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Moving in a complex world: how surface mechanics and proprioception influence locomotion

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Moving in a complex world: how surface mechanics and proprioception influence locomotion

DISSERTATION

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for the degree of

DOCTOR OF PHILOSOPHY

in Biological Sciences

by

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Dissertation Committee:
Associate Professor Emanuel Azizi, Chair
Associate Professor Monica Daley
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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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ABSTRACT OF THE DISSERTATION

Moving in a complex world: how surface mechanics and proprioception influence locomotion
by
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Moving effectively across a wide range of surface conditions is paramount to acquiring food and finding mates while simultaneously avoiding injury and predation. We used the cane toad, *Rhinella marina*, as a model organism to better understand how the environment shapes our locomotion because of toads' jumping gait which pushes locomotor forces to relative extremes. Their amphibious nature also ensures they encounter a wide variety of surface conditions in their natural habitat. In Chapter 1, we used high speed videography and a moveable landing platform instrumented with a force transducer to measure landing kinematics and kinetics. We found that toads did not alter their landing behavior when landing on compliant surfaces. In Chapter 2, we examined the toad's forelimb muscle activity by implanting electrodes in their *pectoralis, deltoideus*, *anconeus*, and *palmaris longus* muscles. Our results paralleled the findings from Chapter 1, were none of the muscle changed their intensity in preparation for or during landing. In Chapter 3, we tested the idea that hindlimb proprioception was informing forelimb landing behavior by performing sciatic nerve reinnervation which allowed motor control to recover while ablating the stretch reflex. We showed that the elbow joint was significantly affected even 6 months following surgery when hindlimb motor function recovered, suggesting the importance of hindlimb proprioception influencing forelimb landing behavior.

INTRODUCTION

How do we traverse a swamp given the uncertainty of sinking deep into soft mud, or clamber over a rocky hillside that with one misstep could result in catastrophic injury? This is a problem all terrestrial animals face when moving throughout their environment. We must possess control strategies that allow us to effectively navigate a variety of surface conditions while avoiding injury. This dissertation investigates how animals effectively move across compliant surface conditions and the role sensory information plays in affecting that coordinated behavior.

Vertebrates have relatively consistent behavior when locomoting. Generally, we experience reduced impact forces and dissipate less energy on compliant substrates which give way and absorb impact energy as they deform (Ferris & Farley, 1997; Demes et al. 1995; Kerdok et al. 2002). We also extend our limbs and orient them prior to impact to allow them the greatest chance to resist impact forces and perform the work necessary to safely decelerate the body (Azizi et al. 2014, Cox & Gillis, 2017; Demes et al. 1995; McKinley & Pedotti, 1992). This preparatory behavior is driven by the activation of muscles well in advance of contact with the ground (Akella & Gillis, 2011; Santello et al. 2001).

While our nervous system can coordinate the muscles to move effectively, it is not the only mechanism that allows us to deal with obstacles or changes in surface properties. For example, unexpected changes in step height and surface compliance result in similar preparatory behavior but produce altered limb stiffness immediately following the unanticipated impact (Daley et al. 2009; Ferris et al. 1999; Moritz & Farley, 2004). These changes following impact occur much too rapidly for neural feedback to coordinate an effective neuromuscular response to the perturbation, but the intrinsic mechanics of the skeletomuscular system allows the individual to continue moving with minor interruption in their gait. Any significant change in motor control usually occurs during subsequent strides and quickly returns to steady-state conditions within two strides following the perturbation (Daley et al. 2009).

We can tune motor control to better navigate obstacles or different terrain by incorporating sensory feedback while moving. Muscles rely on spindles which are cells wrap around muscle fibers that act as stretch receptors, informing the nervous system of both the magnitude and rate of their length changes. This information is sent to the central nervous system where in the spinal cord it can elicit a myotatic stretch reflex – which most of us are familiar with as the knee-jerk reaction when the doctor taps on our patellar tendon and rapidly stretches our quadricep muscles. The neural signal does not stop at the spinal cord but continues to travel proximally to the brain where it can be perceived as proprioception and potentially be incorporated into higher-level decision-making motor control strategies. However, this added distance to transmit the signal and then determine a response comes at the cost of requiring significantly more time for an effective response than relying on intrinsic mechanics or spinal circuitry alone.

Muscles also possess Golgi tendon organs which sense the force generated by the muscle and provide positive force feedback during stance but limit the maximal tension the muscle can exert for safety reasons (Nichols 2018). While both the Golgi tendon organs and muscle spindles serve to inform the nervous system of the muscle’s activity, it appears muscle spindles are more susceptible to connectivity problems following peripheral nerve damage (Lyle et al. 2016). In, humans this is most often caused by physical injury or complications from conditions like diabetes that damage peripheral nerves. The loss in spindle connectivity mainly affects movements where muscles contract eccentrically, or lengthen (e.g. walking downhill), in which case the lack of spindle feedback can severely disrupt both joint function and overall gait dynamics.

The main objective of my dissertation is to better understand how animals compensate for changes in mechanical surface properties and sensory deficits while locomoting. We used cane toads, *Rhinella marina*, as a model organism to explore how animals coordinate motion on various substrates because their amphibious nature means they traverse a wide range of terrestrial surface conditions in their natural habitat. The unique jumping gait of toads also requires their hindlimbs to generate high-energy propulsion and their forelimbs to rapidly decelerate the body and absorb impact energy. Cane toads extend their forelimbs farther with greater jump distance to effectively stick the landing and deal with greater impact energy (Gillis et al. 2010). The forelimb muscles undergo eccentric contractions during landing which puts toads at greater risk of injury and has received far less attention from the scientific community than the concentric takeoff behavior (Azizi & Abbott, 2013).

Summary of Chapters

In **Chapter 1**, I investigated how surface compliance affects the coordinated landing behavior exhibited by cane toads. The landing surface could move vertically along a linear translation stage and was either attached to springs to create a compliant landing condition or locked in place for a rigid surface. I measured forelimb kinematics and estimated energy dissipated by the arms from high-speed video recordings throughout forelimb-mediated deceleration. Impact kinetics were captured from the left limb using a force transducer positioned under the left half of the mobile landing platform, and all forces were corrected by subtracting off the associated accelerations due to the platform's acceleration. I found that toads do not change their kinematics or kinetics when landing on rigid or compliant surfaces that yield up to

20 percent of their forelimb length under a load equivalent to their body weight. This suggests toads are not responding to sensory information after landing to deal with variation in surface compliance.

In **Chapter 2**, I explored how cane toads were coordinating their forelimb muscles to land effectively on surfaces differing in compliance. I implanted fine-wire electrodes to measure forelimb muscle activity during jumping from the *pectoralis*, *deltoideus, anconeus*, and *palmaris longus*. Again, I found that none of the muscles altered their intensity of activation in preparation for or following landing. My results from Chapter 2 further support my Chapter 1 findings that toads do not attempt to alter their landing strategy based on the stiffness of the landing surface.

Finally, in **Chapter 3** I considered how proprioception from the hindlimbs during takeoff may be influencing landing behavior given that the takeoff platform was always held rigid in our previous experiments. I attempted to ablate proprioception from muscle spindles by performing bilateral sciatic nerve reinnervations, as well as sham surgeries to ensure our disruption of any connective tissue was not the cause for any differences in behavior. The reinnervation procedure significantly reduced the response of the stretch reflex while allowing motor function to be restored, and I observed a significant reduction in the rate of elbow extension while the overall forelimb extension at touchdown was conserved 6 months following surgery. These findings suggest proprioceptive feedback from the hindlimbs during takeoff is important for coordinating forelimb landing behavior.

CHAPTER 1

How substrate compliance affects coordinated landing in the cane toad, *Rhinella marina*

Abstract

The cane toad, *Rhinella marina*, has become a model for studying controlled decelerations; however, the kinematics and kinetics of their landing ability have largely been studied on rigid surfaces. Decelerations cause muscles to contract eccentrically which pose a greater risk of skeletomuscular damage than concentric contractions which have historically received greater attention. We know little about how animals like toads alter their behavior under variable substrate conditions. This study used high-speed videography in conjunction with force plates to determine whether forelimb-mediated landing behavior in cane toads ($N = 8$) varied across four compliance treatments (0, 2.5, 5 and 10 mm BW^{-1}) and when landing on platforms of different inertial mass (123 and 725 g). We observed two distinct landing strategies – a coordinated landing where forelimbs kept the animal's trunk and head from contacting the substrate and a crash-landing strategy where the trunk and/or head smashed into the ground. We found individuals maintained similar kinematic behavior and kinetics across compliance treatments and platform mass.

Symbols & Abbreviations

BW – body weight

COM – center of mass

Disp. - displacement (Fig. 1.1)

ΔLER – change in limb extension ratio, or limb compression during landing (Fig. 1.1)

E_{arms} – energy (or work) dissipated by arms

GRF – ground reaction force (Fig. 1.1)

LER – limb extension ratio, measured from shoulder to metacarpophalangeal joint (Fig. 1.1)

SVL – snout vent length

θ_{mipact} – angle, from vertical, of the center of mass velocity at touchdown (Fig. 1.1)

Introduction

The impact forces and energy associated with terrestrial locomotion are increased in certain contexts like running down-hill, decelerating, or landing on stiff surfaces (e.g. compact Earth, rock, cement, etc.). The musculoskeletal system is responsible for producing forces to counteract these impacts and dissipate mechanical energy upon landing or decelerating. Animals - including humans - generally exhibit reduced impact forces and energetic costs when traversing compliant surfaces (Ferris & Farley, 1997; Demes et al. 1995; Kerdok et al. 2002), which could be attributed to actively modulating muscle activity or through passive mechanical changes in limb posture after impact (McKinley & Pedotti, 1992; Moritz & Farley, 2004). This repeatedly high impact force and energy risk damage to the musculoskeletal system, making it critical for animals to possess control strategies that allow them to locomote safely through real-world environments that vary in surface properties.

In general, coordinated landings for animals and humans are characterized by relatively constant extension of their limb joints in preparation for impact (Cox and Gillis, 2015; Konow & Roberts, 2015; McKinley & Pedotti, 1992). Extending the limbs further as jump or fall length increases affords the individual a greater distance over which to apply force after impact to successfully decelerate their body and avoid injury. Several vertebrates can also alter their limb posture prior to touchdown to reduce torques and peak forces (Azizi et al. 2014; Cox & Gillis, 2017; Demes et al. 1995; McKinley & Pedotti, 1992). For multiple species it appears that visual input is not critical to coordinate landings, and that proprioception and vestibular senses are more important for preparatory behavior prior to impact (Cox & Gillis, 2016; Ekstrom et al. 2018; Santello et al. 2001). The muscles involved with dissipating impact energy tend to activate well before touchdown and increase in activation until impact, driving limb extension (Akella & Gillis, 2011; Ekstrom & Gillis, 2015; Santello et al. 2001). Together these findings suggest animals coordinate muscles across their limb to decelerate their body safely upon impact, and they may be relying on proprioceptive feedback to land effectively on a given surface.

Modulating landing behavior based on surface conditions is useful for animals to reduce their risk of injury as well as potentially take advantage of surface properties like elastic recoil that can cycle energy into subsequent movements. Landings on rigid surfaces produce larger displacements in joint ranges of motion across the limb and cause more rapid flexion of distal joints (McKinely & Pedotti, 1992; Moritz & Farley, 2004). Compliant surfaces may also reduce the peak impact forces which may explain why less joint displacement is needed to adequately decelerate (Demes et al. 1995; Ferris & Farley, 1997). Unknown perturbations in surface properties like step height and compliance elicit changes in limb stiffness without prompting changes in muscle activity suggesting the changes in limb stiffness are largely attributable to intrinsic or passive mechanical properties (Daley et al. 2009; Ferris et al. 1999; Moritz & Farley, 2004). However, when human subjects are aware of surface compliance prior to impact they do alter muscle activation and joint kinematics (McKinley & Pedotti, 1992). This more active change in limb stiffness is likely a consequence of the individual altering muscle co-contraction to maintain overall stiffness of the system (combined surface and limb stiffness) to achieve similar center of mass dynamics (Ferris & Farley, 1997). Thus, when surface compliance is unknown to the individual, they are likely to elicit an intrinsic mechanical response upon landing that may results in reduced impact forces. Trying to coordinate locomotion on compliant surfaces is further complicated by the effects of the inertial properties of the surface which can delay the compression and recoil of a substrate influencing the energy absorbed and returned from the surface, respectively (Reynaga et al. 2019).

Anurans, the clade consisting of frogs and toads, have a saltatory gait that can produce ground reaction forces (GRFs) several times their own body weight. Furthermore, Essner et al. (2010) found that more terrestrial species, like toads, exhibit the most derived and robust landing behavior. The cane toad, *Rhinella marina* (formerly *Bufo marinus*), is thus a useful model for understanding controlled decelerations as individuals can travel distances of up to 1 km per night by performing hundreds of jumps in a short period and likely encounter a wide range of environmental and surface conditions (Alford et al., 2009). The ability to repeatedly decelerate the body upon impact in a coordinated fashion makes the cane toad a superior model for investigating muscular aspects of controlled deceleration. Additionally, the landing phase poses a greater risk of injury to the skeletomuscular system in toads as impact forces are higher than at takeoff and the forelimbs are shorter in length and have less muscle mass than hindlimbs which affords less distance over which to effectively dissipate mechanical energy (Nauwelaerts and Aerts, 2006).

Many studies have probed the effects of surface compliance on landing behavior; however, most controlled landing studies on cane toads have focused on flat, rigid surfaces and few have looked at how these animals respond to variation in the landing surface compliance. While flat, rigid surfaces offer a consistent platform to compare various manipulative treatments, it poorly represents the variation in task-space these animals are likely to encounter in their natural habitat. We were interested in exploring how surface compliance would affect the cane toad’s limb posture, impact forces and energy during landing? If toads were unaware of the landing surface compliance, then we anticipate them exhibiting 1) similar maximal limb extension prior to landing but greater limb compression while decelerating, 2) increased impact forces, impulse, and maximal load rate, as well as 3) greater forelimb work to absorb impact energy on rigid surfaces as compared to landing on more compliant surfaces.

Methods

Animals

Eight cane toads (*N* = 8; 6 females and 2 males), *Rhinella marina* (Linnaeus, 1758; formerly *Bufo marinus*), of 112.0 ± 28.0 g body mass (mean ± s.d.) and 104.9 ± 13.9 mm snout-vent-length (SVL) were obtained from a commercial supplier (Reptile City; Honey Grove, TX, USA). These animals were housed in large aquariums in groups of three. Ambient temperature was maintained between 20°C and 22°C and a 12h-light-12h-dark cycle was maintained. Toads were fed crickets twice weekly and provided fresh water daily. Experimental work was conducted under the guidelines set forth by the University of California Irvine’s Institutional Animal Care and Use Committee protocol AUP-17-170.

Experimental Design

We recorded 260 landing trials within a 70 × 32 × 45 cm (*l* x *w* x *h*) open-top jumping arena, constructed from acrylic walls. Only trials characterized as coordinated landings (*n* = 235) where the head or torso did not contact the ground were used for further analyses, the other trials deemed as crash landings (*n* = 35) were excluded from statistical models. A 10 × 10 cm takeoff platform was mounted on a horizontal, linear track that constrained the height of the takeoff platform to that of the landing platform. The linear track allowed the leading edge of the takeoff platform to be positioned 2.5 times the SVL of the toad away from the center of the landing platform. This served to reduce variation in jump distance and ensured an aerial phase followed by forelimb-mediated landing. The landing platform was constructed with two, 7.5 × 15 cm surface plates to make a 15 × 15 cm surface (Fig. 1.1). A six-axes ATI mini40 force transducer (Orion Township, MI, USA) was placed under one of the two surface plates and a spacer was put under the adjacent plate to achieve the same height. This setup allowed us to capture the ground reaction force from only the left forelimb, and we subtracted off the inertial force from the motion of the force transducer during our analysis. A layer of Crocus N-72 sandpaper was fitted to the top surface of each of the two 7.5 × 15 cm landing surfaces to provide traction for the toads. The landing platform was constrained with an NCR 430 Linear Translation Stage (Thorlabs, Inc.; Newton, NJ, USA) so it could only slide vertically. Springs of various stiffness could also be attached to alter the compliance of the landing platform. All mobile components of the platform had a mass of 725 g. To address the problem of the large inertial mass of this original platform that was almost an order of magnitude more massive than the toads, we constructed a less-massive platform that had a similar design and measured 8 × 8 cm with a total mass of 123 g.

During this experiment four compliance treatments were chosen to produce a given displacement per body weight of toad; 0, 2.5, 5, and 10 mm BW^{-1}. Given that the average cane toad forelimb in this study was approximately 5 cm in length, the 10 mm BW^{-1} condition would result in an approximate platform displacement of 20% of the animal’s forelimb length for each unit of body weight. Toads were weighed each day prior to jumping and we used different combinations of Ultra Precision Extension Springs (McMaster-Carr; Santa Fe Springs, CA, USA) in series to achieve the desired compliance; 137, 183, 228, 367, 672, and 960 N m^{-1} (0.78, 1.04, 1.30, 2.09, 3.82, and 5.47 lbs in^{-1}, respectively). We used Hooke's law (Eqn. 1) and the subsequent equation for determining a functional spring constant of two springs in series (Eqn. 2) to achieve 2.5 ± 0.4 (mean ± SD, $n = 65$ landings), 5.0 ± 0.7 ($n = 65$), and 10.0 ± 0.7 mm BW^{-1} ($n = 65$) conditions for all toads.

$$\textbf{Equation 1:}\quad F = k \cdot \Delta z$$

$$\textbf{Equation 2:}\quad k = \left(\frac{1}{k_1} + \frac{1}{k_2}\right)^{-1}$$

Where F represents the force or body weight of a toad, k is the functional spring constant, Δz is the desired vertical displacement of the platform, and k_1 *and* k_2 *are the spring constants of the actual springs used.*

Rigid trials (0 mm BW^{-1}) were achieved by locking the translation stage with a screw. The four compliance treatments were randomized each day and toads only performed one successful trial for each compliance treatment in a day. A successful trial was defined as having the left forefoot contact only the plate over the force transducer and right forefoot contact the adjacent plate.

Animals were marked with whiteout and a sharpie at the center of rotation of their metacarpophalangeal, radiocarpal (wrist), humeral-ulnar (elbow), glenohumeral (shoulder), and the temporomandibular joints. We also digitized the nostril and natural markings between the sacrum in our video recordings to characterize a body local coordinate system and the COM. Limb markings were used to determine the limb extension ratio (LER) and limb compression during landing (ΔLER) by comparing the ratio of the distance between shoulder and metacarpophalangeal joints to the total limb length between the two joints. Therefore, a ratio of one implies the limb is fully extended and a ratio closer to zero implies the limb is more flexed.

We were also able to measure the direction of the center of mass velocity and its angle from the vertical at touchdown (θ_{impact}) using high-speed video captured at 500 fps in the frontal and sagittal plane using two SC1 Edgertronic high-speed cameras (San Jose, CA, USA). Ground reaction force data was recorded using an AMTI mini-40 6-axes transducer (Waterton, MA, USA), and both force and the camera triggering events were recorded on Igor Pro (WaveMetrics, Inc.; Portland, OR, USA) at 1000 Hz using a National Instruments USB-6229 BNC DAQ (Austin, TX, USA). Video data was also used to measure the platform displacement throughout each trial. Animals were prompted to jump by approach from behind with a hand or a puff of air. Eight toads were jumped onto the 725 g platform ($N = 8$) and five of those eight toads were jumped onto the 123 g platform ($N = 5$). For each toad we recorded five replicate jumps on all four compliance treatments (0, 2.5, 5 and 10 mm BW^{-1}). After experiments were complete, the toads were euthanized in 3 g/L of MS-222, buffered with $NaHCO_3$ to a pH of 7.4 followed by double pithing once the animal was unresponsive.

Data & Statistical Analyses

MATLAB (Mathworks; NA, MA, USA) was used to write a script capable of measuring three-dimensional kinematics and kinetics. Both video angles were calibrated with a 64-point cube and digitized using procedures outlined by Hedrick (2008) in MATLAB to determine 3D location of markers. Digitized data was then interpolated from 500Hz to 1000Hz using Igor Pro. Raw voltage signals recorded from the transducer were initially filtered using a FIR low pass filter in Igor Pro, with both the end of the pass band and start of the reject band being set to 0.01 and the number of FIR filter coefficients as 113 for each of the six channels prior to calculating forces. Fundamental frequencies of each platform were determined by forcing oscillations without additional mass, and a Butterworth filter set to reduce frequencies ±20 Hz about the fundamental frequency was applied in MATLAB. The force associated with the inertial acceleration of the platform mass was also estimated using video tracking and subtracted out from our analyses of each compliant trail. Collision Theory was initially explored as a possible explanation for individual variation but was quickly rejected as impact angle did not exhibit strong correlations with any variable (Pearson Correlation, $| r | < 0.41$). The change in energy from redirecting the COM velocity vector was also determined to be several orders of magnitude below that associated with the landing for each trial, suggesting collision theory does not apply well to the forelimb-only mediated landing behavior.

Three-way random block ANOVAs, with type three sum of squares, were used to test for significant differences between compliance treatments, individuals, and platform inertial mass with compliance and plate mass as fixed effects and individuals as a random effect. The critical value, α, was adjusted to 6.25e-3 using a Bonferroni correction as eight separate ANOVAs were performed. Any further analysis within factors was achieved with Tukey Honest Significant Difference post-hoc tests with a critical value of 0.05.

Results

Cane toads performed controlled landings across the four compliance treatments we investigated (Table 1.1; Fig. 1.2). The maximum plate displacement significantly increased with compliance treatment (Table 1.2; Fig. 1.3; ANOVA, p = 1.26e-8), suggesting our compliance treatments significantly altered the displacement depth of the landing platform. The impact angle from vertical (θ_{impact}) did not change with compliance (ANOVA, $p = 0.471$). Neither the maximum limb extension ratio (LER) nor the change in limb extension ratio (ΔLER) throughout forelimb-mediated landing differed significantly between compliance trials (Fig. 1.4; ANOVA, $p = 0.893$ & $p = 0.874$, respectively). This supports our prediction that the maximal limb extension

– which generally occurs at touchdown – would not differ across compliance treatments. However, our observations do not support the prediction that toads would have greater limb compression (ΔLER) on rigid surfaces. The peak ground reaction force (GRF) did not differ significantly with compliance as we expected (Fig. 1.5; ANOVA, p = 0.865). This finding does not support our initial hypothesis that peak impact force would be higher on rigid surfaces. Impulse during forelimb-mediated landing also did not significantly differ between compliance treatments (Fig. 1.5; ANOVA, p = 0.129). This result does not support our prediction that the impulse during landing would be greater on more rigid surfaces. The maximum load rate also did not significantly change across the compliance treatments we investigated (Fig. 1.5; ANOVA, p = 0.833), which does not support our hypothesis that the maximal load rate would be greater as surface conditions became more rigid. The energy dissipated by the forelimbs (E_{arms}) during landing did not significantly change as a function of compliance (Fig. 1.6; ANOVA, p = 1.61E-26). This finding fails to support our prediction that the forelimbs would dissipate more energy when landing on more rigid surfaces.

Landing platform inertial mass was incorporated because it was apparent that the toads were not always capable of displacing the original platform – with a mass of 725 g – to appropriate displacements given the platform stiffness. The less massive platform – of 123 g – did reduce the inertial delay of the platform moving to the maximal displacement, which usually occurred within a few milliseconds of the maximal impact force (Fig. 1.7C). Furthermore, the maximum displacements of the two platforms did not significantly differ (Table 1.1; Fig. 1.4; ANOVA, p = 0.077). Furthermore, while the maximum platform displacement was the only that exhibited a significant interaction between compliance treatment and the platform inertial mass, none of the interactions between the two plate masses differed significantly for the same compliance treatment (Table 1.3). Therefore, the reduction in mass by almost an order of magnitude – which made it comparable to the average body mass of the toads in this study – did not serve to significantly alter the coordinated landing behavior (Table 1.1).

Discussion

We originally hypothesized that if toads were unaware of the landing surface compliance, then they would exhibit 1) similar maximal limb extension prior to touchdown yet greater limb compression during landing, 2) increased peak impact force, impulse, and maximal load rate, and 3) greater forelimb work to dissipate mechanical energy when landing on rigid surfaces compared to more compliant surfaces. We found cane toads did not alter their maximal forelimb extension across compliance treatment and their limb compression during forelimb-mediated landing also did not significantly differ across compliance treatments. Additionally, we observed no significant difference in peak impact force, impulse, or maximal loading rate across compliance treatments. Finally, we found the forelimbs did not increase the amount of work to decelerate the COM on more rigid surfaces during forelimb-only mediated deceleration as we initially anticipated.

Conserved Behavior Across Compliance

The maximal LER is likely under greater influence from preparatory behavior during the aerial phase of the jump as it occurs right at or shortly after touchdown. The consistent maximal LER across compliance may be explained by the random block design of compliance treatments as the toads were unaware of the landing surface condition for a given trial. This may lead them to exhibiting similar preparatory motor behaviors resulting in similar mechanics prior to impact (Moritz & Farley, 2004). Additionally, we constrained jump distance to 2.5 times the individual’s SVL which may affect the LER prior to touchdown since forelimb extension has been found to depend on jump distance (Cox & Gillis, 2015; Gillis et al. 2010). Our results suggest that when the surface stiffness is unknown, a toad will likely prepare to land on a rigid surface as this is the most energetically demanding substrate they may encounter. However, while the ΔLER is determined in part by the maximal LER it is intriguing that toads exhibit similar overall displacement of the forelimb regardless of compliance. This finding contradict what Ferris and Farley (1997) found in humans, where people will increase leg displacement (similar to ΔLER) when jumping on more rigid surfaces. Conversely, McKinely and Pedotti (1992) found that people conserve their general strategy to land on different surfaces while exhibiting minor shifts in individual joint kinematics and muscle activity when surface stiffness was altered. This may help to explain why our observations at the limb-level did not differ between compliance treatments and suggests further investigation is needed into the role individual joints of the forelimb play in decelerating the animal upon impact. Our results suggest cane toads adopt a strategy that conserves overall forelimb displacement regardless of compliance and thus they do not take full advantage of the potential to transfer energy to a compliant surface by increasing stiffness of their forelimbs. It may be that cane toads do not have a strong pressure to select for energy cycling via elastic structures considering their forelimbs act mainly as brakes during landing – absorbing the impact energy – while their hindlimbs act as the motors for jumping (Nauwelaerts and Aerts, 2006). The use of different limbs for energy absorption and production is not what we observed in humans and other bipeds where the same limb(s) act both as brakes and motors during locomotion and therefore can store energy in elastic structures during impact to use during the subsequent takeoff.

Impact forces pose a significant risk to injury for animals landing from a jump considering the forces at touchdown tend to be significantly greater than those during takeoff

(Nauwelaerts & Aerts, 2006; Sohnel et al. 2020). Previous work has shown that cane toads specifically do align their forelimbs with their COM velocity during the arial phase and position their hindlimbs to reduce torques at impact and the likelihood of toppling over (Cox & Gillis, 2017; Azizi et al. 2014). The risk of toppling is exacerbated by unknown surface conditions like compliance, and yet our toads did not show significant changes in their landing kinetics due to surface compliance. Work done in several species of arboreal primates found results that better align with our initial hypothesis that landing on compliant surfaces – in this case branches – does reduce the impact forces associated with landing (Demes et al. 1995). Furthermore, humans jumping down onto rigid surfaces produced higher forces than on compliant mats (Ferris & Farley, 1997; McKinley & Pedotti, 1992). However, gymnasts landing from heights of 69cm produced lower peak vertical force on the rigid surface compared to compliant surfaces and delayed the time of peak vertical force on rigid surfaces (McNitt-Gray et al. 1994). These contradictory findings of McNitt-Gray et al. (1994) may suggest the influence of landing surface compliance is task-dependent and in this case significantly influenced by drop height and potentially skill level given that the participants are gymnasts likely pushing the limit of safe drop height.

Compliant surfaces did not reduce the amount of forelimb work as we originally hypothesized which would have allowed the animal to dissipate energy by maintaining a stiff forelimb and deforming the compliant platform. This strategy of increasing limb stiffness does decrease the energetic demand on muscles to dissipate energy upon landing in humans (Devita & Skelly, 1992). Additionally asking subjects to land with more rigid posture increases the energy stored during landing on compliant surfaces and reduces the amount of energy demand for the subsequent jump (Arampatzis et al. 2001). However, it is unlikely that toads are absorbing and storing elastic energy in their forelimbs to then use during the subsequent jump since the hindlimbs are largely responsible for providing the energy for propulsion.

Conserved Behavior Across Platform Inertial Mass

It is worth stressing that none of the variables investigated significantly differed between the two inertial masses of the platforms. Our results illustrate that the initial platform's mass of 725 grams was almost an order of magnitude larger than the toads in this study, yet they still produced similar maximal displacements as the less massive 123 gram platform in the approximately 100 ms timeframe of forelimb-mediated landing. This suggests cane toads are coordinating similar landing behavior across compliance treatments – up to 20 percent of their forelimb length per unit body weight – even with minimal inertial delay. Moritz & Farley (2003) found similar results that people alter their limb mechanics to overcome surface damping and maintain similar spring-mass COM dynamics. Further work by this group suggests that the altered mechanics are largely driven by changes at the ankle joint, suggesting distal, joint-level modifications may play a role in coordinating landings on damped and compliant surfaces (Moritz et al. 2004).

Strategies Reflect Coordination

Individual variation may illustrate differences in strategy or level of skill in performing a task. Where some toads exhibited greater coordination and kept their trunk from contacting the substrate, other individuals limited their maximal LER and thus had little ΔLER before allowing their torso to crash into the platform (Fig. 1.8A). It is also worth noting from the trials where individuals exhibited crash landing behavior (*n* = 35) the energy dissipated by the forelimbs is reduced (Fig. 1.8D). With little energy being transmitted to the substrate or dissipated by the forelimbs at impact, the bulk of the impact energy must be absorbed by other parts of the body upon impact. In many of the crash landings we noticed the viscera of the abdomen collided with the substrate and were forced to stretch, potentially risking damage to internal organs in addition to musculoskeletal tissue. Bijma et al. (2016) reported a similar distinction between two landing behaviors in a tree frog where one was characterized as a toe pad landing with the animal catching the branch with its feet's adhesive pads, or an abdomen landing where the torso makes first contact with the stick and limbs wrap around the branch. The abdomen landing, just like the crash landings we describe here, may risk injury to the torso and viscera. However, given the relatively smaller size of amphibians crash landing clearly is not as catastrophic as tripping would be for larger vertebrates.

Limitations

We recognize that randomizing compliance treatments makes the landing surface stiffness unknown to the animal prior to impact and may influence the preparatory and landing behavior of the forelimbs. We furthermore only had toads jump off a rigid platform, and previous work suggests hindlimb proprioception plays a role in mediating landing behavior (Cox et al. 2018). Additionally, elbow extension has been shown to increase prior to landing when jumps are of greater distance (Cox and Gillis, 2015), and our experimental setup restricted jump distance to approximately 2.5 times the animal's SVL. These results may explain why we did not observe major differences in forelimb kinematics across compliance considering hindlimb proprioception and jump distance would be relatively consistent regardless of the landing platform's compliance.

Conclusion

In summary, we have shown that forelimb-mediated landing behavior in cane toads remains conserved in kinematics and kinetics across compliance treatments and platform inertial mass. We also observed two distinct strategies that relate to the level of coordination in executing a coordinated landing where toads keep their torso and head from crashing into the substrate. It is still unclear whether the robust landing behavior is a result of feedforward control strategies or reliant on sensory input from other sources like the hindlimbs during takeoff. Future investigation into the motor recruitment during preparation for and during landing may help to shed light on whether these animals are predicting surface conditions and actively adjusting behavior after contact with surfaces.

Acknowledgements

I would like to thank the undergraduate researchers involved – Estefania Flores, Gloria Huynh and Ming Gong – for helping to digitize video footage.

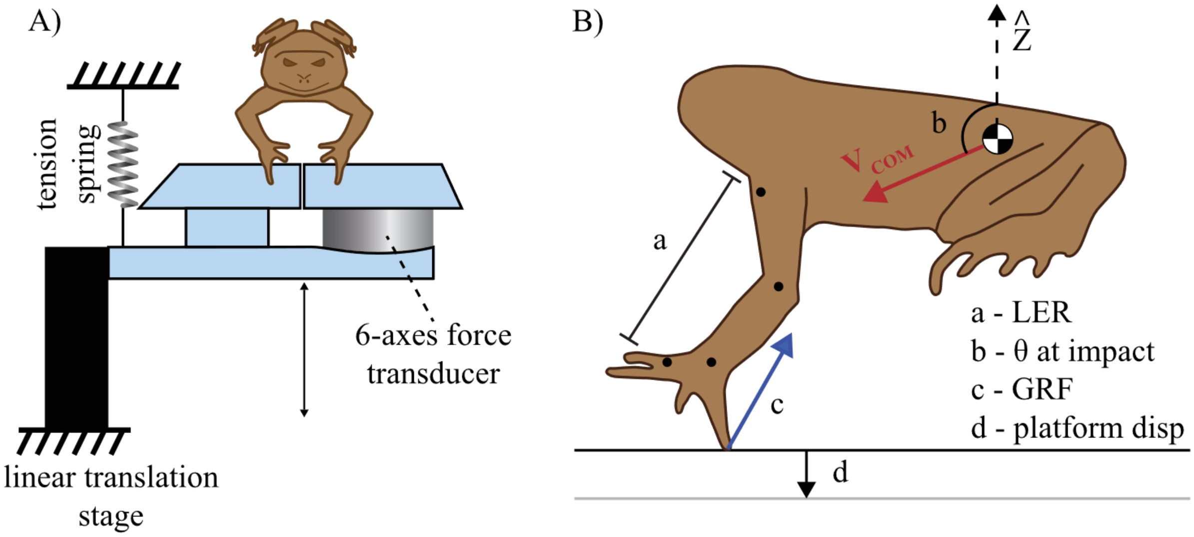

Figure 1.1. Experimental setup of landing surface. A) Diagram shows the landing platform which could be adjusted in stiffness by attaching various springs in series. A 6-axes force transducer was positioned under one half of the landing surface, so forces were only captured from the animal's left forelimb during landing. The platform could only move vertically along a linear translation stage when unconstrained and could be locked in-place using a bolt to make the platform rigid. B) Illustration of variables of interest including the limb extension ratio (a; LER), angle of COM velocity vector at impact relative to the vertical direction ($\hat{z}$; illustrated with b in diagram above), ground reaction force (c), and the platform displacement (d).

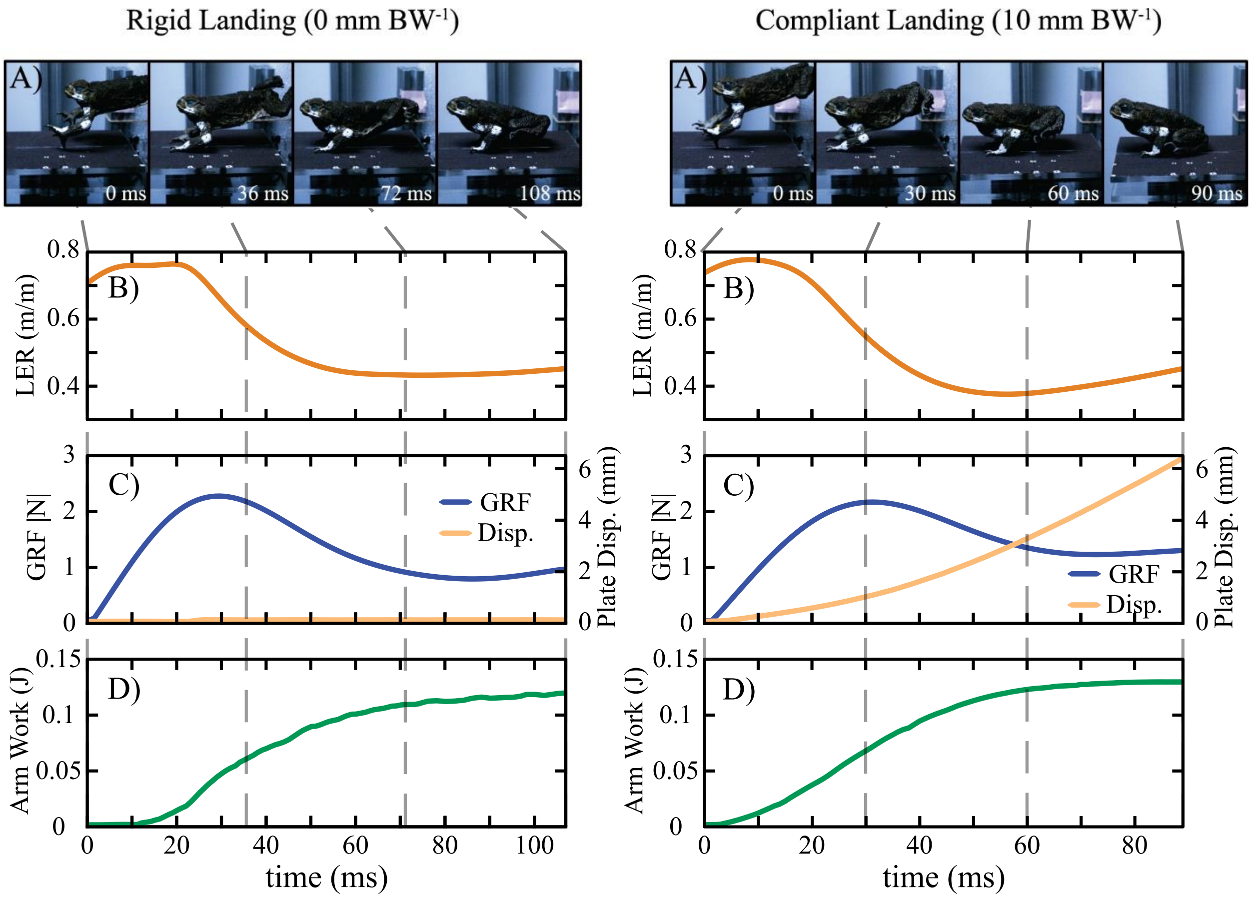

Figure 1.2. Comparing rigid and compliant landings. Representative comparison between rigid (0 mm BW^{-1}; left panels) and compliant landing (10 mm BW^{-1}; right panels). Within each panel A) shows a series of video frames from initial touchdown through the end of forelimb-only mediated landing when the body or hindlimbs make contact, B) provides the limb extension ratio for the forelimb, C) gives the magnitude of the ground reaction force in blue and the platform displacement in light orange, and D) depicts the cumulative work performed by the forelimbs during the landing period. Both trials consist of the same animal on the more massive landing platform.

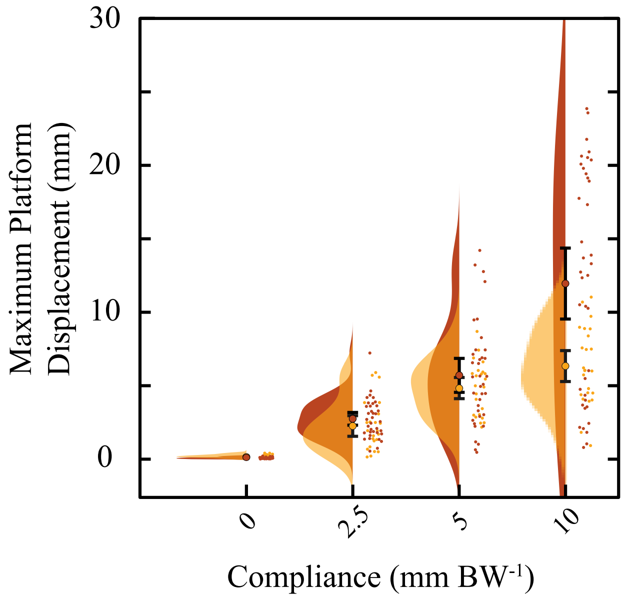

Figure 1.3. Maximal platform displacement across compliance treatments. Platform displacement significantly increased with compliance treatment (ANOVA, *p* = 1.26e-8). Distributions for each compliance treatment are provided just to the left, mean values are the large dots at the right side of the distribution and have error bars showing the 95% CI of the mean, and individual jump trials shown as the scatter plot to the right of each treatment group. Yellow represents the less massive platform and dark orange represents the more massive platform.

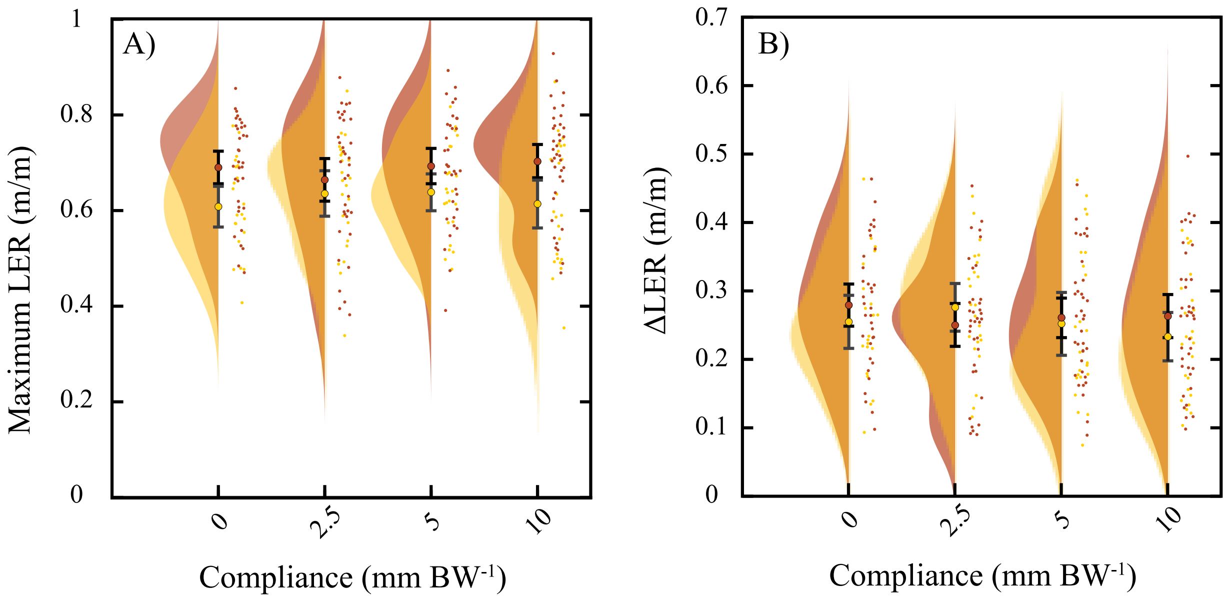

Figure 1.4. Limb kinematics during forelimb-mediated landing. A) Shows the maximal limb extension ratio (LER) during the landing, and B) conveys the change in limb extension ratio (ΔLER) during landing. Distributions for each compliance treatment are illustrated vertically to the left, with the mean value as the large circle at the right side of the distribution with error bars showing the 95% CI of the mean, and individual trials shown as the scatter plot to the right of the treatment group. Yellow represents the less massive platform and dark orange is the more massive platform. In both cases there was no significant difference between compliance treatments or between the two platform masses (ANOVA, $p > 6.25e-3$).

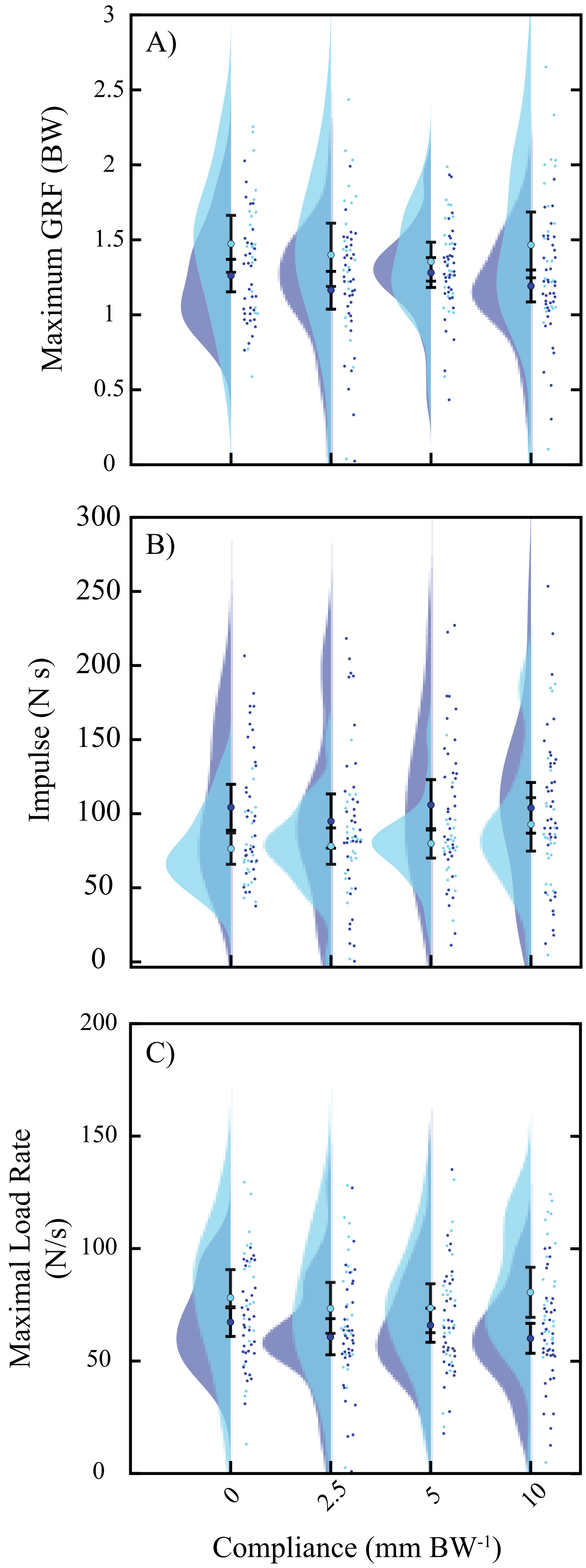

Figure 1.5. Kinetics during forelimb mediated landing. A) maximum ground reaction force in units of body weights (GRF), B) impulse, and C) maximal load rate all did not significantly differ across compliance or between plate masses (ANOVA, $p > 6.25e\text{-}3$). Distributions for each compliance treatment are provided just to the left, with mean values represented by the larger dot to the right of each distribution and having error bars showing the 95% CI of the mean, and individual jumps represented by the scatter plot to the right of each distribution. Light blue represents the less massive platform while dark blue represents the more massive platform.

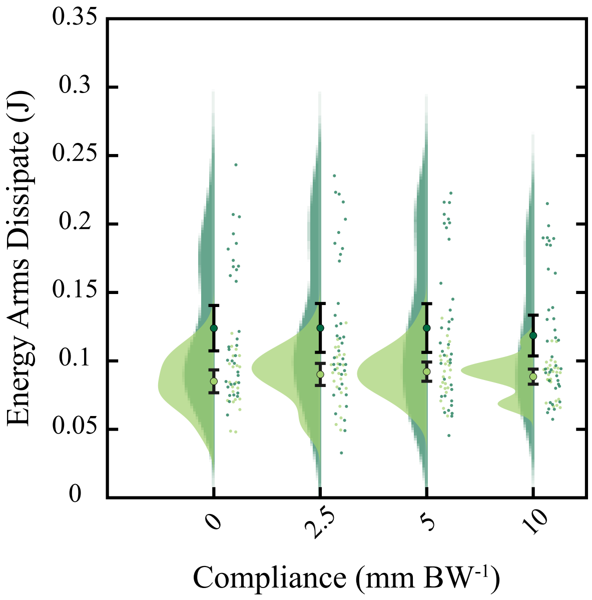

Figure 1.6. Energy dissipated by forelimbs during forelimb-mediated landing. Forelimbs did not significantly alter the amount of energy dissipated while landing across surface compliance or inertial mass (ANOVA, $p > 6.25e\text{-}3$). Distributions for each compliance treatment are provided just to the left, with mean values and the 95% CI of the mean represented by the large dot and error bars to the right of each distribution, and individual jumps are shown to the right of each distribution as a scatter plot. Light green represents the less massive platform and dark green represents the more massive platform.

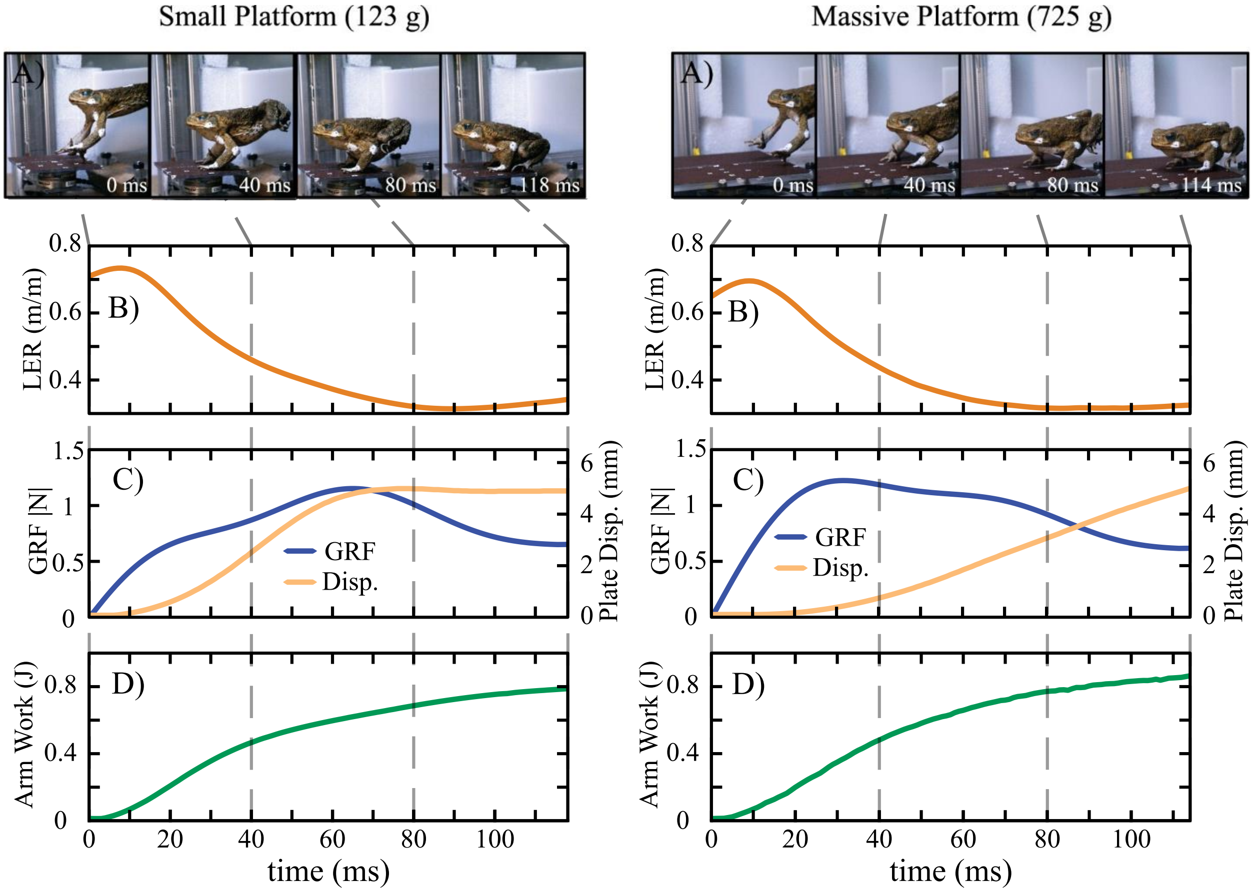

Figure 1.7. Comparing the effects of platform mass on landing behavior. Representative comparison between less massive platform (123 g; left panels) and more massive platform (725 g; right panels). Within each panel A) shows a series of video frames from initial touchdown through the end of forelimb-only mediated landing when the body or hindlimbs make contact, B) provides the limb extension ratio for the forelimb, C) gives the magnitude of the ground reaction force in blue and platform displacement in light orange, and D) depicts the cumulative work performed by the forelimbs during the landing. Both trials consist of the same animal landing on the same compliance treatment (5 mm BW^{-1}).

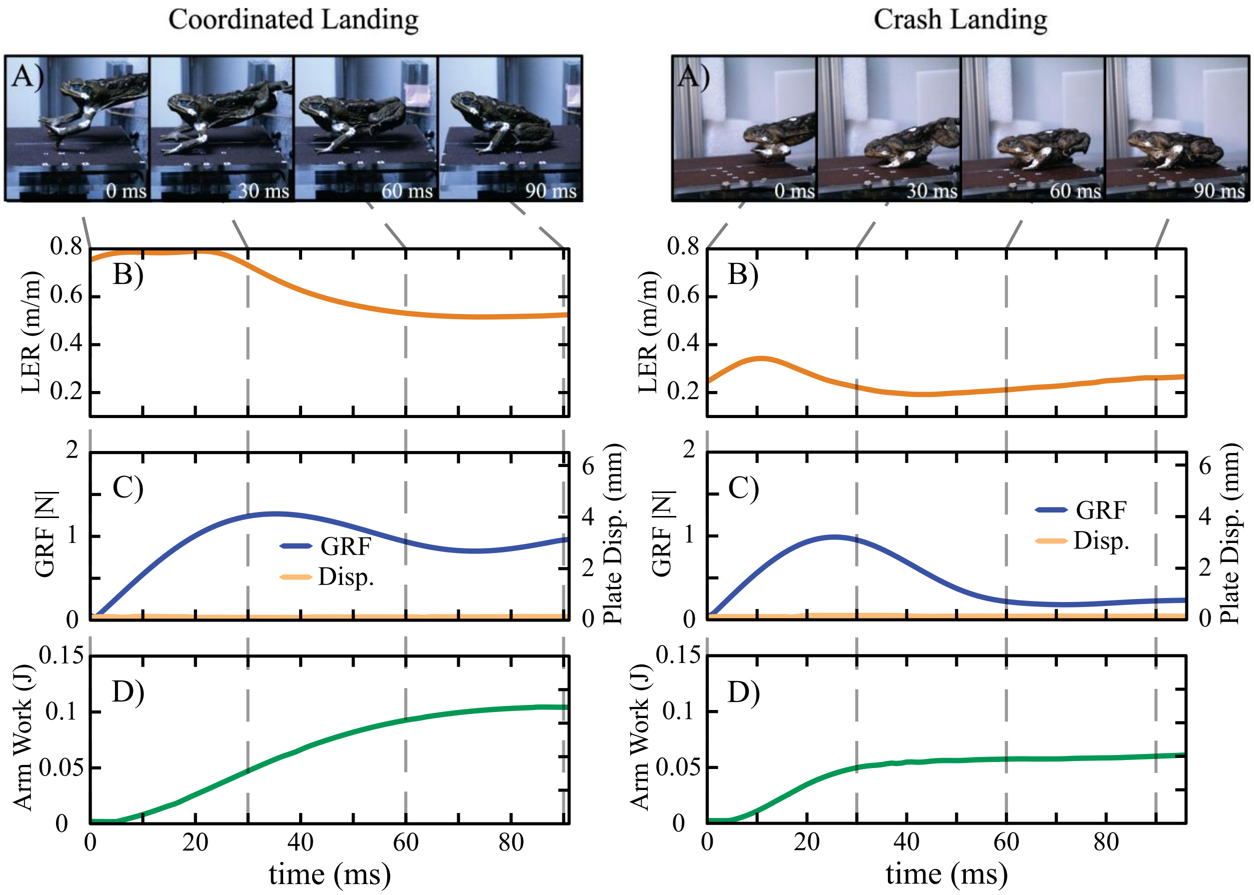

Figure 1.8. Comparing coordinated and crash landing behaviors. Representative comparison between coordinated (left panels) and crash landing (right panels). Within each panel A) shows a series of video frames from initial touchdown through the end of forelimb-only mediated landing when the body or hindlimbs make contact, B) provides the limb extension ratio of the forelimb, C) gives the magnitude of the ground reaction force in blue and the platform displacement in light orange, and D) depicts the cumulative work performed by the forelimbs during the landing. Trials consist of two different toads landing on the same compliance treatment (0 mm BW^{-1}) and on the more massive platform.

Table 1.1. Statistical results from ANOVA tests performed. P-values for respective dependent variables (left-most column) for each of the fixed effect independent variables (top row) and corresponding interaction terms in the ANOVA model. Bolded p-values indicate results below the critical value of 6.25e-3 using a Bonferroni correction to account for the multiple ANOVAs being performed. Compliance had 3 *dof*, Plate Mass had 1 *dof*, the interaction between compliance and plate mass (C:P) had 3 *dof*, the interaction between compliance and individual (C:I) had 21 *dof*, the interaction between plate mass and individual (P:I) had 7 *dof*, and there were 199 *dof* for the error for every independent variable below.

Variable	Comp.	Plate Mass	C:P	C:I	P:I
Max. Plate Disp.	*F* = 33.23 **_p_ = 1.26e-8**	*F* = 4.28 *p* = 0.077	*F* = 6.10 **_p_ = 5.42e-4**	*F* = 4.11 **_p_ = 5.44e-8**	*F* = 14.27 **_p_ = 6.13e-15**
Max. LER	*F* = 0.20 *p* = 0.893	*F* = 3.91 *p* = 0.088	*F* = 2.85 *p* = 0.039	*F* = 1.59 *p* = 0.055	*F* = 6.39 **_p_ = 8.71e-7**
ΔLER	*F* = 0.23 *p* = 0.874	*F* = 0.97 *p* = 0.356	*F* = 2.62 *p* = 0.052	*F* = 1.88 *p* = 0.014	*F* = 2.89 *p* = 6.67e-3
Max. GRF	*F* = 0.24 *p* = 0.865	*F* = 3.25 *p* = 0.113	*F* = 0.64 *p* = 0.591	*F* = 0.89 *p* = 0.601	*F* = 3.80 **_p_ = 6.86e-4**
Impulse	*F* = 1.67 *p* = 0.192	*F* = 2.72 *p* = 0.143	*F* = 1.42 *p* = 0.237	*F* = 0.86 *p* = 0.649	*F* = 31.17 **_p_ = 6.48e-29**
Max Load Rate	*F* = 0.29 *p* = 0.833	*F* = 1.50 *p* = 0.259	*F* = 0.75 *p* = 0.522	*F* = 1.36 *p* = 0.139	*F* = 5.76 **_p_ = 4.31e-6**
$\mathbf{E_{arms}}$	*F* = 0.50 *p* = 0.684	*F* = 4.55 *p* = 0.070	*F* = 1.31 *p* = 0.274	*F* = 1.43 *p* = 0.107	*F* = 90.65 **_p_ = 2.00e-58**
$\boldsymbol{\theta_{impact}}$	*F* = 0.86 *p* = 0.471	*F* = 3.63 *p* = 0.098	*F* = 1.11 *p* = 0.346	*F* = 1.28 *p* = 0.190	*F* = 6.79 **_p_ = 3.09e-7**

Table 1.2. Tukey HSD post-hoc comparisons across surface compliance. P-values for each dependent variable (left-most column) that exhibited a significant difference among compliance treatments. Bolded values are below the post-hoc critical value of 0.05.

	Compliance Comparisons (mm BW^{-1})					
Variable	**0 – 2.5**	**0 – 5**	**0 – 10**	**2.5 – 5**	**2.5 – 10**	**5 – 10**
Max. Plate Disp.	**1.11e-3**	**4.91e-13**	**4.87e-26**	**2.47e-5**	**1.22e-17**	**4.97e-6**

Table 1.3. Tukey HSD post-hoc compliance and plate mass interactions for maximal platform displacement. Units of platform mass (PM) are grams and compliance (Comp) are millimeters per unit body weight. Bolded *p*-values are below the post-hoc critical value of 0.05.

	PM	**123**				**725**			
PM	Comp	0	2.5	5	10	0	2.5	5	10
123	0	-	-	-	-	-	-	-	-
	2.5	0.186	-	-	-	-	-	-	-
	5	**2.63E-08**	**4.17E-03**	-	-	-	-	-	-
	10	**1.40E-13**	**2.44E-07**	0.873	-	-	-	-	-
725	0	1	0.124	**6.62E-08**	**1.35E-12**	-	-	-	-
	2.5	0.301	1	**2.69E-03**	**1.44E-07**	0.173	-	-	-
	5	**8.46E-06**	0.187	1	0.054	**7.8E-06**	0.124	-	-
	10	**3.00E-18**	**1.20E-11**	**1.79E-03**	1	**2.1E-17**	**5.4E-12**	**3.43E-05**	-

CHAPTER 2

How cane toads coordinate their muscles to stick the landing regardless of surface compliance

Abstract

Animals must coordinate their limbs effectively across a wide range of surface conditions to avoid slipping and becoming injured or being caught by a predator. It is therefore in the animal's best interest to coordinate their muscles to take advantage of differences in surface properties that may allow them to reduce their energy inputs and continue to move effectively. We report here on the preparatory and landing muscle activity of the *deltoideus*, *pectoralis*, *anconeus*, and *palmaris longus* muscles in the forelimb of cane toads when jumping onto surfaces of varying stiffness. We found that cane toads do not alter the intensity of muscle activity during the preparatory (aerial) or landing phases of jumps when the landing surface is made compliant. Additionally, we found the toads did not adjust their preparatory activity following landing on different surface stiffnesses. These results may suggest toads coordinate their muscles in preparation for a firm landing, or they are responding to sensory information during takeoff from a firm platform to predict the unknown landing surface properties.

Introduction

Coordinating deceleration is a critical component of terrestrial locomotion, because movements like jumping and running downhill place a significant demand on the skeletomuscular system to absorb and dissipate both impact forces and energy. During landing, the individual coordinates their limbs to slow their body and reduce the risk of injury. Locomoting in real-world environments where surface conditions are variable and the surface properties are potentially unknown prior to touchdown further challenges the individual's motor control to adequately prepare for and coordinate landings.

Hopping and jumping are movements that can generate extreme forces and impact energies upon landing, and therefore minor changes in environmental conditions or task constraints can require altered motor control strategies. In the face of perceived changes in drop height or surface stiffness, animals and humans modulate anticipatory muscle activity to land effectively and likely reduce the risk of injury (Birn-Jeffery & Daley 2012; Ferris & Farley, 1997; Santello, 2005). Furthermore, Santello (2005) proposes three potential mechanisms for altering anticipatory muscle activity to cope with a perceived perturbation while falling based on activation onset prior to touchdown and the rate of activation increase. Both animals and humans coordinate their limbs to conserve spring-mass properties of the body's center of mass even when an unknown environmental perturbation is presented (Daley & Biewener, 2006; Daley et al. 2009; Ferris & Farley 1997; McNitt-Gray et al. 1994; Moritz & Farley, 2004; Santello, 2005). Conservation of spring-mass dynamics is likely due to mechanisms requiring post-landing feedback including stretch reflexes as well as passive mechanical reactions as the limbs compress (Daley & Biewener, 2006; Daley et al. 2009; Moritz & Farley, 2004; Santello, 2005).

Skill also affects anticipatory and landing behavior in both humans and animal models. More advanced, or skilled, horse and dog jumpers exhibit stiffer limbs upon impact compared to novice conspecifics (Cassiat et al. 2004; Söhnel et al. 2020). This trend is also apparent in volleyball players, a group proficient in jumping, who exhibited a greater range of motion in their ankles when landing on rigid surfaces compared to compliant surfaces, while an unskilled jumping group showed intermediate joint excursions on both surface conditions (McKinely & Pedotti 1992). The reduced joint excursions exhibited from more rigid and erect posture of advanced individuals may allow them to reduce their risk of soft-tissue injury since muscles would not undergo as extreme stretching during eccentric contractions during landing (Söhnel et al. 2020). McKinely & Pedotti (1992) also observed no change in the onset of muscle activity between skilled and unskilled jumpers but found greater latency in activation when landing on compliant surfaces in both groups, suggesting skilled jumpers are not tuning the activation onset to coordinate more effective landings. Together these results strongly imply individual variation in skill can impact limb stiffness and the potential risk of skeletomuscular injury.

Anurans - frogs and toads - are a good model for exploring controlled deceleration and landing behavior as their saltatory gait and longer hindlimb than forelimb length increase the demand placed on their forelimbs to dissipate the energy produced by their hindlimbs during takeoff (Nauwelaerts & Aerts, 2006). Toads specifically exhibit more derived and consistent landing behavior within the anuran clade because they spend more time in terrestrial habitats and traverse greater distances out of water (Essner et al. 2010; Reilly et al. 2016; Llewelyn et al. 2010). Furthermore, some toads appear to be less skilled than others and occasionally perform uncoordinated crash landings where the torso or head is allowed to contact the substrate, which increases in prevalence when vestibular and proprioceptive feedback is ablated (Cox et al. 2018). Anurans appear to rely on visual information to plan jumps, however they are more influenced by proprioceptive and vestibular sensory inputs in preparation for and during landings (Cox et al. 2018; Kamada et al. 2017). Therefore, we expect them to modulate their motor patterns after touchdown or in preparation for landing after experiencing different surface properties.

We intend to explore how the cane toad, *Rhinella marina*, alters muscle activation patterns when landing on surfaces that vary in stiffness. Previous work with cane toads found their forelimb muscles have constant onset times prior to touchdown but increase activation with jump distance (Akella & Gillis, 2011; Ekstrom & Gillis 2015; Gillis et al. 2010). Much of the previous work has focused on the anconeus, an elbow extensor, which is known to undergo eccentric contractions and serve as a break during landing yet does not activate a fixed time after takeoff or prior to landing (Azizi & Abbott 2013; Gillis et al. 2014; Cox & Gillis 2020). Based on this background knowledge we hypothesized toads would not alter anticipatory muscle activity prior to touchdown but would exhibit reduced muscle intensity following impact on more compliant landing surfaces.

Methods

Animals

Five adult cane toads (*Rhinella marina*, 4 females and 1 male, body mass: 99.0 ± 20.4 g and snout-vent length: 110.2 ± 12.9 mm; mean ± SD) were obtained from a commercial supplier and housed in individual terrariums. We fed them crickets twice a week as well as provided fresh water daily. Ambient temperature was maintained between 21 -22°C and they were kept on a 12 h light: 12 h dark cycle. We performed all work in accordance with the University of California Irvine Institutional Animal Care and Use Committee (IACUC) AUP-17-170.

Surgical Procedure

We anesthetized toads by soaking them in a chilled bath of 2 g L^{-1} MS-222 buffered to a pH of 7.4 using sodium bicarbonate ($NaHCO_3$) until a toe pinch elicited no response, generally after 10 to 15 minutes. The activation and timing of the *palmaris longus* (wrist flexor), the long head of the *anconeus* (elbow extensor), *pectoralis sternalis* (shoulder adductor), and *deltoideus scapularis* (shoulder extensor) were recorded by implanting custom fine-wire bipolar electrodes into each muscle on the left forelimb and a ground lead placed subcutaneously on the back using techniques outlined in Akella & Gillis (2011) (Duellman & Trueb, 1994; Fig. 2.1). Following each surgery, we subcutaneously injected the toad with 2 mg kg^{-1} Carprofen to reduce inflammation and speed recovery. Toads were then rinsed with dechlorinated water and partially submerged in their water dish with a 250 W heat lamp directed on them for up to two hours post-surgery until they recovered from anesthesia. All toads recovered for at least 24 hrs before performing any jump trials.

Jumping Procedures

The surgically implanted 12-pin connector for recording muscle activity was adhered to the center of the toad's back via superglue and was then connected to a 2.85 m cable running each channel through an A-M Systems Differential AC Amplifier Model 1700 which amplified each signal 1000-fold. We measured ground reaction forces with a six-axes ATI mini40 force transducer mounted in the landing platform, and all forces and electromyographic voltages were recorded at 1000 Hz using NIDAQ and Igor Pro Software. High-speed video was simultaneously captured at 500 fps for each jump using three SC1 Edgertronic Cameras set up in the sagittal and frontal plane as well as a location between sagittal- and frontal-planes to capture the full trajectory of the jump from initial movement to hindlimb touchdown. Markings were painted on with whiteout and a sharpie was used to mark the center of rotation of the metacarpophalangeal, radiocarpal (wrist), humeral-ulnar (elbow), glenohumeral (shoulder), and temporomandibular joints. Additional points tracked included the nostril and the tip of the 12-pin connector which was used as a proxy to estimate the movement of the toad's center of mass (COM).

A total of 165 jump trials were collected from the five toads in a 70 × 32 × 45 cm ($l \times w \times h$) open-top acrylic arena. Toads jumped from a 10 × 10 cm rigid platform placed twice the distance of the individual toad's snout-vent length onto an 8 × 8 cm landing platform of 123 g that was mounted on a Hiwin MGN5 linear guideway. Ultra-precision extension springs of various stiffness - 137, 183, 228, 367, 672, and 960 N m^{-1} – were obtained from Gardner Springs and mounted in series to produce displacements of 2.5 ± 0.3 or 5.0 ± 0.4 mm BW^{-1} for each toad. Rigid landings (0 mm BW^{-1}) were achieved by using a screw to lock the platform in place. We prompted toads to jump by approach from behind with an open palm, and we randomized the order of landing compliance treatments (0, 2.5 and 5 mm BW^{-1}). We collected up to twelve jumps at each compliance treatment over the course of five days. After a toad had completed 12 jumps at each compliance treatment or had reached five days post-surgery the individual was euthanized in 3 g L^{-1} MS-222 buffered to a pH of 7.4 followed by double pithing. We then dissected the toad to verify proper electrode placement and that the electrode remained in the muscle of interest throughout the experiment. All electrodes were found to be properly placed and still anchored except for in two individuals that had their pectoralis electrodes withdrawn prior to dissection. These two individuals' pectoralis muscle activities were excluded from our analyses as we could not confirm proper initial placement nor the trials for which the electrode was embedded within the muscle tissue.

Data & Statistical Analyses

All trials were categorized by landing behavior as either a crash landing or coordinated landing. We defined a crash landing as the toad allowing the head or anterior half of the torso to contact the substrate upon landing, whereas a coordinated landing involved the forelimbs adequately decelerating the body and maintaining the head and anterior half of the torso above the substrate. The high-speed videos were also used to digitize marker locations in 3D space using Hedricks (2008) DLTdv5 MATLAB script. Joint positions were then interpolated from 500 Hz to 1000 Hz in a custom MATLAB script that also aligned kinematics temporally with corresponding electromyography and force data. We used a bandpass filter with minimal cutoff frequency of 70Hz and maximal cutoff frequency of 700Hz for the EMG signals, this ensured we removed low-frequency movement artifacts and electrical noise while including most frequencies associated with electromyography recordings (von Tscharner, 2000). Additionally, all muscle electromyography recordings were rectified after filtering and normalized to the maximal excitation voltage recorded for that specific electrode to allow for comparisons of electromyographic intensities between individuals. We further divided the intensities by the temporal duration of the specific phase of the jump; either occurring during the aerial phase or during the landing phase of the jump to obtain a normalized measure of intensity without units of excitation voltage or time.

We used a random block design, one-way ANOVA with type three sum of squares to test for significant differences between independent variables. Our independent variables included: i) compliance – with three treatments (0, 2.5 and 5mm BW^{-1}), ii) individual, iii) landing behavior – either crashing or coordinated, and iv) previous compliance – with the same three treatment groups as compliance. All these independent variables were treated as fixed effect factors. We treated individual toads as a fixed effect to help discern differences individuals exhibited during jumping that may have caused the vastly different proportions of crash landings we observed between different toads. One final ANOVA model was performed without landing behavior as an independent variable but as the dependent variable to determine whether rates of crashing differed significantly between i) compliance treatments, ii) individuals, and iii) previous compliance treatments. As multiple ANOVAs were calculated we used a Bonferroni correction to reduce our chances of making a type I error, thus the critical value for rejecting the null hypothesis was determined to be $\alpha = 0.05/15 = 0.003$.

Results

Compliance

We observed a significant maximal displacement of the platform between compliance treatments (ANOVA, $p = 2.55\mathrm{E}{-7}$). However, it appears the compliance treatment of 2.5 mm BW^{-1} did not produce a significantly different maximal displacement than the rigid (0 mm BW^{-1}) treatment (Tukey HSD; $p = 0.202$). This may be due to reduced vertical impulse that we observed for crashing trials and therefore reduced platform acceleration and displacement. The 5mm BW^{-1} compliance condition produced significantly greater maximal landing platform displacement than both 0 and 2.5 mm BW^{-1} conditions (Tukey HSD; $p = 1.00\mathrm{E}{-7}$ and $p = 1.20\mathrm{E}{-4}$, respectively). We saw no significant difference in any of the four normalized muscle intensities in the aerial or landing phases with respect to landing surface compliance (ANOVA, $p > 0.003$; Fig. 2.2, Fig. 2.3, & Table 2.1). This result suggests the toads were not attempting to predict or alter their motor behavior as a result of surface stiffness upon landing. The vertical impulse associated with landings also did not differ significantly across surface compliance (ANOVA, $p = 0.826$), suggesting that compliance at landing does not alter force generation during forelimb-mediated landing. Similarly, we observed no significant difference in the average limb extension ratio (LER) at contact (ANOVA, $p = 0.493$) or the change in the limb extension ratio (ΔLER) during landing (ANOVA, $p = 0.843$). This implies toads are not altering extension of their limbs prior to impact due to the anticipated surface stiffness, nor are they modulating the amount of flexion they allow due to the surface compliance. This pattern also holds for the forelimb stiffness which did not differ significantly with landing surface compliance (ANOVA, $p = 0.441$), providing additional support suggesting cane toads are not altering initial configuration or stiffness of their arms which could affect the ΔLER. We also looked at the pitch of the head during landing to ensure the toads were coming in with similar impact angles across treatments and found no significant difference across the compliance treatments (ANOVA, p = 6.11E-2). Landing behavior also did not significantly differ with surface compliance (ANOVA, p = 0.750), suggesting the toads had similar rates of coordinated landings and crashes across the compliance conditions.

Individuals

We did not observe a significant difference in platform displacements between individuals (ANOVA, p = 0.105), suggesting our use of spring constants sufficiently produced normalized compliance conditions of 2.5 and 5 mm displacement per body weight of each toad. Normalized muscle intensities differed significantly between individuals for most muscles of interest during both the aerial and landing phase (ANOVA, $p < 0.003$; see Table 2.1), except the pectoralis while airborne and the deltoideus during landing (ANOVA, $p > 0.003$; see Table 2.1). The vertical impulse also differed significantly between individuals (ANOVA, p = 2.63E-9), this difference in vertical impulse seems to be attributed to the time over which forelimb-mediated landing occurs (Fig. 2.4). We also noticed that individuals varied in their initial LER at touchdown (ANOVA, p = 4.03E-9) as well as the ΔLER during forelimb-mediated landing (ANOVA, p = 3.20E-9). Furthermore, when we used landing behavior as a dependent variable in our model, we found that individuals significantly differed in their landing behavior (ANOVA, p = 2.71E-6; Fig. 2.5). Individual variation in landing behavior was quite extreme with one toad that only performed coordinated landings – not allowing its head or torso to contact the landing surface - whereas another individual crashed in a majority of their landings and the proportion of crashing increased as the initial LER at contact decreases (Fig. 2.5). Limb stiffness does not differ between individuals (ANOVA, p = 0.294). Individuals landed with significantly different pitch at touchdown (ANOVA, p = 4.62E-9).

Landing Behavior

In our second statistical model, we used landing behavior as a fourth dependent variable to determine whether crashing (allowing the torso or head to contact the substrate) or performing a coordinated landing (not allowing torso or head to contact substrate) affects our interpretation of the data. We found no significant difference in maximal platform displacement based on the landing behavior (ANOVA, $p = 0.522$), suggesting that even during crash landings the toads displaced the platform similarly to when they land in a coordinated manner. Additionally, normalized muscle intensities only varied between landing behavior for the *pectoralis* muscle during the aerial phase (ANOVA, $p = 1.14\text{E-}3$) and did not significantly differ for the other three muscle during the aerial phase or any of the four muscles during the landing phase (ANOVA, $p > 0.003$; Fig. 2.6**,** Table 2.1). The LER at initial contact does significantly differ between crashes and coordinated landings (ANOVA, $p = 2.63\text{E-}3$, Fig. 2.7). This suggests toads are not modulating their muscle activity prior to or after touchdown to keep from crashing into the ground, instead they are likely crashing because they have not adequately extended their limbs prior to contact. However, we the ΔLER does not differ between the two landing behaviors (ANOVA, $p = 0.432$). While our results are not significantly different between landing behaviors, we observed a trend towards reduced vertical impulse in crash landings (ANOVA, $p = 6.37\text{E-}3$; Fig. 2.7). The limb stiffness during landing also did not differ significantly with landing behavior (ANOVA, $p = 0.386$). Finally, the pitch at touchdown was not significantly different between crashing and coordinated landing suggesting landing pitch is not critical for avoiding crash landings (ANOVA, $p = 1.87\text{E-}2$).

Previous Compliance

No variable discussed in this study varied significantly with the compliance treatment of the previous jump (ANOVA, $p > 0.003$; see Table 2.1). This strongly suggests that cane toads are not planning their subsequent landing based on the surface they had immediately landed on.

Discussion

We observed no change in muscle activity, limb kinematics, or impact kinetics as a function of surface compliance in this study. Our findings suggest we achieved significantly different displacement treatment at 5 mm BW^{-1}, which produces an approximate displacement of 10% of the forelimb length. Furthermore, the compliance condition from the previous landing had no effect on any of these variables. We did notice differences in landing behavior between individual toads, particularly in their limb kinematics after contact and their rate of crashing.

Compliance

Our results suggest cane toads do not alter their motor activity or landing kinematics as a result of surface compliance upon impact. Many vertebrates - including toads and humans - activate their muscles for longer periods prior to impact when they are aware of greater jump height or distance, this extends the limbs further prior to touchdown and suggests jump distance may be more important for coordinating landing behavior than surface compliance (Gillis et al. 2014; Cox & Gillis 2015; Cox & Gillis 2016; Konow & Roberts 2015; McKinley et al. 1983; Santello 2005). Additionally, we found toads did not alter their arm stiffness which is contrary to how people change their leg stiffness based on known and expected changes in surface compliance during hopping and running (Ferris et al. 1998; Ferris & Farley 1997; Moritz & Farley 2003). However, when the surface compliance is unexpectedly stiffened people exhibit intermediate leg stiffness and it may be that toads appear to exhibit the same limb stiffness as a result of the compliance treatment being unknown prior to touchdown (Moritz & Farley 2004).

Cane toads may be using a feedforward motor control strategy for landing that is robust in the face of landing surface compliance.

It is possible we did not capture the behavior affected by sensory feedback when landing on compliant surfaces. Daley et al. (2009) showed the EMG intensity of the guinea fowl gastrocnemius when running over a drop perturbation does not change until the subsequent step. Therefore, the altered muscular activity may have occurred when the toad prepared for a subsequent jump by pitching its body upward using forelimb musculature (Wang et al. 2014). The singular nature of the jumps we recorded does not ensure the animal is performing continuous bounding or hopping locomotion where landing performance would have a greater impact on the subsequent jump performance. While we found toads did not rely on sensory information from the immediately previous compliance treatment, that does not rule out their ability to integrate sensory information from several of their more recent jumps.

Individual Variation

Because arm stiffness was consistent across individuals, it suggests toads are activating their forelimb muscles similarly in preparation for landing and may be relying on passive stretching and possibly activation of the stretch reflex after touchdown to help dissipate landing energy (Gillis et al. 2014; Santello, 2005). While passive and reflexive properties do not require skill, we observed marked differences in the rate certain individuals crashed (allowing their torso and-or head to contact the substrate when landing). While more advanced vertebrate jumpers exhibit stiffer limbs upon landing (Söhnel et al. 2020; Cassiat et al. 2004; McKinely & Pedotti 1992), limb stiffness appears to be a poor predictor of landing performance in toads which exhibit similar arm stiffness between individuals and when landing coordinately versus crashing. We found the forelimb extension at touchdown was a better predictor of individual landing behavior as both arm stiffness and impulse remain relatively constant throughout forelimb-mediated landing across compliance conditions. More flexed postures at touchdown risk greater flexion of joints and force muscles to contract eccentrically at greater lengths, posing a greater risk of crashing and soft-tissue damage (Söhnel et al. 2020).

Landing Behavior

Landing behavior is dramatically affected by the initial forelimb extension at touchdown. The only muscle that significantly differed in EMG intensity was the pectoralis during the aerial phase suggesting elbow extension during the aerial phase when the toad is preparing to land may be important for coordinating landings. However, because the elbow joint is positioned between the two largest segments of the forelimb (the humerus and radio-ulna) there may be a greater demand to coordinate extension and resist compression with the anconeus (elbow extensor) upon landing as greater joint excursions risk crashing. Toads continuously extend their forelimb prior to touchdown with their anconeus and therefore naturally increase limb extension during longer jumps (Akella & Gillis 2011). Additionally, toads jumping without visual input exhibit greater elbow extension with jump distance suggesting the importance for mediating elbow extension to effectively land and the need for visual cues to adequately predict and tune arm extension for landing (Cox et al. 2018). Furthermore, toads with ablated vestibular systems crash during most of their jumps, and toads lacking hindlimb proprioception also exhibit severely reduced elbow extension (Cox et al. 2018). It appears toads may utilize proprioceptive and/or other sensory cues during takeoff to predict conditions and prepare for landing.

Landing strategies must also accommodate body and hindlimb position to effectively coordinate landing behavior. Toads actively align their forelimbs with the impact angle and likely employ passive, elastic mechanisms to drive hindlimb flexion in order to coordinate their body position for landing and prevent toppling over upon impact (Azizi et al. 2014; Cox & Gillis 2017; Schnyer et al. 2014). While our study did not find a significant difference in head pitch between the two landing behaviors, body pitch at contact may better predict crashing due to the pitch induced from hindlimbs being more extended at touchdown. Additionally, we recognize that toads are a projectile when jumping and their impact conditions may be largely attributed to initial takeoff conditions. Forelimbs likely play a critical role in establishing these takeoff conditions. While forelimb muscles do not significantly contribute to propulsive forces during takeoff, anurans can use their forelimbs to exert normal forces and adjust their takeoff angle (Akella & Gillis 2011; Wang et al. 2014). Our results suggest landing behavior is unaffected by feedback from landing surface compliance, but forelimb preparatory behavior prior to takeoff may change as a result of the sensory information from a previous landing event which was not considered in this study.

Conclusion

One of the major conclusions from our study was that landing substrate compliance does not affect cane toad muscle activity or arm stiffness as it does in other vertebrate's gaits. Toads also fail to alter their preparatory landing behavior based on their immediately previous landing experience. Finally, landing behavior varied widely between individuals which indicates variation in skill level and differences in individual's risk of injury while locomoting. The major limitations of this work were *i*) that our design limited us to only consider how the immediately previous compliance treatment may affect the subsequent jump and it is possible toads integrate sensory information over several of their previous jumps and therefore exhibit altered behavior when continuously jumping on a compliant surface and *ii*) the takeoff conditions were always the same – rigid – which provide toads sensory cues that make them prepare for a rigid landing.

Moving forward we plan to address these limitations by exploring the role of proprioceptive feedback from hindlimbs during takeoff.

Acknowledgements

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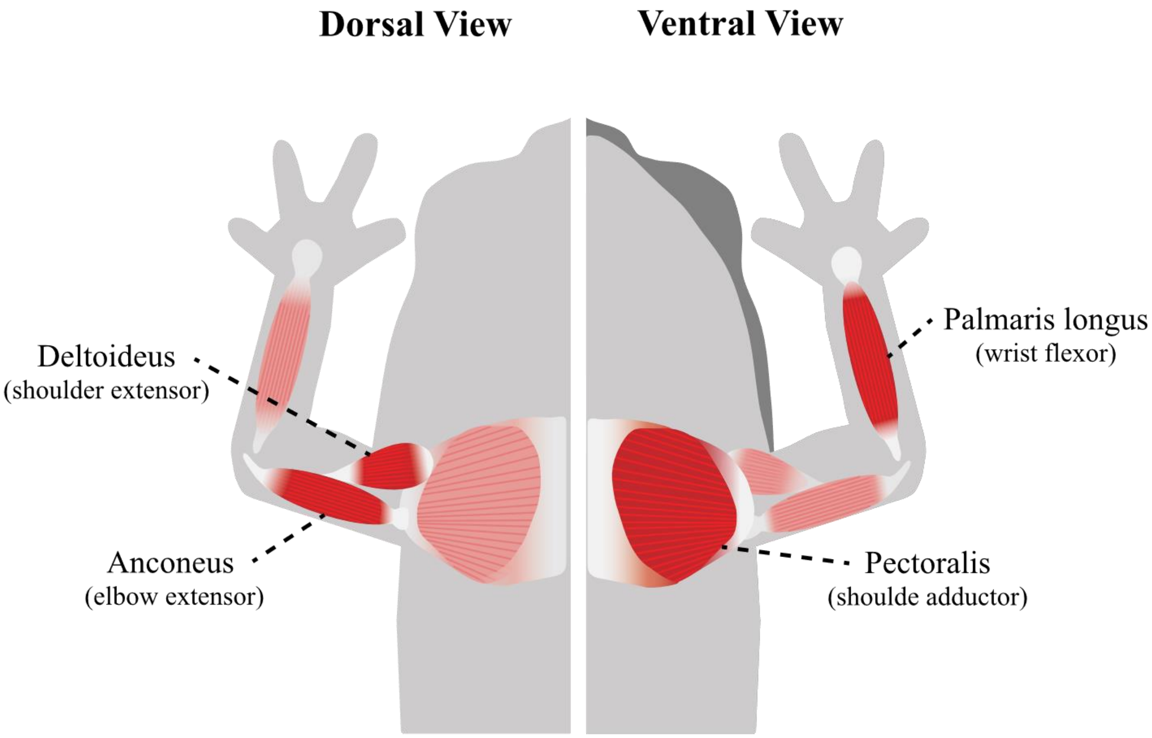

Figure 2.1. Muscles of interest. The above diagram illustrates the four major muscles of interest from the dorsal (left) and ventral (right) sides of the toad's left forelimb. The muscles of interest included the *deltoideus scapularis* (shoulder extensor), the long head of the *anconeus* (elbow extensor), *pectoralis sternalis* (shoulder adductor), and the *palmaris longus* (wrist flexor). Muscles in more vivid color are in the foreground while dulled coloration represents muscles in the background of the specified orientation.

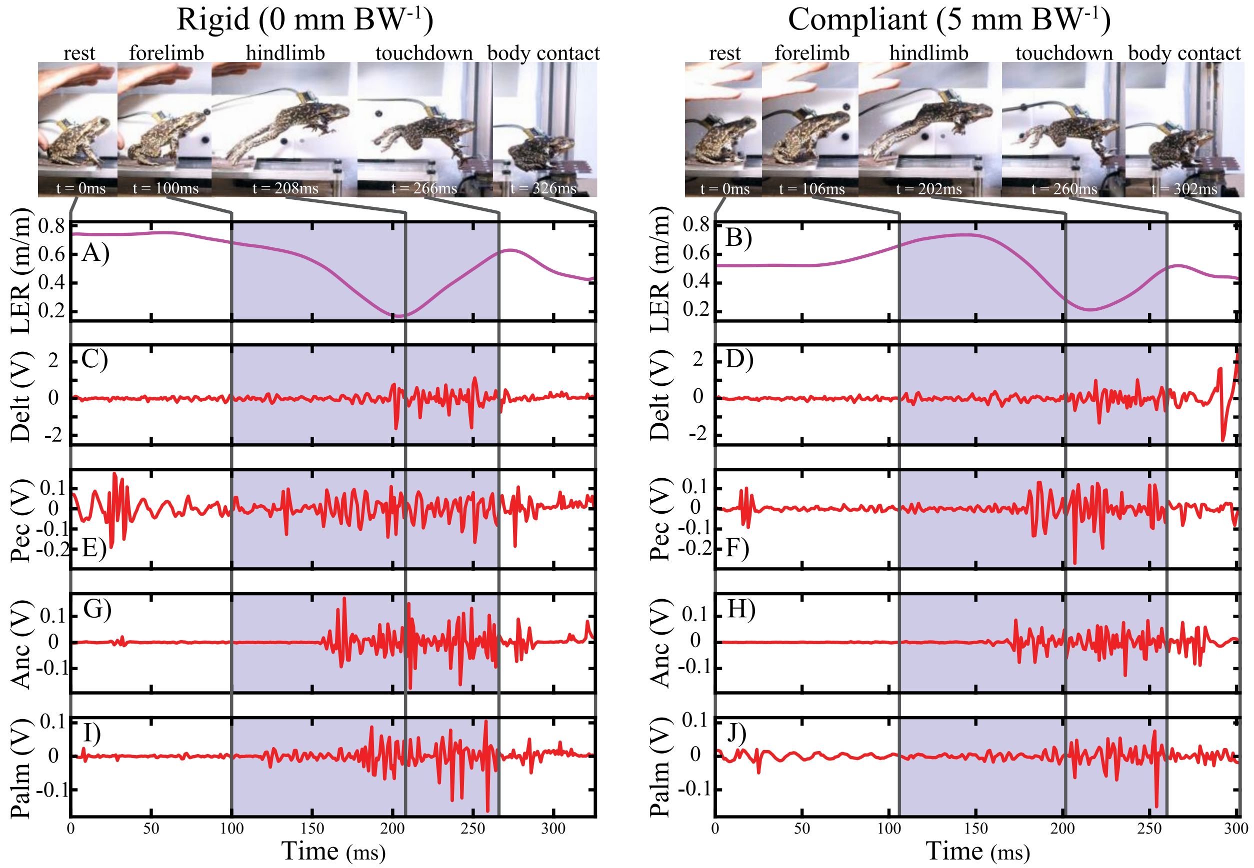

Figure 2.2. Representative timeseries of cane toad landing on rigid (left) and compliant (right) surfaces. Images with corresponding times in milliseconds are shown for start of movement, forelimb and hindlimb liftoff as well as forelimb and hindlimb touchdown, respectively. These frames are marked by vertical black lines along the plots, where the blue region indicates when the forelimbs were airborne. From top-down graphs show the forelimb limb extension ratio (LER), and excitation voltages for the deltoideus, pectoralis, anconeus, and palmaris longus through the time of movement initiation until hindlimb touchdown. Representative traces are from the same individual toad landing on 0 mm BW^{-1} (left) and the most compliant, 5 mm BW^{-1} (right).

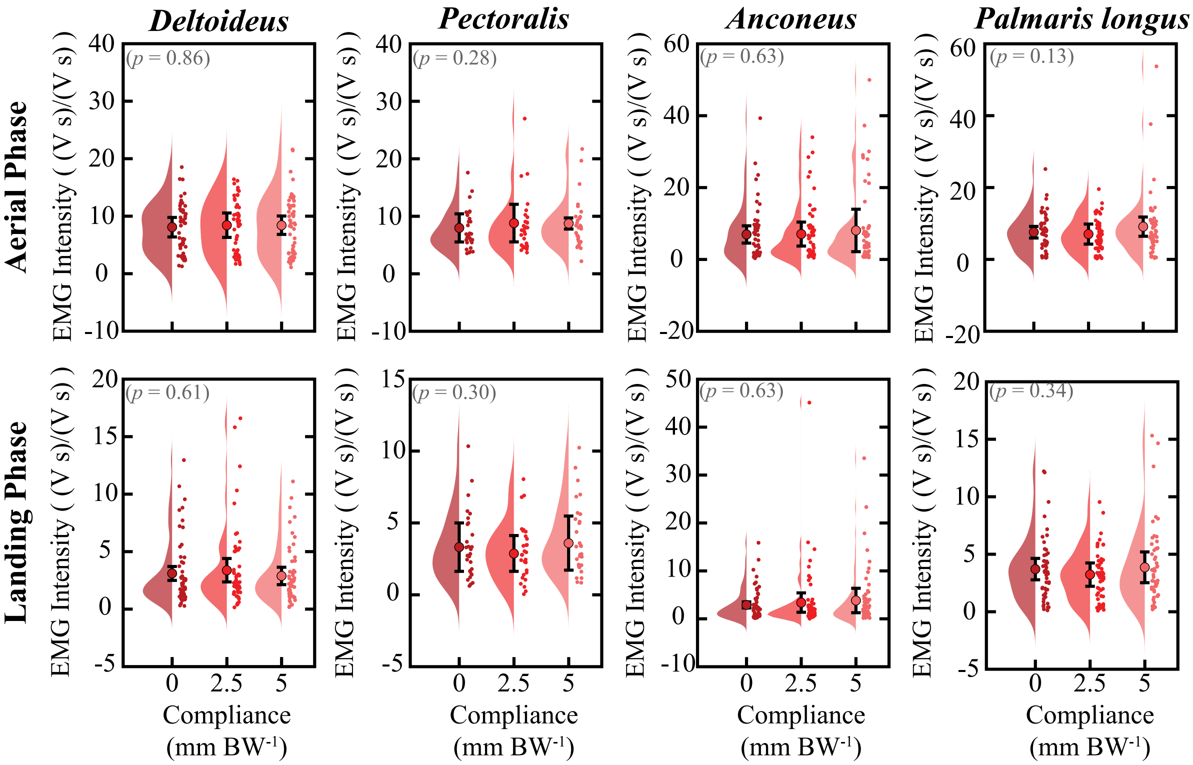

Figure 2.3. Normalized muscle activity across compliance treatments (*N* = 5). Shown are the muscle intensities normalized to maximal excitation voltages and the time of each associated phase of motion, none of which differed significantly as a function of landing surface compliance. The top row, from left-to-right, are the intensities of the deltoideus, pectoralis, anconeus, and palmaris longus while the forelimb is airborne. The bottom row, from left-to-right, are the intensities of the deltoideus, pectoralis, anconeus, and palmaris longus during forelimb-mediated landing. Center marks represent the median, boxes show 1st and 3rd quartiles, and whiskers give most extreme data not considering outliers. ANOVA results are provided on the top right of each plot and significant values ($p < 0.003$) are darkened.

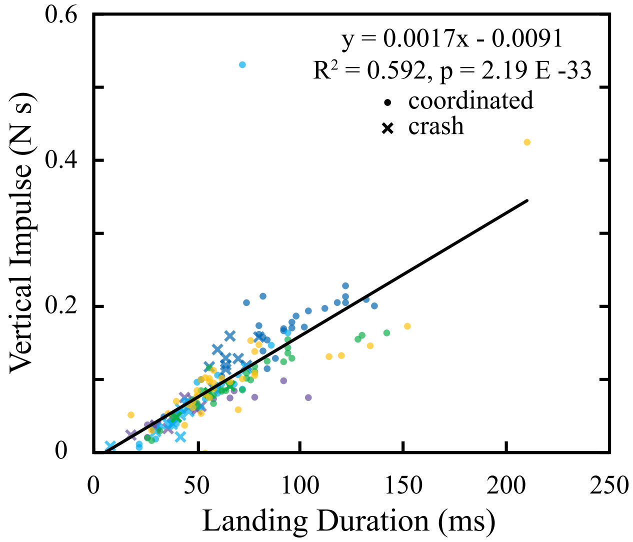

Figure 2.4. Vertical impulse of cane toad landings (*N* = 5). The plot of vertical impulse (N s) versus time (ms) shows all 165 jumps. Individuals are represented by separate colors and symbols indicate the landing behavior with a cross for crashing and circle for coordinated landing. We see a trend of significantly greater vertical impulse as landing time increases (linear regression, *p* = 2.19E-33), and crashes tend to be characterized by both short landing times and low vertical impulses. Linear regression trendline is plotted in solid black and all statistics are provided in the upper right corner.

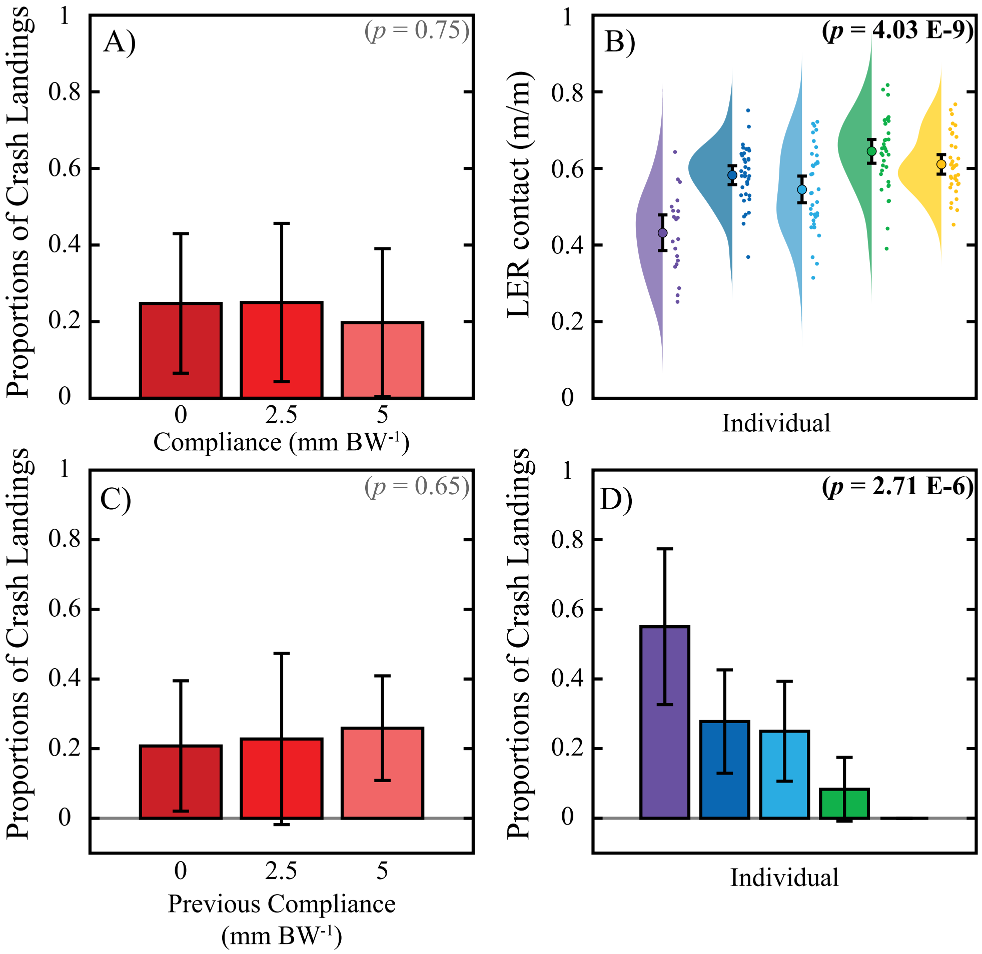

Figure 2.5. Proportions of crash landings ($N = 5$). All probability of crashing graphs are shown with the rates of crashing in red and coordinated landings in blue. For the right most graphs showing individual variation, individuals are plotted in the same order from left-to-right for both graphs. In the top-left graph we show the probability of crashing with respect to compliance treatment which did not significantly differ. The bottom-left graph illustrates the probability of crashing with respect to the previous compliance and this also did not significantly differ. The bottom-right graph the probability of crashing between the five toads was found to significantly differ. From the top-right graph toads with a higher probability of crashing tend to have reduced forelimb limb extension ratio (LER) at touchdown. Center marks in the box and whisker plot represent the median, boxes show 1st and 3rd quartiles, and whiskers give most extreme data not considering outliers. ANOVA results are provided on the top right of each plot and significant values ($p < 0.003$) are darkened.

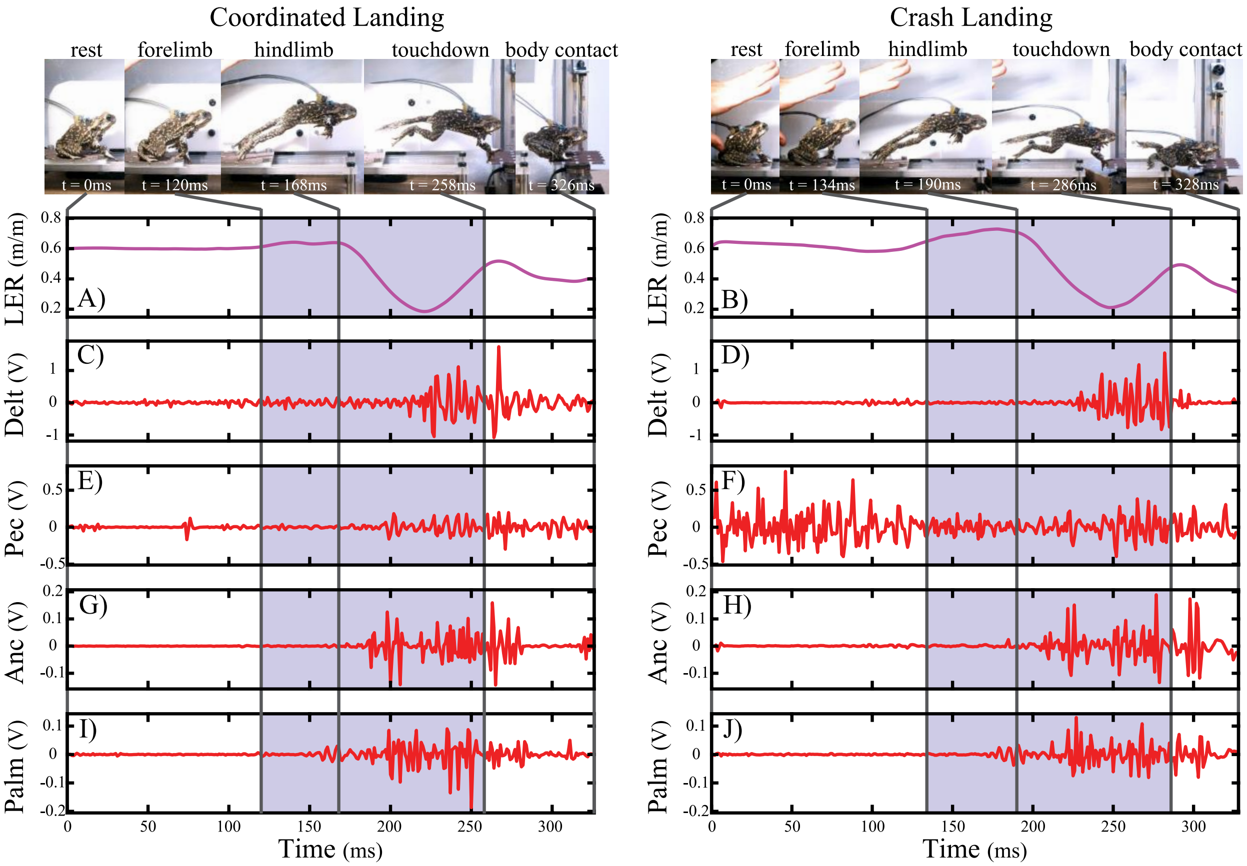

Figure 2.6. Representative time series of a coordinated landing (left) and crash landing (right). Images with corresponding times in milliseconds are shown for start of movement, forelimb and hindlimb liftoff, as well as forelimb and hindlimb touchdown – respectively. These frames are marked by vertical black lines along the plots, where the blue region indicates when the forelimbs were airborne. From top-down graphs show the forelimb limb extension ratio (LER), and excitation voltages for the deltoideus, pectoralis, anconeus, and palmaris longus throughout the time from movement initiation until hindlimb touchdown. Representative traces are from separate trials from the same toad landing on 2.5 mm BW^{-1} for both trials.

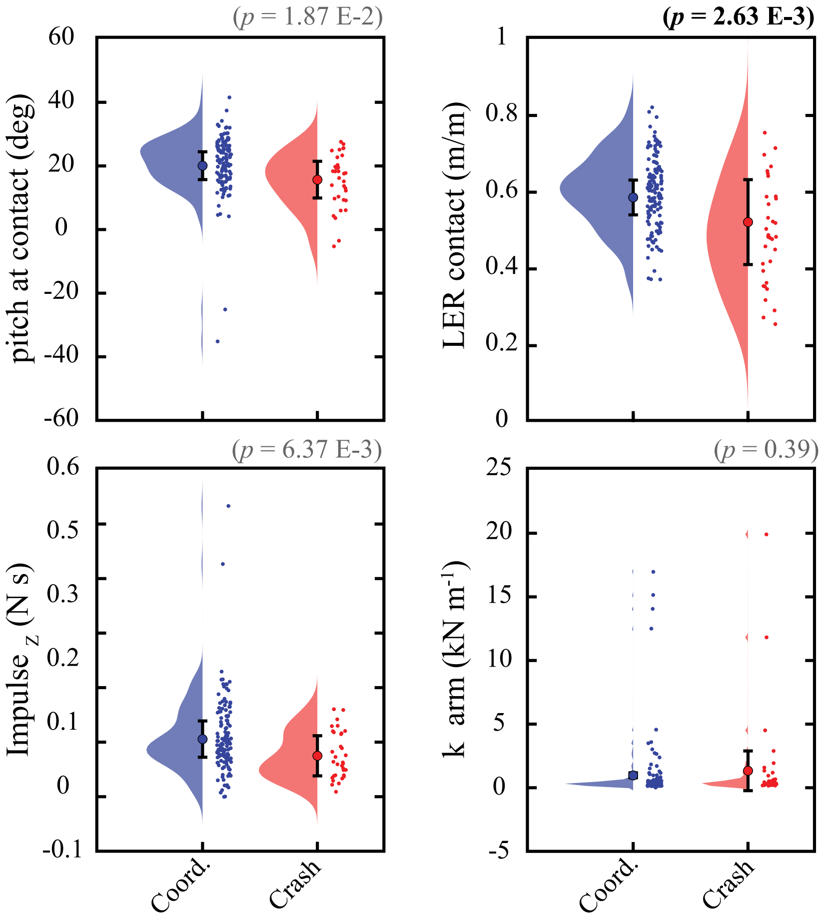

Figure 2.7. Differences attributed to landing behavior in the cane toad (*N* = 5). Top-left graph illustrates there is no significant difference in head pitch at touchdown between coordinated and crash landings. The top-right plot shows a significantly reduced limb extension ratio (LER) for the forelimb at touchdown. The bottom-left graph shows a trend toward reduced vertical impulse for crash landings (critical value, α = 0.003). Bottom-right plot conveys no difference in forelimb stiffness during landing between the two behaviors. All plots show coordinated landing behavior in blue and crash landings in red, with corresponding ANOVA results at the top-right corner of each plot with significant values darkened.

Table 2.1. Statistical results from all ANOVA tests performed. Bolded values signify *p*-values that suggest a significant difference with a critical value of $0.00\overline{3}$. *F*-statistics and the number of degrees of freedom (*df*) are also provided, with the degrees total degrees of freedom in parentheses following variable name.

Variable	*Compliance*	*Individual*	*Landing Behavior*	*Previous Compliance*
Max Plate Disp. ($df = 163$)	$df = 2$, $F = 16.78$ **$p = 2.55\times10^{-7}$**	$df = 4$, $F = 1.95$ $p = 0.105$	$df = 1$, $F = 0.41$ $p = 0.522$	$df = 2$, $F = 1.07$ $p = 0.347$
I_{Del} (aerial) ($df = 162$)	$df = 2$, $F = 0.15$ $p = 0.864$	$df = 4$, $F = 6.14$ **$p = 1.31\times10^{-4}$**	$df = 1$, $F = 0.90$ $p = 0.343$	$df = 2$, $F = 0.28$ $p = 0.757$
I_{Pec} (aerial) ($df = 88$)	$df = 2$, $F = 1.31$ $p = 0.275$	$df = 2$, $F = 1.55$ $p = 0.218$	$df = 1$, $F = 11.38$ **$p = 1.14\times10^{-3}$**	$df = 2$, $F = 1.08$ $p = 0.343$
I_{Anc} (aerial) ($df = 161$)	$df = 2$, $F = 0.46$ $p = 0.630$	$df = 4$, $F = 11.10$ **$p = 6.30\times10^{-8}$**	$df = 1$, $F = 4.34$ $p = 3.89\times10^{-2}$	$df = 2$, $F = 0.13$ $p = 0.882$
I_{Pal} (aerial) ($df = 156$)	$df = 2$, $F = 2.03$ $p = 0.134$	$df = 4$, $F = 5.76$ **$p = 2.45\times10^{-4}$**	$df = 1$, $F = 0.02$ $p = 0.899$	$df = 2$, $F = 0.98$ $p = 0.376$
I_{Del} (landing) ($df = 161$)	$df = 2$, $F = 0.49$ $p = 0.612$	$df = 4$, $F = 1.23$ $p = 0.299$	$df = 1$, $F = 3.41$ $p = 0.0667$	$df = 2$, $F = 0.73$ $p = 0.484$
I_{Pec} (landing) ($df = 87$)	$df = 2$, $F = 1.22$ $p = 0.300$	$df = 2$, $F = 10.28$ **$p = 1.07\times10^{-4}$**	$df = 1$, $F = 0.93$ $p = 0.338$	$df = 2$, $F = 0.72$ $p = 0.492$
I_{Anc} (landing) ($df = 160$)	$df = 2$, $F = 0.46$ $p = 0.634$	$df = 4$, $F = 2.48$ $p = 4.64\times10^{-2}$	$df = 1$, $F = 0.11$ $p = 7.43$	$df = 2$, $F = 0.49$ $p = 0.615$
I_{Pal} (landing) ($df = 155$)	$df = 2$, $F = 1.09$ $p = 0.338$	$df = 4$, $F = 5.99$ **$p = 1.71\times10^{-4}$**	$df = 1$, $F = 1.97$ $p = 0.162$	$df = 2$, $F = 1.54$ $p = 0.218$
Vertical Impulse ($df = 163$)	$df = 2$, $F = 0.19$ $p = 0.826$	$df = 4$, $F = 13.26$ **$p = 2.63\times10^{-9}$**	$df = 1$, $F = 7.65$ $p = 6.37\times10^{-3}$	$df = 2$, $F = 3.58$ $p = 3.03\times10^{-2}$
LER (contact) ($df = 163$)	$df = 2$, $F = 0.71$ $p = 0.493$	$df = 4$, $F = 12.96$ **$p = 4.03\times10^{-9}$**	$df = 1$, $F = 9.72$ **$p = 2.17\times10^{-3}$**	$df = 2$, $F = 1.23$ $p = 0.295$
ΔLER (landing) ($df = 163$)	$df = 2$, $F = 0.17$ $p = 0.843$	$df = 4$, $F = 13.14$ **$p = 3.10\times10^{-9}$**	$df = 1$, $F = 0.62$ $p = 0.431$	$df = 2$, $F = 2.13$ $p = 0.122$
Limb Stiffness ($df = 163$)	$df = 2$, $F = 0.82$ $p = 0.441$	$df = 4$, $F = 1.24$ $p = 0.294$	$df = 1$, $F = 0.76$ $p = 0.386$	$df = 2$, $F = 0.18$ $p = 0.835$
Pitch (landing) ($df = 163$)	$df = 2$, $F = 2.85$ $p = 6.11\times10^{-2}$	$df = 4$, $F = 12.87$ **$p = 4.62\times10^{-9}$**	$df = 1$, $F = 5.65$ $p = 1.87\times10^{-2}$	$df = 2$, $F = 0.88$ $p = 0.418$
Crashing ($df = 163$)	$df = 2$, $F = 0.29$ $p = 0.750$	$df = 4$, $F = 8.60$ **$p = 2.71\times10^{-6}$**	N/A	$df = 2$, $F = 0.43$ $p = 0.652$

CHAPTER 3

Hindlimb proprioception informs preparatory forelimb coordination during landing in toads

Abstract

Animals move across a wide range of surface conditions in real world environments to acquire resources and avoid predation. Animals rely on several mechanisms including intrinsic mechanical responses, central pattern generators, and neural commands that require sensory feedback to effectively navigate various surfaces. Proprioception plays a critical role in providing sensory feedback and informing motor control strategies across legged vertebrate locomotion, which is apparent in cases where it is lacking due to injury or diabetic peripheral neuropathy. Here we tested the hypothesis that proprioception from hindlimb muscles is important for coordinating forelimb landing behavior in the cane toad. We performed bilateral sciatic nerve reinnervations to ablate the stretch reflex from distal hindlimb muscles while allowing for motor neuron recovery. We found that the rate of elbow extension was significantly reduced in magnitude after hindlimb proprioception was ablated while overall forelimb extension was conserved. Additionally, the duration of activation in the elbow extensor muscles was significantly shorter in reinnervated animals. Our results suggest toads developed a novel motor control strategy for landing following reinnervation that preserved limb-level dynamics. These findings indicate a role for proprioceptive feedback to coordinate complex movement. However, proprioception may not be essential to coordinate effective locomotion following injury given the functional redundancy and plasticity of the neuromuscular system.

Introduction

Terrestrial locomotion requires animals and humans to navigate complex terrain that may require different control mechanisms to ensure safe and efficient locomotion. Unknown conditions pose the risk of injury, losing out on a meal, or being caught by a predator if the individual is unable to coordinate an effective response to that condition. A response to an unexpected perturbation may be coordinated through intrinsic mechanical properties (Daley et al. 2009), or from sensory feedback to alter limb coordination and return the body to steady-state locomotion (Moritz & Farley 2004). Here, we explore the role of proprioception in coordinating safe and efficient locomotion and how the loss of proprioceptive feedback may alter movement.

Peripheral nerve transection and reinnervation has been used as an experimental approach to knock out proprioception after complete motor recovery. This intervention has been shown to produce locomotor phenotypes consistent with peripheral nerve damage which can occur after injury or from diabetic peripheral neuropathy (Boulton 2005; Menz et al. 2004). Peripheral nerve reinnervation is characterized by a loss of sensitivity to muscle length which is likely caused by a sustained loss in muscle spindle connectivity. Reinnervation causes a significant loss in muscle spindle Ia sensory afferents responsible for the myotatic stretch reflex which persists throughout recovery (Alvarez et al. 2011; Bullinger et al. 2011; Carr et al. 2010; Cope et al. 1994). By investigating the role of proprioception in locomotor control we can better understand how animals and humans integrate sensory feedback to move effectively.

Several changes to muscle function occur in response to the reinnervation and subsequent loss of proprioception. Immediately following nerve reinnervation, muscle EMG amplitude is significantly reduced and generally does not recover to pre-surgery amplitudes; although, it increases throughout recovery in proportion to the reinnervation of endplates at the neuromuscular junction (Gordon & Stein 1982; Vannucci et al. 2019). Decerebrate cats alter the magnitude of EMG activity in both their MG and LG muscles when the MG is lengthened, conveying the role of length and/or force sensory inputs from muscles influencing motor control (Donelan & Pearson 2004). Furthermore, Yakovenko et al. (2004) through modeling found the role of Ia (spindle) and IIa (Golgi tendon organ) afferents is dependent on the relative strength of the central control associated with the behavior, with loss of feedback having greater effects on behaviors requiring little CPG or central activation and in severe cases resulted in the model being unable to support body weight during steady state locomotion. However, intermuscular feedback from force-sensing Golgi tendon organs remain intact following nerve reinnervation, suggesting that the loss in connectivity of muscle spindles is likely driving the loss of autogenic sensory feedback and the changes in locomotor behavior following reinnervation (Lyle et al. 2016). Pyridoxine induced Ia afferent ablation also results in reduced joint stiffness in cats and reduced range of motion in the distal hindlimb joints of developing chicks, further suggesting the role of muscle spindle feedback on coordinating proper joint mechanics and indicating a potentially greater reliance on stretch feedback from distal limb muscles in the intact animal (Pearson et al. 2003; Sharp & Bekoff 2015). Observed changes following nerve reinnervation are thought to be due to a lasting loss in spindle connectivity rather than changes in muscle properties since the electrical properties of spindles remain unchanged and motor units recover tension at a similar rate to whole muscle tension (Gordon & Stein 1982; Bullinger et al. 2011).

Several approaches have been shown to result in effective peripheral nerve reinnervation. Methods using laser welding, suturing, or gluing all resulted in similar overall functional recovery and recovery rates (Bhatt et al. 2017; Gordon & Stein 1982; Nunes e Silva et al. 2012; Vela et al. 2020). Nunes e Silva et al. (2012) showed nerve-to-nerve (end-to-end or end-to-side) connection is far superior than nerve-to-muscle reinnervation and the use of fibrin or other glues does not significantly improve functional recovery. Most vertebrate models regain locomotor function between 6 weeks and 6 months following reinnervation (Abelew et al. 2000; Bhatt et al. 2017; Carr et al. 2010; Howard et al. 2000; Vannucci et al. 2019). Exercise can be used to accelerate the recovery with earlier initiation of exercise providing greater recovery than delayed exercise programs (Boeltz et al. 2013; Brandt et al. 2015). Therefore, a simple reinnervation using suture accompanied by an exercise recovery regimen can expect similar results to experiments performed with more intricate surgical procedures.

The loss of proprioception resulting from peripheral reinnervation affects some locomotor behaviors more than others. For example, gait characteristics are more significantly affected locomoting downhill, where reinnervated muscle(s) are likely undergoing eccentric contractions, compared to walking uphill or level walking (Abelew et al. 2000; Livingston & Nichols 2014a; Livingston & Nichols 2014b; Maas et al. 2007). Additionally, the stiffness of joints that reinnervated muscles span are reduced, which is again more apparent in tasks where the muscle is likely lengthened relative to steady state conditions (Abelew et al. 2000; Chang et al. 2009; Gordon et al. 2020; Livingston & Nichols 2014a; Maas et al. 2007). Examining the effects of nerve reinnervation in eccentric behaviors where muscles are lengthening and performing negative work is therefore likely to shed more light on the influence of muscle spindle sensory feedback on motor control and coordination.

Frogs and toads have muscle spindles that behave similarly to those of other vertebrates and likely exhibit similar motor control deficits following nerve transection (Jahn 1968; Proske & Stuart 1985; Gray 1957). Most anurans use a saltatory gait where the extension of long powerful legs propel the animal into the air. Since hindlimb muscles are not likely to be stretched during take-off (Azizi and Roberts 2010), takeoff performance is unlikely to be affected by proprioceptive ablation. However, many anuran species have relatively shorter forelimbs used to dissipate mechanical energy during landing, which poses a greater risk of injury if the arms are unable to dissipate the energy generated by the legs (Nauwelaerts & Aerts 2006). Terrestrial species that use jumping as a primary mode of locomotion appear to have well developed and coordinated forelimb behavior to achieve safe and efficient decelerations at landing (Essner et al. 2010; Gillis et al. 2014). The controlled deceleration is achieved through the flexion of forelimbs at impact where eccentric contraction of extensor muscles dissipate the energy (Gillis et al. 2010). Cane toads recruit their elbow extensors earlier during the aerial phase of longer jumps resulting in a more extended elbow at impact as jump distance increases (Gillis et al. 2010; Azizi and Abbott 2013; Gillis et al. 2014). This preparatory recruitment which is tuned to the energy that needs to be dissipated at impact is thought to be informed by vestibular and/or proprioceptive information during take-off (Cox & Gillis 2016). However, a direct test of this hypothesis has not been made in freely jumping animals.

In this study we investigate the role of hindlimb proprioception in informing forelimb coordination during landing in the cane toad, *Rhinella marina*. We use bilateral sciatic nerve reinnervation to ablate the myotatic stretch reflex while motor function is regained. Jumping and landing behavior is compared with and without proprioception. If cane toads rely on proprioception from the hindlimbs to coordinate forelimb landing behavior and we successfully ablate the stretch reflex, then we predict that 1) an overall reduction in the rate of elbow extension prior to landing 2) delayed onset of elbow extension during the aerial phase, and 3) reduced extension of the forelimb at touchdown.

Methods

Animals

Eleven cane toads (6 females and 5 males; body mass: 220.2 ± 85.3 g; snout vent length: 127.0 ± 14.4 mm; mean ± SD) were obtained from a commercial supplier and housed in individual terrariums. Toads were fed crickets twice a week and provided fresh water daily and the room temperature was maintained between 21 and 22°C with a 12h-light-12h-dark cycle. All research was conducted in accordance with the University of California Irvine's Institutional Animal Care and Use Committee (IACUC) AUP-19-155.

Stretch Reflex Test

The myotatic or stretch reflex of the plantaris muscle was tested using a rig that induced rapid ankle flexion and therefore induced rapid plantaris extension (Fig. 3.1). Using high-speed videography at 1964 fps, we measured the joint angle throughout the perturbation and immediately following. We used a bipolar electrode inserted into the plantaris muscle to measure the electrical excitation of the motor units within the muscle as the ankle was being rapidly flexed and during its subsequent extension. The stretch reflex was measured in each toad prior to any surgical intervention ($N = 11$), after a sham surgery for four individuals ($N = 4$), in addition to 1 week ($N = 10$), 3 months ($N = 9$) and 6 months ($N = 8$) following the nerve reinnervation surgery for all surviving toads. The stretch reflex was also tested post-mortem ($N = 11$) as an additional control for movement artifacts in electromyographic signals since this would be the only signal recorded in an animal where the nervous system is no longer funcationing. Three replicate flexion trials were collected at each of these study timepoints. The first animal passed prior to any surgical procedures from unknown causes, the second toad was euthanized for welfare reasons prior to the 3 month time point due to an eye infection that did not respond to treatment, and a third animal was lost prior to the 6 month time point due to an unknown cause.

Nerve Reinnervation Procedure

Nerve reinnervation surgeries began with soaking the individual in a chilled bath of 1.5 g L^{-1} MS-222 buffered to a pH of 7.4 using sodium bicarbonate ($NaHCO_3$) for 15 to 25 minutes until the animal was unresponsive – determined by a loss of both their righting reflex and toe pinch response. Surgical incisions were made along the ventral surface of each thigh parallel with the femur. Connective tissue was cut and muscle tissue separated to locate the sciatic nerve. Once the sciatic nerve was located for sham surgeries – conducted on four toads (2F and 2M) – the nerve was returned intact and the skin was sutured (size 4-0) to allow the skin to heal. All other surgeries were conducted by transecting the sciatic nerve proximally to the branching of the tibial and peroneal nerves and sutured (size 6-0) to allow for contact and reinnervation. The four individuals that underwent sham surgery recovered for one week prior to jumping trials and following jumping immediately underwent surgery to transect and suture the sciatic nerve as done with the other seven toads. After each surgery toads were injected with 2 mg Carprofen per kg of body mass to reduce inflammation and nociception post-operation. Animals were then monitored closely for the next 48 hrs for any signs of complication. After providing one week of recovery, each toad was prompted to exercise for 2 min twice a week by walking or jumping at their preferred speed on the hand-powered treadmill to encourage reinnervation and accelerate functional recovery.

Electromyography Implantation Procedure

We used the same anesthetic and preparatory procedures to implant electrodes as we detailed for the nerve reinnervation surgery previously. Our custom bipolar electrodes were constructed from fine silver wire that was inserted through the dorsal surface of the animal's back and fed under the skin to the *anconeus* (elbow extensor) and *plantaris* (ankle extensor) muscles. A ground wire was also submerged subcutaneously on the animal's dorsal side. Following surgery all toads were injected with 2 mg kg^{-1} body mass Carprofen and allowed to recover for at least 24 hrs prior to any jump trials.

The cable used to connect the toad to the animal and record muscle activity was 63g and 196cm in length. All signals recorded from the toads were fed into an A-M Systems Differential AC Amplifier Model 1700 which amplified the muscle signals 1000-fold before recording them into Igor Pro software via NIDAQ recording at 10 kHz.

Jumping Procedure

Toads were placed on a hand-powered treadmill surrounded by plexiglass walls (43 x 19 x 30 cm; *l* x *w* x *h*; Fig. 3.2) that moved at an approximate speed of 10 cm s^{-1} via entrainment to a metronome. The animals were recorded using high-speed video cameras filming at a rate of 100 fps and angled approximately 30° in either direction from the sagittal plane. Videos were taken prior to and after nerve reinnervation surgeries and toads that underwent EMG electrode implantation were filmed at 250 fps. Toads were prompted to jump by approaching from behind with a hand or gently prodding from behind. Filming occurred over 60 second intervals pre-surgery (N = 10 toads, 200 jumps), 1 wk post-sham (N = 4; 80 jumps), and 6 mo post-reinnervation surgery with EMG (N = 7, 264 jumps). If toads produced less than 10 good jumps - defined by the toads not coming into contact with the sides of the jumping arena - then a second 60 second jump trial was recorded during that same day.

Analysis & Statistics

We digitized video data of both the myotatic stretch reflex tests as well as continuous jumping trials using DLTdv8 freeware (Hedrick, 2008). All kinematic measurements as well as EMG analysis were performed with custom MATLAB code. We filtered our continuous jumping kinematic traces using 7th order Savitzky-Golay Filters with a frame length of 49 (or length of the vector if it was shorter than 49 frames). We rectified the raw EMG signals from the stretch reflex data and used a 0.1 second window that started 0.15 s prior to any ankle motion to quantify the baseline level of noise and its variation. We set an activation threshold of the average rectified background noise plus two standard deviations (threshold = mean + 2 SD). We could then quantify the intensity of activity as well as duration of time the muscle was active (2SD above the average baseline noise) during ankle flexion and extension. The raw EMG signals we recorded from jumping trials were also rectified and then we applied a simple moving average with a window length of 9 frames (approximately 1 ms given our 10 kHz sampling rate). The onset and offset times for both muscles were visually marked for each jumping trial and the intensity was calculated as the area under the rectified trace during the interval of interest.

We employed linear mixed effect models (LME) with individuals treated as a random effect and condition (pre-op, sham, 6 mo post-op) as a fixed effect. We compared this model against the null model only containing individuals as a random effect. The AIC value was always lower for the model including experimental condition as a fixed effect - suggesting that the model including the condition has greater explanatory power – therefore, we used this model for our analyses. For our electromyography data which we only collected 6 months post-op, we compared our results to values reported for intact toads in the literature with a one-sample Student’s *t*-test. This allowed us to determine whether the timing and duration of activation were significantly different from what others have found in in-tact cane toads. We used a Bonferroni correction to account for the multiple statistical tests being performed (both linear mixed effects and *t*-tests) and therefore used a critical value of 2.78e-3 (0.05/18) for these tests. Any linear mixed effect models that were found to have a condition with a significant difference were further analyzed using Tukey Honest Significant Difference tests with a critical value of 0.05.

Results

We tested for the presence of the myotatic stretch reflex prior to surgery, 1 week following the sham surgery, and throughout the recovery from the nerve transection surgery to illustrate the sustained loss of the stretch reflex (Fig. 3.3). The ratio of *plantaris* EMG intensity during ankle extension relative to ankle flexion significantly decreased following never-transection surgery (post 1wk, $N = 10$; 3mo, $N = 9$; 6 mo, $N = 8$; post-mortem, $N = 11$) compared to the intact ratio (pre-surgery, $N = 11$; Fig. 3.3A; LME, $p = 8.89\text{e-}7$). The ratio of intensity following the sham surgery ($N = 4$) also significantly differed from all timepoints following the nerve reinnervation surgery suggesting the stretch reflex remained intact following our sham procedure. All timepoints following the nerve transection surgery, including post-mortem, had average ratios near one (range: 1.16 - 1.98) with all of their 95% confidence intervals for the mean encompassing a ratio of one which is expected from random noise during both phases of ankle motion. We observed a similar pattern for the ratio of EMG duration in the *plantaris* during ankle extension relative to flexion (Fig. 3.3B, LME, $p = 2.62\text{e-}5$). Again, the intact animal activated the *plantaris* significantly longer during extension than flexion as compared to all the time points following the nerve reinnervation. While the average ratio of activation duration following the sham surgery was greater than all timepoints following reinnervation surgery, the difference was not significant. These data suggest we successfully ablated the myotatic stretch reflex through our nerve transection surgeries throughout the course of our 6 month recovery period.

We observed significant differences in forelimb behavior in preparation for landing (Fig 3.4). The most pronounced difference was in the mean rate of elbow extension during the aerial phase which was significantly reduced 6 months following nerve transection (248.0 ± 29.3 deg s^{-1}; mean ± 95% CI of the mean & henceforth referred to as 95% CI_{μ} for brevity) relative to the intact condition (326 ± 91.6 deg s^{-1}; mean ± 95% CI_{μ}; Table 3.1, Fig 3.4A; LME, p = 5.26e-47). The toads also had significantly greater elbow extension at touchdown in both the sham (70.7 ± 4.4 degrees; mean + 95% CI_{μ}) and 6 months post transection (68.3 ± 10.7 degrees; mean + 95% CI_{μ}) conditions compared to the intact time point (62.7 ± 9.7 degrees; mean + 95% CI_{μ}; Fig 3.4B; LME, p = 8.76e-18). We found that the onset time of elbow extension following takeoff was also significantly later for the sham and 6 month post-surgery conditions compared to control toads (Fig. 3.4C; LME, p = 1.31e-5). The onset time of elbow extension prior to touchdown was not found to be significantly different between any of the conditions analyzed (Fig. 3.4D; LME, p = 0.0146). This data supports our initial hypothesis that the rate of elbow extension would be reduced following hindlimb reinnervation. We also found support with the delayed onset of elbow extension relative to takeoff; however, we observed the same trends after our sham surgery and did not find the elbow extension was delayed relative to touchdown. Furthermore, our result of greater elbow extension at touchdown following nerve reinnervation fails to support our initial prediction of reduced limb extension upon impact.

Performing nerve reinnervation surgeries are extremely invasive as the sciatic nerve runs along the femur underneath most of the musculature and connective tissue in the thigh. In an attempt to illustrate any effect this procedure had on hindlimb function during jumping we found that the average rate of hindlimb extension during takeoff did not significantly differ between the pre-surgery (0.085 + 0.118 lengths s^{-1}; mean ± 95% CI_{μ}), sham (0.199 + 0.115 lengths s^{-1}; mean ± 95% CI_{μ}) surgery or 6 months post-transection surgery (0.097 + 0.107 lengths s^{-1}; mean ± 95% CI_{μ}; Table 3.1, Fig 3.5A; LME, $p = 0.183$). However, we found that the maximal rate of hindlimb extension during takeoff was significantly increased 6 months following nerve transection (2.24 ± 0.22 lengths s^{-1}; mean ± 95% CI_{μ}) compared to both the intact (1.72 ± 0.36 lengths s^{-1}; mean ± 95% CI_{μ}) and sham conditions (1.63 ± 0.25 lengths s^{-1}; mean ± 95% CI_{μ}; Fig 3.5B; LME, $p = 2.75e-9$). Furthermore, the instantaneous rate of hindlimb extension at takeoff was significantly increased after both the sham (0.306 + 0.682 lengths s^{-1}; mean ± 95% CI_{μ}) and nerve transection surgeries (0.590 ± 0.359 lengths s^{-1}; mean ± 95% CI_{μ}) compared to the intact jumps (-0.381 ± 0.462 lengths s^{-1}; mean ± 95% CI_{μ}; Fig. 3.5C; LME, $p = 8.26e-8$). Although we report specific shifts in hindlimb performance during takeoff, the jumping behavior we observed in these animals remained largely similar 6 months after we performed the nerve transection surgery (Table 3.1, Fig. 3.6).

We additionally measured the electromyographic activity of the *anconeus* and *plantaris* muscles 6 months following nerve reinnervation surgery. We found the *plantaris* - the primary ankle extensor - initially became active 0.230 ± 0.017 s (mean ± 95% CI_{μ}) prior to takeoff. The *anconeus* - the primary elbow extensor - had an activation duration of 0.0614 + 0.0054 s (mean ± 95% CI_{μ}) during the aerial phase, and initially became active -0.0284 + 0.0058 s (mean ± 95% CI_{μ}) prior to hindlimb liftoff.

Discussion

Forelimb Behavior

We found that cane toads significantly reduced their rate of elbow extension in preparation for landing following sciatic nerve reinnervation. This result supports our initial prediction and the Cox et al. (2018) findings regarding reduced rate of elbow extension following sciatic nerve transection. Toads initiated elbow extension at a later time following takeoff after nerve reinnervation, which supports our prediction of a delayed onset of elbow extension. Sciatic nerve transection immediately alters forelimb landing behavior in the cane toad, although it also inhibits motor control of the hindlimbs and normal takeoff behavior (Cox et al. 2018). Because our experimental manipulation allowed for motor recovery while maintaining a sustained loss of the stretch reflex in the distal hindlimb, our results suggest feedback from muscle spindles in the *hindlimb* is critical for modulating *forelimb* landing behavior.

Reinnervated toads reduced their preparatory elbow extension while achieving greater elbow extension at touchdown. This suggests toads developed a new strategy for landing following sciatic nerve reinnervation. While we observed this same behavior following the sham surgeries, we believe these similar behavioral trends are due to separate mechanisms. We selected the four largest toads for our sham surgeries because they were our first surgeries and the process of locating and identifying the sciatic nerve would be easier in larger individuals. This selection bias likely skewed variables including jump distance which was only significantly greater after the sham surgeries. This is likely because the larger toads were capable of jumping greater distances. Furthermore, toads exhibit greater limb extension at touchdown during longer jumps (Cox & Gillis, 2015; Fig. 3.7). While the sham toads jumped further and likely had altered elbow mechanics from greater average jump distance, the seven toads jumping after nerve reinnervation jumped comparable distances to their intact state and still produced significantly reduced rates of elbow extension with greater elbow extension at touchdown. This suggests that toads changed their preparatory landing behavior following sciatic nerve reinnervation.

Elbow extension determines the orientation of the two longest forelimb segments, and therefore greater extension of the elbow likely equates to greater overall limb extension. Toads may have altered the coordination of the forelimb joints following nerve reinnervation to potentially conserve the center of mass dynamics at impact. This aligns with research showing cats conserved their hip kinematics and limb contact angles following reinnervation in distal limb muscles (Abelew et al. 2000; Chang et al. 2009; Maas et al. 2007). Chang et al. (2009) reported greater flexion of the distal joints (another common effect of reinnervation) that was compensated for by greater extension in the hip of all their cats. Additionally, we only report on a single time point after relatively complete recovery of motor function. Chevallier et al. (2004) found that salamanders following spinal transection initially exhibit increased variability in step duration and forelimb-hindlimb coupling during walking which both gradually converged back to the intact condition over time. This re-development of intact behavior may explain why we observed an increase in overall forelimb extension 6 months following surgery even with a significant reduction in the rate of elbow extension. Furthermore, we only report here on data collected 6 months following transection and Chevallier et al. (2004) suggests we might expect the kinematic data collected 3 months following transection (not reported here) to have greater variation as muscles reinnervate and neural circuits are potentially in the process of reorganization to accommodate a loss in hindlimb proprioception.

Upstream neural reorganization within the central nervous system is likely occurring following nerve damage. Spinal transections result in regeneration of neural networks that restore behavior but are anatomically and functionally reorganized from their intact state (Haspel et al. 2021; Parker 2017). While our sciatic nerve transection in the hindlimbs is not as severe an alteration to the structure of the central nervous system as a spinal transection, our finding a shift in the elbow joint mechanics of the forelimb strongly suggests upstream changes to the circuits coordinating landing behavior. Frogs share synergies between swimming and jumping behaviors to simplify control across movement modalities (Cheung et al. 2005). Therefore we hypothesize that our reinnervation may affect one or more of the synergies associated with the takeoff and landing phase of a jump. Because the central nervous circuitry - likely in the spinal cord - is cut off from complete proprioceptive information during takeoff, the circuitry may be reorganized in order to effectively coordinate a landing with the forelimbs in the absence of proprioceptive information from the whole hindlimb.

Hindlimb Behavior

In addition to assessing alterations in forelimb mechanics, we showed the average rate of hindlimb extension was unaffected by either the sham or reinnervation surgery. Although this was not the case for the maximum rate of hindlimb extension or the instantaneous rate of extension at takeoff, the first decreased only following reinnervation and the latter increased for both surgical interventions. However, using maximal performance is likely a less accurate comparison due to sample size constraints and the inherent variability associated with voluntary behavior (Dakin et al. 2020). The significant increase in hindlimb extension at takeoff following both sham and reinnervation surgeries may be a result of disrupting the physical and elastic properties of the hindlimb during take-off which may be essential for engaging the elastic mechanisms associated with jump propulsion in addition to hindlimb retraction after takeoff (Astley & Roberts 2012; Astley & Roberts, 2014; Reynaga et al. 2019; Schnyer et al. 2014). This could have implications for the cane toad's ability to coordinate an effective landing as they alter hindlimb retraction to effectively minimize torques on their forelimbs upon impact (Azizi et al. 2014).

Electromyography

Electromyography was recorded in the *anconeus* (primary elbow extensor) and *plantaris* (primary ankle extensor) muscles during jumping in the toads at 6 months following their nerve transection procedures. We compared our findings on the activation timing of *plantaris* to results reported for the same muscle in the same species in Gillis & Biewener (2000). We found that the *plantaris* muscle was active for significantly longer during takeoff following sciatic reinnervation (one-sample *t*-test, *p* = 1.35e-15; Fig. 3.8A). The almost two-fold increase in the duration of plantaris activation in toads following sciatic nerve reinnervation may be a compensatory strategy for potentially reduced contraction velocity (Gordon et al. 2020). Toad hindlimbs are also in a relatively ineffective mechanical position during takeoff and initiating movement from the ankle may therefore demand greater duration of plantaris activation following reinnervation if its contraction velocity is reduced (Reynaga et al. 2019). We also showed a significant reduction in the duration of *anconeus* activation during the aerial phase in toads 6 months post-surgery compared to the in-tact toads from Cox et al. (2018; one-sample *t*-test, *p* = 5.74e-31) and Cox & Gillis (2020; one-sample *t*-test, *p* = 3.61e-18; Fig. 3.8B). The onset of anconeus activation during the aerial phase in the post-surgery condition differed significantly from those reported in Cox & Gillis (2020; one-sample *t*-test, *p* =3.08e-10) but not from Cox et al. (2018; one-sample *t*-test, *p* = 0.011; Fig. 3.8C). Our results suggest proprioception from the distal hindlimb influences the duration of anconeus activation, which may help to explain our observation of the reduced rate of elbow extension following reinnervation. If the anconeus is active for a shorter duration, this could result in a decreased average rate of elbow extension.

Limitations

While we observed only minor changes in limb-level hindlimb dynamics during takeoff, there is a chance that muscles distal to the transection site were cross-reinnervated - motor neurons reinnervated different muscles than they innervated originally before transection (Abelew et al. 2000). Cross-reinnervation of motor units limits our ability to draw accurate comparisons between reinnervated and intact animals if the motor commands sent to specific muscles in the hindlimb are significantly altered from the intact state. A second limitation was the site of our sciatic nerve transection being located distally to the nerve branches that innervate thigh musculature, which left proprioceptive feedback from thigh musculature intact. Cheung et al. (2005) suggests the semitendinosus and other hamstring muscles are important for providing feedback for modulating synergies in frogs, feedback from these muscles would not be disrupted from our approach. However, this limitation does not detract from the significant changes in forelimb behavior we observed due to proprioceptive ablation in distal hindlimb muscles.

Conclusion

Through bilateral reinnervation of the sciatic nerve in the hindlimb of cane toads, we successfully ablated the myotatic stretch reflex distal to the transection site and illustrated shifts in forelimb preparatory and landing behavior that suggests the neural control networks responsible for coordinating jumps in toads had been reorganized. Observed changes in behavior are consistent with peripheral nerve damage across vertebrates - including cases of nerve damage in humans brought on by injury or diabetic peripheral neuropathy. Clearly the nervous system is capable of reorganization after losing proprioceptive feedback in order to effectively coordinate movements, but whether this mechanism of neural reorganization is directly responsible for the changes we observed is still unknown. Future efforts should further our understanding of how the nervous system compensates for a loss in proprioceptive feedback and track the changes in the organization of spinal circuitry and/or synergy structures following peripheral nerve reinnervation.

Acknowledgements

We would like to thank the undergraduate researchers that digitized videos from this project including Marielle Almazan, Keen Lee and Kim Nguyen.

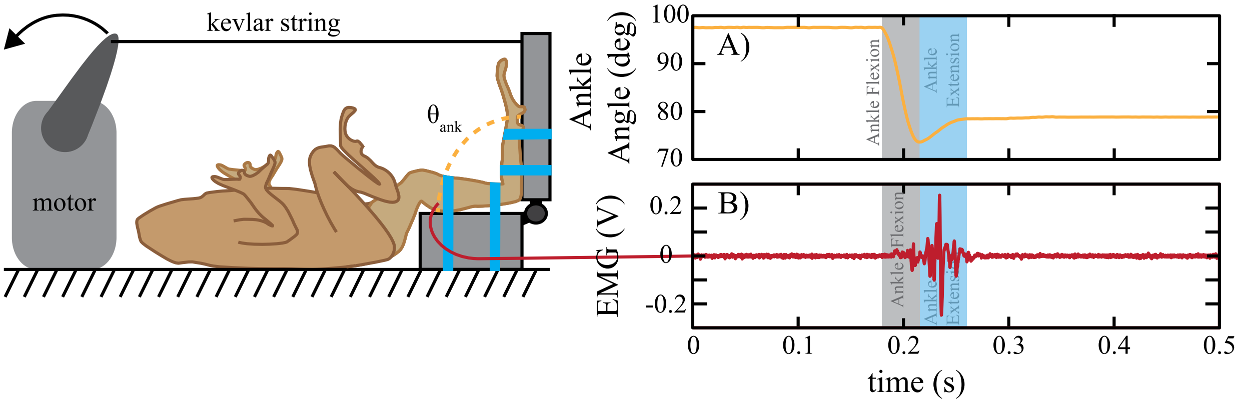

Figure 3.1. Stretch reflex experimental setup. Toads were laid in a supine position with their left hindlimb secured in place to a hinged platform using twist-ties (blue straps in diagram). The hinged apparatus was fixed to the table surface (bottom) which rigidly secured the shank while the foot was secured to the mobile portion of the apparatus which allowed the ankle to freely flex and extend. Measurement of A) the ankle joint angle (θ_{ank}) and B) plantaris muscle activity was recorded via a bipolar electrode. The gray plot background indicates ankle flexion and blue background ankle extension, data is from a representative trial of an intact individual prior to any manipulations (pre).

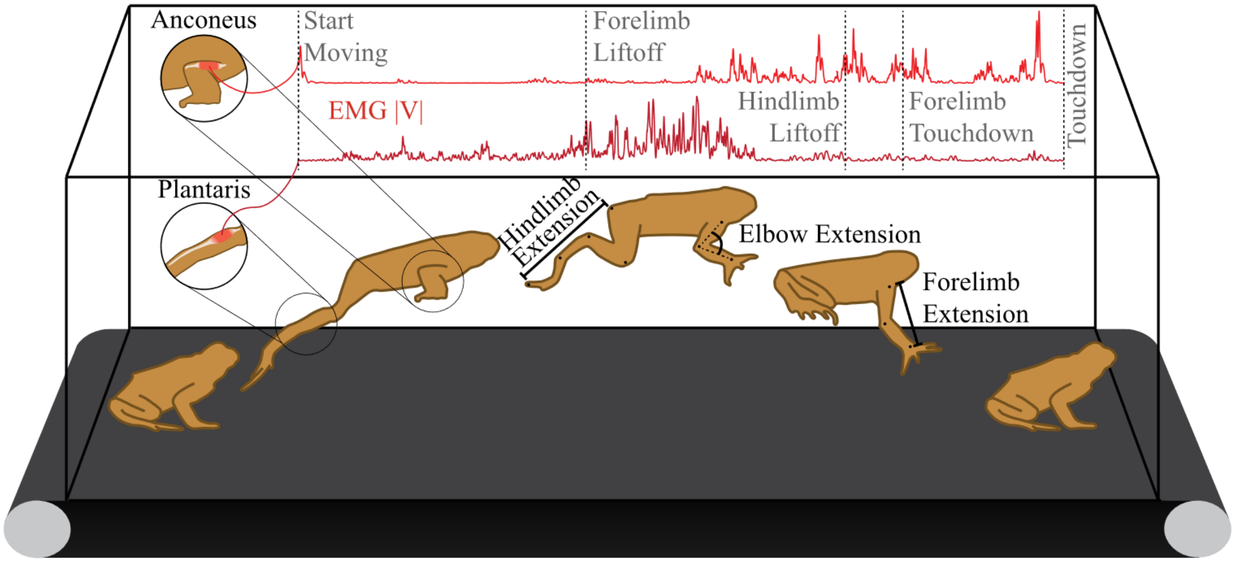

Figure 3.2. Continuous jumping experimental setup. Toads were prompted to jump along a treadmill. Videos were used to track hindlimb and forelimb extension throughout each jump and illustrations convey how those values were attained. Electromyography was recorded from the anconeus and plantaris muscles six months following nerve transection and a representative, rectified trace for each muscle is depicted with key times noted along the series.

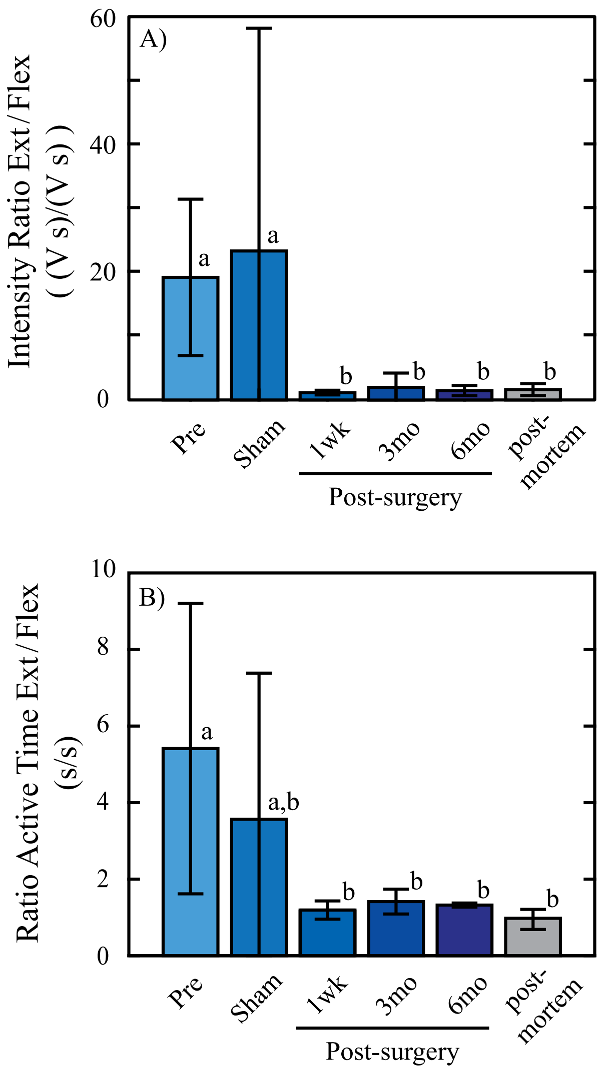

Figure 3.3. Measuring myotatic stretch reflex in in-tact (pre; *N* = 11), after sham surgery (sham; *N* = 4), 1 week (*N* = 10), 3months (*N* = 9), and 6 months (*N* = 8) following nerve transection as well as post-mortem (*N* = 11). A) Shows the ratio of electromyography intensity occurring during ankle extension relative to the intensity occurring during ankle flexion, and B) illustrates the ratio of time the plantaris is active during ankle extension relative to time the plantaris is active during ankle flexion. Bars represent condition means, error bars convey the 95% CI of the mean, and lowercase letters found above bars that do not match signify a significant difference between condition means.

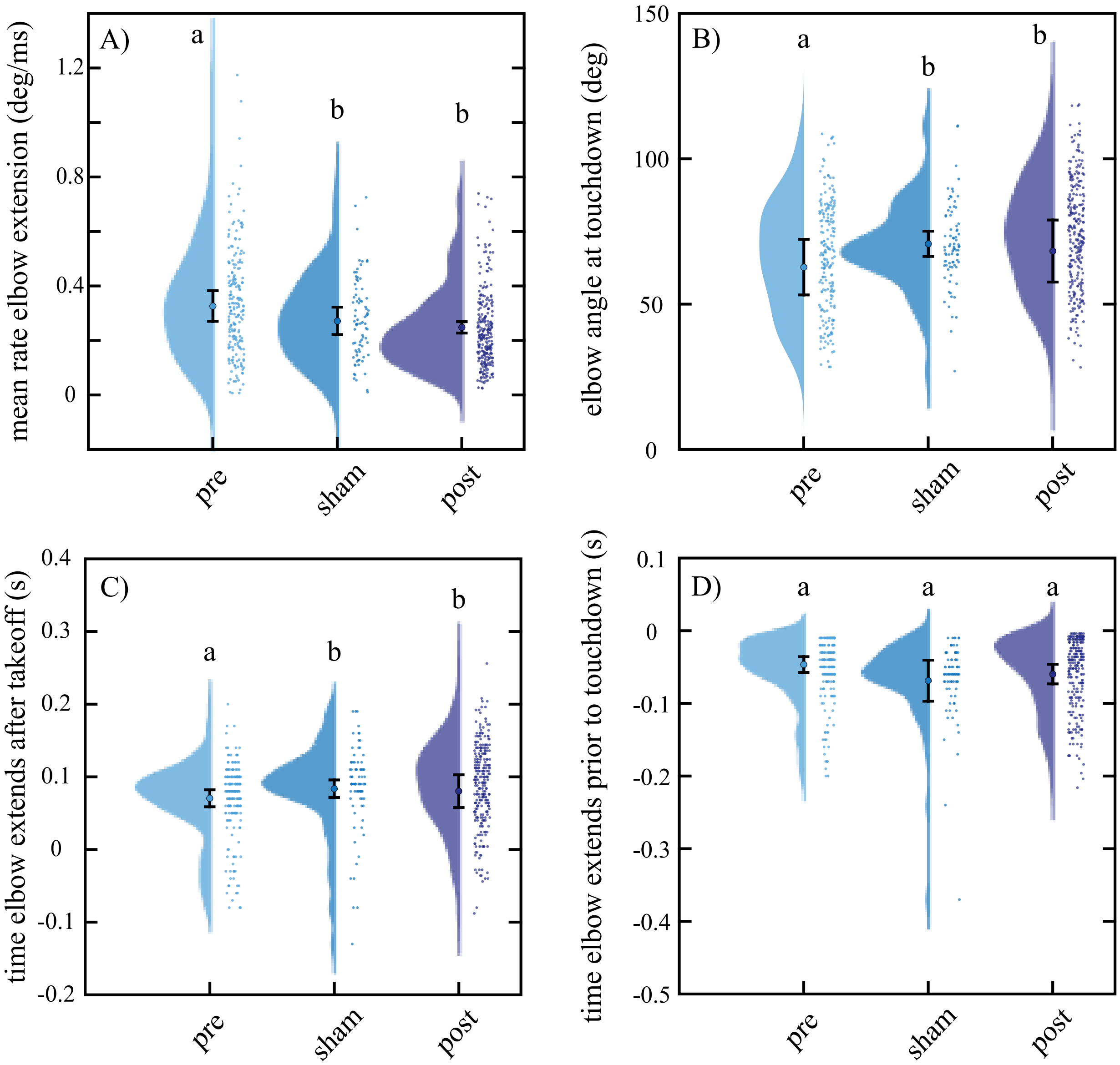

Figure 3.4. Forelimb coordination before surgery (pre; *N* = 10, 200 jumps), after a sham surgery (sham; *N* = 4, 80 jumps), and 6 months following nerve transection (post; *N* = 7, 264 jumps). A) The mean rate of elbow extension, B) the elbow angle at touchdown, C) the time following hindlimb takeoff when the elbow first extends, and D) the time prior to touchdown when the elbow first extends during the aerial phase. Clouds and small points to the right with the same color represent the distribution and all jumps recorded for that condition, respectively. The larger central circle represents the condition mean after accounting for individual variation and error bars convey the 95% CI of the mean. Lowercase letters above distributions that differ represent statistically significant differences in the mean.

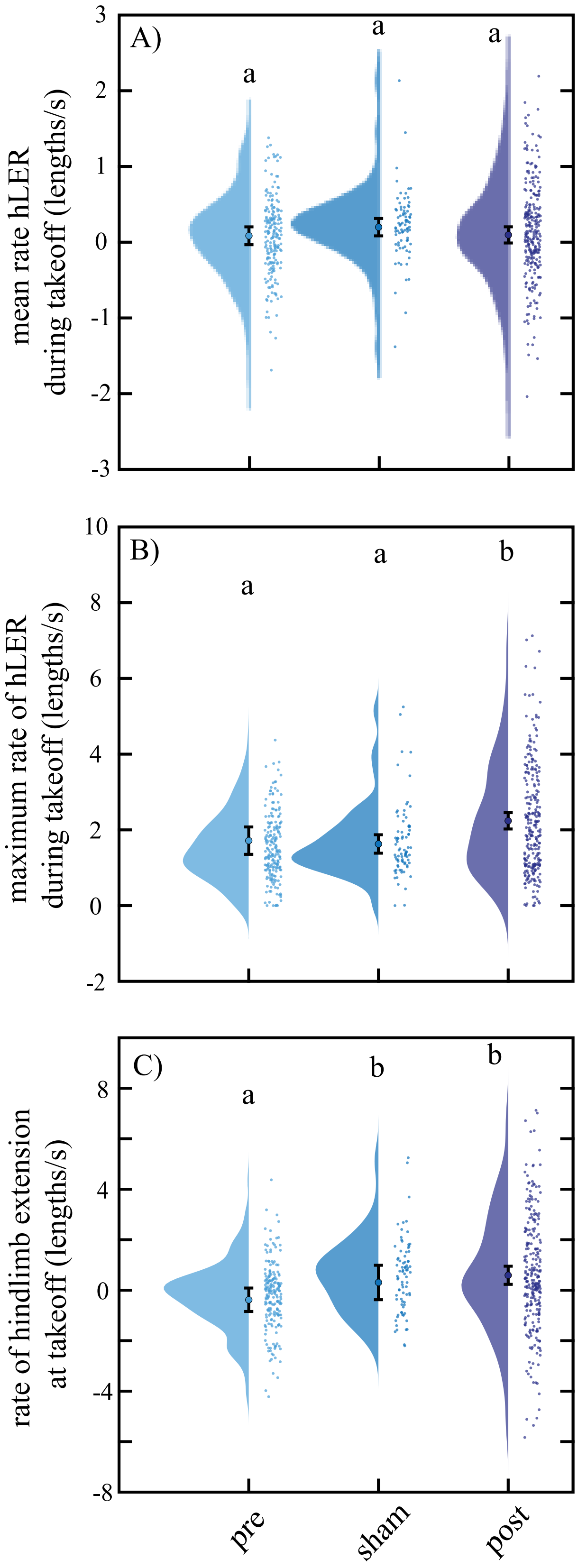

Figure 3.5. Hindlimb coordination before surgery (pre; *N* = 10, 200 jumps), after sham surgery (sham; *N* = 4, 80 jumps), and 6 months following transection (post; *N* = 7, 264 jumps). A) The mean rate of hindlimb extension during takeoff, B) the maximum rate of hindlimb extension during takeoff, and C) the instantaneous rate of hindlimb extension at the time of hindlimb takeoff. Clouds and small points to the right with the same color represent the distribution and all jumps recorded for that condition, respectively. The larger central circle represents the condition mean after accounting for individual variation and error bars convey the 95% CI of the mean. Lowercase letters above distributions that differ represent statistically significant differences in the mean.

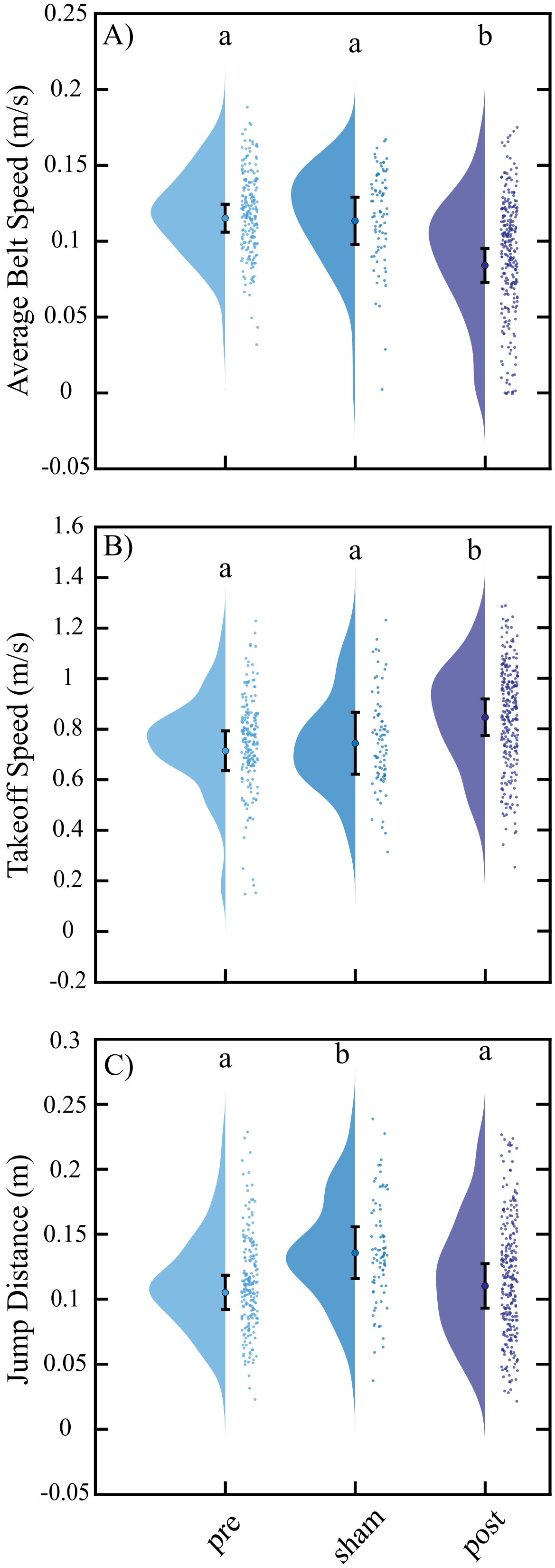

Figure 3.6. Jump conditions before surgery (pre; *N* = 10, 200 jumps), after sham surgery (sham; *N* = 4, 80 jumps) and 6 months following nerve transection (post; *N* = 7, 264 jumps). A) The average treadmill belt speed during the jump, B) instantaneous speed of COM at time of hindlimb takeoff, and C) jump distance. Clouds and small points to the right with the same color represent the distribution and all jumps recorded for that condition, respectively. The larger central circle represents the condition mean after accounting for individual variation and error bars convey the 95% CI of the mean. Lowercase letters above distributions that differ represent statistically significant differences in the mean.

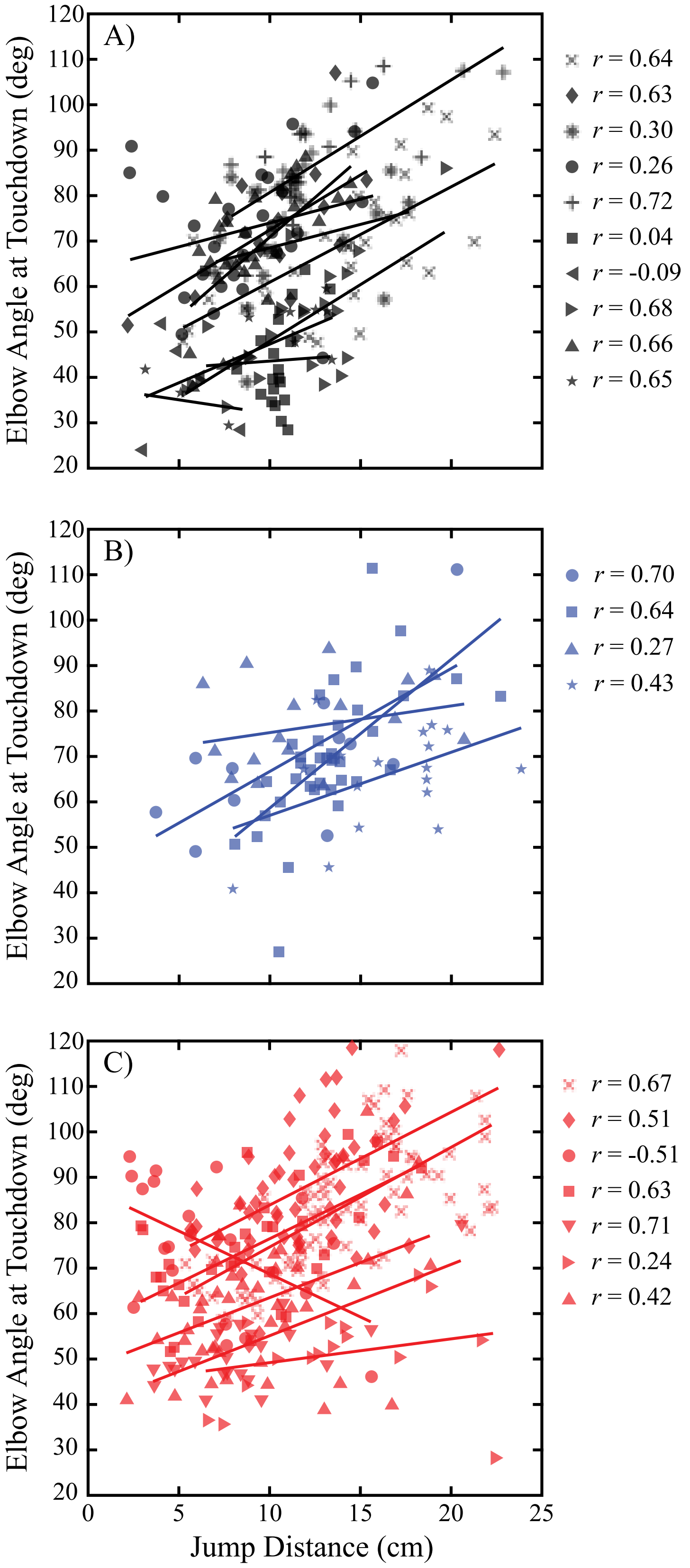

Figure 3.7. Correlation between jump distance and elbow angle at touchdown. The average correlation coefficient relating jump distance and the elbow extension angle at touchdown is greater than zero pre-surgery (A, black, $N = 10$, 200 jumps; One-sample *t*-test, $p = 1.03\text{e-}3$) and following sham surgery (B, blue, $N = 4$, 80 jumps; One-sample *t*-test, $p = 0.0146$). However, while it appears most toads exhibit a positive correlation with elbow extension at touchdown relative to jump distance the average correlation coefficient did not significantly differ from zero 6 months following (post) nerve reinnervation surgery (C, red, $N = 7$, 261 jumps: One-sample *t*-test, $p = 0.0567$). In each plot symbols represent different toads and symbols are conserved across panels for those individuals. Linear regression lines are plotted to help convey relationship for each toad, and Pearson's Correlation Coefficient, *r*, for each individual is listed next to each plot.

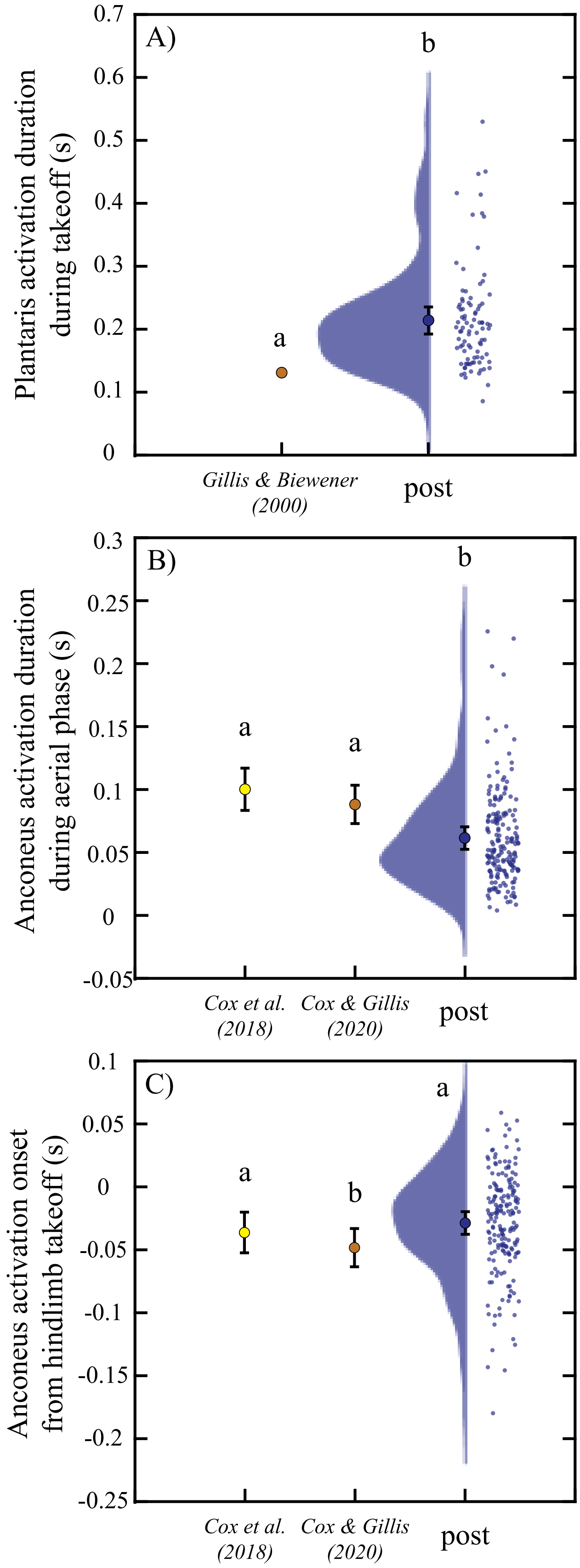

Figure 3.8. Muscle activity six months following nerve transection (post). A) Duration of plantaris ($N = 6$; 91 jumps) activity prior to takeoff, B) the duration of anconeus ($N = 6$; 187 jumps) activation during the aerial phase, and C) the onset time of the anconeus ($N = 6$; 187 jumps) following hindlimb takeoff. Clouds and small points to the right with the same color represent the distribution and all jumps recorded, respectively. The larger central circle represents the condition mean after accounting for variation within an individual and error bars convey the 95% CI of the mean. Lowercase letters above datasets that differ represent statistically significant differences in the mean (one sample *t*-test, $p < 2.76\text{e-}3$).

Table 3.1. Summary of findings comparing intact toads (Pre, *N* = 10, 200 hops) with animals after sham surgery (Sham, *N* = 4, 80 hops) and 6 months following nerve reinnervation surgery (Post, *N* = 7, 264 hops). From left-to-right, columns list the variable of interest, the treatment condition, the mean, 95% confidence interval of the mean, the standard deviation for that condition, and statistics including *F*-statistic and *p*-value from LME for the independent variable. There were 2 *dof* for Condition and 541 *dof* for the error.

Variable	*Condition*	*Mean*	*95% CI*	*SD*	*Statistics*
Mean Rate Elbow Extension (deg/s)	**Pre**	326.0	±56.7	91.6	*F* = 19.6
	Sham	271.2	±51.0	52.0	*p* = 5.86e-9
	Post	248.0	±21.7	29.3	
Elbow Angle at Touchdown (deg)	**Pre**	62.7	±9.7	15.6	*F* = 33.0
	Sham	70.7	±4.4	4.5	*p* = 3.07e-14
	Post	68.3	±10.7	14.5	
Time Elbow Extends After Takeoff (s)	**Pre**	0.0705	±0.0117	0.0189	*F* = 11.5
	Sham	0.0836	±0.0121	0.0123	*p* = 1.31e-5
	Post	0.0802	±0.0226	0.0305	
Time Elbow Extends Prior to Touchdown (s)	**Pre**	-0.0466	±0.0109	0.0175	*F* = 4.26
	Sham	-0.0688	±0.0283	0.0288	*p* = 1.46e-2
	Post	-0.0599	±0.0136	0.0184	
Mean Rate Hindlimb Extension During Takeoff (length/s)	**Pre**	0.085	±0.118	0.190	*F* = 1.70
	Sham	0.199	±0.115	0.117	*p* = 0.183
	Post	0.097	±0.107	0.145	
Max Rate Hindlimb Extension During Takeoff (length/s)	**Pre**	1.715	±0.362	0.584	*F* = 20.5
	Sham	1.627	±0.246	0.251	*p* = 2.75e-9
	Post	2.238	±0.215	0.291	
Rate Hindlimb Extension at Takeoff (length/s)	**Pre**	-0.381	±0.462	0.746	*F* = 13.2
	Sham	0.306	±0.682	0.696	*p* = 2.53e-6
	Post	0.590	±0.359	0.485	
Time Hindlimb Begins Flexing After Takeoff (s)	**Pre**	0.2433	±0.0264	0.0426	*F* = 35.6
	Sham	0.2794	±0.0324	0.0331	*p* = 2.94e-15
	Post	0.2775	±0.0291	0.0393	
Average Belt Speed (m/s)	**Pre**	0.1152	±0.0092	0.0149	*F* = 45.7
	Sham	0.1134	±0.0156	0.0159	*p* = 4.54e-19
	Post	0.0840	±0.0112	0.0151	
Takeoff Speed (m/s)	**Pre**	0.6223	±0.0788	0.1272	*F* = 77.29
	Sham	0.6519	±0.1231	0.1256	*p* = 2.99e-30
	Post	0.7547	±0.0719	0.0970	
Jump Distance (m)	**Pre**	0.1050	±0.0131	0.0211	*F* = 32.6
	Sham	0.1355	±0.0199	0.0203	*p* = 4.47e-14
	Post	0.1101	±0.0171	0.0231	

CONCLUSION

In Chapter 1 we found that cane toads do not alter their forelimb kinematics, impact forces or the energy dissipated by the forelimbs when landing on rigid or compliant surfaces that depress up to 20-percent of the forelimb length for every unit body weight. Furthermore, in Chapter 2 we observed that none of the four forelimb muscles we examined altered their activity intensity as a result of the landing surface compliance. Taken together these results from Chapters 1 and 2 suggest cane toads are not relying on sensory feedback from the forelimbs following touchdown to effectively coordinate landing behavior.

A limitation of both Chapter 1 and 2 was that the takeoff platform was always held rigid while only the landing platform's stiffness was altered. Our findings in the context of this limitation provides three potential explanations for why we may not have observed changes in the landing behavior in the first two chapters; i) a rigid landing is likely the most demanding and therefore if the landing condition is unknown it would be safer for the toad to prepare for a rigid landing to reduce the risk of crashing, ii) toads experienced a rigid surface during takeoff and therefore expect a rigid landing, or iii) toads employ a singular landing strategy that produces robust landing behavior across the surface compliances we tested and therefore the toads have no need to alter their behavior. In Chapter 3 we attempted to test the second possibility that toads rely on proprioceptive feedback from their hindlimbs to inform their forelimbs for landing.

Our results from Chapter 3 illustrated a significant reduction in the rate of elbow extension prior to landing 6 months following nerve reinnervation surgery. However, the overall limb extension at touchdown recovered by 6 months allowing the toads to land effectively even with reduced elbow extension. This strongly suggests that sensory afferents from muscle spindles in the hindlimb are important, but not critical, for mediating forelimb landing behavior in cane toads. Clearly the plasticity of the neuromuscular system is sufficient to allow limb-level behavior to recover even while individual joints may remain affected following injury.

Ultimately this research conveys the importance of proprioception when locomoting effectively and how a model organism deals with a variety of surface conditions. Most terrestrial animals must successfully navigate diverse surfaces in their environment while avoiding injury. Although it appears that even following injury we may have the capacity to develop novel locomotor strategies that allow us to effectively accomplish a task after losing key sensory perceptions.

REFERENCES

Abelew, T. A., Miller, M. D., Cope, T. C., & Nichols, T. R. (2000). Local loss of proprioception results in disruption of interjoint coordination during locomotion in the cat. *Journal of neurophysiology*, *84*(5), 2709-2714.

Akella, T. and Gillis, G. B. (2011). Hopping isn't always about the legs: forelimb muscle activity patterns during toad locomotion. *J. Exp. Zool. 315*(1), 1-11.

Alvarez, F. J., Titus-Mitchell, H. E., Bullinger, K. L., Kraszpulski, M., Nardelli, P., & Cope, T. C. (2011). Permanent central synaptic disconnection of proprioceptors after nerve injury and regeneration. I. Loss of VGLUT1/IA synapses on motoneurons. *Journal of neurophysiology*, *106*(5), 2450-2470.

Alford, R. A., Brown, G. P., Schwarzkopf, L., and Phillips, B. L. (2009). Comparisons through time and space suggest rapid evolution of dispersal behaviour in an invasive species. *Wildlife Res. 36*(1), 23-28.

Arampatzis, A., Brüggemann, G., and Klapsing, G. M. (2001). Let stiffness and mechanical energetic processes during jumping on a sprung surface. *Med. Sci. Sports Exerc. 33*(6), 923-931.

Astley, H. C., & Roberts, T. J. (2012). Evidence for a vertebrate catapult: elastic energy storage in the plantaris tendon during frog jumping. *Biology letters*, *8*(3), 386-389.

Astley, H. C., & Roberts, T. J. (2014). The mechanics of elastic loading and recoil in anuran jumping. *Journal of Experimental Biology*, *217*(24), 4372-4378.

Azizi, E., and Abbott, E. M. (2013). Anticipatory motor patterns limit muscle stretch during landing in toads. *Biology Letters*, *9*(1), 20121045.

Azizi, E., Larson, N. P., Abbott, E. M., and Danos, N. (2014). Reduce torques and stick the landing: limb posture during landing in toads. *J. Exp. Biol. 217*(20), 3742-3747.

Bhatt, N. K., Khan, T. R., Mejias, C., & Paniello, R. C. (2017). Nerve transection repair using laser-activated chitosan in a rat model. *The Laryngoscope*, *127*(8), E253-E257.

Bijma, N. N., Gorb, S. N., and Kleinteich, T. (2016) Landing on branches in the frog *Trachycephalus resinifictrix* (Anura: Hylidae). *J. Comp. Physiol. 202*(4), 267-276.

Birn-Jeffery, A. V. and Daley, M. A. (2012). Birds achieve high robustness in uneven terrain through active control of landing conditions. *J. Exp. Biol. 215*(12), 2117-2127.

Boeltz, T., Ireland, M., Mathis, K., Nicolini, J., Poplavski, K., Rose, S. J., ... & English, A. W. (2013). Effects of treadmill training on functional recovery following peripheral nerve injury in rats. *Journal of neurophysiology*, *109*(11), 2645-2657.

Boulton, A. J. (2005). Management of diabetic peripheral neuropathy. *Clinical diabetes*, *23*(1), 9-15.

Brandt, J., Evans, J. T., Mildenhall, T., Mulligan, A., Konieczny, A., Rose, S. J., & English, A. W. (2015). Delaying the onset of treadmill exercise following peripheral nerve injury has different effects on axon regeneration and motoneuron synaptic plasticity. *Journal of neurophysiology*, *113*(7), 2390-2399.

Bullinger, K. L., Nardelli, P., Pinter, M. J., Alvarez, F. J., & Cope, T. C. (2011). Permanent central synaptic disconnection of proprioceptors after nerve injury and regeneration. II. Loss of functional connectivity with motoneurons. *Journal of neurophysiology*, *106*(5), 2471-2485.

Carr, J., Chao, L., & Biewener, A. (2010). The effects of denervation and self-reinnervation in the guinea fowl lateral gastrocnemius. *Lateral*, *26*(24), 22.

Cassiat, G., Pourcelot, P., Tavernier, L., Geiger, D., Denoix, J. M., and Deguerce, D. (2004). Influence of individual competition level on back kinematics of horses jumping a vertical fence. *Equine Vet. J. 36*(8), 748-753.

Chang, Y. H., Auyang, A. G., Scholz, J. P., & Nichols, T. R. (2009). Whole limb kinematics are preferentially conserved over individual joint kinematics after peripheral nerve injury. *Journal of Experimental Biology*, *212*(21), 3511-3521.

Cheung, V. C., d'Avella, A., Tresch, M. C., & Bizzi, E. (2005). Central and sensory contributions to the activation and organization of muscle synergies during natural motor behaviors. *Journal of Neuroscience*, *25*(27), 6419-6434.

Chevallier, S., Landry, M., Nagy, F., & Cabelguen, J. M. (2004). Recovery of bimodal locomotion in the spinal-transected salamander, *Pleurodeles waltlii*. *European Journal of Neuroscience*, *20*(8), 1995-2007.

Cope, T. C., Bonasera, S. J., & Nichols, T. R. (1994). Reinnervated muscles fail to produce stretch reflexes. *Journal of neurophysiology*, *71*(2), 817-820.

Cox, S. M. and Gillis, G. B. (2015). Forelimb kinematics during hopping and landing in toads. . *Exp. Bio. 218*(19), 3051-3058.

Cox, S. M. and Gillis, G. B. (2016). Sensory feedback and coordinating asymmetrical landing in toads. *Biol. Lett. 12*(6), 20160196.

Cox, S. M. and Gillis, G. B. (2017). Evidence toads may modulate landing preparation without predicting impact time. *Biol. Open 6*(1), 71-76.

Cox, S. M. and Gillis, G. B. (2020). The integration of sensory feedback in the modulation of anuran landing preparation. *J. Exp. Biol. 223*(3).

Cox, S. M., Ekstrom, L. J., and Gillis, G. B. (2018). The influence of visual, vestibular, and hindlimb proprioceptive ablations on landing preparation in cane toads. *Integr. Comp. Biol. 58*(5), 894-905.

Dakin, R., Segre, P. S., & Altshuler, D. L. (2020). Individual variation and the biomechanics of maneuvering flight in hummingbirds. *Journal of Experimental Biology*, *223*(20), jeb161828.

Daley, M. A. and Biewener, A. A. (2006). Running over rough terrain reveals limb control for intrinsic stability. *PNAS 103*, 15681-15686.

Daley, M. A., Voloshina, A., and Biewener, A. A. (2009). The role of intrinsic muscle mechanics in the neuromuscular control of stable running in the guinea fowl. *J. Physiol. 587*(11), 2693-2707.

Demes, B., Jungers, W. L., Gross, T. S., and Fleagle, J. G. (1995). Kinetics of leaping primates: influence of substrate orientation and compliance. *Am. J. Phys. Anthropol. 96*(4), 419-429.

Devita, P. and Skelly, W. A. (1992). Effect of landing stiffness on joint kinetics and energetics in the lower extremity. *Med. Sci. Sports Exerc. 24*(1), 108-115.

Donelan, J. M., & Pearson, K. G. (2004). Contribution of force feedback to ankle extensor activity in decerebrate walking cats. *Journal of neurophysiology*, *92*(4), 2093-2104.

Duellman, W. E. and Trueb, L. (1994). Biology of amphibians. *JHU press*.

Ekstrom, L. J. and Gillis, G. B. (2015). Pre-landing wrist muscle activity in hopping toads. *J. Exp. Biol. 218*(15), 2410-2415.

Ekstrom, L. J., Panzini, C., & Gillis, G. B. (2018). Vision fine-tunes preparation for landing in the cane toad, Rhinella marina. *Biol. Lett. 14*(9), 20180397.

Essner, R. L., Suffian, D. J., Bishop, P. J., and Reilly, S. M. (2010). Landing in basal frogs: evidence of saltational patterns in the evolution of anuran locomotion. *Naturwissenschaften 97*(10), 935-939.

Ferris, D. P. and Farley, C. T. (1997). Interaction of leg stiffness and surface stiffness during human hopping. *J. Appl. Physiol. 82*(1), 15-22.

Ferris, D. P. and Farley, C. T. (1998). Running in the real world: adjusting leg stiffness for different surfaces. *Proc. Royal Soc. B, 265*(1400), 989-994.

Ferris, D.P., Liang, K., & Farley, C. T. (1999). Runners adjust leg stiffness for their first step on a new running surface. *J. Biomech. 32*(8), 787-794.

Gillis, G. B., & Biewener, A. A. (2000). Hindlimb extensor muscle function during jumping and swimming in the toad (Bufo marinus). *Journal of Experimental Biology*, *203*(23), 3547-3563.

Gillis, G. B., Akella, T., and Gunaratne, R. (2010). Do toads have a jump on how far they hop? Pre-landing activity timing and intensity in forelimb muscles of hopping *Bufo marinus*. *Biol. Lett. 6*, 486-489.

Gillis, G., Ekstrom, L., and Azizi, E. (2014). Biomechanics and control of landing in toads. *Integr. Comp. Biol. 54*(6), 1136-1147.

Gordon, T., and Stein, R. B. (1982). Time course and extent of recovery in reinnervated motor units of cat triceps surae muscles. *The Journal of Physiology*, *323*(1), 307-323.

Gordon, J. C., Holt, N. C., Biewener, A., & Daley, M. A. (2020). Tuning of feedforward control enables stable muscle force-length dynamics after loss of autogenic proprioceptive feedback. *Elife*, *9*, e53908.

Gray, E. G. (1957). The spindle and extrafusal innervation of a frog muscle. *Proceedings of the Royal Society of London. Series B-Biological Sciences, 146*(924), 416-430.

Haspel, G., Severi, K. E., Fauci, L. J., Cohen, N., Tytell, E. D., & Morgan, J. R. (2021). Resilience of neural networks for locomotion. *The Journal of Physiology*, *599*(16), 3825-3840.

Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspiration Biomim. 3*(3), 034001.

Howard, C. S., Blakeney, D. C., Medige, J., Moy, O. J., & Peimer, C. A. (2000). Functional assessment in the rat by ground reaction forces. *Journal of biomechanics*, *33*(6), 751-757.

Jahn, S. A. A. (1968). Response of isolated muscle spindles to single transient stretches. *Acta Physiologica Scandinavica*, *72*(4), 441-455.

Kerdok, A. E., Biewener, A. A., McMahon, T. A., Weyand, P. G., & Herr, H. M. (2002). Energetics and mechanics of human running on surfaces of different stiffnesses. *J. Appl. Physiol.*

Konow, N., & Roberts, T. J. (2015). The series elastic shock absorber: tendon elasticity modulates energy dissipation by muscle during burst deceleration. *Proc. Royal Soc. B. 282*(1804), 20142800.

Livingston, B. P., & Nichols, T. R. (2014a). Effects of Reinnervation of the Biarticular Shoulder-Elbow Muscles on Joint Kinematics and Electromyographic Patterns of the Feline Forelimb during Downslope Walking. *Cells Tissues Organs*, *199*(5-6), 423-440.

Livingston, B. P., & Nichols, T. R. (2014b). Effects of reinnervation of the triceps brachii on joint kinematics and electromyographic patterns of the feline forelimb during level and upslope walking. *Cells Tissues Organs*, *199*(5-6), 405-422.

Llewelyn, J., Phillips, B. L., Alford, R. A., Schwarzkopf, L., and Shine, R. (2010). Locomotor performance in an invasive species: cane toads from the invasion front have greater endurance, but not speed, compared to conspecifics from a long-colonised area. *Oecologia*, *162*(2), 343-348.

Lyle, M. A., Prilutsky, B. I., Gregor, R. J., Abelew, T. A., & Nichols, T. R. (2016). Self-reinnervated muscles lose autogenic length feedback, but intermuscular feedback can recover functional connectivity. *Journal of Neurophysiology*, *116*(3), 1055-1067.

Maas, H., Prilutsky, B. I., Nichols, T. R., & Gregor, R. J. (2007). The effects of self-reinnervation of cat medial and lateral gastrocnemius muscles on hindlimb kinematics in slope walking. *Experimental brain research*, *181*(2), 377-393.

Menz, H. B., Lord, S. R., St George, R., & Fitzpatrick, R. C. (2004). Walking stability and sensorimotor function in older people with diabetic peripheral neuropathy. *Archives of physical medicine and rehabilitation*, *85*(2), 245-252.

McKinley, P. and Pedotti, A. (1992). Motor strategies in landing from a jump: the role of skill in task execution. *Exp. Brain. Res. 90*(2), 427-440.

McNitt-Gray, J. L., Yokoi, T., & Millward, C. (1994). Landing strategies used by gymnasts on different surfaces. *Journal of applied biomechanics*, *10*(3), 237-252.

Moritz, C. T., & Farley, C. T. (2003). Human hopping on damped surfaces: strategies for adjusting leg mechanics. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1525), 1741-1746.

Moritz, C. T., & Farley, C. T. (2004). Passive dynamics change leg mechanics for an unexpected surface during human hopping. *Journal of Applied Physiology*, *97*(4), 1313-1322.

Moritz, C. T., Greene, S. M., & Farley, C. T. (2004). Neuromuscular changes for hopping on a range of damped surfaces. *Journal of Applied Physiology*, *96*(5), 1996-2004.

Nauwelaerts, S. and Aerts, P. (2006). Take-off and landing forces in jumping frogs. *J. Exp. Biol. 209*(1), 66-77.

Nichols, T. R. (2018) Distributed force feedback in the spinal cord and the regulation of limb mechanics. *Journal of neurophysiology*, *119*(3), 1186-1200.

Nunes e Silva, D., Antoniolli da Silva, A. C. M. B., Aydos, R. D., Viterbo, F., Pontes, E. R. J. C., Odashiro, D. N., José de Castro, R., & Augusto, D. G. (2012). Nerve growth factor with fibrin glue in end-to-side nerve repair in rats. *Acta Cirurgica Brasileira*, *27*, 325-332.

Parker, D. (2017). The lesioned spinal cord is a "new" spinal cord: evidence from functional changes after spinal injury in lamprey. *Frontiers in Neural Circuits*, *11*, 84.

Pearson, K. G., Misiaszek, J. E., & Hulliger, M. (2003). Chemical ablation of sensory afferents in the walking system of the cat abolishes the capacity for functional recovery after peripheral nerve lesions. *Experimental brain research*, *150*(1), 50-60.

Proske, U., & Stuart, G. J. (1985). The initial burst of impulses in responses of toad muscle spindles during stretch. *The Journal of physiology*, *368*(1), 1-17.

Reilly, S. M., Montuelle, S. J., Schmidt, A., Krause, C., Naylor, E., and Essner Jr, R. L. (2016). Functional evolution of jumping in frogs: Interspecific differences in take-off and landing. *J. Morphol. 277*(3), 379-393.

Reynaga, C.M., Eaton, C.E., Strong, G.A., and Azizi E. (2019). Compliant substrates disrupt elastic energy storage in jumping tree frogs. *Integr. Comp. Biol. 59*(6), 1535-1545.

Santello, M. (2005). Review of motor control mechanisms underlying impact absorption from falls. *Gait Posture*, *21*(1), 85-94.

Santello, M., McDonagh, M. J., & Challis, J. H. (2001). Visual and non-visual control of landing movements in humans. *The Journal of physiology*, *537*(1), 313-327.

Schnyer, A., Gallardo, M., Cox, S., and Gillis, G. (2014). Indirect evidence for elastic energy playing a role in limb recovery during toad hopping. *Biol. Lett. 10*(7), 20140418.

Sharp, A. A., & Bekoff, A. (2015). Pyridoxine treatment alters embryonic motility in chicks: implications for the role of proprioception. *Developmental psychobiology*, *57*(2), 271-277.

Söhnel, K., Rode, C., de Lussanet, M. H., Wagner, H., Fischer, M. S., & Andrada, E. (2020). Limb dynamics in agility jumps of beginner and advanced dogs. *Journal of Experimental Biology*, *223*(7), jeb202119.

von Tscharner, V. (2000). Intensity analysis in time-frequency space of surface myoelectric signals by wavelets of specified resolution. *J. Electromyogr. Kines. 10*(6), 433-445.

Vannucci, B., Santosa, K. B., Keane, A. M., Jablonka-Shariff, A., Lu, C. Y., Yan, Y., MacEwan, M., & Snyder-Warwick, A. K. (2019). What is Normal? Neuromuscular junction reinnervation after nerve injury. *Muscle & nerve*, *60*(5), 604-612.

Vela, F. J., Martínez-Chacón, G., Ballestín, A., Campos, J. L., Sánchez-Margallo, F. M., & Abellán, E. (2020). Animal models used to study direct peripheral nerve repair: a systematic review. *Neural Regeneration Research*, *15*(3), 491.

Wang, Z., Ji, A., Endlein, T., Samuel, D., Yao, N., Wang, Z., and Dai, Z. (2014). The role of fore-and hindlimbs during jumping in the Dybowski's frog (*Rana dybowskii*). *J. Exp. Zool. 321A*, 324-333.

Yakovenko, S., Gritsenko, V., & Prochazka, A. (2004). Contribution of stretch reflexes to locomotor control: a modeling study. *Biological cybernetics*, *90*(2), 146-155.