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TWISTING AND BENDING: THE FUNCTIONAL ROLE OF SALAMANDER LATERAL HYPAXIAL MUSCULATURE DURING LOCOMOTION

WALLACE O. BENNETT, RACHEL S. SIMONS* AND ELIZABETH L. BRAINERD‡

Department of Biology and Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts Amherst, 611 North Pleasant Road, Amherst, MA 01003-9297, USA

*Present address: Department of Biology, Monroe Community College, Rochester, NY 14623, USA
‡Author for correspondence (e-mail: brainerd@bio.umass.edu)

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Summary

The function of the lateral hypaxial muscles during locomotion in tetrapods is controversial. Currently, there are two hypotheses of lateral hypaxial muscle function. The first, supported by electromyographic (EMG) data from a lizard (*Iguana iguana*) and a salamander (*Dicamptodon ensatus*), suggests that hypaxial muscles function to bend the body during swimming and to resist long-axis torsion during walking. The second, supported by EMG data from lizards during relatively high-speed locomotion, suggests that these muscles function primarily to bend the body during locomotion, not to resist torsional forces. To determine whether the results from *D. ensatus* hold for another salamander, we recorded lateral hypaxial muscle EMGs synchronized with body and limb kinematics in the tiger salamander *Ambystoma tigrinum*. In agreement with results from aquatic locomotion in *D. ensatus*, all four layers of lateral hypaxial musculature were found to show synchronous EMG activity during swimming in *A. tigrinum*. Our findings for terrestrial locomotion also agree with previous results from *D. ensatus* and support the torsion resistance hypothesis for terrestrial locomotion. We observed asynchronous EMG bursts of relatively high intensity in the lateral and medial pairs of hypaxial muscles during walking in tiger salamanders (we call these 'α-bursts'). We infer from this pattern that the more lateral two layers of oblique hypaxial musculature, Mm. obliquus externus superficialis (OES) and obliquus externus profundus (OEP), are active on the side towards which the trunk is bending, while the more medial two layers, Mm. obliquus internus (OI) and transversus abdominis (TA), are active on the opposite side. This result is consistent with the hypothesis proposed for *D. ensatus* that the OES and OEP generate torsional moments to counteract ground reaction forces generated by forelimb support, while the OI and TA generate torsional moments to counteract ground reaction forces from hindlimb support. However, unlike the EMG pattern reported for *D. ensatus*, a second, lower-intensity burst of EMG activity ('β-burst') was sometimes recorded from the lateral hypaxial muscles in *A. tigrinum*. As seen in other muscle systems, these β-bursts of hypaxial muscle coactivation may function to provide fine motor control during locomotion. The presence of asynchronous, relatively high-intensity α-bursts indicates that the lateral hypaxial muscles generate torsional moments during terrestrial locomotion, but it is possible that the balance of forces from both α- and β-bursts may allow the lateral hypaxial muscles to contribute to lateral bending of the body as well.

Key words: locomotion, salamander, Urodela, *Ambystoma tigrinum*, functional morphology, biomechanics, hypaxial muscle.

Introduction

Despite a growing body of knowledge about the kinematics and motor control patterns of epaxial and appendicular muscle systems during locomotion in salamanders (Ashley-Ross, 1995; D'Août et al., 1996; Ashley-Ross and Lauder, 1997; Delvolvé et al., 1997; Frolich and Biewener, 1992; Gillis, 1997), relatively little is known about the role of hypaxial muscles during locomotion. Only recently has an extensive morphological analysis of the variation in salamander lateral hypaxial muscles been published (Simons and Brainerd, 1999), and only one study has used electromyographic (EMG) results to examine the function of hypaxial muscles during locomotion (Carrier, 1993). Salamanders are, however, a particularly interesting group in which to study hypaxial muscle function. Vertebrates ranging from fishes to tetrapods all share basic characteristics in the organization of lateral hypaxial muscles (Carrier, 1990), and salamanders provide a potentially useful model for understanding the transition from aquatic to terrestrial habitats (Frolich and Biewener, 1992).

The general arrangement of the oblique hypaxial musculature is conserved across tetrapods. At least one layer in which muscle fibers slope from craniodorsal towards caudoventral and at least one layer in which muscle fibers slope

from cranioventral towards caudodorsal are always present (Carrier, 1993; Simons and Brainerd, 1999; Brainerd and Simons, 2000). In the tiger salamander *Ambystoma tigrinum*, four lateral hypaxial muscles are present: m. obliquus externus superficialis (OES), m. obliquus externus profundus (OEP), m. obliquus internus (OI) and m. transversus abdominis (TA) (Fig. 1). The four hypaxial layers may be grouped, on the basis of similar fiber angles, into lateral and medial pairs: the laterally situated OES and OEP muscles slope from craniodorsal towards caudoventral and the more medially situated OI and TA muscles slope from cranioventral towards caudodorsal (Fig. 1) (Simons and Brainerd, 1999).

During swimming, the lateral hypaxial muscles of salamanders have been found to contribute to lateral bending of the body (Carrier, 1993). In a study of *Dicamptodon ensatus*, synchronous bursts of EMG activity from the lateral hypaxial muscles on the flexing side of the body were found (Fig. 2A). During terrestrial locomotion, however, Carrier hypothesized that the primary function of the lateral hypaxial muscles in salamanders would be to stabilize the body against torsional loads generated by ground reaction forces (on the basis of previous results from a lizard, *Iguana iguana*; Carrier, 1990). This 'torsion hypothesis' may be summarized as follows. When tetrapods walk over land, they support themselves off the substratum using diagonally opposed limbs. The ground reaction forces directed through the diagonally opposed limbs cause the pectoral and pelvic girdles to rotate, twisting the long axis of the body in the process (Fig. 3). To counteract the torsion caused by the supporting limbs, oblique orientations of the hypaxial muscles are required. The torsion hypothesis predicts that the two lateral layers, which slope from craniodorsal towards caudoventral, counteract the torsion caused by the forelimbs, while the medial layers on the opposite side of the body, whose fibers slope from cranioventral towards caudodorsal, counteract the torsion imposed by the hindlimbs (Fig. 3; Carrier, 1990; Carrier, 1993).

Support for the torsion hypothesis in salamanders comes from EMG data collected from the hypaxial muscles of *D. ensatus* (Carrier, 1993). During walking, *D. ensatus* showed asynchronous bursts of activity in the medial and lateral pairs of hypaxial muscles (Fig. 2C). When the left hindlimb was in contact with the ground, the medial hypaxial muscle pair (OI and TA) on the left side of the trunk was active.

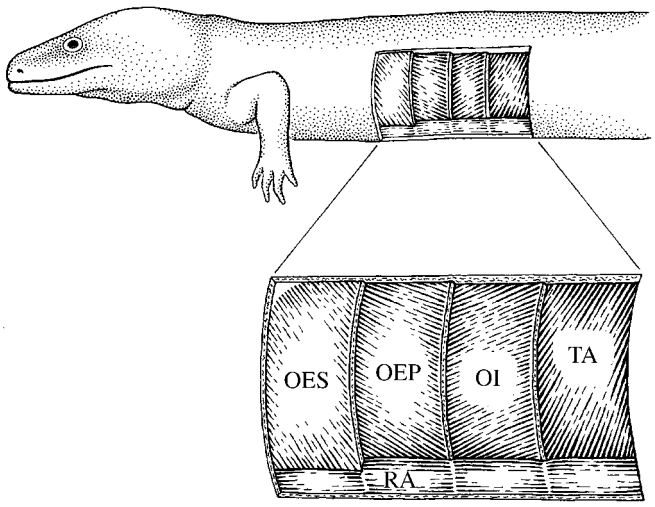

Fig. 1. Left lateral view of the hypaxial musculature of an adult *Ambystoma tigrinum.* Muscle fibers in the lateral pair (OES and OEP) slope from craniodorsal towards caudoventral, whereas muscle fibers in the medial pair (OI and TA) slope from cranioventral towards caudodorsal. OES, m. obliquus externus superficialis; OEP, m. obliquus externus profundus; OI, m. obliquus internus; TA, m. transversus abdominis; RA, m. rectus abdominis. The figure is taken from Simons and Brainerd (Simons and Brainerd, 1999) (with permission). Copyright 1999 Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

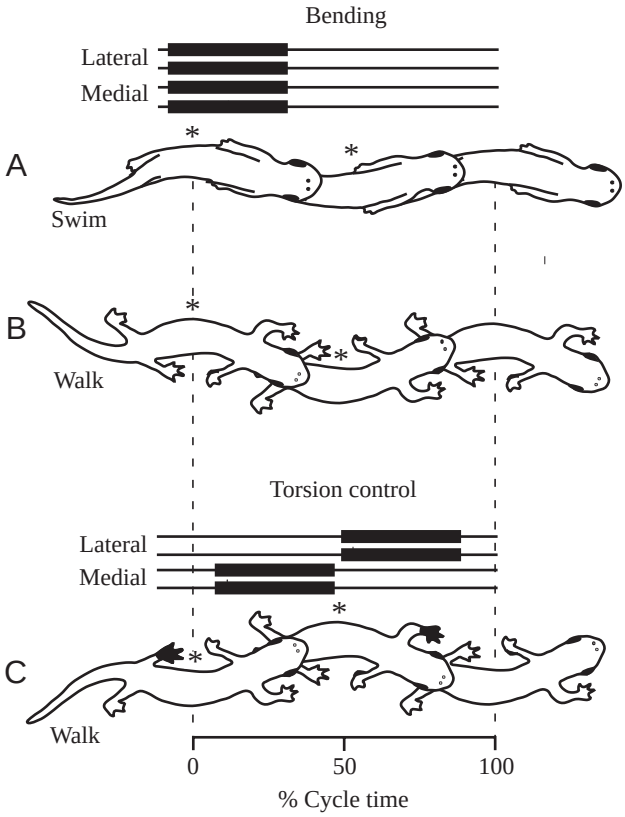

Fig. 2. Schematic representation of two hypothetical functions of the lateral hypaxial muscles during locomotion (after Carrier, 1993). The first hypothesis is that the hypaxial muscles function to bend the body during swimming (A) and walking (B) through the synchronous action of the lateral and medial pairs of hypaxial musculature. A second hypothesis (C) is that the hypaxial muscles function to counteract long-axis torsion of the body during walking. The torsion hypothesis predicts that the lateral and medial pairs of hypaxial muscles will be active asynchronously on one side of the body during walking. Filled bars represent bursts of muscle activity recorded from the lateral and medial hypaxial muscle pairs on one side of the body. Darkened feet in (C) mark the limbs generating the torsional moments countered by the indicated muscle activity. *Denotes the side of the body with hypothetical muscle activity.

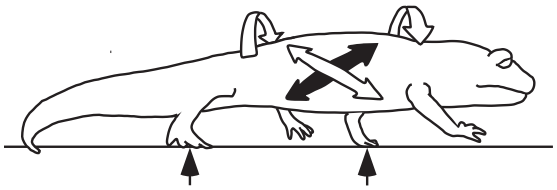

Fig. 3. Torsion control during terrestrial locomotion in salamanders. During walking, the ground reaction forces directed through the diagonally opposed limbs cause the pectoral and pelvic girdles to rotate (curved arrows over the trunk). The oblique orientations of the lateral hypaxial muscles are hypothesized to prevent torsion caused by the limbs. In this example, the medial muscles (open arrow, m. obliquus internus and m. transversus abdominis) on the salamander's right side are active to counteract torsion caused by the hindlimb, while the lateral pair (filled arrow, m. obliquus externus superficialis and m. obliquus externus profundus) of hypaxial muscles on the left side of the trunk is active to counteract the torsion caused by the forelimb (figure modified from Carrier, 1993).

Simultaneously, on the right side of the body, the superficial pair (OES and OEP) was active, thus counteracting the torsion induced by the ground reaction forces transmitted through the forelimb (Carrier, 1993). A similar, asynchronous pattern of hypaxial muscle activity has also been observed in a lizard, *Iguana iguana*, during relatively slow-speed walking (Carrier, 1990).

In conflict with the torsion hypothesis, electromyographic (EMG) data collected from one hypaxial muscle during relatively high-speed locomotion in lizards indicates that this muscle, the external oblique, functions primarily to bend the body (Ritter, 1995; Ritter, 1996). In both *I. iguana* and *Varanus salvator*, Ritter found that the external oblique is active at an appropriate time to produce body bending rather than functioning in torsion control (Fig. 2B). He concluded that the epaxial muscles, rather than the hypaxial muscles, function to control long-axis torsion (Ritter, 1995; Ritter, 1996).

Further evidence supporting the 'bending hypothesis' of hypaxial muscle function comes from denervation experiments of the lateral hypaxial muscles of *D. ensatus* (O'Reilly et al., 2000). When the nerves controlling the lateral hypaxial muscles were transected, *D. ensatus* showed a significant decrease in the amplitude of lateral bending. These results and those from Ritter's work (Ritter, 1995; Ritter, 1996) on lizards suggest that the lateral hypaxial muscles function primarily to bend the body during terrestrial locomotion, but more complete studies including more species, a range of locomotor speeds and EMG data from more than one of the hypaxial muscles are required to draw firm conclusions about the functions of the hypaxial muscles during locomotion in tetrapods.

Given this controversy, the goal of the present study was to determine whether the patterns of EMG activity reported for *D. ensatus* would also occur in another salamander species, *A. tigrinum*, belonging to a different family. Might the asynchronous pattern recorded in *D. ensatus* be peculiar to that species, or is the pattern found in a salamander from a different family as well? On the basis of the previous studies of *Iguana iguana*, *Varanus salvator* and *Dicamptodon ensatus* discussed above, we predict two possible outcomes of our study: (i) the hypaxial muscles function to bend the body during both swimming (Fig. 2A) and walking (Fig. 2B) or (ii) the hypaxial muscles function to bend the body while swimming (Fig. 2A) and to stabilize the trunk against torsion during walking (Fig. 2C). Another possible outcome, although not previously observed in other studies, is that the hypaxial muscles may function to bend and twist the body simultaneously during terrestrial locomotion.

Materials and methods

Animal husbandry

Five adult tiger salamanders (*Ambystoma tigrinum* Green) were purchased from commercial dealers or collected in Coconino County, Arizona, USA. The tiger salamanders had a mean body mass of 64.0±12.7 g (range 49–80 g) and a mean snout–vent length of 12.3±0.91 cm (range 11.0–13.5 cm; means ± S.D.). Animals were housed in separate aquaria on wet paper towels at room temperature (21–23 °C). They were fed three times per week on a diet of crickets supplemented with vitamins and minerals. The Institutional Animal Care and Use Committee at the University of Massachusetts Amherst (approval no. 19-10-07) approved all animal care and use procedures.

Surgical procedures

Electromyographic (EMG) activity was measured using patch electrodes during swimming and walking from the four lateral hypaxial muscles of *A. tigrinum*: Mm. obliquus externus superficialis (OES), obliquus externus profundus (OEP), obliquus internus (OI) and transversus abdominis (TA) (Fig. 1). Patch electrodes were used for two reasons: first, patches help to minimize potential effects of cross-talk between muscles (Loeb and Gans, 1986; Carrier, 1990) and, second, patch electrodes could be sutured directly to the muscles, which helps reduce the low-frequency motion artifact generated during locomotion (Brainerd and Monroy, 1998; Bennett et al., 1999).

Patch electrodes were constructed and implanted following procedures outlined previously (Carrier, 1990; Carrier, 1993; Loeb and Gans, 1986). All electrodes were constructed from reinforced silastic sheeting (Dow Corning, 0.25 mm thick), fine silver wire (outer diameter 51 μm; Cooner Fine Wire, CA, USA) and liquid silicone. Care was taken to construct the electrodes similar to one another to minimize variation between patches. The electrode wires leaving the patches were hand-twisted and connected to lightweight shielded wires *via* miniature connectors. The salamanders were anesthetized using a 1 g l^{-1} solution of tricaine methanesulfonate (MS 222), and the electrodes and shielded cables were sutured to the hypaxial muscles and dorsal surface of the animal, respectively. During surgery, the salamanders were placed

onto a bed of ice, which proved to be an effective method of keeping the animals anesthetized over the long periods required to complete the electrode implantations. Following all experiments, the animals were anesthetized, and electrode placement was verified by dissection (as the electrodes were removed).

In each experiment, EMG activity in at least two of the four hypaxial muscles was recorded using single- or double-sided patch electrodes. In two individuals, recordings were made from all four hypaxial muscles on one side of the body during both walking and swimming. In these individuals, a double-sided patch electrode placed between the OI and OEP muscles was used together with medially facing TA and OES patches. In a third individual, muscle activity from the TA, OI and OES muscles was recorded simultaneously from both sides of the body using medially facing TA and OI electrodes and laterally facing OES patch electrodes. In the remaining two animals, medially facing electrodes recorded activity in just two muscles, the TA and OEP in one animal and the TA and OES in the other.

Electrical signals from the hypaxial muscle electrodes were amplified 10 000× through Grass P511J amplifiers. Signals were filtered in the amplifier with a 60 Hz notch filter and a bandpass filter set between 100 Hz and 5 kHz. All signals were digitized at 4000 samples s^{-1} with a GW Instruments data-acquisition system and Superscope II software. Prior to analysis, low-frequency noise was digitally filtered from all the EMG signals using a custom-designed 100 Hz high-pass filter (WLFDAP; Zola Technologies, Atlanta, GA, USA).

Locomotion experiments

A 122 cm×15 cm×15 cm watertight trackway was constructed for swimming and walking trials. For swimming, the trackway was filled with 6–8 cm of water at room temperature (21–23 °C). Prior to the swimming experiments, the salamanders were given 30 min to acclimate to the water. For walking, the trackway was covered with moist paper towels and the walls were lubricated with a water-based lubricant (KY Jelly). The lubricant helped to reduce the friction caused by the animal occasionally walking along the wall. For simplicity, we use the term 'walk' for terrestrial locomotion in salamanders in this paper, but we recognize that salamanders may actually be using a range of walking and trotting gaits.

A gentle stream of water from a spray bottle was used to motivate the salamanders to swim and walk. Only two salamanders both swam and walked steadily with mild or no spraying. The remaining individuals would not swim steadily, so swimming data were not collected. During all experiments, the electrode leads were held above the salamander so that locomotion could occur as freely as possible. Following each swimming and walking trial, the animals were allowed to rest for several minutes before more data were collected.

Video recordings of locomotion (60 fields s^{-1}) and live EMG traces from the computer screen were simultaneously recorded to videotape using video overlay hardware (TelevEyes/Pro Digital Vision, Inc., Dedham, MA, USA). Because the VHS video recorded both the real-time EMG traces and video of the salamander locomoting, we were able to synchronize the digital EMG in the Superscope II files with the kinematic variables of footfall and maximum body bending.

Quantitative analysis

Within a single muscle and a single locomotor cycle, two EMG bursts were sometimes present. These bursts were distinguishable by their relative timing and frequency of occurrence. One burst of muscle activity was always present in the same part of the locomotor cycle. We call this obligate burst of muscle activity an 'α-burst'. A second burst of muscle activity was sometimes, but not always, present. When present, we refer to the second burst as a 'β-burst'. Cycles with muscle activity that lasted throughout the locomotor cycle are referred to as having 'continuous bursts.' In this study, we noted cycles with continuous bursts of EMG activity but did not include them in our quantitative analysis of EMG burst timing. Only those locomotor cycles for which clear video data were obtained, in which one or two distinguishable bursts of activity were present and in which the salamanders swam or walked steadily were analyzed.

Superscope II software (GW Instruments, Sommerville, MA, USA) was used to display EMG traces, and onset and offset times of EMG bursts were marked manually (i.e. patterns were detected by eye, without strict quantitative criteria). In total, 73 strides for swimming and 97 strides for walking were quantified, but the means and standard deviations reported here represent the number of individuals, not the number of cycles (N=2 individuals for swimming, N=3 individuals for walking). We chose not to combine the EMG data gathered from cycles of different individuals because of the inherent variability contained within each salamander and electrode.

Because of considerable variation in locomotor cycle duration within and among salamanders, kinematic and EMG timing variables were standardized to cycle duration for visual comparison. We did not, however, use standardized muscle onset times in any of the statistical tests. We defined a locomotor cycle as beginning when the salamander was bent maximally to one side and as ending when the animal bent maximally in the same direction again. Footfall times and EMG onsets were measured relative to the beginning of a locomotor cycle (maximal bending).

Statistical analyses

A paired t-test was used to test for significant differences in EMG intensity and duration between α- and β-bursts within each hypaxial muscle for swimming and walking. Locomotor cycles with both α- and β-bursts of EMG activity in one or more of the hypaxial muscles were identified, and low-frequency noise was filtered out. For each α- and β-burst, the rectified integrated area (EMG intensity, mV s) was measured and divided by the duration of muscle activity (in s).

On the basis of previous work (Carrier, 1993), we predicted that the onset times of the TA and OI should be significantly

different from the onset times of the OES and OEP during walking but not during swimming. To test for these differences, one-way analyses of covariance (ANCOVAs) with Fisher pair-wise *post-hoc* tests were performed for each individual during swimming and walking (with locomotor cycle duration as the covariate and muscle as the fixed factor). Significance levels for the multiple pair-wise comparisons were Bonferroni/Dunn-corrected; onset times were not considered significantly different unless the *P*-value was less than 0.008.

Results

Body bending and gait

In *Ambystoma tigrinum*, the kinematics of body bending differ qualitatively and quantitatively in swimming *versus* walking. During swimming, a traveling wave of lateral undulation passes down the body while the limbs are held tightly back against the body. In contrast, during walking, *A. tigrinum* employs a standing wave pattern of body bending with the pectoral and pelvic girdles functioning as nodes.

The mean duration of locomotor cycles during swimming was consistently shorter than the mean duration of locomotor cycles during walking (Table 1). Note that these durations are for swimming in a linear trough and walking on a linear trackway; they therefore represent the locomotor speeds voluntarily selected by the salamanders. Only two individuals exhibited steady swimming behavior, and a significant difference in absolute locomotor cycle duration between these two salamanders was observed (one-way ANOVA, $P<0.05$). Three individuals walked steadily in the trackway and one of these, individual C, used locomotor stride durations that were approximately three times longer than those of individuals A and B (Table 1).

Analysis of 97 walking strides normalized to stride duration indicates that the fore- and hindlimbs have a duty factor of 56±17 % and 57±20 % (means ± S.D.) of the stride, respectively. We found significant individual variation in duty factor and the phase relationship between limb contact and body bending (one-way ANOVA, $P<0.05$). More detailed kinematic analyses of walking and swimming for *A. tigrinum* are described elsewhere (Frolich and Biewener, 1992).

Table 1. *Locomotor cycle duration during voluntary swimming and walking in* Ambystoma tigrinum

Individual	Swimming duration (ms)	Walking duration (ms)
A	390±82 (21)	499±71 (28)
B	307±100 (52)	609±113 (31)
C	–	1520±419 (38)

*Cycle duration is defined as the time between two successive maximum body flexions in the same direction. The sample size (*N*) is the number of strides analyzed for each individual.

Values are means ± S.D. (*N*).

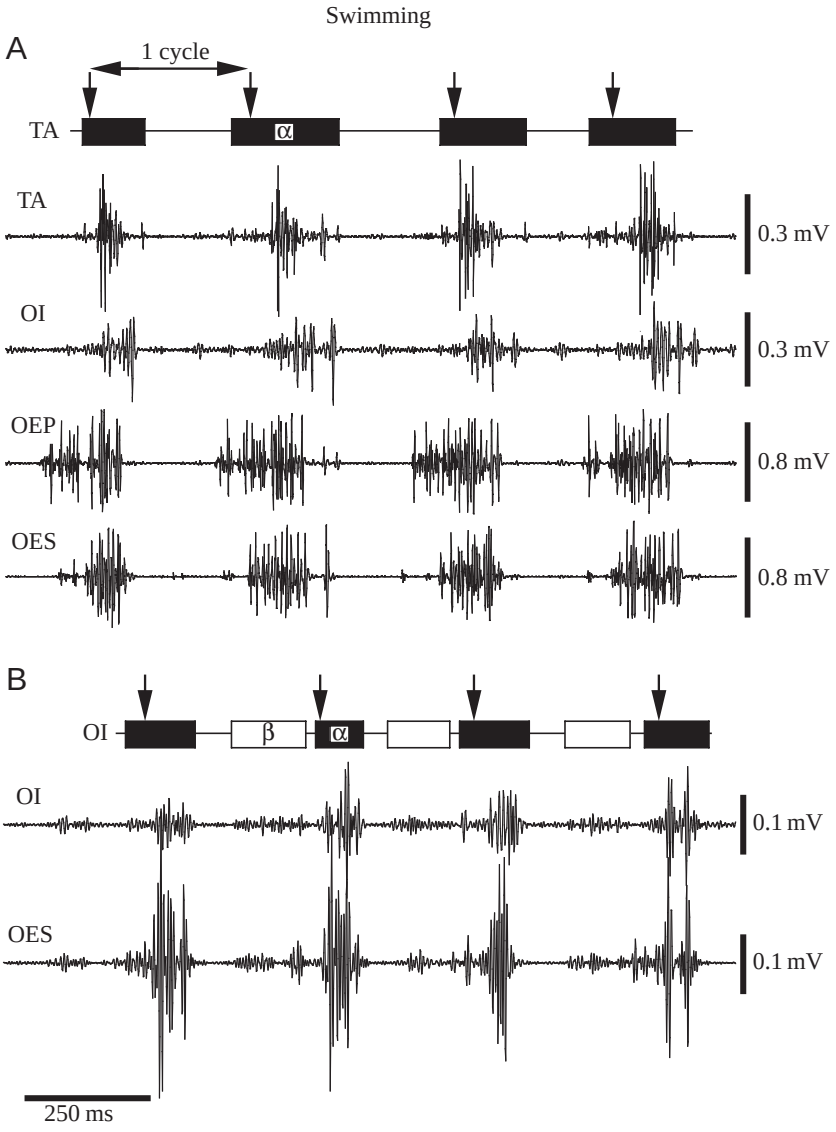

Fig. 4. Representative electromyographic (EMG) activity from the lateral hypaxial muscles of *Ambystoma tigrinum* during swimming. (A) An example of locomotor strides with only the α-bursts present in the TA, OEP and OES muscles. (B) Electromyographic activity recorded from the same individual as A with α- and β-bursts present in the OI and OES. A new locomotor cycle begins at each arrow. Filled and open bars represent our scoring of the α- and β-bursts, respectively, for the corresponding traces. OES, m. obliquus externus superficialis; OEP, m. obliquus externus profundus; OI, m. obliquus internus; TA, m. transversus abdominis.

α- and β-bursts of muscle activity

On the basis of previous results from *Dicamptodon ensatus* (Carrier, 1993), we predicted that each lateral hypaxial muscle in *A. tigrinum* would exhibit only one burst of muscle activity during each locomotor cycle. However, we observed that the lateral hypaxial muscles of *A. tigrinum* often exhibited two distinct bursts of EMG activity within a single locomotor cycle (Fig. 4B, Fig. 5A). One of these bursts, which we refer to as an 'α-burst', is present in every locomotor cycle and is similar in relative timing to the single burst of muscle activity reported for *D. ensatus* (Carrier, 1993). Fig. 4A provides an example of locomotor cycles in which the TA, OEP and OES hypaxial muscles exhibit only α-bursts.

A second burst of muscle activity, which we refer to as a 'β-burst', is present in some of the muscles during some locomotor cycles. When β-bursts are present, the hypaxial muscles display both α- and β-bursts together during a single locomotor cycle (Fig. 4B, Fig. 5A).

We also observed a third pattern of activity in which the α- and β-bursts were not separated by a silent period (Fig. 5B). In this pattern, bursts of EMG activity were nearly continuous throughout the locomotor cycle. The percentages of locomotor cycles in which each of the three EMG patterns (α-bursts only, α- and β-bursts, and continuous bursts) occurred in each of the three individuals during walking and swimming are given in Table 2.

Paired *t*-tests comparing α- and β-burst intensity from each of the lateral hypaxial muscles during swimming and walking indicate that the intensity of α-bursts is significantly higher than the intensity of β-bursts ($P<0.0001$). Fig. 6 shows the results for one individual. A similar analysis for a second salamander showed the same overall relationship between α- and β-burst intensity, with the exception of the TA during swimming in which the intensity of β-bursts was higher than the intensity of α-bursts ($P=0.01$).

Muscle activity patterns during swimming

During swimming, all four lateral hypaxial muscles show α-bursts during the first part of the swimming cycle, defined here as the part of the cycle in which the body begins to bend towards the side of electrode implantation (Fig. 7). Analysis of 73 swimming cycles from two tiger salamanders revealed that the TA and OI show α-burst activity during 31 % and 33 % of the total swimming cycle, and the OEP and OES show α-burst activity during 35 % and 38 % of the total cycle, respectively.

The onset times of the α-bursts (relative to maximum body bending) for three of the four muscles were not significantly different from each other (Table 3; one-way ANCOVA with locomotor cycle duration as the covariate and Fisher pair-wise *post-hoc* tests). However, we did find significant differences in the onset times of α-bursts for the OES compared with onset of α-bursts in the TA, OI and OEP muscles (Table 3). Of the four lateral hypaxial muscles, the onset of α-bursts was earliest in the OES muscle (Fig. 7). EMG onset time was also found to covary significantly with locomotor cycle duration (ANCOVA, $P<0.05$ for the two swimming individuals).

The relative onset times and durations of the lower-intensity β-bursts are also shown in Fig. 7. These bursts occurred in the second half of the locomotor cycle as the animal was bending away from the side in which the electrodes were implanted. β-bursts were not observed in every muscle during every locomotor cycle (see Table 2 for percentages of cycles in

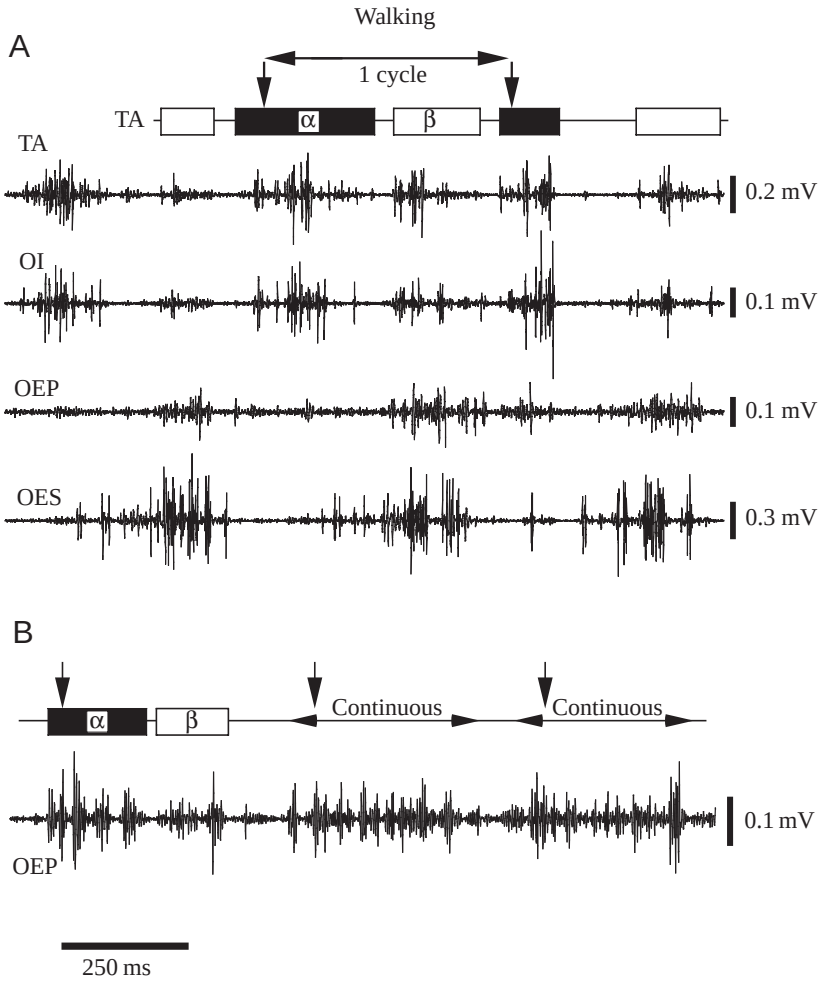

Fig. 5. Representative electromyographic (EMG) activity from the lateral hypaxial muscles of *Ambystoma tigrinum* during walking. (A) EMG activity in which α- and β-bursts occur together within a single walking stride. (B) EMG activity recorded from the same individual in which α- and β-bursts are not separated by silent periods during two strides (continuous bursts). A new locomotor cycle begins at each arrow. Filled and open bars represent our scoring of the of α- and β-bursts, respectively, for the corresponding traces. OES, m. obliquus externus superficialis; OEP, m. obliquus externus profundus; OI, m. obliquus internus; TA, m. transversus abdominis.

Table 2. *Percentage of locomotor cycles in which each of the three observed EMG activity patterns occurs in each muscle layer during walking and swimming*

	Individual A		Individual B		Individual C
	Walking (%) (*N*=115)	Swimming (%) (*N*=60)	Walking (%) (*N*=115)	Swimming (%) (*N*=89)	Walking (%) (*N*=100)
TA_{α}	7	21	18	83	46
$TA_{\alpha+\beta}$	74	73	74	17	48
TA_{cont}	19	6	8	0	6
OI_{α}	8	93	18	100	26
$OI_{\alpha+\beta}$	82	7	65	0	62
OI_{cont}	10	0	17	0	12
OEP_{α}	54	66	30	66	–
$OEP_{\alpha+\beta}$	29	34	52	34	–
OEP_{cont}	17	0	18	0	–
OES_{α}	29	40	9	80	37
$OES_{\alpha+\beta}$	55	57	56	20	59
OES_{cont}	16	3	35	0	4

The three EMG activity patterns observed are α-bursts only, both α- and β-bursts, and continuous bursts (cont).
TA, m. transversus abdominis; OI, m. obliquus internus; OEP, m. obliquus externus profundus; OES, m. obliquus externus superficialis.

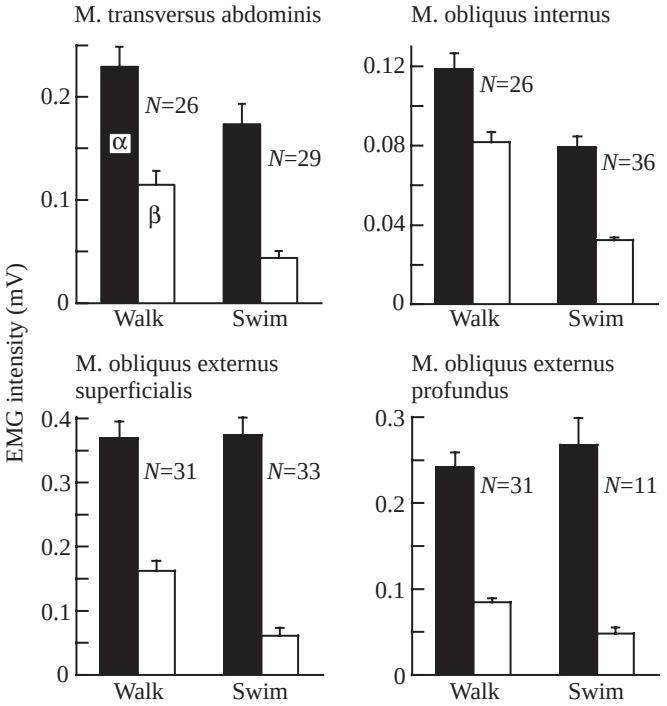

Fig. 6. Bar chart of α- and β-burst intensities from the lateral hypaxial muscles of one tiger salamander (individual A) during walking and swimming. For this individual, the intensity of the α-bursts is significantly higher than the intensity of the β-bursts in all four lateral hypaxial muscles during both swimming and walking (paired *t*-test, $P<0.0001$). The muscle burst intensity was measured as the rectified integrated area divided by the duration of the burst. Filled and open columns represent the mean burst intensity + S.E.M. of the α- and β-bursts, respectively.

which β-bursts occurred). In most cases, the β-bursts were significantly shorter in duration than the α-bursts (paired *t*-test, $P<0.025$), the exception being the TA in individual B (Fig. 7).

Muscle activity patterns during walking

During terrestrial locomotion, the TA and OI muscles show α-bursts in the first half of the stride cycle, defined here as the part of the stride in which the body begins to bend away from the side of electrode implantation. In contrast, the OES and OEP show α-bursts primarily in the second half of the cycle (Fig. 8). As predicted for walking (Carrier, 1993), the onset times of α-bursts in the TA and OI are significantly different from the onset times of α-bursts in the OES and OEP (Table 3; one-way ANCOVA with locomotor cycle duration as the covariate and Fisher pair-wise *post-hoc* tests). EMG onset time was found to covary significantly with locomotor cycle duration in two of three of the walking individuals (ANCOVA, $P<0.05$).

In the TA and OI muscles, the onset of α-bursts is associated with the hindlimb support phase of the locomotor cycle, whereas the onset of α-bursts in the OES and OEP is associated with forelimb support (Fig. 8). During walking, the TA and OI show α-bursts of muscle activity during 39 % and 36 % of the total stride cycle and the OEP and OES show α-bursts during 40 % and 42 % of the total cycle, respectively.

Fig. 8 also shows the relative onset times and durations of the lower-intensity β-bursts. These bursts occur most consistently in the TA and OI muscles, although the OES and OEP do sometimes show β-bursts (see Table 2 for percentages of β-bursts). In most of the muscles evaluated, the duration of β-burst activity was not significantly different from the duration of α-burst activity (Fig. 8). However, the intensity of

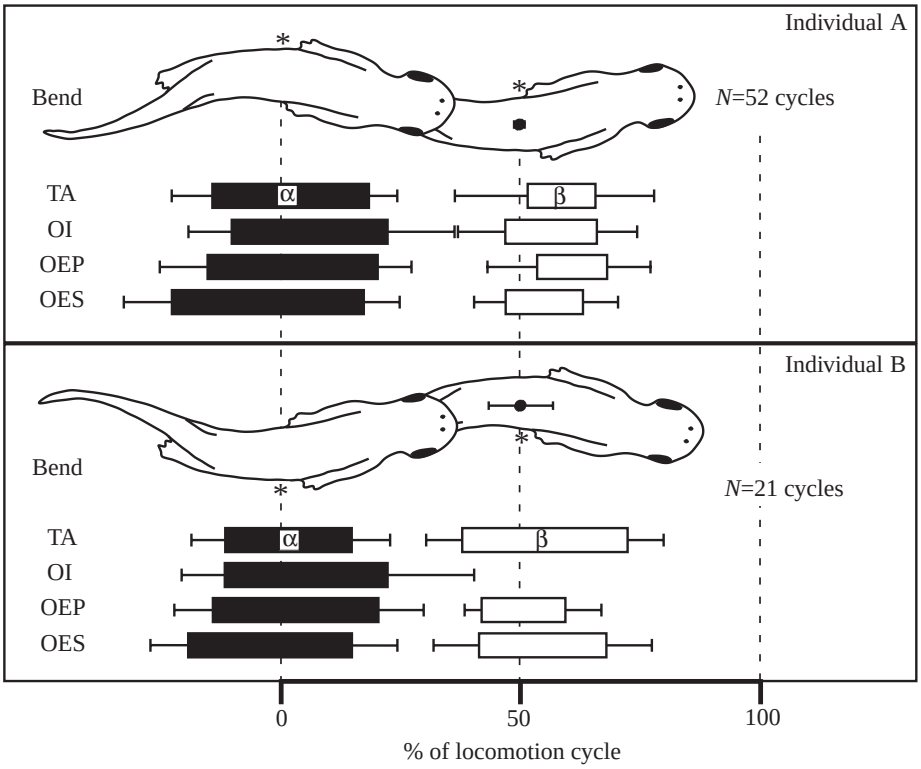

Fig. 7. Summary diagram showing the onset and duration of lateral hypaxial muscle EMG activity relative to maximum body bending in two *Ambystoma tigrinum* during swimming. Values are means ± S.D. for α-burst (filled bars) and β-burst (open bars) onset and duration times, which are calculated from values normalized to locomotor cycle duration. α-bursts occur during every swimming cycle (obligate), whereas β-bursts are more variable and do not necessarily occur in every swimming cycle (facultative). The S.D. of the normalized time of maximum opposite bending is plotted on a point in the center of the body. Sample sizes (*N*): individual A, TA_α=52, TA_β=20, OI_α=52, OI_β=40, OEP_α=52, OEP_β=26, OES_α=52, OES_β=3; individual B, TA_α=21, TA_β=21, OI_α=20, OEP_α=21, OEP_β=4, OES_α=21, OES_β=8. OES, m. obliquus externus superficialis; OEP, m. obliquus externus profundus; OI, m. obliquus internus; TA, m. transversus abdominis. *Denotes the side of the body on which the electrodes were implanted.

the β-bursts was generally less than half that of the α-bursts (Fig. 6).

Discussion

The lateral hypaxial musculature of *Ambystoma tigrinum* exhibits different patterns of muscle activity during swimming *versus* walking. During swimming, all four lateral hypaxial muscle layers are active simultaneously on the flexing side of the body. In contrast, during walking, the medial hypaxial layers (m. obliquus internus, OI, and m. transversus abdominis, TA) are active out of phase with the more lateral layers (m. obliquus externus superficialis, OES; and m. obliquus externus profundus, OEP). We also found that many swimming and walking strides included two distinct bursts of EMG activity: an α-burst, which is consistent with the EMG pattern reported previously for *Dicamptodon ensatus* during walking and swimming (Carrier, 1993); and a β-burst not previously reported for the lateral hypaxial muscles of salamanders.

α-burst muscle activity during swimming

During swimming, all four layers of lateral hypaxial musculature in *A. tigrinum* are active simultaneously to bend (flex) the body towards the active side (Fig. 4, Fig. 7). α-

Table 3. *Pair-wise comparisons of α-burst onset times (relative to maximum body bending) between the four lateral hypaxial muscle layers*

	Swimming		Walking		
Muscle pair	Individual A	Individual B	Individual A	Individual B	Individual C
TA, OI	0.06	0.96	0.20	0.53	0.48
TA, OEP	0.52	0.27	<**>	<**>	–
TA, OES	**	*	<**>	<**>	<**>
OI, OEP	0.013	0.29	<**>	<**>	–
OI, OES	**	*	<**>	<**>	<**>
OEP, OES	**	0.09	0.93	0.01	–

Significance was assessed by one-way ANCOVA with Fisher pair-wise *post-hoc* tests (with locomotor cycle duration as covariate). Significance levels were Bonferroni/Dunn-corrected for multiple comparisons; comparisons are not significant unless $P<0.008$.

<> symbols indicate those muscle pairs that we predicted would have significantly different onset times on the basis of previous results from *Dicamptodon ensatus* (Carrier, 1993).

TA, m. transversus abdominis; OI, m. obliquus internus; OEP, m. obliquus externus profundus; OES, m. obliquus externus superficialis.

*$P<0.008$; **$P<0.0008$.

burst activity occurs in every stride cycle and begins just prior to the beginning of flexion towards the side of electrode placement. The pattern and timing we report for α-bursts from *A. tigrinum* are in agreement with the activity patterns observed from the hypaxial muscles of *D. ensatus* during swimming (Carrier, 1993). Our results from *A. tigrinum* support the hypothesis (Carrier, 1993) that the four lateral hypaxial muscle layers act synergistically to bend the body during swimming in salamanders.

A number of studies have examined the EMG activity patterns of epaxial, lateral hypaxial and limb muscles during salamander swimming (Carrier, 1993; D'Août et al., 1996; Delvolvé et al., 1997; Frolich and Biewener, 1992). The combined results of these studies provide an integrated view of muscle activity in these different muscle groups. During swimming, salamanders hold their limbs close to the body and bend using symmetrical lateral undulations that travel from anterior to posterior at increasing amplitude (Carrier, 1993; D'Août et al., 1996; Delvolvé et al., 1997; Frolich and Biewener, 1992; Gillis, 1997). To produce this body bending, salamanders activate the epaxial musculature in a traveling wave from anterior to posterior (D'Août et al., 1996; Delvolvé et al., 1997; Frolich and Biewener, 1992). In addition to the epaxial contribution, synergistic activation of the lateral hypaxial muscles also helps to bend the body during swimming (*A. tigrinum*, present study; *D. ensatus*, Carrier, 1993) while tonic activation of the limb muscles holds the appendages close to the body (Delvolvé et al., 1997).

Because we recorded EMG activity from only one mid-trunk position for each lateral hypaxial muscle layer, we were unable to determine whether traveling waves or standing waves of EMG activation from the hypaxial muscles are used during swimming. It would be interesting to determine whether several electrodes placed along the hypaxial muscles would detect a traveling wave of EMG similar to that

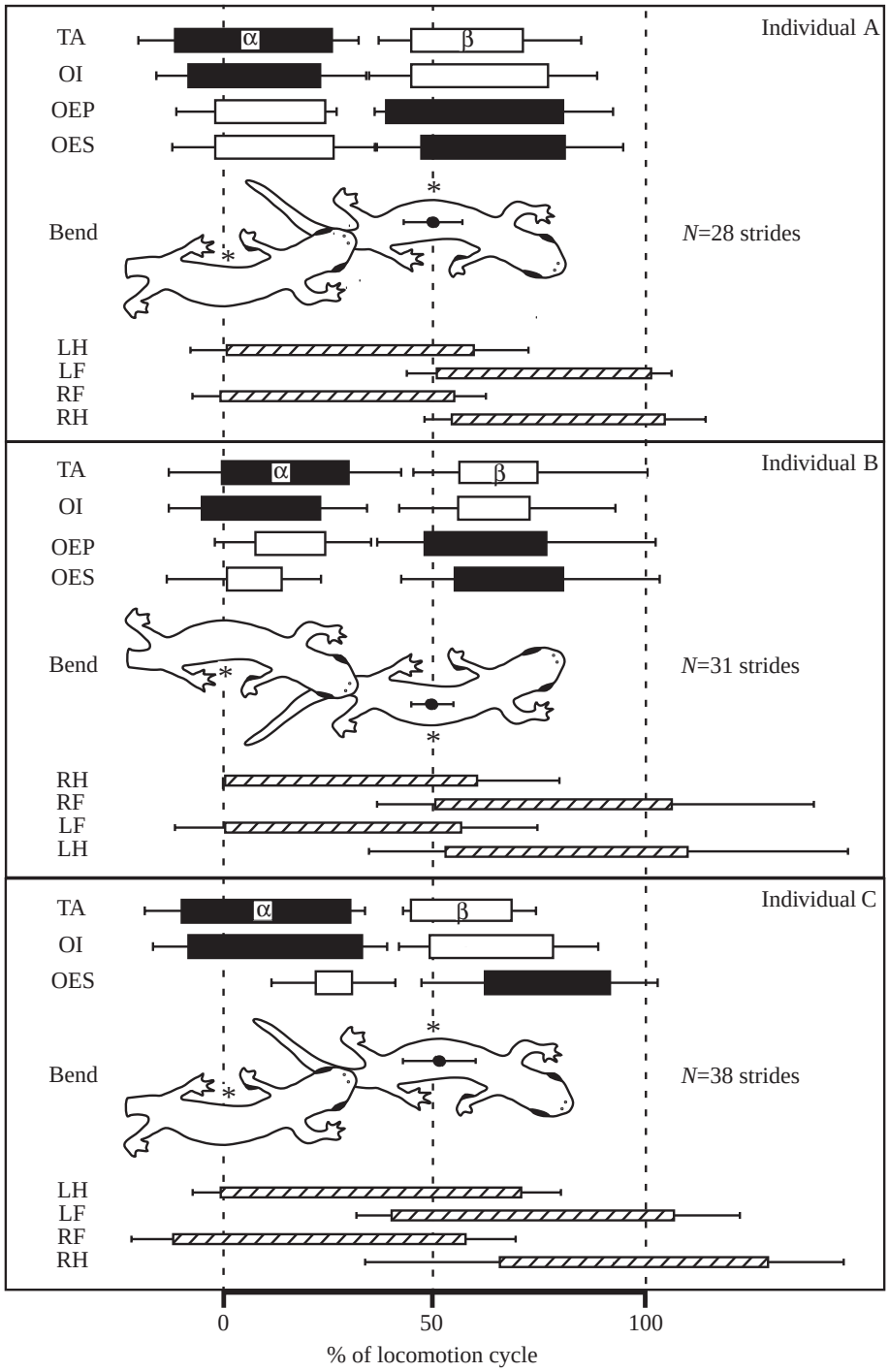

Fig. 8. Summary diagram showing the onset and duration of lateral hypaxial muscle activity relative to maximum body bending and footfall pattern in three *Ambystoma tigrinum* during walking. Means and standard deviations for α-bursts (filled bars), β-bursts (open bars) and limb onset and offset times are calculated from values normalized to stride duration. α-bursts occur during every locomotor cycle, whereas β-bursts are facultative. The S.D. of the normalized time of maximum opposite bending is plotted on a point in the center of the body. LH, left hindlimb; LF, left forelimb; RH, right hindlimb; RF, right forelimb. Sample sizes (*N*): individual A, TA_α=28, TA_β=25, OI_α=27, OI_β=27, OEP_α=28, OEP_β=23, OES_α=26, OES_β=26; individual B, TA_α=30, TA_β=28, OI_α=31, OI_β=31, OEP_α=21, OEP_β=27, OES_α=31, OES_β=31; Individual C, TA_α=37, TA_β=6, OI_α=38, OI_β=28, OES_α=38, OES_β=10. OES, m. obliquus externus superficialis; OEP, m. obliquus externus profundus; OI, m. obliquus internus; TA, m. transversus abdominis. *Denotes the side of the body on which the electrodes were implanted.

seen in the expaxial muscles of swimming salamanders (D'Août et al., 1996; Delvolvé et al., 1997; Frolich and Biewener, 1992).

α-burst muscle activity during walking

The patterns of α-burst activation we observed during walking in *A. tigrinum* are similar to the patterns found in *D. ensatus* (Carrier, 1993). Unlike swimming, the TA and OI muscles are active together on the extending side of the body at the same time as the OEP and OES muscles are active together on the flexing side of the body during walking (Fig. 5, Fig. 8). The onset of α-burst muscle activity from the TA and IO muscles is associated with hindlimb support and body extension, while the EOP and EOS muscles are active on the opposite side of the body during forelimb support and body flexion (Fig. 8). These data from *A. tigrinum* are consistent with the hypothesis (Carrier, 1993) that the lateral hypaxial muscles of salamanders act to counteract torsional forces translated to the trunk by the limbs during walking.

The finding that two salamanders from two families (Ambystomatidae and Dicamptodontidae) both show a motor pattern consistent with torsion control suggests that the lateral hypaxial muscles may act to control torsion during terrestrial locomotion in many salamanders. However, the extent to which other tetrapods use hypaxial muscles for torsion control remains unclear. In dogs, the oblique intercostal muscles function to stabilize the rib cage and trunk against ground reaction forces, but not in a pattern consistent with torsion control (Carrier, 1996). In green iguanas (*I. iguana*), the oblique lateral hypaxial muscles provide stabilization of the trunk by counteracting long-axis torsion generated by ground reaction forces during walking (Carrier, 1990). However, other studies of monitor lizards and green iguanas during high-speed locomotion indicate that the external oblique is not active to stabilize the trunk, but instead contributes primarily to lateral bending of the body (Ritter, 1996).

Articulations between vertebrae would not be expected to resist torsion during terrestrial locomotion in salamanders. Even in snakes and some lizards with relatively more complex vertebral articulations, torsion about the long axis of the body is possible (Moon, 1999). Given that torsion does not appear to be prevented by bony articulations in salamanders or squamates, it is possible that one function of the lateral hypaxial muscles may be to prevent torsion.

β-burst muscle activity during swimming and walking

Not previously reported for swimming or walking is the second, lower-intensity burst of EMG activity that we observed in the hypaxial muscles (β-bursts). The β-bursts reported here for *A. tigrinum* are similar to the secondary (or facultative) bursts recorded from the axial and appendicular muscles of other amphibians and birds in that they vary in burst intensity and duration compared with the primary EMG burst (Ashley-Ross, 1995; Ashley-Ross and Lauder, 1997; Delvolvé et al., 1997; Goslow et al., 1989). As was found for the secondary and primary bursts of other vertebrates, β-bursts are lower in intensity and more variable in occurrence in *A. tigrinum* than are α-bursts.

During swimming and walking, a β-burst occurs between two successive α-bursts (Fig. 4, Fig. 5, Fig. 7, Fig. 8). During swimming, this lower-intensity activity suggests that the muscles on the extending side of the body are undergoing active lengthening and are exerting forces in opposition to bending. During walking, some of the torsional moments generated by the α-burst activity may be countered by the β-burst activity. The function of this antagonistic coactivation is unclear, but we speculate that it may increase body stiffness, improve coordination and contribute to bending during walking.

The β-burst activity observed in the lateral hypaxial muscles of *A. tigrinum* may produce increases in muscle and body stiffness. Increasing body stiffness through bilateral coactivation of muscles has been demonstrated to occur in several fishes (Long, 1998; Long and Nipper, 1996; Westneat et al., 1998). In eels (*Anguilla rostrata*), activation of the axial muscles has been shown to increase body stiffness by as much as three times (Long, 1998). Other studies on fishes have found that simultaneous contraction of locomotor muscles on both sides of the fish can stiffen the body, which may enhance force transmission during fast-starts (Westneat et al., 1998).

Coactivation of the lateral hypaxial musculature during locomotion may also help *A. tigrinum* with body control. Coactivation of muscles is a common strategy that allows animals to achieve controlled movement (e.g. lobster *Homarus americanus*, Ayers and Davis, 1977; salamanders *Dicamptodon tenebrosus* , Ashley-Ross and Lauder, 1997; cats, Buford and Smith, 1990; newt *Pleurodeles waltl*, Delvolvé et al., 1997). Humans often coactivate the antagonistic muscles that act about the wrist and ankle joints to achieve controlled and stable movements (Nielsen, 1998). In a newt, *Pleurodeles waltl*, double bursts of EMG activity recorded during locomotion from the anterior and posterior regions of the epaxial muscles and from several limb muscles are thought to help with motor control (Delvolvé et al., 1997). The double bursts of EMG activity observed in *A. tigrinum* could help the animal achieve roll, yaw and pitch control during swimming and control body flexion during walking.

Although the overall pattern of α-burst activation during walking is consistent with the torsion control hypothesis (Fig. 2C), some of the α and β muscle activity could also contribute to lateral bending. Lateral hypaxial muscles are active on the flexing side of the body (Fig. 8, second half of the cycle, β-bursts in TA and OI and α-bursts in OES and OEP), and if these muscles generate larger bending moments than the contralateral muscles, then the hypaxial muscles could contribute to bending as well as torsion control. Direct evidence supporting the hypothesis that the lateral hypaxial muscles may contribute to body bending comes from denervation experiments of the lateral hypaxial muscles of *D. ensatus* (O'Reilly et al., 2000). In that study, it was found that, when the lateral hypaxial muscles were made inactive, *D.*

ensatus exhibited reduced lateral bending (O'Reilly et al., 2000). If the lateral hypaxial muscles do indeed contribute to body bending during walking, then it is likely that they contribute to both bending and torsion control, with torsion control being effected by the greater amplitude of the α-bursts relative to the β-bursts.

Concluding remarks

The lateral hypaxial musculature of *A. tigrinum* is an example of a muscle group that achieves multiple functions by varying the pattern and timing of muscle activation. During swimming, the lateral hypaxial muscles act synergistically to bend the body. However, during walking, these muscles show an alternating EMG activity pattern that is consistent with the torsion control hypothesis (Carrier, 1993). During both swimming and walking, we also see a lower-intensity β-burst within each cycle. These β-bursts may increase body stiffness, provide fine motor control and contribute to body bending. In agreement with a recent summary of muscle function during aquatic and terrestrial locomotion (Biewener and Gillis, 2000), we conclude that the lateral hypaxial muscles of *A. tigrinum* show changes in recruitment pattern to accommodate both aquatic and terrestrial habitats.

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