Indirect evidence for elastic energy playing a role in limb recovery during toad hopping

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Elastic energy is critical for amplifying muscle power during the propulsive phase of anuran jumping. In this study, we use toads (Bufo marinus) to address whether elastic recoil is also involved after take-off to help flex the limbs before landing. The potential for such spring-like behaviour stems from the unusually flexed configuration of a toad’s hindlimbs in a relaxed state. Manual extension of the knee beyond approximately 90° leads to the rapid development of passive tension in the limb as underlying elastic tissues become stretched. We hypothesized that during take-off, the knee regularly extends beyond this, allowing passive recoil to help drive limb flexion in mid-air. To test this, we used high-speed video and electromyography to record hindlimb kinematics and electrical activity in a hindlimb extensor (semimembranosus) and flexor (iliofibularis). We predicted that hops in which the knees extended further during take-off would require less knee flexor recruitment during recovery. Knees extended beyond 90° in over 80% of hops, and longer hops involved greater degrees of knee extension during take-off and more intense semimembranosus activity. However, knee flexion velocities during recovery were maintained despite a significant decrease in iliofibularis intensity in longer hops, results consistent with elastic recoil playing a role.

1. Introduction

The importance of elastic energy in augmenting muscle power and saving metabolic energy has been a consistent theme crucial to our understanding of terrestrial locomotion (see [1] for a recent review). In diverse animals, running [2], hopping [3] and even walking [4] use cyclical, spring-like actions of tendons stretching and recoiling to provide positive work for propulsion. Ballistic movements like jumping also rely on elastic strain energy, which may be stored in various tissues, and released rapidly to amplify the power available at take-off [5–7].

In the examples above, the release of elastic energy from deformed tissues is important in the propulsive phase of locomotion. However, in most forms of terrestrial locomotion, there is also a recovery phase in which the limb is repositioned for the next propulsive event (e.g. the swing phase of walking and running). The energetic cost of the recovery phase can be high [8], but little is known about whether elastic energy is also used to augment muscle actions during this phase of terrestrial locomotion.

Cane toads (Bufo marinus) are an excellent model for exploring the potential role of elastic energy during the recovery phase of locomotion. Unlike in many anuran jumpers, toad hindlimbs rapidly flex back towards the body immediately after take-off. In addition, toad hindlimbs maintain a relatively flexed configuration in a relaxed state [9] in which the femur and tibiofibula are nearly perpendicular to one another, and extension beyond this develops passive tension in the limb, as underlying tissues including major flexor muscle–tendon units are stretched (electronic supplementary material, figure S1). We propose...
a model in which limb extension during take-off stretches such elastic tissues, effectively loading a tension-spring, which in turn recoils, helping to flex the limb during recovery (figure 1a). To address this, we test two hypotheses. First, we hypothesize that during take-off, toads regularly extend their knees beyond 90°, stretching major limb flexors and tendons and developing passive tension within the limb. Second, because the recoil of these flexor muscle–tendon units could provide energy for subsequent limb flexion, we hypothesize that when hindlimbs extend further during take-off, flexor muscles driving recovery movements will be recruited less intensely.

2. Material and methods

(a) Animals
Five cane toads, *B. marinus* (two male and three female), ranging in mass from 137 to 253 g (mean = 177 g) were used for kinematic analysis and electromyography (EMG) recordings. All animals were housed in groups of three to four in large aquaria and fed crickets several times weekly.

(b) Electromyography and kinematic data collection
EMG data were collected from *m. semimembranosus* and *m. iliofibularis*, which although biarticular, act largely in hip extension and knee flexion, respectively [10], and are superficial and easy to access. To implant electrodes, toads were anaesthetized in a solution of MS-222 (1.5 g l$^{-1}$). Once anaesthetized, 2–3 cm skin incisions were made on the dorsal surface of the thighs to expose the muscles. Bilateral implants were used to increase the likelihood of getting extensor and flexor data from the same limb. Bipolar electrodes were made and implanted as described in detail in previous work [11]. EMG signals were amplified 1000× with Grass P511 preamplifiers using a notch filter at 60 Hz. Signals were digitized at 5000 Hz using an Axon Instruments’ Digidata 1322A 16-bit A/D converter and saved onto a personal computer.

For kinematic data collection, small squares of white cardboard (approx. 3 × 3 mm) were glued to the skin over the hip, knee and ankle joints. Once recovered from anaesthesia, animals
were placed in a well-lit enclosure (64 × 107 cm) and underwent 9–15 hopping trials. Videos of hops were taken with two Fastec HiSpec 1 high-speed video cameras at 500 fps. For three-dimensional spatial calibration, a 64-point calibration cube (21 × 21 cm) was digitized in MATLAB using DLTdv5 [12]. Videos were synchronized with EMG signals using a 5 V trigger that stopped video recording and was included on its own channel with the EMG data. After hopping trials, toads were euthanized by overnight submersion in MS-222 (1.5 g l−1). Post-mortem dissections were used to confirm electrode placements.

(c) Data analysis
Hop videos were analysed to identify the timing of the onset of movement, take-off and landing. Hindlimb joint markers were digitized in MATLAB using DLTdv5 [12] and analysed with customized scripts to calculate angular excursions and velocities of the knee as well as the distance of each hop. Maximum knee angle, maximum knee extension velocity and maximum knee flexion velocity were determined for each hop and regressed against hop distance using linear regression in R [13]. We also regressed maximum knee flexion velocity (during recovery) against maximum knee angle (during take-off) to directly test our second hypothesis.

EMG data were analysed using customized MATLAB scripts in which the muscle activity burst timing was identified visually for each hop and the rectified, integrated area (intensity) of the EMG signal was calculated. For the iliobibularis, the first 60 ms of the EMG signal was used to calculate burst intensity. Given the lag between activation and force development, this interval adequately captures activity important for generating forces during rapid limb flexion. To control for differences in electrode construction and placement between individuals, each muscle’s intensities were normalized to the largest value recorded in each animal. Linear regressions in R were used to test each animal for relationships between hop distance and muscle recruitment intensity. When multiple, but not all, individuals showed significant relationships, Fisher’s method for combining and analysing p-values from multiple independent tests was employed to assess the generality of the result [14].

3. Results
(a) Kinematics
A total of 56 hops were analysed. All hops involved hindlimb extension during the propulsion phase, followed by hindlimb flexion during the recovery phase in preparation for landing (figure 1a,b). In 82% of hops, the knees extended beyond 90° during take-off (figure 2a). In all five animals, there was a significant positive relationship between hop distance and the maximum knee angle reached at take-off (figure 2a). Maximum knee extension velocity also increased significantly with hop distance in all five animals (figure 2b). Knee flexion began immediately after take-off (figure 1b), and maximum knee flexion velocity showed no relationship with hop distance in four of five animals (figure 2c).

(b) Electromyography data
In all hops, a relatively short burst of semimembranosus activity was followed by a longer burst of iliobibularis activity (figure 1c). Semimembranosus activity began near the onset of hop movement and there was a significant positive relationship between hop distance and semimembranosus intensity in four of five animals (figure 2d; Fisher’s method: p < 0.001). Iliobibularis activity began 32 ± 4 ms (mean ± s.d.) before the onset of knee flexion, but the relationship between hop distance and iliobibularis intensity was significant and negative in three of five animals (figure 2e; Fisher’s method: p < 0.001). Thus, longer hops, which use more knee extension during take-off, involve lower levels of iliobibularis recruitment during recovery, without any concomitant reduction in knee flexion velocity.

4. Discussion
Our results support both hypotheses proposed in the introduction. First, in most hops (82%), knees extend beyond 90° (figure 2e), where passive tension begins to develop in a manually extended limb (electronic supplementary material, figure S1). Second, less intense knee flexor activity is required to flex the knees during long hops, in which knee extension during take-off is greatest (figure 2).

Why in longer hops are knee flexion velocities maintained during recovery despite decreasing recruitment intensities in the knee-flexing iliobibularis? We propose that the answer, at least in part, is an increased contribution of elastic recoil of tissues that are stretched more during the take-off phase of longer hops. We have shown that the degree of knee extension increases with hop distance and that passive forces increasingly develop in the hindlimb when it is manually extended to levels observed in normal hopping (figure 2a and the electronic supplementary material, figure S1). These forces develop as tissues such as the flexor muscles themselves begin to be stretched, storing elastic energy. As a result, as hops increase in length, animals rely less on active muscle work to flex the knee during recovery and more on the release of this elastic energy to maintain performance.

The iliobibularis is not the only knee flexor in the hindlimb, and perhaps our results might be explained by contributions from the semitendinosus, which has a greater moment arm for knee flexion [10], and could compensate for the decreased recruitment levels in the iliobibularis. However, preliminary results indicate that semitendinosus recruitment parallels that in the iliobibularis—recruitment levels decrease in longer hops (electronic supplementary material, figure S2). It is also possible that differences in the rate and degree of knee flexor stretching during take-off could lead to conditions in which lower stimulation levels still lead to high forces, via force enhancement, and future work should explore this possibility. Nevertheless, our result that hops in which more knee extension occurs during take-off involve reduced knee flexor recruitment levels, without a loss of knee flexion velocity, is consistent with elastic energy playing a role in limb recovery, especially in longer hops.

Although elastic recoil has been invoked as a prominent mechanism powering the propulsive phase of anuran jumping [6–7], this is, to our knowledge, the first evidence supporting a role for elastic recoil during recovery. In cane toads, which string together many hops to cover long distances [15], the ability to use less muscle energy to reposition the limbs with every hop may increase energy savings during long-distance dispersion.

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Figure 2. Effects of hop distance on various kinematic and EMG parameters. Different symbols represent different animals, and each point is a single hop. Regression lines are present when relationships are significant at $p < 0.05$, and for all animals, $R^2$-values are provided in the legend as well as the $p$-value, when significant: *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$. (a) In all five animals, knees extended more during take-off in longer hops. (b) In all five animals, knee extension velocities were greater in longer hops. (c) In one animal, knee flexion velocity increased with hop distance; in all others, there was no such effect. (d) In four of five animals, semimembranosus EMG intensity increased with hop distance; when all five $p$-values were assessed using Fisher’s method, the result was significant ($p < 0.001$). (e) In three of five animals, iliofibularis EMG intensity decreased with hop distance; when all five $p$-values were assessed using Fisher’s method, the result was significant ($p < 0.001$).
References


