RESEARCH ARTICLE

The integration of sensory feedback in the modulation of anuran landing preparation

Suzanne M. Cox[1],*,§ and Gary B. Gillis[2]

ABSTRACT

Controlled landing requires preparation. Mammals and bipedal birds vary how they prepare for landing by predicting the timing and magnitude of impact from the integration of visual and non-visual information. Here, we explore how the cane toad *Rhinella marina* – an animal that moves primarily through hopping – integrates sensory information to modulate landing preparation. Earlier work suggests that toads may modulate landing preparation using predictions of impact timing and/or magnitude based on non-visual sensory feedback during takeoff rather than visual cues about the landing itself. We disentangled takeoff and landing conditions by hopping toads off platforms of different heights while measuring electromyographic (EMG) activity of an elbow extensor (m. anconeus) and capturing high-speed images to quantify whole body and forelimb kinematics. This enabled us to test how toads integrate visual and non-visual information in landing preparation. We asked two questions: (1) when they conflict, do toads correlate landing preparation with takeoff or landing conditions? And (2) for hops with the same takeoff conditions, does visual information alter the timing of landing preparation? We found that takeoff conditions are a better predictor of the onset of landing preparation than landing conditions, but that visual information is not ignored. When hopping off higher platforms, toads start to prepare for landing later when takeoff conditions are invariant. This suggests that, unlike mammals, toads prioritize non-visual sensory feedback about takeoff conditions to coordinate landing, but that they do integrate visual information to fine-tune landing preparation.

KEY WORDS: Cane toad, Landing, Control, Kinematics, Non-visual sensory feedback

INTRODUCTION

A controlled landing requires preparation. Mammals (cats, monkeys, humans; Lacour et al., 1978; Prochazka and Schofield, 1977; Santello and McDonagh, 1998), bipedal birds (Konow et al., 2011) and anurans (frogs and toads; Ekstrom and Gillis, 2015; Gillis et al., 2010, 2014) prepare for impact forces by stiffening joints in the landing limbs through activation of the underlying musculature before ground contact. The timing and intensity of this pre-landing electromyographic (EMG) activity across a range of conditions can shed light on the control strategy a species uses to prepare for landing. For instance, in mammals, limb EMG activity begins at a fixed duration before touchdown but activation intensity increases with drop height when vision is available and accurate (Santello et al., 2001). This suggests that mammals predict the timing and intensity of impact to tune landing preparation. However, when visual information is unavailable and drop height is inconsistent, EMG onsets are correlated to the time of takeoff, not the time of landing (Liebermann and Goodman, 2007; Liebermann and Hoffman, 2005; Thompson and McKinley, 1995). Nevertheless, after repeated drops from a consistent height in these conditions, mammals return to a landing preparation strategy correlated to the timing and intensity of landing (Liebermann and Hoffman, 2005; Magalhães and Goroso, 2009; Santello et al., 2001). This suggests that mammals primarily rely on visual feedback to predict landing from drops or jumps, although proprioceptive or vestibular information from past events can also suffice when accurate (Santello et al., 2001). Further, it also implies that when predictions of impact are not possible, mammals are able to shift their landing control strategy from correlating with impact conditions to correlating with takeoff conditions (Santello and McDonagh, 1998).

Landing preparation has been well studied in mammals, and a growing number of recent studies of anuran locomotion have also begun to focus on landing (Akella and Gillis, 2011; Azizi and Abbott, 2013; Azizi and Roberts, 2014; Cox and Gillis, 2016, 2017; Ekstrom and Gillis, 2015; Essner et al., 2010; Gillis et al., 2014; Reilly and Jorgensen, 2011; Reilly et al., 2015, 2016; Roberts et al., 2011). However, studies on landing preparation in the most well studied anuran, the cane toad *Rhinella marina*, suggest they may rely on a control strategy that does not mirror what is found in mammals. On the one hand, EMG analyses of elbow (Akella and Gillis, 2011; Gillis et al., 2010, 2014) and wrist (Ekstrom and Gillis, 2015) antagonists demonstrate that, as in mammals, pre-landing EMG activity is tuned to hop distance, starting later and showing greater intensity in longer hops. But unlike mammals, toads do not begin landing preparation a fixed duration before touchdown, which is the key piece of evidence that suggests that mammals use predictions of landing to tune landing preparation. Beginning preparations a fixed duration before a future event implies prediction of that event. Instead, toads begin landing preparation neither a fixed duration after takeoff nor before landing, but consistently vary the timing of onset with hop duration. While this suggests that toads, like mammals, may use predictions of impact time and magnitude to modulate landing preparation, it leaves open many other possibilities.

Further evidence suggests that, if toads do predict impact, they do not primarily rely on vision to do so. For instance, toads adjust the timing of landing preparation between contralateral forelimbs when their body rolls from level after takeoff, but not when the landing surface is tilted at an angle (Cox and Gillis, 2016), suggesting that toads prioritize vestibular over visual information during landing preparation. Further, ablating vestibular or proprioceptive feedback in jumping toads drastically alters or entirely eliminates landing

[1]Organismic and Evolutionary Biology Graduate Program, University of Massachusetts, Amherst, MA 01003, USA. [2]Department of Biology, Mount Holyoke College, Hadley, MA 01075, USA.
*Present address: 29b Rec Hall, Pennsylvania State University, University Park, PA 16802, USA.

§Author for correspondence (smc288@psu.edu)

S.M.C., 0000-0002-9704-0716; G.B.G., 0000-0002-8553-7821

preparation (Cox et al., 2018), whereas blinded toads were able to successfully coordinate landing, albeit with greater timing variability (Cox et al., 2018; Ekstrom et al., 2018). Thus, one possibility is that toads may successfully land hop after hop without taking into account visual cues about landing surface orientation or height. In short, the little evidence available suggests that cane toads may accomplish their great feats of migration (Phillips et al., 2007) by relying on a landing control strategy that assumes the world is, to the best approximation, flat. In other words, like mammals in conditions where they cannot predict landing, toads may tune landing to takeoff conditions. Given the dependence on visual cues for landing preparation in all other animals tested thus far (Kamibayashi and Muro, 2006; Lacour et al., 1978; Lee et al., 1993; Liebermann and Goodman, 1991; Santello, 2005; Santello and McDonagh, 1998; Santello et al., 2001), this suggests that toads may have evolved a control strategy to coordinate landing that is distinct from mammals and birds.

Given the potential novelty of this strategy, we chose to specifically test the hypothesis that toads modulate landing based on takeoff rather than landing conditions. On flat ground, the results of either approach may be indistinguishable, but when hopping from one level to another, non-visual information about takeoff conflicts with visual information about landing. To test whether toads integrate visual information to tune landing preparation, we hopped toads off platforms of three different heights while measuring the intensity and timing of landing preparation. With this setup we asked two questions: (1) do takeoff or landing conditions best predict when toads begin to prepare for impact? And (2) does visual information alter the timing of landing preparation? In other words, do toads coordinate landing as if the world were flat, and impact will occur at the same height they took off from (i.e. takeoff conditions are used to predict landing preparation), or do they take into account different landing heights using vision and prepare accordingly (i.e. landing conditions are important)? The hypothesis that toads use a motor control strategy that tunes landing preparation to takeoff conditions will be falsified if either landing conditions are a better predictor of landing preparation than takeoff or if toads change the timing or intensity of landing preparation when only visual information could predict a later or larger impact.

MATERIALS AND METHODS
Animals
Six female cane toads, *Rhinella marina* (Linnaeus 1758) ranging in mass from 63 to 170 g were used for kinematic analysis and electromyography (EMG) recordings. All animals were housed in groups of three to four in large aquaria on a 12 h:12 h light:dark cycle and fed crickets several times weekly. All experimental work was approved by Mount Holyoke College's IACUC.

Electromyography and kinematic data collection
EMG data were collected from the m. anconeus, an elbow extensor known to show distance-dependent pre-landing onset timing (Gillis et al., 2010). To implant electrodes, toads were anesthetized in a solution of MS-222 (1.5 g l^{-1}). Once anesthetized, 1–2 cm skin incisions were made along each humerus to expose the muscle. Bilateral implants were used to increase the likelihood of successful data collection. Bipolar electrodes were made and implanted as described in detail in previous work (Gillis et al., 2010). EMG signals were amplified 1000× with Grass P511 preamplifiers using a notch filter at 60 Hz. Signals were digitized at 5000 Hz using a NIDAQ 16-bit A/D converter and a custom LABVIEW program and saved onto a personal computer.

Toads alter both the timing of pre-landing EMG activity and the beginning of elbow extension with hop distance (Cox et al., 2018; Gillis et al., 2014). For completeness, we collected both EMG data and forelimb kinematics. For kinematic data collection, small squares of white cardboard (∼3×3 mm) were glued to the skin bilaterally at the wrist and elbow joints as well as at mid-point of the humerus (markers at the shoulder joint are readily obscured). In addition, four markers were also used to form a T along the back of the animal as described in detail in previous work (Cox and Gillis, 2015). Once recovered from anesthesia, animals were placed in a well-lit enclosure (64×107 cm) and, in a random order, hopped off three different height platforms (0 cm, 41 hops; 6 cm, 36 hops; 10 cm, 31 hops) lined with felt to ensure purchase. Between 3 and 12 hops per condition per toad were included in the analysis (6.8± 3.8 hops per treatment per toad; mean±s.d.) for a total of 102 hops. Videos of hops were recorded with two Fastec HiSpec1 high-speed video cameras at 500 fps. For three-dimensional spatial calibration, a 64-point calibration cube (21×21 cm) was digitized in MATLAB using DLTdv5 (Hedrick, 2008). Videos were synchronized with EMG signals using a 5 V trigger pulse that stopped video and EMG recording and was included on its own channel with EMG data. After hopping trials, toads were euthanized by overnight submersion in MS-222 (1.5 g l^{-1}). Post-mortem dissections were used to confirm electrode placements.

Data analysis
Landing preparation in toads is characterized by both distance-dependent amounts of elbow extension and activation of the underlying forelimb musculature at touchdown (Cox and Gillis, 2015, 2017; Cox et al., 2018; Ekstrom et al., 2018; Gillis et al., 2010). But landing preparation at touchdown is not sufficient to determine whether the motor control strategy is altered. Modulation of pre-landing EMG activity and elbow configuration at touchdown can be achieved without varying the timing or intensity of muscle activation. A toad could start elbow extension and muscle activation at the same time relative to liftoff and maintain the same rate of elbow extension and increase of activation intensity throughout the hop. With this strategy, long hops provide more time to generate greater levels of activation or elbow extension without modulating the motor control strategy. Thus, to discriminate changes in motor control, we chose to primarily focus our analysis on how toads changed the timing of landing preparation (the onset and duration of pre-landing EMG activity or elbow extension) since the timing of pre-landing EMG activity for the muscle instrumented varied more consistently with hop duration than amplitude (Gillis et al., 2010). For completeness, we also evaluated metrics of the intensity of both pre-landing EMG activity and the rate of elbow extension 60 ms after liftoff. To quantify how variations in motor control influence landing preparation at touchdown, we also measured EMG intensity over a 60 ms window before landing as well as the elbow's configuration (degree of extension) at touchdown.

Video analysis
Videos were analyzed to identify the timing of the onset of movement, takeoff and landing for each limb (Fig. 1A,B). The onset of movement was defined as the moment the toad's vertical velocity first topped 5 cm s^{-1}. Three-dimensional coordinates of the forelimbs were calculated with MATLAB software (Hedrick, 2008). Data were smoothed with a quintic spline interpolation, and elbow angle, the timing of extension onset (EE$_{onset}$) and extension velocity (EE$_{v}$), as well as the trajectory and velocity of the animal during the hop, were calculated as described elsewhere (Cox and Gillis, 2015) (Fig. 1C).
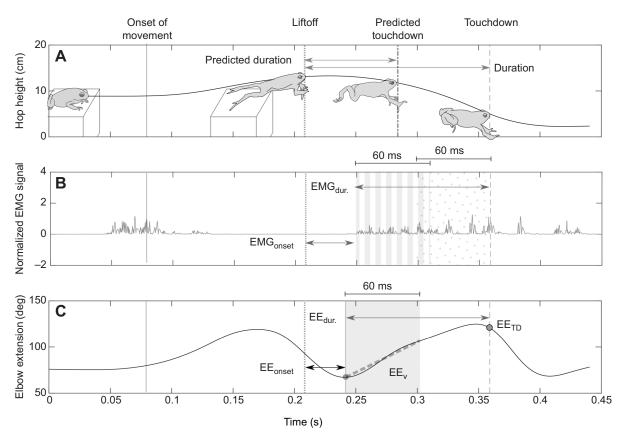
Fig. 1. Visual representation of variables used to analyze landing preparation in the cane toad *Rhinella marina*. (A) Hop height versus time for a typical hop off a platform with predicted and actual touchdown times identified. (B) Corresponding m. anconeus rectified and normalized EMG signal with onset (EMG$_{onset}$) and duration (EMG$_{dur.}$) identified. The regions used to evaluate EMG intensity after onset (striped area) and before touchdown (dotted region) are highlighted. (C) Elbow extension throughout the hop with elbow extension onset (EE$_{onset}$), duration (EE$_{dur.}$) and value at touchdown (EE$_{TD}$) indicated. The onset of elbow extension in preparation for landing is marked by a solid grey circle. Elbow extension velocity during 60 ms after onset (EE$_v$) is indicated by dashed grey line.

Predictions of touchdown times

The time touchdown would have occurred if animals were hopping on flat ground was calculated with equations of motion from the vertical velocity of the center of mass (Vz_{LO}) and height of the marker on the tip of the animal's snout at takeoff (h_{LO}). Time of touchdown (t_{TD}) was the sum of the time of liftoff (t_{LO}), the time to rise to peak height (t_R) and the time to fall back to starting height (t_F):

$$t_{TD} = t_{LO} + t_R + t_F. \qquad (1)$$

Starting height was the height of the tip of snout before hop initiation. The time to peak height (t_R) was found from the vertical component of the velocity of the center of mass at liftoff (Vz_{LO}) and acceleration due to gravity. Given that the vertical velocity at peak height is zero:

$$t_R = Vz_{LO}/g. \qquad (2)$$

The time to fall back to start height (t_F) was calculated from the total height to fall from peak height (h_P) back to starting height (h_S). Given that height rise is ($\frac{1}{2}$)at^2, the peak height was the sum of the takeoff height (h_{LO}):

$$h_P = h_{LO} + \frac{g t_R^2}{2}. \qquad (3)$$

The time to fall, then, is:

$$t_F = \sqrt{\frac{2h_P}{g}}. \qquad (4)$$

While several approaches to calculating time of flight were tested, including limb angle at touchdown and equations to estimate anuran jumps (Marsh, 1994), this approach best fit our data for flat hops (Fig. 2B). We evaluated how well our predictions lined up with impact time by comparing predicted to actual impact times for flat hops (flat hops from Group 1) by fitting two mixed models, a full model with predicted touchdown time as the response variable and actual touchdown time as the fixed effect and a null model with no fixed effect. In both models, individuals were a random factor. The R^2 value for the full model was calculated from the relative likelihood of each model determined by their AIC values (Nakagawa).

Electromyography and kinematics

EMG activity was analyzed using customized MATLAB scripts in which the onset timing of pre-landing muscle activity (EMG$_{onset}$) was identified visually for each hop (Fig. 1B) and the rectified, integrated area, or intensity, of the EMG signals for the first 60 ms after onset (EMG$_I$, Fig. 1B) and the last 60 ms before landing (EMG$_{TD}$) was calculated. To control for differences in electrode construction and placement between individuals, each muscle's EMG intensities were normalized to the largest intensity value
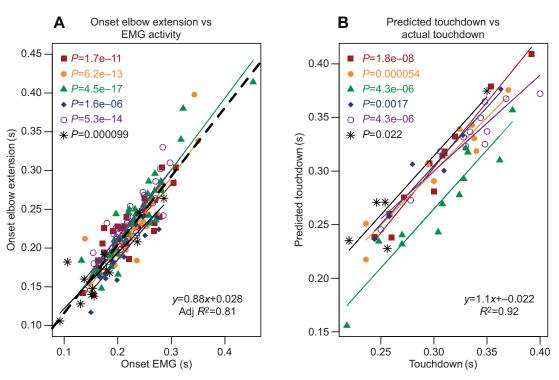
Fig. 2. Correlation between pre-landing EMG activity and elbow extension, and predicted and actual touchdown for toads hopping on level ground.
(A) Onset timing of pre-landing EMG activity and the beginning of elbow extension. (B) Timing of touchdown as predicted by takeoff conditions for toads hopping on level ground and actual touchdown times. Data are color-coded by animal. For both plots, regression lines for each individual are included when fit is significant. The equation of the regression line and R^2 values are for data for all individuals combined. In A, the dashed black line is a regression line for data from all animals.

observed for each animal. The onset of elbow extension was determined using methods described elsewhere (Cox and Gillis, 2015) and elbow extension was calculated at touchdown (EE_{TD}). The rate of elbow extension (EE_v) was the average rate of change of elbow extension during the 60 ms after the onset of elbow extension. Data were filtered to only include signals from the limb that touched down first.

Statistics
Onset comparison
Since previous work demonstrated that sensory ablations influenced the timing of pre-landing EMG activity and the onset of elbow extension differently (Cox et al., 2018), we further tested whether these two metrics of the beginning of landing preparation coincided

when all sensory information was available but conflicting. The correlation between the onset of pre-landing EMG activity and elbow extension was evaluated across all hops (173 total hops, Group 1, Table 1) by two methods. First, a linear model with EMG onset as the independent variable and elbow extension as the dependent variable was fitted to data from all hops and the R^2 of the fit calculated (https://www.r-project.org/). Second, we tested whether the onset of pre-landing EMG and elbow extension were similarly influenced by height by fitting two mixed linear models: a null model with no fixed effect and a full model with platform height as a fixed effect (Bates et al., 2015) with the difference between EMG and elbow extension onset as the dependent variable. In all models, individual toads were included as random effects. The P-value for each full model was computed with a likelihood ratio

Table 1. Information on the four groups used in the different analyses

	N	Total hops	Hops per toad	Duration (min, max; ms)	Mean duration (ms)	Predicted duration (ms)
Group 1: Onset vs EMG comparison						
Flat	6	56	9.3±3.2	−94, 142	12±55	
Low	6	59	9.8±4.2	−80, 141	40±53	
High	6	58	9.7±4.1	−112, 160	54±62	
Group 2: Does onset correlate with takeoff or touchdown?						
Platform (low+high)	5	55	14.6±6.3	−74, 160	63±51	25±0.052
Group 3: Does vision alter onset across different takeoff conditions?						
Flat	5	51	10±2.7	−80, 142	13±55	59±53
Platform (low+high)	5	73	15±6.3	−74, 160	63±51	25±52
Group 4: Does vision alter onset across similar takeoff conditions?						
Flat	5	30	6±4.1	−80, 78	−16±43	23±34
Low	5	31	6.2±2.7	−8, 126	52±40	22±42
High	5	30	6±2.5	−40,160	78±49	19±35

The number of hops per toad, the duration and predicted duration are all given as means±s.d.

Table 2. Landing preparation events by treatment for hops with similar predicted durations

	Flat	Low	High	P
Predicted duration (s)	0.023±0.034	0.022±0.042	0.019±0.035	0.92
Duration (s)	**0.24±0.024**	**0.3±0.035**	**0.32±0.025**	**1.7e−16**
EMG_{onset} (s)	**−0.048±0.019**	**−0.025±0.028**	**−0.011±0.032**	**1e−05***
$EMG_{dur.}$ (s)	**0.088±0.019**	**0.12±0.022**	**0.14±0.022**	**1e−14**
EMG Int 60	0.46±0.17	0.54±0.19	0.49±0.16	0.16
EMG Int 60 TD	0.56±0.2	0.49±0.23	0.51±0.19	0.4
EE_{onset} (s)	**−0.042±0.025**	**−0.023±0.031**	**−0.0097±0.034**	**0.00044**
$EE_{dur.}$ (s)	**0.081±0.018**	**0.12±0.022**	**0.14±0.019**	**2.2e−18**
EE_v (deg ms^{-1})	−0.54±0.14	−0.58±0.12	−0.61±0.14	0.14
EE_{TD} (deg)	99±13	116±7.9	121±7.1	6.4e−09*

Toads alter the onset, but not intensity of landing preparation with platform height. Values are given as means of individual means±s.d. P-values marked with an asterisk required non-parametric comparisons between groups. Bold values highlight significant relationships. EMG_{onset}, m. anconeus EMG signal onset; $EMG_{dur.}$, m. anconeus EMG signal duration; EMG Int 60, integrated m. anconeus EMG signal 60 ms after EMG onset; EMG Int 60 TD, integrated m. anconeus EMG signal 60 ms before touchdown; EE_{onset}, elbow extension onset; $EE_{dur.}$, elbow extension duration; EE_v, elbow extension velocity during 60 ms after onset; EE_{TD}, elbow extension at touchdown.

test between the full and reduced model. If pre-landing EMG activity and elbow kinematics were modulated by the same feedback mechanism, we would expect the difference in their onset timings to be consistent across hop durations and not vary with hop height. For each model, the assumption of data linearity and homogeneity of variance were checked before running models. The data met assumptions in all cases.

Takeoff or landing
Hops off platforms were filtered to only include those that had a takeoff angle within the range seen during flat hops (12–48 deg from horizontal, 55 hops, Group 2, Table 1). We used two linear mixed effects models (lme4; Bates et al., 2015) to test whether takeoff conditions (predicted touchdown) or touchdown best predicted the beginning of landing preparation (Broström and Holmberg, 2011). In both models, individual toads were included as random effects, the onset of EMG activity (EMG_{onset}) was the dependent variable and either predicted or actual touchdown from liftoff was the fixed effect. The relative predictive power of each model was evaluated with a likelihood ratio test (Table 3).

Influence of vision
To isolate the influence of visual information, we performed two analyses. In the first, we again filtered hops to include only hops with predicted touchdown within the range found for flat hops, but in this analysis we included hops on flat ground as well as hops off platforms (124 hops, Group 3, Table 1). With this dataset, we evaluated whether

hopping from a platform altered the timing of landing preparation across a range of hop durations. We did this by evaluating whether the relationship (slope and intercept) between predicted touchdown time and EMG onset was different for hops on flat ground and those off platforms. We generated a mixed model with individual toad as the random effect, onset from takeoff as the dependent variable and predicted time of touchdown from takeoff and treatment (flat or platform) as the fixed effects. We also included the interaction between takeoff and treatment. To evaluate whether platform height altered the slope, we performed an ANOVA on the fit model and computed a P-value for the interaction effect between treatment and onset time. To test whether the intercept of the regression changed with platform height, we calculated the P-value for the influence of treatment on the onset timing from the ANOVA F-value and degrees of freedom. If toads modulate landing preparation based on sensory feedback about takeoff conditions, we would expect the relationship between takeoff and the beginning of landing preparation to be unaffected by platform height; neither the slope nor intercept would change between treatment conditions.

In the second analysis, hops were filtered such that the range of predicted touchdown time did not vary between treatments (eliminating some of the shorter hops off high platforms and longer hops on flat ground (Group 4, Table 1). The resulting predicted hop durations covered a narrow range with the mean value invariant across platform heights (negative hop durations occurred when forelimbs touched down before hind limbs left the ground). The influence of platform height on the onset, intensity (or rate) and

Table 3. Mixed model results

A. Onset EMG vs elbow extension (Group 1)	d.f.	Response variable: $EMG_{onset}-ElbowExt_{onset}$	
Platform height	5	AIC=−828	P=0.53
Null	3	AIC=−831	
B. All platform hops (Group 2)	**d.f.**	**Response variable: EMG_{onset} from liftoff**	
Predicted duration	**4**	**AIC=−337**	**Likelihood=8.1e6**
Duration	4	AIC=−306	Likelihood=1
C. All hops (Group 3)	d.f.	Response variable: EMG_{onset} from liftoff	
Predicted duration	**1**	**F=177.3**	**P=2e−16**
Treatment (platform vs flat)	**1**	**F=59.8**	**P=3e−12**
Predicted duration×Treatment	1	F=0.69	P=0.41

Onset of elbow extension (A) is not significantly different from the onset of EMG activity. Duration as predicted from takeoff conditions (B) correlates with the onset of EMG activity better than touchdown time. For hops with similar takeoff conditions (C), platform height (Treatment) did not alter the slope of the relationship between the onset of EMG activity and takeoff; there was no interaction effect. But the intercept of the line did change; when hopping off platforms, toads started to prepare for landing earlier than when hopping on flat ground (Treatment is significant). Bold values highlight significant relationships.
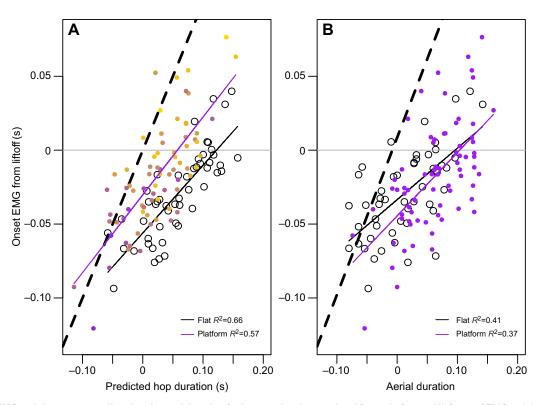
Fig. 3. Onset of EMG activity versus predicted and actual duration for hops on level ground and from platforms. (A) Onset of EMG activity vs predicted hop duration. (B) Onset of EMG activity vs actual hop duration. Open circles indicate hops on level ground; filled circles represent hops from platforms. In A, color of filled circles represents actual duration. Thick dashed line represents touchdown time. Regression lines are shown for each group with corresponding color. Onset of EMG activity begins neither a fixed duration from liftoff (i.e. has a slope of zero) nor a fixed duration before landing (parallel with dashed black line). The relationship between onset and hop duration (slope) was not significantly different in hops on flat ground (black line) and off platforms (purple line). But when hopping off platforms, toads began onset earlier in relation to takeoff conditions (predicted duration); in A, the purple regression line does not have a different slope than the black, but it is shifted up. Onset of EMG activity is better predicted by predicted duration (A) than by actual duration (B) for all treatments.

magnitude at landing of pre-landing EMG activity and elbow extension were each evaluated with ANOVAs of individual means between treatments and significance was set with a Bonferroni correction for multiple tests (0.0045, Table 3). Again, our hypothesis predicts no variation in onset or intensity of landing preparation across differences in platform height.

RESULTS
Onset comparison
Pre-landing EMG activity and elbow extension were highly correlated (Fig. 2). On average, EMG activity preceded elbow extension by 0.70 ± 2.4 ms and this relationship did not vary between platform heights (Table 3A, $P \geq 0.5$). All results are given as means of individual means±s.d.

Predicted versus actual touchdown
Touchdown time for hops on flat ground was within 7.1 ± 15 ms of predicted time for hops that averaged 240 ± 23 ms in duration. A linear regression between the two fit with an adjusted R^2 of 0.91 (slope 1.1, intercept 0.022, Fig. 2B).
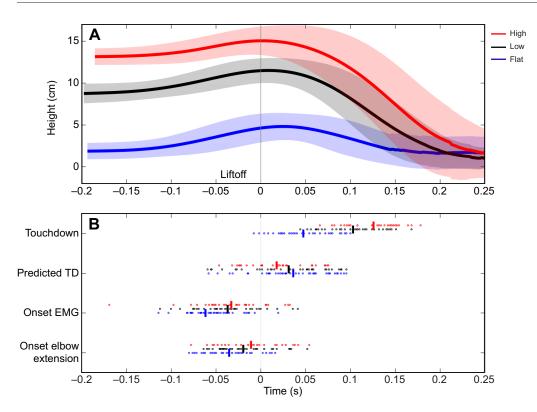
Onset versus hop duration
Given the tight correlation between the onset of EMG activity and elbow extension, we limited this analysis to only evaluate the onset of EMG activity. We found that, in line with our hypothesis, takeoff conditions (predicted hop duration) explained the onset of landing preparation better than landing conditions (actual hop duration, Table 3B, Fig. 3A,B).
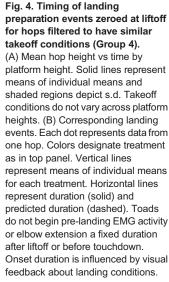
Influence of vision
Across a range of hop durations, the relationship (slope) between onset of pre-landing EMG activity and predicted hop duration did not change when toads hopped off platforms (there was no interaction effect), but contrary to our prediction, toads began landing preparation later when hopping off platforms than they did on flat ground (different intercept: Fig. 3A, Table 3B).

When filtering data to include hops whose takeoff conditions were invariant across platform heights, hop durations increased with hop height (Table 2, Fig. 4); thus, we successfully disentangled takeoff from landing conditions. Under these conditions in which differences in impact time and magnitude could only be inferred from visual feedback, we found that the onset and duration of landing preparation (both pre-landing EMG activity and elbow extension) changed with platform height (Table 2, Table 3C, Fig. 4). The differences in onset timing were much smaller than the differences in duration such that toads began preparation later in longer hops and prepared for impact over a longer duration (Table 2, Fig. 4). This also contradicted our prediction based on the hypothesis that toads only rely on sensory feedback about takeoff conditions to tune landing preparation.

While the timing varied with height, neither the prelanding EMG intensity 60 ms after takeoff (EMG_{60}) or before landing (EMG_{TD}) nor the extension velocity 60 ms after liftoff (EE_v), varied with platform height (Table 2, Fig. 4) Thus, while they started earlier, toads did not change the intensity of pre-landing EMG activity nor the rate of elbow extension. As a result of extending elbows at the same rate for a longer duration, toads landed with elbows more extended in longer hops (Table 2, Fig. 4).

Fig. 4. Timing of landing preparation events zeroed at liftoff for hops filtered to have similar takeoff conditions (Group 4). (A) Mean hop height vs time by platform height. Solid lines represent means of individual means and shaded regions depict s.d. Takeoff conditions do not vary across platform heights. (B) Corresponding landing events. Each dot represents data from one hop. Colors designate treatment as in top panel. Vertical lines represent means of individual means for each treatment. Horizontal lines represent duration (solid) and predicted duration (dashed). Toads do not begin pre-landing EMG activity or elbow extension a fixed duration after liftoff or before touchdown. Onset duration is influenced by visual feedback about landing conditions.

DISCUSSION

In this study we asked two questions in an attempt to illuminate the motor control strategy toads use to coordinate landing: (1) do takeoff or landing conditions best predict landing preparation? And (2) in hops with similar takeoff conditions, does available visual information about differences in landing conditions alter impact preparation? In relation to the first question, we found that takeoff rather than landing best predicted when toads began to prepare for impact. This is in line with our hypothesis that toads solely rely on sensory feedback about takeoff to tune landing coordination. However, contrary to our predictions, we also found that toads alter the timing of landing preparation when only visual information could predict variations in touchdown time.

The incongruity between the results that toads do integrate visual information and that landing preparation is still best correlated to takeoff conditions suggests that the framework underlying our assumptions should be re-evaluated. Specifically, this pair of results is inconsistent if we assume that toads adjust landing preparation based on predictions of impact and that vision is the most informative form of sensory feedback about landing conditions. We would expect that, like humans and other mammals (Santello et al., 2001), if visual information is more accurate, it would be prioritized over vestibular or proprioceptive feedback. If this were the case and toads predict impact to coordinate landing, we would expect that the start of landing preparation would correlate with the actual time of impact rather than less accurate takeoff conditions. But this is not what we found: our results suggest either that toads are very bad at making predictions of hop duration or that they are not making predictions at all.

Our hypothesis was based on two assumptions. First, it assumes that visual feedback provides more accurate information abound landing conditions than other forms of sensory feedback. And studies suggest that toads have a highly developed visual system that is able to successfully deal with a range of spatial problems. For instance, toads discriminate between prey and predator based on

visual cues (Borchers and Ewert, 1979; Ewert, 1974) and display size constancy (Ewert, 1987). They plan routes around obstacles, heading towards gaps in obstructions when available (Collett, 1982; Lock and Collett, 1979), and are able to position themselves to jump through openings. They step up or down to reach surfaces at different heights, and jump accurately to static objects. And, most relevant for this study, toads discriminate chasm width and depth to choose between leaping over narrow chasms, stepping down into shallow ones and turning away from others (Lock and Collett, 1979). Thus, it appears that toads accurately judge the layout of the land before them and have reasonable spatial awareness.

Yet, other details of their optical system may limit how toads use visual information. First, unlike mammals, toads do not seem to integrate visual information during motion. Instead, they plan a route before starting out and do not adjust the direction of an approach once underway, even if the goal (prey or obstacle gap) moves (Lock and Collett, 1979). It appears that toads primarily use visual information while stationary; in fact, they often close their eyes during a hop (our observations) or while walking (Lock and Collett, 1979). Toads may trade off the ability to integrate visual information while moving for enhanced night vision. While stationary, these nocturnal animals can temporally integrate visual signals over several seconds to achieve good low-light sensitivity (Haldin et al., 2009). Since toads do not move their eyes in their sockets (Collett, 1977), they also have less of a need to evolve the cognitive hardware to discriminate self from object motion if they only rely on vision while still. Thus, the visual system of toads limits its effectiveness in predicting impact time from optic flow during a hop.

Further, the visual information about surface height toads receive while preparing for a hop may be less than accurate. Toads do not possess a fovea in the eye (Schaeffel et al., 1994). And while the refractive index of the lens changes vertically across the eye, it does not change enough to bring the much closer ground into focus when the toad is looking ahead (Schaeffel et al., 1994). As Collett (1993) suggested, while their vision is sufficient to distinguish and catch

moving prey it may not be sufficient to accurately judge distances. Thus, while toads may be able to categorize a chasm as 'walkable', 'jumpable' or 'not jumpable', they may not have the visual acuity to make the spatial distinctions necessary to predict hop duration. Prediction of impact time would require integrating information about both the drop and the takeoff conditions, but further comparisons between the accuracy of hops jumping down onto stationary objects and those horizontal or slightly raised may shed light on this question. Thus, while visual feedback may provide humans the most accurate information about impact, the same may not be true for anurans. Our results may not be inconsistent if toads rely on generally more accurate vestibular or proprioceptive information to predict impact.

Yet, it is also worth questioning the assumption that, like mammals during drop-landings, toads make predictions of impact to modulate landing preparation at all. A comparison between the experimental conditions under which most mammalian studies of landing preparation (drop-landings) are conducted and those of toad hopping reveal some striking differences that could suggest that drop-landing motor control strategies may not be the best model for understanding toad landing preparation. During drop landings, in which subjects step off or are dropped from a platform, there is no accurate vestibular or proprioceptive information produced during the drop that could predict drop height or impact time (Lacour et al., 1978; Santello, 2005; Vidal et al., 1979). Given that landing preparation begins before touchdown, visual feedback is the only sensory modality that could provide information about landing in a drop-landing test. In contrast, during self-initiated hops, takeoff effort and angle determine maximum height from the takeoff surface. On flat surfaces, this accurately correlates with hop duration. If toads, in fact, do not process visual information while moving, the available sensory information between these two tasks is exactly opposite. As a result, drop landing may not be the most appropriate comparison for hop landings. We suggest, instead, that motor control during self-initiated movements in mammals like walking, running or hopping may be better comparisons for toad motor control during landing.

In vertebrates, a great deal of the motor control responsible for driving rhythmic motions like locomotion is sub-cortical, driven by spinal central pattern generators (Duysens and Van de Crommert, 1998; Katz and Katz, 2016; MacKay-Lyons, 2002). These pattern generators initiate a sequence of motor commands to produce a pre-programed motion. While some spinal circuits are entirely closed looped, many are modulated by descending sensory afferents and regulate the prioritization of sensory information (Rossignol et al., 2006). Given that cane toads primarily bound as a form of locomotion (Reilly et al., 2015), stringing together a long series of sequential hops, we propose that motor control during locomotion of other vertebrates may be a better model of toad motor control during landing than drop-landings in mammals.

We hypothesize, then, that anuran landing preparation is, likewise, driven by a spinal central program that is initiated in response to hindlimb extension and modulated, at least in part, by visual and vestibular information. We suggest that toads utilize vision to plan a hop, carefully controlling the trajectory to reach a target location, while landing preparation is a reflexive response to the resulting hindlimb extension. This possibility is consistent with the results of several studies. First, it provides a more generous interpretation of the data from this study. Rather than concluding that toads predict impact, but poorly, our results are better explained if landing preparation is a preprogrammed set of movements that is responsive to, but not very sensitive to visual feedback. Second, spinal circuits

drive other anuran motions, so it is feasible that they also drive forelimbs in preparation for landing. For instance, bilateral extension of the hindlimbs during swimming in frogs is driven by spinal circuits (Rauscent et al., 2007; Sillar et al., 2008) and modulated by sensory feedback (Stehouwer and Farel, 1981). Likewise, the wiping reflex persists in spinalized anurans (Berkinblit et al., 1995). Third, results from studies exploring toad landing preparation in the absence of different forms of sensory feedback are consistent with hindlimb extension triggering landing preparation. Loss of proprioceptive information from the hindlimbs or vestibular feedback during takeoff disrupted landing preparation more acutely than the loss of visual information (Cox et al., 2018). In fact, removing hindlimb proprioception entirely eliminated stereotypical forelimb extension patterns prior to landing (Cox et al., 2018) despite accurate vestibular and visual information. In contrast, a bilateral labyrinthectomy dramatically altered the coordination of the timing of different aspects of landing preparation, but did not eliminate them (Cox et al., 2018). More striking, blinded toads were indistinguishable from sighted controls during landing with only a minor increase in the variability of event timing (Cox et al., 2018; Ekstrom et al., 2018). These results imply that toads, unlike mammals, are not able to use visual or vestibular information to compensate for lack of proprioceptive feedback. This suggests that proprioception may play an essential role in landing coordination in toads and is consistent with the hypothesis that hindlimb proprioceptive afferents initiate a pre-landing motor control sequence that is adjusted and modified via vestibular and visual feedback.

It is important to note that the combination of evidence from studies of sensory conflicts and ablations is not sufficient to conclude that toad landing preparation is primarily driven by spinal circuits. We suggest several further experiments that could more precisely test this hypothesis. Our first suggestion is a decerebrated preparation with stimulation of the hindlimb extensors. The presence of forelimb extension and antagonist activation in these conditions would support the hypothesis of a spinal reflex driving landing preparation. Second, the study that removed proprioceptive feedback from toad hindlimbs also removed efferent signals (Cox et al., 2018). Thus, the toads were unable to activate hindlimb musculature to generate a hop and needed to be propelled to produce the same landing conditions as seen in a range of self-generated hops. This could have induced other variations from normal hop conditions that muddied the results. Thus, to test the more specific hypothesis that proprioceptive feedback drives forelimb motor control, one could study landing preparation after blocking only afferent proprioceptive hindlimb signals while toads self-initiate hops. This could be achieved, at least in part, by transecting and then re-attaching the sciatic and femoral nerves to block the stretch reflex (Alvarez et al., 2011). If toads still prepare forelimbs for landing in the absence of a stretch reflex, it would suggest either that other proprioceptive feedback is used or that a motor control program may drive both motions via efferent copy (Subramanian et al., 2019). If, instead, all forelimb preparation is eliminated, it would support the hypothesis that hindlimb extension is a trigger of forelimb landing preparation; however, this approach is limited to 1A proprioceptive afferents (Alvarez et al., 2011). A further study that stimulated hindlimb-specific types of proprioceptive afferent neurons could more precisely distinguish which, if any, type of proprioceptive receptors produce the motor sequence toads use to control landing.

References

Akella, T. and Gillis, G. B. (2011). Hopping isn't always about the legs: forelimb muscle activity patterns during toad locomotion. *J. Exp. Zool. A Ecol. Genet. Physiol.* **315**, 1-11. doi:10.1002/jez.643

Alvarez, F. J., Titus-Mitchell, H. E., Bullinger, K. L., Kraszpulski, M., Nardelli, P. and Cope, T. C. (2011). Permanent central synaptic disconnection of proprioceptors after nerve injury and regeneration. I. loss of VGLUT1/IA synapses on motoneurons. *J. Neurophysiol.* **106**, 2450-2470. doi:10.1152/jn.01095.2010

Azizi, E. and Abbott, E. M. (2013). Anticipatory motor patterns limit muscle stretch during landing in toads. *Biol. Lett.* **9**, 20121045. doi:10.1098/rsbl.2012.1045

Azizi, E. and Roberts, T. J. (2014). Geared up to stretch: pennate muscle behavior during active lengthening. *J. Exp. Biol.* **217**, 376-381. doi:10.1242/jeb.094383

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1-48. doi:10.18637/jss.v067.i01

Berkinblit, M. B., Sidorova, V. Y. and Tkach, T. V. (1995). Wiping reflex in the intact and spinal frogs. *Neurophysiology* **27**, 222-229. doi:10.1007/BF01053337

Borchers, H.-W. and Ewert, J.-P. (1979). Correlation between behavioral and neuronal activities of toads Bufo Bufo (L.) in response to moving configurational prey stimuli. *Behav. Process.* **4**, 99-106. doi:10.1016/0376-6357(79)90026-3

Broström, G. and Holmberg, H. (2011). Generalized linear models with clustered data: fixed and random effects models. *Comput. Stat. Data Anal.* **55**, 3123-3134. doi:10.1016/j.csda.2011.06.011

Collett, T. (1977). Stereopsis in toads. *Nature* **267**, 349-351. doi:10.1038/267349a0

Collett, T. S. (1982). Do toads plan routes? A study of the detour behaviour ofBufo viridis. *J. Comp. Physiol.* **146**, 261-271. doi:10.1007/BF00610246

Collett, T. S. (1993). Do frogs use retinal elevation to measure the distance of a barrier? *J. Comp. Physiol. A* **172**, 109-113. doi:10.1007/bf00214720

Cox, S. M. and Gillis, G. B. (2015). Forelimb kinematics during hopping and landing in toads. *J. Exp. Biol.* **218**, 3051-3058. doi:10.1242/jeb.125385

Cox, S. M. and Gillis, G. B. (2016). Sensory feedback and coordinating asymmetrical landing in toads. *Biol. Lett.* **12**, 20160196. doi:10.1098/rsbl.2016.0196

Cox, S. M. and Gillis, G. (2017). Evidence toads may modulate landing preparation without predicting impact time. *Biol. Open* **6**, 71-76. doi:10.1242/bio.022707

Cox, S. M. and Gillis, G. (2020). Data from: The integration of sensory feedback in the modulation of anuran landing preparation. *Dryad Dataset* https://doi.org/10.5061/dryad.tb2rbnzws.

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**, 894-905. doi:10.1093/icb/icy059

Duysens, J. and Van de Crommert, H. W. A. A. (1998). Neural control of locomotion; part 1: the central pattern generator from cats to humans. *Gait Posture* **7**, 131-141. doi:10.1016/S0966-6362(97)00042-8

Ekstrom, L. J. and Gillis, G. B. (2015). Pre-landing wrist muscle activity in hopping toads. *J. Exp. Biol.* **218**, 2410-2415. doi:10.1242/jeb.113985

Ekstrom, L. J., Panzini, C. and Gillis, G. B. (2018). Vision fine-tunes preparation for landing in the cane toad, Rhinella marina. *Biol. Lett.* **14**, 20180397. doi:10.1098/rsbl.2018.0397

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**, 935-939. doi:10.1007/s00114-010-0697-4

Ewert, J.-P. (1974). The neural basis of visually guided behavior. *Sci. Am.* **230**, 34-42. doi:10.1038/scientificamerican0374-34

Ewert, J.-P. (1987). Neuroethology of releasing mechanisms: prey-catching in toads. *Behav. Brain Sci.* **10**, 337. doi:10.1017/S0140525X00023128

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. doi:10.1098/rsbl.2009.1005

Gillis, G., Ekstrom, L. and Azizi, E. (2014). Biomechanics and control of landing in toads. *Integr. Comp. Biol.* **54**, 1136-1147. doi:10.1093/icb/icu053

Haldin, C., Nymark, S., Aho, A., Koskelainen, A. and Donner, K. (2009). Rod phototransduction determines the trade-off of temporal integration and speed of

vision in dark-adapted toads. *J. Neurosci.* **29**, 5716–5725. doi:10.1523/JNEUROSCI.3888-08.2009

Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspir. Biomim.* 3, 034001. doi:10.1088/1748-3182/3/3/034001

Kamibayashi, K. and Muro, M. (2006). Modulation of pre-programmed muscle activation and stretch reflex to changes of contact surface and visual input during movement to absorb impact. *J. Electromyogr. Kinesiol.* **16**, 432-439. doi:10.1016/j.jelekin.2005.09.004

Katz, P. S. and Katz, P. S. (2016). Evolution of central pattern generators and rhythmic behaviours. *Phil. Trans. R. Soc. B* **371**, 20150057. doi:10.1098/rstb.2015.0057

Konow, N., Azizi, E. and Roberts, T. J. (2011). Muscle power attenuation by tendon during energy dissipation. *Proc. R. Soc. B* **279**, 1108-1113. doi:10.1098/rspb.2011.1435

Lacour, M., Xerri, C. and Hugon, M. (1978). Muscle responses and monosynaptic reflexes in falling monkey. Role of the vestibular system. *J. Physiol.* **74**, 427-438.

Lee, D., Davies, M. and Green, P. (1993). Visual control of velocity of approach by pigeons when landing. *J. Exp. Biol.* **180**, 85-104.

Liebermann, D. G. and Goodman, D. (1991). Effects of visual guidance on the reduction of impacts during landings. *Ergonomics* **34**, 1399-1406. doi:10.1080/00140139108964880

Liebermann, D. G. and Goodman, D. (2007). Pre-landing muscle timing and post-landing effects of falling with continuous vision and in blindfold conditions. *J. Electromyogr. Kinesiol.* **17**, 212-227. doi:10.1016/j.jelekin.2006.01.011

Liebermann, D. G. and Hoffman, J. R. (2005). Timing of preparatory landing responses as a function of availability of optic flow information. *J. Electromyogr. Kinesiol.* **15**, 120-130. doi:10.1016/j.jelekin.2004.07.005

Lock, A. and Collett, T. (1979). A toad's devious approach to its prey: a study of some complex uses of depth vision. *J. Comp. Physiol. A* **131**, 179-189. doi:10.1007/BF00619078

MacKay-Lyons, M. (2002). Central pattern generation of locomotion: a review of the evidence. *Phys. Ther.* **82**, 69-83. doi:10.1093/ptj/82.1.69

Magalhães, F. H. and Goroso, D. G. (2009). Preparatory EMG activity reveals a rapid adaptation pattern in humans performing landing movements in blindfolded condition. *Percept. Mot. Skills* **109**, 500-516. doi:10.2466/pms.109.2.500-516

Marsh, R. L. (1994). Jumping ability of anuran amphibians. *Adv. Vet. Sci. Comp. Med.* **38B**, 51-111.

Phillips, B. L., Brown, G. P., Greenlees, M., Webb, J. K. and Shine, R. (2007). Rapid expansion of the cane toad (Bufo marinus) invasion front in tropical Australia. *Austral. Ecol.* **32**, 169-176. doi:10.1111/j.1442-9993.2007.01664.x

Prochazka, A., Schofield, P., Westerman, R. A. and Ziccone, S. P. (1977). Reflexes in cat ankle muscles after landing from falls. *J. Physiol.* **272**, 705-719. doi:10.1113/jphysiol.1977.sp012068

Rauscent, A., Le Ray, D., Cabirol-pol, M.-J., Sillar, K. T., Simmers, J. and Combes, D. (2007). Development and neuromodulation of spinal locomotor networks in the metamorphosing frog. **100**, 317-327. doi:10.1016/j.jphysparis.2007.05.009

Reilly, S. M. and Jorgensen, M. E. (2011). The evolution of jumping in frogs: morphological evidence for the basal anuran locomotor condition and the radiation of locomotor systems in crown group anurans. *J. Morphol.* **272**, 149-168. doi:10.1002/jmor.10902

Reilly, S. M., Montuelle, S. J., Schmidt, A., Naylor, E., Jorgensen, M. E., Halsey, L. G. and Essner, R. L. (2015). Conquering the world in leaps and bounds: Hopping locomotion in toads is actually bounding. *Funct. Ecol.* **29**, 1308-1316. doi:10.1111/1365-2435.12414

Reilly, S. M., Montuelle, S. J., Schmidt, A., Krause, C., Naylor, E. and Essner, R. L. (2016). Functional evolution of jumping in frogs: Interspecific differences in takeoff and landing. *J. Morphol.* **277**, 379-393. doi:10.1002/jmor.20504

Roberts, T. J., Abbott, E. M. and Azizi, E. (2011). The weak link: do muscle properties determine locomotor performance in frogs? *Phil. Trans. R. Soc. B* **366**, 1488-1495. doi:10.1098/rstb.2010.0326

Rossignol, S., Dubuc, R., Gossard, J.-P. and Dubuc, J. (2006). Dynamic sensorimotor interactions in locomotion. *Physiol. Rev.* **86**, 89-154. doi:10.1152/physrev.00028.2005

Santello, M. (2005). Review of motor control mechanisms underlying impact absorption from falls. *Gait Posture* **21**, 85-94. doi:10.1016/j.gaitpost.2004.01.005

Santello, M. and McDonagh, M. J. (1998). The control of timing and amplitude of EMG activity in landing movements in humans. *Exp. Physiol.* **83**, 857-874. doi:10.1113/expphysiol.1998.sp004165

Santello, M., McDonagh, M. J. N. and Challis, J. H. (2001). Visual and non-visual control of landing movements in humans. *J. Physiol.* **537**, 313-327. doi:10.1111/j.1469-7793.2001.0313k.x

Schaeffel, F., Hagel, G., Eikermann, J. and Collett, T. (1994). Lower-field myopia and astigmatism in amphibians and chickens. *J. Opt. Soc. Am.* **11**, 487. doi:10.1364/JOSAA.11.000487

Sillar, K. T., Combes, D., Ramanathan, S., Molinari, M. and Simmers, J. (2008). Neuromodulation and developmental plasticity in the locomotor system of anuran amphibians during metamorphosis. *Brain Res. Rev.* **57**, 94-102. doi:10.1016/j.brainresrev.2007.07.018

Stehouwer, D. J. and Farel, P. B. (1981). Sensory Interactions with a central motor program in anuran larvae. *Brain Res.* **218**, 131-140. doi:10.1016/0006-8993(81)91296-8

Subramanian, D., Alers, A. and Sommer, M. A. (2019). Corollary discharge for action and cognition. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* **4**, 782-790. doi:10.1016/j.bpsc.2019.05.010

Thompson, H. W. and McKinley, P. A. (1995). Landing from a jump: the role of vision when landing from known and unknown heights. *Neuroreport* **6**, 581-584. doi:10.1097/00001756-199502000-00043

Vidal, P. P., Lacour, M. and Berthoz, A. (1979). Contribution of vision to muscle responses in monkey during free-fall: Visual stabilization decreases vestibular-dependent responses. *Exp. Brain Res.* **37**, 241-252. doi:10.1007/BF00237711