-frequency contractions of *Drosophila* flight muscles (Dickinson and Lighton, 1995), and in the limb muscles that produce rapid accelerations in small mammals such as kangaroo rats (Biewener et al., 1981).

It is possible to estimate the potential range of energy storage if tailshaker muscle is assumed to act as a simple spring that obeys Hooke's Law (Alexander, 1988): strain energy=$Fx/2$, where strain energy is in Joules, F is muscle force in N (derived from Moon et al., 2002), and x is the magnitude of active lengthening in m from this study. The strain energy can then be divided by the energy used to shake the rattle (from Moon et al., 2002) to estimate energy recycling. The muscles on one side of the tail are stretched by the force from the contralateral muscles. We estimated strain energy storage and recoil using the amount of active lengthening after accounting for the ECC delay, and assuming that the muscles are stretched by maximal force. Under these conditions, tailshaker muscle could store and recycle 9.0×10^{-9} J or 0.04% of the energy required to rattle at 10°C and up to 5.5×10^{-7} J or 0.78% at 30°C. Thus, although this model greatly simplifies the muscle mechanics, it supports the inference that energy storage is very limited in tailshaker muscle.

Third, this very limited energy storage and recoil is quantitatively consistent with a simple model of contractile energetics (Conley and Lindstedt, 2002). The energetic analysis showed that the major factor keeping the cost of contraction low is a small number of cross-bridge cycles per contraction; energy recycling is not necessary to account for the low cost of contraction.

Energy recycling versus energy reducing strategies

Muscles that recycle elastic strain energy typically exert high forces that produce large joint displacements, do considerable work, and appear to have high efficiency (Biewener, 1998; Ettema, 1996; Heglund and Cavagna, 1987; Minetti et al., 1999; Roberts et al., 1997; Woledge et al., 1985). In contrast, synchronous muscles that contract at very high frequencies appear to be 'energy reducers' rather than 'energy recyclers' (Conley and Lindstedt, 2002). Rattlesnake tailshaker muscles sustain high-frequency contractions with low

metabolic energy use by contracting with minimal strains and by generating very low forces, work and power (Conley and Lindstedt, 1996, 2002; Moon et al., 2002). These features indicate that tailshaker muscles, and perhaps other high-frequency muscles, primarily minimize cross-bridge cycling and reduce metabolic energy input rather than recycle mechanical energy output.

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