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# EPAXIAL MUSCLE FUNCTION DURING LOCOMOTION IN A LIZARD (*VARANUS SALVATOR*) AND THE PROPOSAL OF A KEY INNOVATION IN THE VERTEBRATE AXIAL MUSCULOSKELETAL SYSTEM

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## Summary

**The pattern of electromyographic activity in the epaxial muscles of walking and running lizards (water monitors, *Varanus salvator*) was quantified with high-speed video and synchronized electromyography. Muscle denervation experiments were performed and ground reaction forces were recorded to evaluate hypotheses of muscle function. Water monitors exhibit unilateral, uniphasic activation of the epaxial muscles ipsilateral to rear limb support. The iliocostalis and longissimus dorsi muscles are active throughout the support phase of the rear limb, while the transversospinalis is active only during the second half of the support phase. The timing of muscle activity does not support the presumed locomotor function of the epaxial muscles of lizards (lateral bending of the trunk). Bilateral denervation of the two most lateral epaxial muscles (iliocostalis and longissimus dorsi) caused no significant change in the amplitude of lateral trunk bending. These two epaxial muscles do not appear to be necessary for lateral bending of the trunk, nor do they appear to be involved in resisting lateral bending of the trunk. On the basis of the data reported in this study, as well as a re-evaluation of previously existing data, it is proposed that the locomotor function of the epaxial muscles of lizards is to stabilize the trunk against the vertical component of the ground reaction forces; i.e. the epaxial muscles function in a postural role during locomotion in lizards, as they do in other amniotes. This proposed postural role of the epaxial muscles may represent a key innovation of amniotes, made possible by the loss of an aquatic larval stage in their common ancestor.**

Key words: water monitor, *Varanus salvator*, lizard, locomotion, epaxial muscles, lateral bending, muscle denervation, key innovation.

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## Introduction

The trunks of vertebrates are involved in locomotion in a variety of ways; they may bend laterally (as in most ectotherms), sagittally (as in most mammals) or not at all (as in birds). Although the locomotor role of the trunk varies considerably, some generalizations may be made. Of the two major groups of axial muscles (epaxial and hypaxial), the epaxial muscles have historically been assumed to be the main locomotor muscles (but see Carrier, 1990, 1993). Comparisons between higher-level vertebrate taxa reveal gross differences in epaxial muscle activation patterns and epaxial muscle function during locomotion. These generalizations do not reflect all possibilities, but indicate apparent trends and obvious differences. During locomotion in fishes (Williams, 1986), amphibians (Frolich and Biewener, 1992) and snakes (Jayne, 1988*a*,*b*), there is alternating activity between left- and right-side epaxial muscles. This activity may be described as unilateral (only one side is active at any one point along the trunk) and uniphasic (one period of activity per side per locomotor cycle). The epaxial muscles produce lateral bending of the trunk in these taxa. In contrast, birds and mammals exhibit a bilateral, biphasic activation pattern during walking and trotting, resulting in a period of synchronous left- and right-side activity associated with the support phase of each rear foot. It has been proposed that this activity provides postural stability to the trunk, which is achieved by reducing, rather than producing, movements of the trunk (birds, Gatesy and Dial, 1993; mammals, Tokuriki, 1973*a*,*b*, 1974; Carlson *et al*. 1979; English, 1980; Thorstensson *et al*. 1982). During galloping in mammals, when the rear limbs move together, the epaxial muscles show a uniphasic pattern, though activation is still bilateral (Tokuriki, 1974; English, 1980). The epaxial muscles are believed to assist sagittal bending of the trunk during galloping (Tokuriki, 1974; English, 1980). Birds have much of the vertebral column fused within the trunk, so that only a very limited degree of intrinsic movement is possible.

These gross differences in epaxial muscle activation pattern and function co-vary such that two distinct sets of character states result (Fig. 1). The basal vertebrate condition is a

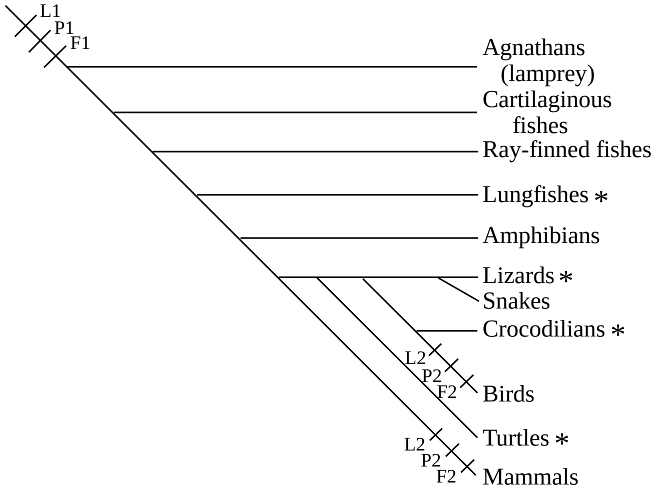

Fig. 1. A simplified vertebrate cladogram with character states representing gross aspects of epaxial muscle activity pattern and function during normal, sustained locomotion (i.e. excluding locomotor behaviors such as fast-starts in fish). Laterality (L) indicates whether the epaxial muscles show alternating left/right activity during locomotion (unilateral, L1) or synchronous activity of both left- and right-side muscles (bilateral, L2). Phase (P) indicates whether the epaxial muscles on one side of the animal exhibit one (uniphasic, P1) or two (biphasic, P2) periods of activity per locomotor cycle. Function (F) indicates whether the epaxial muscles are believed to function in the production of lateral bending (F1) or in the production of sagittal bending or provision of postural stability (F2). The presence of a ‘2’ character state does not necessarily mean that the ‘1’ character state has been replaced. For instance, mammals may show biphasic or uniphasic activation of the epaxial muscles, depending on whether they are using symmetrical or asymmetrical gaits, respectively. An asterisk indicates that no data are available for this group.

unilateral, uniphasic activation of the epaxial muscles that produces lateral bending of the trunk. The derived condition is a bilateral, biphasic activation pattern that provides postural stability to the trunk, or a bilateral, uniphasic activation pattern that produces sagittal bending of the trunk. Once these differences in axial function are placed in a phylogenetic context, we may begin to ask questions not only about the axial systems of specific organisms and groups of organisms but also about aspects of the evolution of the axial musculoskeletal system. A phylogenetic context also identifies taxa whose investigation may contribute most to our understanding of a given evolutionary pattern; i.e. where are the gaps in our comparative database?

The first goal of this study is to fill such a gap by quantifying the activity of the epaxial muscles during locomotion in a lizard. Lizards are a key clade for studies of locomotion because of their intermediate position among tetrapods; although they are fully terrestrial amniotes, they retain the sprawled limb posture of their anamniote ancestors. The second goal of this study is to evaluate the function of the epaxial muscles. The epaxial muscles of lizards are believed to produce lateral bending of the trunk, although there are no data to support this assumption. Therefore, investigation of epaxial muscle function begins by testing this assumption. Finally, results from this study are considered in the broader context of vertebrate evolution.

## Materials and methods

### Animals

The species used in this study was the water monitor *Varanus salvator* Cantor. Animals were housed communally in a large pen (2 m×2 m×4 m), on a 12 h:12 h L:D cycle. The pen was maintained at 26 °C, and the lizards were allowed to thermoregulate behaviorally *via* heat lamps. Lizards were given free access to water and were fed dead mice once per week. Nine animals were used in this study; three for quantification of epaxial electromyography (EMG), four for muscle denervation experiments, one for an *in situ* muscle stimulation procedure and one was killed for initial dissection of the epaxial muscles after fixation in 10 % formalin. Snout–vent lengths (in cm) and masses (in g) of the three animals used for EMG analysis were 36, 900; 39, 960; and 38, 900, respectively. Snout–vent lengths (in cm) and masses (in g) of the four animals used for the muscle denervation experiments were: 33, 560; 33, 640; 38, 1000; and 40, 1000, respectively.

### Video-taping

Video-taping was performed at 120 frames $s^{-1}$ with a Peak Performance Technologies high-speed video camera and VCR. Lizards ran on a stationary trackway 0.6 m wide and 9.7 m long, with 0.6 m high sides. The middle 2.4 m of the trackway had an acrylic side-wall, the ‘working-section’ of the trackway. The animals had sufficient trackway in front of and behind the working-section so that they often moved at constant speed while in the working-section. A ‘locomotor trial’ encompassed the time during which the animal was visible in the working-section of the trackway, which usually consisted of two or three locomotor cycles for a running animal. The animal’s average forward speed was computed from movements of a kinematic marker glued to its dorsal midline at the level of the pectoral girdle (point 1, Fig. 2A). Trials chosen for analysis were those in which the standard deviation of the speed of the animal while in the working-section of the trackway was 10 % or less of the average speed for that trial. A 1.8 m long mirror was suspended over the working-section of the track, oriented at 45 ° to the horizontal, which permitted simultaneous lateral and dorsal views of the animal to be recorded. A light-emitting diode in the field of view was used to synchronize the video record to EMG and/or force records.

### Kinematic analyses

Kinematic analyses were performed with Peak Performance Technologies video analysis software (version 5) running on an IBM-compatible computer. Footfall events (time of placement and lift-off for all feet) were quantified and synchronized with trunk kinematics and EMG and force

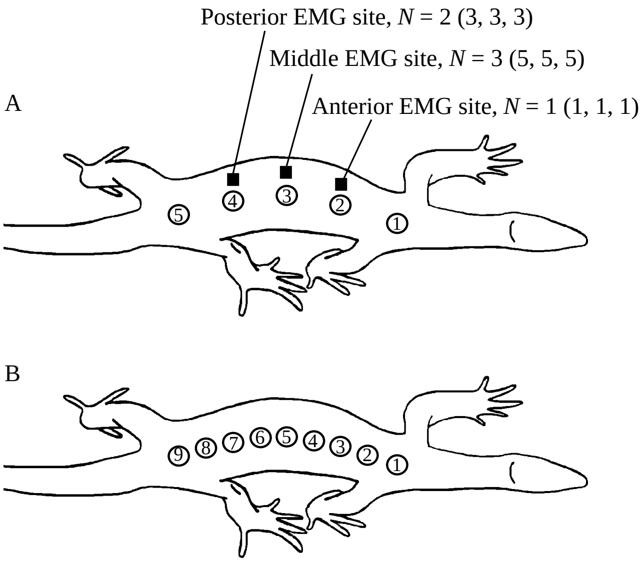

Fig. 2. Diagrammatic lizard trunks showing placement of kinematic markers (numbered circles) and EMG electrodes (squares) for all treatments. (A) Placement of kinematic markers and EMG electrodes for the three animals used for electromyographic recordings. All three animals had five kinematic markers glued to the dorsal midline, which were used to quantify three angles along the trunk; the anterior (between points 1, 2 and 3), middle (between points 2, 3 and 4) and posterior (between points 3, 4 and 5) angles. The vertices of these three angles (points 2, 3 and 4) were the three sites of electrode placement, also termed anterior, middle and posterior. The sample sizes (*N*) at each electrode site indicate the total number of animals with electrodes implanted at that site, with the total number of electrodes implanted in the transversospinalis, longissimus dorsi and iliocostalis muscles, respectively, in parentheses. Bilateral implants were made at the middle and posterior sites. (B) Placement of kinematic markers for denervation experiments. The two animals used in the initial denervation experiments had only three markers, at positions 1, 5 and 9, so that a single angle described bending of the entire trunk between the limb girdles. The two animals used for the fine-scale denervation experiments had nine kinematic markers, which divided their trunk into seven angles. These angles are numbered sequentially from one to seven, with angle one formed by points 1, 2 and 3, angle two formed by points 2, 3 and 4, etc..., to angle seven, which is formed by points 7, 8 and 9.

events. Both the amplitude and timing of lateral bending were calculated by digitizing 1 cm$^2$ reflective markers glued to the dorsal midline of the animals. The total number of reflective markers on an animal varied, but the markers were always evenly spaced between the pectoral and pelvic girdle, with the pectoral girdle being the most anterior point and the pelvic girdle the most posterior point (see Fig. 2 for details). As the animals move, any angle formed by the trunk in the horizontal plane oscillates above and below 180 °. Amplitude of bending is reported as maximum resultant angles, which are the absolute values of the maximum deviations from 180 °. Additionally, a given side of the trunk (left or right) may be described as oscillating between times of maximum concavity and maximum convexity. This is the convention used to report the timing of lateral trunk bending and it is always reported relative to a kinematic or electromyographic event on the same side of the trunk.

### *Force plate recordings*

A Kistler force plate (model 9281B) mounted flush in the center of the working-section of the trackway was used to record ground reaction forces from two animals, simultaneously with EMG recordings. Force plate recordings were made using Bioware software, running on an IBM-compatible computer. The output from the force plate was sampled at 2 kHz. The times of peak forces (vertical, lateral, deceleration and acceleration of horizontal force) were recorded and synchronized with EMG and kinematic data.

### *Muscle stimulation procedure*

*In situ* muscle stimulation was performed on one animal to confirm the pattern of innervation of the individual muscle fascicles that collectively make up the iliocostalis (IC) and longissimus dorsi (LD) muscles. The animal was anesthetized with Ketamine hydrochloride injected intramuscularly (150 mg kg$^{-1}$ initial dosage, with supplements as needed). An 8 cm long, mid-dorsal incision was made in the skin mid-way between the pectoral and pelvic girdles. By dissecting along the connective tissue plane that separates the transversospinalis (TS) and LD muscles, a single nerve trunk was isolated, along with the fascicles of the LD and IC muscles that the nerve trunk appeared to innervate (based on previous dissections). The nerve trunk was placed on a silver stimulating electrode. Bipolar recording electrodes were inserted into three LD fascicles; the one apparently innervated by the nerve trunk and the two immediately anterior and posterior to it. These electrodes were made from 100 $\mu$m diameter silver wire (California Fine Wire Co.). The insulation was removed from the terminal 0.5 mm of the wires and the two bared tips were separated by 0.5 mm. Patch electrodes (see below for construction) were sutured to three IC fascicles; the IC fascicle apparently innervated by the nerve trunk and the two immediately anterior and posterior to it. The nerve trunk was stimulated until a contraction was apparent, to determine a threshold stimulus. The nerve trunk was then stimulated at twice the threshold level while EMGs were simultaneously collected from the three LD or the three IC muscle fascicles.

### *Electromyography*

Bipolar patch electrodes (Loeb and Gans, 1986) were used to record the activity of the TS, LD and IC muscles. The electrodes were constructed from Dow Corning Silastic sheeting (500 $\mu$m thick) and Teflon-insulated, multi-stranded, stainless-steel wire (280 $\mu$m diameter, California Fine Wire Co.). Individual patches were approximately 4 mm×6 mm. The bared portions of the wires were approximately 1 mm long, and the bared wires were separated from each other by approximately 1 mm. Animals were anesthetized as described above, and small incisions were made lateral to the dorsal midline, overlying the site of interest. Both the TS and the LD are contained in distinct connective tissue sheaths. Incisions were made in these sheaths, the electrode was placed between the muscle and the sheath, and the sheath was sutured closed. Because the IC was closely adherent to the overlying skin, a small ‘pocket’ was dissected between the skin and muscle for electrode placement. The IC electrodes were immobilized by suturing them to the underlying muscle fascia. The wire leads from the electrodes were passed subcutaneously to a single exit point on the dorsal midline and soldered into gold connector pins mounted in insulated Amphenol nine-pin plugs. Signals were transmitted *via* lightweight conductors (Cooner Wire) to Grass P511 preamplifiers, amplified 5000 times and bandpass-filtered between 100 and 1000 Hz (the effective sampling rate was 0.5 kHz). The signals were stored on a Macintosh Quadra 700 computer, using Labview software, or on an IBM-compatible computer, using Bioware software. The three animals from which EMGs were recorded differed in the number and position of electrodes implanted (see Fig. 2A for details).

### *Muscle denervations*

Portions of the two most lateral epaxial muscles (the IC and LD) were surgically denervated bilaterally in four animals. The branches of the dorsal rami that supply motor and sensory innervation to the epaxial muscles and the overlying skin lie just deep to the epaxial muscles. Animals were anesthetized as described above and a mid-dorsal skin incision was made, which was approximately as long as the portion of the trunk to be denervated. To gain access to the dorsal rami, a small incision was made in the connective tissue overlying the junction of the TS and LD muscles, superior to an adjacent rib. These two muscles were spread along their common connective tissue plane, the branches of the dorsal ramus that supply the LD and IC were located and the nerve (or nerves) was transected. Care was taken not to disturb the associated blood supply. A 2–3 mm piece of the nerve was removed to retard regeneration. This process was repeated bilaterally until the desired number of segments had been denervated (see below, and Fig. 2B). The effect of the denervation was tested by comparing the maximum amplitude of lateral bending in pre-denervation locomotor trials with that in post-denervation trials.

Initial denervation experiments were performed on two animals (the smaller two of the four noted above). In both of these animals, bending of the entire trunk between the limb girdles was quantified with a single angle (points 1, 5 and 9 in Fig. 2B). Both pre- and post-denervation lateral bending data were collected over a range of speeds (0.3–3.6 $\mathrm{m\,s^{-1}}$). After gathering pre-denervation lateral bending data, the middle eight vertebral segments between the pectoral and pelvic girdles were bilaterally denervated. After recovery, post-denervation data were obtained. These bending data (all maximum resultant angles from all trials) were logarithmically transformed (to linearize them), and the effect of denervation was tested by analysis of covariance (ANCOVA), with speed as the covariate.

The results from these initial denervations suggested that more fine-scale measurements were required, and two additional animals were denervated (the larger two of the four noted above). Both of these animals had nine reflective markers placed on their trunks, dividing the trunk between the pectoral and pelvic girdles into seven angles (see Fig. 2B). One animal had angle five denervated, the other had both angles four and five denervated. These denervations were performed such that none of the IC or LD muscle fascicles that spanned any part of the indicated angles was left intact. Since these fascicles span multiple vertebral segments, portions of the trunk anterior and posterior to the indicated angles were also denervated to ensure complete denervation of the indicated angle(s). The animal with angle five denervated also had EMG electrodes implanted in the TS, LD and IC muscles adjacent to the site of denervation, in order to confirm the denervation. Pre- and post-denervation bending data (all maximum resultant angles from all trials) were collected at a single walking speed and a single running speed, and two three-way ANCOVAs (one for walking, one for running) were used to test the effects of denervation. For these analyses, both treatment (normal *versus* denervated) and angle number (one to seven) were taken as fixed factors. Individual was taken as a random factor. The *F*-value for the treatment effect was calculated by dividing the treatment mean square by the treatment × individual interaction mean square.

### *Parameter calculation*

The data recorded in this study were subjected to various calculations to facilitate comparison of kinematic, electromyographic and force plate data at different speeds. Calculations were also performed to express one variable relative to another, in order to evaluate hypotheses of muscle function.

The temporal relationship between lateral bending of the trunk and rear foot support (‘bending–footfall delay’, or $t_{\mathrm{bf}}$) was calculated as:

$$t_{\mathrm{bf}} = (t_{\mathrm{c}} - t_{\mathrm{r}})/t_{\mathrm{d}} \times 100\,\%\,, \tag{1}$$

where $t_{\mathrm{c}}$ is the time of maximum concave curvature, $t_{\mathrm{r}}$ is the time of ipsilateral rear foot strike and $t_{\mathrm{d}}$ is the cycle duration (all in ms). Cycle duration is the elapsed time between $t_{\mathrm{r}}$ and the subsequent ipsilateral rear foot strike and was determined from the video tapes. A value of zero for $t_{\mathrm{bf}}$ would indicate that the trunk is maximally concave at the time of ipsilateral rear foot strike. Negative values indicate that maximal concavity occurs before rear foot strike, positive values indicate that maximal concavity occurs after rear foot strike. The middle trunk angle (Fig. 2A) was used to make this calculation.

The times of onset and offset of EMG activity ($t_{\mathrm{on}}$ and $t_{\mathrm{off}}$, respectively, in ms) were standardized across speeds by expressing them relative to $t_{\mathrm{r}}$, as a percentage of $t_{\mathrm{d}}$; relative EMG onset:

$$(\%t_{\mathrm{on}}) = (t_{\mathrm{on}} - t_{\mathrm{r}})/t_{\mathrm{d}} \times 100\,\%\,, \tag{2}$$

and relative EMG offset:

$$(\%t_{\mathrm{off}}) = (t_{\mathrm{off}} - t_{\mathrm{r}})/t_{\mathrm{d}} \times 100\,\%\,. \tag{3}$$

The duration of muscle activity ($t_{\mathrm{dur}}$, in ms) was similarly standardized: relative EMG duration:

$$(\%t_{\mathrm{dur}}) = t_{\mathrm{dur}}/t_{\mathrm{d}} \times 100\,\%\,. \tag{4}$$

The times of peak force production ($t_{\mathrm{pf}}$, in ms) may also be expressed in relative terms: relative time of peak force:

$$(\%t_{\mathrm{pf}}) = (t_{\mathrm{pf}} - t_{\mathrm{r}})/t_{\mathrm{d}} \times 100\,\%\,. \tag{5}$$

Two parameters were calculated to evaluate the hypothesis that the epaxial muscles produce lateral bending of the trunk: onset–bending delay (in ms) = $t_{\mathrm{on}} - t_{\mathrm{c}}$ and offset–bending delay (in ms) = $t_{\mathrm{off}}$ − time of maximum concave curvature. If the epaxial muscles produce lateral bending, both of these parameters should be close to zero. For example, if the left IC produces lateral bending, it should begin activity ($t_{\mathrm{on}}$) at approximately the same time as maximum convexity ($t_{\mathrm{c}}$) of the left side of the trunk and cease activity ($t_{\mathrm{off}}$) at approximately the time of maximum concavity of the left side of the trunk.

## Results

### *Anatomy*

The three epaxial muscles are each composed of serially arranged muscle fascicles of varying lengths and orientations (Fig. 3). A detailed exploration of the epaxial muscles of lizards, including variation between lizard taxa, may be found in Gasc (1981). X-ray photographs of one animal shows 21 vertebrae (inclusive) between the pectoral and pelvic girdles, all of similar size.

The most medial of the epaxial muscles, the TS, is the most internally complex and is composed of muscle fascicles that connect vertebrae to vertebrae. The main divisions of the TS are the multifidus, spinalis and semispinalis fascicles. The most medial and deepest of these three is the multifidus. The patch electrodes probably did not record activity from these muscle fascicles. The fascicles of the spinalis are lateral and dorsal to the multifidus. They originate on the post-zygapophyseal wing of a vertebra, course posteriorly and medially, and insert *via* a discrete tendon onto the neural spine of a vertebra four segments posterior to their origin. The semispinalis is the most lateral and superficial of the muscle fascicles of the TS. Fascicles of the semispinalis originate from the post-zygapophyseal wing of a vertebra, course anteriorly and medially, and insert by a strong tendon onto the neural spine of a vertebra six segments anterior to the vertebra of origin. The patch electrodes were in direct contact with the semispinalis portion of the TS, although they may also have recorded signals from the spinalis.

The LD is completely enclosed in a strong connective tissue sheath. Fascicles of the LD have an antero-lateral orientation and span 4–5 vertebral segments. They originate from the post-zygapophyseal wings of the vertebrae and insert on the dorsal surfaces of the ribs. Each LD fascicle is composed of a well-defined muscle belly, with a discrete tendon of origin and insertion. A portion of the tendon of insertion also contributes to a longitudinal connective tissue sheet that divides the LD and IC muscles.

The IC fascicles also span 4–5 vertebrae. They originate from the dorsal surfaces of the ribs and from the above-mentioned aponeurosis that divides the LD and IC muscles. The points of insertion of the IC are the dorsal surfaces of the ribs. Like the subunits of the LD, the IC fascicles have an antero-lateral orientation. The individual muscle fascicles are

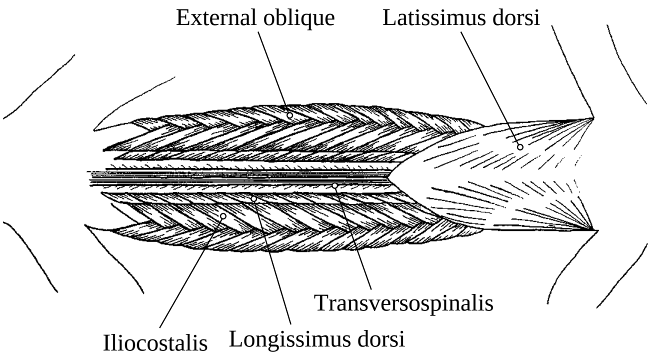

Fig. 3. A diagrammatic representation of the superficial anatomy of the epaxial muscles of *Varanus salvator*. Anterior is to the right.

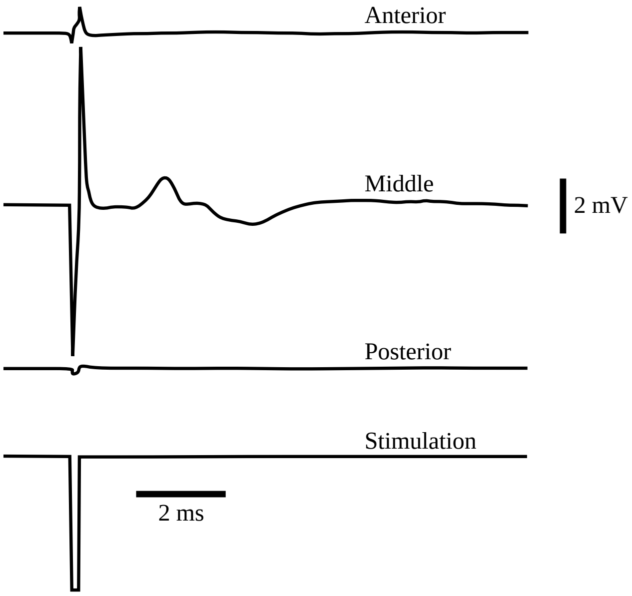

Fig. 4. Representative data from the muscle stimulation procedure. The top three traces are from recording electrodes implanted in three consecutive muscle fascicles of the longissimus dorsi; the bottom trace is a direct record of the stimulation (0.2 ms duration). The nerve trunk that appeared to innervate the middle muscle fascicle (based on gross dissection) was stimulated. A stimulation artifact is apparent in all three muscle fascicles, but EMG activity is only present in the middle fascicle, beginning approximately 2 ms after stimulation. Muscle fascicles are innervated by a single dorsal ramus. The voltage scale bar is applicable to the top three traces. The time scale bar applies to all four traces.

thin and broad and are accurately described as strap muscles. This muscle is closely adherent to the overlying skin. In none of the specimens dissected were the IC muscle fascicles oriented transversely (cf. Gasc, 1981). There are no apparent regional specializations of the epaxial muscles within the trunk between the limb girdles.

### *Muscle stimulation*

Stimulation of a given nerve trunk results in visible contraction and EMG activity only from the muscle fascicle that appears to be innervated by that nerve trunk (Fig. 4). Each fascicle of the LD and IC muscles is innervated by a nerve trunk from a single dorsal ramus; these muscle fascicles are not multiply innervated in the trunk region between the limb girdles. Furthermore, the pair of LD and IC fascicles that are innervated by a single nerve trunk originate on a common vertebral segment and also insert on a common vertebral segment, 4–5 segments anterior to their point of origination.

### *Epaxial muscle activity*

All three of the animals used in the EMG analyses had electrodes implanted in the middle site (Fig. 2A); therefore, the following discussion of the pattern of muscle activity is based on data from that site. Data from the anterior and posterior sites (Fig. 2A) were not appreciably different from the general pattern seen in the middle site.

The epaxial muscles exhibited a unilateral, uniphasic activity pattern during locomotion, with activity ipsilateral to rear foot support (Fig. 5). There was a variable tendency for a short pause within the IC burst (e.g. Fig. 5A, left IC). These pauses varied within and between individuals, and even within trials. Therefore, muscle activity is described as uniphasic, because it was most typical to see one main burst of activity per locomotor cycle.

$\%t_{\text{on}}$ and $\%t_{\text{off}}$ of all three muscles showed a constant relationship to ipsilateral rear foot strike, regardless of speed, over the range measured (0.5–3.5 m s$^{-1}$, Fig. 6). Least-squares regression of $\%t_{\text{on}}$ *versus* speed gave $F$ and $P$ values of: 1.99, 0.17 (IC); 0.01, 0.91 (LD) and 0.59, 0.45 (TS), respectively. Least-squares regression of $\%t_{\text{off}}$ *versus* speed gave $F$ and $P$ values of: 1.07, 0.31 (IC); <0.001, 0.99 (LD) and 0.19, 0.67 (TS), respectively. Data from left- and right-side epaxials from all individuals were pooled to calculate the means and standard deviations of $\%t_{\text{on}}$ and $\%t_{\text{off}}$ (Fig. 6). Periods of support by the rear feet are also shown in Fig. 6, and the pattern of $\%t_{\text{on}}$ and $\%t_{\text{off}}$ is shown for both the right and left sides of the animal. Using these conventions, rear foot strike occurred at 0 % and 50 % of the cycle. Beginning the locomotor cycle with the right rear foot strike, the mean (± S.D.) $\%t_{\text{on}}$ of the three right-side

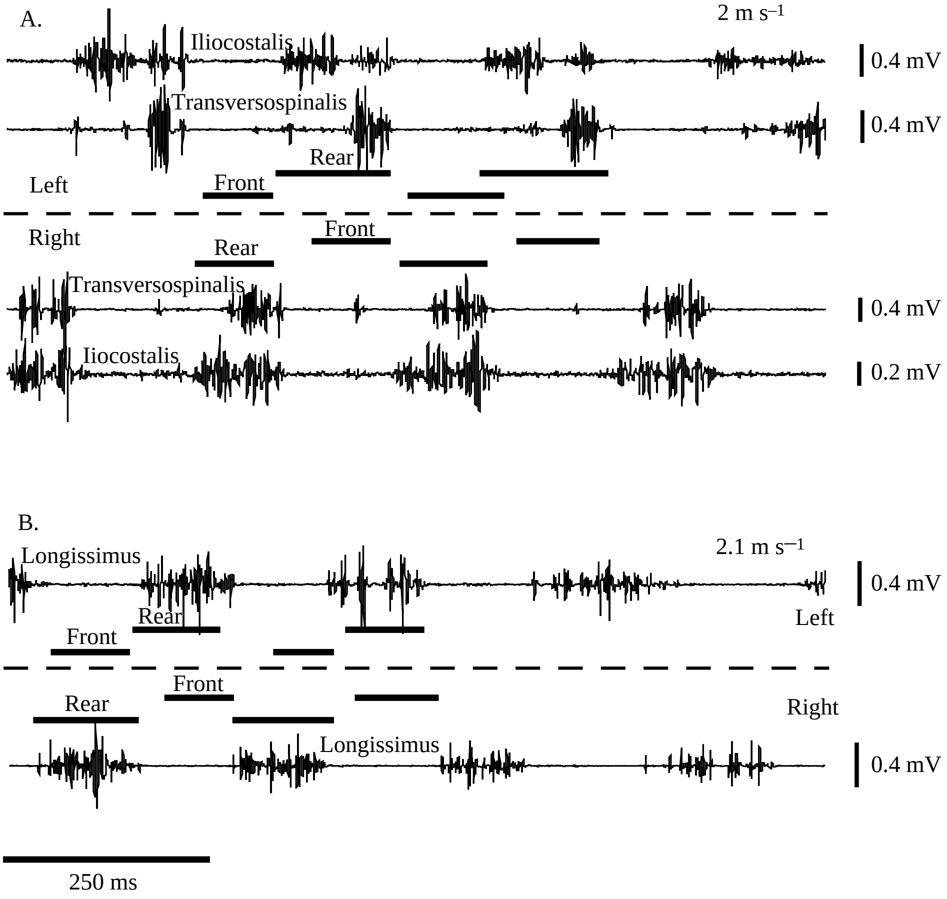

Fig. 5. EMG recordings from two locomotion trials from the same lizard, recorded from the middle EMG site (see Fig. 2A). All three epaxial muscles are active ipsilateral to rear limb support. (A) Recordings from the transversospinalis and iliocostalis muscles. (B) Recordings from the longissimus dorsi muscle. In both A and B, the central dashed line separates the animal's left side from its right side (above and below the line, respectively). Just lateral to the central dashed line is a footfall diagram, with dark bars representing periods of support by the foot indicated. The forward speed of the animal (in m s$^{-1}$) is indicated above each trace. The horizontal scale bar indicating time applies to all records.

epaxial muscles was −4±7 % (IC), 1±6 % (LD) and 21±15 % (TS). The negative value for IC $\%t_{\mathrm{on}}$ indicates that it was active before the right rear foot strike. Onset of activity of the IC and LD was approximately synchronous with ipsilateral rear foot strike. The TS onset time was approximately half-way through ipsilateral rear foot support and was more variable than that of either the IC or LD. The mean (± S.D.) $\%t_{\mathrm{off}}$ of the right-side epaxial muscles was 46±8 % (IC), 48±5 % (LD) and 46±8 % (TS). All three of the epaxial muscles ceased activity at approximately the end of the support phase of the ipsilateral rear foot.

Given that $\%t_{\mathrm{on}}$ and $\%t_{\mathrm{off}}$ are independent of speed over the range measured, it follows that $\%t_{\mathrm{dur}}$ was also independent of speed. Mean values (± S.D.) of $\%t_{\mathrm{dur}}$ were 50±10 % (IC), 49±6 % (LD) and 25±16 % (TS). Both the IC and LD were active throughout the support phase of the ipsilateral rear foot, while the TS was active only during the second half of the support phase of the ipsilateral rear foot (Fig. 6).

### *Ground force production*

The forces that a running animal exerts on the ground can be divided into three components. The horizontal component of the ground force results in both deceleration and acceleration in the direction of travel and may be conveniently divided into deceleration and acceleration phases. The lateral component also acts in the horizontal plane, but at an angle of 90 ° to the direction of travel. The vertical component resists the force of gravity. During normal locomotion, the magnitudes of all components of the ground forces increase regularly until the time of peak force and then decrease

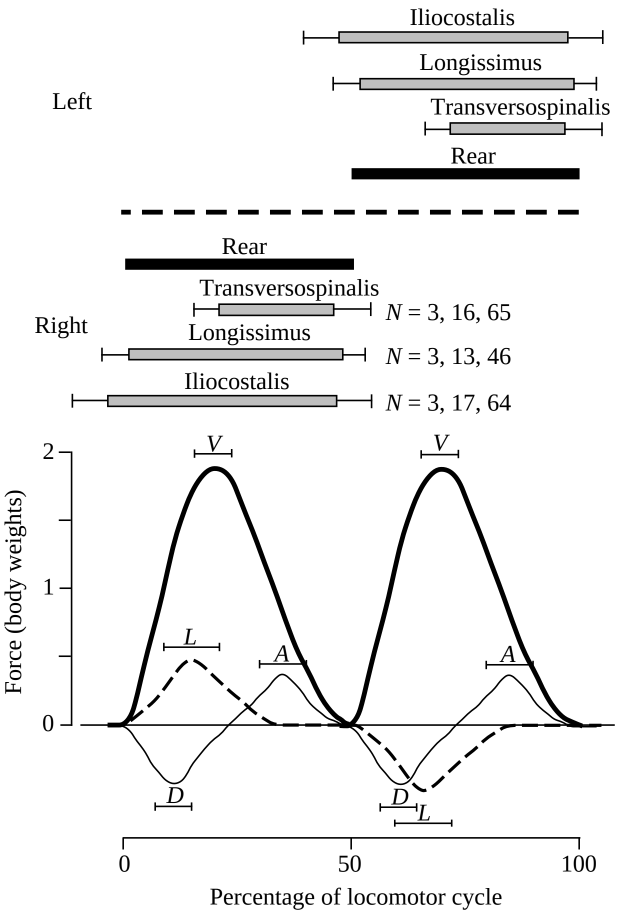

Fig. 6. A summary diagram of EMG activity and ground reaction forces, relative to rear foot support. Relative times of EMG onset and offset, and relative times of peak forces, did not vary with speed over the range recorded (0.5–3.5 $\mathrm{m\,s^{-1}}$ for EMG recordings, 0.6–2.8 $\mathrm{m\,s^{-1}}$ for force recordings). The central dashed line separates the left and right sides of the animal, dark bars representing periods of rear foot support are lateral to the central dashed line, and the EMG data are lateral to these. Mean times of relative peak forces are superimposed on the representative force traces, at the bottom of the figure. The force traces represent a typical trot and are the summed forces from a pair of feet, first right rear and left front, then left rear and right front. The vertical force is represented by a heavy solid line, the horizontal force by a thin solid line, and the lateral force by a dashed line. Abbreviations for peak forces are as follows: *D*, deceleration of horizontal force; *A*, acceleration of horizontal force; *L*, lateral force; *V*, vertical force. EMG data are from the middle site of electrode implantation (see Fig. 2A). The thin horizontal bars at the end of each EMG bar represent 1 S.D. of the relative EMG onset and offset times and are calculated using pooled data from three animals. The number of individuals, trials and locomotor cycles used to calculate the mean times of relative onset and offset for each muscle are given to the right of the right-side EMG data. The thin horizontal lines below the relative times of peak ground forces represent 1 S.D. of the relative times of peak force production. Means and standard deviations for the relative times of peak forces were calculated from data from two individuals, 21 trials and 32 locomotor cycles. See text for calculations of relative times of EMG onset and offset and relative times of peak force production.

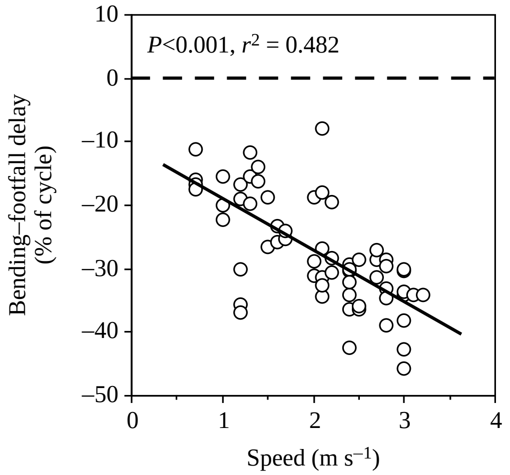

Fig. 7. Graph representing the bending–footfall delay as a function of speed, calculated using data from the middle trunk angle (points 2, 3 and 4 of Fig. 2A). The temporal relationship between trunk bending and foot support varies linearly with speed. The bending–footfall delay represents the percentage of the locomotor cycle between maximum concavity of the trunk and ipsilateral rear foot strike. As speed increases, there is a progressively larger percentage of the locomotor cycle separating the time of maximum concavity and the time of ipsilateral rear foot strike. These data are from three individuals, 43 trials and 154 locomotor cycles.

regularly (Fig. 6). Values reported are %$t_{pf}$ of the summed forces from a pair of supporting feet; either right rear and left front, or left rear and right front. Description of a 'mean' force trace begins with right rear foot strike. Peak horizontal deceleration occurs first, giving a mean (± S.D.) value of 11±4 %. Peak lateral force occurs next, at 15±6 %. Both front and rear feet produce a lateral force, but the resultant force is always lateral to the rear foot that is in contact with the ground. Peak vertical force occurs next, at 20±4 %. Finally, peak horizontal acceleration occurs at 35±5 %. Mean values for %$t_{pf}$ during the period of left rear foot support are the right-side values plus 50 %.

### *Lateral trunk bending*

Values of $t_{bf}$ are always negative (Fig. 7), indicating that maximum concave curvature of the trunk occurs before ipsilateral rear foot strike. The significant relationship between speed and $t_{bf}$ (least-squares regression, $P<0.001$, $r^2=0.482$) indicates that, as speed increases, an increasing percentage of the locomotor cycle elapses between the time of maximum concave curvature and ipsilateral rear foot strike.

Both the onset–bending delay and the offset–bending delay of the IC and LD muscles are significantly affected by speed (Fig. 8). As speed increases, there is a progressively shorter delay between the onset of muscle activity and the time of maximum convex trunk curvature and between the offset of muscle activity and maximum concave trunk curvature. The situation is different for the onset–bending delay of the TS, in which the delay is not significantly affected by speed, being approximately 3 ms. The offset–bending delay for the TS is

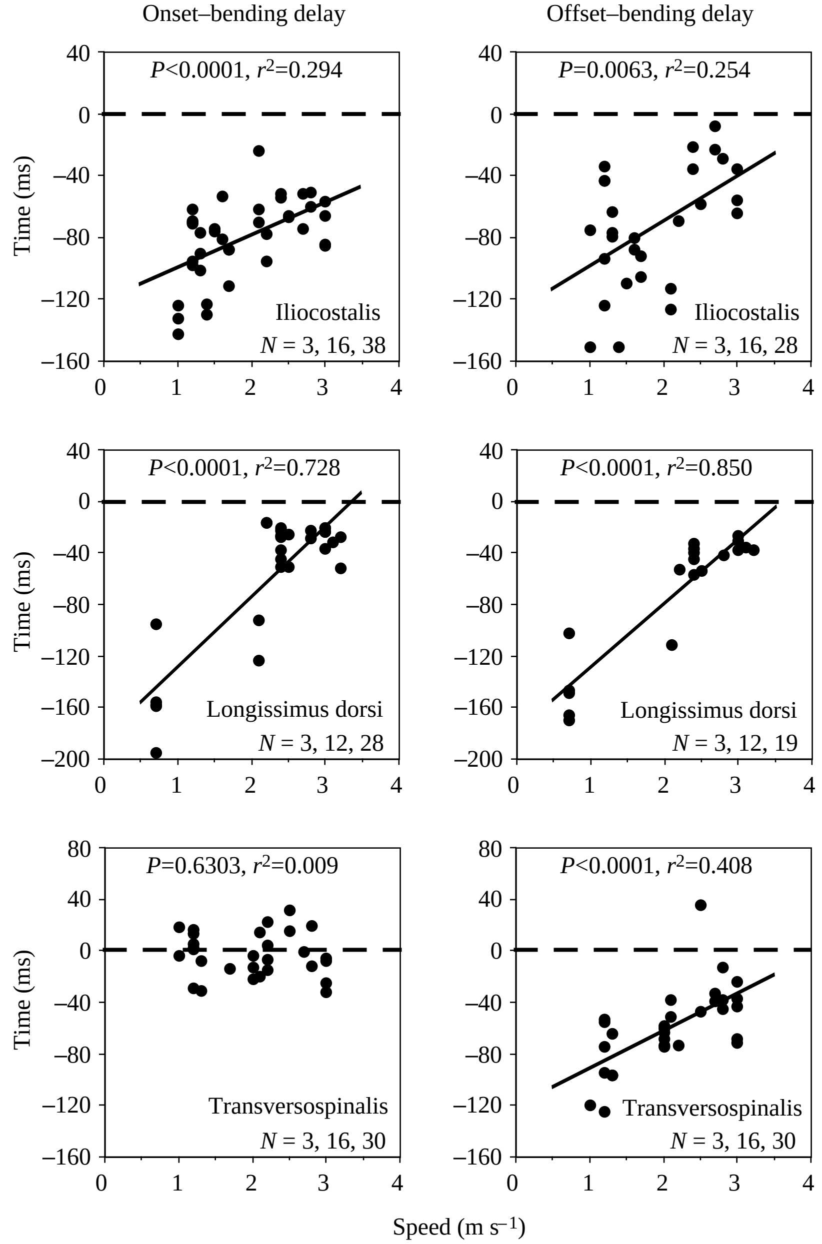

Fig. 8. Graphs of onset–bending delay (left panel) and offset–bending delay (right panel) as a function of speed, for each epaxial muscle, calculated using data from the middle site. These data indicate the amount of time elapsed between EMG activity onset and ipsilateral maximum convex curvature of the trunk, or EMG activity offset and ipsilateral maximum concave curvature of the trunk. Negative values indicate that muscle onset or offset occurs prior to maximum curvature. The horizontal dashed line in each graph indicates zero. Least-squares regression lines for each set of delays against speed are shown. In all cases, the hypothesis that the epaxial muscles produce lateral bending predicts that the data points will be centered around zero, or some small negative value. Furthermore, the hypothesis predicts that delays should be independent of speed. Only the onset–bending delay data from the transversospinalis muscle fit the prediction of the null hypothesis. Sample sizes indicated on each graph are for individuals, trials and locomotor cycles, respectively.

significantly affected by speed and shows the same pattern as the IC and LD muscles (Fig. 8), with a decrease in delay with increasing speed. Therefore, only one (TS onset–bending delay) of the six calculated parameters indicates an invariant relationship between epaxial muscle activity and lateral trunk bending.

### *Denervation experiments*

The EMGs recorded from the denervated animal showed that the denervations were successful in eliminating muscle activity in the LD and IC. Signals from the TS of this individual were qualitatively similar to those obtained from intact animals (Fig. 9).

The initial denervation experiments indicated no significant effect of the denervation on maximum amplitude of lateral bending ($F$=1.5, $P$=0.24). However, these data do show a significant decrease in lateral bending associated with increased speed, from a high value of approximately 25 ° at 0.5 $\mathrm{m\,s^{-1}}$ to a low of approximately 10 ° at 3.5 $\mathrm{m\,s^{-1}}$ (Fig. 10A). This decrease in bending is apparent in both the pre- and post-denervation data and is statistically significant ($F$=210.0, $P$<0.0001 for the covariate, speed).

Data from the fine-scale denervation experiments showed the same basic pattern seen in the initial denervation experiments; there was no significant treatment effect (walking, $F$=0.24, $P$=0.71; running, $F$=2.96, $P$=0.34), but as speed increased, bending amplitude decreased (Fig. 10B). These data further showed that the decrease in bending with speed was most pronounced in the mid-trunk region, and less pronounced (or not seen) in the more anterior and posterior regions of the trunk. For analyses of both walking and running, significant individual effects (walking, $F$=7.53, $P$=0.007; running, $F$=14.09, $P$<0.001) as well as angle effects (walking, $F$=20.1, $P$<0.001; running, $F$=130.7, $P$<0.001) were found.

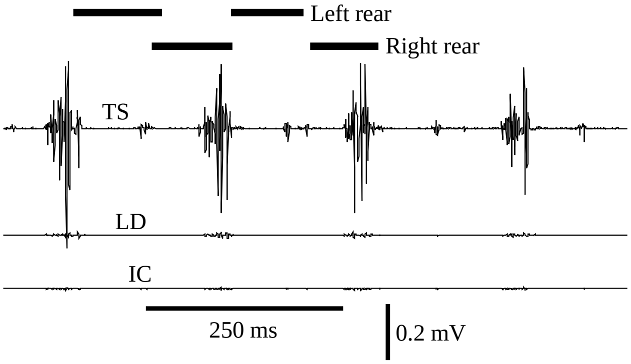

Fig. 9. EMG recording from a denervated animal, confirming the denervation of the iliocostalis (IC) and longissimus dorsi (LD) muscles. The bars above the traces represent periods of support by the indicated foot. EMGs are from right-side muscles. Electrodes were positioned mid-way between the pectoral and pelvic girdles. The timing of activity in the transversospinalis (TS) is similar to data from intact animals (compare Fig. 5). The scale bars apply to all three traces.

## Discussion

Analyses of the muscle onset and offset times relative to the timing of trunk bending do not support the hypothesis that the epaxial muscles produce lateral bending during locomotion (Fig. 8). If the epaxial muscles produced lateral bending, one would predict that the onset of muscle activity would either be coincident with the time of maximum convexity or would precede it by a small, constant amount of time. The force generated by muscle contraction should cause the bending to reverse direction. In this case, values for onset–bending delay should be zero or some small negative number. This prediction was not supported in either the IC or LD muscles, because the delay was not constant for these muscles and was often large (Fig. 8). The delay showed maximum values of 150 and 200 ms in the IC and LD muscles, respectively. In the TS, however, the delay was as predicted for a lateral bending muscle, with a mean of 3 ms and no relationship with speed (Fig. 8). If the epaxial muscles produced lateral bending, the time of muscle offset should be coincident with maximum concave curvature, or any delay should be small and constant. If these muscles produce lateral bending, they should be active until approximately the time of maximum concavity and then cease activity. This would result in values of offset–bending delay close to zero. The recorded delays between muscle offset and maximum concave curvature, however, all showed a significant relationship to speed and were large at low speeds (Fig. 8). In all three muscles, the delay became progressively shorter with increasing speed.

It is possible that the onset–bending delay represents a period of initial ‘braking’ of lateral bending by the epaxial muscles, before the reversal in bending direction. This would be a situation analogous to that found in flying birds, where it has been reported that as much as half of the activity of the main upstroke muscle (the supracoracoideus) occurs before the beginning of upstroke (Dial *et al*. 1988, 1991). If the epaxial muscles are initially braking lateral bending, we would expect braking to be most important at high speeds, when there is less time to reverse bending in the trunk. However, the onset–bending delays reported here are counter to those expected for this braking function, in that the longest delays were at the slowest speeds (Fig. 8).

The muscle denervation experiments provide a direct test of the hypothesis that the epaxial muscles produce lateral bending. These experimental treatments resulted in neither a decrease nor an increase in the maximum amplitude of lateral bending (Fig. 10). One possible explanation for this apparent lack of effect is that compensation occurs in regions of the trunk anterior and/or posterior to the denervated region. By dividing the trunk into more angles, as was carried out for the second two animals (Fig. 10B), compensation should become apparent as an increase in curvature anterior and/or posterior to the denervated area. This did not occur (Fig. 10B). This lack of increase in bending after denervation is further evidence against the possibility that the epaxial muscles act to ‘brake’ lateral bending, as an increase in bending would be expected if this were the case. The denervation experiments provide

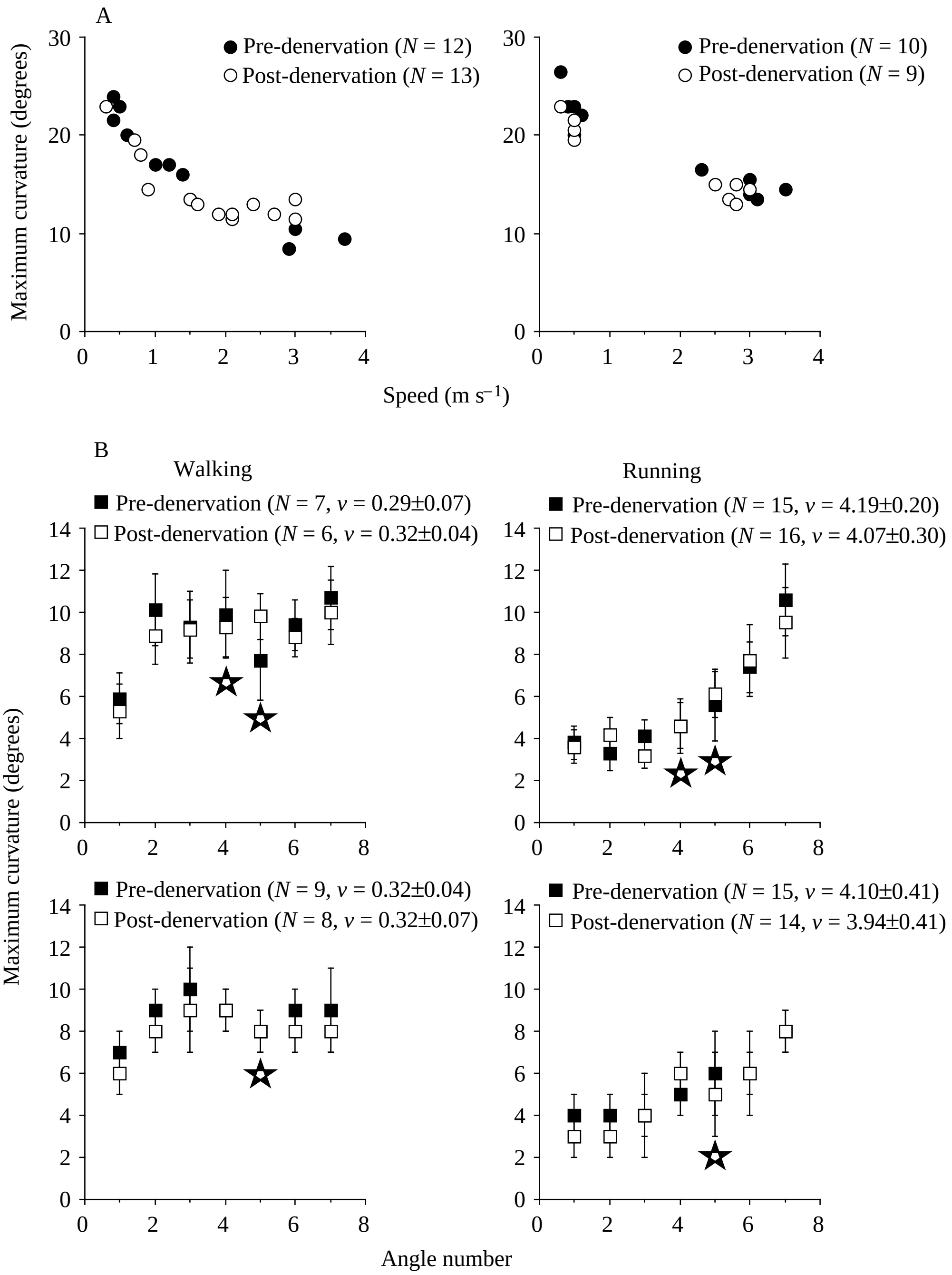

Fig. 10. Graphs comparing lateral bending data before and after epaxial denervation treatments. All treatments indicate no significant difference in lateral trunk bending due to denervation. (A) Initial denervation experiment. Pre- and post-denervation lateral bending data from two animals collected over a range of speeds. The angle calculated describes bending of the whole trunk between the limb girdles (points 1, 5 and 9, Fig. 2B). The legend gives the number of pre- and post-denervation trials. Each point is a mean value for a trial, which typically consisted of 2–3 locomotor cycles. Analysis of covariance showed no significant difference between normal and post-denervation maximum curvature. (B) Fine-scale denervation experiments. Pre- and post-denervation lateral bending data from two animals, at walking (left panels) and running (right panels) speeds. The animal's trunk between the limb girdles was divided into seven angles (indicated on the *x*-axis), with angle one being most anterior and angle seven being most posterior (see Fig. 2B for details). The legend gives the number of pre- and post-denervation trials, and the mean (± S.D.) speed (*v*, in m s$^{-1}$). The curvature values are mean maximum values from the number of trials indicated. Each trial typically consisted of 2–3 locomotor cycles. Error bars give ±1 S.D. Stars indicate the angle(s) over which the longissimus dorsi and iliocostalis muscles were denervated. Some mean values for maximum curvature are identical before and after denervation, so that only one square is visible for that angle. Analysis of variance indicated no significant difference between pre- and post-denervation maximum curvature values.

strong evidence that neither the IC nor the LD muscles are involved in the production of or resistance to lateral bending. The TS was not denervated, but the anatomy of this muscle (it would have the smallest moment-arm of the epaxial muscles, owing to its medial position), its duration of activity and its offset time relative to lateral bending all argue against the production of lateral bending by this muscle.

Lateral bending does occur during lizard locomotion, but the temporal relationship found between footfalls and trunk bending is unexpected. The $t_{\mathrm{bf}}$ data indicate a changing relationship between movements of the trunk and movements of the limbs with increasing speed (Fig. 7) and suggest that increases in stride length due to lateral trunk bending may occur only at very low speeds (when values of $t_{\mathrm{bf}}$ approach zero). For lateral bending to maximally increases stride length, the trunk should be maximally concave at approximately the time of ipsilateral rear foot strike, allowing for maximum forward extension of the rear foot and resulting in $t_{\mathrm{bf}}$ values of

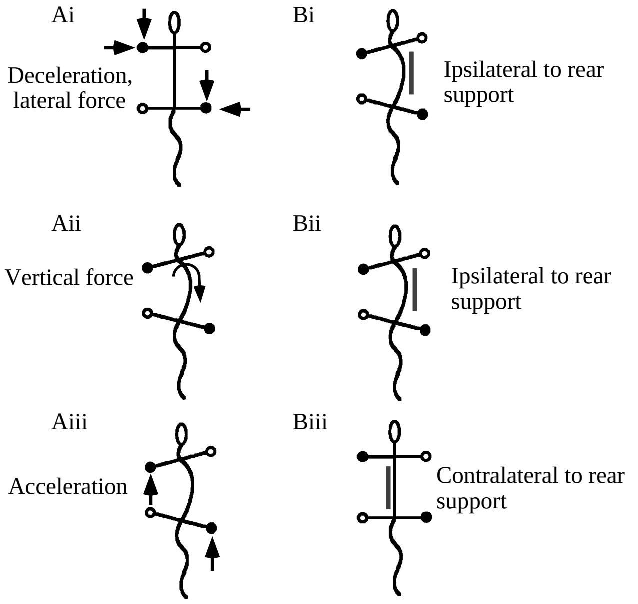

Fig. 11. Simplified models depicting the net effect of ground reaction forces on the trunk of a running lizard. The left side (A) shows the forces, their principal line of action (arrows) and the basic configuration of the trunk when the maximum force occurs. Support-phase feet are indicated by filled circles, swing-phase feet by open circles. The right side (B) shows the net effect these forces should have on the animal's trunk and indicates when the epaxial muscles would have to be active to resist these effects, given a unilateral, uniphasic pattern of activity.

approximately 0 %. However, the data show a range of $t_{\text{bf}}$ values between −10 % and −40 % (Fig. 7). Furthermore, there is a significant relationship between $t_{\text{bf}}$ and the speed of the animal, so that the delay becomes an increasingly larger percentage of the locomotor cycle as speed increases. As $t_{\text{bf}}$ becomes larger than zero (either more negative or more positive), lateral bending of the trunk is able to contribute less to stride length. In fact, values of $t_{\text{bf}}$ larger than 25 % would mean that the trunk is actually convex on the side of rear foot strike, and lateral trunk bending should, in this case, cause a decrease in stride length. A detailed consideration of the role of lateral bending during locomotion in lizards is beyond the scope of this paper. However, the apparent dynamic relationship between limb support and trunk bending (Fig. 7), and the significant decrease in maximum bending amplitude seen with increased speed (Fig. 10), should be taken into account when considering the possible function of lateral bending during lizard locomotion.

One obvious question left unanswered by this study is how trunk bending is produced during locomotion. Bending of the trunk could be produced passively by horizontally and laterally oriented forces acting on the limb girdles (Fig. 11), but the magnitudes of these forces are least when bending magnitude is greatest (i.e. at low speeds), making this unlikely. Recordings from the hypaxial muscles of walking iguanas indicate that several hypaxial muscles with an approximately longitudinal orientation (e.g. the external oblique, rectus abdominis) exhibit activity that is appropriately timed for the production of lateral bending (Carrier, 1990). Work is currently under way to evaluate the possibility that the hypaxial muscles produce lateral bending in water monitors.

Although the epaxial muscles do not appear to produce lateral bending of the trunk, they do show a regular pattern of activity during locomotion, suggesting that they perform some locomotor function. The only kinematic parameter whose relationship with epaxial muscle activity is not affected by speed is the time of rear foot strike. The LD and IC muscles both begin activity approximately synchronously with rear foot strike and cease activity approximately 50 % of the locomotor cycle later. The TS begins activity later in the locomotor cycle, but ceases activity at approximately the same time as the LD and IC muscles (Fig. 6). The correlation between epaxial muscle activity and time of rear foot strike suggests the possibility that the epaxial muscles are postural; i.e. they function to counteract some effect of the ground reaction forces that the animal experiences as it walks or runs.

Effects of the ground reaction forces on the trunk are predictable, given knowledge of when the peak forces occur, the general configuration of the trunk as it experiences these forces and the general line of action of the forces (Fig. 11A). All forces with a horizontal orientation (acceleration, deceleration and lateral forces) will tend to cause rotation of the limb girdles in a horizontal plane, which should result in lateral bending of the trunk (Fig. 11Ai,iii). Some portion of the vertical force is translated into long-axis torsion because the vertical force pulls the animal towards the ground while lateral bending of the trunk shifts the center of mass towards the unsupported side of the pectoral girdle. Therefore, the animal will tend to fall to the unsupported side of the pectoral girdle, resulting in long-axis torsion in the trunk (Fig. 11Aii).

Using these predictions, one may determine whether the observed timing of epaxial muscle activity is appropriate for resisting the effects of these forces (Fig. 11B). The possibility shown in Fig. 11Biii can be excluded, because the epaxial muscles would have to be active contralateral to rear limb support to counteract the acceleration ground force, and observations contradict this. The observed muscle activity is generally consistent with both Fig. 11Bi and Fig. 11Bii. However, if the epaxial muscles function to counteract lateral bending produced by the deceleration and lateral forces (Fig. 11Ai), an increase in lateral bending after epaxial denervation would be expected and this was not found (Fig. 10). Furthermore, the timing of muscle activity relative to peak deceleration and lateral forces (Fig. 11Ai) is not as predicted. The IC and LD are active throughout rear foot support, which means they would be active at least twice as long after peak deceleration and lateral forces than before them (Fig. 6). A more reasonable expectation is for the muscles to be active for approximately equal amounts of time before and after the time of peak force, as is the case for peak vertical force (Fig. 6).

The results of the denervation experiments do not support the possibility shown in Fig. 11Bii. If the role of the epaxial muscles is to counteract the effects of the vertical component of the ground forces then, after denervation, the animals should not perform at pre-denervation levels. Contrary to this, all denervated animals were capable of running at pre-denervation speeds (Fig. 10A). The lack of a significant, measurable effect of denervation may be due to compensation in the sagittal plane. In fact, the zygapophyseal orientations of lizard vertebrae are such that the trunk passively resists sagittal bending to a greater degree than lateral bending (Rockwell *et al*. 1938). It may be possible, therefore, that the intact portions of the epaxial muscles anterior and posterior to the denervated segment, in concert with the structure of the vertebral column, allowed the animals to compensate for the denervated portions of the trunk. Of the possibilities discussed here, the hypothesis most consistent with the available data is that the epaxial muscles of lizards are postural and function to counteract the effects of the vertical component of the ground forces that the animals experience as they run.

### *Comparisons with other tetrapods*

We may consider the results of this study in broader terms by comparing the data obtained here with similar data available for other vertebrate groups. Throughout these comparisons, the results of a few or even a single study of an organism must be taken as representative of a fairly large and diverse vertebrate taxon. These comparisons are also complicated by differences among taxa in preferred temperatures (e.g. salamanders *versus* lizards), possible differences in sensory mechanisms (e.g. anamniotes *versus* amniotes) and differences in size (e.g. 80 g salamanders *versus* 1 kg lizards *versus* 20 kg dogs). However, these comparisons appear to indicate elemental changes in axial function between taxa and, therefore, are taken to reflect fundamental evolutionary changes in the vertebrate axial system. Examination of the anatomy, axial kinematics and epaxial muscle activation patterns of salamanders, lizards and mammals indicates that, while in some respects lizards are intermediate between amphibians and mammals, in other respects they tend to be more similar to mammals than to amphibians.

The anatomy of the epaxial muscles of lizards is more similar to that of other amniotes than to that of salamanders. The epaxial muscles of salamanders are myomeric, so that adjacent vertebral segments are spanned by a longitudinally oriented block of muscle of only one vertebral segment in length. The epaxial muscles of lizards, however, are differentiated into three discrete muscles, as are the epaxial muscles of mammals. These gross differences in muscular anatomy are mirrored in gross differences in the spinal motor pools of these muscles. In amniotes, there is an isomorphic ‘mapping’ of motoneurons within the spinal cord, so that motoneurons innervating a particular axial muscle form a discrete motor pool within the spinal cord (Smith and Hollyday, 1983; Fetcho, 1986*b*). In all anamniotes studied, this mapping does not occur (Wallén *et al*. 1985; Fetcho, 1986*a*; Fetcho and Reich, 1992). On both a gross anatomical and a neuroanatomical level, the epaxial muscles of lepidosaurs are more similar to mammalian epaxial muscles than to salamander epaxial muscles. Assuming that some degree of covariance between form and function exists, it is more logical to predict similar functions for the epaxial muscles of lizards and mammals than for lizards and salamanders.

There are also gross differences in the kinematics of lateral bending among salamanders, lizards and mammals. Increased speed in the Pacific giant salamander *Dicamptodon tenebrosus* is correlated with increased amplitude of lateral bending, especially in the girdle regions (Ashley-Ross, 1994). Lizards, conversely, show a decrease in lateral bending with increasing speed (Daan and Belterman, 1968; this study). This decreased amplitude is most apparent at mid-trunk (Fig. 10). The pattern of lateral bending in the trunk of a basal mammal, the American opossum *Monodelphis domestica*, is qualitatively similar to the pattern seen in lizards. Pridmore (1992) has reported that the American opossum exhibits significant lateral bending during walking and shows a reduction in lateral bending when it switches to a trot. Although salamanders and lizards both bend laterally during locomotion, there are significant qualitative and quantitative differences in their patterns of lateral bending. Lizards and opossums show a similar pattern of decreasing amplitude of lateral bending in the trunk with increasing speed.

Another major difference between salamanders and lizards is obvious in the gross activation pattern of the epaxial muscles relative to foot support. The epaxials of trotting tiger salamanders *Ambystoma tigrinum* are active contralateral to rear limb support (Frolich and Biewener, 1992), while the epaxial muscles of lizards are active ipsilateral to rear limb support (Fig. 5). Preliminary data from the epaxial muscles of the green iguana *Iguana iguana* also show activity ipsilateral to rear limb support (D. Ritter, unpublished data). Comparison with other amniotes is complicated by the prevalence of bilateral activity in the epaxial muscles of birds and mammals (e.g. English, 1980; Gatesy and Dial, 1993). Data from dogs, however, indicate that the thoracic epaxials often exhibit unilateral activity during trotting. When unilateral activity is recorded, it is ipsilateral to rear limb support (Nassar and Carrier, 1992), as is found for lizards. Again, this aspect of epaxial muscle activity is, in some respects, more similar to the mammalian than to the amphibian condition. In all of the above comparisons, lizards are most accurately described as intermediate between salamanders and mammals.

### *Evolutionary scenario*

If the functional role of the epaxial muscles of lizards is to provide postural support to the trunk (the F2 character state in Fig. 1), then this may be a trait that was present in the common ancestor of all amniotes. At this position, the proposed postural role of the epaxial muscles may be considered a ‘key innovation’ (see Liem, 1973; Liem and Osse, 1975) in the evolution of the locomotor system of vertebrates. Furthermore, if the epaxial muscles of lizards are postural, their function in lateral bending in snakes must be considered to be a derived condition of that group (*sensu* Gans, 1986; cf. Ritter, 1992). The hypothesis that the switch to a postural role for the epaxial muscles is a key innovation raises two general questions: (1) what do taxa with this key innovation 'gain' over those taxa without it and, (2) how is this new function incorporated into a pre-existing functional complex?

By employing the epaxial muscles in a postural role, ancestral amniotes and their descendants may have been able to increase their terrestrial locomotor speed by increasing the magnitude of the peak vertical forces that they were capable of withstanding. As a limbed animal increases its speed, its feet are in contact with the ground for shorter periods, leading to an increase in the peak vertical force that the animal experiences. Animals must be able to resist these increasing peak vertical forces in order to increase locomotor speed. Lizards are certainly capable of greater maximum speeds than salamanders, whether measured in relative or absolute terms (lizards, Huey and Hertz, 1982; Huey and Bennett, 1987; this study: salamanders, Edwards, 1976; Ashley-Ross, 1994). Evidence of a postural limit on terrestrial speed for salamanders is provided by the observation that, when salamanders exceed some maximum speed, they fall to the ground in a uncoordinated fashion (Ashley-Ross, 1994). Enhanced terrestrial performance may have facilitated the movement of early amniotes into the terrestrial environment. Indeed, it has been suggested that the peculiar structure of the neural spines in the vertebral column of some of the earliest truly terrestrial tetrapods was associated with the use of the epaxial muscles in dorsiflexion of the trunk (Sumida, 1987, 1989, 1990). Frey (1984) has suggested that the epaxial muscles of crocodilians also function in a postural role. Thus, while this proposal of a key innovation in the axial system is speculative, it does have several complementary lines of support.

Examples of key innovations usually invoke duplication of parts as the means of removing constraints on a given part of a functional system (see Lauder and Liem, 1989, and references therein). The proposed innovation of new epaxial muscle function in amniotes, however, probably did not occur by duplication of parts, but by a change in the basic ecology of the in-group clade (amniotes) relative to the out-group clade (anamniotes). Anamniotes, at least in their larval state, swim through their aquatic environment using lateral bending of the trunk and tail. These lateral movements are powered, at least in part, by the epaxial muscles. Concomitant with the loss of an aquatic larval stage in the ancestor of amniotes, perhaps, was the loss of a need to employ the epaxial muscles in swimming. At the same time, early amniotes were becoming more fully terrestrial and may have been subject to enhanced selection for terrestrial locomotor performance. This scenario is highly speculative. However, it fits well with existing data and generates several testable hypotheses. For example, running lizards should be capable of successfully counteracting greater peak vertical forces than running salamanders, while swimming salamanders should be capable of greater peak velocities than swimming lizards.

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