

RESEARCH ARTICLE

Three-dimensional trajectories affect the epaxial muscle activity of arboreal snakes crossing gaps

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ABSTRACT

The need for long-axis support is widespread among non-aquatic vertebrates and may be particularly acute for arboreal snakes when many vertebrae span sizable gaps between branches with diverse orientations. Hence, we used brown tree snakes (*Boiga irregularis*) bridging gaps to test how three-dimensional trajectories affected muscle activity and whether these motor patterns differed from those for the locomotion of terrestrial snakes and movements of other vertebrates. We used five trajectories: pitch angles of 90, 0 and –90 deg (downward) when yaw=0 deg, and 90 deg yaw angles to the left and right when pitch=0 deg. We recorded movement and electromyograms from the three largest epaxial muscles, which from dorsal to ventral are the semispinalis-spinalis (SSP), longissimus dorsi (LD) and iliocostalis (IL). Overall, the SSP had extensive bilateral activity, which resembled the motor pattern during the dorsiflexion of sidewinding snakes. Unlike any previously described terrestrial snake locomotion, bilateral activity of the LD and IL was also common during gap bridging. The largest amounts of muscle activity usually occurred for horizontal gaps, and muscle activity decreased markedly as soon as the snake's head touched the far edge of the gap. Snakes had the least amount of muscle activity for pitch=–90 deg. While turning sideways, muscles on the convex side had less activity when turning compared with the concave side. Hence, the orientation relative to gravity profoundly affected muscle activity during gap bridging, and these complex three-dimensional movements involved several previously undescribed variants of axial motor pattern.

KEY WORDS: *Boiga irregularis*, Cantilever, Arboreal, Electromyography, Locomotion, Coactivation

INTRODUCTION

Arboreal habitats have considerable three-dimensional complexity as well as sizable gaps between branches, and snakes are one of many groups of animals with several evolutionary lineages that have independently acquired anatomical and behavioral specializations well suited for locomotion in trees (Cartmill, 1985). The ability to cross gaps can be advantageous for allowing animals to take more direct routes to a destination, and the elongate body of snakes is one key factor that facilitates reaching across large gaps (Temerin and Cant, 1983). Yet, gaps can only be bridged if the relevant anatomical structures are rigid enough to prevent collapsing. Activation of muscle provides a mechanism for increasing stiffness.

The need to support the vertebral column is widespread in diverse non-aquatic animals with and without limbs. For example, generalized tetrapods commonly must support the vertebral column between two points (pectoral and pelvic girdles), whereas during bipedal running, animals such as kangaroo rats and lizards have cantilevered loading of the trunk and tail anterior and posterior to the pelvic girdle, respectively. Similarly, snakes crossing from one branch to another transiently experience both suspension between two points and cantilevered loading, in addition to an impressive diversity of loading regimes that are associated with moving in three dimensions. Hence, the diversity and magnitudes of axial loading regimes have been taken to an interesting extreme in arboreal snakes.

Despite having some advantages, a more elongate shape exaggerates some effects of changing the orientation of the long axis of the body relative to gravity. For example, when a cube and an elongate rectangular prism with equal volume are balanced on the edge of a table so that one-half of their length extends beyond the edge, the center of mass for the unsupported portions will be farther from the edge for the rectangular prism than the cube. Because the relevant lever arm and buckling torque are proportional to the length of the unsupported fraction, they will be greater for the more elongate object. Similarly, when equal fractions of the mass of the elongate object extend beyond a supporting ledge, the lever arm contributing to the buckling torque will be much longer when the long axis of the object is horizontal rather than vertical. Indeed, the gap-bridging performance of snakes is reduced for horizontal compared to vertical trajectories (Byrnes and Jayne, 2012; Hoefer and Jayne, 2013). These aforementioned factors and findings suggest that snakes use their axial muscles differently when they bridge gaps to destinations with different three-dimensional locations, but relevant experimental data are presently lacking.

In common with the generalized vertebrae body plan, the axial muscles of snakes are segmented (Gasc, 1974). Hence, a key issue is how the activity of such serially homologous structures is coordinated as the animals move. A challenge for understanding this is that, unlike the myomeres of most fishes, individual axial muscle segments in snakes often have tendons that span several vertebrae (Fig. 1). Furthermore, several slender colubroid species that are arboreal specialists have convergently evolved tendons that are longer than those in any non-arboreal taxa (Jayne, 1982). Despite plausible proposed benefits of tendinous elongation for preventing sagging between branches and bridging gaps (Gasc, 1974; Jayne, 1982; Jayne and Riley, 2007; Lillywhite and Henderson, 1993), no previous study has determined how such long muscles are used in either of these ecological contexts. Hence, our electromyographic study of gap bridging focused on three of the longest and largest epaxial muscles that often comprise well over half of the total cross-sectional area of axial muscles (Hoefer and Jayne, 2013; Ruben, 1977), and from dorsal to ventral these muscles are the semispinalis-spinalis (SSP), the longissimus dorsi (LD) and the iliocostalis (IL) (Fig. 1).

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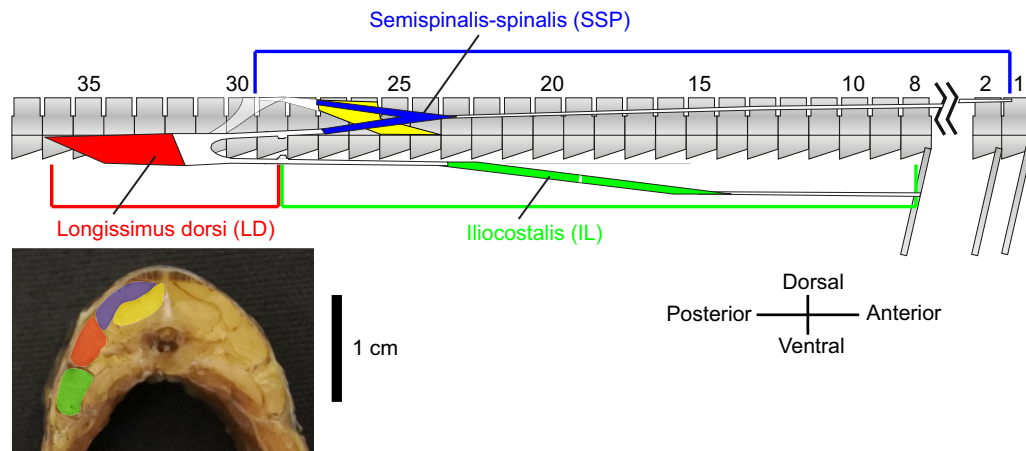


Fig. 1. Major epaxial muscles of *Boiga irregularis* at mid-body. The schematic figure is modified from a previous study (Jayne and Riley, 2007), in which more detailed anatomical descriptions are provided. The contractile tissue portions of individual segments are colored, whereas tendons are white. The thin black horizontal line indicates the location of the pre- and post-zygophyses. The numbering of the vertebrae begins at the most anterior extent of the SSP, and the horizontal colored lines indicate the longitudinal extent of each muscle used to define time intervals in the kinematic analysis (Fig. 2). The fibers of the more dorsal head of the semispinalis-spinalis (SSP) attach posteriorly to a triangular tendon that also gives rise to the fibers of the multifidus muscle (yellow), which is deep to the SSP. Midway along the length of the tendon that connects the ventral head of the SSP to the longissimus dorsi (LD), a diffuse portion of tendon contributes to a tendinous sheet that attaches to the zygapophyses. The LD also has a diffuse tendinous portion that extends dorsal and superficial to the SSP before attaching to the neural spines, and another between the LD and the iliocostalis (IL) extends medially and contributes to a tendinous sheet that separates the LD and IL. The inset is a cross-section from 30% snout–vent length (SVL) of a *B. irregularis* with SVL=160 cm.

Previous experiments have determined the *in vivo* patterns of muscle activity that vertebrates, including snakes, use to bend the vertebral column and generate propulsive forces. Similar to the axial motor patterns of swimming fishes, snakes performing lateral undulatory locomotion have an alternating unilateral activity of the SSP, LD and IL at a given longitudinal location, and this pattern of activity is propagated posteriorly (Jayne, 1988a; Moon and Gans, 1998). Unlike the planar movements of terrestrial lateral undulation, a sidewinding snake arches its back as it lifts the body above the ground using the apparently derived motor pattern of bilaterally activating the SSP (Jayne, 1988b). Overall, the activity of the SSP, LD and IL muscles during terrestrial lateral undulation and sidewinding locomotion is consistent with active shortening of the muscle fibers, but some isometric activity probably occurs during concertina locomotion as the snake forms a region of static contact by wedging itself against the sides of a tunnel (Jayne, 1988b). Thus, snakes have some plasticity of the axial motor pattern in response to structural variation in the environment, and previous electromyographic studies also provide insights for predicting some aspects of motor pattern for snakes bridging gaps.

We varied the three-dimensional trajectories for bridging gaps to test how body orientation affects the activity of the major epaxial muscles in snakes. Based on its anatomical location (Fig. 1) and role during sidewinding, we expected the SSP to have bilateral activity to prevent downward buckling at the edge of the gap of all horizontal trajectories and to actively dorsiflex the vertebral column when snakes went up vertically. For gaps with horizontal turns, we expected unilateral activity in the LD and IL to make a region of the body more concave toward the side with muscle activity. We expected horizontal trajectories to have the most SSP activity. Given some precedents for snakes using gravity to move downward passively (Astley and Jayne, 2007), we expected the least amount of muscle activity for the gap with a downward vertical trajectory. We also sought to compare the axial motor patterns used during gap bridging with those used for other behaviors of snakes as well as a wide variety of other vertebrates for which moving and supporting the vertebral column is critically important.

MATERIALS AND METHODS

Experimental subjects

We studied brown tree snakes, *Boiga irregularis* (Merrem 1802), for several reasons. Firstly, the gap-bridging performance, arboreal locomotion and axial anatomy of this species are well known (Byrnes and Jayne, 2012; Hoefer and Jayne, 2013; Jayne et al., 2015; Jayne and Riley, 2007). Secondly, brown tree snakes are relatively slender for a given length (Hoefer and Jayne, 2013; Jayne and Riley, 2007) and the tendons of the SSP are very long (Fig. 1), both of which typify specialized arboreal colubroid snake species. Finally, compared with some extremely attenuate colubrid arboreal snakes in the genera *Ahaetulla*, *Dendrelaphis* and *Chrysopelea*, brown tree snakes attain large enough sizes that implanting EMG electrodes in single axial muscle segments is facilitated.

We performed experiments with a total of eight individuals, but we only quantitatively analyzed the results for the seven individuals for which the most electrodes remained in place and for which all of the gaps were crossed. These seven individuals were adults with similar snout–vent lengths (SVL) (mean=145 cm, range=120–164 cm) and mass (mean=529 g, range=381–638 g). We collected all snakes in Guam in 2010 and 2011 and brought them to the University of Cincinnati, where all experiments were performed during 2014 and 2015. All snakes were housed in a variety of cages on 12 h:12 h light:dark cycles, with incandescent bulbs that allowed the snakes to regulate a daytime body temperature between 25 and 33°C. During all experiments, the body temperatures of the snakes were between 29 and 31°C, which is within the range of active field body temperatures for this species (Anderson et al., 2005). We captured and imported all snakes with permits (MA214902-0; MA3500A-0) from the US Fish and Wildlife Service, and all research, procedures and husbandry were in compliance with the Institutional Animal Care and Use Committee of the University of Cincinnati requirements (protocol no. 07-01-08-01).

Experimental setup and procedures

We used pairs of artificial branches to create gaps. Both the initial perch upon which the snake was placed and the destination perch on

the far side of a gap were made of PVC pipes with a diameter of 5 cm, and their 57 cm lengths were oriented horizontally. As in previous experiments on gap bridging in this species, we covered the pipes with Nashua 394 duct tape (Berry Plastics, Franklin, KY, USA) to create a rougher surface and reduce slipping compared with an uncovered pipe (Hoefer and Jayne, 2013). The initial perch was always 1.5 m above the floor. Both perches had two length-wise rows of wooden pegs (diameter 6 mm, length 2.5 cm), with a pair of 5 cm long pegs at the end of the initial perch nearest the gap and a pair of 10 cm long pegs at the end of the destination perch nearest the gap. All pegs were oriented approximately 45 deg upward relative to the horizontal, with 10 cm between each adjacent pair.

We arranged the two perches to create five different trajectories required to cross the gap. Three of these treatments had a yaw angle of 0 deg and pitch angles of 90 deg (straight up), 0 deg (horizontal) and –90 deg (straight down). The two remaining treatments had pitch angles of 0 deg and yaw angles of 90 deg to the left and right. All of the gap distances were constant for each individual snake, but the gap distances among individuals varied slightly as they were approximately 5 cm longer than the distance from the snout of the snake to the most anterior electrode location (approximately 23% SVL). We used gap distances that were approximately 75% of the maximal expected gap distance for a horizontal trajectory of the snakes for a particular size (Jayne and Riley, 2007) so that a substantial effort would be required but the snake could still bridge the gap repeatedly. We randomized the order of perch orientations among different individuals, and snakes bridged the gap for each perch orientation a minimum of five times before we proceeded with the next treatment.

In an attempt to obtain the maximal amplitudes of muscle activity, we performed a control experiment during which the snakes performed concertina locomotion in a tunnel 9 cm wide as we held the tail of the snakes and pulled backwards with sufficient force to prevent forward progress.

Kinematics

In the regions of interest, we marked every other mid-dorsal scale of the snakes with pieces of Scotchlite reflective tape (3M, St Paul, MN, USA) that were 4×2.5 mm (Movie 1). We obtained simultaneous video images (30 images s⁻¹) of dorsal and lateral views using either Vixia HF G10s (Canon, Melville, NY, USA) or Lumix DMC FZ200 (Panasonic, Newark, NJ, USA) HD video cameras with resolutions of 1920×1080 pixels. To synchronize the two views, we used a 1 s square wave voltage to simultaneously actuate LEDs that were visible to both cameras.

We used MaxTRAQ 2D software (Innovision Systems, Columbiaville, MI, USA) to perform frame-by-frame motion analysis of each trial. For each trial and each muscle segment, we recorded the times at which: (1) the anterior portion of the muscle segment entered the gap, (2) the posterior end of the muscle entered the gap, (3) the head of the snake first touched the destination perch, (4) the anterior end of the muscle touched the edge of the destination perch and (5) the posterior end of the muscle left the gap (Fig. 2A). We then used successive pairs of these times to define the time intervals that were used for further analysis. We chose the location of the most posterior electrode site so that it lacked an interval when the entire longitudinal extent of a muscle segment was within the gap and the anterior end of the snake was not touching the destination perch (Fig. 2B). Hence, event 3 was lacking for this far posterior electrode site.

Electromyography

We made bipolar EMG electrodes with 0.05 mm diameter, poly-coated, stainless steel wire (California Fine Wire Company, CA,

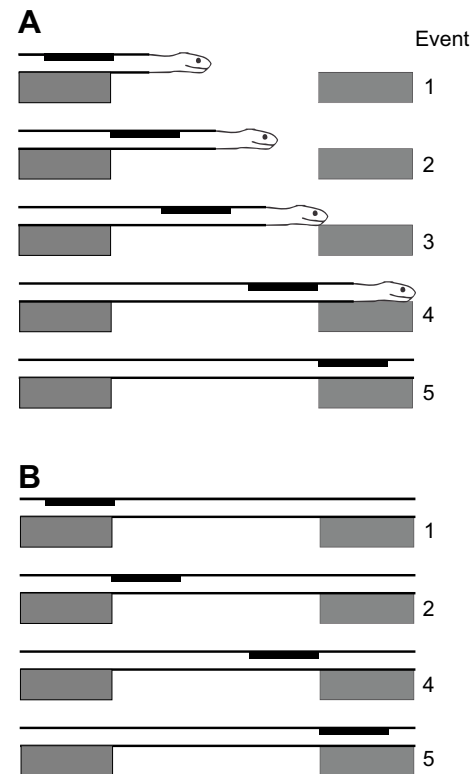


Fig. 2. Schematic view of the events used to define time intervals as the epaxial muscle segments crossed the gap. The black rectangle within the snake represents the longitudinal extent of a single muscle segment of interest (Fig. 1), and the gray rectangles represent the two perches. Unlike the anterior site (A), the muscles in the far posterior site (B) were never confined to the gap before the anterior end of the snake touched the destination perch. The figure is not drawn to scale.

USA) using the same overall methods as in previous electromyographic studies of snake locomotion (e.g. Jayne, 1988a). We used 26 gauge hypodermic needles to insert a total of 12 electrodes percutaneously into the SSP, LD and IL muscle segments (Fig. 1) on both the left and right sides of the snake at two longitudinal locations. For all individuals, the more anterior electrode location resulted in the entire longitudinal extent of the implanted muscles transiently being in between the edges of the gap before the head of the snake touched the destination perch (Fig. 2A, between events 2 and 3). For four individuals, the muscles with electrodes at the posterior location (approximately 30 vertebrae posterior to the anterior site) usually experienced the same sequence of events as the anterior site (Fig. 2A). However, for three individuals, the posterior site was located approximately 50 vertebrae posterior to the anterior site. Consequently, when the most posteriorly located muscles with electrodes were in between the edges of the gap, the anterior end of the snake was already touching the destination perch (Fig. 2B, event 2). After the completion of a successful experiment, we euthanized the snakes using an overdose of sodium pentobarbital. Post-mortem dissections confirmed the exact location of each EMG electrode.

We amplified the EMGs using Grass P511 pre-amplifiers (Grass Technologies, Warwick, RI, USA) with a gain of 2000 and high- and low-pass filter cut-off frequencies of 30 Hz and 10 kHz, respectively. We converted the analog EMGs to digital data using a sampling rate of 4 kHz with LabChart 7 software and a PowerLab 16SP A/D converter (ADInstruments, Colorado Springs, CO,

USA). In addition to the 12 channels of EMGs, one channel displayed the square-wave voltage that drove the synchronizing flash of the LED.

Data analysis

After rectifying the voltages and correcting for the amplification of the digital EMGs in LabChart, we exported the data into Microsoft Excel to perform further analyses using bins with a duration 0.01 s. To remove baseline noise, we chose an appropriate threshold voltage (usually between 10 and 20 μV) and converted any values below that threshold to zero before further analysis. For each electrode we then calculated relative values of rectified integrated area (RIA) per bin during gap bridging by dividing each RIA per bin by the maximal RIA of any bin that was observed during any of the gap-bridging or control experiments.

We grouped the bins into different time intervals defined by the head and muscle positions relative to the gap (Fig. 2). For each time interval within each trial, we calculated average relative activity per bin as the total relative RIA of all bins divided by the number of bins with $\text{RIA} > 0$. We also calculated the percentage of bins with activity (%BWA) within a behavioral time interval (Fig. 2) by dividing the number of bins with activity by the total number of bins in the respective interval.

We used R (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria) to perform four-way mixed-model ANOVAs in which either average relative activity per bin or %BWA were the dependent variables. These analyses were limited to one longitudinal location and one side of the snake where the electrodes in the three non-homologous muscles remained in place for all of the treatments. One analysis was performed for the three treatments for the three pitch angles where yaw angle = 0 deg and another analysis was performed for the three yaw angles where pitch angle = 0 deg. All of these ANOVAs used trajectory angle ($N=3$), muscle ($N=3$) and behavioral time interval ($N=4$ or 3) as fixed and crossed factors, whereas individuals were a random, crossed factor. Hence, the F -tests for main effects divided the mean squares of the main effect by the mean squares of the two-way interaction term between the main effect and individuals (Scheffe, 1959).

RESULTS

Muscle activity

We first describe the events for snakes bridging a straight horizontal gap to provide a frame of reference before discussing how snakes bridged gaps with turns in either the horizontal or vertical planes. All three muscles (SSP, LD and IL) on both sides of the anterior site had extensive activity that began when their anterior end first entered the gap (Fig. 3A, event 1; Movie 1) and continued until the head touched the destination perch (Fig. 3A, time 3). Activity of both the SSP and LD ceased or decreased substantially soon after the head first touched the destination perch (Fig. 3A, event 3), and little high-amplitude or regular activity occurred thereafter until the posterior ends of the muscles reached the destination perch (Fig. 3A, time 5). In contrast to the SSP and LD, substantial activity of the IL continued on both sides of the snake after the head made first contact with the destination perch (Fig. 3A, event 3) and this activity continued until the posterior ends of the IL were supported by the destination perch.

Close examination of the EMGs during the early stages of crossing the gap revealed additional important details. First, on a fine time scale, the bursts of activity of homologous muscles on the left and right sides at the same longitudinal location were out of phase with each other such that when a left segment was active, the

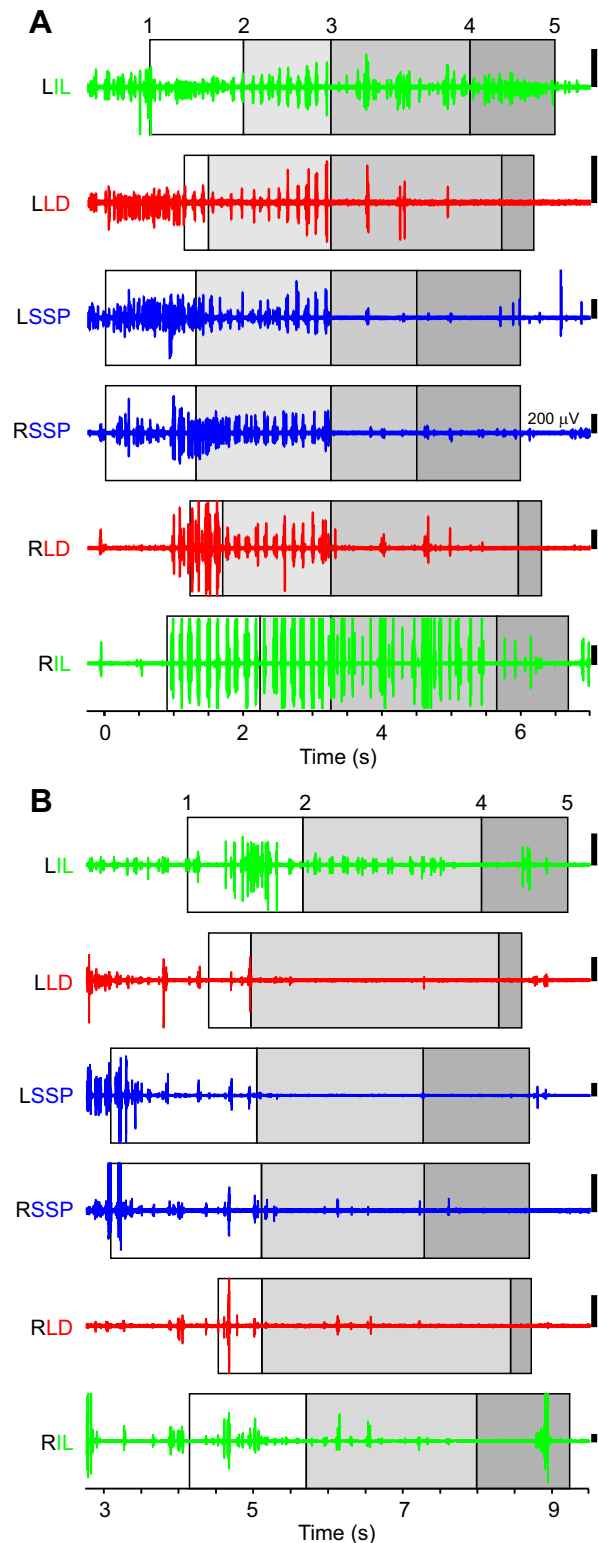


Fig. 3. Electromyograms (EMGs) from two longitudinal locations for crossing a gap where pitch and yaw angles are 0 deg. The EMGs of the SSP, LD and IL are blue, red and green, respectively. Within each panel, muscles on the left (L) and right (R) sides are the top and bottom three rows, respectively. For both the anterior (A) (vertebra 50) and far posterior (B) (vertebra 80) sites, the numbers and shaded rectangles indicate the sequence of events as a muscle segment crosses the gap (Fig. 2). Note for the posterior site, when the most anterior end of the muscle began to enter the gap, the head and neck were already supported by the destination perch. The most anterior extent of all of the implanted muscles entered the gap at 0 s.

right segment was not (Fig. 4A). Second, the fine-scale bursts from the contractile tissue of the three non-homologous muscles on a given side and a given longitudinal location were nearly synchronous (Fig. 4A). Finally, these small-scale EMG bursts commonly occurred with frequencies of approximately 5–10 Hz (Fig. 4A).

Comparisons of the timing of muscle activity between the anterior and posterior sites verified that the axial motor pattern during gap bridging propagated posteriorly, and this was often easiest to discern for the SSP segments, which often had a distinct

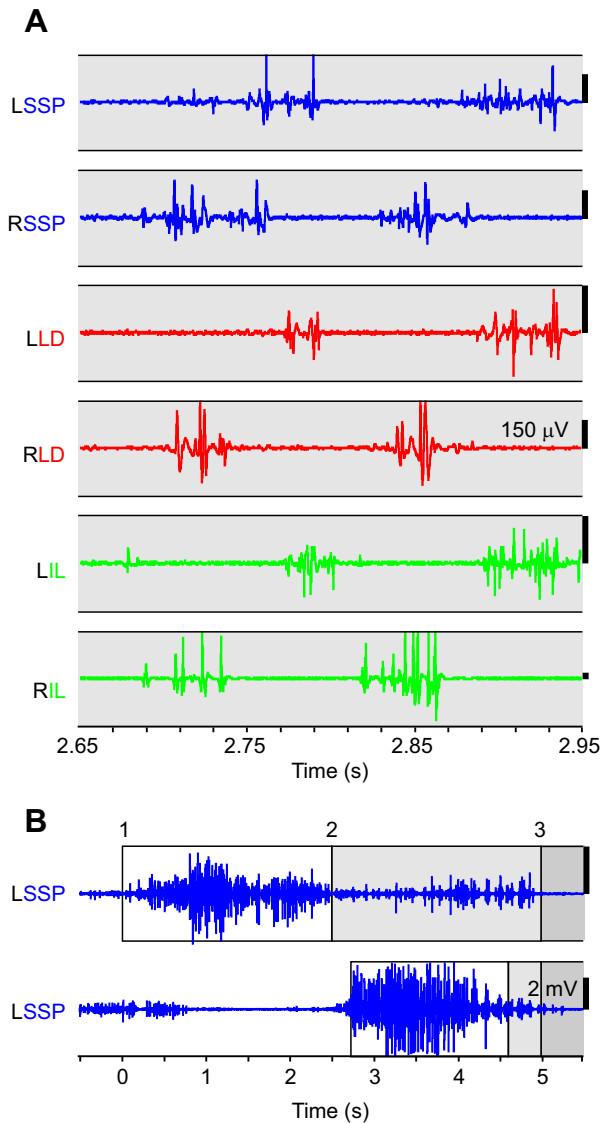


Fig. 4. Fine-time-scale view of EMGs from the anterior location of a single snake while crossing a straight horizontal gap. (A) The EMGs of the SSP, LD and IL are blue, red and green, respectively. Within each pair of EMGs from homologous muscles, the left (L) and right (R) sides are the top and bottom rows, respectively. For the time interval illustrated, all of the muscle segments were completely within the gap before the head touched the destination perch (Fig. 2A, between events 2 and 3). For all of the EMGs, 0 s occurred when the most anterior extent of all of the implanted muscles began to enter the gap. The EMGs are from the same sites, snake and trial as in Fig. 3A. (B) EMGs from SSP muscle segments on the left side of a different snake at one site (bottom) that was 30 vertebrae posterior to another site (top). Homologous events at the posterior site lagged behind those at the more anterior site.

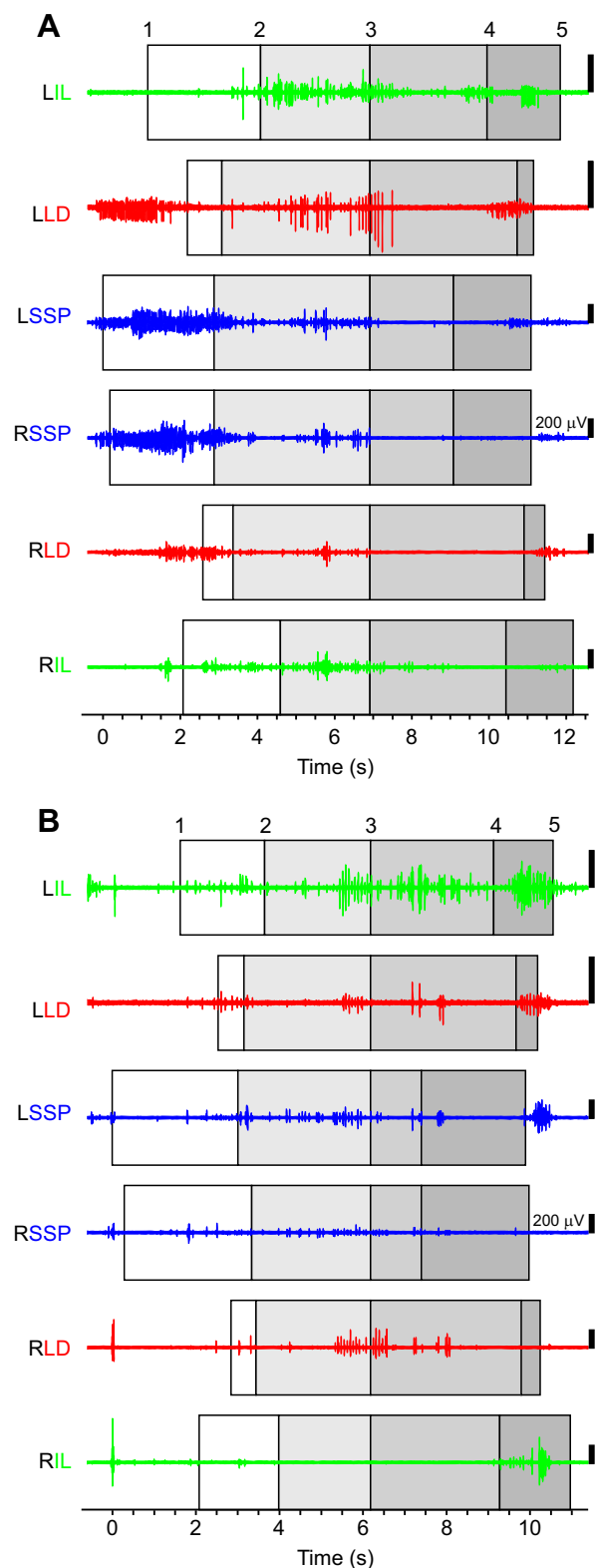


Fig. 5. EMGs from the anterior location of a single snake while crossing upward and downward gaps. The EMGs of the SSP, LD and IL are blue, red and green, respectively. The EMGs are for crossing gaps vertically upward (A) and vertically downward (B). Within each panel, muscles on the left (L) and right (R) sides are the top and bottom three rows, respectively. The numbers and shaded rectangles indicate the sequence of events as a muscle segment crosses the gap (Fig. 2A). For all of the EMGs, 0 s occurred when the most anterior extent of all of the implanted muscles began to enter the gap. The EMGs are from the same sites and snake as in Fig. 3A.

onset of activity as their anterior end first entered the gap (Fig. 4B). For example, for sites approximately 30 vertebrae apart in the trial illustrated in Fig. 4B, the onset of activity of the more posterior SSP segment lagged behind that of the more anterior segment, and the cessation of major activity of the more anterior segment coincided almost exactly with the onset of major activity of the more posterior site (Fig. 4B). Hence, approximately 30 adjacent segments of the SSP were simultaneously active, and this large longitudinal region of activity traveled posteriorly within a large region of the anterior portion of the snake.

The overall pattern of muscle activity differed between the most posterior location (Fig. 3B) and the two more anterior locations that we used to record EMGs. Namely, between events 1 and 2, the SSP and LD activity at the most posterior site was usually much less and quite irregular compared with the sustained high-amplitude activity when these muscle were located more anteriorly (Fig. 3A). For both the anterior and the most posterior sites, the beginning of event 2 coincided with the posterior end of the muscle segment entering the gap. Immediately thereafter, when the muscle segment was entirely within the gap, the SSP and LD of the most posterior site had effectively no activity, whereas the homologous muscles at the more anterior site continued to have prolonged major activity (Fig. 3B versus A). The reductions of muscle activity at the more posterior locations are consistent with a reduction in torque experienced at the edge of the initial perch that could result from the anterior portion of the snake contributing to the support of the part of the snake that was within the gap.

Effects of pitch angle

Similar to the anteriorly located SSP muscles while bridging a horizontal straight gap, the SSP muscles while bridging an upward vertical gap had prolonged and substantial activity between events 1 and 2 (Fig. 5A; Movie 1). By contrast, the activity of the IL between events 1 and 2 when going up a vertical gap was small or absent (Fig. 5A) compared to its sizable activity when crossing a horizontal gap (Fig. 3A, times 1–2). Compared with activity when crossing the horizontal gap (Fig. 3A), the activity of both the SSP and LD was greatly diminished shortly after event 2 (Fig. 5A) as the body of the snake was supported at the edge of the initial perch and the vertebrae flexed dorsally to elevate the head of the snake towards the destination (Fig. 5A).

Compared with all other trajectories, the activity of the SSP, LD and IL was small to non-existent when crossing gaps that were vertically downward (Fig. 5B versus Figs 3A and 5A; Movie 1). Hence, much of the movement vertically downward appeared to be passive.

Table 1. Results from two ANOVAs testing the effects of pitch angle on muscle activity

Effect	d.f.	Dependent variables	
		%BWA	Relative RIA
Pitch (P)	2, 12	5.6 (0.018)	1.7 (0.216)
Time (T)	3, 18	17.5 (<0.001)	1.9 (0.160)
Muscle (M)	2, 12	3.5 (0.064)	3.6 (0.060)
P×T	6, 36	20.4 (<0.001)	2.1 (0.081)
T×M	6, 36	10.6 (<0.001)	1.7 (0.141)
P×M	4, 24	0.97 (0.443)	0.25 (0.906)
P×T×M	12, 72	10.7 (<0.001)	1.5 (0.134)

Values are *F* (*P*). *n*=7 individuals and a total of 40, 39 and 31 trials for –90, 0 and 90 deg pitch, respectively. %BWA, percentage of bins with activity within a time interval; RIA, rectified integrated area per bin scaled relative to the maximum value observed.

Results from our ANOVAs further support conclusions regarding the effects of pitch angle on muscle activity. Many of the differences in the amount of activity within a given time interval resulted from varying amounts of time when activity was absent, and %BWA accounts for this. For %BWA at the anterior site, the main effects of both pitch angle and time interval were significant, and all the two- and three-way interaction terms involving time interval were also significant (Table 1). The main effect of pitch angle on %BWA arose from the overall amount of muscle activity being least for downward, greatest for horizontal and intermediate for the vertical upward trajectory (Fig. 6). The main effect of time interval on %BWA resulted from the greatest overall muscle activity occurring in interval 1 and the least amount of muscle activity occurring in interval 3 (Fig. 6). One example of an interactive effect was the amounts of activity varied little among time intervals for the vertical downward gap, whereas for the other trajectories the most activity usually occurred in the first time interval (Fig. 6). In addition, for the horizontal and vertical upward gaps, both the SSP and LD had similar and substantial variation among the four time intervals, whereas the IL had rather uniform activity across all of the corresponding time intervals (Fig. 6). The ANOVA did not detect any significant variation for the relative rectified integrated area per bin (for bins where activity was present) (Table 1).

Except for the downward trajectory, the mean amounts of activity of the SSP and LD (Figs 4 and 6) were consistently the lowest between events 3 and 4 while the head was touching the destination

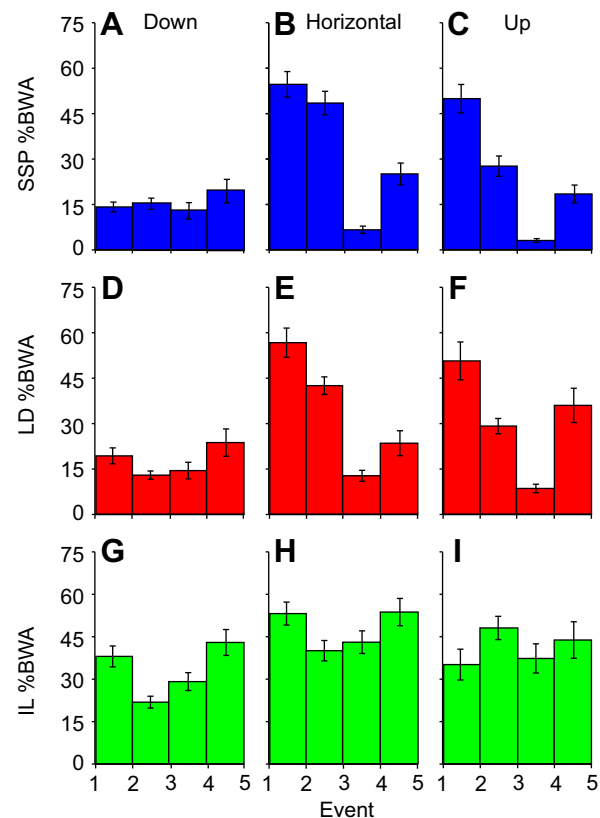


Fig. 6. Effects of pitch angle on the percentage of bins with activity for all muscles at the anterior site for gap trajectories with yaw=0 deg. The columns from left to right are for pitch angles of –90 deg (A,D,G; *n*=40), 0 deg (B,E,H; *n*=39) and 90 deg (C,F,I; *n*=31). The time intervals for the SSP (A–C), LD (D–F) and IL (G–I) during crossing the gap are as defined in Fig. 2A. All mean values are shown ± s.e.m.

and the muscles were confined to the gap. In many trials, effectively no activity of the SSP and LD occurred as these muscles crossed the edge of the destination perch (Fig. 3). However, in some trials, some EMGs between events 4 and 5 suggested that the muscles were pushing laterally against a peg near the edge of the destination (Fig. 5A, left SSP and LD; Movie 1). This sporadic activity (not always on the focal side used for statistical analysis) contributed to the higher mean values of activity between events 4 and 5 compared with those from event 3 to 4.

Effects of yaw angle

At a given point in time, the amplitudes of EMGs from homologous muscles on left and right sides of the snake often differed markedly when the snakes bridged gaps with 90 deg turns sideways (Fig. 7; Movie 1). This differed from trials with yaw=0 deg for which left and right homologous muscles usually had similar amplitudes of activity (Figs 3A and 5). In the trials with yaw=0 deg and pitch=0 deg, the left and right SSP segments both had large amplitudes of activity as soon as their anterior tendons entered the gap (Fig. 3A), whereas initially during a sideways turn the SSP muscle segments on the convex side of the body had little to no activity (Fig. 7A, right SSP; Fig. 7B left SSP) while the SSP on the concave side had large amounts of activity (Fig. 7A, left SSP; Fig. 7B right SSP). Some of the early activity of the SSP, LD and IL muscles (before and near event 1) was consistent with actively flexing the vertebrae toward the inner side of the turn and forming a concave region on the side of the active muscles (Fig. 7). Subsequently (from event 2 to 3), some of the large amplitude muscle activity was consistent with straightening the body as it changed from being convex on the side of muscle activity to being nearly straight as the same region was suspended in the gap (Fig. 7).

For %BWA at the anterior site, the main effects of both yaw angle and time interval were significant, and all the two- and three-way interaction terms involving time interval were also significant (Table 2). The main effect of yaw angle on %BWA arose from the overall amount of muscle activity being greater for the horizontal trajectory without a turn compared with that of both of the turns to either side, and the main effect of time interval resulted mainly from the least amount of activity usually occurring in time interval 3 regardless of yaw angle (Fig. 8). The most obvious interactive effect between time interval and yaw angle was that the proportion of time when muscles were active in intervals 3 and 4 was nearly constant for the SSP and LD (across all three yaw angles), whereas the amounts of activity in time interval 1 were diminished for sideways turns compared with straight trajectories (Fig. 8). The muscle by time interval interaction arose from little variation among the four time intervals for the IL compared with the other two muscles (Fig. 8).

DISCUSSION

Overall, we found considerable plasticity of axial motor patterns associated with: (1) the three-dimensional orientation of the snake, (2) where a muscle segment was relative to the edges of the gap and (3) whether a region was cantilevering or suspended between two points of support. We commonly observed considerable overlap in the activity of homologous muscle on both sides of the snake as well as between non-homologous muscles with the antagonistic functions of dorsal and ventral flexion. The extent to which muscular antagonists are co-activated may reflect some trade-offs between the needs for stability and economy of effort. Comparing the motor patterns used by snakes during gap bridging and other modes of locomotion can clarify what is novel or perhaps merely coopted from a motor pattern that is used in other contexts. Even

broader comparisons with other axial motor patterns of vertebrates can also provide insights into the extent to which very general strategies are used for supporting and moving the multi-segmented long axis that is the defining trait of the vertebrate body plan.

Effects of body posture and position on muscle activity and performance

Many of the variants of muscle activity that we observed corresponded well with: (1) biomechanical considerations for body support, (2) previous findings of how three-dimensional perch location affects gap bridging performance and (3) where some snakes choose to go. For example, the maximal distance performance of snakes bridging gaps in a horizontal plane is less than for gaps with either steep upward or downward trajectories (Byrnes and Jayne, 2012; Hoefer and Jayne, 2013). The highest amplitude EMGs in the SSP and LD at the edge of the initial perch occurred during horizontal trajectories, which implies that considerable muscular effort was required to prevent the body from buckling downward. The maximal gap distance that brown tree snakes can bridge with a horizontal trajectory is only about one-half of that when going straight down (Byrnes and Jayne, 2012), and this species prefers the latter to the former trajectory (Hoefer and Jayne, 2013). The minimal muscle activity at the edge of the initial perch suggests downward movement was nearly passive, and some snakes also use their own weight to slide down steep cylindrical surfaces (Astley and Jayne, 2007).

Brown tree snakes can bridge gap distances straight up nearly as long as those going straight down (Byrnes and Jayne, 2012), and they prefer to go up rather than horizontally (Hoefer and Jayne, 2013). Unlike the self-stabilizing, pendulum-like posture when going vertically downward, snakes going vertically upward resemble a less stable inverted pendulum, which should require more effort both to maintain balance and to lift the weight of the body that is in the gap (Byrnes and Jayne, 2012). For vertical gaps the proximity of supporting surface to the center of mass of the body in the gap greatly reduces the relevant moment arm contributing to either buckling or pitching torques, which in turn should be much smaller than those for horizontal trajectories (Byrnes and Jayne, 2012). The intermediate amounts of muscle activity that we observed for the vertical upward trajectories (Fig. 5A) compared with large and small amounts of activity observed for the horizontal (Fig. 3A) and vertical downward (Fig. 5B) trajectories, respectively, are consistent with these mechanical considerations. Collectively, many of these results for different pitch angles also suggest that these snakes will often choose a ‘path of least resistance’.

Although similar timing and amplitudes of muscle activity occurred on the left and right sides when yaw was zero (Figs 3 and 5), this was not the case when snakes turned horizontally (Fig. 7). Reducing the amount of activity of the primary lateral flexors (LD and IL) on one side while turning horizontally seems beneficial for reducing the effort required to laterally flex or straighten the body on the side with greater muscle activity. However, the early, nearly unilateral activity of the primary dorsiflexor (SSP) (Fig. 7, approximately 2 s after time 1) was unexpected, in part because of the strong bilateral activity known to arch the back of sidewinding snakes (Jayne, 1988b). Compared with bilateral activity, unilateral activity decreases the amount of contractile tissue available to prevent buckling, and this may contribute to the significant decreases in gap-bridging performance that occur with increased yaw angle (Byrnes and Jayne, 2012).

Additional conspicuous variation in muscle activity was associated with the longitudinal location of the muscle within the

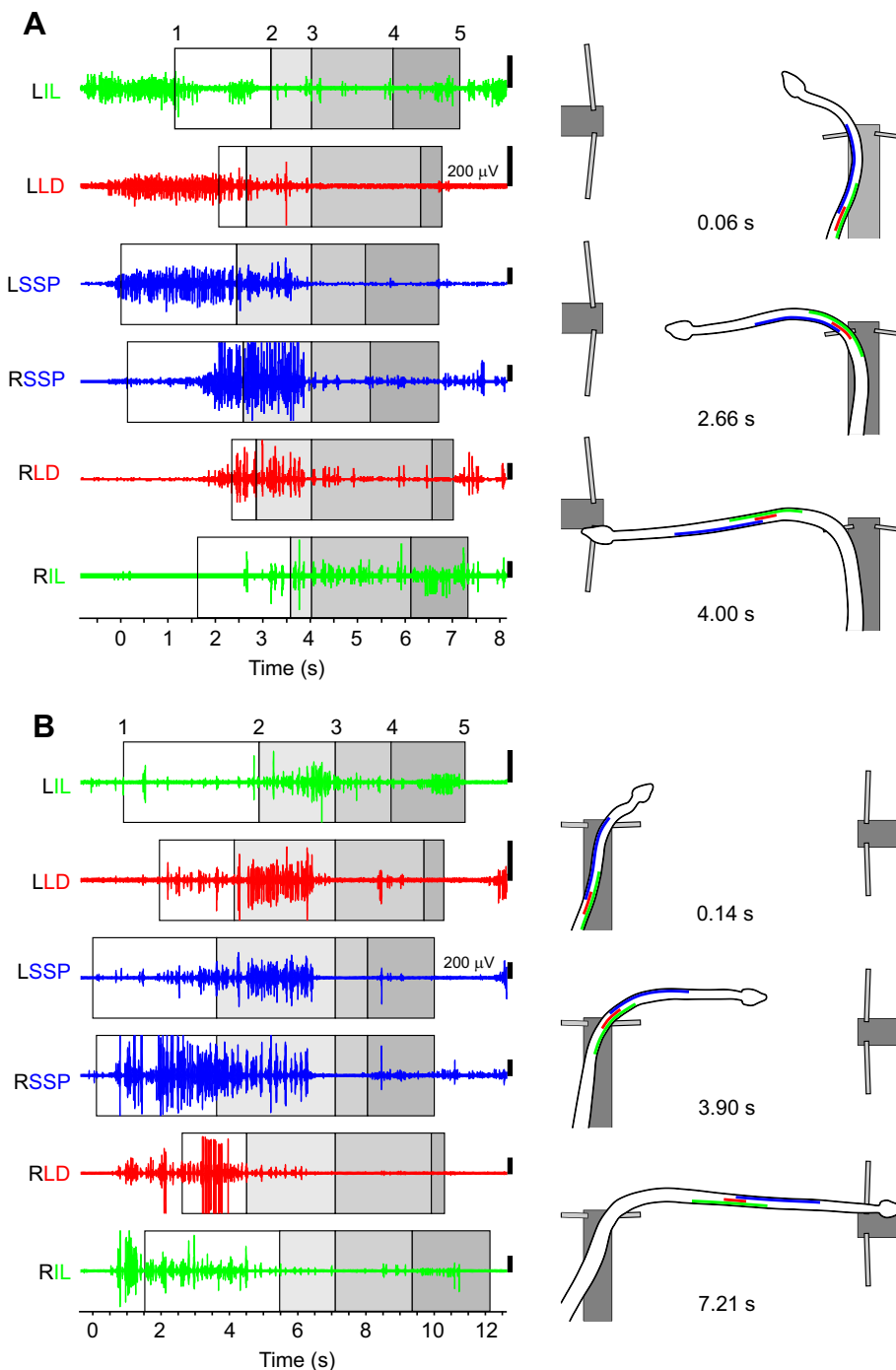


Fig. 7. EMGs from the anterior location of a single snake turning left and right while crossing horizontal gaps. The EMGs of the SSP, LD and IL are blue, red and green, respectively. The EMGs are for crossing horizontal gaps turning left (A) and right (B). Within each panel, muscles on the left (L) and right (R) sides are the top and bottom three rows, respectively. The numbers and shaded rectangles indicate the sequence of events as a muscle segment crosses the gap (Fig. 2A). For all of the EMGs, 0 s occurred when the most anterior extent of all of the implanted muscles began to enter the gap. The EMGs are from the same sites and snake as in Figs 3A and 4. The overhead views to the right of the EMGs in each panel were traced directly from synchronized video images. The colored lines within the snake outline indicate the approximate longitudinal extent of the right epaxial muscle segments from which the EMGs were obtained.

snake and the location of the snake relative to the edges of the gap. Upon entering the horizontal gaps and until the head touched the destination perch (Fig. 2A events 1–3), substantial activity occurred in the muscle segments within the most anterior portion of the snake that was equal to the distance of the gap (Figs 5 and 7). By contrast, when muscles located posterior to the head by more than the gap distance entered the horizontal gaps after the head was already supported (Fig. 2B, times 1–2), similarly large amounts of activity were lacking (Fig. 3B). This is an intriguing example of longitudinal variation in a propagated, axial locomotor motor pattern. Namely, a rather stereotyped high-amplitude pattern of bilateral activity of the SSP, LD and IL was propagated posteriorly (Fig. 4B) for a longitudinal distance only equal to the distance of the

gap rather than along the entire length of the snake (Fig. 3A versus B).

As soon as the head of the snake was supported by the destination perch, the activity of the anteriorly located SSP and LD decreased substantially, but at that point and for a considerable time thereafter the IL usually had sustained high levels of activity (Fig. 3, after 3.5 s). The position of the IL ventral to the vertebral column (Fig. 1) seems well suited for flexing the body ventrally. By contrast, the location of the SSP along the mid-dorsal line (Fig. 1) seems well suited for dorsi-flexion, and EMGs recorded from this muscle in other experiments correlate well with dorsi-flexion of the body as sidewinding snakes lift their bodies above the ground (Jayne, 1988b). An analogous pattern with the snakes using a ventral flexor

Table 2. Results from two ANOVAs testing the effects of yaw angle on muscle activity

Effect	d.f.	Dependent variables	
		%BWA	Relative RIA
Yaw (Y)	2, 12	5.7 (0.018)	3.8 (0.054)
Time (T)	3, 18	21.4 (<0.001)	2.5 (0.094)
Muscle (M)	2, 12	1.3 (0.309)	3.9 (0.050)
Y×T	6, 36	5.9 (<0.001)	1.5 (0.217)
T×M	6, 36	15.7 (<0.001)	1.8 (0.118)
Y×M	4, 24	1.4 (0.278)	0.62 (0.655)
Y×T×M	12, 72	3.1 (0.001)	1.6 (0.123)

Values are $F(P)$. $N=7$ individuals and a total of 37, 39 and 33 trials for concave, straight and convex trajectories, respectively. %BWA, percentage of bins with activity within a time interval; RIA, rectified integrated area per bin scaled relative to the maximum value observed.

(IL) to support the vertebral column when it is horizontal and supported by the two edges of a gap occurs for some training exercises commonly performed by humans. For example, during prone plank exercises the rectus abdominis muscles have very large amounts of activity and the activity of the spinal erectors is small compared with that during back extensions performed on a bench, when the spinal erector muscles have considerable activity and the activity of the rectus abdominis is small (Maeo et al., 2013). This nicely illustrates the different demands of preventing buckling

(ventral flexion) while cantilevering from a single point of support versus preventing sagging (dorsi-flexion) for the length of the body that is between two points of support.

In many musculoskeletal systems, coactivating muscular antagonists provides a mechanism for enhancing stability (Gardner-Morse and Stokes, 1998). Whether or not muscular antagonists are activated simultaneously may reflect some interesting potential trade-offs between stability and economy. Unlike the nearly exclusive reliance on the ventral flexors (IL) to prevent the snake from sagging between two points of support, prior to that when cantilevering across straight horizontal gaps, the left and right SSP, LD and IL all had substantial simultaneous activity (Fig. 3A). Such coactivation of pairs of muscular antagonists for dorsi-ventral flexion (SSP and IL) and lateral flexion (left and right LD) may be a mechanism for maximizing the stability and rigidity of the body, although this may not be the most energetically economical way to support the body.

Comparisons of vertebrate axial motor pattern and function

Many diverse vertebrates including fishes, amphibians and reptiles have retained the ancestral conditions of having equal numbers of axial muscular and skeletal segments and using posteriorly propagated lateral axial bending to generate propulsive forces. In fishes (Jayne and Lauder, 1994) and amphibians (Azizi et al., 2002), the convoluted overall shape of major axial muscle segments can span several vertebrae even though individual muscle fibers are approximately one vertebra long, and these groups have myosepta rather than elongate tendons. By contrast, the axial muscles of snakes commonly have longer contractile tissue and extremely long tendons. In some extreme cases, axial muscle segments span more than 40 vertebrae in slender colubrid snakes that are arboreal specialists (Jayne, 1982), and to the best of our knowledge the 30-vertebrae-long SSP segments that we studied in brown tree snakes (Fig. 1) are the longest axial muscles from which EMGs have been obtained for any species of vertebrate.

Biomechanical modeling suggests that a series of axial muscles spanning only one joint are much less effective for providing stability than a system where individual muscle segments span multiple joints and hence multiple muscle segments cross each joint (Crisco and Panjabi, 1991). Increased stability seems likely to enhance the ability to cross the gaps between branches in arboreal habitats. Furthermore, the combination of gaps between branches and small branch diameters can create very localized loads and hence increase the need to prevent buckling. By contrast, in aquatic environments, the needs for stability and coping with localized loads seem unlikely, and not only do the myomeres of fish span a modest number of vertebrae, so too some specialized aquatic snakes have short SSP segments (Jayne, 1982). Tendinous elongation theoretically could allow force to come from more contractile tissue to act across each joint if more serial homologues could be recruited simultaneously. The alternative of having more contractile tissue directly crossing each joint would result in a heavier animal ill suited for moving on thin and compliant branches.

As is the case for many musculo-skeletal systems, the axial muscles of snakes have considerable redundancy as more than 20 non-homologous axial muscles may cross a given pair of skeletal segments (Gasc, 1974). In addition to the SSP, the multifidus muscle is another dorsiflexor in snakes (Jayne, 1988b), but in brown tree snakes this muscle has a short tendon and a total length only one-fifth that of the SSP (Fig. 1). Thus, the short multifidus could facilitate some arboreal specialists retaining a capacity for some

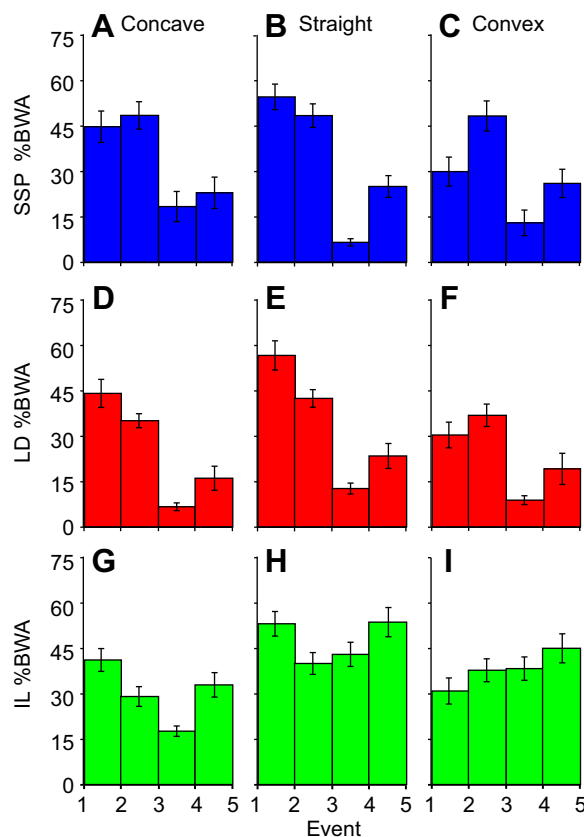


Fig. 8. Effects of yaw angle on the percentage of bins with activity for all muscles at the anterior site for horizontal gap trajectories. The columns from left to right are for turns that initially required the side of the snake with the muscles of interest to be concave toward the side of the muscles (A,D,G; $n=37$), straight (B,E,H; $n=39$) and convex (C,F,I; $n=33$). The progression of events for the SSP (A–C), LD (D–F) and IL (G–I) during crossing the gap is as defined in Fig. 2A. All mean values are shown \pm s.e.m.

very localized dorsiflexion as specialized elongated SSP segments assume a primary role for preventing local deformation.

The considerable lengths and overlapping arrangements of serially homologous axial muscle segments in vertebrates also pose challenges for understanding what comprises a functional unit based on anatomy alone. Theoretically, animals could have a propagated axial motor pattern where muscle segments are activated in isolation one by one if the activity of one muscle segment ceased before the onset of activity in the next more posterior segment. However, whenever axial muscle segments are activated sequentially during the locomotion of vertebrates such as lampreys, bony fishes, salamanders and snakes (Frolich and Biewener, 1992; Jayne, 1988a; Williams et al., 1989), a recurrent finding is that several adjacent, ipsilateral serial homologues have simultaneous activity.

For propagated motor patterns, the number of adjacent ipsilateral axial muscle segments with simultaneous activity equals the duration of the EMG of a single segment divided by the lag time per segment for the onsets of activity. For the propagated motor pattern used by brown tree snakes to support their body at the edge of a gap (Fig. 4B), the combination of EMG duration and the rate of propagation resulted in a number of adjacent muscle segments with simultaneous activity often corresponding almost exactly to the number of vertebrae (30) spanned by an individual SSP segment (Fig. 1). This finding agrees well with a previous suggestion that a possible advantage of having long muscle segments in arboreal snakes is that a greater amount of contractile tissue can be recruited to produce tension in all of the overlapping tendons that cross a single joint between adjacent vertebrae (Jayne and Riley, 2007). For other types of snake locomotion, the number of ipsilateral, adjacent, serially homologous muscle segments with simultaneous activity commonly exceeds the number of vertebrae spanned by an individual muscle segment, and this quantity may vary at a single point in time for different longitudinal locations within a snake that have different wavelengths of bending (Jayne, 1988a). No previous electromyographic studies of propagated motor patterns in snake locomotion have found a number of adjacent simultaneously active ipsilateral segments less than the number of vertebrae spanned by a single segment, but this does occur occasionally for some of the highly localized bending that occurs during concertina locomotion in narrow tunnels (Gasc et al., 1989; Jayne, 1988a,b; Moon and Gans, 1998).

Our observed patterns of muscle activity for snakes bridging gaps in different directions were much more variable than those described previously for diverse modes of snake locomotion. During aquatic and terrestrial lateral undulation and tunnel concertina locomotion, the activity of ipsilateral SSP, LD and IL muscles at a given longitudinal location is unilateral and approximately synchronous, and it usually alternates with the activity of the contralateral muscles (Gasc et al., 1989; Jayne, 1988a, b; Moon and Gans, 1998). Two noteworthy features of the motor pattern of sidewinding snakes are: bilateral activity occurs in the SSP segments where the body is being lifted, and the activity of the SSP can be decoupled from that of the ipsilateral segments of the LD and IL. Similarly, during gap bridging the activity of the SSP on the left and right sides of the snake commonly had extensive temporal overlap, and we often observed SSP activity decoupled from that of the LD. However, the bilateral activity of the LD and IL that we observed during gap bridging has never been observed in any previous study of the motor patterns used during snake locomotion. Another novel finding for the muscle activity during gap bridging was that IL activity could be decoupled from that of both the SSP

and LD at the same longitudinal location. However, we never observed any clear instance of LD activity being decoupled simultaneously from that of both the SSP and IL.

Some of the differential timing of the activity of non-homologous, ipsilateral muscles at a given longitudinal location when the snakes crossed gaps resembles the variable motor patterns associated with the needs of other vertebrates to bend laterally or support the vertebral column. For example, the axial motor pattern of walking versus swimming salamanders differs as a result of some axial muscles having activity that is decoupled from a role in lateral flexion and instead supports and stabilizes the trunk between the limbs (Carrier, 1993; Deban and Schilling, 2009; Frolich and Biewener, 1992). A key innovation in the evolution of vertebrates was acquiring two pairs of limbs that could elevate the trunk above the ground, but this was secondarily lost in limbless taxa such as snakes. Such transitions presumably change the primacy of the need to prevent sagging along the length of the body. Furthermore, similar to the standing of limbed animals, the mechanical demand of preventing sagging between two points of support re-emerges when limbless animals move on discontinuous surfaces such as branches.

Although we commonly observed simultaneous activity of the left- and right-side muscles on a coarse time scale (Fig. 3), the activity of left and right pairs of homologous muscles at a given longitudinal location alternated on a fine time scale (Fig. 4A). Such a pulsed, alternating motor pattern (Fig. 4A) has not been observed in any previous electromyographic study of snake locomotion (Gasc et al., 1989; Jayne, 1988a,b; Moon and Gans, 1998). This fine-scale alternating pattern of activity might reflect reciprocal inhibition, which is a very common feature of the spinal locomotor circuits in vertebrates (Cangiano and Grillner, 2005). Perhaps the pulsed pattern of muscle activation provides a mechanism for reducing the rate of fatiguing. Gap bridging of snakes is often quite prolonged, and we have observed brown tree snakes maintaining a cantilevered position for several minutes. Some of the EMGs obtained from snakes during constriction (Moon, 2000) may have a pulsed pattern. However, the long time scale of these published illustrations complicates determining definitively whether a pulsed pattern occurs and, if so, whether fine-scale activity was unilateral at a single longitudinal location. The constricting behavior of snakes is another example of a prolonged activity (Boback et al., 2012), which may also benefit energetically by using a pulsed pattern rather than continuous pattern of activity.

Our current working hypotheses for the global function of the three major epaxial muscles (SSP, LD and IL) are as follows. The SSP can be used in isolation from either the neighboring ipsilateral LD or IL, and SSP activity can be either unilateral (Fig. 7) or bilateral (Figs 3 and 5A) for dorsi-flexion of the vertebrae or for static body support. For maintaining a rigid static posture, LD and IL activity may also be bilateral. The IL can be used in isolation from the other two muscles for active ventral flexion or to prevent sagging between two points of support. The function of the IL is especially interesting because anatomically this is an epaxial muscle, but it has a function (ventral flexion) similar to that of many hypaxial muscles. All three of these muscles can contribute to lateral flexion (making the body more concave toward the side of the active muscles), but our results and those of all previous studies with EMGs for snakes suggest that the LD appears unable to do so in the absence of activity of either the SSP or the IL.

Many features of propulsive axial motor patterns appear to be widely conserved across diverse ectothermic vertebrates, but most prior work on this topic has studied planar movements of animals traveling in a straight line. In light of this, perhaps it should not be

too surprising that snakes moving with diverse three-dimensional trajectories had a corresponding diversity of axial motor patterns much greater than that which had been previously described. Compared with some other limbless ectothermic vertebrate lineages, such as several clades of lizards and salamanders, caecilians and amphisbaenians, snakes live and move in a much greater diversity of habitats. Perhaps the plasticity of their axial locomotor motor patterns has contributed to this ecological success and diversity.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: B.C.J., R.M.J.; Methodology: B.C.J., R.M.J.; Formal analysis: B.C.J., R.M.J.; Investigation: B.C.J., R.M.J.; Resources: B.C.J.; Writing - original draft: B.C.J., R.M.J.; Writing - review & editing: B.C.J.; Visualization: R.M.J.; Supervision: B.C.J.; Project administration: B.C.J.; Funding acquisition: B.C.J.

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Supplementary information

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