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SYMPOSIUM

The Influence of Visual, Vestibular, and Hindlimb Proprioceptive Ablations on Landing Preparation in Cane Toads

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Synopsis Coordinated landing from a jump requires preparation, which must include appropriate positioning and configuration of the landing limbs and body to be successful. While well studied in mammals, our lab has been using the cane toad (*Rhinella marinus*) as a model for understanding the biomechanics of controlled landing in anurans, animals that use jumping or bounding as their dominant mode of locomotion. In this article, we report new results from experiments designed to explore how different modes of sensory feedback contribute to previously identified features of coordinated landing in toads. More specifically, animals in which vision, hindlimb proprioception, or vestibular feedback were removed, underwent a series of hopping trials while high-speed video was used to record and characterize limb movements and electromyographic (EMG) activity was recorded from a major elbow extensor (anconeus). Results demonstrate that altering any sensory system impacts landing behavior, though loss of vision had the least effect. Blind animals showed significant differences in anconeus EMG timing relative to controls, but forelimb and hindlimb movements as well as the ability to successfully decelerate the body using the forelimbs were not affected. Compromising hindlimb proprioception led to distinctly different forelimb kinematics. Though EMG patterns were disrupted, animals in this condition were also able to decelerate after impact, though with less control, regularly allowing their trunks to make ground contact during landing. Animals with compromised vestibular systems showed the greatest deficits, both in takeoff and landing behavior, which were highly variable and rarely coordinated. Nevertheless, animals in this condition demonstrated EMG patterns and forelimb kinematics similar to those in control animals. The fact that no ablation entirely eliminates all aspects of landing preparation suggests that its underpinnings are complex and that there is no single sensory trigger for its initiation.

Introduction

As tetrapods transitioned to land, water’s buoyant effects were lost and animals’ limbs were confronted with significantly greater loads when interacting with the substrate. Limb skeletons surely helped to resist these increased forces, but limb musculature must have been similarly critical for stiffening joints and maintaining balance as animals left the aquatic environment. Electromyographic recordings from limb muscles of modern, extant tetrapods during walking and running indicate the importance of limb muscle activation during the stance phase of locomotion to support body weight and provide propulsion (Engberg and Lundberg 1969; Goslow et al. 1981; Ashley-Ross 1995; Jenkins et al. 1997; Gillis and Biewener 2001). It is interesting to note that the activation of many large limb muscles critical to generating force during stance occurs well in advance of when the limb makes ground contact (Ibid). This makes sense as it takes time for muscle forces to be developed, and were an animal to wait for mechanical feedback from limb touch-down itself to trigger stance-related muscle activation, it would likely be too late to stiffen joints fast enough to prevent the limb from collapsing after impact (Santello and McDonagh 1998).

Such preparatory activation of limb muscles before impact is especially germane to jumping, where landing forces, and hence the risk of injury, can be quite high. Indeed, previous work on landing from a jump or drop in humans and a range of other mammals has shown that in mid-air, limb muscles are activated in ways that suggest they are tuned to the anticipated time and magnitude of impact (Jones and Watt 1971; Prochazka et al. 1977; Dyhre-Poulsen and Laursen 1984; Santello 2005). Studies that eliminate vision during drop landings in mammals suggest that mammals primarily rely on vision to predict impact, but that with time and under consistent landing conditions, mammals can tune landing via other sensory modalities (Lacour et al. 1978; Craik et al. 1982; Liebermann and Goodman 1991; Thompson and McKinley 1995; Santello and McDonagh 1998; Greenwood and Hopkins 1976). Together this implies that mammals modulate landing preparation by relying on mental models of their position in space and update those models through adjustable weightings of different sensory modalities (Thompson and McKinley 1995; Santello et al. 2001; Santello 2005; Magalhães and Goroso 2009, 2011).

How, then, would an organism that relies on jumping as part of its primary form of locomotion organize motor control of landing preparation? Here we explore motor control of cane toad landing to try to shed light on that question. While humans and many other mammals are good jumpers, perhaps no vertebrate taxon is better suited to jumping than anurans. With their elongated hindlimbs and modified axial skeletons, many frogs and toads use jumping as their dominant mode of locomotion. Recent work across a range of anuran taxa demonstrates broad variation in landing ability (Essner et al. 2010; Reilly et al. 2016). Cane toads (*Rhinella marinus*) possess the most highly coordinated landing behaviors studied to date, and use their forelimbs exclusively to resist impact (Reilly et al. 2015, 2016). Over the last decade we have been using these toads as a model system for studying the biomechanics and control of landing behavior (Gillis et al. 2010; Akella and Gillis 2011; Azizi and Abbott 2012; Azizi et al. 2014; Schnyer et al. 2014; Ekstrom and Gillis 2015; Cox and Gillis 2015, 2016, 2017).

Toad landing

Toads are able to decelerate after a jump in a controlled manner and manage the forces of impact using their forelimbs exclusively (Fig. 1A) (Gillis et al. 2010; Reilly et al. 2015, 2016). Nevertheless, successful landing in toads involves hindlimb actions as well, which help position the center of mass anteriorly for better balance at impact (Azizi et al. 2014). We have identified a number of features that underlie controlled landing in toads, and in this article focus on the following three (Fig. 1A):

(1) Elbow kinematics—toads exhibit distance-dependent forelimb kinematics during hopping. During longer hops, with greater impact forces, animals land with more extended elbows allowing more time and greater distances for controlled deceleration (Cox and Gillis 2015);
(2) Forelimb muscle activity—toads exhibit distance-dependent electromyographic (EMG) activity in forelimb muscles during hopping. Recruitment intensity increases and onset timing gets later in longer hops (Gillis et al. 2010; Ekstrom and Gillis 2015).
(3) Hindlimb positioning—toads undergo rapid retraction of their hindlimbs after takeoff to reposition their center of mass in better alignment with the ground reaction force vector, improving balance at impact (Azizi et al. 2014).

In this article, we present results from experiments in which the sensory modalities of vision, hindlimb proprioception, and vestibular feedback have been compromised to assess their effects on the features of coordinated landing outlined above. Our aim is to both present an evaluation of the role each modality plays in landing preparation and to explore how sensory information is integrated and utilized to tune landing preparation. Specifically, we ask:

(1) Whether any one form of sensory feedback is necessary for landing preparation such that its loss disrupts landing preparation entirely?
(2) Whether any form of sensory feedback is sufficient for landing preparation such that its presence results in no deficits?

Given that toads prioritize non-visual over visual feedback, when the two provide conflicting information about landing conditions (Cox and Gillis 2016), we hypothesize that, unlike mammals, toads do not utilize visual feedback to coordinate landing. Instead, we suggest that landing preparation is triggered by proprioceptive feedback from the hindlimbs during takeoff and modulated by proprioceptive and vestibular feedback. Thus, we predict that a loss of vision will have no influence on landing preparation, loss of hindlimb proprioception will eliminate landing

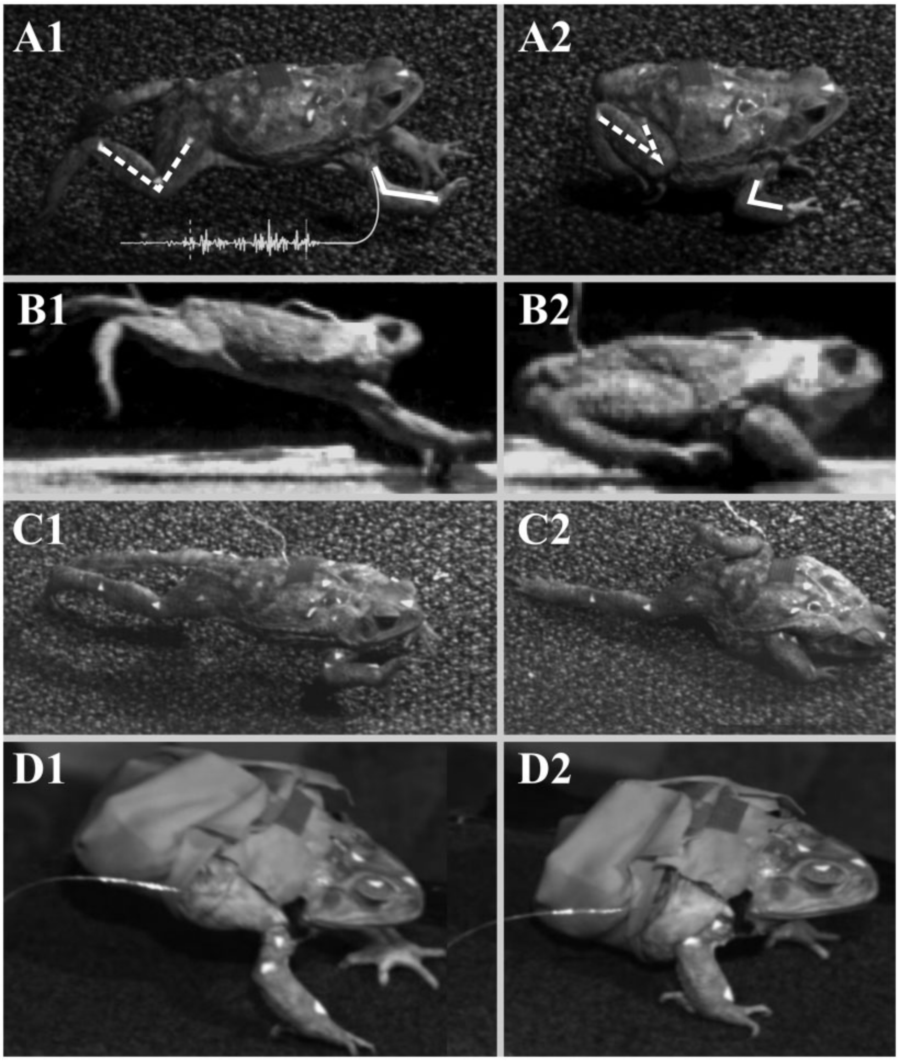

Fig. 1 Comparison between treatments of typical toad limb configurations at touchdown (**A1–D1**) and at the end of forward movement (**A2–D2**). (**A**) Dashed (hindlimb) and solid white (forelimb) line segments highlight digitized points for characterizing limb configurations. In addition, a raw EMG trace is shown coming from the anconeus implant site. Note that elbow configuration at impact, hindlimb retraction and pre-landing EMG activity are all important features of coordinated landing. Toads with (**B**) visual ablation show little disruption to landing preparation. Toads with (**C**) vestibular ablation are unable to land in a coordinated fashion and those with (**D**) hindlimb proprioceptive ablations often cannot use their forelimbs to stop their trunks from contacting the ground during landing.

preparation, and loss of vestibular information will disrupt coordination between components of landing preparation.

Methods

Control animals

Fourteen adult *R. marina* (63–170 g) were used to collect control kinematic or electrographic data for comparison with ablated animals. All animals were obtained from a commercial supplier and housed in groups of two to four in large plastic containers in a holding room maintained at ∼24°C with a 12-h light:12-h dark cycle. They were fed a diet of crickets several times a week and water was always made available. All experimental work was approved by either Mount Holyoke College's or Wheaton College's IACUC and was designed to minimize pain and discomfort. For each ablation experiment, we collected data from 12 to 15 hops from 8 to 10 toads in each condition to collect at least 8 hops from 4 toads in each condition, as per our earlier work (Gillis et al. 2014; Schnyer et al. 2014; Cox and Gillis 2015, 2016; Ekstrom and Gillis 2015). Results presented here represent data from hops that generated both clear digitizable kinematics and clean EMG signals from at least one forelimb. Since all hops from control animals were collected using the same methods, control hops were compiled and include data collected from several experiments. Detailed

Table 1 Individual animals used for each analysis

Animal ID's used in each analysis		
	Kinematics	EMG
Unaltered	6, 9, 10, 19, 20, 21, 22	6, 7, 9, 10, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23
Vision	11, 12	13, 14, 15, 16, 17, 18
Vestibular	6, 7, 8, 9, 10	6, 7, 9, 10
Proprioception	1, 3, 4, 5	1, 2, 3, 4, 5

information on which individual animals were used for which experiments can be found in Table 1.

Data collection

Kinematic data were collected following the methods of Cox and Gillis (2015). In brief, small squares of white cardboard (~3 × 3 mm) were glued to the skin bilaterally at the wrist, elbow, and mid-way along the humerus. In addition, four markers were also used to form a T along the back of the animal. Hopping trials were conducted in a rectangular glass tank (89 × 43 × 43 cm) lined along the bottom with rough felt. Two high-speed cameras (Fastec HiSpec, San Diego, CA, USA) were used to record simultaneous video for 3D kinematic reconstruction (500 frames/s; 1280 × 1024 pixels) and videos were calibrated with a 64-point 3D calibration cube. EMG data were collected following the methods of Gillis et al. (2010). In brief, we inserted fine-wire bipolar electrodes into anconeus muscles bilaterally. While toads modulate muscles acting across both the elbow and the wrist in preparation for landing (Gillis et al. 2010; Akella and Gillis 2011; Ekstrom and Gillis 2015), anconeus (an elbow extensor) was chosen for these studies since it exhibits typical distance-dependent onset timing of pre-landing EMG activity (Gillis et al. 2010) and is easily accessed for instrumentation. Electrical signals were amplified ×1000 and filtered (HPF at 3000 Hz, Low pass filtered at 100 Hz,) by a Model 1700 AmSystem differential AC amplifier and digitized using a National Instruments NI 9205 16-bit A/D converter.

Vision

Animals

Six adult *R. marina* (137–203 g) were used for EMG experiments. Another two animals (64 and 86 g) were used for kinematic measurements. Note that unlike in the other ablation experiments, for vision, completely separate sets of animals were used for recording EMGs and kinematics. Animals were obtained and housed as described above.

Surgical procedures

To remove all visual input, optic nerves were severed bilaterally. Toads were anesthetized by immersion in MS-222 (1.5 g/L). The optic nerves were accessed via a 1–2 cm incision on the soft palate (Gaze and Jacobson 1963) and severed with fine scissors, then the palate incision was sutured closed (6.0 silk). Following completion of the hopping trials, each toad was euthanized by immersion overnight in MS-222 (1.5 g/L) and dissected to confirm complete bilateral optic nerve separation.

Data collection

Kinematic and EMG data were collected as described above for control animals.

Vestibular system

Animals

Five adult *R. marina* (97–146 g) were obtained and housed as described above. All experimental work was approved by Mount Holyoke College's IACUC.

Surgical procedures

To perform labyrinthectomies, animals were anesthetized as previously described. A hole (0.3 mm) was drilled in the parasphenoidal bone along the line bisecting the tympanic membranes and into the cavity holding the membranous labyrinth. The semicircular canals and otolith organs were removed using a small hooked 36 gauge stainless steel wire. Then the hole was filled with wax and the toad was allowed to recover for several hours.

In order to verify bilateral labyrinthectomy, two unilateral labyrinthectomy procedures were performed sequentially, and between them toads were evaluated for postural changes typical of removal of one vestibular organ. Unilateral ablation leads to ipsilateral body lowering and head tilting, while ipsilateral limbs flexed and contralateral limbs extended. While hopping, frogs move in circles to the ablated side. Following successful bilateral labyrinthectomy, symmetry returns, though the head is depressed, as if the animal were trying to burrow (McNally and Tait 1925, 1933). Validation of successful labyrinthectomy included observation of striking postural asymmetry after the first ablation followed by a return to symmetrical postures following the second. Further, following data collection, toads were sacrificed by overnight immersion in MS 222 (1.5 g/L) and skulls were transected along a line bisecting the tympanic membranes to visually confirm that bilateral labyrinthectomy was complete.

Data collection

Kinematic and EMG data were collected as described above.

Proprioception

Animals

Five adult *R. marina* (135–197 g) were obtained and housed as described above.

Surgical procedures

In order to block all proprioceptive signals from the hindlimbs, all animals were anesthetized by immersion in MS-222 (1.5 g/L) and the sciatic and femoral nerves were transected bilaterally. Skin incisions were made along the base of the ilium and fibers of the coccygeoiliacus were split to expose both nerves, which were transected with fine scissors. The skin was then sutured closed and the animal was allowed to recover for several hours. Successful transections blocked both motor and sensory input to the hindlimbs and were marked by hindlimb paralysis.

Data collection

We collected kinematic and EMG data as described above. Because of hindlimb paralysis, toads were unable to hop themselves, and thus were hopped with the assistance of a spring loaded platform (Fig. 2). The device was angled and spring displacement adjusted to generate takeoff angles, Φ, velocities, and hop distances within the range measured by intact toads hopping on flat ground (Φ 0.4°–45.1°, Dist: 9.9–42.9 cm). The device could be adjusted to produce shorter (dashed arrow, Fig. 1, Dist: 12.4 ± 1.4 cm, Φ_S: 29.2 ± 6°) or longer hops (solid arrow, Fig. 1, Dist: 19.4 ± 1.7 cm, Φ_L: 37.1 ± 3°). A spandex sling was wrapped around the hindlimbs to keep them close to the body during the aerial phase to mimic unaltered animals and help them maintain appropriate body angles as they approached landing.

Data Analysis (similar for all treatments)

Kinematics

We analyzed all hops to identify the time of takeoff and touchdown. Markers on the forelimb and back were then digitized in each frame between these time points and 3D coordinates calculated with Matlab software (Hedrick 2008). Data were smoothed with a quintic spline interpolation, and elbow flexion/extension angle was calculated as the angle in the plane of the forelimb between the cords formed by the markers along the humerus and those at the wrist and elbow, as previously described (Cox and Gillis 2015). Elbow extension velocity was the change in elbow extension between the onset of elbow extension and touchdown. The pitch of the animal was calculated as the angle between two points along the animal's back and the horizontal. Only kinematics from the forelimb that touched down first were used in the analysis (Cox and Gillis 2016). Hop duration was the difference in time between landing and takeoff, and was used as a surrogate for distance (negative values indicate short hops in which the forelimbs touched down before the hindlimbs left the ground). The onset of elbow extension (determined as previously described (Cox and Gillis 2017)), elbow angle at touchdown, and the maximum pitch of the body were calculated for every hop. Finally, hindlimb configuration at touchdown was categorized for every hop as either "retracted" or "extended" and we noted whether the toad's head or trunk contacted the ground during landing.

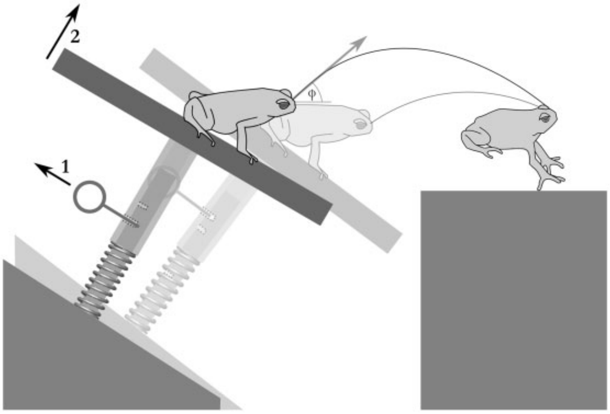

Fig. 2 Spring-loaded apparatus to launch toads after ablation of hind limb proprioception. The angle (Φ) and spring loading were adjusted to generate takeoff conditions comparable to hops of different distance (Φ_1 dashed line; Φ_2, solid line) on flat ground. Device was loaded by compressing the platform and engaging the latch. With the removal of the latch (**1**), the platform accelerated upwards (**2**) to generate takeoff forces necessary to launch the toad into a hop.

Electromyography

Anconeus EMG signals were synchronized with videos using a 5-V trigger pulse that stopped video recording and was included on its own channel with EMG data. We analyzed EMG activity using customized MATLAB scripts in which the onset of pre-landing muscle activity was identified visually for each hop as described elsewhere (Schnyer et al. 2014).

Statistics (similar for all treatments)

Distance-dependence

For each ablation, we evaluated the influence of distance on three metrics of landing preparation that are tuned with distance in unaltered frogs: (1) onset timing of pre-landing EMG activity; (2) onset timing of elbow extension, and (3) the elbow angle at touchdown. For each metric and treatment (12 total, 1 unaltered, 3 ablations), we fit two mixed linear models: a null model with no fixed effect and a full model with the metric as a fixed effect. In all models, individual toads were included as random effects. The P-value for each model was computed with a likelihood ratio test between the full and reduced model. All analyses were conducted in R (R Core Team 2015). Significant relationships are represented by a regression line in Fig. 3. Marginal R^2 values for the full model calculated in R (Nakagawa and Schielzeth 2013) and listed in legends of Fig. 3.

Comparing ablations to the unaltered condition

In order to isolate the influence of each sensory system on landing preparation, we also compared metrics of landing preparation between hops from unaltered toads and those from animals with each ablation (Table 2). For each ablation, we fit two mixed linear models for each landing metric, a null model with hop duration as fixed effect, and a full model with both treatment and hop duration as fixed effects. Including hop duration as a fixed effect in both models allowed us to eliminate any cofounding distance-dependent effects. In all models, individual toads were included as random effects again, and models were compared as described above. Metrics of landing preparation that were significantly altered by an ablation are listed in bold in Table 2. We used a conservative threshold of $P \leq 0.001$ to account for the large number of tests conducted.

Results

Unaltered hops

Kinematics

Sample size: 71 hops from seven animals (10 ± 3 hops/toad). Aerial durations ranged from −21 to 152 ms (mean: 55 ± 29 ms).

General behavior

Unaltered toads initiate hops by rapid coordinated hindlimb extension that propels the animal up and forward into the air. Following takeoff, hindlimbs immediately begin to retract, as the animal's body rotates back toward the ground and its forelimbs are protracted and extended to brace for impact. After impact, toads decelerate the body exclusively with the forelimbs, pivoting their suspended body ventrally as they lower their hindlimbs to the ground (Fig. 1A).

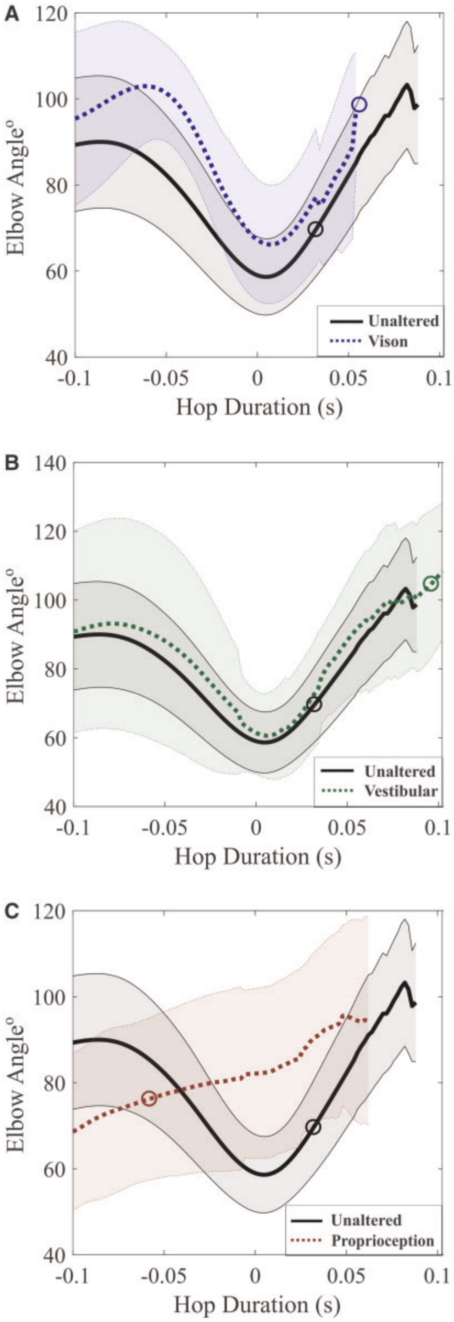

Fig. 3 Comparison of elbow extension before landing between unaltered hops (solid lines) and toads with ablated (dashed lines) (**A**) visual, (**B**) vestibular, and (**C**) hindlimb proprioceptive sensory feedback. Lines depict means and shaded regions one standard deviation around the mean at each time-step. Data zeroed at the onset of elbow extension. Open circles show average touchdown time. Visual and vestibular feedback does not significantly alter pre-landing elbow kinematics while the pattern of elbow flexion and extension is entirely lost after hindlimb proprioceptive ablations.

Elbow angle

Control animals underwent a stereotypical sequence of elbow movements characterized first by a large bout of elbow flexion as the animal took off followed

Table 2 Average values ± SD for kinematic and EMG variables for all treatments

		Ablations		
	Unaltered toads	**Visual**	**Vestibular**	**Proprioception**
Elbow ext at TD	110 ± 12	94 ± 12	105 + 9	96 + 17
Ext velocity	564 ± 111	381 ± 122	561 + 87	**225.5 + 54**
Onset elbow ext	−0.035 ± 0.025	−0.052 + 0.004	−0.113 ± 0.048	0.047 + 0.03
Duration ext	0.09 ± 0.011	0.047 + 0.016	0.1 ± 0.04	0.067 + 0.024
Percentage bilateral hindlimb retract	100	100	21	**NA**
Percentage of landings with head impact	0	0	93	21
Percentage of landings with trunk impact	13	16	100	94.2
Onset EMG	−0.036 ± 0.031	**−0.016 + 0.042**	−0.09 + 0.07	0.004 + 0.081
Duration EMG	0.10 ± 0.032	0.105 + 0.04	0.078 ± 0.064	0.126 + 0.10

Note: Bold values represent significant differences ($P \leq 0.001$) from the unaltered state.

by an even larger amount extension as the animal approached and braced for impact (Fig. 3, black traces). Distance affected elbow kinematics such that animals began to extend their elbows later in longer hops, and reached a more extended elbow configuration at touchdown with increased hop distance (Fig. 4A, B).

Hindlimb configuration

In all 71 hops toads immediately began to retract their hindlimbs following takeoff (Table 2).

EMG

Sample size: 188 hops from 14 animals (13 ± 5.9 hops/toad). Aerial durations ranged from 28 to 178 ms (mean: 64 ± 30 ms). Control animals underwent a stereotypical pattern of muscle activity characterized by a burst of EMG activity at the start of the hop followed by a second burst that turned on ~100 ms before impact, on average (Fig. 5A; Table 2) and extended through the early parts of the landing phase of the hop. Like with the kinematic patterns, hop distance affected muscle activity onset timing. Specifically, anconeus EMG activity began later in longer hops (Fig. 4C).

Visual ablation

Kinematics

Sample size: 20 hops from two animals (10 ± 4.2 hops/toad). Aerial durations ranged from −90 ms to 88 ms (mean: 2 ± 8 ms).

General behavior

Toads without vision were much more reluctant to hop and, when they did, traveled shorter distances; mean aerial durations were 60 ms shorter in blind hops than unaltered hops on average. Blinded toads showed no obvious changes in takeoff or landing kinematics. Hops began with coordinated hindlimb extension as the trunk and forelimbs were propelled up and forward. Once aerial, hindlimbs retracted as forelimbs protracted and elbows extended in preparation for landing. Forelimbs exclusively decelerated the animals and the head and trunk remained suspended in air as hindlimbs were slowly rotated to the ground (Fig. 1B).

Elbow angle

Although the sample size is small, our data suggest that blind toads underwent a similar sequence of stereotyped forelimb movements compared with control animals. Elbow kinematics included a major bout of flexion as the animal took off, followed by even more extension before impact (Fig. 3B). There was no effect of vision loss on the onset timing of elbow extension (Table 2) and blind animals continued to exhibit distance-dependent patterns of elbow extension, in which toads began extending their elbows later in longer hops (Figure 4B). Distance did not have an effect on elbow configuration at impact in blind animals (Fig. 4A).

Hindlimb configuration

As with sighted animals, hindlimbs immediately retracted following takeoff in all hops (Table 2).

EMG

Sample size: 124 hops from 6 animals (21 ± 3 hops/toad). Aerial durations ranged from 2 to 232 ms (mean: 89 ± 11). Blind toads underwent stereotypical bursts of anconeus pre-landing activity that began significantly later than in sighted toads (Fig. 5; Table 2). Blind animals still exhibited distance-dependent EMG onset timing, in which anconeus

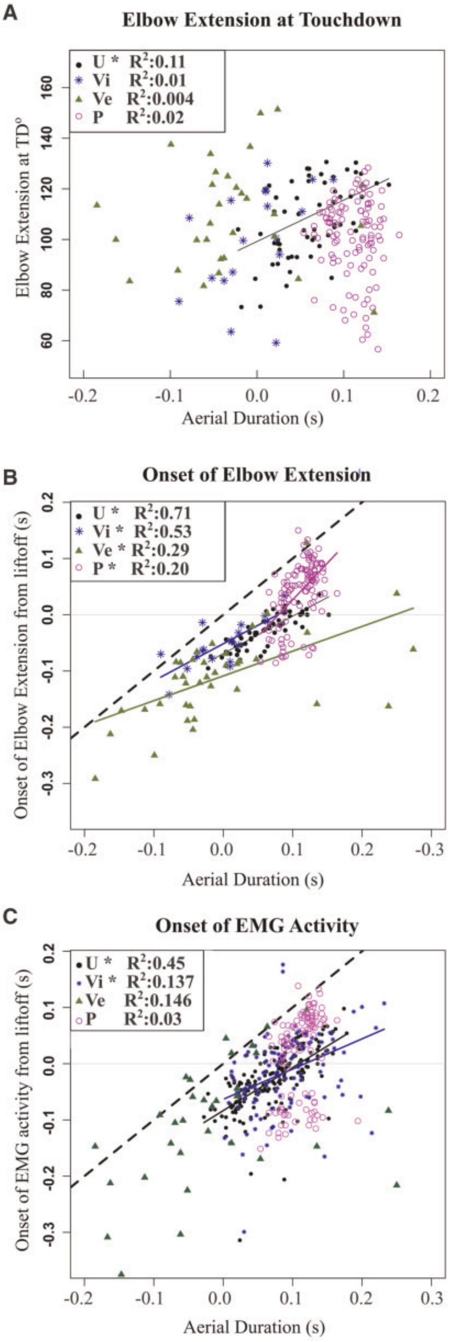

Fig. 4 Plots of kinematic and EMG variables versus aerial duration for all treatments to test for distance-dependence. In all panels, symbols reflect individual hops and different symbol types denote different treatments. Unaltered hops (U): filled circles; visual ablation (Vi): stars; vestibular ablation (Ve): triangles; proprioceptive ablation (P): open circles. R^2 values provided and * denotes a significant relationship with distance ($P \leq 0.001$). Note that aerial duration is zero at takeoff. (**A**) Elbow extension at touchdown as a function of aerial duration. Unaltered toads exhibit distance-dependence and land longer hops with more extended elbows. Ablating any of the sensory systems results in a loss of this relationship and considerably more variation in elbow configuration at impact. (**B**) The onset of elbow extension in preparation for landing (relative to takeoff) exhibits distance dependence in all treatments, starting later in longer hops in all cases. (**C**) Onset timing of pre-landing EMG activity relative to takeoff. Both unaltered toads and blinded toads modulate the timing of pre-landing EMG activity with hop duration. Ablating either the vestibular system or hindlimb proprioception disrupts this relationship.

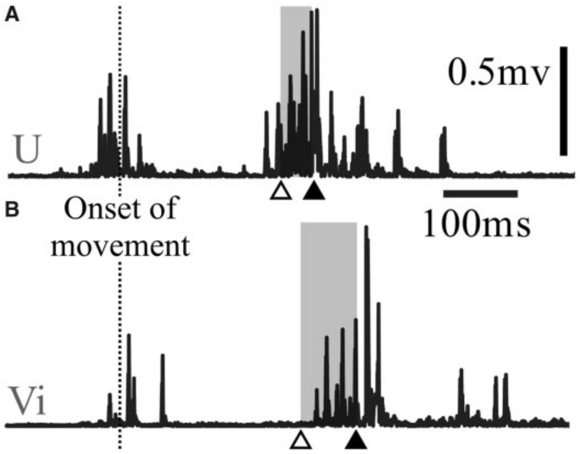

Fig. 5 Representative traces of EMG recordings from the anconeus of the unaltered (**A**) and blind (**B**) cane toads during a short hop. The onset of movement (dotted line), liftoff (open triangle), touchdown (filled triangle), and aerial phase (shaded gray region) are indicated.

activity began later in longer hops (Fig. 4C), yet with greater variability.

Vestibular ablation

Kinematics

Sample size: 49 hops from 5 animals (9 ± 5 hops/toad). Aerial durations ranged from −185 to 274 ms (mean: −6 ± 30 ms).

General behavior

After vestibular ablation, toads were very reluctant to hop. Hopping movements were highly variable and involved a much wider range of takeoff angles than observed in unaltered animals. Most often toads took off at shallow angles (Fig. 1C) and were unable to prevent their trunk and head from making ground contact after impact (Fig. 1C; Table 2). In other hops, animals took off with steep takeoff angles (up to fully vertical), failing to orient their body properly before touchdown, and landed with their hindlimbs first (Fig. 6). Unlike in other treatments, following vestibular ablation, animals often exhibited a lack of bilateral coordination involving limbs moving asymmetrically and animals rolling in mid-air and occasionally even landing on their backs. Hops with very high takeoff angles or severe

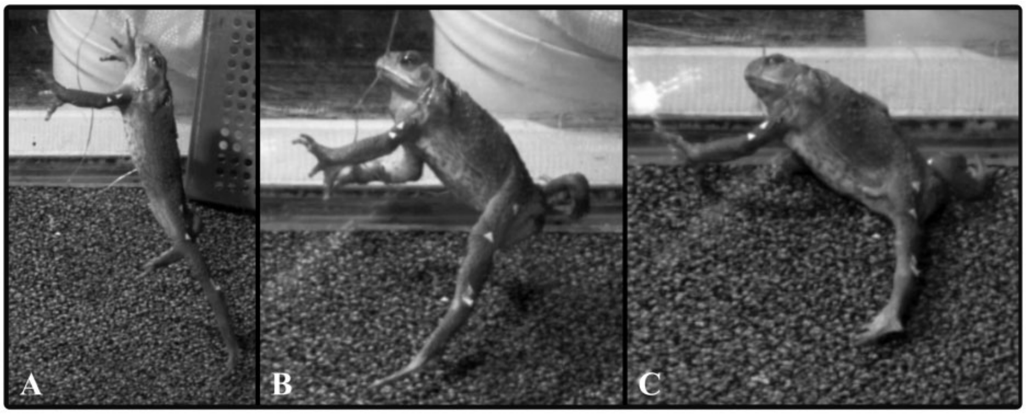

Fig. 6 Landing sequence of a high hop from a toads after labyrinthectomy at (**A**) liftoff, (**B**) touchdown, and (**C**) the end of forward movement. Forelimbs protract and extend but are not oriented such that they can absorb the impact.

rotations were unable to be digitized in our set-up and were thus often omitted from analysis.

Elbow angle

Despite the highly variable behavior described above, the elbows consistently underwent excursions typical of unaltered animals, consisting of an initial phase of flexion, followed by a phase of elbow extension (Fig. 3B). Distance affected the onset timing of elbow extension (Fig. 4B), with toads extending their forelimbs later in longer hops, like in unaltered animals. It is important to note that regardless of these preserved elbow kinematics, toads routinely failed to position the limbs appropriately relative to the ground to absorb impact (Figs. 1C and 5).

Hindlimb configuration

In only 8 of 49 hops did both hindlimbs begin to retract immediately after takeoff (Table 2). In the majority of hops, one or both hindlimbs remained extended well after landing.

EMG:

Sample size: 32 hops from 4 animals (8 ± 2 hops/toad). Aerial durations ranged from −185 to 250 ms (mean: −14 ± 36). EMG activity in toads with vestibular ablations was highly variable. They often exhibited the typical bursts of activity at the onset of movement, followed by a period of inactivity and another burst before landing, as is seen in unaltered toads. But the timing of the pre-landing EMG activity varied widely (and not consistently) with hop duration.

Hindlimb proprioception ablation

Kinematics

Sample size: 109 hops from 4 animals (27 ± 7.1 hops/toad). Aerial durations ranged from 58 to 242 ms (mean: 127 ± 33 ms).

General behavior

Because animals were launched into the air by a device rather than via self-propulsion, the animal's pitch was significantly different during both takeoff and landing from unaltered hops (Table 2). Rather than subtending a range of pitch angles from "head up" (positive pitch) during takeoff to "head down" (negative pitch) when approaching landing, as is common during unaltered hops, toads maintained a negative pitch throughout the hop. This was by design to ensure landing at similar pitch orientations as in unaltered animals. While toads always landed on their forelimbs in this condition, toads were usually unable to maintain the balance necessary after impact to pivot on their forelimbs and lower their hindlimbs to the ground without their trunk (but not their head) contacting the substrate (Fig. 1D; Table 2).

Elbow angle

Forelimb kinematics was drastically altered in animals under this condition. Unlike other treatments, there was no stereotyped sequence of initial elbow flexion followed by extension prior to impact (Fig. 3C). Instead, at the start of a hop, elbows began in a more flexed configuration and extended slowly throughout the first half of the hop, followed by more significant extension as the animal approached impact (Fig. 3C). This latter bout of elbow extension was significantly slower than in control animals (Table 2). Despite these differences, like control hops, elbows began the more significant extension phase later in longer hops (Fig. 4B) and toads landed with comparable elbow extensions (Table 2).

Hindlimb configuration

Because sensory input and motor output to the hindlimbs were ablated in this condition, this variable was not quantified.

EMG

Sample size: 114 hops from 5 individuals (23 ± 14 hops/toad). Aerial durations ranged from 54 to 242 ms (mean: 127 ± 33). EMG signals were characterized by an initial burst that occurred right as the launch began, probably in response to the rapid acceleration. A second burst, more associated with landing preparation, began 105 ms before impact, on average (Table 2). Onset timing was not affected by distance, and instead was simply highly variable (Fig. 4C).

Discussion

We found that, in preparation for landing, cane toads do not respond in a cohesive manner to the alteration of sensory feedback. Rather than adopting an alternative landing control strategy with disrupted sensory feedback, the coordination and tuning of landing preparation becomes highly variable. No form of sensory feedback is sufficient to maintain unaltered landing preparation nor does the loss of any sensory modality entirely disrupt all aspects of landing preparation. Instead, the loss of each sensory modality alters landing preparation in a different manner, though animals appear most sensitive to a loss of vestibular information and least sensitive to a loss of visual feedback.

Vision

Vision is important for fine-tuning landing preparation and is not essential for the gross kinematic patterns required for coordinated landing. Similar to unaltered toads, blinded animals landed most hops exclusively using their forelimbs, but showed more variability in both muscle activation and kinematic patterns. Given the minimal impact of losing vision, toads must rely on other senses for coordinating fundamental movements associated with landing preparation. This is in contrast to the more dominant role vision plays in landing preparation in mammals where it is heavily relied upon to update an internal model of the animal's present or predicted location in space (Santello et al. 2001). Our results and others suggest that toads instead use vision to help with fine-tuning. Very recent work on landing in tree frogs (*Hyla japonica*), also demonstrates that vision is not critical for controlling basic forelimb kinematics employed in preparation for landing (Kamada et al. 2018). Further work measuring the effects of vision loss on patterns of landing forces in anurans is required to better understand its role in controlling the mechanics of impact, which may suffer consequences, despite the similar forelimb kinematics.

Vestibular feedback

Eliminating vestibular feedback had the most dramatic effects on toad hopping, leading to landings that were largely uncontrolled with the body and head crashing into the substrate after impact. This greater influence of vestibular over visual feedback on landing preparation is consistent with earlier work that showed that when feedback from the two sensory systems conflicted, cane toads prioritized vestibular information (Cox and Gillis 2016).

Effects of bilateral labyrinthectomies on the motor control of locomotion have been studied in a variety of mammalian systems including primates and cats. The lack of coordination we observed in toads without vestibular feedback is similar to results in mammals. In labyrinthectomized primates, landing from a leap is not coordinated, and animals do not even extend their limbs as they approach landing (Dow 1938). Cats are able to execute jumps and land with extended forelimbs, but, they collapse upon impact (Thompson and McKinley 1995). Like in toads, forelimb EMG activity in labyrinthectomized cats is not substantially different from in unaltered animals, but the forelimbs are often unable to prevent the trunk from hitting the ground (McKinley and Smith 1983). This emphasizes the complexity of landing coordination and the limitations of visual feedback in isolation of vestibular input.

Proprioception

Unlike for vestibular and visual feedback, there are no direct experiments that we know of in which hindlimb proprioception is eliminated to study its effects on jumping and landing. Instead, in studies demonstrating that animals can execute coordinated landing without vision, authors invoke proprioceptive feedback as a potential explanatory factor (Craik et al. 1982; Santello et al. 2001). Our results from animals after proprioceptive ablation indicate that elbow kinematics are greatly affected in both pattern (Fig. 3C) and velocity (Table 2) as animals braced for impact, yet timing of onset of pre-landing EMG activity is not (Table 2). It is not clear whether these variations are due to changes in starting position or a lack of landing preparation. Functional consequences were notable as most hops resulted in landings in which animals could not use their forelimbs to decelerate their bodies exclusively, and instead, the body contacted the ground shortly after forelimb impact (Table 2). Yet, unlike toads that lacked vestibular information, without proprioception toads were still able to use their forelimbs to brace enough to stop their heads from hitting the ground, suggesting some amount of successful preparatory bracing.

A major limitation was that our approach required ablating both sensory feedback and motor output to the hindlimbs, forcing us to artificially launch animals in a jump-like trajectory, an approach that more closely mirrors the drop landings most common in mammal landing studies, but is still more artificial than other ablation experimental designs. Thus, the extent to which the kinematic differences observed reflects actual responses to a lack of feedback from the hindlimbs during takeoff, versus the artificial nature of the launch itself, is unknown. However, until we have data from animals in which just the sensory neurons are ablated, we can hypothesize that a lack of hindlimb proprioception might have a major impact on the coordination of forelimb movements in preparation for landing.

Sensory feedback and the motor control of landing in toads

The results of this series of experiments suggest two primary conclusions. First, we find no clear evidence that toads tune landing preparation through predictions of impact as is observed in mammals. Mammals are thought to rely on predictions because under normal conditions they begin landing preparation a fixed duration before a future event (touchdown), which implies successful prediction of that event (Santello and McDonagh 1998); If landings are not predictable (from visual information or repetition), mammals switch landing control strategies and instead begin to brace for landing a fixed duration after takeoff (Santello et al. 2001). We see no such clear pattern in toad landing. Consistent with earlier work, toads do not begin landing preparation a fixed duration before touchdown, nor do toads show the disruption to landing preparation after a loss of visual feedback that we would expect if they primarily used visual information to predict landing conditions. This leaves open the possibility that toads instead make predictions based on sensory feedback from the takeoff event itself. Yet, in contrast to our predictions, loss of hindlimb proprioception about takeoff does not eliminate landing preparation. This suggests that bracing of forelimbs before impact is not simply a central pattern generated movement triggered by proprioception feedback during takeoff, as we hypothesized. The complex response of toads to each ablation suggests that the control of landing, instead, is triggered or coordinated through the integration of visual, vestibular, and proprioceptive feedback.

Second, these results suggest that landing preparation is more complex than initially assumed. The highly altered hops of toads after labyrinthectomies, especially, emphasize that more than the three highlighted individual aspects laid out in the introduction are necessary for a coordinated landing. In addition, a good landing depends upon a “good” takeoff requiring (1) coordination between hindlimbs to maintain a level body during a hop, and (2) takeoff angles that are high enough to allow time for forelimbs to move into position to absorb impact but low enough that hindlimb retraction will apply enough angular velocity to pitch the animal forward to land on its forelimbs. Additionally, in order for forelimbs to act as brakes to absorb impact, they need to be oriented properly in relation to the momentum of the hop, as was suggested by Cox and Gillis (2017) but made particularly obvious by the results from our vestibular ablations.

In conclusion, landing preparation in toads involves the coordinated movement of the entire body and is influenced by the loss of all three of the sensory modalities studied here. The vestibular and proprioceptive systems appear especially important in coordinating essential components of takeoff and landing, though visual information may be important for fine-tuning the behavior.

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